gpen

melbourne, australia

2012

MONASH University
Pharmacy and Pharmaceutical Sciences

Table of Contents

Introduction

Meeting Schedule

Podium Presentations

Poster Presentations

Short Courses

Delegate List

Miscellaneous

Welcome to GPEN 2012

Dear GPEN 2012 participants,

It is with great pleasure that we welcome you to the 9th biennial meeting of the Globalization of Pharmaceutics Education Network (GPEN). We are proud to announce that this meeting in Melbourne, the first to be held in the Southern Hemisphere, has attracted over 270 attendees from around the world. This comprises graduate students, postdoctoral fellows and faculty representatives from 50 member institutions across five continents and two newly invited institutions from Australia, in addition to industrial representatives from Australia and abroad.

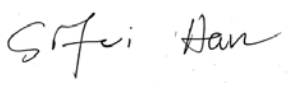
GPEN 2012 will include 50 podium presentations and 86 poster presentations by graduate students and postdoctoral fellows. We are also excited to host eight short courses, chaired by local and international faculty experts, which span a wide array of topics relevant to current and future pharmaceutical scientists.

The GPEN 2012 Career Centre will occur concurrently with the short course sessions, providing students and postdoctoral fellows with the opportunity to discuss potential career opportunities and goals with the participating industrial representatives.

We are also very pleased to have Monash's own Professor Nigel Bunnett deliver the GPEN 2012 keynote lecture, entitled 'Great expectations: a cautionary tale of the hopes and disappointments of developing treatments for inflammation and pain'. This will be followed by the Welcome Reception, the first of our social events which we have developed to ensure that GPEN 2012 provides to our attendees a nice balance between great science and great social interactions.

We realise that many of you have made significant efforts to travel to Australia and we are very thankful that you have taken this opportunity. We encourage you to enjoy the sights of our beautiful city and country, and if there is anything that we can do to assist you during GPEN 2012, please do not hesitate to ask one of our friendly GPEN 2012 Organising Committee members.

We wish you a very pleasant stay and hope you have a wonderful experience at GPEN 2012 and in Australia.



Sifei Han
Student Chair
GPEN 2012 Organising Committee

Joseph A. Nicolazzo
Faculty Chair
GPEN 2012 Organising Committee

GPEN 2012 Organising Committee

Top row: Sifei Han (Student Chair), Dr Joseph Nicolazzo (Faculty Chair)
Middle row: Gordon Lee, Yijun Pan, Orlagh Feeney, Gemma Nassta, Annette Dahlberg
Bottom row: Caroline Le, Gemma Ryan, Durgesh Tiwari

The GPEN 2012 Organising Committee is comprised of talented postgraduate students from the Faculty of Pharmacy and Pharmaceutical Sciences, Monash University at various stages of their Ph.D. candidature. In addition, the committee received valuable advice and guidance from the faculty chair Dr Joseph Nicolazzo.

About the Meeting Hosts

The Faculty of Pharmacy and Pharmaceutical Sciences, Monash University has an established reputation in world-class research and education. The key research initiatives within the faculty are the Monash Institute of Pharmaceutical Sciences (MIPS) and the Centre for Medicine Use and Safety (CMUS).

MIPS is one of the largest, best equipped and most experienced groups of pharmaceutical scientists in Australia. Over 160 scientists and 120 Ph.D students undertake basic and translational drug discovery, drug delivery and drug development research in new, state-of-the-art laboratories at the Monash Parkville campus. The contemporary and collaborative organisational structure of MIPS enables research to occur where core scientific disciplines meet. MIPS staff includes international leaders in G protein-coupled receptor biology, translational medicinal chemistry, structural biology, lead candidate optimisation and drug delivery.

CMUS is focused on delivering innovative research which optimises the health outcomes for individuals and communities in Australia and around the world. Based in the Monash University Parkville campus and the Pharmacy department of The Alfred hospital, CMUS integrates academic and applied research to address medicine use and safety issues in the home, community, hospitals, residential aged-care facilities and their interfaces. CMUS aims to deliver high-quality and timely research by combining key components of in-house expertise with existing multidisciplinary collaborators. The ultimate goal of CMUS is to optimise the safe and effective use of medicine.

Keynote Lecture

Wednesday, 28th November 2012
Lecture Theatre 3
5:00 pm - 6:10 pm

"Great expectations: a cautionary tale of the hopes and disappointments of developing treatments for inflammation and pain"

Professor Nigel Bunnett, B.Sc, Ph.D.
Professor of Pharmacology and Medicine
NHMRC Australia Fellow
Theme Leader - Drug Discovery Biology
Monash Institute of Pharmaceutical Sciences

Prof. Nigel Bunnett was educated at Cambridge University where he was awarded a Ph.D. degree in 1981. He spent the next 30 years of his career on the West Coast of the United States, as a post-doctoral fellow at the University of California, Los Angeles, and then as an Assistant Professor at the University of Washington, Seattle. In 1987 he joined the University of California, San Francisco (UCSF), and he remained there for almost 25 years, becoming Professor of Surgery and Physiology, Vice Chair of Surgery, and Director of the UCSF Center for the Neurobiology of Digestive Diseases. Prof. Bunnett relocated to Monash University, Melbourne in 2011, where he holds appointments as National Health and Medical Research Council (NHMRC) Australia Fellow and Professor of Pharmacology and Medicine at Monash Institute of Pharmaceutical Sciences.

Prof. Bunnett's research focuses on understanding the mechanisms of inflammation and pain which underlie diseases of global relevance. He is particularly recognised for his work on defining the functions and regulation of G protein-coupled receptors and transient receptor potential ion channels, two major classes of cell-surface proteins that are essential for the transmission of inflammation and pain. Prof. Bunnett's work has been reported in over 280 research papers, reviews and chapters, and is funded by the NHMRC, Australian Research Council and National Institutes of Health (NIH). His contributions have been recognised by awards that include an Australia Fellowship, an NIH MERIT Award, the Novartis Neurogastroenterology Award, the Jansen Award for Basic Research in Gastroenterology, and the Victor Mutt Award for Research in Regulatory Peptides. Throughout his career, Prof. Bunnett has been committed to medical education, and he has received numerous awards in recognition of his dedication to teaching.

Acknowledgements

The Organising Committee sincerely expresses their gratitude to everyone who contributed to the success of the GPEN 2012 meeting. Particular thanks go to:

- Professor Ron Borchardt and the GPEN Executive Committee and Board of Directors (Professors Kenneth Audus, Per Artursson, Peter Swaan, Yoshi Takakura, Dhiren Thakker, Patrick Augustijns, and Jay Sisco)
- Ms Nancy Helm (University of Kansas)
- Professor Bill Charman (Dean, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University)
- Professor Nigel Bunnett (Keynote speaker)
- Faculty of Pharmacy and Pharmaceutical Sciences Professional Staff (Ms Marian Costelloe, Ms Deborah Horne, Mrs Jessica Rostas, Mrs Gianni Iliopoulos, and Mr Luke Campbell)
- All judges of oral and poster presentations
- All industry observers
- All short course chairs
- All faculty and industrial delegates participating in short courses
- GPEN 2010 Planning Committee (University of North Carolina, USA)

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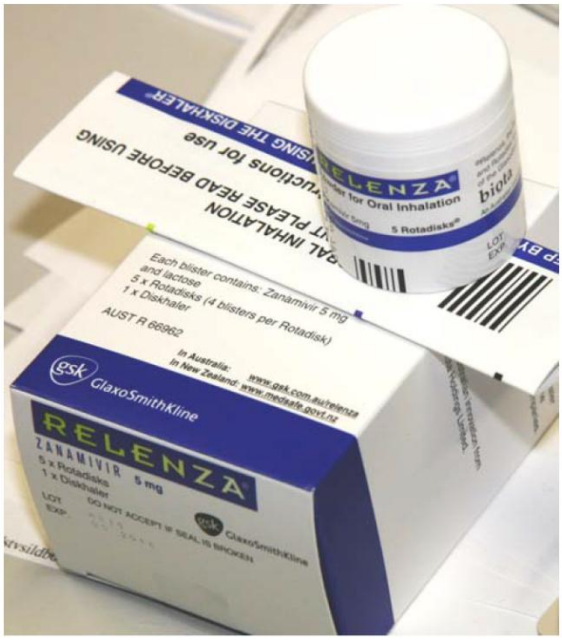
Biota has a wealth of experience in medicinal chemistry, virology, microbiology, drug characterisation and non-clinical/clinical development. Building from the proud history of class leading innovative products for the diagnosis and treatment of influenza Biota has discovered and developed an exciting channel extending into antivirals for other respiratory viruses to blood borne viruses and beyond, to the field of antibacterials.



The company's staff is dominated by expert teams of scientists, all with higher degrees and strong experience in drug discovery and development. Its commercial partners include Daiichi-Sankyo (long acting neuraminidase inhibitors for influenza) and GlaxoSmithKline 'GSK' (Relenza).

Biota's foundations are innovative research and development conducted by teams of dedicated professionals. The scientists are focused on the discovery, development and registration of new medicines for the treatment of human infectious diseases, including those caused by the ever increasing burden of drug resistant infectious agents.

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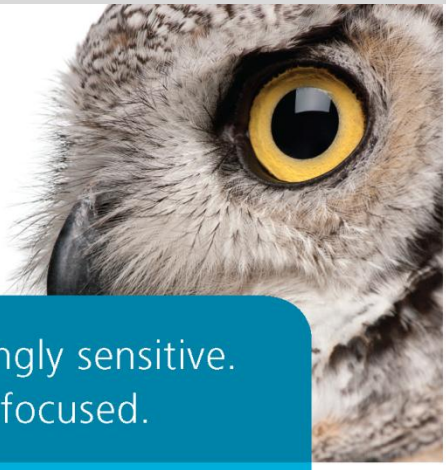

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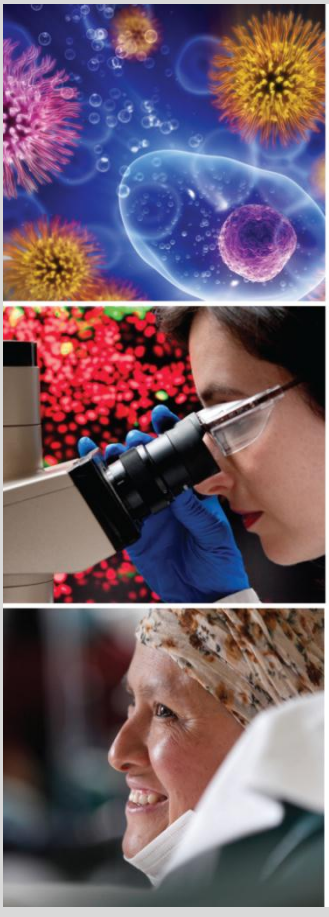

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Schedule

Meeting Overview

Wednesday 28th November	**Thursday** 29th November	**Friday** 30th November	**Saturday** 1st December	**Sunday** 2nd December
	9:00 AM - 9:10 AM Opening Comments	9:00 AM - 12:30 PM Morning Short Courses and Podium Presentations/ Career Centre	9:00 AM - 12:10 PM Podium Presentations	9:15 AM - 5:00 PM Optional Social Activity
	9:10 AM - 1:00 PM Podium Presentations			
		12:30 PM - 1:30 PM Lunch	12:10 PM - 1:30 PM Lunch and Poster Presentations	
	1:00 PM - 2:20 PM Lunch and Poster Presentations	1:30 PM - 5:00 PM Afternoon Short Courses and Podium Presentations/ Career Centre	1:30 PM - 4:40 PM Podium Presentations	
3:00 PM - 5:00 PM Registration	2:20 PM - 6:10 PM Podium Presentations		4:40 PM - 5:00 PM Conference Closing and Awards Ceremony	
5:00 PM - 6:10 PM Keynote Lecture				
6:15 PM - 9:00 PM Welcome Reception	6:30 PM - 9:30 PM BBQ Dinner	7:00 PM - 11:00 PM Conference Banquet	7:00 PM - 09:30 PM Optional Social Event	

Note: Poster sessions will occur during lunch breaks on all three days, however judging will only occur on either Thursday or Saturday.
The Career Centre will occur concurrently with the short courses on Friday.

Wednesday Schedule

Time	Location	Event
3:00 PM	Cossar Hall	**Registration**
5:00 PM	Lecture Theatre 3	**Keynote Lecture** *Professor Nigel Bunnett,* *Monash University, Australia*
6:15 PM	Cossar Hall	**Welcome Reception**
9:00 PM	Courtyard outside Cossar Hall	**Bus departure for hotels***

* Shuttle buses will be provided after the Welcome Reception to transport delegates back to hotels. Buses will depart at 9:00 PM and will stop at four conference hotels (IBIS on Therry St, Jasper, Novotel and IBIS on Little Bourke St) in Melbourne CBD.

Thursday Schedule

Time	Location	Event
9:00 AM	Lecture Theatre 3	**Opening remarks**
9:10 AM	Lecture Theatre 3	**Podium presentations**
10:50 AM	Cafeteria	**Morning tea**
11:20 AM	Lecture Theatre 3	**Podium presentations**
1:00 PM	Cafeteria/Cossar Hall	**Lunch/poster presentations**
2:20 PM	Lecture Theatre 3	**Podium presentations**
4:00 PM	Cafeteria	**Afternoon tea**
4:30 PM	Lecture Theatre 3	**Podium presentations**
6:10 PM	Courtyard outside Cossar Hall	**Walk to Visy Park**
6:30 PM	Visy Park, Royal Parade, Carlton North	**BBQ Dinner**
8:45 PM, 9:30 PM	Visy Park, Royal Parade, Carlton North	**Bus departure for hotels***

* Shuttle buses will be provided after the BBQ dinner. The first fleet of buses will depart at 8:45 PM and will return for the second trip, departing at approximately 9:30 PM. All buses will stop at the four conference hotels (IBIS on Therry St, Jasper, Novotel and IBIS on Little Bourke St) in Melbourne CBD.

Friday Schedule

Time	Location	Event
9:00 AM		**Morning Short Courses commence**
	Lecture Theatre 1	**<u>Morning Short Course 1:</u> Barriers and Novel Approaches for Effective Delivery of Biotherapeutics** *Professor Wim Hennink, Utrecht University and Professor Claus-Michael Lehr, Saarland University*
	Lecture Theatre 2	**<u>Morning Short Course 2:</u> Novel Approaches for Solid State Characterisation** *Professor Thomas Rades, University of Copenhagen*
	Lecture Theatre 3	**<u>Morning Short Course 3:</u> Pharmacokinetic-Pharmacodynamic Modelling in Drug Safety and Efficacy** *Professor Carl Kirkpatrick, Monash University*
	Lecture Theatre 5	**<u>Morning Short Course 4:</u> Lead Optimisation in Drug Discovery and Development** *Professor Susan Charman, Monash University*
10:30 AM	Cafeteria	**Morning tea**
11:00 AM	Short Course venues, as listed above	**Morning Short Courses continue**
12:30 PM	Cafeteria	**Lunch**

Friday Schedule (Continued)

Time	Location	Event
1:30 PM		**Afternoon Short Courses commence**
	Lecture Theatre 1	**Afternoon Short Course 1: Approaches for Enhancing Oral Delivery of Poorly-Water Soluble Drugs** *Professor Chris Porter, Monash University*
	Lecture Theatre 2	**Afternoon Short Course 2: Formulation and Stability of Proteins, Antibodies and Vaccines** *Professor Christian Schöneich, University of Kansas*
	Lecture Theatre 3	**Afternoon Short Course 3: Imaging Modalities for Assessing Drug Delivery and Disposition** *Dr Erica Sloan, Monash University and Professor Jashvant Unadkat, University of Washington*
	Lecture Theatre 5	**Afternoon Short Course 4: Transporters as Mediators of Drug Disposition in Health and Disease** *Professor Kim Brouwer, University of North Carolina and Professor Peter Swaan, University of Maryland*
3:00 PM	Cafeteria	**Afternoon tea**
3:30 PM	Short Course venues, as listed above	**Afternoon Short Courses continue**
5:00 PM		**Short Courses conclude**
7:00 PM	RACV Club, 501 Bourke Street, Melbourne	**Conference Banquet**
10:30 PM, 11:00 PM	RACV Club, 501 Bourke Street, Melbourne	**Bus departure for hotels***

* Shuttle buses will be provided after the conference banquet. The first fleet of buses will depart at 10:30 PM and will return for the second trip, departing at approximately 11:00 PM. Please see the sign in the front of each bus to find the relevant bus, as buses will be heading to different hotels.

Saturday Schedule

Time	Location	Event
9:00 AM	Lecture Theatre 3	**Podium presentations**
10:20 AM	Cafeteria	**Morning tea**
10:50 AM	Lecture Theatre 3	**Podium presentations**
12:10 PM	Cafeteria/ Cossar Hall	**Lunch/poster presentations**
1:30 PM	Lecture Theatre 3	**Podium presentations**
2:50 PM	Cafeteria	**Afternoon tea**
3:20 PM	Lecture Theatre 3	**Podium presentations**
4:40 PM	Lecture Theatre 3	**Awards presentation and closing remarks**
7:00 PM	The Mercat, 456 Queen Street, Melbourne	**Optional social event**
9:30 PM, 10:15 PM, 11:00 PM	The Mercat, 456 Queen Street, Melbourne	**Bus departure for hotels***

* Shuttle buses will be provided after the social event. The first bus will depart at 9:30 PM and will return for the second trip departing at approximately 10:15 PM, followed by the third trip departing at approximately 11:00 PM. All buses stop at five conference hotels (IBIS on Therry St, Jasper, Novotel, IBIS on Little Bourke St and Vibe) located in Melbourne CBD or Parkville.

Sunday Schedule (Optional Day Trip)

Time	Location	Event
9:15 – 9:30 AM	381 Royal Parade, Parkville	**Bus boarding**
9:30 AM - 4:00 PM	Healesville Sanctuary and Yarra Valley Wineries	**Sanctuary tour, lunch, wine tasting**
4:00 PM	Bus stop at wineries	**Bus departure and travel back to 5 hotels in Melbourne**

Bus will depart Monash University Parkville Campus at 9:30 AM and will return at approximately 5:00 PM.

Podium Presentations

Podium Presentations: Session 1

Date: Thursday 29th November 2012

Time: 9:10 am - 1:00 pm

Location: Lecture Theatre 3

Time	Presenter	Presentation Title
9:10 AM	O-TH-01 **Haruki Higashino** *Setsunan University* *Japan*	Effect of supersaturation on oral absorption of BCS Class II drugs: Quantitative evaluation by successive in vitro dissolution and in vivo absorption studies in rat
9:30 AM	O-TH-02 **Jitinder Singh Wilkhu** *Aston University* *United Kingdom*	Understanding the transit of bilosomes and antigen via the oral route
9:50 AM	O-TH-03 **Daniel Wolak** *University of Wisconsin* *USA*	Probing diffusion limitations on antibody distribution in brain using real-time optical imaging
10:10 AM	O-TH-04 **Jonathan Sockolosky** *University of California-San Francisco* *USA*	Recombinant proteins with short peptide extensions that bind to human FcRn undergo receptor-mediated recycling and transcytosis
10:30 AM	O-TH-05 **Jong-Suep Baek** *Chungnam National University* *Korea*	Paclitaxel loaded SLN modified with 2-hydroxypropyl-beta-cyclodextrin for overcoming P-glycoprotein function in multidrug-resistant breast cancer cells
10:50 AM	***Morning tea***	
11:20 AM	O-TH-06 **Nicole Payton** *University of Colorado* *USA*	Degradation of liposomal formulations during lyophilization
11:40 AM	O-TH-07 **Hristo Zlatev** *University of Eastern Finland* *Finland*	Uptake and intracellular distribution of bisphosphonates

Time	Presenter	Presentation Title
12:00 PM	O-TH-08 **Anita Nair** *University of Frankfurt Germany*	Can multisource immediate release tenofovir disoproxil fumarate products qualify for BCS based biowaivers?
12:20 PM	O-TH-09 **Isabelle Kohler** *University of Geneva Switzerland*	Capillary electrophoresis in bioanalysis: Application to transferrin in chronic alcohol abuse
12:40 PM	O-TH-10 **Secil Caban Toktas** *Hacettepe University Turkey*	Preparation and characterization of Z-DEVD-FMK and basic fibroblast growth factor (bFGF) loaded chitosan nanoparticles

Podium Presentations: Session 2

Date: Thursday 29th November 2012

Time: 2:20 pm – 6:10 pm

Location: Lecture Theatre 3

Time	Presenter	Presentation Title
2:20 PM	O-TH-11 **Helena Shifrin** *The Hebrew University of Jerusalem* *Israel*	Rivastigmine, a cholinesterase inhibitor, reduces intestinal inflammation by indirect activation of alpha7 cholinergic receptors
2:40 PM	O-TH-12 **Astrid Subrizi** *University of Helsinki* *Finland*	Generation of human embryonic stem cell-derived retinal pigment epithelium on biopolymer coated polyimide membranes
3:00 PM	O-TH-13 **Randall Logan** *University of Kansas* *USA*	Studies on the mechanisms and consequences of intracellular drug-drug interactions involving lysosomes
3:20 PM	O-TH-14 **Niels Vande Casteele** *KU Leuven* *Belgium*	Therapeutic drug and immunogenicity monitoring of the monoclonal antibody infliximab to personalize treatment of inflammatory bowel disease patients: Results on the optimization phase of the TAXIT trial
3:40 PM	O-TH-15 **Sweta Modi** *University of Kentucky* *USA*	Optimization of liposome release kinetics of a corticosteroid phosphate based on pH and lipid composition
4:00 PM	***Afternoon tea***	
4:30 PM	O-TH-16 **Fatma Goksin Bahar** *Kumamoto University* *Japan*	Characterization of plasma hydrolases and their species differences
4:50 PM	O-TH-17 **Mitsuru Ando** *Kyoto University* *Japan*	Complete elimination of hepatitis C virus by sustained nonviral gene delivery of human interferon γ in human hepatocyte chimeric mice
5:10 PM	O-TH-18 **Ousama Rachid** *University of Manitoba* *Canada*	A preclinical study of new-generation, rapidly-disintegrating taste-masked epinephrine sublingual tablets for the treatment of anaphylaxis

Time	Presenter	Presentation Title
5:30 PM	O-TH-19 **Brittany Avaritt** *University of Maryland* *USA*	Mechanisms of PAMAM dendrimer-mediated tight junction modulation
5:50 PM	O-TH-20 **Yajun Liu** *University of Michigan* *USA*	Investigation of pH distribution in degrading microspheres of hydrophilic poly(d,l-lactide-co-hydroxymethyl glycolide) by confocal laser scanning microscopy

Short Course Student Presentations

Date: Friday 30th November 2012

Time: Morning Short Courses

Time	Presenter	Presentation Title
10:10 AM Morning Short Course 1	O-F-01 **Jayanant Iemsam-arng** *University of London* *United Kingdom*	Biocompatible delivery systems for nucleic acid therapies
11:00 AM Morning Short Course 1	O-F-02 **Boris Strehlow** *Philipps University* *Germany*	The chicken chorioallantoic membrane. A model for angiogenesis, transepithelial drug delivery and xenograft research
10:10 AM Morning Short Course 2	O-F-03 **Sirigul Thongrangsalit** *Chulalongkorn University* *Thailand*	Development of bromocriptine mesylate tablet using solid-smedds as drug delivery system
11:00 AM Morning Short Course 2	O-F-04 **Line Hagner Nielsen** *University of Copenhagen* *Denmark*	Amorphous furosemide salt exhibits higher dissolution rate and stability compared to amorphous furosemide acid
10:00 AM Morning Short Course 3	O-F-05 **Daniel Gonzalez** *University of Florida* *USA*	Breath testing to assess definative adherence to oral and vaginal medications
11:00 AM Morning Short Course 3	O-F-06 **Wyatt Roth** *Purdue University* *USA*	Assessment of juvenile pigs to serve as human pediatric surrogates for preclinical formulation pharmacokinetic testing

Short Course Student Presentations

Date: Friday 30th November 2012

Time: Afternoon Short Courses

Time	Presenter	Presentation Title
2:45 PM Afternoon Short Course 1	O-F-07 **Mary Kleppe** *University of Connecticut* *USA*	Comparison of biorelevant media and USP compendial media on solution-mediated phase transformation of an amorphous drug during dissolution
3:30 PM Afternoon Short Course 1	O-F-08 **Ursula Thormann** *University of Basel* *Switzerland*	Vehicle elicited improvement of intestinal absorption of a phytopharmaceutical compound in the Caco-2 model via increased stability and reduced metabolism
2:40 PM Afternoon Short Course 2	O-F-09 **Riccardo Torosantucci** *Leiden University* *The Netherlands*	Chemical modifications in aggregates of recombinant human insulin induced by metal-catalyzed oxidation: covalent cross-linking via Michael addition to tyrosine oxidation products
3:30 PM Afternoon Short Course 2	O-F-10 **Saranya Chandrudu** *University of Queensland* *Australia*	Development of lipopeptide vaccine candidate against group A streptococcus
2:50 PM Afternoon Short Course 3	O-F-11 **Helene Marie** *Paris-Sud* *France*	Magnetic targeting of magnetic-fluid-loaded liposomes (MFLs) to brain for MRI diagnosis and treatment of glioblastoma
3:30 PM Afternoon Short Course 3	O-F-12 **Yongjun Liu** *Shandong University* *China*	Theranostic nanoparticles for the diagnosis and treatment of HCC
2:35 PM Afternoon Short Course 4	O-F-13 **Taku Kasai** *Kanazawa University* *Japan*	A role of prostaglandin transporter in regulation of intercellular PGE2 levels under inflammatory conditions
3:30 PM Afternoon Short Course 4	O-F-14 **Kazumasa Kobayashi** *Tokyo University* *Japan*	Functional characterization of organic cation transport in HEK293 cells

Podium Presentations: Session 3

Date: Saturday 1st December 2012

Time: 9:00 am – 12:00 pm

Locations: Lecture Theatre 3

Time	Presenter	Presentation Title
9:00 AM	O-S-01 **Rajendar K Mittapalli** *University of Minnesota* *USA*	Mechanisms limiting distribution of novel BRAF inhibitors to the brain: Impact on the treatment of melanoma brain metastases
9:20 AM	O-S-02 **Sifei Han** *Monash University* *Australia*	Targeted delivery of a model immunomodulator mycophenolic acid to the lymphatic system using triglyceride mimetic prodrugs
9:40 AM	O-S-03 **Amit Kumar Jain** *NIPER* *India*	Co-administration of CoQ10 nanoparticles with tamoxifen nanoparticles for augmented antitumor efficacy and reduced hepatotoxicity
10:00 AM	O-S-04 **Parind Mahendrakumar Desai** *National University of Singapore* *Singapore*	Importance of strain recovery phenomenon in tablet disintegration
10:20 AM	***Morning tea***	
10:50 AM	O-S-05 **Sai Praneeth Reddy Bathena** *University of Nebraska* *USA*	Profiling of bile acids and their sulfate metabolites in human urine by LC-MS/MS
11:10 AM	O-S-06 **Melanie Nicol** *University of North Carolina* *USA*	Development of a mucosal tissue culture model as a preclinical tool to evaluate antiretroviral (ARV) candidates for HIV pre-exposure prophylaxis (PrEP)
11:30 AM	O-S-07 **Sara Hanning** *University of Otago* *New Zealand*	Rheological characterisation of lecithin-based emulsions for potential use as a saliva substitute in patients with xerostomia
11:50 AM	O-S-08 **Marius Hittinger** *Saarland University* *Germany*	Establishment & characterization of an advanced in vitro co-culture model of the air-blood barrier

Podium Presentations: Session 4

Date: Saturday 1st December 2012

Time: 1:30 pm - 4:40 pm

Locations: Lecture Theatre 3

Time	Presenter	Presentation Title
1:30 PM	O-S-09 **Wanessa Silva Garcia Medina** *University of Sao Paulo* *Brazil*	Characterization of cell penetration of ZnPcSO4 delivered by nanoparticles of hexagonal liquid crystalline phase
1:50 PM	O-S-10 **Farzana Alam** *Seoul National University* *Korea*	Polyethylene glycol (PEG) conjugated heparin-taurocholate derivative (LHT7) and protamine nanocomplex enhances anti-tumor activity by inhibiting angiogenesis
2:10 PM	O-S-11 **Erik Serrao** *University of Southern California* *USA*	A symmetric region of the HIV-1 integrase dimerization interface is essential for viral replication
2:30 PM	O-S-12 **Mattias Ivarsson** *ETH-Zurich* *Switzerland*	Toxin-targeted polymeric binders for the treatment of Clostridium difficili infection
2:50 PM	***Afternoon tea***	
3:20 PM	O-S-13 **André Mateus** *Uppsala University* *Sweden*	A new method for determination of intracellular unbound drug concentrations in simple cell cultures: Impact of active uptake via OATP1B1
3:40 PM	O-S-14 **Nate Larson** *University of Utah* *USA*	Tumor hyperthermia to increase the delivery of macromolecular chemotherapeutics
4:00 PM	O-S-15 **Negar Babae** *Utrecht University* *The Netherlands*	Novel anti-angiogenic miRNA reduces angiogenesis and tumor growth in mouse tumor model
4:20 PM	O-S-16 **Justin Lutz** *University of Washington* *USA*	Fluoxetine strongly inhibits CYP2D6 and CYP2C19 but does not inhibit CYP3A4 in vivo: A cocktail study

Podium Abstracts

O-TH-01

EFFECT OF SUPERSATURATION ON ORAL ABSORPTION OF BCS CLASS II DRUGS: QUANTITATIVE EVALUATION BY SUCCESSIVE IN VITRO DISSOLUTION AND IN VIVO ABSORPTION STUDIES IN RAT

H. Higashino, T. Hasegawa, M. Yamamoto, R. Matsui, Y. Masaoka, M. Kataoka, S. Sakuma and S. Yamashita
Setsunan University, Japan

Objective: The purpose of the present study is to develop a novel experimental system for evaluating the effect of supersaturation on oral absorption of Class II drugs in human.

Methods: Ketoconazole, albendazole and dipyridamole, basic drugs categorized into BCS Class II, were used as model drugs. Impact of supersaturation on the oral absorption in human are estimated from the report of Sugano et al. (Int J Pharm. 398: 73-82 (2010)), in which fraction absorbed (Fa) of these drugs after administration to normal and high gastric pH subjects was calculated. In our study, supersaturation was induced by solvent shift method. The dissolution behavior of both control and co-solvent suspension were studied in fasted state simulated intestinal fluid. In rat study in vivo, each suspension was injected into the duodenum, and then blood samples were collected from jugular vein for 4 hours. The AUC ratio after administration of standard and co-solvent suspension was calculated as an indicator of the effect of supersaturation.

Results: In the case of ketoconazole, a significant difference was observed in the dissolution profiles between two suspensions. Dissolved drug concentration in co-solvent suspension was more than 10 times higher than that in control one during first 5 min. The state of supersaturation was maintained for about 0.5 hour. In rat studies, AUC0-4h ratio after administration of control and co-solvent suspension into duodenum became approximately 17.8, indicating that supersaturation dramatically increased the systemic expose of ketoconazole. This value corresponded well with the reported human Fa ratio. The ratio of AUC0-4h for other two drugs also indicated clearly the effect of supersaturation on their oral absorption.

Conclusion: Combination of in vitro dissolution and in vivo rat absorption studies is a novel and useful system to investigate the impact of supersaturation on the oral absorption of poorly water-soluble drugs in human.

O-TH-02

UNDERSTANDING THE TRANSIT OF BILOSOMES AND ANTIGEN VIA THE ORAL ROUTE

J. S. Wilkhu[1], D. Anderson[2] and Y. Perrie[1]
[1] *Aston University, Birmingham, United Kingdom*
[2] *Variation Biotechnologies INC, Ottawa, Canada*

Objective: Whilst offering a range of advantages, few vaccines can be administered orally due to their degradation in the harsh gut environment and their poor uptake by appropriate target sites, namely M cells located in the Peyer's patches, which are responsible for secretory IgA and other mucosal responses. Therefore within this work we have considered the use of bilosomes to enhance the protection and delivery of sub-unit vaccines and tracked their transit through the GI tract after oral administration.

Method: H3N2 Antigen was radiolabelled with I-125 isotope and was then entrapped into bilosomes [5:4:1 ratio of Monopalmitoyl Glycerol: Cholesterol: Dicetyl Phosphate respectively at 6 µM] vesicles prepared via the homogenisation melt method.

Results: Results show that the blood, spleen, kidneys and liver have trace amounts of antigen recovery with the majority of the antigen being located in the stomach, Small intestine, and the colon and cecum. Upon entrapping the antigen into bilosomes, significantly ($p < 0.05$) more antigen was located in the small intestine and significantly less was located in the colon and cecum. Of the dose administered, in general significantly ($p < 0.05$) more antigen (50.5%) was recovered when formulated with bilosome vesicles compared to the free antigen dose (38%). In terms of the antigen reaching the site of action, antigen recovery at the Peyer's patches and mesenteric lymph tissue show that the presence of the bilosome vesicles results in a higher recovery of antigen within the Peyer's patches with significantly higher ($p < 0.05$) levels of antigen within the mesenteric lymph tissue when delivered using bilosomes.

Conclusion: In conclusion, there is an advantage of associating the H3N2 antigen with the bilosome vesicles as it increases the percentage of antigen recovered within the target site. Studies to consider if these differences translate into enhanced immune responses continue.

O-TH-03

PROBING DIFFUSION LIMITATIONS ON ANTIBODY DISTRIBUTION IN BRAIN USING REAL-TIME OPTICAL IMAGING

D. J. Wolak and R. G. Thorne
University of Wisconsin-Madison, USA

Objective: One hurdle in the use of biotherapeutics to treat central nervous system (CNS) disorders has been limited diffusion through the brain microenvironment to the target site. While there is little information on the diffusion of biotherapeutics in brain, techniques such as integrative optical imaging (IOI) have measured diffusion in brain for molecules such as dextrans, quantum dots, oligonucleotides, and proteins under 100 kDa. The purpose of the current study is to determine the diffusion behaviour of antibodies, which are a promising category of biotherapeutics to treat CNS disorders

Methods: IOI was used to measure the free diffusion coefficient (D) in dilute agarose or the effective diffusion coefficient (D*) 200 µm below the brain surface in anesthetized rats using an open cranial window. Sequential images were obtained following pressure injection of 2 mg/ml Alexa Fluor 488 labeled goat anti-rabbit immunoglobulin G (IgG; Invitrogen). Fluorescence intensity curves were then extracted from the obtained images and fit by the diffusion equation to yield D or D*.

Results: The free diffusion coefficient (37°C) of IgG [D = 6.47 ± 0.57 × 10^-7 cm^2/s (mean ± SD; n = 29)] yields a Stokes-Einstein hydrodynamic diameter of 10.2 nm. Our preliminary findings show IgG diffusion is hindered ~9-fold in brain relative to free solution, with D* = 7.28 ± 1.1 × 10^-8 cm^2/s (mean ± SD; n = 8, N = 3 animals).

Conclusion: Our measurements are the first reported values of in vivo antibody diffusion in healthy adult brain. While the free diffusion measurement matches well with both predicted and literature values, the brain diffusion coefficient shows a higher hindrance than expected from a model. Using our data and previously reported measurements, we can predict brain distribution following intracerebroventricular infusion, allowing comparisons between therapeutic classes and realistic evaluation of strategies to treat CNS disorders.

O-TH-04

RECOMBINANT PROTEINS WITH SHORT PEPTIDE EXTENSIONS THAT BIND TO HUMAN FCRN UNDERGO RECEPTOR-MEDIATED RECYCLING AND TRANSCYTOSIS

J. Sockolosky, M. Tiffany and F. Szoka
University of California, USA

Objective: The importance of therapeutic recombinant proteins in medicine has led to a variety of tactics to increase their circulation time or to enable routes of administration other than injection. Engineering proteins to interact with the human neonatal Fc receptor (FcRn) by genetic fusion to the Fc-domain of immunoglobulin G (IgG) or albumin is a clinically validated approach to improve both protein circulation and delivery. As an alternative approach to improve the pharmaceutical properties of proteins, we have modified their N- and/or C-terminus with a short peptide sequence that interacts with FcRn.

Methods: FcRn binding peptide (FcBP) sequences were genetically fused to the N- and/or C-terminus of model proteins and expressed in E. coli. The interaction between FcBP fusion proteins and FcRn was characterized in vitro through a number of molecular and cell-based assays including surface plasmon resonance, FACS, fluorescence microscopy, and cellular recycling and transcytosis.

Results: The small size and simple structure of the FcBP allows for expression of FcBP fusion proteins in E. coli and results in their pH-dependent binding to FcRn with an affinity comparable to that of the endogenous ligand, IgG. The FcBP fusion proteins are internalized, recycled and transcytosed across cell monolayers that express FcRn. Modification of the FcBP sequence results in FcBP fusion proteins with differential FcRn binding properties and altered transport across cell monolayers.

Conclusion: We demonstrate that proteins can be engineered to interact with FcRn by recombinant fusion of a short FcBP sequence at a proteins N- and/or C-terminus. The FcBP fusion approach may overcome limitations associated with alternative strategies to improve protein half-life, be easy to manufacture, and provide a solution to both the rapid elimination and limited protein delivery routes that may translate to improved therapeutic activity and compliance in the clinic.

O-TH-05

PACLITAXEL LOADED SLN MODIFIED WITH 2-HYDROXYPROPYL-BETA-CYCLODEXTRIN FOR OVERCOMING P-GLYCOPROTEIN FUNCTION IN MULTIDRUG-RESISTANT BREAST CANCER CELLS

J. Baek and C. Cho
Chungnam National University, Daejeon, South Korea

Objective: This study was to evaluate the potential of solid lipid nanoparticles (SLNs) of paclitaxel (PTX) modified with a 2-hydroxypropyl-beta-cyclodextrin system to enhance cellular accumulation of PTX into p-glycoprotein (p-gp)-expressing cells.

Methods: Preparation PTX-loaded-SLNs consisted of lipid (stearic acid) and surfactants (lecithin and poloxamer 188) and were then modified with 2-hydroxypropyl-beta-cyclodextrin by a hot-sonication method. Cell study – Cytotoxicity of SLNs was carried out by MTT assay. P-gp inhibition of MCF-7/ADR cell was confirmed by Western blotting. Cellular uptake of PTX solution, PS and PSC was carried out with(out) vepamail at different time point in MCF-7 and MCF-7/ADR cells. Confocal microscopy of FS and FSC was conducted to evaluate the cellular uptake of PTX from PS and PSC, qualitatively.

Results: In terms of cytotoxicity, PTX-loaded SLNs modified with 2-hydroxypropyl-beta-cyclodextrin showed higher cytotoxicity than other formulations. In particular, the cellular uptake of PTX from PTX-loaded SLNs modified with 2-hydroxypropyl-beta-cyclodextrin was about 5.8- and 1.5-fold higher than that from PTX solution and unmodified PTX-loaded SLNs in MCF-7/ADR cells, respectively. After a 4-h incubation, clear fluorescence images inside cells were observed over time. When PTX-loaded SLNs modified with 2-hydroxypropyl-beta-cyclodextrin were incubated with MCF-7/ADR cells for 4 h, cellular uptake of PTX increased 1.7-fold versus that of PTX in the presence of verapamil.

Conclusion: In this study, PSC was successfully prepared by an optimized ultrasonication method. PSC showed improved cellular uptake in cancer cell lines compared with PTX solution and the PS formulation. These results suggest that PSC may be a promising carrier for an alternative formulation of PTX.

O-TH-06

DEGRADATION OF LIPOSOMAL FORMULATIONS DURING LYOPHILIZATION

N. M. Payton, M. F. Wempe and T. J. Anchordoquy
University of Colorado Denver, USA

Objective: Because liquid formulations of liposomes are prone to chemical degradation and aggregation, these formulations are often lyophilized in order to achieve stability consistent with a marketable product. However, previous studies have indicated that liposomal formulations can also undergo oxidation during lyophilization and prolonged storage in the dried state. The goal of this project is to characterize lipid degradation during lyophilization, and to understand the role of metal contaminants in promoting lipid oxidation.

Methods: To investigate this effect, an unsaturated lipid (DLPC) was lyophilized in sugar formulations and lipid degradation was monitored with HPLC and GC/MS. Our experiments involved lyophilizing lipid samples under different conditions in order to determine the effect of varying iron and sucrose concentrations as well as in which stage of lyophilization lipid degradation was occurring.

Results: The degradation of lipid samples increased with higher iron concentrations. After lyophilization, 99.5% ± 1.3%, 69.2% ± 1.4%, and 28.5% ± 0.14% lipid was remaining in trehalose samples containing 0ppm, 0.2ppm and1.0ppm ferrous iron respectively. Lipid degradation predominantly occurred during the freezing stage of lyophilization. After freezing, 51.5% ± 1.1% lipid remained in trehalose samples containing 0.2ppm ferrous iron whereas after lyophilization (freezing, primary drying and secondary drying) 53.9% ± 0.64% lipid remained. The percent lipid remaining after freezing and lyophilization was not significantly different ($p=0.077$). Sugar concentrations and buffer ionic strength also had a significant effect on metal-catalyzed lipid degradation. The loss of DLPC correlated with the formation of degradation products.

Conclusion: Lipid oxidation during the freezing step of lyophilization can dramatically compromise the stability of lipid-based formulations. Addition of exogenous metals did not accurately mimic metal contaminants in pharmaceutical grade sugars. Transition metal contaminants in pharmaceutical grade sugars have very little effect on lipid oxidation during lyophilization unless they are reduced by other formulation components.

O-TH-07

UPTAKE AND INTRACELLULAR DISTRIBUTION OF BISPHOSPHONATES

H. Zlatev, S. Coimbra de Sousa, S. Arkko, J. Mönkkönen and J. Määttä
University of Eastern Finland, Finland

Objective: The bisphosphonates (BPs) – drugs containing pyrophosphate resembling P-C-P backbone are used to treat osteoporosis and cancer induced bone diseases. Nitrogen containing bisphosphonates (N-BPs) inhibit farnesyl pyrophosphate synthetase (FPPS).

Methods: Inhibition of FPPS leads to accumulation of isopentenyl pyrophosphate (IPP) which can be further used to form ApppI via action of aminoacyl-tRNAsynthetases. IPP can be recognized by cytotoxic γδ-T-cells .ApppI on its side is a toxic molecule that leads to apoptosis. This is initially triggered by inhibition of the mitochondrial adenylyl nucleotide translocase. The mechanisms of uptake of BPs by the tumor cells is incompletely understood as well as their kinetics and mechanisms of intracellular distribution. The aim of this part of our research project is to address these issues. The results will be of help in development of novel drug targeting formulations for BP treatment of malignancies. Different inhibitors such as chlorpromazine, wortmannin, amiloride etc. are used to inhibit endocytosis, and macropinocytosis, respectively in MCF-7 and T47D human breast cancer cell lines and their effect on cell survival and formation of IPP and ApppI will be followed with MTT assay and LC-MS mass spectrometry (EQUIPMENT) analysis. All experiments are done in 3 independent biological replicates . Statistical one way ANOVA analysis will be performed with SPSS software (version 18). Fluorescence-labeled BP analogs will be applied to follow the intracellular fate of the drugs with confocal and STED microscopy.

Results: Our preliminary observations indicate that endocytosis but not mactopinocytosis is used for N-BP uptake by these cells. We have shown in MTT assay that risedronate induce reduction in cell viability ($p < 0.001$) and this reduction in viabitity was 80% abolished when (15 μM) chlorpromazine was used as an inhibitor of the clathrin-dependent endocytosis. Analysis of Intracellular drug kinetics with fluoresecence labeled compounds is currently ongoing.

Conclusion: We postulate that clathrin-dependent endocytosis is the major route of N-BP uptake in these cancer cell lines.

O-TH-08

CAN MULTISOURCE IMMEDIATE RELEASE TENOFOVIR DISOPROXIL FUMARATE PRODUCTS QUALIFY FOR BCS BASED BIOWAIVERS?

A. Nair and J. B. Dressman
Goethe University, Germany

Objective: The biowaiver procedure based Biopharmaceutic Classification System (BCS) uses in vitro dissolution as a surrogate technique to test bioequivalence, thus reducing cost and improving availability of quality generic products. The present study aims at evaluating the eligibility of tenofovir disoproxil fumarate (TDF), a first line antiretroviral drug, for a biowaiver procedure based on the FDA, EMA and WHO guidances.

Methods: Relevant data in literature pertaining to solubility, permeability, pharmacokinetics, toxicity, effect of excipients and bioequivalence of TDF were evaluated. Additionally, solubility testing of tenofovir was performed in Uniprep® vials over the pH range of 1-7.5 at 37°C based on the shake flask method. Dissolution studies of pure tenofovir, the comparator product and a test product were performed in accordance with the WHO guidelines in pH 1.2, 4.5 and 6.8 buffers. Drug contents were analyzed using UV spectrophotometry and concentrations calculated using appropriate standard curves.

Results: Based on the permeability data available in open literature and BCS oriented solubility studies TDF can be classified as a Class III drug. Dissolution studies revealed > 85% drug dissolution in less than 15min thus demonstrating “very rapidly dissolving” properties. Several in vivo studies conducted in volunteers reported TDF formulations to be bioequivalent. The drug has a wide therapeutic index and the risks associated with an in vitro based bioequivalence approval seem minimal.

Conclusion: Tenofovir disoproxil fumarate containing immediate release multisource products are eligible for a BCS based biowaiver application.

O-TH-09

CAPILLARY ELECTROPHORESIS IN BIOANALYSIS: APPLICATION TO TRANSFERRIN IN CHRONIC ALCOHOL ABUSE

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[1] *University of Geneva, Switzerland*
[2] *University Center of Legal Medicine (CURML), Switzerland*

Objective: Capillary electrophoresis (CE) is an efficient separation technique for proteins analysis. However, one major drawback is the potential protein adsorption onto the negatively charged surface of the capillary, which can be overcome using a capillary coating. In this study, such approach was implemented for the analysis of Transferrin (Tf). Tf contains two carbohydrate chains with a total of up to eight sialic acid terminals. Carbohydrate-deficient transferrin (CDT) is the most specific marker for chronic alcohol abuse and encompasses isoforms with zero (asialo-Tf, specific biomarker) and two sialic acid residues (disialo-Tf). Determination of these glycoforms can be achieved by CE with coated capillaries hyphenated to mass spectrometry (MS), the latter increasing both sensitivity and selectivity of the procedure.

Methods: Numerous coatings, i.e., monolayer (PB, PEI, PVA and UltraTrol™ LN), bilayer (PB-DS, PB-PVS) and trilayer (PB-DS-PB) coatings were investigated on Tf standard with optimization of polymers concentration and coating procedure. Various ammonium acetate and formate background electrolytes (BGE) were tested with optimization of concentration (20 to 100 mM) and pH (7.5 to 9.5). Electrospray (ESI) and MS operating conditions, i.e., sheath liquid composition (MeOH, MeCN and isopropanol), nebulizing gas flow rate, drying gas flow rate and temperature were investigated to maximize Tf ionization.

Results: Best results for glycoforms separation were obtained with a PB-DS coating at 10% each. BGE consisted in 20 mM ammonium acetate at pH 8.5. Separation was achieved at 30 kV and 25°C. Sheath liquid was composed of H2O/isopropanol/formic acid 50:50:5 (v/v/v). Nebulizing gas was set at 4 psi, and drying gas at 4 L/min and 350 °C. These conditions allowed for glycoforms discrimination and analysis by CE-MS.

Conclusion: The application of CE-MS in intact protein analysis was demonstrated with Tf. Protein adsorption was prevented with the use of a bilayer capillary coating which also allowed for the discrimination between glycoforms.

O-TH-10

PREPARATION AND CHARACTERIZATION OF Z-DEVD-FMK AND BASIC FIBROBLAST GROWTH FACTOR (BFGF) LOADED CHITOSAN NANOPARTICLES

S. Caban Toktas[1], M. Yemisci[1], Y. Gursoy Ozdemir[1], E. Fernandez-Megia[2], R. Novoa-Carballal[2], R. Riguera[2], Y. Capan[1] and T. Dalkara[1]
[1] *Hacettepe University, Turkey*
[2] *University of Santiago de Compostela, Spain*

Objective: bFGF and caspase inhibitor Z-DEVD-FMK have therapeutical features in stroke treatment but they cannot pass through the BBB alone. The limiting properties of substances will be optimized with nanotechnological approaches.

Methods: Chitosan-polyethylene glycol-biotin (CS-PEG-BIO)/Streptavidin (SA)-CD71 nanoparticles were prepared by drop wise addition of tripolyphosphate (TPP) to the CS-PEG-BIO solution prior to the incorporation of the SA/monoclonal CD71 antibody conjugate. Nanoparticle formation resulted from the interaction between the negative groups of TPP and the positively charged amino groups of chitosan. In order to evaluate the caspase-mediated cell death; appropriate concentration of fluorescent dye (nile red) was loaded into the nanoparticles and screened by using a fluorescence microscope in vivo.

Results: Particle size and zeta potential values of peptide loaded and monoclonal antibody conjugated CS-PEG-BIO nanoparticles were approximately 700 nm and 20 mV, respectively. On systematic administration to mice, CS-PEG-BIO/SA-CD71 nanoparticles were located in the brain parenchyma, outside the vascular compartment, whereas the CD71-free nanoparticles could not penetrate into the brain.

Conclusion: These nanoparticles combine the ability of cationic CS to interact with negative charges on the brain endothelium with the affinity of CD71 for the transferrin receptor to trigger receptor-mediated transport across the brain blood barrier. Based on the results, it can be concluded that these nanoparticles are promising carriers for the transport of active substances into the brain.

O-TH-11

RIVASTIGMINE, A CHOLINESTERASE INHIBITOR, REDUCES INTESTINAL INFLAMMATION BY INDIRECT ACTIVATION OF ALPHA7 CHOLINERGIC RECEPTORS

H. Shifrin and M. Weinstock
The Hebrew University of Jerusalem, Jerusalem, Israel

Objective: Acetylcholinesterase (AChE) inhibitors have been reported to suppress pro-inflammatory cytokine production by increasing the concentration of extracellular acetylcholine that interacts with α7 nicotinic receptors expressed on macrophages and lymphocytes. Macrophages in the large intestine are crucial effectors in inflammatory bowel disease. We investigated the ability of rivastigmine, a pseudo-reversible AChE inhibitor used in the treatment of dementia, to improve the pathology of ulcerative colitis.

Method: Peritoneal macrophages prepared from C57 black mice and RAW 264.7 macrophage cells were incubated with lipopolysaccharide (10 and 5 μg/ml) and carbachol (1-10 uM) with and without rivastigmine (1uM). Rivastigmine was also tested in two animal models of acute colitis. In the first, BALB/c mice were injected i.p. once daily with rivastigmine (1mg/kg) and colitis was induced by 4% DSS in their drinking water for 8 days. In the second model in rats, colitis was induced by rectal administration of DNBS.

Results: Carbachol alone was ineffective but with rivastigmine, which inhibited AChE by 50%, significantly decreased the release of nitric oxide and pro-inflammatory cytokines TNF-α and IL-6. The effect of rivastigmine was abolished by α7 nicotinic receptor blockade. Rivastigmine inhibited AChE in colon by 60%, reduced diarrhea and rectal bleeding, abolished the increase in myeloperoxidase activity and decreased the expression of pro-inflammatory cytokines IL-6 and IL-1β. Rivastigmine treatment also improved the signs of colonic inflammation like crypt dysplasia and submucosal edema. Immunostaining analysis revealed that treated mice showed a lower expression than untreated diseased mice of CD11b+ macrophages in subepithelial layer. In DNBS model,rivastigmine (1 mg/kg) reduced the number and area of ulcers, TNF-α and myeloperoxidase activity.

Conclusion: Study revealed that rivastigmine is able to reduce gastrointestinal inflammation, by acting indirectly on alpha7 nicotinic receptors found on macrophages.

O-TH-12

GENERATION OF HUMAN EMBRYONIC STEM CELL-DERIVED RETINAL PIGMENT EPITHELIUM ON BIOPOLYMER COATED POLYIMIDE MEMBRANES

A. Subrizi[1], H. Hiidenmaa[2], T. Ilmarinen[2], S. Nymark[2], P. Dubruel[3], H. Uusitalo[2], M. Yliperttula[1], A. Urtti[1] and H. Skottman[2]
[1] *University of Helsinki, Finland*
[2] *University of Tampere, Finland*
[3] *Ghent University, Belgium*

Objective: The retinal pigment epithelium (RPE) is a monolayer of post-mitotic, pigmented cells forming the outer blood-retinal barrier that is essential for vision. Degeneration of RPE cells is the cause of loss of macular function in age-related macular degeneration, a common blinding disorder and major public health problem in the industrialized world. The aim of our study is the generation of a functional, stem cell-based and tissue engineered RPE.

Methods: Biopolymer coated polyimide (PI) membranes were used as RPE substrate to promote maturation of human embryonic stem cells (hESC) toward RPE phenotype. Human ESC-derived RPE (hESC-RPE) monolayers were evaluated by observation of pigmentation, morphology, gene and protein expression, and barrier function. Cell functionality was assessed by phagocytosis experiments.

Results: Human ESC-RPE cells regained pigmentation and cobblestone morphology on biopolymer coated PI membranes. Laminin, collagens type I and type IV, CELLStart™, MaxGel™, and HyStem™ supported cell growth and maturation. Gene and protein expression indicated maturation of hESC-RPE monolayers. The cells did not express the pluripotency marker OCT4, while important genes for early-field development PAX6 and RAX were still faintly expressed. Melanogenesis-related genes MITF, PMEL, and TYR, as well as RPE65, involved in visual pigment regeneration, were expressed. RPE-specific genes BEST and PEDF were also detected. At protein level, the localization of MITF, CRALBP, BEST, ZO-1, NaKATPase, and MERTK was studied. The cellular proliferation marker Ki67 was not expressed. The paracellular permeability of 6-carboxyfluorescein and the transepithelial electrical resistance were measured to establish the quality of barrier function and the integrity of the monolayers. Human ESC-RPE internalized photoreceptor outer segment discs during co-culture with rat retina explants.

Conclusion: The PI biomembrane presented in this study promoted growth and maturation of hESC-RPE monolayers. We suggest the suitability of our PI biomembrane as scaffold for hESC-RPE tissue engineering.

O-TH-13

STUDIES ON THE MECHANISMS AND CONSEQUENCES OF INTRACELLULAR DRUG-DRUG INTERACTIONS INVOLVING LYSOSOMES

R. Logan, A. Kong and J. P. Krise
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Objective: The manner in which a small molecular weight drug localizes and distributes inside a highly compartmentalized human cell can strongly influence activity, toxicity and macroscopic pharmacokinetic distribution properties. Many currently approved drugs possess weakly basic properties that make them substrates for extensive sequestration in acidic intracellular compartments such as lysosomes through an ion trapping-type mechanism. Lysosomotropic drugs often have unique pharmacokinetic properties that stem from their extensive entrapment in lysosomes, including an extremely large volume of distribution and long half-life. In this work we describe our efforts to elucidate the mechanisms by which drugs can modify lysosomes in such a way that their drug sequestration capacity for subsequently administered drugs is altered.

Methods: Lysotracker red, a fluorescent lysosomotropic probe, was used to estimate drug-induced changes in the apparent aqueous volume of lysosomes. Drug induced alterations in lysosomal vesicle-mediated trafficking were assessed by measuring the cellular release of lysosome-localized dextran polymers. The induction of autophagy was assessed by Western blot analysis of LC3I/LC3II expression.

Results: We have evaluated a panel of 8 commercially available hydrophobic weakly basic drugs and have shown them to cause 2-3 fold expansion of the apparent aqueous volume of lysosomes. All of the drugs were shown to cause a significant decrease in the cellular efflux of lysosome-associated dextran polymers, suggesting that they interfere with lysosomal efflux pathways. In addition, Western blot analysis indicated these drugs also induced autophagy. Separate evaluations with non-lysosomotropic chemical inducers of autophagy (i.e., rapamycin) demonstrate their propensity to increase lysosomal volume without negatively effecting lysosomal egress pathways.

Conclusion: This work reveals mechanisms by which drugs can alter lysosome-associated properties such that the lysosomal accumulation of secondarily administered drugs is perturbed. We propose that these changes in intracellular distribution are quantitatively important and present a source for variability in drug pharmacokinetics and response.

O-TH-14

THERAPEUTIC DRUG AND IMMUNOGENICITY MONITORING OF THE MONOCLONAL ANTIBODY INFLIXIMAB TO PERSONALIZE TREATMENT OF INFLAMMATORY BOWEL DISEASE PATIENTS: RESULTS ON THE OPTIMIZATION PHASE OF THE TAXIT TRIAL

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Objective: Infliximab, a monoclonal antibody directed against TNF-alpha is used to induce and maintain clinical remission in inflammatory bowel disease (IBD) patients. Despite its beneficial effect, loss of response to the drug is seen in 25-40% of the patients. This can be attributed to low infliximab serum levels. We investigated prospectively in a randomized controlled trial the impact on clinical outcome of adapting the dosing scheme individually, based on infliximab trough serum levels: Trough level Adapted infliXImab Treatment (TAXIT) trial.

Methods: 263 consecutive IBD patients on infliximab maintenance therapy were first dose optimized by mening or prolonging the interval between infusions and/or increasing or decreasing the dose to have a baseline infliximab trough level (TLI) between 3 and 7 µg/ml. After optimization, patients were randomized to an active (treatment based on TLI) or a control (treatment based on symptoms) group. We here report on the results of the optimization phase.

Results: Of the 263 patients, 115 patients (44%) had a TLI between 3 and 7 µg/ml, the remaining 148 patients (56%) were dose optimized: this was successful in 68 patients (46%) with a TLI <3 µg/ml and in 68 patients (46%) with a TLI >7 µg/ml. 12 patients (8%) could not be optimized. Patients with TLI <3 µg/ml had significantly higher C-reactive protein levels (median 2.7 mg/l; inter quartile range 1.1-7.5) versus patients with a TLI between 3 and 7 µg/ml (1.5 mg/l; 0.60-3.8; P<0.001) and a TLI >7 µg/ml (1.2 mg/l; 0.6-4.8; P<0.01). In patients in whom the dose was escalated, there was a significant decrease in CRP (P=0.0008) and Harvey-Bradshaw index (P=0.03).

Conclusion: The results of the optimization phase of the TAXIT trial show that in this large cohort of patients in remission under treatment with maintenance infliximab only 44% have optimal TLI and optimizing patients with TLI <3 µg/ml is beneficial.

O-TH-15

OPTIMIZATION OF LIPOSOME RELEASE KINETICS OF A CORTICOSTEROID PHOSPHATE BASED ON PH AND LIPID COMPOSITION

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Objective: Controlled release of dexamethasone phosphate (DexP) may be desirable to take advantage of its anti-inflammatory and chemosensitizing effects in cancer therapy. This study explores liposomal delivery systems having a range of release kinetics in an attempt to extend previously developed quantitative relationships between lipid bilayer permeability and factors such as intravesicular pH, lipid composition, and permeant size to larger drug molecules.

Methods: Drug loaded liposomes were prepared at varying pH by thin film hydration using DMPC: mPEG DMPE, DPPC: mPEG DPPE and DSPC: mPEG DSPE in the ratio of 95:5 mol%. Drug release kinetics from loaded liposomes were monitored by dynamic dialysis at 37°C by analyzing samples from inside the dialysis tube at various time points using a validated HPLC method.

Results: The bilayer transport of DexP varied as a function of pH across liposomal membranes in gel phase bilayers (DPPC and DSPC). The change in apparent permeability with pH could be attributed to the different permeability coefficients of the neutral and ionic species and changes in the relative fraction of the neutral species with pH. At a particular pH (pH 4), the bilayer permeability varied with bilayer chain length (DMPC>DPPC>DSPC). At the temperature of these release studies (i.e., 37°C), DMPC was in the liquid crystalline phase while DPPC and DSPC were in the gel phase. The logarithm of the permeability coefficients for DexP decreased linearly with the inverse of bilayer free surface area consistent with our previously published model.

Conclusion: Changes in bilayer free surface area and intravesicular pH can be exploited to tune drug release kinetics. A range of release rates of DexP across liposomes corresponding to half-lives from 1 hr to 220 hr were obtained by varying the lipid composition and the intravesicular pH.

O-TH-16

CHARACTERIZATION OF PLASMA HYDROLASES AND THEIR SPECIES DIFFERENCES

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Objective: In the plasma, there are three major classes of hydrolases, carboxylesterase (CES), butyrylcholinesterase (BChE) and paraoxonase (PON). The purpose of this study is to elucidate the species differences through the expression and the activity of these hydrolases in human and several animals' plasma.

Methods: Native polyacrylamide gel electrophoresis (PAGE) was performed to visualize esterase bands via hydrolysis of β-naphthylacetate and butyrylthiocholine. Hydrolysis of *p*-nitrophenylacetate (PNPA), temocapril, oseltamivir, irinotecan, propranolol (PL) derivatives and aspirin were estimated via formation of their hydrolysate by HPLC in the presence and absence of *bis*-[*p*-nitrophenyl] phosphate (BNPP), ethopropazine and EDTA as inhibitors of CES, BChE and PON, respectively.

Results: In native-PAGE, BChE and PON were observed in all species, but were highly expressed in primates and dogs, while CES was abundant in rabbit, mouse, and rat. It is shown by hydrolysis experiment that high activity of mouse and rat plasma is owing to CES, but rabbit CES showed extremely low activities except for PNPA and PL derivatives. Human, monkey, minipig and dog plasma showed low and nearly same activities for all substrates except PL derivatives. In case of valeryl-PL, BChE activity of monkey plasma (63.8μM/min) was higher than that of human (3.2μM/min), even though they share 96% sequence homology. Aspirin was hydrolyzed nearly at same level (19μM/min) by BChE and PON of human and monkey plasma. Interestingly calcium addition increased the BChE activity but not PON activity of human (7-fold) rather than monkey (4-fold). Aspirin activity of human BChE also increased by magnesium but decreased by zinc addition.

Conclusion: The relative abundance and substrate specificities of the hydrolase isozymes showed varieties in different species. These differences and their respective hydrolase activities resulted in species different of plasma hydrolase activities. Especially, BChE isozyme showed high hydrolase activity against a particular substrate. This activity also affected by the divalent cations maybe owing to allosteric effect.

O-TH-17

COMPLETE ELIMINATION OF HEPATITIS C VIRUS BY SUSTAINED NONVIRAL GENE DELIVERY OF HUMAN INTERFERON Γ IN HUMAN HEPATOCYTE CHIMERIC MICE

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Objective: Chronic hepatitis C (CHC) is caused by infection with hepatitis C virus (HCV). Half the patients infected with HCV1b are refractory to interferon (IFN) α-based therapy. Recent studies have shown that IFNγ is as effective as IFNα in inhibiting HCV replication in cultured cells. In this study, we examined whether sustained nonviral gene delivery of IFNγ is effective in inhibiting HCV replication in human hepatocyte chimeric mice.

Methods: A plasmid expressing human IFNγ was constructed. The expression profile of IFNγ was examined after hydrodynamic injection of the plasmid to normal and chimeric mice at a dose of 0.12 or 250 μg/mouse respectively. The cells transfected by this gene transfer method was evaluated using a plasmid expressing enhanced green fluorescent protein to visualize transduced cells. Chimeric mice were injected intravenously with HCV1b to obtain HCV1b infected mice. The serum HCV RNA and IFNγ of these infected mice were measured for the evaluation of anti-HCV effects of sustained gene delivery of IFNγ.

Results: An injection of the IFNγ-expressing plasmid resulted in sustained transgene expression, and the therapeutic level of IFNγ (> 4,200 pg/ml) was maintained for more than two months. Confocal microscopy analysis revealed that human hepatocytes of chimeric mice were transfected with the plasmid by the method. The serum HCV RNA levels of mice infected with HCV1b were significantly ($P<0.05$) reduced to less than 1,000 copies/mL by IFNγ gene transfer, and no HCV RNA was detected in all of the surviving mouse livers at 7 weeks after gene transfer. In contrast, a transiently IFNγ-expressing plasmid induced little effects on the serum HCV RNA of HCV1b-infected mice.

Conclusion: These results indicate that sustained nonviral gene delivery of IFNγ is effective in eliminating HCV in human hepatocyte chimeric mice and, therefore, it can be a promising approach to CHC therapy.

O-TH-18

A PRECLINICAL STUDY OF NEW-GENERATION, RAPIDLY-DISINTEGRATING TASTE-MASKED EPINEPHRINE SUBLINGUAL TABLETS FOR THE TREATMENT OF ANAPHYLAXIS

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Objective: Epinephrine (E) is life-saving treatment in anaphylaxis. In community settings, a first-aid dose of E is injected from an auto-injector (E-auto). E-autos are associated with a number of issues. We developed an E sublingual (SL) tablet formulation as a potential alternative dosage form. The aim of this study was to evaluate the SL bioavailability of E from new-generation tablets.

Methods: New-generation SL E (30 mg and 40 mg) tablets were manufactured by direct compression. Dissolution was evaluated in vitro using our validated apparatus and method. By addition of citric acid, we masked the bitter taste of E. We studied the rate and extent of E absorption from the tablets in our validated rabbit model using a parallel-dose design (n = 5). The positive control was E 0.3 mg IM (EpiPen). The negative control was a placebo SL tablet. Tablets were placed under the tongue for 2 minutes. Blood samples were collected at frequent intervals to 1h. E concentrations were measured using HPLC with electrochemical detection.

Results: SL E tablets resulted in total in vitro release of E within 60 seconds. The mean ± SD maximum plasma concentration (Cmax) of 31.7±10.1 ng/mL at a peak time (Tmax) of 20±7 min, and area under the curve (AUC0-1h) of 678.0±149.0 ng/mL/min after SL E 40 mg did not differ significantly ($p>0.05$) from the Cmax of 27.6±7.0 ng/mL at a Tmax of 30±0 min with an AUC0-1h of 592.0±122.3 ng/mL/min after E 0.3 mg IM in the thigh. The Cmax of 16.7±6.3 ng/mL at a Tmax of 21±6 min with an AUC0-1h of 372.3±48.6 ng/mL/min after SL E 30 mg was significantly higher than after placebo.

Conclusion: E is absorbed rapidly from the new-generation, rapidly-disintegrating, taste-masked SL formulation. These tablets are suitable for Phase I studies in humans and potentially useful in the first-aid treatment of anaphylaxis.

O-TH-19

MECHANISMS OF PAMAM DENDRIMER-MEDIATED TIGHT JUNCTION MODULATION

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Objective: Poly (amidoamine) (PAMAM) dendrimers are capable of crossing the epithelial barrier, making them a promising oral drug delivery vehicle. Transcellular and paracellular transport are both important for dendrimer translocation; however, little is known about how dendrimers affect tight junctions.

Methods: Anionic G3.5 and cationic G4 PAMAM dendrimer-induced tight junction modulation was investigated in Caco-2 cell monolayers by measuring the permeability of [14C]-mannitol in the presence of dendrimers with or without various inhibitors of tight junction regulation. Actin, claudin-1, occludin, and ZO-1 accessibility was visualized using confocal laser scanning microscopy. Western blotting was utilized to determine tight junction protein expression after dendrimer treatment. Live cell confocal microscopy with Fluo-4 AM determined if intracellular calcium release occurred upon dendrimer treatment.

Results: For G3.5, phospholipase C, myosin light chain kinase, and dynamin inhibitors displayed a decrease in [14C]-mannitol permeability; however, this decrease did not occur for G4. A calcium/calmodulin protein kinase II inhibitor caused a decrease in permeability for both dendrimers. These results suggest differences exist in mechanisms of cellular entry and tight junction modulation between the anionic and cationic dendrimers. Tight junction accessibility after treatment increased, verifying that modulation occurred, and tight junction protein expression was not affected by the dendrimers. Calcium imaging revealed that both G3.5 and G4 induced changes in intracellular calcium concentrations in Caco-2 cells.

Conclusion: This is the first study to elucidate the mechanisms of dendrimer-mediated tight junction modulation. Inhibitor studies indicated that calcium has a role in dendrimer paracellular permeability, which was supported by live cell calcium imaging. These results will help guide the design of efficacious and safe dendrimer-based drug delivery systems.

O-TH-20

INVESTIGATION OF PH DISTRIBUTION IN DEGRADING MICROSPHERES OF HYDROPHILIC POLY(D,L-LACTIDE-CO-HYDROXYMETHYL GLYCOLIDE) BY CONFOCAL LASER SCANNING MICROSCOPY

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Objective: The acidification of poly(D,L-lactic-co-glycolic acid) (PLGA) during biodegradation often results in the instability of encapsulated biomacromolecules. The purpose of study was to investigate the microclimate pH (µpH) distribution in degrading microspheres of a more hydrophilic polyester, poly(D,L-lactide-co-hydroxymethyl glycolide) (PLHMGA), and compare those values with similar PLGA 50/50 formulations.

Methods: Microsphere µpH mapping was performed after incubation under physiological conditions by using a previously validated ratiometric confocal laser scanning microscopic (CLSM) method. The effect of polymer composition and concentration of PLHMGA solution used for the preparation of the microspheres on µpH kinetics were evaluated and compared to that of PLGA formulations. To understand the mechanism for microclimate pH differences, the pH in the release media of microspheres was monitored and the amount of water-soluble acids inside microspheres was extracted and quantified by titration. In addition, the transport of a pH-independent fluorescent probe, bodipy, as a marker of polymer matrix diffusion of µpH-controlling acid degradation products through PLHMGA and PLGA microspheres, was also studied by CLSM.

Results: Confocal µpH maps revealed that PLHMGA microspheres, regardless of copolymer composition, developed mostly neutral µpH (>5.8) compared with microspheres from PLGA showing acidic µpH as low as 4 during 4 weeks of incubation. The pH in the release media of PLHMGA microspheres was higher while and quantity of water-soluble acids inside the microspheres was lower than PLGA counterparts during incubation, strongly suggesting that acids do not incline to accumulate in PLHMGA matrix relative to PLGA. Moreover, the study of diffusion of bodipy showed that bodipy diffuses ~3-7 fold more rapidly in PLHMGA compared to PLGA microspheres, consistent with much more rapid release of acids observed from the hydrophilic polymer during bioerosion.

Conclusion: PLHMGA microspheres are less susceptible to acidification during degradation as compared to similar PLGA formulations. Therefore, PLHMGA systems may be more suitable to deliver acid labile molecules such as proteins.

O-F-01

BIOCOMPATIBLE DELIVERY SYSTEMS FOR NUCLEIC ACID THERAPIES

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Objective: The synthesis and characterisation of a new polymer carrier for gene and siRNA therapy

Methods: The delivery system was synthesised, characterised and complexed with a reporter gene (β-gal plasmid DNA) in phosphate buffer pH 6.0. The resulting complexes were sized and their zeta potential measured (Malvern Zetasizer 3000HS, UK). The complexes were also imaged using transmission electron microscopy and characterised for DNA binding affinity and DNA protection using gel electrophoresis. The in vitro transfection efficiency and biocompatibility of the complexes were determined in the A431 cell line. Additionally, in vivo tumour regression and siRNA downregulation studies in female tumour bearing mice (n=5/group) were carried out.

Results: The gene carrier produced gene-polymer complexes of 200-400 nm in diameter (polydispersity < 0.6). Complexes had a zeta potential of + 19.8 ± 4.4 mV (n=3) and were spherical, fibrillar and toroidal in shape. The new gene delivery complex protects DNA from degradation in serum for up to 2 hours and was as efficient as poly(ethylenimine) (PEI) in transfecting the A431 cell line. However, the new synthetic polymer was more than 1,000 times less cytotoxic than the PEI. In vivo gene medicine, comprising the polymer and the tumour necrosis factor alpha gene, was tumouricidal and when complexed with siRNA, the siRNA polymer complex demonstrated in vivo gene silencing activity.

Conclusion: A new synthetic gene and siRNA delivery polymer has been synthesised. This polymer is biocompatible to cells and is an efficient in vitro and in vivo gene and siRNA carrier

O-F-02

THE CHICKEN CHORIOALLANTOIC MEMBRANE. A MODEL FOR ANGIOGENESIS, TRANSEPITHELIAL DRUG DELIVERY AND XENOGRAFT RESEARCH

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Objective: In the present study we investigated the influence of the superficial sheet of flat peridermal cells (superficial sheet) on drug absorption, Angiogenesis and Xenograft cultivation. The Chorioallantoic Membrane (CAM) is a highly vascularized membrane that supplies the embryo with oxygen and calcium. The CAM model has been used intensively for fundamental and therapeutic research. But its application is limited. The main limitation is the superficial sheet that lays on top of the CAM.

Methods: We analyzed CAMs with and without removed superficial sheets on three different subjects: Drug uptake, Angiogenesis and tumor growth. Drug uptake has been investigated by the use of fluorescent labeled liposomes. Investigating Angiogenesis, CAMs have been treated with Vascular Endothelial Growth Factor (VEGF), a stimulator of blood vessel formation. Concerning tumor growth we used human lymphangiomas from the University Hospital Giessen and Marburg. Light and electron microscopy was used to visualize the differences regarding all three subjects.

Results: CAMs without superficial sheet absorb liposomes more quickly into the lymphatic vasculature. The removal of the superficial sheet has no influence on the stimulated formation of new blood vessels by VEGF. The superficial sheet acts as a protection for the vascularization of the tumor and tumor angiogenesis. The embryo's survival rate did not change significantly after the removal of the superficial sheet.

Conclusion: Without the superficial sheet we get a better uptake of drug carriers, especially liposomes. This is important for the characterization of topically applied drugs that are used in e.g. Photo Dynamic therapy. Tumors that grow on CAM can be used to study their physiology and to investigate new therapies. Proangiogenetic substances like VEGF seem to work independently from the existence of the superficial sheet. The removing of the superficial sheet is easy to do and the experiments with the CAM model remain cheap and effective.

O-F-03

DEVELOPMENT OF BROMOCRIPTINE MESYLATE TABLET USING SOLID-SMEDDS AS DRUG DELIVERY SYSTEM

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Objective: Bromocriptine mesylate (BM) following oral administration was reported to have very low bioavailability. Self-microemulsifying drug delivery system (SMEDDS) has been shown to improve the dissolution of poorly-water soluble drugs and thus enhance the bioavailability of drugs administered orally. Solid-SMEDDS is preferred over liquid SMEDDS due to low production cost, convenience of process control, high stability and reproducibility and better compliance. Therefore, the purpose of this study was to prepare BM as SMEDDS tablet to improve dissolution compared to original tablet.

Methods: Pseudo-ternary phase diagrams were constructed to obtain microemulsions with suitable components. Self-microemulsion was tested by diluting with 250 folds of DI water. Both particle size and morphology were determined. Selected drug loaded SMEDDS liquid was blended with various solid carriers at different ratios to form SMEDDS powders which suitable formulations were then mixed with additives and compressed into tablet. The dissolution and other tests of SMEDDS tablets were performed following USP specification compared to original product.

Results: The particle size and size distribution of selected SMEDDS were 63.60±7.4710 nm and 0.331±0.035 nm, respectively and were not changed after drug loading. TEM photomicrographs showed droplets of spherical shape. Upon adsorption onto solid carriers, the maximum weight ratio of SMEDDS to aerosil200, aerosil300 and neusilinUS2 with satisfactory flowability were 1.5:1, 2.0:1 and 2.5:1, respectively. These SMEDDS powders could redisperse back into microemulsion with the same droplet size. SMEDDS tablet using aerosil300 as solid carrier showed the highest drug release that the dissolution at 30 minutes was more than 80% which met the USP specification while the similarity and difference factors were 69 and 6, respectively.

Conclusion: SMEDDS of BM was successfully formulated and then transformed into powders with good flowability suitable for further preparation into conventional solid dosage form. Tablet of drug loaded SMEDDS with aerosil300 had dissolution profile similar to original tablet (f1=6, f2=69). Therefore, SMEDDS tablet was satisfactorily applicable as oral drug delivery system for poorly water-soluble drug.

O-F-04

AMORPHOUS FUROSEMIDE SALT EXHIBITS HIGHER DISSOLUTION RATE AND STABILITY COMPARED TO AMORPHOUS FUROSEMIDE ACID

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Objective: To characterise an amorphous furosemide salt and evaluate its stability, solubility, and dissolution rate in comparison to amorphous furosemide acid.

Methods: Amorphous furosemide sodium salt was prepared by spray drying furosemide from a mixture of ethanol 10v/v%, NaOH (molar ratio 1:1 with furosemide) and purified water. An amorphous acid was obtained by spray drying using methanol as solvent. The solubility of the two amorphous forms in simulated gastric and intestinal media was determined using a modified shake-flask method. The dissolution characteristics were studied using the µ-Diss profiler. The stability of the two amorphous forms was investigated using XRPD, and DSC was utilised to investigate the T_g. Information about differences between the amorphous forms was obtained by FTIR.

Results: A significantly higher equilibrium solubility of the amorphous salt in simulated gastric and intestinal media was found compared to the amorphous furosemide acid. The intrinsic dissolution rate was found to be 8.8±0.6 mg/cm^2/min for the amorphous salt and 1.1±0.1 mg/cm^2/min for the amorphous acid hence, a significant difference ($p<0.0001$) was observed. Furthermore, the salt exhibited a higher physical stability than the acid; the amorphous acid was stable for 4 days, whereas the amorphous salt was stable for 291 days at 22°C and 30% RH. The amorphous salt had a T_g of 101.2°C; a value 40°C higher than that found for the amorphous acid. Additionally, differences in the FTIR spectrum between the two forms were found between 1500-1700 cm^{-1} corresponding to the COOH and COO^- functional groups.

Conclusion: Amorphous furosemide sodium salt exhibited a significantly higher solubility and intrinsic dissolution rate compared to amorphous furosemide acid. The amorphous salt was more stable than the acid, probably due to the higher T_g. The enhanced stability, solubility and dissolution characteristics of the amorphous furosemide salt can potentially lead to an enhanced furosemide bioavailability upon oral delivery.

O-F-05

BREATH TESTING TO ASSESS DEFINITIVE ADHERENCE TO ORAL AND VAGINAL MEDICATIONS

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Objective: Poor medication adherence increases drug response variability. The availability of a breath test to measure, monitor and increase medication adherence may facilitate medical decision-making and improve clinical outcomes. Pilot studies were conducted to assess the feasibility of using a breath test to evaluate medication adherence.

Methods: Two separate IRB-approved studies were conducted. In study one, five healthy subjects were administered a size zero hard gel capsule containing 2-butanol, 2-pentanone, and L-carvone on six different occasions. Breath concentrations of 2-butanone and 2-pentanone were measured using a miniature gas chromatograph for 60 minutes. A population pharmacokinetic analysis was conducted using NONMEM (Version 7.2). Study two was a double blind randomized study with 13 healthy subjects. Two taggants, 2-pentyl acetate and 2-butyl acetate, were added to a tenofovir placebo gel and hydroxyethylcellulose placebo gel, respectively. The tenofovir placebo gel was applied to the vagina using a syringe applicator, while the HEC gel was used as a lubricant on a condom and applied into the vagina with a dildo. Sequential breath samples were collected for 75 minutes. A non-compartmental pharmacokinetic analysis was conducted using WinNonlin (Version 5.2).

Results: Using a one-compartment model, the model estimates for the first-order absorption and elimination rate constants were 0.034 and 0.129 $minutes^{-1}$ for 2-butanone, and 0.061 and 0.078 $minutes^{-1}$ for 2-pentanone, respectively. Significant inter-individual variability was observed in each molecule's elimination (2-butanone: 73.7%; 2-pentanone: 56.5%). For study two, concentrations of 2-pentyl acetate, 2-pentanol, and 2-pentanone, could be quantified in breath within 5 minutes. The elimination half-life was 25.2 (±8.9) minutes for 2-pentyl acetate, and 6 (±4.7) minutes for 2-butyl acetate. 2-Butanol and 2-butanone concentrations could not be quantified.

Conclusion: The results of these two pilot studies demonstrate that 2-pentyl acetate and 2-butyl acetate, and/or their metabolites, could be quantified in breath following oral or vaginal administration.

O-F-06

ASSESSMENT OF JUVENILE PIGS TO SERVE AS HUMAN PEDIATRIC SURROGATES FOR PRECLINICAL FORMULATION PHARMACOKINETIC TESTING

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Objective: Pediatric drug development is hampered by biological, clinical, and formulation challenges associated with age-based populations. A primary cause for this lack of development is the inability to accurately predict ontogenic changes that affect pharmacokinetics (PK) in children using traditional preclinical animal models. In response to this issue, our laboratory has conducted a proof-of-concept study to investigate the utility of juvenile pigs to serve as surrogates for children during preclinical PK testing of selected rifampin dosage forms.

Methods: Pigs were surgically modified with jugular vein catheters that were externalized in the dorsal scapular region and connected to an automated blood sampling system. Commercially available rifampin capsules were administered to both juvenile (~20 kg) and adult (~40 kg) pigs to determine relevant PK parameters. Orally disintegrating tablet formulations of rifampin were also developed and administered to juvenile pigs. Plasma samples were prepared from whole blood and analyzed for rifampin content by LC-MS/MS. Porcine PK parameters were determined from the resultant plasma-concentration time profiles and contrasted with published rifampin PK data in human adults and children.

Results: Ontogenic changes in Cmax and AUC values obtained in both juvenile (7.0 ± 2.2 µg/mL; 58.1 µg*h/mL) and adult pigs (28.8 ± 5.6 µg/mL; 188.4 µg*h/mL) correlated very well with age-based changes reported in the literature for human children and adults at the same dose. Additionally, changes in weight normalized CL and VD between the juvenile (0.3 L/h/kg; 1.3 L/kg) and adult pigs (0.1 L/h/kg; 0.4 L/kg) were representative of ontogenic changes in humans. Similar to humans, Tmax (2.0 h) and T1/2 (3.5 h) were unaltered between the different aged pigs.

Conclusion: These results demonstrate the utility of the juvenile porcine model for predicting human PK for rifampin. Furthermore, utilization of juvenile pigs during formulation testing may provide an alternative approach to expedite reformulation efforts during pediatric drug development.

O-F-07

COMPARISON OF BIORELEVANT MEDIA AND USP COMPENDIAL MEDIA ON SOLUTION-MEDIATED PHASE TRANSFORMATION OF AN AMORPHOUS DRUG DURING DISSOLUTION

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Objective: The majority of drugs currently in development are classified as BCS II, requiring various techniques to increase solubility and dissolution rates. One approach to increase solubility is to formulate the BCS II compounds in the amorphous state, where the amorphous solid produces a highly supersaturated concentration during dissolution. However, this supersaturated drug solution is often followed by precipitation (i.e., solution-mediated phase transformation, SMPT). The duration and extent of the supersaturation, which can be directly related to bioavailability, are reportedly dependent not only on the physicochemical properties of the drug compound (e.g. pKa and crystalline properties), but also on the composition of the dissolution medium. It has been shown that some surfactants also change the rate of the SMPT of BCS II drugs and effectively suppress their dissolution rates.

Methods: In this study, SMPT of an amorphous spironolactone, a non-ionizable BCS II compound, in the presence of Simulated Intestinal Fluid without pancreatin, USP (SIFsp), and biorelevant media (both fed and fasted), were investigated. Dissolution rates were measured using a flow-through dissolution apparatus. Images of the amorphous spironolactone compacts were captured in real-time in the flow-through apparatus using a stereomicroscope and polarized light microscope (PLM).

Results: Higher dissolution rates of amorphous spironolactone, 1.5-2.0x greater, were obtained in biorelevant media when compared to compendia media. Furthermore, duration of supersaturation was longer in both biorelevant media vs. SIFsp (~60 min vs. ~15 min). SMPT confirmed by the decrease in dissolution rates as well as images captures with stereomicroscope and PLM.

Conclusion: The duration of supersaturation is greater when amorphous spironolactone was evaluated in fed and fasted biorelevant media vs. compendia media. An increase in duration of supersaturation is predictive of a prolonged increase in higher concentration of drug in intestinal fluid which can result in a greater flux across the intestinal mucosa, potentially resulting in greater bioavailability.

O-F-08

VEHICLE ELICITED IMPROVEMENT OF INTESTINAL ABSORPTION OF A PHYTOPHARMACEUTICAL COMPOUND IN THE CACO-2 MODEL VIA INCREASED STABILITY AND REDUCED METABOLISM

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Objective: The objective of this work was to investigate the intestinal absorption of the phytopharmaceutical compound Nobilin and elucidate the effect of pharmaceutical formulations and of the method of preparation on absorption.

Methods: Aqueous transport media (aq-TMcaco) and two microemulsion formulations with high and medium surfactant-to-fluid lipid ratio in aq-TMcaco were used with two different preparations of Anthemis Nobilis L. in stability and absorption studies. In vitro absorption was determined using the Caco-2 cell culture model and permeation and metabolism parameters were deduced by means of kinetic multi-compartment modeling.

Results: The degradation constant of Nobilin in solution with aq-TMcaco was approximately 0.4 h-1 and degradation occurred mainly by water addition. The two formulations with medium and low lipid content decreased the degradation constant 5-fold and 2-fold, respectively, independently of the plant preparation. A relatively high permeability coefficient of 2.69 * 10-4 cm/s of Nobilin due to transport by diffusion was obtained in aq-TMcaco, which was reduced by the use of formulations. The increased stability of the drug in solution elicited by both formulations and the decrease of drug permeability by these formulations is probably because of the encapsulation of the drug in colloidal particles. A marked degradation of Nobilin in the cells was observed with degradation constant varying between 27.5 and 2.86 h-1 depending on the used preparation. This led to absorption of 33 and 64%, respectively. The degradation pathway in the cells differed from that in solution and yielded conjugation products among others with cysteine and glutathione that were subject to polarized efflux from the cells. In combination with one of the formulations, cellular degradation was further suppressed, increasing drug absorption.

Conclusion: Pharmaceutical formulations improved the stability of Nobilin and the preparation of Anthemis Nobilis L. reduced cellular metabolism which increased absorption. Pharmaceutical formulations improved the stability of Nobilin and the preparation of Anthemis Nobilis L. reduced cellular metabolism which increased absorption.

O-F-09

CHEMICAL MODIFICATIONS IN AGGREGATES OF RECOMBINANT HUMAN INSULIN INDUCED BY METAL-CATALYZED OXIDATION: COVALENT CROSS-LINKING VIA MICHAEL ADDITION TO TYROSINE OXIDATION PRODUCTS

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Objective: To elucidate the chemical modifications in aggregates of recombinant human insulin induced by metal catalyzed oxidation (MCO).

Methods: Insulin was exposed for 3 hours at room temperature to the oxidative system copper(II)/ascorbate. Chemical derivatization with 4-(aminomethyl) benzenesulfonic acid (ABS) was performed to detect 3,4-dihydroxyphenylalanine (DOPA) formation. Electrospray ionization-mass spectrometry (ESI-MS) was employed to localize the amino acids targeted by oxidation and the cross-links involved in insulin aggregation. Oxidation at different pH and temperature was monitored with size exclusion chromatography (SEC) and ESI-MS analysis to further investigate the chemical mechanism(s), to estimate the aggregates content and to quantify DOPA in aggregated insulin.

Results: The results implicate the formation of DOPA and 2-amino-3-(3,4-dioxocyclohexa-1,5-dien-1-yl) propanoic acid (DOCH), followed by Michael addition, as responsible for new cross-links resulting in covalent aggregation of insulin during MCO. Michael addition products were detected between DOCH at positions B16, B26, A14, and A19, and free amino groups of the N-terminal amino acids Phe B1 and Gly A1, and side chains of Lys B29, His B5 and His B10. Fragments originating from peptide bond hydrolysis were also detected.

Conclusion: MCO of insulin leads to aggregation through covalent cross-linking via Michael addition to tyrosine oxidation products.

O-F-10

DEVELOPMENT OF LIPOPEPTIDE VACCINE CANDIDATE AGAINST GROUP A STREPTOCOCCUS

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Objective: Group A streptococcus (GAS) infections result in a number of human diseases, such as potentially life-threatening postinfectious sequelae. To protect against infection with multiple GAS serotypes, we designed and synthesized a fully synthetic self-adjuvanting lipopeptide (LP) GAS vaccine constructs, using stepwise solid phase peptide synthesis (SPPS). In the present study, each lipopeptide was composed of GAS B-cell epitopes (J14 or 88-30 or incorporating both epitopes), a universal CD4+ T-cell helper epitope (P25) and an immunostimulant lipid moiety. Immunological evaluation of LPs was carried out following intranasal administration of Swiss outbred mice. Further physico-chemical characterization was done including dynamic light scattering (DLS), transmission electron microscopy (TEM) and circular dichorism spectroscopy (CD) studies.

Methods: The lipopeptides were synthesized using Boc-SPPS. The lipopeptides were self-assembled into nanoparticles and the particle size was analyzed by DLS. CD spectroscopy was performed to analyse the peptide conformation. The immunogenicity of the nanoparticles was tested in mice and antibody titres were analysed using ELISA.

Results: Intranasal immunization with LP-88/30-J14 resulted in systemic IgG antibody response in outbred Swiss mice. Interestingly, 88/30 specific IgG response was significantly higher in the LP analogue containing the J14 epitope (LP-88/30-J14) in comparison to a lipopeptide incorporating the 88/30 epitope only (LP-88/30). DLS measurement showed LP-88/30-J14 formed nanoparticles of a smaller size (10 nm) than LP-88/30 (100 nm). LP-J14 exhibited same size and induce same J14 specific IgG response as LP-88/30-J14 suggesting incorporation of the J14 epitope had a significant influence on the size of lipopeptides. CD studies showed that none of the tested lipopeptides formed a typical conformational secondary structure.

Conclusion: The current study demonstrated the capacity of our lipopeptidic nanoparticulate-system to provide adjuvant activity to more than one GAS peptide epitope for eliciting systemic IgG antibodies. Choice of peptide epitopes can also mediate nanoparticulate size of lipopeptides and consequent immune response. These findings are particularly encouraging for the development of a broad-spectrum lipopeptide GAS vaccine.

O-F-11

MAGNETIC TARGETING OF MAGNETIC-FLUID-LOADED LIPOSOMES (MFLS) TO BRAIN FOR MRI DIAGNOSIS AND TREATMENT OF GLIOBLASTOMA

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Objective: Currently the management of brain cancers remains a real challenge in oncology mainly due to late diagnosis. The difficulty of total resection of glioblastomas and the presence of blood-brain-barrier which significantly reduces the efficacy of tumor-targeted chemotherapy are the most notable obstacles. MFLs offer an attractive alternative as theranostic systems, indeed efficient MRI contrast agents which can serve as drug delivery reservoirs able to be guided under magnetic field gradient. The challenge here is to target glioblastomas implanted in the striatum of mice by using this hybrid nanosystem after its intravenous administration followed by its selective accumulation into the tumors by applying an attractive magnetic force produced by an external magnet.

Methods: Rhodamine-labelled MFLs were prepared by hydration of a thin lipid film with suspension of maghemite nanocrystals, followed by sequential extrusion and gel exclusion chromatography to remove non-entrapped iron oxide. Targeting experiments were conducted on U87 glioblastomas bearing mice with or without an external magnet (138 T/m) onto the head. In-vivo MR Images were acquired at 7 Tesla, histological analyses performed by confocal fluorescence microscopy and iron oxide concentration in the tumors determined by ex-vivo electron spin resonance.

Results: MRI and histological analyses clearly revealed that the applied magnetic field gradient efficiently accumulates the MFLs in the tumors while, in healthy brain tissue, the liposomes mainly remained within the blood stream. ESR analysis showed a recovered amount of iron oxide increased more than 1,84 times in the magnet-exposed glioblastomas. The magnetic force exerted on the MFLs concentrates them in the tumor vessels where they extravasate to the tissue through enhanced EPR process.

Conclusion: Taking into account the ability of the vesicle structure to incorporate anticancer drugs, our results in their whole demonstrate that magnetic targeting of MFLs should be an efficient strategy for glioblastoma treatment advantageously combined with MRI diagnosis and monitoring.

O-F-12

THERANOSTIC NANOPARTICLES FOR THE DIAGNOSIS AND TREATMENT OF HCC

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Objective: To design and prepare a multifunctional nano-vector for the diagnosis and treatment of human hepatocellular carcinoma (HCC).

Methods: A novel biodegradable block polymer Poly (lactic acid)-poly (ethylene glycol)-poly (l-lysine) (PLA-PEG-PLL) which contained many free side chain amino groups were designed and synthesized. Diethylenetriaminepentaacetic acid (DTPA) and biotin were linked to the amino groups of PLA-PEG-PLL to form two modified materials: PLA-PEG-PLL-DTPA and PLA-PEG-PLL-biotin, respectively. The paclitaxel(PTX)-loaded nanoparticles (NPs) were prepared by solvent diffusion method with mixed PLA-PEG-PLL-DTPA and PLA-PEG-PLL-biotin where all the active groups (DTPA and biotin) were distributed on the surface of PTX-loaded NPs. Then Gd ions were chelated to the surface DTPA groups of the PTX-loaded NPs by incubating PTX-loaded NPs with GdCl3 to obtain Gd, PTX-loaded NPs. The biotinylation alpha-fetoprotein (AFP) antibodies were linked to the surface biotin groups of Gd, PTX-loaded NPs by avidin –biotin reaction to obtain target Gd, PTX-loaded NPs.

Results: The target Gd, PTX-loaded NPs showed spherical morphology, positive surface charge, and uniform particle size distribution. The encapsulation efficiency and drug loading were 88.76±1.64% and 1.59±0.06%, respectively. The target Gd, PTX-loaded NPs showed high in vitro cytotoxicity and could be internalized by HepG2 cells and the internalization was both concentration and time depended. The target Gd, PTX-loaded NPs exhibited higher T1 relaxivity and higher cellular uptake rate compared to non-target Gd, PTX-loaded NPs. The in vivo MRI results indicated that the target Gd, PTX-loaded NPs showed significantly signal intensity enhancement at the tumor site compared with non-target Gd, PTX-loaded loaded NPs and Gd-DTPA injection (Magnevist®) in H22 tumor-bearing mice and the imaging time was significantly prolonged from less than an hour (Magnevist®) to 12 h. Meanwhile, the target Gd, PTX-loaded NPs showed significantly higher anti-tumor activity compared to PTX injection (Taxol®) and non-target Gd, PTX-loaded NPs.

Conclusion: These results demonstrated that the target Gd, PTX-loaded loaded NPs had great potential for theragnostic application on HCC.

O-F-13

A ROLE OF PROSTAGLANDIN TRANSPORTER IN REGULATION OF INTERCELLULAR PGE2 LEVELS UNDER INFLAMMATORY CONDITIONS

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Objective: Prostaglandin E2 (PGE2) is a principal mediator of inflammation. PGE2 is basically impermeable to cell membranes at physiological pH and needs solute carrier; however it remains unclear how intercellular concentration of PGE2 is regulated under the condition of inflammatory. We, therefore, aimed to study a role of prostaglandin transporter (PGT/SLCO2A1) in regulating PGE2 release in response to pro-inflammatory stress in human pulmonary BEAS-2B cells.

Methods: PGT-mediated PGE2 transport was examined by Xenopus laevis oocytes expressing PGT. In BEAS-2B treated with lipopolysaccharide (LPS) for 24 hours, the effect of PGT inhibitors on PGE2 efflux was investigated by assessing its extracellular concentration of PGE2. Data are given as the mean of values obtained in at least three individual experiments with standard error (SEM). Student's t-test ($p < 0.05$) was performed to analyze statistical significance.

Results: In the BEAS-2B cells exposed to LPS (0.1µg/mL), mRNA expression of a pro-inflammatory cytokine IL-6 increased approximately 8-fold compared to the untreated cells, and cumulated PGE2 in extracellular medium was also increased at least 3-fold. Four PGT inhibitors (e.g. BCG, BSP, TGBz and PGB1) were tested for their inhibitory potential to PGT-mediated PGE2 uptake by and efflux out of Xenopus laevis oocytes expressing PGT. BCG, BSP and PGB1 almost abolished the uptake, while TGBz had the least effect showing a reduction in uptake by 40%. The three inhibitors except for TGBz were comparable to reduce PGT-mediated efflux of PGE2 by about 80 %. Similarly, released PGE2 from the cells decreased in the presence of BCG and BSP by 45% and 44%, respectively, while TGBz increased the concentration by 71%, suggesting that PGT mediates PGE2 release from BEAS-2B cells.

Conclusion: The present study shows a role of PGT in regulating extracellular concentration of PGE2 by controlling influx and release of PGE2 induced by pro-inflammatory signals.

O-F-14

FUNCTIONAL CHARACTERIZATION OF ORGANIC CATION TRANSPORT IN HEK293 CELLS

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Objective: The blood-brain barrier (BBB), formed a tight monolayer of the brain capillary endothelial cells, restricts the transfer of drugs to the central nervous system (CNS). It is a major obstacle to the delivery of drugs to their target in the CNS. Recently, it is suggested that the penetration of some cationic drugs into the CNS should involve transporter since the BBB permeability of amantadine, clonidine, diphenhydramine and oxycodon is saturable, and inhibited by other cationic drugs. The putative transporter will improve the CNS penetration of drugs. However, until now, the molecular entity of this transporter remains unknown. The purpose of this study is to identify uptake transporter of cationic drugs.

Methods: In vitro transport studies using amantadine were conducted in HEK293 cells. Expression of transporters is confirmed by RT-PCR and Western blot analysis. siRNA and plasmide were introduced into the cells using Lipofectamine RNAiMAX and Fugene HD according to the manufactures protocols.

Results: Uptake of amantadine in HEK293 cells was saturable with K_m value of 215±55 μM, and inhibited by olanzapine and pyrilamine with K_i of 2.1 and 6.7 μM, respectively. The uptake of amantadine was increased 2.5-fold by a preincubation with 1 mM of amantadine and pyrilamine (p<0.05). Among the orphan transporters, which belong to SLC16 and SLC22, mRNA expression of SLC16A9, SLC16A12 and SLC22A23 was detected in HEK293 cells by RT-PCR. Transfection of siRNA against *SLC16A9* caused a significant reduction in the expression of SLC16A9 protein (19% of the control, p<0.05) along with the uptake of amantadine for 10 min (63% of the control value, p<0.05). Expression of Slc16A9 in the crude membrane fraction prepared from mouse BBB enriched fraction was detected by Western blot analysis.

Conclusion: HEK293 cells express the putative uptake transporter of amantadine endogenously, which accept pyrilamine as substrate. Knockdown study suggests SLC16A9 as a candidate transporter for the amantadine uptake in HEK293 cells, and in the blood-brain barrier.

O-S-01

MECHANISMS LIMITING DISTRIBUTION OF NOVEL BRAF INHIBITORS TO THE BRAIN: IMPACT ON THE TREATMENT OF MELANOMA BRAIN METASTASES

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Objective: Brain metastases represent a devastating consequence of melanoma primarily due their effects on both mortality and quality of life. The incidence of melanoma is escalating. About 75% of the patients diagnosed with metastatic melanoma are found to have brain metastases at autopsy. Several selective BRAF inhibitors have been developed to target the valine 600 to glutamic acid substitution ($BRAF^{V600E}$) which is most commonly found in metastatic melanoma. Clinical trials with BRAF inhibitors are showing remarkable activity in peripheral disease. However, the CNS delivery and efficacy of these targeted agents against brain metastases remains unknown. Thus, the objective of the current study was to investigate the brain distribution of the novel BRAF inhibitors vemurafenib, PLX4720, and dabrafenib.

Methods: Intracellular accumulation, bidirectional transport, and the competition assays for the three BRAF inhibitors were conducted in MDCKII cells that overexpress either murine bcrp or human P-gp. *In vivo* pharmacokinetic studies to examine brain distribution were performed in FVBn (WT), *Mdr1a/b*$^{-/-}$, *Bcrp1*$^{-/-}$ and Mdr1*a/b*$^{-/-}$*/Bcrp1*$^{-/-}$ (TKO) mice. The concentrations of vemurafenib, PLX4720, and dabrafenib from all the studies were determined using a sensitive LC-MS/MS method.

Results: The intracellular accumulation was significantly lower in the transfected cell lines compared to wild-type control for all three molecules. Bidirectional flux studies revealed greater transport in B-to-A direction compared to A-to-B direction due to active efflux by both P-gp and Bcrp. The steady-state B/P ratio of vemurafenib was ~81-fold higher in TKO mice compared to WT mice. The AUC_{Brain}/AUC_{plasma} ratio of dabrafenib was ~6-fold higher in TKO mice compared to WT mice after a single oral dose. Interestingly, there was no significant difference found in the brain distribution of PLX4720 between WT and TKO mice.

Conclusions: The brain distribution of vemurafenib and dabrafenib is severely restricted at the blood-brain barrier due to active efflux by both P-gp and bcrp. The preliminary data suggest that additional efflux transporter(s) may be playing a role in the BBB efflux of PLX4720. These initial data suggest that dabrafenib has greater brain penetration when compared to vemurafenib and PLX4720, which may have eventual clinical significance in treating melanoma brain metastases.

O-S-02

TARGETED DELIVERY OF A MODEL IMMUNOMODULATOR MYCOPHENOLIC ACID TO THE LYMPHATIC SYSTEM USING TRIGLYCERIDE MIMETIC PRODRUGS

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Objective: Enhanced delivery of immunomodulators to the lymphatic system provides an opportunity to increase drug exposure to lymphocytes and may selectively improve the treatment of certain immune system diseases. By harnessing the metabolic pathways of dietary lipids which are inherently absorbed through the lymphatic system in the form of lipoproteins, the current study aims to target the delivery of an immunosuppressant mycophenolic acid (MPA) to the intestinal lymphatic system via a series of bio-inspired triglyceride mimetic prodrug derivatives: three ester-spacer linked (C4es, C5es, C6es); two ether-spacer linked (C4et, C6et); or directly linked (MPA-TG).

Methods: Lymphatic drug transport was examined in mesenteric lymph-cannulated rats following intraduodenal infusion of MPA or prodrugs in lipid formulations containing 40mg oleic acid. Biotransformation of the prodrugs was assessed via incubation with rat digestive fluid and by examination of the prodrug derivatives in mesenteric lymph by HPLC-MS.

Results: Compared with parent MPA, lymphatic transport was enhanced by 97 fold for MPA-TG (no spacer), 51-71 fold for ester-spacer linked, and only 2-fold for ether-spacer linked compounds. Hydrolysis of all triglyceride mimetics in rat digestive fluid was rapid, with instantaneous release of corresponding monoglyceride forms. Subsequently, monoglyceride forms of MPA-TG and ester-spacer linked prodrugs were re-esterified with oleic acid, most likely originating from the co-administered formulation, in the enterocyte prior to incorporation into lymph lipoprotein assembly and transport pathways. In contrast, no re-esterified products were found for the ether-spacer prodrugs. In addition, the monoglyceride forms of ester-spacer prodrugs were labile to further luminal degradation, which might explain their slightly inferior lymphatic transport compared to MPA-TG.

Conclusion: The type of spacer between MPA and glyceride backbone played an important role in dictating lymphatic uptake by affecting metabolic stability of intermediate hydrolytic products in the gastrointestinal lumen and specific recognition of monoglyceride-like substrates by re-esterification enzymes in the enterocyte. This study provides insights into effective design of lymphotropic prodrugs, and exemplifies the potential use of drug delivery principles to guide medicinal chemistry strategies for immune-related therapeutic indications.

O-S-03

CO-ADMINISTRATION OF COQ10 NANOPARTICLES WITH TAMOXIFEN NANOPARTICLES FOR AUGMENTED ANTITUMOR EFFICACY AND REDUCED HEPATOTOXICITY

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Objective: To explore the oral antitumor efficacy and toxicity of tamoxifen (Tmx) loaded PLGA nanoparticles in DMBA induced breast cancer model and effect of co-administration of CoQ10 loaded PLGA nanoparticles on same.

Methods: Tmx and CoQ10 loaded PLGA nanoparticles (Tmx-NPs; CoQ10-NPs) were prepared by emulsion solvent-evaporation method with PVA and DMAB as the stabilizes respectively. NPs were freeze dried with 5 % trehalose as cryoprotectant and evaluated for their particle size and entrapment efficiency. Median effect analysis of the MTT assay was implemented to evaluate the efficacy of the combination against MCF-7 cell lines. In vivo antitumor efficacy after oral administration of combination of Tmx-NPs and CoQ10-NPs were investigated in DMBA induced female Sprague Dawley (SD) rats. Tumor volume was measured continuously and animals were sacrificed at the end of 28 days. Different toxicity markers were evaluated in plasma (TNF- α, IL-6, ALT, AST) and liver tissue (MDA).

Results: Tmx-NPs and CoQ10-NPs were prepared and found to have particle size 164.58 ±3.81 nm, 81.45±2.45 nm and entrapment efficiency 85.23±2.31%, 93.26±2.89% respectively. Cell cytotoxicity experiments revealed ~13.37-fold dose reduction index for Tmx-NPs upon combination with CoQ10-NPs. The combination showed strong synergism with CI ~0.09 when evaluated using Calcusyn, Biosoft USA. The combination further significantly reduced the tumor growth (35.45±2.45%) as compared to Tmx-NPs alone (41.56±2.34%) whereas untreated group showed an increase in tumor size up to 158.66%. Co-administration of Tmx-NPs and CoQ10-NPs also showed significant reduction in hepatotoxicity of Tmx which was indicated by the decreased level of biochemical parameters (ALT, AST and MDA) and inflammation indexes (IL-6 and TNF-α) as compared to the free drug.

Conclusion: Co-administration of CoQ10-NPs with the Tmx therapy could be employed as tool for augmented breast cancer efficacy and reduced toxicity of Tmx.

O-S-04

IMPORTANCE OF STRAIN RECOVERY PHENOMENON IN TABLET DISINTEGRATION

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Objective: Disintegration is one of the most important characteristics of tablets for ensuring the bioavailability of active pharmaceutical ingredients. The major mechanisms proposed for disintegrant action include swelling, capillary action and strain recovery. However, the role of disintegrant particle deformation and subsequent strain recovery has not been well studied. The objective of this study was to understand the importance of strain recovery phenomenon in tablet disintegration.

Methods: Disintegrant particles were either compacted using the universal testing machine or entrapped as free particles in a stainless steel mesh. The disintegration of compacts and wetting effect on free disintegrant particles were visualized using high speed video imaging. MATLAB was used to analyse the acquired images. Break down behavior of compacts, prepared with selected disintegrants, was determined at different compression forces to evaluate recovery of compaction strain. Particle sizes of compacted and free particles were also determined using laser diffractometry and optical microscopy.

Results: Sodium starch glycolate demonstrated highest swelling property (~ 6.5x and 12x increase in compact and particle area, respectively) while microcrystalline cellulose compacts had the highest capillary action (flow rate, ~11.5 mm/s) among all the disintegrants studied. No significant swelling (~1.1x increase) and capillary action (flow rate, ~1.5 mm/s) were observed with crospovidone for free and compacted particles respectively. Disintegrability of crospovidone compacts increased with increase in compression pressure, suggesting the importance of strain recovery for crospovidone disintegrant action.

Conclusion: High speed video imaging, followed by image analysis had provided an in-depth understanding of the disintegrant behaviors of free and compacted disintegrant particles upon wetting. The role of deformation and recovery under actual compaction conditions were examined. It was concluded that recovery of energy of strain deformation was the major mechanism for crospovidone disintegrant action.

O-S-05

PROFILING OF BILE ACIDS AND THEIR SULFATE METABOLITES IN HUMAN URINE BY LC-MS/MS

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Objective: Sulfation is the dominant pathway of bile acids (BAs) elimination and detoxification in humans. Several lines of evidence indicate the role of sulfation as a defensive mechanism for protecting the liver by decreasing the toxicity of BAs accumulated during hepatobiliary diseases. Individual BAs and their sulfate metabolites (BA-sulfates) vary markedly in their physiological and pathological roles. To this end we developed LC-MS/MS method for the simultaneous quantification of individual BAs and BA-sulfates in human urine to understand their role in the prognosis of hepatobiliary diseases.

Methods: BAs and BA-sulfates were extracted from human urine by solid phase extraction. The chromatographic separation was carried out on a ACQUITY UPLC® BEH C18 column (1.7 µm, 150 x 2.1 mm I.D.) with 80% methanol/20% acetonitrile (Mobile Phase A) and 7.5 mM ammonium acetate (Mobile Phase B), adjusted to pH 7.0 using 10 M ammonium hydroxide at a flow rate of 0.2 mL/min and in a total run time of 32 min. The MS/MS analysis were performed in negative mode on Applied Biosystems/MDS SCIEX 4000 Q TRAP® quadrupole linear ion trap mass spectrometer with an electrospray ionization (ESI) source.

Results: The limit of quantification was 1ng/ml for all the analytes and the method was linear over the range of 1-1000 ng/ml. The validated method is selective, sensitive, accurate (<15% RE), and precise (<15% RSD). This LC-MS/MS method was applied to simultaneously quantify BAs and BA-sulfates in male and female healthy control subjects. More than 85% of the total bile acids are present in sulfate form suggesting that sulfation is the major pathway of BA elimination in humans.

Conclusion: A specific, sensitive, accurate, and reliable LC-MS/MS method was developed for quantification of BAs and BA-sulfates. This LC-MS/MS assay will be applied in support of studies investigating the role of BAs and BA-sulfates as biomarkers for hepatobiliary diseases.

O-S-06

DEVELOPMENT OF A MUCOSAL TISSUE CULTURE MODEL AS A PRECLINICAL TOOL TO EVALUATE ANTIRETROVIRAL (ARV) CANDIDATES FOR HIV PRE-EXPOSURE PROPHYLAXIS (PREP)

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Objective: Better predictive models are required to identify ideal candidates for HIV prevention. We optimized a mucosal tissue culture system for this purpose.

Methods: Mucosal tissue was prepared as 3-mm3 explants. Explants (n=8 donors) were incubated in tenofovir (TFV) or maraviroc (MVC) for 72h, transferred to gelfoam rafts, and harvested over 96h. From 10 donors, TFV intracellular active metabolite (TFV-dp) was measured at 24h and viability determined by MTT. All drug concentrations were determined by LC-MS/MS. Immunohistochemistry (IHC) quantified number/distribution of HIV-infectable cell populations (CD3: total T-cells; CD4: T-helper; CD68: monocytes; CD1a: dendritic) at baseline in 11 vaginal, 10 cervical, and 4 rectal tissues. Changes in these populations over time were evaluated in 12 tissue specimens. Median data are presented.

Results: TFV and MVC equilibrated in tissue within 9h (MVC conc. 82%, and TFV conc. 68% of media). MVC and TFV tissue elimination was biphasic (alpha t1/2 = 2.8 and 3.3h; beta t1/2 = 75 and 53h, respectively). TFV-dp correlated with MTT viability. Over 48h, mucosal epithelium uniformly deteriorated, yet localization of HIV-infectable populations at the mucosal/submucosal junction were preserved. Variability in baseline populations was highest in cervical tissue (CV% = 75, 79, and 88% for CD3, CD4 and CD68 staining, respectively), moderate in vaginal tissue (48, 57, and 80%), and lowest in rectal tissue (44, 66, and 15%). By 7d in culture, the median loss of CD3 and CD4 cells was 73 and 77%, respectively.

Conclusion: Residual ARV concentrations are detected up to 4 days. To avoid falsely positive infection data, MTT baseline screening is critical for tissue selection. High variability in target cell populations likely explains high variability in infectivity published by others, and IHC should assist in interpretation of results. With significant loss of target cells, infectivity assays must target activity prior to 7d in culture.

O-S-07

RHEOLOGICAL CHARACTERISATION OF LECITHIN-BASED EMULSIONS FOR POTENTIAL USE AS A SALIVA SUBSTITUTE IN PATIENTS WITH XEROSTOMIA

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Objective: Xerostomia is a condition in which there is a reduction or absence of saliva production, causing difficulties swallowing, speaking and eating, and an increased incidence of dental caries and periodontal disease. This study aimed to investigate the rheological properties of oily formulations for potential use as saliva substitutes in patients with xerostomia.

Methods: Compositions (10g) comprising 1:1 w/w lecithin/propylene glycol mix (SM), water (W) and rice bran oil (RBO) or isopropyl myristate (IPM) were prepared by stirring. These were assessed by light microscopy and rheology, and ternary phase diagrams constructed to summarise results. Rheological properties were investigated using a rotational rheometer (AR2000, T.A. Instruments) with parallel plate and 40 mm solvent trap steel plate geometry. Steady shear was varied from 0 to 50 s^{-1} and oscillatory rate from 0.1 to 10 Hz. Storage (G') and loss (G") modulus were used to determine tanδ, the ratio of liquid-like to solid-like behaviour (G"/G').

Results: For compositions containing RBO, frequency-dependent viscoelasticity was observed at SM concentrations of 20-30% w/w. Liquid-like properties dominated at frequencies <5 Hz (tanδ >1) while elasticity dominated at higher frequencies (tanδ <1). A positive spike in tan δ occurred in formulations containing greater than 10% W and 30% SM, which may suggest an abrupt physical change occurs in these compositions as frequency increases. Tanδ < 1 occurred if SM was greater than 30% for all RBO formulations and in IPM formulations when the ratio of IPM:W was less than 1.5, suggesting the higher surfactant concentration results in higher elasticity.

Conclusions: Rheological properties were influenced by oily formulation composition, frequency and shear stress. Frequency-dependent formulations are of particular interest as the viscosity that dominates at low frequencies may improve lubrication of the oral cavity at rest whereas elasticity at higher frequencies may improve retention during swallowing and speaking.

O-S-08

ESTABLISHMENT & CHARACTERIZATION OF AN ADVANCED IN VITRO CO-CULTURE MODEL OF THE AIR-BLOOD BARRIER

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Objective: The deep lung is characterized by a thin air-blood barrier (ABB) and a huge surface. Therefore, it is one of the most interesting routes for drug delivery. To achieve a better understanding of the pulmonary route with regard to bioavailability and toxicity of drugs, the use of animal testing for research is common to date. In order to minimize these animal studies according to the 3R-principle (Replacement, Reduction and Refinement), in vitro models are now in focus of research. Thus, the aim of our work is the establishment of a complex in vitro co-culture model to mimic the human in vivo situation of the ABB.

Methods: The barrier functions, such as macrophage clearance and integrity of the epithelial cell layer, are essential to investigate drug delivery of different formulations. Hence, this model is based on a co-culture of human primary alveolar epithelial type (AT) I-like cells and human primary alveolar macrophages. Therefore, culture conditions have to be optimized. A more detailed investigation of the inhalation process is then carried out by usage of the PADDOCC (Pharmaceutical Aerosol Deposition Device On Cell Cultures). This instrument simultaneously allows the aerosolization, deposition and absorption of pulmonary formulations on in vitro systems.

Results: As a first step cell densities and respective culture media were optimized to improve the barrier properties with regard to TEER (>1000 Ohm*cm²) and expression of tight junctions. Second, the differentiation of ATII to ATI-like cells was determined via immunohistochemical methods. Third, live cell imaging could show the behaviour of macrophages on the epithelial cell layer and moreover, particle uptake experiments were performed using the PADDOCC.

Conclusion: In the context of drug delivery this novel primary human in vitro co-culture model will allow the evaluation of new drugs and drug carriers for pulmonary applications in the future.

O-S-09

CHARACTERIZATION OF CELL PENETRATION OF ZNPCSO4 DELIVERED BY NANOPARTICLES OF HEXAGONAL LIQUID CRYSTALLINE PHASE

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Objective: Nanodispersed systems of monoolein-based hexagonal phase have been studied in recent years as a topical delivery system. The aim of this research was to investigate the in vitro topical delivery of ZnPcSO4 into culture cell by nanoparticles of hexagonal liquid crystalline phase for topical Photodynamic Therapy (PDT) of skin cancer.

Methods: The hexagonal crystalline phases were prepared with a sufficient amount of monoolein, poloxamer, oleic acid, and aqueous phase (citrate buffer, pH 6.0). The mean diameter and particle size distribution of the nanodispersion were determined by dynamic light scattering. The formulation was incubated with fibroblastic cell culture (L929) for 4 h, the in vitro cell penetration was assayed by fluorescence microscopy (Axioskop 2 plus, Carl Zeiss, Göttingen, Germany) with (filter Set 50, Carl Zeiss). The cytotoxicity of the ZnPcSO4 nanodispersion was evaluated by the spectrophotometric MTT assay. The spectrometric method was applied for the quantifications by measuring the absorbance values at 570 nm with a microplate reader. The cellular viability was calculated as the percentage of viable cells compared to the control group.

Results: Particle size results showed that all samples were in the nanometric range (140 to 200 nm) associated with the ideal size distribution. Polydispersive index was lower than 0.21. Zeta potential surface was average value of -23.8 mV. The in vitro penetration results showed that the nanodispersion system promoted a greater penetration of ZnPcSO4. It was observed an increase in the intensity of the emission light retained by the ZnPcSO4 at a specific wavelength (640 and 730 nm). The results revealed cell viability above 99% for the nanodispersion containing or not ZnPcSO4.

Conclusion: These results may suggest a potential delivery system for topical PDT since, its effectiveness depends on the delivery and accumulation of ZnPcSO4 in the target cells.

O-S-10

POLYETHYLENE GLYCOL (PEG) CONJUGATED HEPARIN-TAUROCHOLATE DERIVATIVE (LHT7) AND PROTAMINE NANOCOMPLEX ENHANCES ANTI-TUMOR ACTIVITY BY INHIBITING ANGIOGENESIS

F. Alam and Y. Byun
Seoul National University, Korea

Objective: Targeting tumor angiogenesis with nano-sized materials is a classical approach for chemotherapy. Our nano-complex or nanoplex (NP) consists of biomimic material, Polyethylene glycon (PEG) conjugated low molecular weight heparin-taurocholate derivative (P-LHT7) with protamine, which is designed to form a physical complex as well as target to deliver to tumor angiogenic environment.

Methods: This NP was prepared by dissolving PEG (10 kDa) conjugated LHT7 with protamine and chronologically, dialyzed and lyophilized. The size had been measured with zeta-potential with DLS and confirmed by image with its stability in PBS and serum. In SD rat model, the plasma profile was measured with therapeutic dose 5 mg/kg and biodistribution and organ and tumor accumulation were measured in tumor bearing mice model with Cy5.5 labeled NP. Finally its anti-tumor efficacy was conducted at first in murine cancer model with different dose-intervals. And once per week and twice per week dose interval dosage regimen had been chosen for human xenograft breast cancer model.

Results: This NP is a physical complex with size ~ 200 nm with proper stability. The plasma concentration profiles showed sustain release with around 6 hr half-life. After 24 hr, the accumulation of the complex in tumor had been detected by confocal images in the tumor mass. In biodistribution, the labeled P-LHT7 could be detected up to5 days after single administration in the body. The once and twice per week interval showed statistically significant decrease of tumor volume compared with control in antitumor model (both in SCC7 and MDA-MB231 cell lines).

Conclusion: The common concern of NPs are their side effects including off-side organ accumulation which containing can’t avoid, rather those can be just minimized. Using biomimic compounds like, heparin and protamine, are useful for minimizing the off-target effects and conjugation of PEG helps of increased retaintion in the body with proper efficacy.

O-S-11

A SYMMETRIC REGION OF THE HIV-1 INTEGRASE DIMERIZATION INTERFACE IS ESSENTIAL FOR VIRAL REPLICATION

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[2] *KU Leuven, Belgium*

Objective: HIV-1 integrase (IN) is an important target for contemporary antiretroviral drug design research. Historically, efforts at inactivating the enzyme have focused upon blocking its active site. However, it has become apparent that new classes of allosteric inhibitors will be necessary to advance the antiretroviral field in light of the emergence of viral strains resistant to contemporary clinically used IN drugs.

Methods: Through site-directed mutagenesis, we determined that all but one substitution made at a highly symmetric region of the HIV-1 IN dimeric interface abolished the enzyme's in vitro enzymatic activity. We used a novel AlphaScreen® assay to study the contribution of mutated residues to IN multimerization – a platform that will be indispensible for future identification of IN multimerization-disrupting inhibitors. We went further to generate IN mutants in the NL4.3 viral clone and showed that dimeric interface mutant viruses were non-infectious.

Results: In this study we have characterized the importance of a close network of IN residues, distant from the active site, as critical for the obligatory dimerization of the enzyme and viral replication as a whole. Specifically, we have determined that six residues within a highly symmetrical region at the IN dimerization interface, composed of a four-tiered aromatic interaction flanked by two salt bridges, is indispensible for HIV-1 replication. Additionally, we have utilized a novel assay to examine IN oligomerization and have determined that there is a very low tolerance for amino acid substitutions along this region. Even conservative residue substitutions led to ablation of IN multimerization, resulting in an inactive viral enzyme and a non-replicative virus.

Conclusion: We have shown that there is a very low tolerance for amino acid variation at the symmetrical dimeric interfacial region characterized in this study, and therefore drugs designed to target the amino acid network detailed here could be expected to yield a significantly reduced number of drug-resistant escape mutations compared to contemporary clinically-evaluated antiretrovirals.

O-S-12

TOXIN-TARGETED POLYMERIC BINDERS FOR THE TREATMENT OF CLOSTRIDIUM DIFFICILI INFECTION

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Objectives: The current use of antibiotics to treat Clostridium difficile infection is inadequate due to high rates of mortality and recurrence, as well as rising pathogen resistance. New therapies to combat the infection are therefore urgently needed. We have developed polymers that bind specifically to the receptor-binding domain (RBD) of the toxins secreted by C. difficile, which cause disease symptoms. The polymers inhibit uptake of the toxins by cells and reduce toxin-mediated cytotoxicity.

Methods: Random copolymers of N-2-hydroxypropyl methacrylamide and azidotriethyleneglycol methacrylamide containing varying proportions of each monomer were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. The trisaccharide α-Gal-(1,3)-β-Gal-(1,4)-β-Glc known to bind the RBD of toxin A was conjugated to a linker with an alkyne group at the extremity, which was used to functionalize the polymers via Cu(I)-catalyzed alkyne-azide “click” cycloaddition. The polymers were characterized by NMR, GPC and FTIR before being tested for their ability to protect HT-29 cells from toxin-mediated cytotoxicity.

Results: Polymers with 5-20 % (mol/mol) azide monomer content and molecular weights of 30-40 kDa were successfully synthesized and fully functionalized with the trisaccharide ligand by “click” cycloaddition. Challenging HT-29 cells with 4 ng/mL toxin A lead to 80 % cell rounding whereas adding 1 mg/mL of 10 % trisaccharide-functionalized polymer to 4 ng/mL toxin A before challenging the cells significantly reduced the proportion of rounded cells to 30 % ($P < 0.05$). Adding the same concentration of a polymer functionalized with a non-specific ligand (lactose) to toxin A did not significantly reduce the proportion of rounded cells.

Conclusion: The high solubility, specificity and low toxicity of the polymers developed in this project make them an ideal starting point for the optimization of a novel therapeutic product for the treatment of Clostridium difficile infection.

This work was supported by an ETH Zurich Research Grant (ETH-08-10-3).

O-S-13

A NEW METHOD FOR DETERMINATION OF INTRACELLULAR UNBOUND DRUG CONCENTRATIONS IN SIMPLE CELL CULTURES: IMPACT OF ACTIVE UPTAKE VIA OATP1B1

A. Mateus, P. Matsson and P. Artursson
Uppsala University, Sweden

Objective: To develop an experimental method to measure intracellular unbound drug concentrations and to assess the impact of the human Organic Anionic-Transporting Polypeptide 1B1 (OATP1B1) on this parameter.

Methods: Steady-state accumulation of total drug (i.e. the ratio between total drug concentration in cells and that in buffer; Kp) was measured for a set of 30 drugs (including 12 OATP1B1 substrates) in HEK293 cells stably transfected with OATP1B1 or with the corresponding empty vector. In parallel, equilibrium dialysis was used to determine binding to the corresponding cell homogenates, allowing calculation of the fraction unbound in cells (fu,cell). The unbound accumulation ratio (Kpuu) could then be determined from the product of these two parameters (Kpuu=fu,cell x Kp) in both cell lines.

Results: For passively transported compounds (non-substrates of OATP1B1), Kpuu was indistinguishable between empty vector-transfected cells and cells transfected with OATP1B1. In contrast, for substrates of OATP1B1 an up to 12-fold higher Kpuu was observed in the OATP1B1-transfected cell line, indicating transporter-mediated intracellular accumulation of unbound drug. Further, the Kpuu of OATP1B1 substrates was highly dependent on the substrate concentration, with Kpuu values in the OATP1B1-transfected cell line approaching those in the control cells at higher concentrations. This reflects saturation of the transporter and an ensuing dominance of passive diffusion for substrate translocation across the membrane.

Conclusion: We describe a straightforward methodology to measure unbound intracellular drug concentrations, and use it to assess the impact of an uptake drug transporter on this parameter. We anticipate that the developed method will contribute to improved predictions of intracellular drug metabolism and activity, by providing an experimental measure of intracellular unbound drug concentrations (which determine the rates of metabolism by detoxifying enzymes and the binding to intracellular targets).

O-S-14

TUMOR HYPERTHERMIA TO INCREASE THE DELIVERY OF MACROMOLECULAR CHEMOTHERAPEUTICS

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University of Utah, USA

Objective: Gold nanorod (GNR) mediated plasmonic photothermal therapy (PPTT) was utilized to deliver localized tumor hyperthermia in vivo in an attempt to increase the delivery of heat shock targeted HPMA copolymers. A combination of hyperthermia and heat shock targeted HPMA copolymer-drug conjugates was also evaluated in vitro to determine if the combination therapy was antagonistic, additive, or synergistic.

Methods: The tumor accumulation of radiolabeled heat shock targeted HPMA copolymers was investigated in DU145 human prostate cancer bearing mice in combination with GNR mediated PPTT. Conjugates carrying the anticancer agentsgeldanamycin, docetaxel, or cisplatin were synthesized, characterized, and their in vitro cytotoxicity in combination with hyperthermia investigated. Combined effects were classified as antagonistic, additive, or synergistic by combination index analysis.

Results: Tumors receiving GNR mediated PPTT prior to administration of HPMA copolymers showed acute increases in accumulation as compared to unheated tumors. High tumor concentrations were not maintained for periods of time greater than approximately 4 hours. When heat shock targeted HPMA copolymers were utilized, significant increases in tumor concentration and total tumor exposure were achieved, with elevated concentrations in the tumor maintained for greater than 12 hours. Heat shock targeted HPMA copolymer-drug conjugates were synthesized and characterized for their molecular weight, molecular weight distribution, drug loading, and targeting moiety content. In combination with in vitro hyperthermia, conjugates carrying cisplatin were additive in their effects with combination index values close to 1.0. Heat shock targeted conjugates carrying geldanamycin or docetaxel demonstrated significant synergism in combination with hyperthermia (combination index values ranging from 0.5 – 0.6), whereas non-targeted control conjugates demonstrated additive effects (combination index values close to 1.0).

Conclusion: A combination strategy of localized hyperthermia, induced via GNR mediated PPTT, can increase the tumor delivery and cytotoxicity of heat shock targeted HPMA copolymer-drug conjugates.

O-S-15

NOVEL ANTI-ANGIOGENIC MIRNA REDUCES ANGIOGENESIS AND TUMOR GROWTH IN MOUSE TUMOR MODEL

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[5] *Hubrecht Institute, Cancer Genomics Center and University Medical Center Utrecht (UMCU), Netherlands*
[6] *Laboratory Clinical Chemistry & Haematology, Netherlands*

Objective: MicroRNAs (miRNAs) are implicated in regulation of tumor angiogenesis. In tumor endothelium, miRNA expression profiles are out of balance. Restoring this imbalance by treatment with anti-angiogenic miRNAs to inactivate the endothelium offers new therapeutic approaches in angiogenesis.

A lentiviral miRNA expression library identified 6 candidate miRNAs involved in angiogenesis. Novel anti-angiogenic miRNAs were identified based on their anti-proliferative behavior by using synthetic miRNA mimics which dose dependently inhibited cell viability. Anti-angiogenic activity of the miRNA was tested in 2D tubeformation and 3D sprouting assays. Differentially expressed genes obtained after mimic transfection in primary endothelial cells and subsequent RNA-seq analysis was combined with miRNA-target predictions to identify the miRNA's target genes. Based on these *in vitro* anti-angiogenic properties, the miRNA was studied *in vivo* to assess inhibition of angiogenesis and effect on tumor growth in a mouse neuroblastoma model. Tumor growth rates of treated mice were significantly reduced as was their tumor vasculature. Systemic administration, via a delivery system resulted in similar reductions.

Conclusion: We successfully identified miRNA with anti-angiogenic properties *in vitro* and *in vivo.* More importantly miRNA was successfully delivered to the tumor after systemic administration, which offers a promising novel anti-angiogenic therapeutic approach.

O-S-16

FLUOXETINE STRONGLY INHIBITS CYP2D6 AND CYP2C19 BUT DOES NOT INHIBIT CYP3A4 IN VIVO: A COCKTAIL STUDY

J. Lutz, J. E. Sager, C. Davis, K. L. Kunze and N. Isoherranen
University of Washington, USA

Objective: Fluoxetine and its metabolite, norfluoxetine, inhibit multiple cytochrome P450 enzymes (CYP2D6, CYP3A4, and CYP2C19) via different mechanisms (reversible or time-dependent inhibition) in vitro. In vivo, fluoxetine is a strong inhibitor of CYP2D6, but the magnitude of CYP2C19 and CYP3A4 inhibition by fluoxetine is unclear. Based on the in vitro data, fluoxetine is predicted to be a strong (>5-fold AUC change) inhibitor of CYP3A4 and a moderate inhibitor (2- to 5-fold AUC change) of CYP2C19 and CYP2D6. The objective of this study was to determine the magnitude of CYP3A4, CYP2D6 and CYP2C19 inhibition by fluoxetine in vivo using a probe cocktail approach.

Methods: The effect of fluoxetine on the disposition of a cocktail of dextromethorphan, omeprazole, midazolam and caffeine as well as lovastatin was determined in 10 healthy volunteers who were CYP2D6 and CYP2C19 extensive metabolizers as well as CYP3A5 non-expressors. The cocktail was administered on days 1 and 16 and lovastatin on days 3 and 18. Fluoxetine was administered once daily: 20 mg on day 5 and 60 mg on days 6 through 18.

Results: Fluoxetine decreased dextromethorphan CYP2D6-mediated clearance by 99% and omeprazole CYP2C19-mediated clearance by 93%, whereas no significant change in midazolam or lovastatin CYP3A4-mediated clearance was observed.

Conclusion: The rank order of P450 inhibition magnitude by fluoxetine predicted from in vitro data (CYP3A4 > CYP2C19 ≈ CYP2D6) is in disagreement to that observed in vivo (CYP2D6 ≈ CYP2C19 > CYP3A4) when measured simultaneously. This data shows that even when hepatocyte exposure of inhibitor is constant for all P450s, significant prediction gaps can occur. Additionally, the data shows that in vitro inhibition may translate to in vivo inhibition in a mechanism or P450-dependent manner. Finally, the study shows that caution must be exercised when coadministering fluoxetine with a drug that is eliminated by CYP2D6, CYP2C19 or both.

Podium Index by Presenting Author

Surname	Initial	Oral #	Page #
Alam	F	O-S-10	76
Ando	M	O-TH-17	49
Avaritt	B	O-TH-19	51
Babae	N	O-S-15	81
Baek	J	O-TH-05	37
Bahar	F	O-TH-16	48
Bathena	S	O-S-05	71
Vande Casteele	N	O-TH-14	46
Chandrudu	S	O-F-10	62
Desai	P	O-S-04	70
Gonzalez	D	O-F-05	57
Han	S	O-S-02	68
Hanning	S	O-S-07	73
Higashino	H	O-TH-01	33
Hittinger	M	O-S-08	74
Iemsam-arng	J	O-F-01	53
Ivarsson	M	O-S-12	78
Jain	A	O-S-03	69
Kasai	T	O-F-13	65
Kleppe	M	O-F-07	59
Kobayashi	K	O-F-14	66
Kohler	I	O-TH-09	41
Larson	N	O-S-14	80
Liu	Y	O-F-12	64
Liu	Y	O-TH-20	52
Logan	R	O-TH-13	45
Lutz	J	O-S-16	82
Marie	H	O-F-11	63
Mateus	A	O-S-13	79
Medina	W	O-S-09	75
Mittapalli	R	O-S-01	67
Modi	S	O-TH-15	47
Nair	A	O-TH-08	40
Nicol	M	O-S-06	72
Nielsen	L	O-F-04	56
Payton	N	O-TH-06	38
Rachid	O	O-TH-18	50
Roth	W	O-F-06	58
Serrao	E	O-S-11	77
Shifrin	H	O-TH-11	43
Sockolosky	J	O-TH-04	36

Surname	Initial	Oral #	Page #
Strehlow	B	O-F-02	54
Subrizi	A	O-TH-12	44
Thongrangsalit	S	O-F-03	55
Thormann	U	O-F-08	60
Toktas	S	O-TH-10	42
Torosantucci	R	O-F-09	61
Wilkhu	J	O-TH-02	34
Wolak	D	O-TH-03	35
Zlatev	H	O-TH-07	39

Poster Presentations

Poster Presentations: Session 1

Date: Thursday 29th November 2012

Time: 1:00 pm – 2:20 pm

Location: Cossar Hall

Presenter	Poster Title
P-TH-01 **Cordula Stillhart** *University of Basel* *Switzerland*	Real-time monitoring of drug precipitation during in vitro lipolysis of lipid-based drug delivery systems
P-TH-02 **Siti Zahliyatul Munawiroh** *Chulalongkorn University* *Thailand*	Dry powder nanostructured lipid carrier (NLC) chitosan-based of bromocriptine mesylate: Preparation and physicochemical characterization
P-TH-03 **Shelley Durazo** *University of Colorado* *USA*	Regression analyses of mitochondrial delivery of drug molecules with negative, neutral, and positive charges
P-TH-04 Johan Boetker *University of Copenhagen* *Denmark*	Insight into dissolution processes using UV imaging, Raman spectroscopy and numerical analysis
P-TH-05 **Sarah Gordon** *University of Copenhagen* *Denmark*	Biorelevant characterisation of the dissolution behaviour of furosemide polymorphs by UV imaging and flow through Raman spectroscopy
P-TH-06 **Martha Kampp Thomsen** *University of Copenhagen* *Denmark*	The proton-coupled amino acid transporter hPAT1 is the main transporter involved in vigabatrin uptake in intestinal Caco-2 cells
P-TH-07 **Emil Meng-Lund** *University of Copenhagen* *Denmark*	pH dependent bioavailability study of buccal bioadhesive tablets containing metoprolol in conscious and anesthetised mini-pigs
P-TH-08 **Satu Arkko** *University of Eastern Finland* *Finland*	Resistance of PC-3 prostate cancer cells to n-bisphosphonate induced gamma-delta T-cell cytotoxicity

Presenter	Poster Title
P-TH-09 **Miriam Dadparvar** *University of Frankfurt* *Germany*	Freeze-drying of HI-6-loaded albumin nanoparticles for antidote therapy against organophosphate poisoning
P-TH-10 **Burçin Yavuz** *Hacettepe University* *Turkey*	Determination and analytical method validation of cyclosporin A from ocular implants
P-TH-11 **Joseph Fanous** *The Hebrew University of Jerusalem* *Israel*	The pharmacokinetic properties and activity evaluation of a novel orally available anti-HIV drug candidate
P-TH-12 **Melina Malinen** *University of Helsinki* *Finland*	Three-dimensional liver cell cultures for drug testing
P-TH-13 **Noora Sjöstedt** *University of Helsinki* *Finland*	In vitro based pharmacokinetic simulations of quinidine brain transport
P-TH-14 **Takuya Shimizu** *Kanazawa University* *Japan*	Interaction of breast cancer resistance protein (BCRP/ABCG2) with PDZ adaptor PDZK1 and therapeutic agents in small intestine
P-TH-15 **Christopher Kuehl** *University of Kansas* *USA*	NanoClusters enhance dissolution rates of poorly soluble drugs
P-TH-16 **Monica Vialpando** *KU Leuven* *Belgium*	Agglomeration of mesoporous silica prepared in a high-shear mixer: A comparison between melt and steam granulation
P-TH-17 **Kayoko Ohura** *Kumamoto University* *Japan*	Evaluation of synovial esterase for development of soft-drugs
P-TH-18 **Yuki Umemori** *Kumamoto University* *Japan*	Pharmaceutical properties of molecular-targeting peptides “microantibody” against granulocyte colony-stimulating factor (G-CSF) receptor
P-TH-19 **Priyanka Ghosh** *University of Maryland* *USA*	Effect of formulation pH on transport and pore closure in microneedle enhanced transdermal drug delivery
P-TH-20 **Kyoung Ah Min** *University of Michigan* *USA*	Measurement of molecular transport across the normal human bronchial primary epithelial cell monolayer at a single cell level
P-TH-21 **Mohammad Azad** *Monash University* *Australia*	Concentration- and time-dependent apoptosis induced by polymyxin B in rat proximal tubular cells

Presenter	Poster Title
P-TH-22 **Soon-ee Cheah** *Monash University* *Australia*	In vitro PK/PD and mathematical models to combat antibiotic resistance
P-TH-23 **Orlagh Feeney** *Monash University* *Australia*	Digestion modulating formulations as a platform to enhance the utility of lipid based drug delivery systems.
P-TH-24 **Caroline Le** *Monash University* *Australia*	Sympathetic nervous system activation promotes breast cancer progression by VEGF-driven lymphangiogenesis in primary tumours
P-TH-25 **Dharmini Mehta** *Monash University* *Australia*	Drug transport across the blood-brain barrier in Alzheimer's disease
P-TH-26 **Stephane Phan** *Monash University* *Australia*	Linking structure and composition of lipid based drug formulations during digestion
P-TH-27 **Tomas Sou** *Monash University* *Australia*	The effect of L-leucine on particle formation, morphology and hygroscopicity of spray-dried formulations for inhalation
P-TH-28 **Durgesh Tiwari** *Monash University* *Australia*	A modified three day water maze protocol to test spatial memory deficits in mice
P-TH-29 **Hywel Williams** *Monash University* *Australia*	Ionic liquids provide unique opportunities for oral drug delivery: First in vivo and in vitro evidence of utility
P-TH-30 **Hui Li Ng** *National University of Singapore* *Singpore*	Synthesis and antiproliferative activity of methoxy- and methylthio-substituted chalcones
P-TH-31 **Luqi Zhang** *National University of Singapore* *Singpore*	A missing link: the interaction between ApoE and LRP5 in the pathogenesis of Alzheimer's disease
P-TH-32 **Chester Costales** *University of North Carolina* *USA*	Insights into a novel intestinal absorption mechanism of the antidiabetic drug metformin
P-TH-33 **James Huckle** *University of North Carolina* *USA*	Prodrugs of DTPA as orally bioavailable radionuclide decorporation agents

Presenter	Poster Title
P-TH-34 **Jasper Chiu** *University of Otago* *New Zealand*	Arginine tagging of polymeric nanoparticles to enhance cellular uptake?
P-TH-35 **Jan Kusterer** *Philipps University* *Germany*	Combination of sulphur compounds from central asian allium species – new approach to obtain higher antibacterial and antifungal activity
P-TH-36 **Emad Malaeksefat** *Saarland University* *Germany*	Impact of chitosan-PLGA and further cationic polymer nanoparticles on cell growth
P-TH-37 **Diogo Rivelli** *University of Sao Paulo* *Brazil*	Sun protection factor and cutaneous permeation of hydroethanolic extract of Ilex paraguariensis
P-TH-38 **Hiroaki Hatano** *Setsunan University* *Japan*	Evaluation of the relative contribution of influx/efflux trasporters on the intestinal absorption of fexofenadine in rat
P-TH-39 **Wen Wang** *University of South Australia* *Australia*	A pharmacokinetic study of 5-fluorouracil loaded into a novel sterically stabilized liposomal formulation using microdialysis
P-TH-40 **Vincent Forster** *ETH-Zurich* *Switzerland*	Scavenging liposomes: A novel modality for biodetoxification
P-TH-41 **Hui Xin Ong** *University of Sydney* *Australia*	Absorption profiling of inhaled liposomal ciprofloxacin nanoparticles using isolated perfused lung
P-TH-42 **Elsa Lilienberg** *Uppsala University* *Sweden*	Improved pharmacokinetic understanding of two formulations for intra-arterial injection
P-TH-43 **Peter van Meer** *Utrecht University* *The Netherlands*	What you should know about the future of nonhuman primates in drug development

Poster Presentations: Session 2

Date: Saturday 1st December 2012

Time: 12:10 pm – 1:30 pm

Location: Cossar Hall

Presenter	Poster Title
P-S-01 **Marc Anthony Yago** *University of California-San Francisco* *USA*	Use of betaine HCl to improve dasatinib absorption in healthy volunteers with pharmacologically-induced hypochlorhydria
P-S-02 **Minho Park** *Chungnam National University* *Korea*	Preparation and characterization of BSA-loaded PLGA nanoparticles for preformulation of exenatide-loaded PLGA nanoparticle
P-S-03 **Kelly Forney-Stevens** *University of Connecticut* *USA*	Optimization of a Raman microscopic technique to efficiently detect amorphous-amorphous phase separation in freeze-dried protein formulations
P-S-04 **Philip Christophersen** *University of Copenhagen* *Denmark*	Preparation and characterization of solid lipid micro-particles for oral delivery of proteins
P-S-05 **Linda G. Jensen** *University of Copenhagen* *Denmark*	Visualization of API precipitation in simulated gastric media by polarized light microscopy
P-S-06 **Sebastian Kapp** *University of Copenhagen* *Denmark*	Protein adsorption to hydrophobic nanospheres
P-S-07 **Pernille Barbre Pedersen** *University of Copenhagen* *Denmark*	Rheological characterization of fasted human gastric fluid
P-S-08 **Benjamin Weber** *University of Florida* *USA*	A clinical trial simulation tool for the suppression of the endogenous cortisol release after administration of inhaled corticosteroids

Presenter	Poster Title
P-S-09 **Astrid Muehlstein** *University of Frankfurt* *Germany*	Development of positively charged poly(lactic acid) and poly(lactic co glycolic acid) nanoparticles by addition of stearylamin
P-S-10 **Orit Amsalem** *The Hebrew University of Jerusalem* *Israel*	Development of novel nano delivery systems of hydrophilic biomacromolecules for improved therapy
P-S-11 **Leena-Stiina Kontturi** *University of Helsinki* *Finland*	An injectable vehicle for chondrocyte delivery in cartilage tissue engineering
P-S-12 **Manuela Raviña Fernandez** *University of Helsinki* *Finland*	Liposomal siRNA delivery to retinal pigment epithelial cells
P-S-13 **Eva Tuominen** *University of Helsinki* *Finland*	Pharmacokinetic ocular modeling: Predicting drug concentrations in the vitreous after drug delivery system administration
P-S-14 **Jessica Haywood** *University of Kansas* *USA*	Novel oxidative side chain cleavages in peptides and proteins exposed to light
P-S-15 **Warangkana Pornputtapitak** *University of Kansas* *USA*	Nanocluster budesonide formulation enhances drug delivery in mechanical ventilation
P-S-16 **Mo Dan** *University of Kentucky* *USA*	Hyperthermia and anti-PECAM-1 surface modification increase Fe_3O_4 nanoparticle flux across the blood brain barrier
P-S-17 **Keiichiro Tanaka** *Kumamoto University* *Japan*	Effect of conversion rate of active drug from prodrug on the subsequent bidirectional-transport in epithelial cell
P-S-18 **Zhizhi Sun** *University of Manitoba* *Canada*	Characterization of cellular uptake and permeability of aminosilane coated iron oxide nanoparticles in a cell culture model of the blood-brain barrier
P-S-19 **Hairat Sabit** *University of Maryland* *USA*	Exploring the role of transmembrane domain ii on the function of the human apical sodium-dependent bile acid transporter (hASBT)
P-S-20 **Ameya Kirtane** *University of Minnesota* *USA*	Polymeric nanoparticles for tumor targeted delivery of tylocrebrine

Presenter	Poster Title
P-S-21 **David Barling** *Monash University* *Australia*	Analysis and control of dry powder mixing processes using a fine cohesive pigment in an inhalation grade lactose
P-S-22 **Annette Dahlberg** *Monash University* *Australia*	The lymphatic system plays a significant role in the subcutaneous absorption and pharmacokinetics of the monoclonal antibody trastuzumab in rats
P-S-23 **Liang Jin** *Monash University* *Australia*	The impact of systemic inflammation and bacterial infection on the brain uptake of colistin
P-S-24 **Gordon Lee** *Monash University* *Australia*	The expression profile and ligand binding affinity of fatty acid-binding proteins at the blood-brain barrier
P-S-25 **Gemma Nassta** *Monash University* *Australia*	Probing the mechanisms for degradation of oxytocin in aqueous solutions
P-S-26 **Gemma Ryan** *Monash University* *Australia*	Preferential lymphatic access of doxorubicin after subcutaneous administration of a pegylated dendrimer when compared to liposomal and solution formulations
P-S-27 **Thomas Taylor** *Monash University* *Australia*	Heats of dilution of two modified β-cyclodextrins
P-S-28 **Natalie Trevaskis** *Monash University* *Australia*	Targeting the sphingosine-1-phosphate receptor in the mesenteric lymphatics
P-S-29 **Priti Bahety** *National University of Singapore* *Singapore*	Gas chromatography/time-of-flight mass spectrometry metabotyping of docosahexaenoic acid-treated alzheimer's disease cell model
P-S-30 **Kheng-Lin Tan** *National University of Singapore* *Singapore*	Proteasome inhibition by curcumin analogs prevents nuclear receptor co-repressor (N-CoR) degradation and triggers the unfolded protein response (UPR)-induced apoptosis
P-S-31 **Bala Vamsi Krishna Karuturi** *University of Nebraska* *USA*	A mucosal peptide vaccine composed of EP67, a response-selective peptide agonist of the C5a receptor, and ctl epitopes protects against primary mucosal challenge with murine cytomegalovirus
P-S-32 **Tianxiang (Kevin) Han** *University of North Carolina* *USA*	Intestinal cation-selective transporters involved in the apical uptake, accumulation and absorption of metformin

Presenter	Poster Title
P-S-33 **Gina Song** *University of North Carolina* *USA*	Association of CC chemokine ligand-2 (CCL2/MCP-1) and CC chemokine ligand-5 (CCL5/RANTES) with the pharmacokinetics (PK) and pharmacodynamics (PD) of pegylated liposomal doxorubicin (pld) in patients with recurrent ovarian cancer
P-S-34 **Katie Owens** *University of Otago* *New Zealand*	Development of a structural population PKPD model to describe the effect of paracetamol on the international normalised ratio (INR)
P-S-35 **Khairunnisa Ghaffar** *University of Queensland* *Australia*	Synthesis and characterization of levofloxacin conjugates
P-S-36 **Kely Medeiros Turra** *University of Sao Paulo* *Brazil*	3D-pharmacophore mapping of a set of novel β-N-biaryl ether sulfonamide-based hydroxamates
P-S-37 **Jooho Park** *Seoul National University* *Korea*	Development of very low molecular heparin derivatives for oral anticancer therapy
P-S-38 **Laizhong Chen** *Shandong University* *China*	The first ratiometric fluorescent probe for aminopeptidase N
P-S-39 **Martha Pastuszka** *University of Southern California* *USA*	Development of a thermally responsive intracellular switch
P-S-40 **Michael Dolton** *University of Sydney* *Australia*	Clinical utility of posaconazole therapeutic drug monitoring
P-S-41 **Kensuke Aida** *Tokyo University* *Japan*	Role of ubiqutination in the intracellular sorting of hepatocanalicular ABC transporters
P-S-42 **Yan Zhou** *University of Utah* *USA*	Selective inhibitory effect of HPMA copolymer-cyclopamine conjugate on prostate cancer stem cells
P-S-43 **Jenna Voellinger** *University of Washington* *USA*	Pharmacogenetic profiling of the WiCell(r) human embryonic stem cell lines

Poster Abstracts

P-TH-01

REAL-TIME MONITORING OF DRUG PRECIPITATION DURING IN VITRO LIPOLYSIS OF LIPID-BASED DRUG DELIVERY SYSTEMS

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Objective: The digestion of lipid-based drug delivery systems (LBDDS) can impair their solubilization capacity, implicating the risk of undesired drug precipitation. In vitro models with new analytical tools are required to better characterize the biopharmaceutical behavior of LBDDS. The aim of the study was to evaluate Raman spectroscopy as a novel real-time method to monitor drug precipitation during in vitro lipolysis.

Methods: A model LBDDS containing fenofibrate at different drug loads was digested using an in vitro model described in the literature (Sek L. et al., J Pharm Pharmacol, 2002). We employed a multi-fiber Raman probe to collect the spectra during the simulated digestion experiment. As a reference, the concentrations of dissolved and precipitated drug of LBDDS with different drug loading were determined after lipolysis inhibition, sample ultracentrifugation, and HPLC analysis of the aqueous and the pellet phase (standard method).

Results: The LBDDS was extensively digested and the solubilization capacity of the vehicle dropped rapidly in the course of hydrolysis. Thus, approximately 85 % of fenofibrate precipitated within 30 min of digestion. A partial least square regression analysis was performed based on a set of Raman spectra ($R2 > 0.9$) and the concentration of precipitated fenofibrate was determined over the entire lipolysis experiment. As a result, the spectroscopic method provided a description of fenofibrate crystallization kinetics with high temporal resolution.

Conclusion: Raman spectroscopy proved to be a valuable tool for real-time analysis of drug crystallization during in vitro lipolysis. The technique bears great potential to gain a better understanding of parameters affecting oral bioavailability of LBDDS, which is ultimately the basis for developing effective formulations.

P-TH-02

DRY POWDER NANOSTRUCTURED LIPID CARRIER (NLC) CHITOSAN-BASED OF BROMOCRIPTINE MESYLATE: PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION

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Objective: The present work describes the development of dry powdered Nanostructured Lipid Carrier (NLC) chitosan-based as oral drug delivery system for bromocriptine mesylate (BM) using spray drying technique.

Methods: NLC were prepared using hot high pressure homogenizer technique and then spray dried with and without chitosan base. The conditions of spray drying process were 115¬0C (inlet temperature),330 mL/h (feed rate) and 3% w/w (feed concentration). The physical properties of NLC-BM powder and redispersed NLC-BM were characterized by evaluating the average size, morphology, chemical interaction and crystallography using nanozetasizer, scanning electron microscope (SEM), transmission electron microscope (TEM), Fourier transform infrared (FTIR) spectrometer and wide angle X-ray diffractometer (WAXRD), respectively. The drug entrapment efficiency and the drug retention were evaluated using HPLC analyses.

Results: NLC-BM with average particle diameter of 84.66±1.63 nm and zeta potential of 3.16±0.57 meV were obtained. After spray drying, the morphology of the obtained powder was fairly spherical shape with 0.3-1.3 micron size. Upon redispersion, the particle size was 322.0±15.27 and 612.60±62.80 nm without and with chitosan base, respectively. FTIR spectra and WAXRD analysis of the obtained powder with chitosan base showed no significant shifting of BM peaks and amorphous system, respectively. The drug entrapment efficiency (DEE) of this powder was 90.09±4.96 % while the drug retention was 52.26±2.10 and 62.44±0.76 % without and with chitosan base, respectively.

Conclusion: Spray drying was potential technique to obtain NLC as dry powder of amorphous, spherical particles with 0.3-1.3 µm size which could easily be redispersed into NLC. BM had no chemical reaction with other ingredients in NLC systems during processing. Drug retention of the obtained spray dried powder was significantly improved with incorporation of chitosan as base. Thus, dry powdered NLC chitosan-based is potential vehicle for BM oral delivery application.

P-TH-03

REGRESSION ANALYSES OF MITOCHONDRIAL DELIVERY OF DRUG MOLECULES WITH NEGATIVE, NEUTRAL, AND POSITIVE CHARGES

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Objective: Mitochondria are essential organelles of the cell and have critical roles in both cell death and survival. Therefore, it is not surprising that mitochondria contribute significantly to the pathology and progression of several degenerative diseases. The purpose of this study was to investigate the mitochondrial delivery of 20 small molecules and develop a model equation to predict mitochondrial drug delivery.

Methods: Cassettes of 8 cationic drugs (beta-blockers), 6 neutral drugs (corticosteroids), or 6 anionic drugs (NSAIDs) were incubated at 37°C in the presence and absence of valinomycin, a depolarizer of membrane potential, with mitochondria isolated from Sprague Dawley rat brain. Drugs in each cassette were quantified in supernatant and mitochondrial fractions using high throughput liquid chromatography tandem mass spectrometry assays. Mitochondrial purity was assessed using western blot to determine the presence of mitochondrial proteins and the absence of two contaminant proteins: lactate dehydrogenase and CNPase. Mitochondrial integrity was assessed using a JC-1 dye assay. Multiple linear regression analysis and statistics were conducted using SPSS software to determine the most significant physiochemical parameters amongst Log P, Log D, Log α (polarizability), Log MW, Q (charge), and Log PSA (polar surface area). The level of significance was set to a p value of <0.05.

Results: Mitochondria isolated from brain were pure and the integrity was maintained for at least up to 1 h after isolation. Percent uptake was in the order: cationic>neutral and anionic and uptake was the highest for the most lipophilic compound in each cassette. Depolarization of the membrane potential only reduced the uptake of the most lipophilic cationic compounds. Log D contributed the most to the model equation for percent uptake when all compounds were used to generate the model ($R=0.8$).

Conclusion: Mitochondrial uptake is driven by lipophilicity and charge, wherein cationic lipophilic compounds have the highest percent uptake. Further, the delivery of cationic lipophilic compounds is significantly higher when the mitochondrial membrane is polarized.

P-TH-04

INSIGHT INTO DISSOLUTION PROCESSES USING UV IMAGING, RAMAN SPECTROSCOPY AND NUMERICAL ANALYSIS

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Objective: To provide a deeper insight into the dissolution processes using an enhanced experimental approach for dissolution testing combined with numerical analysis.

Methods: Paracetamol, amorphous amlodipine besilate and amlodipine besilate dihydrate were used as model compounds. UV imaging was performed with an Actipix SDI300 dissolution imaging system (Paraytec Ltd, York, UK). Raman spectra were acquired with a Raman spectrometer (Control Development Inc., South Bend, IN, USA). Multivariate data analysis (MVDA) of the Raman spectra was performed using PLS toolbox version 6.0.1 (Eigenvector Research Inc., Wenatchee, WA, USA). Numerical analysis was developed in the Comsol finite element software (COMSOL Multiphysics, Stockholm, SE).

Results: UV imaging visualized the initially fast dissolution rate of a high-energy thermodynamically unstable formulation of amorphous amlodipine besilate. Furthermore, the UV imaging technique displayed that the dissolution rate of this amorphous amlodipine besilate formulation was decreasing over time. Raman spectroscopy of the samples enabled kinetic profiling of the conversion of the amorphous form to the monohydrate form of amlodipine besilate. MVDA analysis of an in-situ Raman experiment of an amorphous amlodipine besilate sample displayed that there was a good agreement between the change in Raman spectra over time and the decrease in the dissolution rate of the amorphous besilate samples. Furthermore, a numerical model was developed for the UV imaging geometry using paracetamol as a model compound. The numerical model showed a good agreement with the experimentally obtained effluent dissolution rates.

Conclusion: UV imaging can be used for visualization of the dissolution process and, as confirmed by Raman spectroscopy, for obtaining indications of recrystallization. Multivariate data analysis in combination with Raman spectroscopy showed that there was a high agreement between the recrystallization kinetics and the observed decrease in dissolution rate of amorphous amlodipine besilate samples. Numerical analysis displayed that there was good agreement between the calculated and experimentally obtained effluent dissolution rates.

P-TH-05

BIORELEVANT CHARACTERISATION OF THE DISSOLUTION BEHAVIOUR OF FUROSEMIDE POLYMORPHS BY UV IMAGING AND FLOW THROUGH RAMAN SPECTROSCOPY

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Objective: To investigate and compare the dissolution behaviour of different polymorphic forms of furosemide under physiologically relevant conditions, by employing two different imaging techniques, UV imaging and Raman spectroscopy.

Methods: Amorphous acid and amorphous sodium salt forms of furosemide were prepared by spray drying. The dissolution behaviour of the prepared amorphous forms, together with that of crystalline furosemide, was investigated using UV imaging. Drug compacts within the UV imaging cell were subjected to a flow of simulated intestinal media (containing 10 mM sodium taurocholate as a bile salt and 2.5 mM phosphatidylcholine as a phospholipid, pH 6.5). A similar flow cell setup was also employed in conjunction with in situ Raman spectroscopy, to investigate the occurrence of solid form transformations at the surface of drug compacts during dissolution. The solid form of furosemide remaining in the compacts following Raman experiments was identified using X-ray powder diffraction (XRPD).

Results: The UV imaging setup was found to be entirely compatible with the use of biorelevant media. Dissolved furosemide could be readily detected from UV images as absorbance downstream from the sample compact surface. Such 'tails' of dissolved furosemide exhibited a greater degree of thickness and intensity in the case of amorphous furosemide salt as compared to both crystalline and amorphous furosemide acid, indicative of a greater rate and extent of dissolution of this form. In situ Raman spectroscopy and XRPD showed however that the amorphous furosemide salt recrystallised to the crystalline salt form during dissolution experiments.

Conclusion: Detailed information related to the biorelevant dissolution behaviour of furosemide polymorphs was gained using a combination of UV imaging and in situ Raman spectroscopy. Despite undergoing recrystallisation, amorphous furosemide salt showed an enhanced dissolution rate as compared to the crystalline furosemide and amorphous furosemide acid, making this form a promising candidate for future work.

P-TH-06

THE PROTON-COUPLED AMINO ACID TRANSPORTER HPAT1 IS THE MAIN TRANSPORTER INVOLVED IN VIGABATRIN UPTAKE IN INTESTINAL CACO-2 CELLS

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Objective: Many drug substances are substrates for membrane transporters, which influence their intestinal absorption. Vigabatrin is a drug substance used in the treatment of epilepsy, mainly for treatment of infantile spasms. Vigabatrin is a substrate for the human proton-coupled amino acid transporter hPAT1. The aim of the project was to identify if transporters are involved in cellular uptake of vigabatrin in Caco-2 cells.

Methods: The uptake rate of vigabatrin was measured in Caco-2 cells at pH 6.0 or 7.4 for 15 min after application of 0.1 – 25.0 mM vigabatrin. The inhibitory effect of selected amino acids and -derivatives on the apical vigabatrin uptake in Caco-2 cells was investigated. Vigabatrin samples were analyzed using liquid chromatography (LC) coupled to a mass selective detector (MSD).

Results: The uptake rate of vigabatrin in Caco-2 cells was pH-dependent. The uptake of vigabatrin was saturable at pH 6.0 with a Michaelis constant, Km of 12.4 ± 3.6 mM and a maximal flux, Jmax of 1.9 ± 0.2 nmol•min-1•cm-2. The presences of hPAT1 ligands significantly inhibited the uptake of vigabatrin in Caco-2 cells at pH 6.0, whereas hPAT1 non-ligands did not.

Conclusion: The saturability of the uptake at pH 6.0 indicates involvement of a carrier-mediated process in vigabatrin absorption. The pH-dependency of the vigabatrin uptake suggests that the uptake may be driven by a proton-coupled transporter. The Km-value is comparable to Km- values for other hPAT1 substrates (1-20 mM). The interaction of hPAT1 ligands with the uptake is a further indication of hPAT1 involvement in vigabatrin absorption. It is concluded that the proton-coupled amino acid transporter hPAT1 is the main transporter responsible for absorption of vigabatrin in Caco-2 cells.

P-TH-07

PH DEPENDENT BIOAVAILABILITY STUDY OF BUCCAL BIOADHESIVE TABLETS CONTAINING METOPROLOL IN CONSCIOUS AND ANESTHETISED MINI-PIGS

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Objective: Drug delivery in the oral cavity for systemic effects has numerous advantages compared to e.g. oral administration, for example bypassing the liver metabolism, avoiding acid and enzymatic degradation in the gastrointestinal tract. The degree of ionisation of the compound is affecting the lipophilicity and therefore the possible bioavailability. Therefore, the aim of this study was to evaluate possible effects of pH on the buccal absorption of metoprolol from bioadhesive tablets dosed to both conscious and anesthetised mini-pigs.

Methods: Four male Göttinge mini-pigs (weight from 12.2 to 26.0 kg) were tested in a non-randomised cross-over study. Metoprolol formulations were administrated to both anesthetised and conscious mini-pigs including: 1) IV 2) oral-solution 3) buccal tablet pH 8.9 4) buccal tablet pH 6.2. The animals were anesthetised with 0.1 mg/kg Zoletil® and the anaesthesia maintained by additional administration of Zoletil®. Blood samples were collected and processed before analysing by ultra performance liquid chromatography and a mass spectrometer.

Results: The results showed a significant change in bioavailability of metoprolol when comparing the two buccal tablets groups with the resulting pH of 6.2 and 8.9, both in the conscious (7.2±1.4% vs. 11.4±0.7%, mean ± SEM, n=4) and the anesthetised (4.3±1.6% vs. 21.7±4.1%) mini-pigs. Also, the tmax decreased significantly in the conscious-buccal groups compared to the anesthetised-buccal groups. The oral groups showed a minor bioavailability (<3.3±0.2%), indicating the absorption in the buccal groups has taken place in the oral cavity and was not a reflection of swallowing of tablet and subsequently oral absorption.

Conclusion: The degree of ionisation and thereby the lipophilicity of a given compound has shown to have an impact on the resulting bioavailability obtained from buccal drug delivery. Also, this animal study has shown that the anesthesia can have an impact on both the bioavailability as well as the time to onset after buccal delivery.

P-TH-08

RESISTANCE OF PC-3 PROSTATE CANCER CELLS TO N-BISPHOSPHONATE INDUCED GAMMA-DELTA T-CELL CYTOTOXICITY

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Objective: The nitrogen-containing bisphosphonate zoledronic acid (ZOL), a potent inhibitor of farnesyl pyrophosphate synthase (FPPS), blocks the mevalonate pathway, leading to intracellular accumulation of isopentenyl pyrophosphate / triphosphoric acid 1-adenosin-5'-yl ester 3-(3-methylbut-3-enyl) ester (IPP/ApppI) metabolites. IPP/ApppI accumulation in ZOL- treated cancer cells may be recognized by Vγ9Vδ2 T cells as tumour phosphoantigens. Human breast cancer cells have been previously shown to have marked differences in ZOL induced IPP/ApppI production.

Methods: In this study we investigated the anticancer activities of Vγ9Vδ2 T cells and the intracellular IPP/ApppI production in ZOL-treated prostate cancer cell lines (PC-3 and Du-145), human renal carcinoma cell line (Caki-2) and human glioblastoma cell line (U87MG). Cytotoxicity of Vγ9Vδ2 T cells against cancer cells was measured by MTT assay and IPP/ApppI production of cancer cells after ZOL treatment by HPLC-ESI-MS. Cellular uptake of ZOL was measured with ^{14}C-labeled ZOL and the activity of the mevalonate pathway after ZOL treatment by Western blot. All experiments were performed at least as three independent biological repeats with similar results. The significance of results was assessed with one-way ANOVA with Tukey´s or Dunn´s multiple comparison test.

Results: Of the cell lines treated only the viability of Du-145 was reduced 20% with ZOL treatment only ($p < 0.001$). Addition of Vγ9Vδ2 T cells to ZOL treated cancer cell cultures reduced the viability of Du-145, Caki-2 and U87MG cells 85-95% ($p < 0.001$ for each), whereas no effect was seen on PC-3 cells.We found that the potency of Vγ9Vδ2 T cells to kill cancer cells was dependent on ZOL-induced intracellular IPP production, which was not comparable to the drug uptake by the cells. The intracellular ZOL concentration was 5-7 pmol/mg protein with no significant differences between cell lines, whereas IPP (200-400 pmol/mg protein) was detected in Du-145, Caki-2 and U87MG cells (no significant differences between these cell lines) but only barely detectable trace amounts of IPP was found in PC-3 cells. However FPPS was still inhibited in all of these cells as detected by western blot for unprenylated Rap1A.

Conclusion: This study confirms that Vγ9Vδ2 T cell-mediated cytotoxicity is efficient in the cancer cell types with high IPP production.

P-TH-09

FREEZE-DRYING OF HI-6-LOADED ALBUMIN NANOPARTICLES FOR ANTIDOTE THERAPY AGAINST ORGANOPHOSPHATE POISONING

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Objective: Organophosphorus (OP) compounds are used worldwide as agricultural pesticides and pose a threat if used as chemical warfare agents. The standard treatment of intoxication with OP compounds includes the administration of oximes acting as acetylcholinesterase reactivating antidotes in order to counteract a cholinergic crisis. However, the blood-brain barrier restricts the rapid transport of these drugs from the blood into the brain in therapeutically relevant concentrations. To enable brain-targeting, HI-6 dimethanesulfonate was bound to albumin nanoparticles followed by freeze-drying of the resulting suspensions in order to prolong the stability of these formulations. Different cryoprotectors such as lactose, mannitol, sucrose and trehalose were tested.

Methods: Freeze-dried nanoparticle suspensions were reconstituted in water prior to evaluation of drug binding via ion pair HPLC. Respective particle sizes, polydispersity, and zeta potentials were determined by photon correlation spectroscopy. The release of non- and freeze-dried HI-6-nanoparticles was further studied using a dialysis membrane.

Results: All samples showed a narrow size distribution. Sucrose and trehalose were superior to other cryoprotectors in terms of particle characteristics. Drug binding increased with increasing percentages of cryoprotector. Maximum drug binding was found to be 112.3 µg HI-6 per mg of nanoparticles for mannitol followed by 88.5 µg HI-6 per mg nanoparticles for sucrose. Release studies proved HI-6 to be released from the nanoparticulate suspension completely within 120 minutes.

Conclusion: This study proves the possibility to freeze-dry HI-6-loaded albumin nanoparticles. Binding HI-6 to albumin nanoparticles with subsequent freeze-drying is a promising approach to ease handling and prolong stability of the formulations. Stable drug-loaded nanoparticles where obtained with all cryoprotectors. Sucrose was shown to be superior to the other cryoprotectors, keeping small particle sizes, and high drug binding. HI-6 proved to be released rapidly from the nanoparticles enabling a fast onset of the therapeutic antidote effect.

P-TH-10

DETERMINATION AND ANALYTICAL METHOD VALIDATION OF CYCLOSPORIN A FROM OCULAR IMPLANTS

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Objective: For the treatment of severe dry eye disease, cyclosporin A (CsA), the first one of the new generation immunomodulatory drugs. The purpose of this study was to develop a new specific HPLC method used for the quantitation of released CsA from ocular implants and to validate this analytical method.

Methods: The reversed-phase procedure utilized a C18 column and UV detection at 210 nm. The mobile phase contained acetonitrile and purified water (80:20 v/v). The flow rate was 1 mL/min for 14 minutes with an injection volume of 50 µL at 65˚C. The method was validated to meet requirements for a global regulatory filing and this validation included specificity, range, linearity, precision, accuracy, LOD and LOQ.

Results: Specificity was examined by analyzing dissolution medium and solutions of polymers which will be used to prepare CsA loaded nanoparticles and implants. Absence of interference was demonstrated. Six concentrations between the range of 7.5–100 µg/mL of CsA solutions prepared to show the linearity of the method. The R^2 for the regression line is 0.992 with a slope of 96.265 and a y-intercept of -447.07. Six samples prepared at the same concentration (40 µg/mL) to evaluate method precision and coefficient of variation (CV%) was 2.0%. Six injections of the same sample (40 µg/mL) were made to evaluate instrument precision and CV% was 0,44%, Intermediate precision was evaluated to show the variation between the days. Samples (40 µg/mL) prepared on three separate days and CV% was 1.75%. Results considered acceptable. Recovery values were found to be 103.64%, 103.91% and 98.05% for samples at 10, 40 and 100 µg/mL (n=3). LOD was 0.016 µg/mL and LOQ was 0.055 µg/mL.

Conclusion: The results indicated that the method shows good performance with respect to specificity, rage, linearity, precision, accuracy, LOD and LOW in order to establish the suitability of the analytical method.

P-TH-11

THE PHARMACOKINETIC PROPERTIES AND ACTIVITY EVALUATION OF A NOVEL ORALLY AVAILABLE ANTI-HIV DRUG CANDIDATE

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Objective: The initial infection step of HIV is mediated through the binding of the gp120 viral envelope glycoprotein to the first extra-cellular domain of the trans-membrane CD-4 receptor expressed at the surface of the target cell. Based on cycloscan approach, the non-continuous active region of CD-4 was converted to a small macrocyclic CD-4 mimetic lead that has two chiral centers, having four diastereomers. The current investigation examined the pharmacokinetics and activity of these four diastereomers.

Methods: The intestinal permeability was assessed by Caco2 model, Ussing chamber system and pharmacokinetic study. Multinuclear activation of a galactosidase indicator (MAGI) assay was used to assess activity of entry inhibition. The metabolic stability of the compounds was assessed by incubation with intestinal brush border membrane vesicles (BBMVs) and liver microsomes. All the in-vitro experiments were performed in triplicates and the pharmacokinetic evaluation was performed on 5 male Wistar rats. One-tailed Student t test was used for univariate analysis (For multivariate analysis ANOVA is required, to be followed by Tukey HSD or Newman-Keuls test). Where indicated, $p<0.05$ was considered statistically significant.

Results: The results indicate that only one of the diastereomers (S/S), named CG-1, have both the best intestinal permeability (Papp=1.8E-05) and entry inhibition activity (ED50< 8 μM). The permeability screening revealed a unique transporter-mediated absorption pathway of CG-1, suggesting a significant role of the molecule's conformation on the mechanism of absorption. The oral bioavailability of CG-1 in rats was 7.3% where the low bioavailability was attributed, in part, to first pass metabolism (confirmed by liver microsomes assay). Ritonavir, anti-HIV drug, elevated the oral bioavailability of CG-1 to 21%.

Conclusion: CG-1 has promising anti HIV-1 entry inhibitory properties combined with sufficient oral bioavailability, particularly when it is co-administered with ritonavir. Thus, CG-1 should be further evaluated as a potential oral treatment for HIV.

P-TH-12

THREE-DIMENSIONAL LIVER CELL CULTURES FOR DRUG TESTING

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Objective: To generate methodologies on the 3D culture of hepatic cells and eventually to generate better cellular models for the ADME research.

Methods: In this study, we established 3D hepatic cell cultures with aid of biomaterial hydrogels. A novel plant derived nanofibrillar cellulose (NFC) hydrogel was tested against the commercial cell growth supporting hydrogels. Structural properties of NFC hydrogel were investigated. Viability, morphology and the liver-specific functions of hydrogel embedded cells were reviewed.

Results: Structure, rheology and permeability experiments established that NFC hydrogel has interesting physicochemical features for cell culturing and tissue engineering. NFC supported cellular polarization, and differentiation of human hepatic cells, HepaRG and HepG2. Both HepG2 and HepaRG formed 3D multicellular spheroids similar to those seen in hyaluronan-gelatin-polyethylene glycol diacrylate hydrogel and in peptide nanofiber hydrogels. Enhanced formation of filamentous actin between apical cells asserted the in vivo-like polarity of HepaRG spheroids. In addition, 3D HepaRG cell cultures showed the process of differentiation.

Conclusion: This study shows that a single component NFC hydrogel supports the 3D liver cell culture as such, importantly without any added bioactive components. We conclude that 3D culturing with hydrogels improved liver specific polarity making this culturing system a promising approach to organotypic cell culture applications in biomedicine and drug discovery.

P-TH-13

IN VITRO BASED PHARMACOKINETIC SIMULATIONS OF QUINIDINE BRAIN TRANSPORT

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Objective: This study addresses the feasibility of predicting brain concentrations of p-glycoprotein substrate quinidine in silico, using in vitro data to describe brain transport. The aim was to test how different estimates of passive permeability and Michaelis-Menten parameters (Vmax and Km) affect the brain concentrations predicted by the pharmacokinetic simulation.

Methods: A literature review was undertaken to find and compare published in vitro and in situ parameter values describing quinidine transport into the brain. Using this data and Matlab, pharmacokinetic simulations were performed to predict the previously published ratio of unbound quinidine concentrations in brain and plasma (Kp,uu) in rats. A three compartment (plasma, endothelial cell and brain) model from the literature, including p-glycoprotein efflux described by Michaelis-Menten kinetics, was used.

Results: Published in vitro Caco-2 and MDR1-MDCKII monolayer estimates of quinidine Vmax and Km are highly variable (12.9 – 5670 fmol/s/cm^2 and 0.23 – 27 µM, respectively), resulting in significant differences in the simulated Kp,uu values (0.0026 – 0.77). Most published passive permeability values calculated from the in vitro monolayer experiments were within a 3-fold range of the in situ brain perfusion data. Therefore in these cases substitution of in situ with in vitro permeability led only to a moderate change in Kp,uu predictions. Two simulated Kp,uu estimates were close to the experimental observation (0.11 and 0.16 versus 0.17), although input parameter values were very different. This demonstrates that the underlying intrinsic parameter values cannot be determined based on these simulations.

Conclusion: Apparent Michaelis-Menten parameters are too inaccurate and variable to give reliable predictions with the used model. A scaling factor for Vmax is critical both for the validation of in vitro models and extrapolation to in vivo. Simulations should be repeated with other compounds to gain more information about the reliability of in vitro assays and data handling.

P-TH-14

INTERACTION OF BREAST CANCER RESISTANCE PROTEIN (BCRP/ABCG2) WITH PDZ ADAPTOR PDZK1 AND THERAPEUTIC AGENTS IN SMALL INTESTINE

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Objective: BCRP/ABCG2 is ATP-binding cassette transporter involved in the efflux of various types of substrates in small intestine and other organs. Despite its essential role in oral absorption of therapeutic agents, the interacting partner protein for BCRP in small intestine has not yet been identified. Here, we focused on PDZ adaptor PDZK1, which regulates apical membrane localization of various solute carrier influx transporters by direct association, as a possible regulatory protein for BCRP with an aim to clarify whole picture of membrane transport of therapeutic agents.

Methods: Pdzk1 gene knockout (pdzk1-/-) mice were used to examine pharmacokinetic and immunolocalization analyses. MDCKII cells stably expressing both BCRP and PDZK1 (MDCKII/BCRP/PDZK1) were constructed.

Results: Western blot and immunohistochemical analyses revealed that expression and localization of BCRP on apical membranes in small intestine is much reduced in pdzk1-/-, compared with wild-type mice. This was consistent with higher expression of BCRP on apical membranes in MDCKII/BCRP/PDZK1 cells, compared with MDCKII/BCRP cells, demonstrating that PDZK1 is essential for apical localization of BCRP. Pull-down and immunoprecipitation studies revealed direct association between BCRP and PDZK1, supporting the regulation of BCRP by PDZK1. To understand relevance of this protein-protein interaction with oral absorption of therapeutic agents, plasma concentration profile of cimetidine, a substrate of BCRP was examined after oral administration and found to be higher in pdzk1-/-, compared with wild-type mice. This was supported by higher basal-to-apical cimetidine transport in MDCKII/BCRP/PDZK1 compared with MDCKII/BCRP cells. This double transfectant cell line, MDCKII/BCRP/PDZK1 could be useful tool to identify BCRP substrates since a certain therapeutic agents was found to be a BCRP substrate in MDCKII/BCRP/PDZK1, but not in MDCKII/BCRP.

Conclusion: This is the first evidence that PDZK1 is an interacting protein for both influx and efflux transporters in vivo in small intestine and affects oral drug absorption.

P-TH-15

NANOCLUSTERS ENHANCE DISSOLUTION RATES OF POORLY SOLUBLE DRUGS

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Objective: Poorly water soluble drugs comprise approximately 50% of all new drug molecules inhibiting their bioavailability and impeding their use in formulations. Nanoparticle agglomerates, called NanoClusters, can increase the dissolution of poorly solvated compounds by decreasing the particle size thus increasing surface area. Budesonide and danazol, two poorly soluble steroids, are the model compounds utilizing the NanoCluster approach to increase their dissolution rate.

Methods: NanoCluster powder was made using a Netzsch Minicer wet mill with samples collected between 5 and 25 hours and lyophilized to create a dry powder. Dissolution rates were evaluated using a Distek 2100A Dissolution Tester with temperature control unit using 0.25% SDS as dissolution medium. Lyophilized NanoCluster powder was characterized through Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD), and BET surface area analysis.

Results: SEM confirmed that milling created agglomerated nanoparticles, and HPLC analysis showed less than 3% degradation for all used NanoCluster products. Milled budesonide NanoClusters had relatively constant dissolution compared to the micronized material, which took 15 minutes to reach similar levels, while micronized danazol took the entire hour to reach the dissolution level of the NanoClusters. BET analysis indicated the NanoClusters had an increased surface area of 8 to 10 times for budesonide and 10 to 15 times for danazol compared to the micronized stock materials. DSC and PXRD data revealed budesonide and danazol structure within NanoClusters is unchanged compared to stock material. Observed differences appear to be minimal and possibly attributed to the change in crystalline particle size and not conversion to an amorphous or different crystalline form.

Conclusion: The increased dissolution rate of the NanoCluster agglomerates is hypothesized to be from increased surface area. This work shows that NanoClusters could provide an alternative approach to increase the dissolution of poorly water soluble compounds.

P-TH-16

AGGLOMERATION OF MESOPOROUS SILICA PREPARED IN A HIGH-SHEAR MIXER: A COMPARISON BETWEEN MELT AND STEAM GRANULATION

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Objective: Due to the risk of unwanted drug release during processing, it has been previously shown that the use of solvents should be avoided when processing mesoporous silica. Therefore, the objective was to evaluate both melt and steam granulation techniques as an approach to eliminate or minimize the use of solvents during down-stream processing for immediate release tablet formulations.

Methods: All granulates were prepared in a Rotolab® laboratory scale high-shear mixer. Itraconazole was selected as the poorly soluble compound which was loaded into both non-ordered Syloid® 244 and ordered COK-12 using the incipient wetness procedure. For melt granulation, Poloxamer 188 (Lutrol® micro 68) was selected as binding agent and both Polyvinylpyrrolidone [Kollidon® 25 (PVP)] and Hydroxy Propyl Methyl Cellulose [Methocel® E5 (HPMC)] were selected for steam granulation. To determine the influence of granulate composition, various tablet formulation were evaluated using Heckel Plots, tablet hardness and thickness, porosity, particle size, and in vitro drug release. Characterization techniques include bulk and tapped density, nitrogen physisorption, X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), laser diffraction, powder flow, high-performance liquid chromatography (HPLC), x-ray tomography, and scanning electron microscopy (SEM).

Results: Morphological comparison to the unprocessed silica material reveals an increase in particle size, which improved the powder properties based on Carr Index values. XRPD results indicate that slight drug release did occur during melt granulation which was not observed with steam. Despite melt granulation showing unwanted drug release from the pores, in vitro drug release remains greatly enhanced when compared to crystalline itraconazole. Furthermore, granulates prepared with steam resulted in better compression/compaction properties.

Conclusion: The comparison between melt and steam granulation was assessed on both ordered and non-ordered mesoporous silica material. While granulates prepared by steam resulted in better tablet properties, it was demonstrated that both melt and steam granulation are appropriate techniques for enhancing the flowability, compression and compaction properties of mesoporous silica material for immediate release tablet formulations.

P-TH-17

EVALUATION OF SYNOVIAL ESTERASE FOR DEVELOPMENT OF SOFT-DRUGS

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Objective: Intra-articular treatment of arthritis or osteoarthritis involves short- and long-acting corticosteroids and hyaluronate. Corticosteroids are effective, but have severe side effects including cartridge damage and bacterial infection by repetitive injection. A soft-drug with long-term retention in synoviocytes is useful for avoidance of side effects by causing its rapid hydrolysis to inactive metabolite in synovial fluid or blood. In order to design soft-drugs, it is important to know esterases in synovial fluid and synoviocytes. In addition to human synovial fluid and synoviocyte, we also studied rabbit synoviocyte since it is useful animal model of arthritis.

Methods: Synovial fluid was obtained from arthritis patients. Human synoviocyte (HFLS) and rabbit synoviocyte (HIG-82) were cultured, and confluent cells were provided to prepare homogenates. Native PAGE gels were stained for hydrolase activity. Paraoxon, diisopropylfluorophosphate (DFP) and bis(p-nitrophenyl) phosphate (BNPP) were used as esterase inhibitors.

Results: Plasma esterases, such as paraoxonase associated with high-density lipoprotein and butyrylcholinesterase tetramer, were detected in synovial fluid. Human HFLS and rabbit HIG-82 cells showed two and three esterase bands, respectively, with different mobilities of their plasma esterases. Two bands in human HFLS were inhibited by high concentration of paraoxon and were resistant to DFP and BNPP, suggesting the possibility of A-esterase, inhibited competitively by paraoxon. One band with relatively high activity in rabbit HIG-82 showed similar property of human esterases, and the other two bands were inhibited by low concentration of paraoxon, however DFP and BNPP did not affect one band, while inhibited the other band, like B-esterase.

Conclusion: High-molecular weight plasma esterases are present in human synovial fluid due to vascular hyperpermeability. This study showed a possibility of presence of A-esterase in human synoviocyte, while both A- and B-esterase in rabbit synoviocyte. The different expression of esterase between synovial fluid and synoviocytes facilitates the development of soft-drugs.

P-TH-18

PHARMACEUTICAL PROPERTIES OF MOLECULAR-TARGETING PEPTIDES "MICROANTIBODY" AGAINST GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) RECEPTOR

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Objective: Antibodies are successful reagents in molecular targeting therapy, but their use is limited due to the biophysical properties and the cost to manufacture. Prof. Fujii has developed a helix-loop-helix peptide, "MicroAntibody", as an alternative-binding molecule with non-immunoglobulin domain. MicroAntibody is composed of three regions, N-terminal alpha-helix (14 amino acids), flexible connecting loop (7 Gly residues) and C-terminal alpha-helix (14 amino acids). Since the MicroAntibody is folded into a helix-loop-helix structure by hydrophobic and electrostatic interactions between amino acid residues positioned inside the helices, outside residues of the C-terminal helix are randomized to give a library of the conformationally constrained peptides. In this study, we evaluated the stability and the protein binding of MicroAntibody generated by bio-panning of the phage-displayed library against granulocyte colony-stimulating factor (G-CSF) receptor.

Methods: Adsorption of MicroAntibody to polyethylene tube and its solubility were measured in water and PBS. The stability of MicroAntibody was determined in PBS and human or rat plasma, and its protein binding was measured by ultrafiltration method.

Results: The concentration MicroAntibody was measured by HPLC connected with ODS column flowed by 0.1% TFA/ acetonitrile (0- 50% gradient) at 205 nm. Its retention time is 22.6 min with limitation of 0.7 µM and 5.2 µM in PBS and plasma, respectively. MicroAntibody was easily soluble and stable in aqueous solution, and slightly adsorbed to polyethylene tube. Half-life of MicroAntibody was 37 and 52 days in human and rat plasma, respectively, indicating it is resistant to plasma esterases. Interestingly, MicroAntibody bound to plasma protein about 40 % in both human and rat plasma due to binding to Albumin (HSA: 40%, RSA: 30%).

Conclusion: We propose that MicroAntibody is a good candidate for clinical application due to its biophysical properties.

P-TH-19

EFFECT OF FORMULATION PH ON TRANSPORT AND PORE CLOSURE IN MICRONEEDLE ENHANCED TRANSDERMAL DRUG DELIVERY

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Objective: The aim of this research was to obtain a mechanistic understanding of transport and pore closure across microneedle (MN) treated skin towards the development of a 7 day transdermal patch system. Naltrexone, the model compound, exhibits a solubility maximum around pH 5.0, and solubility optimization is crucial to MN-enhanced flux. However, it is known from literature that wound healing is slower at pH 7.4, thus making pH 7.4 potentially desirable from the perspective of prolonging micropore lifetime.

Methods: Current studies evaluated the effect of 6 different pH values on naltrexone transport across MN treated skin in vitro. Impedance spectroscopy was used in pig studies to look at the effect of formulation pH (3 formulations) on pore closure kinetics under occlusion in vivo.

Results: The in vitro flux values indicated that there was a significant advantage of using low pH formulations for flux enhancement across MN treated skin ($p<0.05$) while flux values across intact skin were not significantly different from each other ($p>0.05$). Compartmental analysis of permeability across the microchannel pathway and intact skin pathway revealed that permeability of naltrexone didn't change across the microchannel pathway but permeability across the intact skin pathway increased with increase in formulation pH. The in vivo data (pH 5.5, pH 6.5 and pH 7.4) indicated that there was no significant difference in the rate of micropore closure due to formulation pH after the first 24 hours ($p>0.05$).

Conclusion: It can be concluded from in vitro and in vivo data that a lower pH formulation will be beneficial for the development of the 7 day patch system due to flux enhancement and no significant contribution from higher pH values towards pore lifetime enhancement. Permeability values indicate that transport across the microchannel pathway is independent of the ionization state of the molecule, while higher concentrations of unionized molecule at higher pH values lead to increased permeability across the intact skin pathway. Therefore, increased ionization can definitely be expected to alter flux by altering only the solubility.

P-TH-20

MEASUREMENT OF MOLECULAR TRANSPORT ACROSS THE NORMAL HUMAN BRONCHIAL PRIMARY EPITHELIAL CELL MONOLAYER AT A SINGLE CELL LEVEL

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Objective: To model drug absorption in the lung, normal human bronchial primary epithelial cells (NHBE) offer advantages over cancer-derived bronchial epithelial cell lines. However, differentiated NHBE cells form multilayers of varying thickness, which makes interpretation of mass transport and permeability measurements difficult. Therefore, we decided to develop experimental conditions to suppress NHBE multilayer formation, in order to assay mass transport and permeability across single NHBE cell in monolayer cultures.

Methods: To suppress multilayer formation, mixed Calu-3:NHBE cell cultures were differentiated on Transwell inserts in air-liquid interfaced conditions. Cells were incubated with Hoechst 33342, LysoTracker Green, and MitoTracker Red, followed by confocal imaging and three-dimensional reconstructions. Quantitative imaging analyses allowed distinguishing Calu-3 and NHBEs in mixed cultures. Transcellular transport and intracellular accumulation assays were performed with propranolol (a model drug) in the mixed cell culture and analyzed by LC/MS. Biophysical model with the optimized cellular parameters were constructed to predict the pharmacokinetics of propranolol at a single cell level.

Results: Mixed Calu-3/NHBE cells in a 1:1 ratio formed stable cell monolayers with low paracellular permeability and high TEER values. Morphometric analyses showed that a single NHBE cell has about 2.6 fold larger surface area and volume than a Calu-3. Based on the propranolol transport measurements together with the fractional areas covered by Calu-3 and NHBEs, a NHBE cell showed 1.74 fold higher mass transport rate and 1.65 fold larger intracellular accumulation than a Calu-3. With the model, active drug efflux rates in Calu-3 were calculated (Vmax = 9.03e4 molecules/sec/cell, Km = 50 μM) while NHBE did not show a measurable P-gp activity.

Conclusion: Mixed cell monolayer cultures were developed to study the transport of small molecule drugs across differentiated NHBE cells. Measurements of drug transport across NHBE cells in monolayer cultures facilitate analysis of transcellular transport mechanisms of the inhaled drug molecules.

P-TH-21

CONCENTRATION- AND TIME-DEPENDENT APOPTOSIS INDUCED BY POLYMYXIN B IN RAT PROXIMAL TUBULAR CELLS

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Objective: The dose-dependent nephrotoxicity of polymyxin B, one of the clinically available polymyxins, is a major limitation for dose escalation for treatment of infections caused by multidrug-resistant Gram-negative superbugs. The mechanism(s) of polymyxin B-induced nephrotoxicity is poorly understood. The objective of the present study was to characterise the polymyxin B-induced apoptosis in rat kidney proximal tubular cells (NRK-52E).

Methods: NRK-52E cells were exposed to a range of concentrations (0.25, 0.50, 0.75, 1.00, 1.25, 1.5, 1.75, 2.00 and 4.00 mM) of polymyxin B for 24 hr and to polymyxin B 2.00 mM for 1, 6, 12, 16, 20 and 24 hr. Apoptosis was determined by fluorescent activated cell sorting (FACS) with annexin V-Alexafluor 488 and propidium iodide. The concentration that produced fifty percent of maximal apoptosis (EC50) after 24 hr of incubation was determined. DNA breakage in polymyxin B treated NRK-52E cells was also detected using TUNEL assay.

Results: Polymyxin B induced apoptosis in a concentration- and time-dependent manner. The EC50 (95% CI) was 1.04 (0.91 to 1.19) mM. After treatment with 2.0 mMpolymyxin B, the % of apoptotic cells at 1, 6, 12, 16, 20 and 24 hr were 4.33 ± 2.97, 10.90 ± 4.69, 29.80 ± 7.53, 69.17 ± 8.50, 88.83 ± 3.51 and 89.63 ± 3.74. Polymyxin B-induced apoptosis was also confirmed by positive TUNEL assay results.

Conclusion: Our study is the first to demonstrate that polymyxin B induces apoptosis kidney cells. Further studies on the mechanism of polymyxin-induced apoptosis and its role in polymyxin nephrotoxicity are being conducted.

P-TH-22

IN VITRO PK/PD AND MATHEMATICAL MODELS TO COMBAT ANTIBIOTIC RESISTANCE

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Objective: Antibiotics have enabled many advances in modern medicine. However, the recent emergence of bacterial 'superbugs' and the decline of antimicrobial drug discovery have led to a severe global medical challenge. The objectives of this study were: (1) To examine, in an in vitro one-compartment PK/PD model (IVM), the benefit of loading and front-loading polymyxin B dosing regimens against Acinetobacterbaumannii. (2) To develop new mathematical approaches to analyse in vitro experimental data.

Methods: Antibacterial effect of polymyxin B against A. baumannii ATCC17978 and emergence of resistance were examined in an IVM (simulated half-life of 12 h and Css of 3 mg/L). Further, a new empirical mathematical model was developed for interpretation of in vitro experimental data.

Results: Polymyxin B achieved significant (>5 Log10) bacterial killing within 1 h for dosage regimens featuring loading and front-loading doses. The absence of such doses significantly decreased bacterial killing (<2 Log10 killing over 96 h). The development of resistance was suppressed by front-loading regimens, with no resistant bacteria observed at 96 h (c.f. 24 – 72 h for other regimens). In addition, a novel mathematical approach was successfully applied to the analysis of experimental data, providing insights into polymyxin activity not discernible using current analysis techniques.

Conclusion: Results from the IVM highlight the important link between rapid attainment of target polymyxin B concentrations and both bacterial killing and resistance prevention. Further, the application of in vitro experiments and novel mathematical approaches provide means to understand antimicrobial activity and aid development of effective dosing regimens.

P-TH-23

DIGESTION MODULATING FORMULATIONS AS A PLATFORM TO ENHANCE THE UTILITY OF LIPID BASED DRUG DELIVERY SYSTEMS.

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Objective: Lipid-based formulations (LBFs) can improve the oral absorption of poorly water-soluble drugs by delivering the drug to the gastro-intestinal tract in a pre-dissolved, molecularly dispersed form. However, LBFs are rapidly digested by intestinal lipases, causing a digestion rate dependent loss in solubilisation capacity, in turn creating the risk of drug precipitation and reduced bioavailability. The aim of the present study was to evaluate the effect of PEGylated surfactants on the in vitro digestion of medium-chain triglycerides (MCT) and subsequently to evaluate drug absorption *in vivo*.

Methods: An in vitro digestion model was used to examine the influence of PEGylated surfactants on the digestion of MCT. The time required for 10% of the MCT to be digested (T10%) was estimated by linear interpolation and plotted as a function of the chain length of the PEG surfactant component. The solubilisation capacity of the aqueous digestion phases was evaluated by assay of drug content by HPLC. Drug absorption was examined in carotid-artery cannulated male SD rats following oral administration of digestion modulating formulations containing danazol at 80% saturated solubility.

Results: The presence of PEGylated surfactants altered the digestion of co-formulated MCT. Surfactants containing short and long PEG chains were poor inhibitors of digestion. However for intermediate PEG-chain length surfactants, digestion inhibition, presumably by formation of a PEG mantle around the lipidic micellar core, was effective in increasing the time for 10% of MCT to digest by 25 minutes. The solubilisation capacity of the digestion aqueous phases correlated with the digestion inhibitory effects of the PEGylated surfactants with short chain PEG surfactants losing up to 85% solvent capacity after 15 min. In contrast, the intermediate PEG-chain surfactants (digestion modulators) lost only 20% solvent capacity.

Conclusion: PEGylated surfactants are effective in modulating MCT digestion rate and maintaining drug solubilisation capacity post-digestion. The potential to delay digestion mediated changes in solubilisation capacity may provide an improved platform for oral lipid based drug delivery.

P-TH-24

SYMPATHETIC NERVOUS SYSTEM ACTIVATION PROMOTES BREAST CANCER PROGRESSION BY VEGF-DRIVEN LYMPHANGIOGENESIS IN PRIMARY TUMOURS

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Objective: Stress-induced sympathetic nervous system (SNS) activation has been shown to promote metastasis of breast cancer to distant organs, including lymph nodes (LNs). However, little is known about the mechanisms that underlie stress-enhanced metastasis to LNs. This study therefore aims to compare the effect of physiological SNS activation on lymphangiogenesis-linked gene expression and lymphatic system function throughout breast cancer progression.

Methods: To activate SNS to physiological levels, mice were subject to 2 hours of daily restraint stress for a total of 21 days. *Ex vivo* bioluminescence imaging was used to track the impact of stress on tumour progression. The effect of stress on lymphatic vessel density and collector diameter was determined using lyve-1 immunostaining and intra-tumoural injection of patent blue V dye respectively. RT-PCR was used to analyse stress-induced changes in gene expression.

Results: Chronic physiological stress increased metastasis to LNs by >14-fold ($p < 0.005$). The effect of stress was mimicked with beta-agonist isoprenaline, which resulted in >40-fold increase in LN metastasis compared to control ($p < 0.005$). Stress increased both lymphatic vessel density and vessel diameter by 60% when compared to control ($p < 0.01$). Gene expression analysis showed lymphangiogenesis-linked genes *Vegf-b* and *-d* were up-regulated in primary tumours of stressed animals compared to control (all, $p < 0.01$).

Conclusions: These findings indicate that SNS activation may promote LN metastasis by inducing lymphangiogenesis and lymphatic vessel dilation to allow tumour cell dissemination to LNs. Ongoing studies are dissecting the functional molecular and cellular dynamics that occur in the primary tumour to support SNS regulation of lymphangiogenesis. Findings of these studies will provide a mechanistic foundation for translation of beta-blockers and other SNS antagonists into the cancer clinic.

P-TH-25

DRUG TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER IN ALZHEIMER'S DISEASE

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Objective: There are various blood-brain barrier (BBB) related pathological changes reported in Alzheimer's disease (AD) but less is known about what impact such BBB alterations have on the ability of therapeutic agents to enter the central nervous system.The purpose of this study, therefore, was to systematically assess the BBB transport of various probe compounds (with different mechanism of transport) in a relevant animal model of AD.

Method: Marker ^{3}H- and ^{14}C-labelled compounds were perfused through the left ventricle of 18 month 3×Tg (TG) and wild type (WT) mice at a rate of 2 mL/min. After a 4 min perfusion, cortex, hippocampus and perfusate concentrations were determined by liquid scintillation counting, and cortex/hippocampus-to-perfusate concentration (C:P/H:P) ratios calculated. To further understand the observed *in vivo* transport alterations, molecular characterization of the BBB was performed, with, collagen-IV and brain microvascular P-glycoprotein (P-gp) expression assessed by immunohistochemistry and western blot, respectively.

Results: C:P and H:P ratio of the paracellular marker ^{14}C-sucrose was not significantly different between WT and TG mice. BBB transport of the passive diffusion markers ^{3}H-diazepam and ^{3}H-propranolol were significantly ($p<0.05$) decreased by 54-60 % in TG mice relative to WT mice, whereas the P-gp substrates (^{3}H-digoxin, ^{3}H-loperamide and ^{3}H-verapamil) exhibited no significant difference in their BBB transport between genotypes. There was significant thickening of the basement membrane as seen by 33 % increase in collagen-IV staining in brain slices of TG mice, and a 20 % reduction in P-gp expression in the isolated microvessels of TG mice.

Conclusion: Consistent with that observed clinically, the BBB paracellular route is maintained in TG mice. The BBB transport of passively-diffusing compounds is reduced in TG mice likely as a result of increased cerebrovascular membrane thickness. In contrast, the BBB transport of P-gp substrates appears unaffected in TG mice, as the reduced expression of P-gp is compensated by a thickened basement membrane. These studies are the first to systematically address the impact of AD on BBB transport phenomena, suggesting this disease significantly alters disposition of therapeutics into the brain.

P-TH-26

LINKING STRUCTURE AND COMPOSITION OF LIPID BASED DRUG FORMULATIONS DURING DIGESTION

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Objective: There is an increasing need to understand the colloidal species formed in the gastrointestinal tract following oral administration of lipid based drug formulations. During digestion, colloidal species are generated which are able to solubilise poorly water soluble drugs, and thus enhance absorption. Of current strong interest in lipid based drug delivery is the capability of medium chain triglycerides to reportedly support supersaturated drug during digestion, via formation of a so-called 'vesicular phase'. However, the structure of the apparent vesicular phase has never been demonstrated. Captex 355 was studied to represent a lipid previously shown to support the supersaturation effect. The nanostructure of the self-assembly colloidal species formed by Captex 355 under lipolysis conditions in simulated gastrointestinal fluids was studied via small angle X-ray scattering (SAXS) and cryo-TEM imaging.

Methods: In vitro digestion studies were performed using a pH-stat auto titrator. Lipids were added to fasted simulated intestinal fluid pH 6.5 in the thermostatted digestion vessel at 37 °C. Pancreatin was added to initiate digestion and titrated with NaOH to maintain the system at pH 6.5. SAXS measurements were performed at the Australian Synchrotron to monitor changes in nanostructure in real-time during digestion. The digestion medium was pumped through a quartz capillary which was fixed in the X-ray beam. Cryo-TEM measurements were performed at Bio21.

Results: Dynamic SAXS measurements taken during digestion show that initially micelles were present, and after 25 min, a lamellar phase was formed. This phase was confirmed using cryo-TEM, where the presence of bilamellar, unilamellar vesicles, indicative of a dispersed lamellar phase, and micelles were present. The digestion profile showed two distinctive regimes, depicting digestion in the micellar phase, and digestion in the lamellar phase separately. Captex 355 is a mixture of C8 and C10 triglycerides and the lattice dimensions scales as expected. This structural change was found to be reproducible and the structure-titration link in this system was therefore established. It is consequent interest to determine whether this dynamic colloidal behaviour translates to an in vivo scenario, potentially leading to increased drug absorption and bioavailability.

Conclusion: These findings are consistent with dynamic SAXS studies reported recently using long-chain and medium-chain commercial triglycerides. The results provide our first insights into likely structure formation for ultimate correlation to nanostructure during dynamic lipolysis studies in vitro and in vivo.

P-TH-27

THE EFFECT OF L-LEUCINE ON PARTICLE FORMATION, MORPHOLOGY AND HYGROSCOPICITY OF SPRAY-DRIED FORMULATIONS FOR INHALATION

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Objective: In the present study, co-spray-drying of various excipients was investigated as a potential formulation strategy for the production of a multi-component dry powder formulation with a stable amorphous glassy matrix which is essential in the solid-state stabilisation of biomacromolecules. Several formulations containing mannitol, trehalose and/or glycine were spray-dried with different concentrations of leucine as a particle formation and moisture protection enhancing agent.

Methods: The particle size distribution of the powders was determined by laser scattering using the Malvern Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK) equipped with a Scirocco cell and a Scirocco 2000 dry powder dispersion unit. Particle morphology was examined using a scanning electron microscope (Phenom™, FEI company, USA).

Results: The effect of leucine in assisting particle formation appears most pronounced in the mannitol/glycine formulation where the decrease in size from 13.48 µm (indicating fused agglomerates) to 2.63 µm is observed upon addition of leucine 10 % w/w. The particle size of all the formulations slightly decreased as the proportion of leucine increased from 10 to 20 % w/w. The morphology result suggests multiple combinations of small molecule excipients appear to be susceptible to fusion and more hygroscopic than the individual components. Further increase in leucine concentration above 20 % w/w produced increasingly wrinkled particles. However this was not shown to provide significant aerosol delivery benefit.

Conclusion: The addition of leucine appeared to facilitate particle formation in all the spray-dried formulations. Scanning electronic micrographs revealed a transition of morphology upon increasing concentration of leucine from fused particles, to distinctive spherical particles, to highly wrinkled particles in some of these formulations. The results show that leucine between 10 and 20 % w/w is sufficient to assist particle formation while higher concentrations of leucine beyond 20 % w/w appear to provide little benefit in terms of surface moisture protection and particle separation.

P-TH-28

A MODIFIED THREE DAY WATER MAZE PROTOCOL TO TEST SPATIAL MEMORY DEFICITS IN MICE

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Objective: The water maze (WM) task is one of the most reliable tests to evaluate spatial navigation memory in mice. The available protocols suffer the disadvantage of being time consuming as mice often require many days of training with multiple trials a day. This study validated a novel three day WM protocol of 60 second trials which could be used to effectively assess memory in C57BL/6 mice.

Methods: An immunotoxin model of (acquired) memory deficits in mice was used. 6-8 week old male C57BL/6 mice (n = 24) were treated with bilateral intracerebroventricular injections of saline (control) or mu-p75-saporin toxin (0.4µg/µl/mouse) to induce cholinergic neuronal lesions. The mice were trained in the WM paradigm using four visible platform (cued) trials on day 1 and the latency to the platform was recorded. After 24 hours (day two, spatial trials) acquisition of the tasks was assessed using three hidden platform trials. An additional probe trial (no platform) was performed on day three to assess the long term (reference) memory. Neuronal lesions were assessed using choline acetyltransferase (ChAT) immunohistochemistry.

Result: A significant difference in learning the task was observed during cued and spatial trials, with toxin-treated mice taking longer to reach the platform than control mice (two way ANOVA; $p<0.01$). Performance on the probe trial was also significantly reduced in toxin-treated mice, compared to control mice (t-test; $p<0.05$), indicating memory loss in toxin-injected mice and better learning in the saline-treated controls. Immunohistochemistry demonstrated a reduction in the number of ChAT immunopositive cells in the medial septum of toxin-treated mice as compared to controls, whilst GABAergic neurones were unaffected, supporting a correlation between the cholinergic neuronal loss and memory impairments that is detected in this WM protocol.

Conclusion: The modified WM protocol provides a fast, easy and reliable way to assess cognitive deficits in mice, which may prove useful in high throughput screens to assess the potential of novel memory enhancing compounds in the future.

P-TH-29

IONIC LIQUIDS PROVIDE UNIQUE OPPORTUNITIES FOR ORAL DRUG DELIVERY: FIRST IN VIVO AND IN VITRO EVIDENCE OF UTILITY

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Objective: To evaluate the potential utility of ionic liquids (ILs) as novel oral drug delivery vehicles for poorly water-soluble drugs (PWSD).

Methods: Custom-made ILs comprising a cation based on natural substances, nicotinic acid and trigonelline, and paired with hydrophobic triflimide [NTf2]-, hydrophilic [N(CN)2]- or amphiphilic alkyl sulfate [CXSO4]- anions were synthesized in-house. Solvency of ILs towards drugs danazol, itraconazole, fenofibrate was assessed at 37oC. Self-emulsifying drug delivery systems (SEDDS) consisting of 60% (w/w) Cremophor® EL, 10% ethanol and 30% IL or 30% lipid (soybean oil, MaisineTM, 1:1) with incorporated danazol (at 25 mg/g) were administered orally to fasted Sprague-Dawley rats.

Results: Increasing alkyl chain length, and therefore, the hydrophobicity of the IL cation increased solvency towards danazol. Danazol solubility in an IL consisting of 3-hexoxycarbonyl-1-hexylpyridinium [hhcpy]+ (cation) and [NTf2]- (anion) was 26.8 mg/g. Substituting the hydrophobic [NTf2]- for the more hydrophilic [N(CN)2]- increased danazol solubility to >96 mg/g. This danazol solubility was >20-fold higher than the solubility in soybean oil and exceeds the solubility in commonly used cosolvents. ILs also provided a remarkable solubility advantage over lipids in the case of itraconazole, but not for the more lipophilic fenofibrate. Danazol exposure in rats from SEDDS containing the amphiphilicoctadecylsulfate [C18SO4] anion was high, and consistent with the exposure using a 'gold-standard' lipid-containing SEDDS. In contrast, absorption from SEDDS containing the hydrophobic [hhcpy][NTf2] was no greater than the absorption from a crystalline drug suspension. Differences in IL utility in vivo correlated with their ability to interact with bile salt/phospholipid micelles, suggesting that drug absorption was dependent on the type of colloids formed by ILs.

Conclusion: Custom-made ILs were synthesized and showed very high solvent properties towards PWSD. Following first evidence of in vivo utility, this work highlights a new potential application of ILs as improved oral drug delivery vehicles for PWSD.

P-TH-30

SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF METHOXY- AND METHYLTHIO-SUBSTITUTED CHALCONES

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Objective: Chalcones are natural or synthetic compounds which display a wide variety of biological activities including antioxidant, anticancer, anti-inflammatory, antimicrobial, antiprotozoal and antihyperglycemic activities. The chemical structure of this group of compounds consists of two phenyl rings (ring A and B) connected by a three-carbon α,β-unsaturated carbonyl chain. In a bid to further our understanding of the effect of ring substituents on the antiproliferative activity of chalcones, a series of methoxy- and methylthio- substituted chalcones were synthesized and their antiproliferative activities were evaluated.

Methods: The chalcones were synthesized via Claisen-Schmidt condensation reaction. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the antiproliferative activity of these compounds against the MCF-7 breast cancer and HCT-116 colon cancer cell lines.

Results: A total of 13 methoxy-substituted chalcones and 5 methylthio-substituted chalcones were synthesized and evaluated for antiproliferative activity. Most of the compounds exhibited moderate antiproliferative activity against both cell lines, with GI50 values of methoxy-substituted chalcones ranging from 7.1μM to 32.3μM and GI50 values of methylthio-substituted chalcones ranging from 13.4μM to more than 100μM. Methoxy substitution on the 2'-position of ring A resulted in chalcones with the best antiproliferative activity. Compound 4 was the most potent against both MCF-7 and HCT-116 with a GI50 of 7.1μM against MCF-7 and 7.3μM against HCT116.

Conclusion: This study has identified that methoxy substitution on the 2'-position of ring A of the chalcone scaffold is beneficial for antiproliferative activity. This study has also demonstrated the feasibility of using the methylthio group as a bioisostere of the methoxy group in selected chalcones.

P-TH-31

A MISSING LINK: THE INTERACTION BETWEEN APOE AND LRP5 IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objective: 1. To identify the role of low-density lipoprotein receptor-related protein 5 (LRP5) in modulating Wnt signaling in SH-SY5Y human neuroblastoma cells.
2. To determine the binding affinity of LRP5 to Apolipoprotein E (ApoE) isoforms.
3. To investigate the effect of the interaction between ApoE isoforms and LRP5 on Wnt signaling and tau phosphorylation.

Methods: SH-SY5Y cells were stably transfected with expression plasmids for human wild-type LRP5 (SY5Y-LRP5) and a dominant negative form containing only the extracellular domain of LRP5 (SY5Y-EA4). The mRNA and protein levels of β-catenin and its downstream target in SY5Y-LRP5 and SY5Y-EA4 were determined by Quantitative Real-Time PCR and Western Blotting. The TCF/LEF-dependent TOPglow reporter activity was measured using dual luciferase assay. Knockdown of LRP5 in SH-SY5Y with siRNA was carried out and the levels of β-catenin and Cyclin D1 were examined. HEK293T cells were transfected with both LRP5 and ApoE isoforms and subjected to co-immunoprecipitation to determine the interaction. SY5Y-LRP5 was transfected with ApoE isoforms and Wnt activity was determined via luciferase assays.

Results: We showed that LRP5 overexpression increased β-catenin protein level and enhanced Wnt signaling while LRP5 knockdown decreased β-catenin protein level, Wnt signaling and its downstream targets such as Cyclin D1 and Axin2. We identified that LRP5 interacted with ApoE isoforms (ApoE2, 3, 4) in HEK293T cells when overexpressed. The interaction between LRP5 and ApoE isoforms activated Wnt signaling to a great extent compared to LRP5 transfection alone.

Conclusion: LRP5 interacts with ApoE isoforms and the interaction stimulates Wnt signaling in human neuroblastoma cells. Characterization of this deregulation of Wnt signaling by the interaction would provide a novel perspective to identify unique therapeutic targets to treat AD.

P-TH-32

INSIGHTS INTO A NOVEL INTESTINAL ABSORPTION MECHANISM OF THE ANTIDIABETIC DRUG METFORMIN

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Objective: Metformin is well-absorbed (oral bioavailability 40–60%) despite its hydrophilicity and positive charge at physiologic pHs; however, its intestinal absorption mechanisms are unknown. Based on efficient apical uptake/efflux and poor basolateral efflux in Caco-2 cells, our laboratory proposed that metformin oral absorption is predominantly paracellular and augmented by its enterocytic accumulation and recycling between the lumen and enterocytes via apical uptake/efflux cation-selective transporters. This study aims to provide evidence for transporter-mediated metformin intestinal accumulation and enhanced absorption in a mouse model.

Methods: Gene expression of intestinal mouse organic cation transporters (mOct) 1-3, plasma membrane monoamine transporter (mPmat), and multidrug and toxin extrusion (mMate) 1 was evaluated by qRT-PCR. Stable transporter-transfected cell lines were generated to characterize metformin uptake kinetics and transporter inhibitor potencies. Transporter-mediated metformin intestinal accumulation/absorption was investigated using a pan inhibitor administered intraperitoneally 30min before oral co-administration of [14C]metformin (3.5mg/kg) and inhibitor. Metformin intestinal accumulation was measured at various time points. Portal bioavailability with or without inhibitor was determined by quantifying metformin portal and systemic AUCs.

Results: Mouse intestine showed high expression of mOct1 and mMate1, ~100-fold lower mOct3 and mPmat, and poor expression of mOct2. As high as ~25% of orally-administered metformin accumulated within enterocytes. Desipramine (2.7mg/kg), a pan transporter inhibitor identified from in vitro studies, reduced metformin intestinal accumulation and systemic AUC0-4hrs by ~35% (at 45 and 60min) and 75% ($p<0.01$), respectively. Effect of desipramine on metformin intestinal absorption will be deconvoluted from these and portal sampling data.

Conclusion: Metformin intestinal accumulation suggests transporter-mediated apical uptake and inefficient basolateral egress. Reduction of metformin intestinal accumulation and systemic AUC by desipramine demonstrates that cation-selective transporters are involved, and that intestinal accumulation contributes to increased absorption. These and previous data showing low basolateral and high apical efflux rates in metformin-preloaded mouse intestinal tissue strongly support the novel absorption mechanism of metformin.

P-TH-33

PRODRUGS OF DTPA AS ORALLY BIOAVAILABLE RADIONUCLIDE DECORPORATION AGENTS

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Objective: The increasing threats of nuclear terrorism have made the development of medical countermeasures a priority for international security. Injectable formulations of diethylenetriaminepentaacetic acid (DTPA) have been approved by the FDA, however an oral formulation is more amenable in a situation involving mass casualties. Therefore, the objective of our research program is to develop and characterize DTPA esters and assess their oral bioavailability and radionuclide decorporation efficacy.

Methods: The penta- and di-ethyl ester prodrugs (C2E5 and C2E2, respectively) were synthesized and their solubility, lipophilicity and stability determined. Single dose pharmacokinetics and relative oral bioavailability of C2E5 and C2E2 were evaluated in Sprague Dawley rats. Oral dose-range finding toxicology studies were performed to identify no-observed-adverse-effect-levels (NOAEL). Multiple daily doses of C2E5 and C2E2 were evaluated for 7 days to determine efficacy in rats using the wound model (IM contamination with 241Am nitrate).

Results: C2E5 is a viscous liquid whereas C2E2 is a solid. A greater fraction of C2E5 was absorbed orally. Pharmacokinetic analysis following C2E5 administration revealed the presence of tri- and di-ethyl esters as the predominant metabolites. In contrast, unmetabolized C2E2 was the predominant compound identified in the circulation after C2E2 administration along with lower levels of the mono-ester metabolite and DTPA. Toxicology studies identified NOAELs of 114 and 800 mg/kg/day for C2E5 and C2E2, respectively. Tolerance appears to be related to the extent of de-esterification. Total decorporation in rats was found to be 15.3 ± 4.7 %, 21.6 ± 1.3 % and 32.3 ± 3.7 % for control, 100 mg/kg C2E5 and 84 mg/kg C2E2 (molar equivalents), respectively.

Conclusion: Both C2E5 and C2E2 enhance 241Am decorporation. Although C2E5 provides greater efficacy than C2E2, a lower NOAEL and complex metabolism complicate its further development. Due to its larger therapeutic index and ease of formulation, C2E2 has been selected for investigational new drug application-enabling studies.

P-TH-34

ARGININE TAGGING OF POLYMERIC NANOPARTICLES TO ENHANCE CELLULAR UPTAKE?

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Objective: Cell-penetrating peptides (CPPs), such as polyarginine, have been shown to aid bioactive penetration into cells. However, conjugating polyarginine to the bioactive moiety may alter its bioactivity. An alternative strategy is to decorate nanoparticulate carriers containing the bioactive with CPPs. A nano-sized particle with multiple arginine molecules on its surface could further enhance cellular uptake compared to that of utilizing an unmodified nanoparticulate system. The aim of this study was to make and characterize novel poly(ethyl-cyanoacrylate) PECA nanoparticles tagged with polyarginine via histidine anchoring and to determine if this enhanced cellular uptake.

Methods: Nanoparticulate formulations were prepared from a microemulsion template. Polyarginine of different chain lengths attached to histidine (RH, RRH, RRRR-aca-H, RRRRRRRRH) were added to the aqueous phase prior to polymerization. Resultant nanoparticles were analyzed for covalent association between the arginine peptide and the wall of PECA nanoparticles using MALDI-TOF. Nanoparticle formulations that were covalently R-tagged, were selected for further size and zeta potential characterization via photon correlation spectroscopy (PCS). Uptake of R-tagged nanoparticles loaded with FITC-dextran into Caco-2 cells was quantified using flow cytometry.

Results: R-tagging with RH and RRH resulted in covalent binding with the PECA nanoparticle wall, shifting the zeta potential from -14mV to -3 mV and +18 mV, respectively. The size of nanoparticles was approximately 200 nm for both R-tagged nanoparticles and did not differ from unmodified nanoparticles. Unmodified nanoparticles and R-tagged nanoparticles showed 16% and 18% cellular uptake, respectively, compared with 2.5% uptake of unencapsulated FITC-dextran.

Conclusion: R-tagging can be achieved with PECA nanoparticles in a single step polymerization via histidine anchoring. R-tagged nanoparticles did not show significant improvement in cellular uptake compared to that of unmodified nanoparticles. The surface density of arginine on the nanoparticles may be critical for cellular uptake. The next step is to investigate the relationship between arginine loading of nanoparticles and cellular uptake.

P-TH-35

COMBINATION OF SULPHUR COMPOUNDS FROM CENTRAL ASIAN ALLIUM SPECIES – NEW APPROACH TO OBTAIN HIGHER ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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Objective: *Allium* species contain high amounts of cysteine derivatives (up to 5% of dry weight).The secondary metabolites of these compounds which are mainly formed by the enzyme alliinase show several pharmaceutical effects. Very interesting are the highly antibacterial and antifungal effects of these compounds.

Methods: With the analytical methods HPLC, HPLC MS/MS, IR and NMR, and the derivatisation with o-phthaldialdehyde and 2-methyl-propanethiole, some interesting compounds were identified and quantified (triple determination) in *Allium* samples. New secondary metabolites were identified with MS/MS, IR and NMR after extraction of homogenized *Allium* extracts with ethyl acetate.

Results: The cysteine sulphoxides marasmin and homoisoalliin were detected in *Allium* species of Central Asia for the first time. Marasmin was found in a maximum concentration of 1.08 % ± 0.03 % (related to fresh weight) in samples of *Alliumsuworowii*, while homoisoalliin was found in *Alliumtripedale* samples with a maximum amount of 0.087% ± 0.002%. New unique secondary metabolites of homoisoalliin like the di-(1-S-sulfoxymethyl-butyl)-disulfide were found in *Allium tripedale*. A high amount of the rare cysteine-pyridine N-oxide was detected in *Alliumaltissimum* samples (0.609% ± 0.005%). Until now, no species or subgenus was found where all cysteine derivatives were detected in high concentrations.

Conclusion: The alliinase is a very stable enzyme with a high acticity, while the secondary metabolites are very unstable. Therefore it makes more sense to combine the prodrugs (cysteine derivatives) with the enzyme in a gastro resistant formulation than to apply the secondary metabolites. A lyophilised mixture of extracts from several Allium species could be incorporated in a capsule; or a formulation based on the pure compounds and isolated alliinase can be used. The extract formulation would add other pharmaceutical relevant compounds like flavonoids and saponines to the drug. The pure compounds formulation is more reproducible, due to synthetically produced cysteine derivatives and pure isolated alliinase. The production of these capsules is very simple and cheap. They might contribute in the pharmaceutical treatment for example in Central Asia where Allium species have already been in use in folk medicine for several hundred years.

P-TH-36

IMPACT OF CHITOSAN-PLGA AND FURTHER CATIONIC POLYMER NANOPARTICLES ON CELL GROWTH

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Objective: Previous studies showed that Chitosan coated PLGA nanoparticles (Cs-PLGA NPs) without any active ingredient interfered with tumorigenicity but interestingly enough did not stop proliferation in vitro. Therefore, this study aims to investigate the key parameter for the loss of tumorigenicity.

Methods: Cs-PLGA NPs were generated using the emulsion-diffusion-evaporation method. A cationic starch derivative was used in the same NP generation method replacing chitosan as coating material. As non-carbohydrate polymer we chose Eudragit E100, a dimethylaminoethyl methacrylate copolymer. The Eudragit solubility in organic solvent required the development of an ultrasound based NP generation method. The NP characterization was done by dynamic light scattering (DLS) and scanning electron microscopy (SEM). In addition cytotoxicity tests (LDH and MTT) were used to compare the viability of NCI-H460 cells after incubation with the different NPs (Cs-PLGA E100-PLGA, Starch-PLGA). Beyond the level of acute toxicity, the aim is to look for changes in gene expression, which could have impact in the long-term without being visible in the short term experiment.

Results: Produced Cs-PLGA NPs had a mean size of ~150 nm and a zeta potential of ~+30 mV. A ratio of 1:6.6 Eudragit E100:PLGA and a ultrasound impulse of ~20W was found to be the optimum condition to produce E100-PLGA NPs similar to Cs-PLGA NPs. For the starch-PLGA NP a degree of substitution of 42,2% was necessary to achieve a zeta potential near +28mV. SEM confirmed the particle sizes measured by DLS as well as comparable morphology. Cytotoxicity tests are still in progress but points towards E100-PLGA as most cytotoxic NP.

Conclusion: A small test set of NPs was successfully generated, which is comparable in size and zeta potential to Cs-PLGA NPs but made from diverse cationic polymers. These NPs should allow the evaluation of the impact of cationic NPs on cells and tissues.

P-TH-37

SUN PROTECTION FACTOR AND CUTANEOUS PERMEATION OF HYDROETHANOLIC EXTRACT OF ILEX PARAGUARIENSIS

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Objective: *Ilex paraguariensis* (IP), known yerba mate, is used in Latin America as aqueous infusion. Among its biological properties is the antioxidant activity, suggesting a role in prevention/treatment of oxidative stress associated diseases, such as photoaging. Recently we described that chlorogenic acid (CA) (IP extract major component) possesses also remarkable *in vitro* sun protection factor (*iv*SPF) (Rivelli *et al.*, 2010). Due to its hydrophilic characteristic, CA hardly permeates the skin what hinders access of this compound to deeper skin layers. Formulations containing plant extracts with both sunscreen and antioxidant activity and acting in the superficial and deep layers of skin are of interest to protect and reverse the deleterious effects of UV radiation.

Methods: IP leaves were percolated with ethanol:water (50%) and then evaporated and freeze-dried. The extract was incorporated (10% final concentration) into three formulations (gel-cream (GC), cream (CR) and cream-nanoemulsion (CN)). These formulations were assayed for *iv*SPF in Vitroskin, PMMA and Transpore tape matrices and for cutaneous permeation. Franz diffusion cell using 500 µm pig ear skin as membrane and ethanol 30% as receptor fluid (32^{o}C) were employed. Concentration of CA and caffeine in stratum corneum (SC), epidermis plus dermis (EP+D) and receptor fluid (RF) were measured.

Results: SPF results showed that the three formulations were equivalent, but these values differ among the matrices (around 10 for Transpore and PMMA and 100 for Vitroskin). CA (CN-2.2µg/cm^2,CR-2.2µg/cm^2,GC-0.8µg/cm^2) and caffeine (CR-1.5µg/cm^2,CN-1.1µg/cm^2,GC-0.6µg/cm^2) accumulated in EP+D and in the SC (CA - CR-5.0µg/cm^2,GC-3.9µg/cm^2,CN-1.2µg/cm^2 and caffeine CR-1.8µg/cm^2,GC-1.6µg/cm^2,CN-0.6µg/cm^2) for the three formulations, but only reached the RF for CN ($flow_{(CA)}$=3.5µg/cm^{2}h|$flow_{(caffeine)}$=2.0µg/cm^{2}h) and CR ($flow_{(CA)}$=1.3µg/cm^{2}h|$flow_{(caffeine)}$=0.4µg/cm^{2}h).

Conclusion: Our results demonstrate that IP extract has considerable *iv*SPF, the differences attributed to the interaction of the formulation with the matrices and viscosity of the sample. Permeation studies showed a penetration profile dependent of formulation polarity, with cream and cream-nanoemulsion showing the best permeation profile.

P-TH-38

EVALUATION OF THE RELATIVE CONTRIBUTION OF INFLUX/EFFLUX TRASPORTERS ON THE INTESTINAL ABSORPTION OF FEXOFENADINE IN RAT

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Objective: The purpose of this study is to clarify the role of organic anion transporting polypeptide (oatp) and P-glycoprotein (P-gp) in the intestinal absorption of fexofenadine in rat.

Methods: Naringin (NAR) (2.9 mg/kg) and zosuquidar (ZSQ) (30 mg/kg) were used as inhibitors of oatp and P-gp, respectively. Absorbability of fexofenadine (FEX) from rat jejunum was evaluated by in situ closed loop method in the absence and presence of inhibitors. Oral bioavailability (BA) and intestinal availability (Fa*Fg) of FEX were determined by in vivo oral and intravenous administration studies using portal vein cannulated rat. Dose of FEX was 5 mg/kg and both inhibitors were orally administered at 40 min prior to FEX administration. The concentration of FEX in all samples was quantified with LC/MS/MS.

Results: In the in situ loop study, absorbed fraction of FEX during 30 min was about 16 % in the absence of inhibitors. Addition of NAR did not affect the absorption of FEX significantly. In the in vivo study, no significant differences in Fa*Fg were observed between control (FEX only) and oatp-inhibited (FEX+NAR) groups, corresponding well with the result of loop study. In contrast, in the P-gp inhibited (FEX+ZSQ) group, Fa*Fg increased more than 6-fold. The oral BA of FEX in human was reported as about 30 %, whereas rat showed only 1.5 % of BA in this study. Since Fa*Fg of FEX was calculated as about 14% in rat, these results suggest the high first-pass uptake of FEX in rat liver, probably by oatp.

Conclusion: This study indicated that, in rat, the contribution of oatp to the intestinal absorption of FEX was relatively small and the low oral BA of FEX could be attributed to the potent efflux transport mediated by P-gp and the high hepatic uptake by oatp.

P-TH-39

A PHARMACOKINETIC STUDY OF 5-FLUOROURACIL LOADED INTO A NOVEL STERICALLY STABILIZED LIPOSOMAL FORMULATION USING MICRODIALYSIS

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Objective: Liposomes are recognized as effective carriers for anticancer drugs as they can prolong plasma retention and increase the tumour distribution of encapsulated drugs. Sustained release of 5-fluorouracil (5-FU) has been reported when incorporated into liposomes as a complex with selected metals. This study investigated the impact of such liposomes on the site-specific delivery of 5-FU into tumour tissue relative to healthy tissue using microdialysis sampling technology.

Methods: Three groups of tumour-bearing Sprague Dawley rats were administered single intravenous doses of (1) a solution of 5-FU (FUConv) or (2) 5-FU-loaded neutral PEG-liposomes (FULip) or (3) 5-FU-copper-polyethylenimine-loaded neutral PEG-liposomes (FU-Complex-Lip). Microdialysis probes were implanted into tumour and subcutaneous healthy tissues of anaesthetized rats to obtain continuous concentration-time profiles of 5-FU. Blood samples (for plasma) were collected at selected times and the concentrations of 5-FU measured by HPLC.

Results: FU-Complex-Lip achieved a 10-fold higher plasma AUC (2163 ± 624 µg*min/mL) than FUConv (210 ± 62 µg*min/mL) ($p<0.0001$) and 4-fold more than FULip (546 ± 114 µg*min/mL) ($p<0.001$). 5-FU levels in tumour were almost 2-times higher following injection of FU-Complex-Lip (237 ± 88 µg*min/mL) than following injection of FUConv (121 ± 44 µg*min/mL) ($p<0.05$). The distribution of 5-FU to healthy tissues was slightly decreased following injection of FU-Complex-Lip (100 ± 33 µg*min/mL) compared with FUConv (137 ± 43 µg*min/mL) ($p>0.1$).

Conclusion: FU-Complex-Lip resulted in superior pharmocokinetics over FUConv. FU-Complex-Lip tends to distribute more into tumour tissue compared with FUConv and FULip thus is promising for the further efficacy and toxicity studies.

P-TH-40

SCAVENGING LIPOSOMES: A NOVEL MODALITY FOR BIODETOXIFICATION

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Objective: Poisoning and drug intoxication can result from causes such as accidental overexposure to noxious substances, therapeutic misadventure, illicit drug overdose, or suicide attempt. In particular, calcium channel blockers (CCBs) toxicities lead to serious mortality due to a dual effect on cardiac and vascular tissues. Conventional supportive measures mainly focus on gastrointestinal decontamination and are ineffective in accelerating blood clearance. To date, no breakthrough has been made in the development of antidote; the only recent innovation in this field is the off-label use of parenteral lipid emulsions. These emulsions can scavenge the drug in situ, restricting its distribution to tissues and decreasing its pharmacological effect. Alas, they are strictly limited to lipophilic compounds, and exhibit low capture capacity. To address these issues, we propose a novel and universal modality for drug detoxification based on liposomes bearing a transmembrane pH gradient.

Methods: Scavenging liposomes were optimized in vitro for their stability and ability to sequester CCBs in serum. Subsequently, the efficacy of the best formulation in reversing the cardiovascular effects of a model CCB (verapamil, 50 mg/kg, per os), was assessed in vivo in Sprague-Dawley rats implanted with telemetric pressure/biopotential transmitters.

Results: In animals orally intoxicated with verapamil, an intravenous injection of the liposomal antidote attenuated the reduction of blood pressure more promptly and intensively than the commercially available lipid emulsions. Areas under diastolic, systolic, and mean pressures curves were significantly reduced by up to 60% and the time to achieve hemodynamic recovery was shortened from 19 h to almost half.

Conclusion: This work confirmed the protective effect of pH-gradient liposomes against cardiovascular failure after CBB intoxication. The proposed detoxifiers greatly outperformed the lipid emulsions and stand as a promising answer to the quest for universal antidote.

P-TH-41

ABSORPTION PROFILING OF INHALED LIPOSOMAL CIPROFLOXACIN NANOPARTICLES USING ISOLATED PERFUSED LUNG

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Objective: Respiratory tract infections together with chronic management of disease for patients with obstructive pulmonary diseases such as cystic fibrosis (CF) require efficacious and safe treatment strategies. Liposomal ciprofloxacin nanoparticles were developed with a view to reduce the burden of current treatment, which involves lengthy treatment times and multiple dosing of concentrated antibiotics due to rapid clearance of the antibiotics from the lungs. In vitro experiments using the air interface Calu-3 model have previously demonstrated the sustained release behavior of the liposomal ciprofloxacin formulations compared to unencapsulated ciprofloxacin, whereas in this study the isolated lung perfusion model was used to measure absorption rates from the lungs.

Methods: Three formulations, including liposomal ciprofloxacin, free ciprofloxacin and a 1:1 mixture of these formulations, were instilled into the isolated perfused Wistar rat lungs. For each formulation, a dose of 500 µg ciprofloxacin in 100 µL was administered via the trachea. Samples of the vascular perfusion buffer were collected at pre-determined time points over a 30 minute interval. Quantification of ciprofloxacin in all samples was performed using high-performance liquid chromatography.

Results: The liposomal ciprofloxacin formulation showed retention of antibiotic in the lungs with less than 15% of the administered dose being transferred to the perfusate in 30 min in comparison to the rapid clearance of free ciprofloxacin which demonstrated ~90% of the antibiotic being cleared from the lungs. Additionally, the mixture formulation showed that about 50% of the drug was transported across the lungs, primarily due to the free ciprofloxacin component and comparable to the mean value for the free and liposomal formulations.

Conclusion: Sustained release from the liposomal nanoparticle formulation compared to free ciprofloxacin was demonstrated clearly using an isolated perfused lung model that provided a reproducible, scalable measure of drug retention that was predictive of effects observed in vivo.

P-TH-42

IMPROVED PHARMACOKINETIC UNDERSTANDING OF TWO FORMULATIONS FOR INTRA-ARTERIAL INJECTION

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Objective: Two different doxorubicin (DOX) formulations injected locally into the hepatic artery are in clinical use as a palliative treatment for primary liver cancer. DOX/lipiodol emulsion (LIP) and the novel DOX compatible Beads (DCB) both reduce the systemic adverse effects of DOX. However, it is unclear which of these formulations achieve the best anticancer effect. In this study, the disposition and metabolism of DOX were investigated locally and systemically with plasma and bile samples from multiple sites, to evaluate the best mechanistic approach to increase the local drug dose.

Methods: The advanced multisampling pig model was used to collect bile, urine and to sample blood from three sites during six hours. DOX was administered intravenously (IV, n=4) or as intra-arterial injection with LIP (n=4) or DCB (n=4). All samples were analyzed for concentrations of DOX and its primary metabolite doxorubicinol (DOXol) with UPLC-MS/MS.

Results: The IV data showed a median terminal half-life of 5.4 h and a liver extraction of 41 % for DOX. 24 % of the dose was eliminated through the bile, whereof 82 % unchanged and 18 % as DOXol. The systemic peak concentration (Cmax) and AUC was significantly lower following DCB. The AUC ratio (bile/plasma) for DOX was 350 (290-400), while it was 2900 (2200-3600) for DOXol. The fraction of DOXol excreted into bile was significantly higher for LIP compared to DCB. As DOX has to enter the hepatocyte to get metabolized, this suggests a higher cellular concentration of DOX was achieved after dosing with LIP.

Conclusion: In conclusion, DOX has an intermediate liver extraction and rapid disposition kinetics following IV administration. The two formulations significantly affected the pharmacokinetics in all compartments and DCB has a slower in vivo release than LIP. It appears that LIP might result in higher DOX concentrations within the liver tissue.

P-TH-43

WHAT YOU SHOULD KNOW ABOUT THE FUTURE OF NONHUMAN PRIMATES IN DRUG DEVELOPMENT

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Objective: Non-human primates (NHP) are often assumed to be the only relevant species to evaluate safety and efficacy of monoclonal antibodies (MAbs). However the scientific value of NHP in their development has never been established.

Methods: We performed a retrospective review to study the value of using NHP to evaluate the safety and efficacy of these products. Uniquely, non-public drug registration files of all MAbs marketed in the European Union were used in this retrospective study.

Results: 33 MAbs have been registered up to 2010, of which 26 used NHP. In only one case, NHP was the only species used. In total, 6045 NHP have been used for non-clinical assessment and cynomolgus is the predominant species for testing (86%). The use of NHP increased as the MAb became more human (ANOVA, $p<0.05$). Aside from diagnostics, there was no difference in the number of NHP used per indication (antineoplastic, immunosuppressive or other, ANOVA, $p<0.05$). Inadequate justification of NHP use and ineffective study design led to a needless increase of NHP use. In particular, the use of chimpanzee was highly uninformative due to poor study design and rationale, technical difficulties and handling. Immunogenicity occurred in 98% of the development programs and was severe in 7 cases (33%), limiting the value of NHP. As a predictive model NHP did not stand out because MAbs only exerted their expected pharmacological action.

Conclusion: Therefore, a reevaluation of the need for routine studies with NHP to develop MAbs is urgently needed.

P-S-01

USE OF BETAINE HCL TO IMPROVE DASATINIB ABSORPTION IN HEALTHY VOLUNTEERS WITH PHARMACOLOGICALLY-INDUCED HYPOCHLORHYDRIA

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Objective: Dasatinib, a targeted small molecule tyrosine kinase inhibitor, has pH-dependent solubility and shows reduced absorption when co-administered with acid-reducing agents. The objective of this clinical study was to investigate whether the coadministration of dasatinib with the re-acidifying agent betaine HCl can mitigate the reduced dasatinib absorption seen in the presence of pharmacologically-induced hypochlorhydria in healthy volunteers.

Methods: For this three-period, crossover pharmacokinetic clinical study, healthy volunteers received each of the following treatments separated by a one week wash-out: (A) 100 mg dasatinib; (B) 100 mg dasatinib after pre-treatment with 20 mg rabeprazole twice daily for three days; (C) 100 mg dasatinib AND 1500 mg betaine HCl after pre-treatment with 20 mg rabeprazole twice daily for three days. For treatments B + C, rabeprazole was also given on the morning of each dasatinib study day. During all treatments, gastric pH was monitored using the Heidelberg pH capsule to confirm gastric pH conditions. Dasatinib plasma concentrations were measured over 22 hours post dasatinib administration.

Results: Rabeprazole significantly lowered dasatinib AUC0-INF and Cmax by 5- and 18-fold, respectively (Relative F = 18±6%). Compared with rabeprazole study days, the co-administration of betaine HCl increased dasatinib AUC0-INF and Cmax by 4- and 9-fold, respectively. Betaine HCl was thus able to restore dasatinib AUC0-INF and Cmax to 80% and 52% of the control (i.e., dasatinib alone study days), respectively. Across all treatment periods, no changes in terminal elimination half-life or time to maximum plasma concentration (Tmax) were observed.

Conclusion: The co-administration of betaine HCl significantly reversed the effects of hypochlorhydria on dasatinib drug exposure. As such, this may be an effective strategy to mitigate potential drug-drug interactions for drugs, such as molecular targeted agents, that exhibit a pH-dependent solubility and are administered orally under hypochlorhydric conditions.

P-S-02

PREPARATION AND CHARACTERIZATION OF BSA-LOADED PLGA NANOPARTICLES FOR PREFORMULATION OF EXENATIDE-LOADED PLGA NANOPARTICLE

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Objective: Poly(D,L-lactide-co-glycolide) nanoparticles (PLGA-NP) have been broadly used as a carrier for drug delivery system of proteins and peptides. However, negative surface charge of PLGA-NP decreases bioavailability under oral administration. Recently, cationically modified PLGA-NP has been introduced as novel carriers for oral delivery sysetem. The characteristics of the nanoparticles, such as surface charge, particle size, and bioadhesion are considered the most important determinants of the effect of these nanoparticles. Our purpose was to introduce and evaluate the physiochemical characteristics and bioadhesion of positively charged PLGA-NP, using bovine serum albumin (BSA) as a model drug, eventually, exenatide.

Methods: First of all, the preliminary study of exenatide was conducted prior to preparation of PLGA-NP. Exenatide was evaluated the stability in the various conditions. While as the nanoparticles using BSA were prepared by a double-emulsion solvent evaporation method. BSA was dissolved 0.03% citric acid solution. PLGA or PLGA/Eudragit RL 100 or RS 100 was dissolved dichloromethane. Above solutions was mixed using ultrasonication then W/O emulsion was obtained. W/O emulsion was added to 1% w/v PVA solution and ultrasonication was conducted. Then organic phase was evaporated and centrifugation was conducted. Supernatant was removed. Freeze-drying was conducted.

Results: Exenatide was unstable in base and long ultrasonication in over 2 min. It was stable in acid and organic solvent such as dichloromethane. Exenatide was analyzed by the HPLC condition of C18 reversed phase analytical column(Phenomenex, 4.6mm x 250mm) with distilled water containing 0.01% Trifluoroaceticacid(TFA) and acetonitrile containing 0.01% TFA of mobile phase at 210nm. The flow rate was maintained at 1ml/min and the column temperature was 55℃. On the other hand, the size, zeta-potential and encapsulation efficiency of negatively charged PLGA-NP using BSA was about 400nm, -4.54mV and 67%, respectively. Positively charged PLGA-NP using PLGA/Eudragit RL 100 or RS 100 was prepared and being evaluated for comparison to those of common negatively charged PLGA-NP.

Conclusion: It was suggested the exenatide loaded PLGA-NP was prepared based on the preparation method PLGA-NP using BSA and could be developed for a sustained-release formulation.

P-S-03

OPTIMIZATION OF A RAMAN MICROSCOPIC TECHNIQUE TO EFFICIENTLY DETECT AMORPHOUS-AMORPHOUS PHASE SEPARATION IN FREEZE-DRIED PROTEIN FORMULATIONS

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Objective: A Raman microscopic technique was optimized to more efficiently detect amorphous-amorphous phase separation in freeze-dried protein formulations by evaluating various instrument settings (focal mode, scan time, line map length, step size, slide type). To determine the practicality of the optimized method, several protein formulations were evaluated for amorphous-amorphous phase separation.

Methods: Formulations containing an excipient (dextran 40K, sucrose, trehalose, isomaltose) and/or a protein (lysozyme, β-lactoglobulin) were freeze-dried at several ratios. Raman spectra were collected along 100-400 µm lines of each sample with a Renishaw Raman inVia confocal microscope using excitation by a 785-nm diode laser. Non-overlapping bands representative of each component of interest were identified. At each point across the line map, the composition was evaluated from the intensity of the non-overlapping band of interest.

Results: Phase separation was successfully detected in standard confocal mode with a scan time of 5 seconds over a 200 µm line map, 2 µm step size and using a gold plated microscope slide. The total experimental time for one experiment was approximately 30 minutes, which was 5 times faster than the originally developed method. The optimal method detected phase separation in the following formulations: Lysozyme-trehalose (1:1), lysozyme-isomaltose (1:1), β-lactoglobulin-dextran (1:1), β-lactoglobulin-dextran (1:3) and β-lactoglobulin-trehalose (1:1). Phase separation was not detected in Lysozyme-sucrose (1:1) and β-lactoglobulin-sucrose (1:1) formulations.

Conclusion: A Raman microscopic technique was optimized to more efficiently evaluate freeze-dried protein formulations for amorphous-amorphous phase separation. The new method successfully detected phase separation in several protein formulations, where phase separation was previously suspected.

P-S-04

PREPARATION AND CHARACTERIZATION OF SOLID LIPID MICRO-PARTICLES FOR ORAL DELIVERY OF PROTEINS

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Objective: Solid lipid micro-particles (SLM) may have an advantage for oral delivery of proteins by upholding a protective barrier against proteases in the gastrointestinal tract. The aim of the present study is to prepare and characterize peptide-loaded SLM and investigate the protein release profile during *in vitro* lipolysis.

Methods: Lysozyme was encapsulated in SLM using a melt dispersion technique. 300mg of lipid was melted at 70°C and 100µL of a lysozyme solution (200mg/mL) was added. The mixture was vortex-mixed before and after addition of 3mL of polyvinyl alcohol (PVA) solution (1%, 70°C). This pre-emulsion was mixed with 150mL of PVA solution (0.1%, 4°C) for 5min. The SLM were filtrated and washed with Milli-Q water and dried under vacuum. Three kinds of lipid excipients were selected, Dynasan 114 (C-14 triglycerides), cetylpalmitate, and Precirol ATO 5 (C-18 blend of mono-, di- and triglycerides, the major component is diglyceride). The size, morphology, encapsulation efficiency (EE) and drug loading (DL) of SLM were investigated.

Results: Cetylpalmitate particles had 90% of the volume distribution ($D_{0.9}$) below 80.9±9.0µm which was in the same range as Dynasan 114 particles ($D_{0.9}$=92.1±8.0µm). Precirol ATO 5 particles were slightly smaller ($D_{0.9}$=60.6±9.6µm). The particles had SPAN values of 1.55±0.03 (Dynasan 114), 2.06±0.29 (Precirol ATO 5) and 1.61±0.16 (cetylpalmitate). Dynasan 114 and Precirol ATO 5 particles were able to incorporate more lysozyme (EE=46.2±3.0% and DL=3.2±0.2% for Dynasan 114 and 35.8±6.2% and 3.2±0.2% for Precirol ATO 5) than cetylpalmitate particles (EE=7.2±0.7% and DL=0.48±0.04%). The particles were all determined to be spherical by light microscopy. Experiments investigating lysozyme release during *in vitro* lipolysis are ongoing.

Conclusion: Lipid excipients affect the size and drug loading ability of solid lipid micro-particles when a melt dispersion technique is used. Dynasan 114 particles were able to incorporate the largest amount of lysozyme with a drug loading of 3.2±0.2%.

P-S-05

VISUALIZATION OF API PRECIPITATION IN SIMULATED GASTRIC MEDIA BY POLARIZED LIGHT MICROSCOPY

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Objective: For an increasing number of APIs optimization of the solid form is used to increase the solubility and dissolution rate of the API. This can lead to a supersaturation in the stomach and if the supersaturation cannot be maintained the API will precipitate. The purpose of this study is to develop a method for visualizing precipitation of APIs in simulated gastric media (SGM) by use of polarized light microscopy.

Methods: Carbamazepine was dissolved in dimethylacetamide (DMA) and 0.5µL was placed on a microscope slide. 19.5µL SGM (0.1M HCl) was added to the DMA/drug solution. A camera attached to a polarized light microscope was used to capture a video of the API precipitation. The morphology, nucleation and size of crystals were analyzed using Motic Images Plus 2.0 and VirtualDub 1.9.11.0.

Results: Using a 5x objective it was possible to film an area of approximately 1875µm x1405µm (n=4). A typical needle growth of the hydrate phase was observed for all precipitated carbamazepine crystals. More than one hundred crystals were observed within the captured area (111±48) and no new crystals could be identified after 6.1±0.8 seconds. The average length of the precipitated crystals was 289±123µm, with 75% being shorter than 350µm, and crystal growth stopped after 28.5±9.6 seconds. The developed microscopy based method makes it possible to analyze nucleation and crystal growth over time. The variation observed in the data may be due to the fact that only a small sample area was filmed by the camera and that nucleation and crystal growth are influenced by how well the DMA/drug solution is mixed with the SGM.

Conclusion: The developed microscopy based method is suitable for visualization of API precipitation in simulated gastric media, and provides a fast method for evaluating API precipitation, using only very small amounts of API and media.

P-S-06

PROTEIN ADSORPTION TO HYDROPHOBIC NANOSPHERES

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Objective: To study the adsorption mechanism of two natively unfolded proteins (beta-casein, alpha-synuclein) onto hydrophobic nanospheres.

Methods: The energetics of the adsorption process was determined by Isothermal Titration Calorimetry in a temperature range of 25 to 47°C. For this purpose, protein was titrated into a suspension of hydrophobic nanospheres. Enthalpy of adsorption, Kd and Gibbs free energy were determined using a custom made software. Structural changes upon adsorption were characterized by circular dichroism. To reduce scattering from the nanospheres, the refractive index was adjusted with glycerol. Hence, each protein was analyzed in 10mM phosphate buffer, and in phosphate buffer adjusted with glycerol with and without nanospheres for appropriate evaluation.

Results: The interaction between protein and the nanospheres is exothermic and occurs in spite of the fact that at pH 7.4 both are negatively charged. The enthalpy of adsorption becomes more favorable with increasing temperature, indicating a negative heat capacity. For beta-casein the Enthalpy of adsorption is determined to -67.3 ± 7.5 kcal/mol on 25°C, while it increases to 89.7 ± 4.0 kcal/mol on 37°C (n=3). The dissociation constant, Kd, is in the µM range, indicating strong binding. The Gibbs free energy and the adsorbed amount of beta-casein (9.0 ± 0.1 kcal/mol and -9.4kcal/mol on 25°C and 37°C, respectively (n=3)) seem to be independent of temperature, while for alpha-synuclein both parameters are by preliminary data suggested to increase in absolute value with increasing temperature.Both proteins seem to become more ordered/structured upon adsorption.

Conclusion: The adsorption process may involve electrostatic forces. The apparent change in structure could be due to a recurrent interaction pattern of charged groups in the protein and the surface. Alternatively, it can stem from the increased hydrogen bonding within the backbone.

P-S-07

RHEOLOGICAL CHARACTERIZATION OF FASTED HUMAN GASTRIC FLUID

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Objective: From Noyes Whitney's equation it is known that an increased viscosity of a medium decreases the dissolution rate of a drug substance. In earlier developed gastric simulated media (FaSSGF) the viscosity of the human gastric fluid has not been taken into considerations. The purpose of this study was to make an overall evaluation of human gastric aspirates (HGA) but focusing on the rheological characteristics to enable eventual optimization of the used FaSSGF.

Methods: Fasted HGA were collected from 20 healthy volunteers during a gastroscopic examination. pH, osmolality, buffer capacity and surface tension were measured. The total protein content and bile salt level were measured using BCA, Protein Assay Kit and a Total Bile Acid kit. Lipase activity was measured by continuous titration of released free fatty acid from tributyrate. Rheological characterization of the aspirates was conducted on a TA AR-G2 rheometer, using cone and plate geometry.

Results: The measured characteristics of the HGA showed that high inter- and intra-variability exist in the HGA. The obtained pH, surface tension, buffer capacity, bile salt concentration, and osmolality are similar to the values found in the literature. The activity of the gastric lipase was 7.4 ± 4.0U/mL (N=6, n=3) and 99.0 ± 45.3U/mL (N=20, n=3), respectively at pH 2.8 and 5.4. Rheological examination of HGA showed non-Newtonian shear-thinning behaviour with predominant elastic behaviour in the linear range. The elastic modulus, G' was 0.008-0.439Pa at an oscillation torque of 0.01mN/m indicating different amounts of elastic components in HGA. At high shear stresses a plateau was observed and the apparent viscosity was measured to be in the range of 1.6 – 6.4mPa·s at a shear rate of 100s-1. The FaSSGF and HCl pH 1.2 have no shear thinning properties and showed lower viscosity (1.1mPa·s). The observed viscosity of the HGA will decrease the intrinsic dissolution rate of drugs.

Conclusion: The apparent viscosity of HGAs is higher than that of HCl and FaSSGF and a predominant elastic behavior was observed, potentially enabling further optimization of the current FaSSGF.

P-S-08

A CLINICAL TRIAL SIMULATION TOOL FOR THE SUPPRESSION OF THE ENDOGENOUS CORTISOL RELEASE AFTER ADMINISTRATION OF INHALED CORTICOSTEROIDS

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Objective: The establishment of bioequivalence of inhaled corticosteroids (ICS) remains challenging. For the pharmacodynamic (PD) assessment of systemic effects of ICS, the European Medicines Agency (EMA) recommends under certain circumstances the evaluation of the effect of ICS on the hypothalamic pituitary adrenocortical (HPA) axis. The preferred PD method of assessing the HPA axis is the assessment of the change from baseline in 24-hour plasma cortisol as measured by AUC and Cmax. The objective of this project is to provide a freely available software tool for clinical trial simulation of the suppression of the endogenous cortisol release after administration of test and reference ICS. This simulation tool will be useful for e.g. evaluating the suitability of the PD approach for comparing systemic safety of ICS and calculating the number of subjects needed to achieve a certain statistical power.

Methods: A previously established compartment model that describes the fate of an ICS while incorporating physiological aspects and variability between and within subjects was used for modeling the pharmacokinetics (PKs). A previously established deterministic model for the suppression of the endogenous cortisol release was applied to link the PKs with the PD response and extended to allow for variability between and within patients. A closed-form expression for the PKPD model was derived to ensure an efficient simulation of the clinical trials.

Results: The clinical trial simulation tool was successfully developed and is available as an extension package to the freely available statistical software R (http://www.r-project.org).

Conclusion: A freely available tool for clinical trial simulation of the suppression of the release of the endogenous cortisol after ICS administration was successfully developed. This tool could be a valuable resource for evaluating the current PD approach (i.e. assessment of the effect of ICS on the HPA axis) for establishment of systemic safety between a test and reference ICS.

P-S-09

DEVELOPMENT OF POSITIVELY CHARGED POLY(LACTIC ACID) AND POLY(LACTIC CO GLYCOLIC ACID) NANOPARTICLES BY ADDITION OF STEARYLAMINE

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Objective: The objective of the present study was to generate positively charged poly(D,L-lactide) and poly(lactide-co-glycolide) nanoparticles (Np). Np can function as carriers for a large number of drugs. In previous investigations, it was shown that binding of the negatively charged molecules such as arylsulfatase A (ASA) to the negatively charged PLA, PLGA, and HSA Np surfaces is rather low which may be due to the electrostatic repulsion. The aim of the present study was to investigate the feasibility of the ASA adsorption enhancement using a positively charged nanoparticle system.

Methods: The nanoparticles were prepared by a high pressure homogenization - solvent evaporation technique. PLA, Resomer® S or H (250 mg) as core polymer and stearylamine in a polymer/stearylamine ratio 20:1, 10:1, and 5:1 (w/w) were dissolved in dichloromethane. To this solution an aqueous solution of human serum albumin was added and the solution was homogenized firstly by Ultra-Turrax and then by a high pressure homogenizer (Avestin, Canada). The resulting nanoparticles were characterised regarding mean particle diameter, polydispersity, and particle surface charge using photon correlation spectroscopy (PCS). The particle morphology was analysed by scanning electron microscopy (SEM Hitachi S-4500).

Results: Addition of stearylamine enabled the preparation of the NP displaying the positive surface charge. Increasing amounts of stearylamine led to the charge increase up to +30 mV; the particle sizes and polydispersity were increasing likewise. Typical micrographs (SEM) of the resulting Np supported the PCS data. The nanoparticles had generally a spherical shape.

Conclusion: The objective of the present study was to investigate the influence of different polymer/stearylamine ratios on the physicochemical properties of the PLA/ PLGA Np. The nanoparticles displayed the positive surface charges that were increasing in parallel with the stearylamine content. The NP were characterized with small sizes and narrow size distribution.

P-S-10

DEVELOPMENT OF NOVEL NANO DELIVERY SYSTEMS OF HYDROPHILIC BIOMACROMOLECULES FOR IMPROVED THERAPY

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Objective: Hydrophilic biomacromolecules (proteins, peptides or siRNA), usually exhibit poor membrane permeability and high sensitivity to environmental conditions (heat, pH, enzymatic degradation). They typically require the use of nanocarriers for efficient intracellular delivery. However, the number of clinically relevant nanocarriers used for such a purpose are scarce. In the present study, double nanoencapsulation was used to protect and release efficiently siRNA.

Methods: The first line of protection was achieved by loading the siRNA into primary nanoparticles (NPs ~100 nm) with the aid of the cationic lipid -1,2-dioleoyl-3-trimethylammonium-propane (DOTAP). The second line of stability was obtained by encapsulating the primary nanoparticles into nanocapsules (NCs) smaller than 1 um, with or without a polyethylene glycol (PEG) moiety anchored to their surface. This new vehicle consists of the biodegradable polymers PLGA (Poly D,L-lactic-co-glycolic acid) and HSA (human serum albumin), both compatible for i.v. administration in humans. These nanocarriers were prepared using a novel technique developed in our lab, carried out below 60° C. Quantification and integrity of the loaded siRNA was determined by HPLC and gel electrophoresis. SEM and laser scattering technologies were used for morphology and size determination. Confocal microscopy was used to observe uptake of primary NPs into carcinoma cells.

Results: Successful formation of spherical, rigid and intact sub-micron NCs, loaded with primary spherical NPs was obtained. An adequate loading of siRNA was achieved on the primary NPs (2 to 10 µg/mg) as well as on the final NCs (0.7 to 3.6 µg/mg). The chemical integrity of siRNA (21 bp, Tm above 75° C) was preserved. Uptake of the primary NPs into A-431 human epithelial squamous carcinoma cells, already after 4h was verified using confocal microscopy.

Conclusion: An original technique where nanoparticles have been nanoencapsulated resulting in a biodegradable delivery system, at temperatures compatible with biomacromolecules, has been developed and is actually being optimized.

P-S-11

AN INJECTABLE VEHICLE FOR CHONDROCYTE DELIVERY IN CARTILAGE TISSUE ENGINEERING

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Objective: Cartilage defects are difficult to treat due to the incapacity of the tissue for self-repair. Cell based tissue engineering is a potential approach to regenerate cartilage tissue. We aim to develop a chondrocyte delivery system for tissue engineering. We chose an injectable hydrogel based on materials that closely resemble native cartilage, i.e. collagen II and hyaluronic acid (HA). To form a sufficiently strong and stable construct after injection, the poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4S-PEG) was used to cross-link the collagen II component. Additionally, to promote the chondrocytic cell phenotype, transforming growth factor b1 (TGFb1) was combined into the vehicle.

Methods: Primary chondrocytes expanded for two weeks in monolayer cultures were encapsulated into the collagen II/HA/4S-PEG hydrogel with TGFb1. The chondrocyte/hydrogel constructs were cultured in vitro for 7 days and studied for cell viability and proliferation, morphology, glycosaminoglycan production and gene expression.

Results: The collagen II/HA/4S-PEG formed a strong and stable hydrogel structure, and the chondrocytes remained viable during the encapsulation process and for the 7-day culture period. Additionally, the encapsulated cells showed spherical morphology characteristic for chondrocytic phenotype. The cells were able to produce glycosaminoglycans into their extracellular matrix, and the expression levels of collagen II and aggrecan, genes specific for differentiated chondrocytes, increased over time.

Conclusion: The results indicate that the injecteble hydrogel system is able to maintain viability and phenotypic characteristics of encapsulated chondrocytes. Hence, it is a potential vehicle for cell based engineering of cartilage tissue.

P-S-12

LIPOSOMAL SIRNA DELIVERY TO RETINAL PIGMENT EPITHELIAL CELLS

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Objective: Knocking down gene expression using small interfering RNA (siRNAs) has raised a great interest for ocular therapeutics. However its polyanionic nature and high molecular weight limits its cellular uptake. Effective and safe delivery systems need to be developed. Recent findings indicate that over secretion of IL-6 by the retinal pigment epithelium during inflammatory stimulus may be an underlying factor in the progression of some retinal pathologies. Inhibition of IL-6 secretion seems a promising strategy for the treatment of inflammatory related retinal diseases.The aim of this work was to develop a liposomal siRNA delivery system targeting IL-6 and evaluate its transfection efficiency in dividing and in polarized filter-cultured retinal pigment epithelium (ARPE-19) cells.

Methods: A liposomal delivery system composed of cationic liposomes (DOTAP/DOPE) and protamine sulphate (PS) was investigated for the delivery of siRNA targeting IL-6. DOTAP/DOPE/PS/siRNA complexes were formulated at a 4:1 charge ratio. The gene knockdown ability of the siRNA delivery system was evaluated in dividing and in polarized filter-cultured ARPE-19 cells. Both of them were stimulated with lipopolysaccharides (LPS) to induce the secretion of IL-6. After transfection, IL-6 levels were determined using an IL-6 ELISA kit.

Results: DOTAP/DOPE/PS/siRNA complexes showed a nanoscale size distribution (216 ± 27 nm). Gel retardation assays showed that the siRNA was efficiently associated. Relative IL-6 secretion with DOTAP/DOPE/PS/siRNA targeting IL-6 in LPS stimulated cells was reduced to 33% ± 10 % ($p<0.05$, compared with LPS treated cells) in dividing ARPE-19 cells. Meanwhile, DOTAP/DOPE/PS/siRNA with a non-specific siRNA did not show any knockdown effect. Contrarily, no gene knock-down could be achieved with DOTAP/DOPE/PS/siRNA targeting IL-6 in polarized filter-cultured ARPE-19 cells.

Conclusion: Efficient siRNA delivery in dividing and polarized filter-cultured ARPE-19 cells needs different requirements. Elucidating these factors will help the rational design of siRNA delivery systems that target the retinal pigment epithelium.

P-S-13

PHARMACOKINETIC OCULAR MODELING: PREDICTING DRUG CONCENTRATIONS IN THE VITREOUS AFTER DRUG DELIVERY SYSTEM ADMINISTRATION

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Objective: Our aim was to develop a pharmacokinetic model, which could predict drug concentrations in the vitreous after administration of drug delivery systems (DDS). The model is expected to augment in the design of ocular DDSs.

Methods: Well-stirred one compartment model was used. The building of the model and the simulations were carried out by Stella (ISEE systems 9.0). In the model the first order drug release is affected by drug solubility, dose, the concentration of free drug in vitreous and the dissolution rate constant in sink conditions. The elimination rate is affected by drug concentration in the vitreous and clearance. Simulations were first conducted to probe the influence of different factors on the concentration profiles. Typical vitreal clearance values from the literature were used.

Results: If a protein solution (1000 µg) is injected in the vitreous (t1/2: 115 h) its duration of action is 40 days, when the required minimum concentration is 2 µg/ml. If the release rate is 0.07 /h at sink condition and the solubility is 1000 µg/ml there is no difference in the protein concentration profile compared to the single protein solution injection. If the release rate is kept the same, but the solubility is changed to 10 µg/ml the duration of action is increased to 460 days. A change in the release rate to 0.0007 /h with a solubility of 1000 µg/ml will increase the duration of action to 220 days. This example illustrates the interactions of release rate, solubility and clearance in defining the in vivo release rate and duration of drug action. The parameter related interactions have been simulated and will be presented for small molecules and proteins.

Conclusion: Drug dose, solubility, release rate and clearance affect the drug concentrations in the vitreous after the DDS administration. The model will be a tool in the DDS design.

P-S-14

NOVEL OXIDATIVE SIDE CHAIN CLEAVAGES IN PEPTIDES AND PROTEINS EXPOSED TO LIGHT

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Objective: To investigate the mechanism of oxidative and photochemical damage to Trp- and disulfide-containing peptides and proteins after UV light exposure.

Methods: Two synthetic model peptides, GGCWGL-GGCGGL and (LGGCWGL)2, connected through a disulfide bond, along with N-acetyl-L-tryptophanamide (NATA), a tryptophan derivative (in the presence of oxidized glutathione), were exposed to light for up to 30 minutes. To study a pharmaceutically relevant protein, an antibody, IgG1 was also subjected to light exposure. Following irradiation, the resultant photoproducts were analyzed by mass spectrometry.

Results: Light exposure resulted in the cleavage of the αC-βC bond of the Trp side chain in both of the model peptides studied. The GGCWGL-GGCGGL peptide was converted to GGCG(OOH)GL, a product containing a Gly residue and a hydroperoxy group in place of the Trp residue, in a 20.7% yield. 1.4% of the (LGGCWGL)2 peptide was converted to LGGC(-2)GGL, a product containing a Gly residue in place of the Trp residue, and a thioaldehyde in place of the Cys residue. These modifications suggest a homolytic cleavage of the Cα-Cβ bond, followed by oxidation or hydrogen tranfer possibly from the Cys residue, respectively. To trap the cleaved Trp side chain, NATA and oxidized glutathione were irradiated, and a crosslink between the glutathione and Trp side chain was observed in a 6.6% yield. In addition to these peptide modifications, a trace amount of Trp 191 in IgG1 was modified to Gly-OOH after irradiation, indicating that the pathway of photodegradation observed in the model peptides studied is also relevant to IgG1.

Conclusion: The conversion of Trp to Gly or Gly-OOH demonstrates the ability of light to modify protein therapeutics through the transformation of a large, aromatic amino acid residue into an small, helix-breaking amino acid that could mediate further conformational changes and/or oxidative damage.

P-S-15

NANOCLUSTER BUDESONIDE FORMULATION ENHANCES DRUG DELIVERY IN MECHANICAL VENTILATION

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Objective: Agglomerates of budesonide nanoparticles (also known as 'NanoClusters') were formulated to optimize drug delivery through ventilator circuits. Budesonide NanoCluster formulations (NC-Bud) were compared to micronized budesonide and were studied when applied through an endotracheal tube. A novel device was designed for introducing drugs into the ventilator circuit and compared to an adapted Monodose® inhaler.

Methods: Budesonide suspensions were prepared by a wet milling technique. Particle size and morphology of budesonide NanoClusters were evaluated by scanning electron microscopy (SEM). Inspiration flow rates and other parameters such as inspiration patterns and inspiration volumes were controlled by a ventilator. Formulations were delivered via the Monodose® inhaler or the novel device and then entrained through commercial endotracheal tubes and analyzed by cascade impaction.

Results: Budesonide formulations showed a higher percent emitted fraction (%EF) compared to budesonide as received (30 L/min) ($p<0.05$). The different inspiration patterns did not affect the powder performance of NC-Bud when applied through 5.0-mm endotracheal tube. The aerosolization of NC-Bud did not change with the inspiration volume (1.5 – 2.5 L) nor with the inspiration flow rate (20 – 40 L/min). The %EF of NC-Bud was not significantly different when applied via the novel inhaler or the Monodose® inhaler ($p<0.05$).

Conclusion: NanoCluster budesonide formulations have a higher efficiency of aerosol delivery compared to budesonide as received when applied through endotracheal tubes. The novel device and the Monodose® inhaler showed the same efficiency on drug delivery in mechanical ventilation but the novel device can fit directly to ventilator tubing connections. The new device combined with NanoCluster formulation technology allowed convenient and efficient drug delivery through endotracheal tubes.

P-S-16

HYPERTHERMIA AND ANTI-PECAM-1 SURFACE MODIFICATION INCREASE FE3O4 NANOPARTICLE FLUX ACROSS THE BLOOD BRAIN BARRIER

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Objective: Test the hypothesis that alternating magnetic field (AMF) induced hyperthermia and anti-PECAM-1 surface coating will increase iron oxide nanoparticles (IONs) flux across the blood brain barrier (BBB) in vitro.

Methods: Citrate IONs (CIONs) and cross-linked nanoassemblies loaded with IONs (CNA-IONs) were synthesized using an aqueous co-precipitation method. Cytotoxicity of CIONs and CNA-IONs was tested using a mouse brain endothelial cell (bEnd.3). IONs and CNA-IONs flux assays were performed using MDCKII cells on Transwell® membranes. A human brain endothelial cell (hCMEC/D3) was used for human anti-PECAM-1 modified IONs (anti-PECAM-1-IONs) flux assays.

Results: The CIONs primary size was 10 to 15 nm (TEM) with a zeta potential at pH 7.4 in PBS of -29.5 ± 3.6 mV. Their volume average diameter in water was 80 nm by dynamic light scattering (DLS). They agglomerated to 400 nm in DMEM medium within a few minutes. CNA-IONs were 28 ± 3 nm in water (DLS) and stable (28 nm) in the DMEM medium for 30 h. CIONs showed toxicity to bEnd.3 cells after 3 h at 50 µg/ml. The CNA-IONs did not show toxicity up to 10 mg/ml after 30 h. Recipient chamber lucifer yellow (LY) was significantly increased after incubation at 43 ºC for 4 h followed by 30 h incubation at 37 ºC. After 30 min AMF, LY and CNA-IONs flux across MDCKII cells significantly increased (recipient chamber less than 1% of the donor chamber). The binding affinity of anti-PECAM-1 on hCMEC/D3 was 32 nM and Bmax was 17 x 105 antibodies per cell. Twenty-seven percentages of Anti-PECAM-1-IOs were in the recipient chamber after 4 h.

Conclusion: CNA-IONs showed better stability and less toxicity than CIONs. AMF-induced hyperthermia significantly increased CNA-IONs flux across the MDCKII monolayer, but not sufficiently as nanocarriers. Anti-PECAM-1-IONs significantly and sufficiently increased IONs flux across the hCMEC/D3 monolayer.

P-S-17

EFFECT OF CONVERSION RATE OF ACTIVE DRUG FROM PRODRUG ON THE SUBSEQUENT BIDIRECTIONAL-TRANSPORT IN EPITHELIAL CELL

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Objective: Prodrug approach is useful to improve bioavailability of a therapeutic active drug. Bioconversion of prodrug in intestinal epithelial cells is significantly responsible for its bioavailability due to bidirectional transport of active parent drug. Previously, we have shown that parent drug extensively converted from prodrug in the epithelial cells is transported into intestinal lumen 3-4 times faster than blood vessel, owing to passive diffusion depending on surface area of epithelial cells. In this study, we evaluated the effect of mucosal concentration of parent drug on its bidirectional transport. The several conversion rate of *p*-amino benzoic acid (PABA) as a model parent drug was obtained from butyl *p*-amino benzoate (butyl-PABA), ethyl *p*-amino benzoate (ethyl-PABA), and procaine.

Methods: *In vitro*hydrolysis rate of butyl-PABA, ethyl-PABA, and procaine was determined in 9000g supernatant (S9) of rat jejunum homogenate, and then intrinsic clearance was calculated.*In situ* single-pass perfusion method was performed by simultaneous perfusion of intestinal lumen and blood vessel to determine absorption of PABA and its derivatives in 10cm of jejunum. Concentrations of PABA and its derivatives were determined by HPLC.

Results: Intrinsic hydrolysis clearance was 26.9±1.43, 6.0±2.69, and 1.45±0.32 µL/min/mg S9 protein for butyl-PABA, ethyl-PABA and procaine, respectively. In the *in situ* experiment, the absorption/secretion ratio of PABA was highest in procaine (7.0±2.7) with slowest hydrolysis. This ratio was followed by ethyl-PABA(2.3±0.83) and butyl-PABA(1.1±0.37), indicating that lower mucosal concentration of PABA lead to high transport into blood vessel. The total absorption of prodrug itself and parent drug was successfully increased by slow hydrolysis of prodrug in epithelial cells.

Conclusion: Hydrolysis of prodrug in epithelial cells greatly affects intestinal absorption. Rapid hydrolysis causes substantial efflux of the parent drug to intestinal lumen, even for a parent drug mediated by basal membrane transporter.

P-S-18

CHARACTERIZATION OF CELLULAR UPTAKE AND PERMEABILITY OF AMINOSILANE COATED IRON OXIDE NANOPARTICLES IN A CELL CULTURE MODEL OF THE BLOOD-BRAIN BARRIER

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Objective: Aminosilane coated iron oxide nanoparticles (AmS-IONPs) have been widely used in constructing complex and multifunctional drug delivery systems. However, suitability of AmS-IONPs for brain related drug delivery applications is unknown. The aim of this study is to determine the effect of surface charge and magnetic field on AmS-IONPs uptake and permeability across a cell culture model of the blood-brain barrier (BBB).

Methods: Cellular accumulation and permeability of IONPs were examined using a mouse brain microvessel endothelial cell line (bEnd.3). A modified Ferrozine assay was used to quantitatively determine iron content in samples.

Results: Less than 6% of negatively charged COOH-AmS-IONPs were taken up by bEnd.3 cells whereas up to 60% of the positively charged AmS-IONPs were taken up in absence of magnetic field. Magnetic field increased the efficiency of AmS-IONPs uptake at high concentration (10ug/mL) and COOH-AmS-IONPs uptake at low concentration (2.5ug/mL). COOH-AmS-IONPs showed 16% flux across bEnd.3 monolayers after 24 hrs in the presence of a magnetic field. Disruption of bEnd.3 permeability with 50mM D-glucose resulted in a further enhancement of flux (24%) after 24 hrs in the presence of a magnetic field. Positively charged AmS-IONP showed minimal permeability (<5%) after 24hrs, and was not influenced by the presence of a magnetic field.

Conclusion: Positively charged AmS-IONP show enhanced uptake profile but negligible permeability across confluent bEnd.3 monolayer compared to the negatively charged COOH-AmS-IONP. Given the negligible cellular uptake of COOH-AmS-IONPs, the enhanced permeability is likely via paracellular diffusion routes. Modification of surface charge influences the cellular uptake and permeability profile of IONPs in a cell culture model of the BBB and may be useful for targeting particular routes of passage through the BBB and the delivery of drugs into the brain.

P-S-19

EXPLORING THE ROLE OF TRANSMEMBRANE DOMAIN II ON THE FUNCTION OF THE HUMAN APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER (HASBT)

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Objective: The apical sodium-dependent bile acid transporter (SLC10A2) plays essential roles in the intestinal reabsorption of bile acids and cholesterol homeostasis. Contrary to a recently published crystal structure of a bacterial homologue of ASBT, our lab previously has proposed a 7 transmembrane (TM) domain topology for hASBT. In this study, we utilized a targeted and systemic analysis to explore the role of TM2 and its constituent amino acids on the expression, stability and function of hASBT.

Methods: Residues of TM2 were mutated to cysteine using C270A scaffold, a methanethiosulfonate (MTS)-insensitive mutant. [3H]-taurocholate (TCA) uptake in hASBT wild type and mutant-transfected cells were determined at physiological (137 mM) and equilibrative (12 mM) sodium concentrations. Membrane expression of each mutant was confirmed via tagging with membrane-impermeant sulfo-NH-SS-biotin. Substituted cysteine accessibility mutagenesis (SCAM) method was used to elucidate the role of each residue in substrate translocation.

Results: SCAM study showed none of the 25 mutated residues in TM2 are solvent accessible, indicating that TM2 is not involved in substrate translocation. Even though protein expression at the plasma membrane were comparable to wild type ASBT, mutations Q75C, F76C, M79C, P80C, G83C, P94C and Q96C abolished TCA uptake. Mutants ranging from G83C to D91C showed higher Na+ sensitivity, and I92C showed lower Na+ sensitivity than C270A. When wild type Asp91 was mutated to Glu or Asn, the mutants showed similar hill coefficients as the wild type; however, the Vmax were significantly reduced. D91G and D91A mutants, on the other hand, gave a hill coefficient of 1 or less, indicating that Asp91 might be involved in interaction with Na+.

Conclusion: We concluded that TM2 may not be directly involved in substrate translocation; given the Na+ sensitivity of some of the residues in TM2, especially Asp91, we further hypothesize that TM2 may play a role in Na+ binding.

P-S-20

POLYMERIC NANOPARTICLES FOR TUMOR TARGETED DELIVERY OF TYLOCREBRINE

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Objective: Many promising anticancer drugs fail in clinical trials owing to their unacceptable systemic toxicity. Additionally, these drugs are often difficult to formulate and need toxic excipients like Cremophor®. Reformulation strategies that can improve the pharmacokinetics and mitigate the non-specific toxicity will allow many such drugs to be reclaimed and decrease the overall anticancer drug development costs. To illustrate, we present here a reformulation approach for tylocrebrine, a phenanthropiperidine alkaloid isolated from T. crebriflora with potent anticancer activity. Its development was discontinued owing to severe CNS toxicities discovered in phase-I clinical trials. Analysis of the structural features of the drug suggests that it likely penetrates the brain efficiently. We propose that reformulating tylocrebrine in targeted polymeric nanoparticles will reduce its brain penetration and mitigate the CNS toxicity and eliminate the need for use of toxic excipients. As many tumor cells overexpress epidermal growth factor receptor (EGFR) on their cell surface, nanoparticles were functionalized with EGFR-targeting peptide.

Methods: Nanoparticles loaded with tylocrebrine and surface functionalized with a carboxyl terminated polyethylene glycol (PEG) were synthesized by solvent evaporation. The terminal carboxyl group was then reacted with the targeting peptide.

Results: The nanoparticles had an optimum balance between particle size (250 nm), drug loading (5% w/w) and peptide conjugation efficiency (38%). Under in vitro conditions (pH 7.4, 37° C), nanoparticles released their cargo over 48 hours. In vitro cytotoxicity studies with A549 lung cancer cells showed that targeted nanoparticles were significantly more potent than non-targeted nanoparticles (IC50 – 24 Vs 64 nM). Increased effectiveness of targeted nanoparticles was likely due to enhanced tumor cell delivery of the encapsulated drug, as evidenced by >10 fold higher cellular internalization of targeted nanoparticles.

Conclusion: Highly loaded targeted nanoparticles with superior efficacy were generated. Future studies will evaluate pharmacokinetics, safety and efficacy of these nanoparticles in mouse tumor models.

P-S-21

ANALYSIS AND CONTROL OF DRY POWDER MIXING PROCESSES USING A FINE COHESIVE PIGMENT IN AN INHALATION GRADE LACTOSE

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Objective: Pharmaceutical product homogeneity is an extremely important factor in powder mixing, especially where as often a small scale of powder is used per dose. To increase product safety and consistency a very fine degree of mixing is desired, which is very difficult to achieve in practice due to the size, density and physical property differences of the constituent powders. Powder flow throughout currenty powder mixing technologies, particularly high shear/vertical axis mixers, is not well understood. This project is directed to gain a better quantitative understanding of the influence of operating conditions on powder mixing in different mixing processes.

Methods: A novel extension to a previous method has been proposed to evaluate the progression of dry powder mixing processes using a mixing sensitive colouring agent. The method proposed measures the change in the colour (hue) and colour intensity of the powder blends over mixing time and method. To evaluate the extent of fine particle dispersion and de-agglomeration, cohesive and free flowing lactose (white) and cohesive micronized iron oxide (dark red) were blended as a bulk and tracer probe respectively.

Results: Blends were manufactured using two different types of powder mixers at a range of processing conditions. Samples were withdrawn from the mixers at regular time intervals and the hue and intensity analysed using a colourimeter. Measuring the change in hue and intensity of micronized iron oxide in a lactose bulk enables the analysis of two distinct mixing behaviours, namely the de-agglomeration of the pigment (transition from red to orange) and the dispersion of a given sized aggregate through the bulk material (increase in the intensity of the blend's hue).

Conclusion: Hue values were assembled to create a series of formulation-specific colour curves which were able to clearly distinguish and group mixers into low and high intensity given their range of values along the same formulation curves. These initial results over a range of powders, mixer scales and processing conditions suggest that the iron oxide tracer method could provide the basis for a novel quantitative approach to producing equivalent mixture qualities as a function of mixer type, scale and operating conditions given a particular formulation

P-S-22

THE LYMPHATIC SYSTEM PLAYS A SIGNIFICANT ROLE IN THE SUBCUTANEOUS ABSORPTION AND PHARMACOKINETICS OF THE MONOCLONAL ANTIBODY TRASTUZUMAB IN RATS

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Objective: Most therapeutic monoclonal antibodies are currently delivered intravenously (IV), although there is significant interest in subcutaneous (SC) administration to allow self-administration and to potentially increase lymphatic exposure. The plasma profiles and pharmacokinetic parameters of therapeutic antibodies have been reported following SC administration, however the specific contribution of the lymphatic system to their absorption and disposition has thus far not been determined. The objective of this study is to characterise the role of the lymphatic system in the absorption of a model monoclonal antibody (trastuzumab) following IV and SC administration.

Methods: Trastuzumab plasma concentrations were measured in non-lymph cannulated and thoracic lymph cannulated rats following SC and IV administration (2 mg/kg). Plasma and lymph concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA). A population pharmacokinetic model was developed and fit to the plasma and lymph concentration data to predict the long term PK behaviour of trastuzumab in rats.

Results: The bioavailability of trastuzumab following SC administration to rats was approximately 80% and the antibody displayed a prolonged circulation half life of 2 weeks following IV and SC administration in non-lymph cannulated rats. Plasma concentrations in lymph cannulated rats, however, were significantly lower with approximately 44% and 27% of the administered dose was recovered in thoracic lymph over 30 h after IV and SC administration, respectively.

Conclusion: This represents the first study where the lymphatic recovery of a monoclonal antibody has been measured by direct quantification in collected lymph. The results of this study demonstrate that the lymphatic system is a major pathway by which monoclonal antibodies are absorbed from SC injection sites.

P-S-23

THE IMPACT OF SYSTEMIC INFLAMMATION AND BACTERIAL INFECTION ON THE BRAIN UPTAKE OF COLISTIN

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Objective: One of the reported adverse effects associated with colistin therapy is neurotoxicity and although the brain uptake of colistin is minimal in healthy animals, whether brain uptake alters during disease remains unclear. The aim of this project, therefore, was to assess the impact of both systemic inflammation and bacterial infection on the brain uptake of colistin.

Methods: Systemic inflammation was induced in male Swiss Outbred mice with three intraperitoneal doses of lipopolysaccharide (LPS, *Salmonella enterica* or *Pseudomonas aeruginosa*, 3 mg/kg) over 24 h. The brain uptake of subcutaneously-administered colistin (40 mg/kg) was then assessed 4 h following LPS or saline treatment, with brain and plasma concentrations determined by HPLC. Colistin brain uptake was also measured 8 h after intramuscular injection of *P. aeruginosa* bacteria (10^5 colony forming units/thigh) or saline. To assess if any alterations to brain uptake resulted from enhanced blood-brain barrier (BBB) paracellular diffusion, the brain uptake of ^{14}C-sucrose and ^{14}C-inulin was assessed in these models. The impact of LPS strains (15 µg/mL) on the expression of the main BBB tight junction protein claudin-5 in human brain microvessel endothelial cells (hCMEC/D3) was also assessed.

Results: Brain uptake of colistin was significantly enhanced (z=2.8) with area under the brain concentration-time curve (AUC_{brain}) values of 4.0 ± 0.3 µg·h/g and 11.7 ± 2.7 µg·h/g for saline and *S. enterica* LPS-treated mice (n=4), respectively. The brain-to-plasma ratios of ^{14}C-sucrose and ^{14}C-inulin were enhanced 2.0 and 1.7-fold, respectively, following *S. enterica* LPS administration. Neither systemic bacterial infection with *P. aeruginosa* nor systemic inflammation induced by *P. aeruginosa* LPS led to increased brain uptake of colistin. Consistent with *in vivo* observations, LPS from *S. enterica* induced a 1.3-fold reduction in claudin-5 expression in hCMEC/D3 cells, whereas LPS from *P. aeruginosa* had no significant effect on claudin-5 expression.

Conclusion: The brain uptake of colistin is increased in the presence of *S. enterica*-induced systemic inflammation but not during *P. aeruginosa*-induced inflammation or infection, suggesting that the BBB paracellular route is differently affected by different bacterial strains.

P-S-24

THE EXPRESSION PROFILE AND LIGAND BINDING AFFINITY OF FATTY ACID-BINDING PROTEINS AT THE BLOOD-BRAIN BARRIER

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Objective: Hydrophobic molecules can readily permeate the luminal cell membrane of brain microvessel endothelial cells (BMECs) either by passive diffusion or through membrane transporters. They then have to diffuse across the cytosol and partition across the abluminal membrane to access the brain parenchyma. Given these hydrophobic molecules generally have limited aqueous solubility, some form of intracellular carrier protein may be essential to shuttle these entities across the cytoplasm of BMECs. Given that fatty acid-binding proteins (FABPs) have been shown to facilitate cellular trafficking of lipophilic molecules in adipocytes and hepatocytes, this study examined the expression of FABPs at the blood-brain barrier (BBB) and the ability of certain FABPs to bind to lipophilic molecules.

Methods: To determine which of the 9 FABP genes are expressed at the BBB, the RNA content of an immortalized human brain endothelial cell line (hCMEC/D3) was extracted and analysed using real time reverse transcriptase polymerase chain reaction (RT-PCR). Similarly, protein expression of FABPs was determined using Western blots. The binding affinity of lipophilic molecules docosahexaenoic acid (DHA) and 1-anilinonaphthalene-8-sulfonic acid (ANS) to FABP5 was also measured by isothermal titration calorimetry (ITC).

Results: Out of the 9 FABP genes, FABP3, FABP4 and FABP5 were expressed on the mRNA level in hCMEC/D3 cells. The expression of FABP3, FABP4 and FABP5 relative to glyceraldehyde 3-phosphate dehydrogenase were 0.68 ± 0.02, 0.62 ± 0.04 and 0.69 ± 0.03, respectively (mean ± SD, n=3). Of these three FABPs, only FABP5 protein was present at a detectable level in hCMEC/D3 cells. Preliminary ITC studies demonstrate that human recombinant FABP5 binds to ANS in the low µM range and the binding affinity of DHA to FABP5 was measured to be 156 ± 33 nM (mean ± SD, n=12).

Conclusion: This study has demonstrated that hCMEC/D3 cells express appreciable levels of FABP3, FABP4 and FABP5 at the mRNA level, and FABP5 at the protein level. Furthermore, FABP5 has the ability to bind to DHA and ANS, and may likely bind to other hydrophobic molecules, facilitating their transport across the BBB.

P-S-25

PROBING THE MECHANISMS FOR DEGRADATION OF OXYTOCIN IN AQUEOUS SOLUTIONS

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Objective: Peptides and proteins which contain disulphide bridges have been shown to undergo degradation via two distinct mechanisms: (i) β-elimination (catalysed by hydroxide ions) and (ii) disulphide interchange (catalysed by the free thiols formed during β-elimination). The stability of a number of cysteine-containing peptides and proteins is dependent on sample pH, where the degradation is faster at high pH conditions (i.e. pH 8) compared to low pH (i.e. pH 2). This corresponds with the initiation ofβ-elimination. The objective of this study was to determine if the disulphide bridge-containing nonapeptide, oxytocin undergoes these specific degradation processes.

Methods: Solutions containing various concentrations of oxytocin (10, 100 and 600IU/mL) were buffered to a range of pH values (pH 2, 4, 6 and 8) and stored at high temperatures (40 and 50°C – control 4°C). Aliquots from each solution were taken from the various storage conditions over a period of time and analysed for free thiol concentration using the Ellman's reagent (DTNB) assay. The concentration of free thiols in each solution was determined photometrically at a wavelength of 412 nm.

Results: The concentration of free thiols in the oxytocin solutions increased over time and was dependent on sample pH, initial oxytocin concentration and storage temperature. The results showed that there was a higher concentration of free thiols measured in oxytocin solutions which were buffered to pH 8 compared to solutions buffered to pH 2 or 4, where there were lower amounts of free thiols detected over time. At higher oxytocin concentrations, there were relatively more free thiols producedover time compared to lower oxytocin concentrations and storage athigher temperatures accelerated the generation of free thiols.

Conclusion: Free thiols are involved in the degradation process of oxytocin where higher concentrations are produced over time with increasing pH, temperature and oxytocin concentrations. These findings suggest that the first step of oxytocin degradation occurs through β-elimination.

P-S-26

PREFERENTIAL LYMPHATIC ACCESS OF DOXORUBICIN AFTER SUBCUTANEOUS ADMINISTRATION OF A PEGYLATED DENDRIMER WHEN COMPARED TO LIPOSOMAL AND SOLUTION FORMULATIONS

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Objective: The lymphatic system is a major pathway by which metastasizing cancers spread throughout the body. Cancers that arrest within lymph nodes are also able to seed further tumours at distal locations. Targeting chemotherapeutic agents towards the lymphatic system and lymph nodes offers an alternative treatment option to surgical lymph node removal and further provides the potential to limit the side effects commonly associated with chemotherapy. Here we directly compare the lymphatic uptake of doxorubicin in a clinically available PEGylated liposomal formulation (Doxil®) and a PEGylated dendrimer containing doxorubicin covalently linked to the dendrimer surface via an acid-labile hydrazine linker after IV and SC administration to rats.

Methods: Male Sprague Dawley rats were cannulated via the right carotid artery to facilitate blood collection, the thoracic lymph duct to facilitate lymph collection, and the right jugular vein to allow intravenous dosing and replacement of fluid lost via the drainage of thoracic lymph. Rats were dosed either IV via the jugular vein cannula or SC into the left heel and blood and lymph samples were collected for 30 h in thoracic duct cannulated rats and up to 7 days in non-lymph cannulated rats. Plasma and lymph were analysed for doxorubicin concentration via HPLC.

Results: Liposomal and dendritic formulations of doxorubicin significantly increased the lymphatic exposure of doxorubicin by ~110 fold and ~490 fold respectively after SC dosing. Interestingly, IV administration of the dendritic formulation also significantly improved lymphatic exposure to doxorubicin when compared to IV administration of doxorubicin (by 330 fold) or the liposomal formulation (by >2 fold), suggesting that the dendritic formulation was able to target the lymphatics from both an SC injection site and from the blood after systemic absorption.

Conclusion: The work demonstrates the potential for colloidal and polymer-based drug delivery systems to improve the exposure of lymph-resident metastases to chemotherapeutic drugs.

P-S-27

HEATS OF DILUTION OF TWO MODIFIED B-CYCLODEXTRINS

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Objective: Since the 1980s there have been many cyclodextrins tested for their potential use as drug delivery systems. The most successful of these are two modified β-cyclodextrins, 2-HP-β-CD and SBE7-β-CD. The reason for continued research in this area is due to problems that cyclodextrins can present when administered, such as kidney irritation or interaction with plasma proteins or cholesterol.

Methods: The dilution phenomena of both 2-HP-β-CD and SBE7-β-CD were observed using Isothermal Titration Calorimetry, Fourier Transformation Infrared Spectroscopy and Circular Dichroism Spectroscopy.

Results: The heat of dilution of 2-HP-β-CD was examined and was found to be highly exothermic. These results were in contrast to those obtained for SBE7-β-CD, which were endothermic at high concentrations and slightly exothermic at low concentrations. These results indicated that the two cyclodextrins had differences in how their self associations formed.

Conclusion: The most likely reason for the difference found was that the charged sulphobutylether group caused Coulombic repulsion between the cyclodextrin molecules, thus weakening self association. The heat of dilution results obtained indicated that 2-HP-β-CD could be dangerous to administer intravenously whereas the results obtained for SBE7-β-CD indicated that it had a very low possibility of damage under intravenous administration.

P-S-28

TARGETING THE SPHINGOSINE-1-PHOSPHATE RECEPTOR IN THE MESENTERIC LYMPHATICS

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Objective: Endogenous sphingosine-1-phosphate (S1P) is an exit signal for lymphocyte egress from lymph nodes. A reversible lymphopenia results from administration of S1P receptor modulators (S1PRM). An S1PRM, fingolimod, was recently approved for the treatment of multiple sclerosis. However, fingolimod has a number of off-target toxicities. An ongoing program in our laboratory investigates the potential to enhance the activity and reduce systemic toxicity of immunomodulators, including S1PRM, through targeted lymphatic delivery. This study represents a proof-of-concept investigation to explore the pharmacodynamic benefit of lymphatic targeted delivery for S1PRM (using fingolimod and SEW2871 as exemplar compounds).

Methods: The logD and soybean oil solubility of S1PRM were measured. S1PRMs (0.5-5 mg/kg) were administered to rats via intravenous or intraperitoneal infusion, or intestinal administration in lipid-formulations or lipid-free formulations. Lymph, plasma and lymph node concentrations of S1PRMs (measured via HPLC-MS) and systemic lymphocyte counts (measured using cytometry) were assessed in mesenteric lymph or carotid artery cannulated rats, lymphatic transport and bioavailability calculated.

Results: The logD and soybean oil solubility of SEW2871 but not fingolimod were consistent with the potential for lymphatic transport. Fingolimod was undetectable in lymph. Surprisingly, lymphatic uptake of SEW2871 was substantial (over 35% of dose) after intestinal administration, irrespective of the formulation co-administered. The impact of SEW2871 on lymphocyte counts was administration route and dose-dependent. SEW2871 was effective at lower doses (1.5-5 mg/kg) than expected given its poor in vitro potency.

Conclusion: As far as we are aware, SEW2871 is the first drug for which co-administration with lipids has been found unimportant in stimulating lymphatic transport. Future studies will assess the mechanisms driving SEW2871 lymphatic transport. Further, lymphatic transport of SEW2871 appears to improve its efficacy following delivery via the oral rather than parenteral routes, and compared to that expected from in vitro potency. These findings highlight the potential to improve pharmacodynamic outcomes through drug design and delivery strategies which optimize immunomodulator access to sites of action in lymphatics.

P-S-29

GAS CHROMATOGRAPHY/TIME-OF-FLIGHT MASS SPECTROMETRY METABOTYPING OF DOCOSAHEXAENOIC ACID-TREATED ALZHEIMER'S DISEASE CELL MODEL

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Objective: Recent evidence has indicated the potential health benefits of docosahexaenoic acid (DHA) in managing Alzheimer's disease (AD). Therefore, elucidation of the role of DHA in prevention and treatment of AD becomes pertinent. To date, lipidomic-based efforts have mostly focused on identifying active lipid mediators via DHA metabolism. The role of DHA in the modification of metabolic pathways perturbed by amyloid precursor protein (APP)-induced mitochondrial dysfunction has, however, not been studied. Objective: To elucidate the metabotypes associated with DHA therapy via gas chromatography time-of-flight mass spectrometry (GC/TOFMS) metabonomic profiling of an AD cell model.

Methods: In this study, GC/TOFMS metabolic profiling of the cell culture media and lysate of Chinese hamster ovary (CHO) and CHO-APP695 cells treated with 25 µM DHA for 24 h was performed. The lysate and supernatant samples were prepared for GC/TOFMS analysis via protein precipitation with methanol and chemical silylation derivatization. Metabolic profiles were analyzed by multivariate data analysis techniques using SIMCA-P+ software.

Results: Unsupervised principal component analysis (PCA) revealed a clustering trend between the treatment and control groups in both lysate and medium samples. Subsequent supervised partial least squares discriminant analysis (PLS-DA) (medium: R2X=0.627, R2Y=0.999 and Q2(cum)=0.909; lysate: R2X=0.364, R2Y=0.992 and Q2(cum)=0.669) and Welch t-test ($p<0.05$) uncovered a list of putative marker metabolites that were significantly different and characterized the metabotypes associated with DHA treatment effect (e.g. elevated levels of succinic acid, citric acid and glycine; decreased levels of cholesterol, zymosterol and arachidonic acid).

Conclusion: GC/TOFMS metabonomics yielded distinct metabotypes associated with normal and APP695 transfected cells. Our study shows that DHA may play a role in mitigating APP-induced impairment in energy metabolism and reducing the levels of inflammatory and pro-inflammatory mediators commonly observed in AD brain. Understanding differences in the metabolic pathways affected by DHA in the normal and diseased cell models provides deeper insight into the mechanism of the protective effects of DHA.

P-S-30

PROTEASOME INHIBITION BY CURCUMIN ANALOGS PREVENTS NUCLEAR RECEPTOR CO-REPRESSOR (N-COR) DEGRADATION AND TRIGGERS THE UNFOLDED PROTEIN RESPONSE (UPR)-INDUCED APOPTOSIS

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Objective: Acute promyelocytic leukemia (APL) results from the fusion of promyelocytic leukemia (PML) and retinoic acid receptor (RAR). The fusion of PML-RARinduces a conformational change in the nuclear receptor co-repressor (N-CoR) protein which triggers a pro-survival pathway ultimately providing APL cells an escape route from cell death. Curcumin, an active ingredient in turmeric, weakens the defense mechanism of APL cells by preventing the degradation of misfolded N-CoR. Accumulation of misfolded N-CoR protein in APL cells induces endoplasmic reticulum (ER) stress and activates unfolded protein response (UPR)–induced apoptosis.

Methods: In the development of a lead compound with higher potency and selectivity for APL cells, a series of 47 curcumin analogs were synthesized. Activity and selectivity profiles were established on APL, non-APL and non-malignant cell lines. Western blot and immunofluorescence were carried out to determine the effect of analogs on levels of endogenous N-CoR. Protein binding assay was carried out to elucidate the protein target of lead compounds.

Results: Herein we report scaffolds with up to 100-fold improved potency on NB4 (APL) compared to curcumin while maintaining 2-fold selectivity for APL over non-APL and 10-fold selectivity over non-malignant IMR90. Treatment of NB4 cells with APL selective analogs induced the accumulation of misfolded N-CoR protein in the cytosol. Cell-based proteasome inhibition assay revealed that these analogs possess significantly greater proteasome inhibitory activity over curcumin. The most potent analog 41 inhibited all three catalytic sites of the proteasome at 1 µM, compared to curcumin at 30 µM. Our study suggests that impeding the degradation of N-CoR by proteasomal inhibition is an important mechanistic event contributing to APL cell death.

Conclusion: This study provides a platform for the rational design of potent and selective targeting novel drugs for APL in the exploration of targeting the UPR-proteasomal pathway in cancer research.

P-S-31

A MUCOSAL PEPTIDE VACCINE COMPOSED OF EP67, A RESPONSE-SELECTIVE PEPTIDE AGONIST OF THE C5A RECEPTOR, AND CTL EPITOPES PROTECTS AGAINST PRIMARY MUCOSAL CHALLENGE WITH MURINE CYTOMEGALOVIRUS

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Objective: Human cytomegalovirus (HCMV) is the primary cause of birth defects as well as morbidity and mortality in transplant recipients in the US. Mucosal vaccines can potentially decrease the risk of HCMV infection but are limited, in part, by the absence of clinically practical mucosal adjuvants that can generate memory CTL. Peptide vaccines that incorporate EP67, a selective peptide agonist of the C5a receptor effectively recruit epitope-specific CTL after systemic immunization. Thus, the objective of this work was to determine whether mucosal immunization with a peptide vaccine that includes EP67 can protect against primary mucosal challenge in a murine model of CMV (MCMV).

Methods: Two partially protective MCMV CTL epitopes in BALB/c, pp89 and M84, were added to the N-terminus of EP67 (CMV-EP67) or scrambled EP67 (CMV-scEP67) via a protease-labile double arginine. Treatments (50 µg each peptide) were co-administered intranasally (IN) in 15 µL PBS on days 0, 7, and 14. Epitope-specific CTL were compared on day-24 by ELISPOT (spleen) and ICS-FACS (spleen and lungs) after stimulating ex-vivo with M84 or pp89. MCMV viremia in tropic organs after primary IN challenge (5x10^3 PFU/50 µL PBS on day-24) was compared by plaque assay.

Results: Compared to naïve or CMV-scEP67, IN immunization with CMV-EP67 (i) increased number of epitope-specific IFN-γ secreting splenoctyes (9±6, 60±9, and 154±25 mean±SD SFU/million splenoctyes, $P<0.01$, One-way ANOVA/Tukey post-test) and (ii) increased percentage of multifunctional IFN-γ+/TNF-α+ memory CTLs (0.2±0.0, 0.4±0.3, and 1.7±0.4 %mean±SD CD8+/CD44Hi, $P<0.05$, One-way ANOVA/Tukey post-test). IN immunization with CMV-EP67 was only associated with the clearance of productive virus from the salivary glands 4 weeks post MCMV challenge.

Conclusion: EP67-based peptide mucosal vaccines can recruit memory CTL capable of protecting against primary mucosal infection with CMV. Thus, EP67-based mucosal vaccines may effectively protect against HCMV or any viral mucosal infection that requires a strong CTL response.

P-S-32

INTESTINAL CATION-SELECTIVE TRANSPORTERS INVOLVED IN THE APICAL UPTAKE, ACCUMULATION AND ABSORPTION OF METFORMIN

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Objective: Metformin is widely prescribed for Type II diabetes. This hydrophilic and charged drug traverses predominantly paracellularly across the human intestinal Caco-2 cellular model. However, metformin oral bioavailability is significantly higher than the paracellular probe mannitol (60-80% vs ~20%). This discrepancy is explained by our hypothesis that functional synergy between apical (AP) cation-selective transporters and paracellular transport enhances the intestinal absorption of metformin. This work aims to determine AP intestinal transporters of metformin, their localization and contributions to its absorption.

Methods: Several cation-transporter specific inhibitors, and transporter-knockdown cells were employed to elucidate transporter involvement in [14C]metformin uptake in Caco-2 cells. Functional and confocal microscopy studies were performed to determine organic cation transporter (OCT)1 localization in Caco-2 cells, human and mouse enterocytes. Chemical inhibition and transporter-knockdown studies in mice to demonstrate transporter-mediated metformin intestinal absorption are ongoing.

Results: OCT1 and plasma membrane monoamine transporter (PMAT) each contribute 20-25% of metformin AP uptake into Caco-2 cells. Additionally, cation-selective serotonin transporter (SERT) and choline transporter (CHT) also contribute to metformin AP uptake. Although AP intestinal localization of PMAT, SERT and CHT is well-established, OCT1 intestinal localization is ambiguous in literature. Functional studies and immunostaining and confocal microscopy confirmed AP localization of OCT1 in Caco-2 cells, and human and mouse enterocytes.

Conclusion: OCT1, PMAT, SERT and CHT, contribute significantly to metformin AP uptake in Caco-2 cells. For the first time, transporters of endogenous amines, SERT and CHT, were shown to transport metformin. Our results unequivocally confirm AP localization of OCT1 in Caco-2 cells, human and mouse enterocytes, and clarify ambiguity in the literature. These results will impact interpretation of intestinal absorption data of cationic compounds. In vivo studies could explain intestinal absorption mechanisms of cationic drugs, and help in discovery/development of drugs with high oral bioavailability.

P-S-33

ASSOCIATION OF CC CHEMOKINE LIGAND-2 (CCL2/MCP-1) AND CC CHEMOKINE LIGAND-5 (CCL5/RANTES) WITH THE PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF PEGYLATED LIPOSOMAL DOXORUBICIN (PLD) IN PATIENTS WITH RECURRENT OVARIAN CANCER

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Objective: Unlike small molecules, nanoparticles (NPs) such as PLD are cleared by the mononuclear phagocyte system (MPS). We have reported a bi-directional interaction between NP anticancer agents and monocytes (MO), the primary cells of MPS. Immune mechanisms underlying this interaction remain unknown. Chemokines play an essential role in migration and activation of MO. These immune mediators may have important effects on the PK and PD of NPs, but have not been evaluated. We investigated the association between chemokines and the PK and PD of PLD in patients with EOC.

Methods: PLD was administered at 40 mg/m^2 alone or 30 mg/m^2 IV with carboplatin (AUC=5). PK studies of encapsulated and released doxorubicin were performed on cycle 1 in 10 patients. Serial blood was collected at 0 h to 672 h post infusion. The serial plasma concentrations (conc) of CCL2, CCL3, CCL4, and CCL5 from 0 h to 672 h were measured using multiplex immunochemistry. Area under the concentration versus time curve (AUC) of PLD and chemokines were calculated via non-compartmental analysis. Progression free survival (PFS) and % decrease in MO at their nadir were assessed.

Results: The CCL5 baseline conc [CCL5: 2174.7 ± 2913.6; CCL2: 242.3 ± 74.6; CCL3: 43.5 ± 17.6; CCL4: 42.9 ± 22.9 pg/ml (P<0 .0001)] and CCL5 AUC0-last [CCL5: 687,128 ± 455,130; CCL2: 123,766 ± 79,865; CCL3: 17,298 ± 16,562; CCL4: 21,955 ± 18,905 pg•h/ml (P< 0.0001)] were higher compared with other chemokines. There was a positive linear relationship between the CCL5 AUC and encapsulated doxorubicin AUC0-∞ (P=0.002) and between the CCL2 AUC and % decrease in MO (P=0.037). There was a non-significant positive linear relationship between the CCL5 AUC and PFS (P=0.16).

Conclusions: These findings indicate that chemokines CCL2 and CCL5 are associated with the PLD PK and PD. These results also suggest that PLD may have a positive feedback stimulus on the MPS via chemokine system. Further evaluation of mechanisms that underlie interaction between monocytes and chemokines and PLD may be useful for individualizing PLD therapy in patients with recurrent ovarian cancer.

P-S-34

DEVELOPMENT OF A STRUCTURAL POPULATION PKPD MODEL TO DESCRIBE THE EFFECT OF PARACETAMOL ON THE INTERNATIONAL NORMALISED RATIO (INR)

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Objective: Paracetamol is one the most common substances taken in overdose. (1, 2) Paracetamol may increase International Normalised Ratio (INR) in paracetamol poisoning without hepatic injury by reducing functional factor VII. (3) The aims of this study were to develop a structural model to describe the PKPD of paracetamol and its effect on INR.

Methods: A total of 167 patients were included in the dataset (31 paracetamol overdose patients, 9 control overdose patients, 20 cross-over clinical trial patients, 107 retrospective paracetamol overdose patients); 109 of the patients were men, the median age (range) was 22 years (13–71). A structural population PKPD model was graphically developed in Phoenix® NLMETM.

Results: 167 patients contributed to a total of 1242 paracetamol plasma observations and INR observations. The pharmacokinetics of paracetamol were best described by a 1-compartment model with first order input and linear disposition. An indirect effect Emax model was used to describe the effect of paracetamol on INR. The population mean estimates of the pharmacokinetic parameters volume of distribution, clearance, absorption rate constant (Ka), and lag time (tlag) were 5.62 L, 2.16 L/h, 0.85 h-1, and 0.87 h, respectively. The population mean estimates of the pharmacodynamic parameters Emax and EC50 were 0.79 (increase in INR) and 0.77 µM, respectively. A mixed inter subject error model and mixed residual error model was used. Covariates investigated in the final model included age, sex, and treatment with antidote N-acetylcysteine (NAC).

Conclusion: Preliminary findings demonstrated that the structural model adequately estimated population PKPD parameters for paracetamol and provided the basis for covariate analyses.

P-S-35

SYNTHESIS AND CHARACTERIZATION OF LEVOFLOXACIN CONJUGATES

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Objective: Levofloxacin is a broad-range antibiotic belonging to the group class of fluoroquinolones. Due to its potent antimicrobial effect, levofloxacin is currently used as a first-line therapy for community-acquired pneumonia. However, levofloxacin resistance occurs worldwide. The conjugation of levofloxacin to specific peptides may overcome antimicrobial resistance. Indolicidin peptide has antimicrobial properties which complements levofloxacin. Tat is a cell penetrating peptide which is found to increase membrane permeability of cell and is commonly used in drug delivery. Thus, we designed and synthesised levofloxacin conjugated with peptide.

Methods: The peptides, indolicidin and Tat were synthesised using Fmoc SPPS. Subsequently, a linker was attached to the peptide forming either an amide or an ester bond. Conjugation of levofloxacin to the peptide via glycine linker was performed using HBTU/DIPEA whereas an additional catalyst, DMAP, was used for conjugation of levofloxacin to the peptide via glycolic acid linker. Conjugates were characterised using ESI-MS and analytical HPLC prior purification by preparative HPLC. Haemolytic assay was carried out following conjugation. Results were analysed using UV-Vis spectroscopy.

Results: The parent peptides (indolicidin and Tat) along with the final conjugates (levofloxacin-glycine-indolicidin, levofloxacin-glycolic acid-indolicidin, levofloxacin-glycine-Tat and levoflxacin-glycolic acid-Tat) were successfully synthesised. The conjugation methods were optimised to attain the final compounds with moderate to high yields (44-80%) and excellent purity (≥95%). Haemolytic assay was performed on the parent peptides, peptide conjugates and levofloxacin. Both indolicidin-levofloxacin conjugates were haemolytic (11% ± 0.05 and 13% ± 0.01) at 100µM. However, these conjugates were not haemolytic at lower concentrations while both Tat-levofloxacin conjugates were not haemolytic (≤0.4% ± 0.01) even at high concentrations of 100µM.

Conclusion: Methods of conjugation of levofloxacin to Tat and indolicidin via ester and amide linkage were established. These new antibiotic derivatives are currently under biological evaluation to determine their antimicrobial activity. The current study has paved a novel method for drug delivery.

P-S-36

3D-PHARMACOPHORE MAPPING OF A SET OF NOVEL B-N-BIARYL ETHER SULFONAMIDE-BASED HYDROXAMATES

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Objective: Matrix metalloproteinases (MMPs) are enzymes involved in the degradation of the extracellular matrix being overexpressed in several cancer types. Here, a receptor-independent (RI) four dimensional (4D) quantitative structure-activity relationships (QSAR) method was applied to a set of forty β-N-biaryl ether sulfonamide-based hydroxamates, reported as potential MMP-2 inhibitor (Yang, et al, 2008), to identify the 3D-pharmacophore map and use this information for designing new MMP inhibitors.

Methods: The 3D molecular models were built up in their neutral forms, and the geometry optimization was carried out employing MM+ force field, without constrains. Partial atomic charges were calculated using the AM1 method (HyperChem 7.51). The energy-minimized molecular models were used as initial structures to perform molecular dynamics simulations of 1 ns [1,000,000 steps, 1 fs step size, T 298 K] (MOLSIM 3.2) to obtain the conformational ensemble profile (CEP) of each inhibitor. The CEPs were aligned (three-ordered atoms; three distinct alignments) in a cubic grid cell with 1.0 Å resolution, and compute the grid cell occupancy descriptors (GCODs) (4D-QSAR 3.0). 4D-QSAR analysis generates numerous trial GCODs, and partial least squares (PLS) regression was used to data reduction fit. The 500 most highly weighted PLS GCODs were used to form the trial basis set for the genetic function approximation (GFA) analysis. The top ten models generated by GFA-MLR (multiple linear regression) and leave-one-out crossvalidation (LOO) method for each alignment were evaluated.

Results: The final best QSAR model (n = 30) presented 5 GCODs and was statistically significant (r2 = 0.93; qLOO2 = 0.88; LOF = 0.11; LSE = 0.04). External validation was performed using a test set (n = 10) and the biological activities of seven compounds were well predicted by the best QSAR model, indicating a suitable power of prediction (70%).

Conclusion: Due to its good predictivity the results can help for designing new MMP-2 inhibitors.

P-S-37

DEVELOPMENT OF VERY LOW MOLECULAR HEPARIN DERIVATIVES FOR ORAL ANTICANCER THERAPY

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Objective: Heparin, a highly sulfated glycosaminoglycan is known as to be a potent injectable anticoagulant drug. Until now heparin has been not used as an anticancer agent due to its anticoagulant property. Our present study was designed to prepare new very low molecular heparin (VLMWHs) and deoxycholic acid conjugates and evaluates their therapeutic effects. Therefore, we synthesized different size of materials and conducted in vitro and in vivo experiments for evaluation of their therapeutic effects.

Methods: VLMWHs were prepared by controlled nitrous acid depolymerization of heparin. Using GPC (Gel permeation chromatography) peak analysis, the average molecular weight and molecular weight distribution was confirmed. The bile acid was conjugated by amide bond. The anticoagulant effect of materials was measured by in vitro FXa (Factor Xa) assay. For anti-angiogenesis effect of these materials, matrigel-plug assay and tubular formation assay on HUVEC (Human umbilical endothelial cell) and in vivo tumor inhibition assay were performed. All data were statistically analyzed by the ANOVA and the p value of less than 0.05 was considered to be statistically significant.

Results: The molecular weight obtained of synthesized VLMWHs was 1400~3200da. By GPC peak analysis the molecular weight distribution of heparin was confirmed. VLMWH and bile acid was successfully conjugated and conjugation ratio was 1.5~3. The FXa assay results showed that anticoagulant activity of heparin was maintained in between 96-98 %, when the molecular weight of heparin is lower than 3000da compare to low molecular weight heparin (LMWH). After bile acid conjugation, materials limited their anti-coagulation effect as 0-5 % compare with LMWH. These VLMWHs showed increased anti-angiogenesis activity in tubular formation assay and matrigel-plug assay. In addition, VLMWHs showed significant anti-cancer effect in mouse tumor inhibition test.

Conclusion: In future, our target is to develop oral delivery of VLMWHs as an anti-angiogenic agent with lower side-effects.

P-S-38

THE FIRST RATIOMETRIC FLUORESCENT PROBE FOR AMINOPEPTIDASE N

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Objective: Aminopeptidase N (APN, EC 3.4.11.2) plays a vital role in tumor invasion, metastasis and angiogenesis. However, so far there are only few optical probes for detecting the activity of APN, which can be affected by sensor concentration and environment. The purpose of this paper was to find a ratiometric fluorescent probe for APN, which allow the measurement of emission intensities at two wavelengths, should provide a built-in correction for environmental effects.

Methods: Probes were synthesized from APN favored amino acids in five steps. Vario skan was used for all fluorescent studies. Kinetic constants and LOD valule were used to evaluate probes. The practical feasibility of the ratiometric fluorescent probes were elucidated by examining the IC50 of Bestatin (an effective inhibitor to APN).

Results: Three of six synthesized probes could be recognized and hydrolyzed by APN. Upon adding APN to the solution of Ala-PABA-7HC in PBS or culture medium, the maximum emission peak experienced a red shift from 390 nm to 450 nm, as well as the ratio of fluorescence intensity (I450/I390) (λex = 330 nm) significantly increased from 0.26 to 3.05 (R = 11.7-fold) in 15 min. LOD of Ala-PABA-7HC to APN (0.3 mIU) was at least 30 times lower than that of commercial substrate L-Leu-p-nitroanilide (>10 mIU). Km of Ala-PABA-7HC to APN (73.70μM) was 16 times lower than that of L-Leu-p-nitroanilide (1180 μM). IC50 obtained with Ala-PABA-7HC was at the equivalent order of magnitude as that with L-Leu-p-nitroanilide in APN inhibitory assay.

Conclusion: One of the probes, Ala-PABA-7HC could provide a convenient and economical avenue to high-throughput screening assay for APN inhibitors. It is also a promising probe in cell-based APN inhibition assay because of the built-in correction ability for environmental effects, which drives the assay be proceed in cell favored culture medium and without removement of cells before reading fluorescent data.

P-S-39

DEVELOPMENT OF A THERMALLY RESPONSIVE INTRACELLULAR SWITCH

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Objective: There remains a strong need to develop new tools for the targeted control of protein-mediated cell biology. To study target molecular pathways and their downstream effectors, a reliable platform is required capable of rapid and reversible arrest of function. While there are multiple ways to switch the activity of target proteins (gene deletion, siRNA, drugs), few of these can rapidly and reversibly activate target proteins inside live cells. To better explore the real-time cellular environment, an ideal intracellular switch would activate in the course of a few minutes.

Methods: Utilizing the inverse phase transition properties of elastin-like polypeptides (ELPs), it is hypothesized that target biomolecules can be reversibly sequestered

Results: To test the ability of ELPs to associate inside cells, we characterized the biophysics of fluorescently tagged ELPs inside live mammalian cells. After probing a library of ELPs of various lengths and sequence, it was determined that the ELPs are highly mobile through the cytosol below their Tt, but immobile once aggregated. After studying the biophysical behavior, and optimizing phase transition to within a few degrees +/- of 37oC, an ELP was fused to the N-terminal end of the clathrin light chain (CLC), a protein essential in clathrin-mediated endocytosis. Preliminary studies suggest that the ELP-CLC fusion protein functions normally below the ELP's Tt, however, above Tt the fusion become insoluble and inhibits clathrin-mediated endocytosis.

Conclusion: This proof of concept study suggests the potential of an ELP as a new technology for studying protein function in real time.

P-S-40

CLINICAL UTILITY OF POSACONAZOLE THERAPEUTIC DRUG MONITORING

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Objective: Posaconazole has an important role in the prophylaxis and salvage treatment of invasive fungal infections (IFI), although its limited absorption remains an important clinical concern. Few studies have examined the utility of monitoring posaconazole plasma concentrations. This study aimed to investigate the factors affecting posaconazole pharmacokinetics, and evaluate the clinical utility of therapeutic drug monitoring (TDM) for posaconazole.

Methods: Patient medical records from 6 Australian hospitals (Dec. 2008 – Dec. 2010) were reviewed to collect information on dosing and clinical outcome for all patients who had ≥1 plasma concentrations measured. Ethics approval was received.

Results: 85 patients received posaconazole during the study period, with 538 plasma concentrations available. Posaconazole concentrations were frequently low in most patients (median 467 ng/mL). Among 72 patients taking posaconazole for prophylaxis against IFI, 12 patients developed fungal infection (17%); posaconazole concentrations were significantly reduced in these patients compared to those who did not develop fungal infection (median 289 ng/mL vs. 485 ng/mL respectively, $P<0.05$). Multivariate analysis of posaconazole concentrations identified a number of significant factors associated with reduced posaconazole exposure, including co-administration with proton pump inhibitors, metoclopramide, phenytoin or rifampicin, the presence of mucositis or diarrhoea, as well as a novel finding of significantly reduced posaconazole exposure in the early post-transplant period in haematopoietic stem cell transplant recipients.

Conclusion: This study is the largest investigation of posaconazole TDM to date. Low posaconazole concentrations are common and are associated with a poor clinical outcome, supporting the utility of monitoring posaconazole concentrations to ensure optimal systemic exposure.

P-S-41

ROLE OF UBIQUTINATION IN THE INTRACELLULAR SORTING OF HEPATOCANALICULAR ABC TRANSPORTERS

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Objective: Bile salt export pump (BSEP/ABCB11) and Multidrug resistance-associated protein 2 (MRP2/ABCC2) mediate the membrane transport of bile acids and glutathione across hepatocanalicular membrane. The impaired post-translational regulations of both transporters are implicated in a disease state called intrahepatic cholestasis. To address this issue, we explore the intracellular sorting mechanism of BSEP and MRP2 focusing on ubiquitination, a reversible post-translational modification, which acts as internalization and/or degradation signal for membrane proteins.

Methods: Co-immunoprecipitation and subsequent immunoblotting were used to examine the ubiquitination status of BSEP/Bsep and MRP2/Mrp2. A cell surface biotinylation method was applied to study the internalization and degradation rate of cell surface-resident BSEP and MRP2.

Results: BSEP/Bsep and MRP2/Mrp2 were ubiquitinated in Flp-In™ T-REx™ 293 cells (293 cells) expressing 3×FLAG-BSEP and MRP2 and rat canalicular membrane fractions. Overexpression of dominant negative forms of ubiquitin (DN-Ub) increased the amount of remaining biotin-labeled 3×FLAG-BSEP after 12 hours incubation from 39.4 ± 9.3 % (mean ± SE) to 90.7 ± 14.4% ($p<0.05$). It decreased the amount of internalized 3×FLAG-BSEP after 3 minutes incubation from 10.1 ± 1.4% to 3.00 ± 0.83% ($p<0.05$) but didn't affect degradation rate of internalized 3xFLAG-BSEP. On the other hand, overexpression of DN-Ub moderately increased remaining biotin-labeled MRP2 after 12 hours incubation from 29.0 ± 4.2% to 53.3 ± 10.9% ($p=0.07$). It didn't affect internalization rate of MRP2 but increased the amount of remaining internalized biotin-labeled MRP2 after 8 hours incubation from 35.2 ± 18.2 % to 123 ± 25 % ($p<0.05$).

Conclusion: Ubiquitination is suggested to control the cell surface-resident BSEP and MRP2 by promoting the internalization of BSEP and the degradation of MRP2 after internalization.

P-S-42

SELECTIVE INHIBITORY EFFECT OF HPMA COPOLYMER-CYCLOPAMINE CONJUGATE ON PROSTATE CANCER STEM CELLS

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Objective: An important reason for the failure of conventional therapies for prostate cancer (PC) is the failure to kill Cancer Stem Cells (CSCs). Evidences have shown the critical role of hedgehog (Hh) signaling pathway in prostate CSC maintenance. Aiming at improving PC treatment outcomes, we designed an HPMA (N-(2-hydroxypropyl)methacrylamide) copolymer-based drug delivery system containing the Hh inhibitor-cyclopamine. The anti-CSC effects of this conjugate were evaluated on PC cells with CSC properties.

Methods: HPMA copolymer-cyclopamine conjugate (P-CYP) was synthesized by RAFT (Reversible Addition-Fragmentation Chain Transfer) copolymerization of HPMA and MA-GFLG-TT (N-methacryloylglycylphenylalanylleucylglycyl thiazolidine-2-thione), followed by conjugation with cyclopamine. The CSC marker changes, prostasphere forming ability, cytotoxicity and apoptosis induction were evaluated following treatments of P-CYP on human RC-92a/hTERT PC cells with telomerase reverse transcriptase transcription. The results were compared with docetaxel (conventional first-line chemotherapeutics) treatment.

Results: The HPMA copolymer precursor containing TT groups was synthesized with Mn 47.7 kDa, Mw 50.8 kDa and polydispersity of 1.07. The conversion of TT groups (6.1 mol%) to cyclopamine was 50%. The CSC marker CD133 was expressed in 5-8% of the RC-92a/hTERT cells. CD133+ cells showed significantly higher prostasphere forming efficiency than CD133- cells. Following P-CYP treatments, percentages of CD133+ CSCs decreased in a dose-dependent manner. Both, the decreases in CD133+ cell percentage and prostaphere forming efficiency were more significant in P-CYP treated groups than in docetaxel groups ($p<0.01$). P-CYP caused 50.6±1.5% decrease in CSC population when the whole cell viability remained above 90%; docetaxel did not reduce the CSC percentage when causing 40% decrease in cell viability. Annexin V assay showed that P-CYP induced CD133+ cell apoptosis.

Conclusion: HPMA copolymer-cyclopamine conjugate designed to eliminate prostate CSCs was synthesized with controlled molecular weight and narrow polydispersity. This conjugate showed selective inhibitory effect on CSCs than on bulk cancer cells in a PC model with CSC properties.

P-S-43

PHARMACOGENETIC PROFILING OF THE WICELL(R) HUMAN EMBRYONIC STEM CELL LINES

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Objective: Predicting response to pharmacotherapy is vital to risk assessment and clinical efficacy. Primary human hepatocytes are commonly used to evaluate liver drug metabolism and toxicity. However, human hepatocytes i.) are source limited, ii.) have high donor variability, iii.) survive short term in culture, and iv.) lack selection for specific genetic profiles. To overcome these limitations, we are investigating pluripotent stem cells as an alternative source for generating hepatocytes. Pluripotent stem cells offer advantages over primary hepatocytes because they have high replicative capacity, providing a limitless source of hepatocytes. Stem cell derived hepatocytes (SCDHs) may also provide an improved in vitro system for evaluating genotype-phenotype relationships of the cytochrome P450 (CYP) enzymes and their impact on drug metabolism and toxicity.

Methods: To demonstrate the utility of SCDHs in pharmacogenetic screening, we genotyped the five commonly used WiCell® human embryonic stem cell lines, H1, H7, H9, H13 and H14. The Affymetrix DMET Plus chip array was used to test 1936 polymorphisms in drug disposition genes.

Results: We identified the H14 line as homozygous for CYP2C9*2 and H1 as heterozygous for CYP2C9*3, polymorphisms known to have clinically significant effects. We are focusing on CYP2D6 to establish a proof-of-concept in utilizing SCDHs for making genotype-phenotype predictions given its highly polymorphic nature. With regard to CYP2D6 gene deletion/duplication, we found that H1 has only one gene copy and also harbors the CYP2D6*41 splicing defect of variable penetrance. The H7 and H14 lines are heterozygous for the CYP2D6*4 splicing defect, a null mutation, meaning these two cell lines also have only one functional copy of CYP2D6. Studies are ongoing to assess the functional implications of these polymorphisms in SCDHs.

Conclusion: Overall, our results demonstrate limited genetic diversity between the WiCell® lines, supporting the need to develop more pluripotent stem cell lines to better evaluate pharmacogenetic effects on drug metabolism.

Poster Index by Presenting Author

Surname	Initial	Poster #	Page #
Stillhart	C	P-TH-01	95
Sun	Z	P-S-18	155
Tan	K	P-S-30	167
Tanaka	K	P-S-17	154
Taylor	T	P-S-27	164
Kapp Thomsen	M	P-TH-06	100
Tiwari	D	P-TH-28	122
Trevaskis	N	P-S-28	165
Tuominen	E	P-S-13	150
Turra	K	P-S-36	173
Umemori	Y	P-TH-18	112
van Meer	P	P-TH-43	137
Vialpando	M	P-TH-16	110
Voellinger	J	P-S-43	180
Wang	W	P-TH-39	133
Weber	B	P-S-08	145
Williams	H	P-TH-29	123
Yago	M	P-S-01	138
Yavuz	B	P-TH-10	104
Zhang	L	P-TH-31	125
Zhou	Y	P-S-42	179

Short Courses

Short Courses Timetable

Date: Friday, 30th November 2012

Location: Various, please check venue of short courses

Morning session (9:00 AM - 12:30 PM)

1. Barriers and Novel Approaches for Effective Delivery of Biotherapeutics
2. Novel Approaches for Solid State Characterisation
3. Pharmacokinetic-Pharmacodynamic Modelling in Drug Safety and Efficacy
4. Lead Optimisation in Drug Discovery and Development

Afternoon session (1:30 PM - 5:00 AM)

1. Approaches for Enhancing Oral Delivery of Poorly-Water Soluble Drugs
2. Formulation and Stability of Proteins, Antibodies and Vaccines
3. Imaging Modalities for Assessing Drug Delivery and Disposition
4. Transporters as Mediators of Drug Disposition in Health and Disease

Morning Short Course 1

BARRIERS AND NOVEL APPROACHES FOR EFFECTIVE DELIVERY OF BIOTHERAPEUTICS

Course Chairs: Professor Wim Hennink (Utrecht University, Netherlands) and Professor Claus-Michael Lehr (Saarland University, Germany)

Location: Lecture Theatre 1

Safe and efficient delivery of biotherapeutics, i.e. molecules based on peptides and nucleotides, is largely hampered by the poor permeability of these typically rather large and hydrophilic molecules across biological barriers. The latter may be represented by the lipoidic membranes that surround individual cells or subcellular compartments. At some higher level of biological complexity, biological barriers are represented by epithelial tissues, such as the skin, the mucosae oft digestive and the respiratory tract or the vascular endothelium oft the brain that represents the so-called blood brain barrier.

At the one hand a biological necessity, these barriers on the other hand represent a major challenge to drug delivery, because they are separating the site of drug administration from the site of drug action. Rational development of advanced drug delivery systems, capable to shuttle pharmacologically active agents to the intended site of action, requires to acknowledge the physico-chemical, molecular and cellular principles governing barrier transport. In this context, cell- and tissue based in-vitro models of relevant biological barriers are most useful tools. However, as a next step such concepts must also be translated into technological approaches for pharmaceutical formulations that must be efficient, safe and producible at reasonable costs.

This course will provide an introduction to the physico-chemical fundaments of barrier transport, as well as to some state-of -the art in vitro models. The second part oft he course will illustrate some innovative approaches for trans-epithelial and intra-cellular drug delivery of macromolecular biotherapeutics.

Morning Short Course 1 Timetable

Time	Lecture and Presenter
9:00 AM	**Introduction** *Professor Wim Hennink, Utrecht University, Netherlands and Professor Claus-Michael Lehr, Saarland University, Germany*
9:10 AM	**Physicochemical concepts of how molecules and nanoparticles cross biological barriers** *Professor David Grainger, University of Utah, USA*
9:40 AM	**Cell culture models of mucosal epithelia (gastrointestinal and lung)** *Professor Claus-Michael Lehr, Saarland University, Germany*
10:10 AM	**Student Podium: Biocompatible delivery systems for nucleic acid therapies** *Ms Jayanant Iemsam-arng, University of London, United Kingdom*
10:30 AM	**Morning tea**
11:00 AM	**Student Podium: The chicken chorioallantoic membrane. a model for angiogenesis, transepithelial drug delivery and xenograft research** *Mr Boris Strehlow, Philipps University, Germany*
11:20 AM	**Oral delivery of heparin for the prevention of thrombosis** *Professor Youngro Byun, Seoul National University, Korea*
11:50 AM	**Delivering challenging molecules across biological barriers** *Professor Ijeoma Uchegbu, University of London, United Kingdom*
12:20 PM	**General Discussion** *Professor Wim Hennink, Utrecht University, Netherlands and Professor Claus-Michael Lehr, Saarland University, Germany*

Morning Short Course 2

NOVEL APPROACHES FOR SOLID STATE CHARACTERISATION

Course Chair: Professor Thomas Rades, University of Copenhagen, Denmark
Location: Lecture Theatre 2

Properties of the solid state of drugs and dosage forms remain a major issue in the development of medicines. For small molecular weight drugs the issue of poor aqueous solubility frequently requires the formulation of the drug in a high energy forms, for example in the amorphous state. Lipophilic drugs can be formulated in lipid drug delivery systems, and although these are mostly lipid solutions, solid state properties of the drug play in important role, for example during the digestion of the delivery systems after administration, when precipitation of the drug can occur. Biologics also are often formulated in the solid state, for example as freeze dried products, or the biological will be in contact with solid matrices and polymers. It is therefore of more importance than ever in the field of drug and dosage form development to have a range of solid state characterisation techniques available, to determine the quality relevant solid state properties of drugs and dosage forms. In this short course we will introduce some important solid state characterisation techniques, with a view on the quality relevant information for the development of high energy forms of drugs and drugs in lipid formulations. We will discuss some important solid state characterisation techniques relevant for the development of biologicals and we will hear about the enormous potential in using synchrotron based techniques for the characterisation of drugs and dosage forms.

Morning Short Course 2 Timetable

Time	Lecture and Presenter
9:00 AM	**Characterisation techniques for solid drugs and dosage forms** *Professor Thomas Rades, University of Copenhagen, Denmark*
9:30 AM	**Multivariate analysis of protein in solid matrices** *Associate Professor Natalie Medlicott, University of Otago, New Zealand*
9:50 AM	**Investigating peptide-polymer interactions using MALDI-TOF** *Dr Arlene McDowell, University of Otago, New Zealand*
10:10 AM	**Student Podium: Development of bromocriptine mesylate tablet using solid-smedds as drug delivery system** *Miss Sirigul Thongrangsalit, Chulalongkorn University, Thailand*
10:30 AM	**Morning tea**
11:00 AM	**Student Podium: Amorphous furosemide salt exhibits higher dissolution rate and stability compared to amorphous furosemide acid** *Miss Line Hagner Nielsen, University of Copenhagen, Denmark*
11:20 AM	**Basic concepts of the Synchrotron and its application in solid state characterization** *Associate Professor Ben Boyd, Monash University, Australia*
11:50 AM	**Studying the coating structure of solid particles using synchrotron techniques** *Dr Felix Meiser, Monash University, Australia*
12:10 PM	**Solid state characterisation in lipid dosage forms** *Associate Professor Annette Müllertz, University of Copenhagen, Denmark*

Morning Short Course 3

PHARMACOKINETIC-PHARMACODYNAMIC MODELLING IN DRUG SAFETY AND EFFICACY

Course Chair: Professor Carl Kirkpatrick, Monash University, Australia
Location: Lecture Theatre 3

The pharmacometrics short course will provide attendees the opportunity to gain an understanding of the area of pharmacometrics and how it is applied from a pre-clinical setting through to phase IV clinical research to evaluate safety and efficacy. There will be emphasis on the utility of pharmacometrics in drug development and academic research to unpack and support research programs. The four speakers in the short-course are world leading researchers and/or clinical drug developers who will share with the attendees their real world experience across a number of clinical areas. The speakers will provide the rationale for adopting pharmacometric approaches to support their research programs, benefits achieved, and synergies with other parts of their research programs. To obtain the most out of this workshop, it would be expected that the attendees for this workshop have an understanding of the time course of drug response i.e. pharmacokinetics and pharmacodynamics.

Morning Short Course 3 Timetable

Time	Lecture and Presenter
9:00 AM	**Introduction** *Professor Carl Kirkpatrick, Monash University, Australia*
9:10 AM	**Overview of pharmacometrics as a discipline with perspectives on its application to safety and efficacy** *Professor Hartmut Derendorf, University of Florida, USA*
9:35 AM	**Pharmacometrics in drug safety and efficacy for anti-infective agents** *Dr Craig Rayner, Roche, Australia*
10:00 AM	**Student Podium: Breath testing to assess definative adherence to oral and vaginal medications** *Dr Daniel Gonzalez, University of Florida, USA*
10:20 AM	**Morning Tea**
11:00 AM	**Student Podium: Assessment of juvenile pigs to serve as human pediatric surrogates for preclinical formulation pharmacokinetic testing** *Dr Wyatt Roth, Purdue University, USA*
11:20 AM	**Why use mechanism-based models and how should they be used for efficacy and safety?** *Dr Jurgen Bulitta, Monash University, Australia*
11:45 AM	**Pharmacometrics to gain insights into the pharmacology of hypoxia-activated anticancer prodrugs** *Dr Kashyap Patel, Monash University, Australia*
12:10 PM	**Panel discussion and questions** *Professor Carl Kirkpatrick, Monash University, Australia*

Morning Short Course 4

LEAD OPTIMISATION IN DRUG DISCOVERY AND DEVELOPMENT

Course Chair: Professor Susan Charman, Monash University, Australia
Location: Lecture Theatre 5

Optimisation of the pharmaceutical properties of new drug candidates has become an essential component of modern drug discovery and plays a critical role in compound design and progression. In recent years, significant progress has been made to reduce the frequency with which new chemical entities (NCEs) fail in clinical development as a result of poor pharmacokinetic properties. This trend stems not only from an increased understanding of the chemical and biological factors contributing to a compound's pharmacokinetic characteristics, but also to the widespread application of screening methodologies to assess physicochemical and metabolic liabilities during lead optimization. Medicinal chemists now rely heavily on early stage assessment of "drug-like" properties; including solubility, permeability, and metabolic stability, to inform compound design and guide lead optimization. Inherent in this process is the need to relate specific chemical properties of candidate compounds to their absorption, distribution, and clearance characteristics and to utilize this information in a predictive manner. In this short course, the presenters will focus on intrinsic pharmaceutical properties which underpin the pharmacokinetic characteristics of drug candidates, and will share their experiences and strategies for assessing these properties during drug discovery and early development.

Morning Short Course 4 Timetable

Time	Lecture and Presenter
9:00 AM	**Introductory comments** *Professor Susan Charman, Monash University, Australia*
9:10 AM	**Biorelevant solubility profiling** *Professor Patrick Augustijns, KU Leuven, Belgium*
9:40 AM	**Permeability and transport profiling** *Professor Per Artursson, Uppsala University, Sweden*
10:10 AM	**Accelerating drug discovery and development using MRM3 experiments for faster and more sensitive analysis** *Dr. Frank Rooney, ABSciex, Australia*
10:25 AM	**Morning Tea**
11:00 AM	**Strategic use of in vitro and in vivo ADME studies in drug discovery and development** *Professor Dhiren Thakker, University of North Carolina, USA*
11:30 AM	**Application of computational models in drug discovery** *Dr. Dan Mudra, Eli Lilly, USA*
12:00 PM	**Case studies: from drug discovery and development to the clinic** *Dr. Bianca Liederer, Genentech, USA*
12:30 PM	**Closing comments** *Professor Susan Charman, Monash University, Australia*

Afternoon Short Course 1

APPROACHES FOR ENHANCING ORAL DELIVERY OF POORLY-WATER SOLUBLE DRUGS

Course Chair: Professor Chris Porter, Monash University, Australia
Location: Lecture Theatre 1

Drug discovery programs are increasingly identifying drug candidates that are highly potent but which also have intrinsically low water solubility. Indeed recent estimates suggest that up to 70% of development candidates may be regarded as 'poorly water soluble'. The reasons for these trends are difficult to identify with certainty – but the increasing use of combinatorial chemistry approaches to synthesis and high throughput cellular screens in biology has likely accelerated the frequency with which highly lipophilic molecules are identified as potential 'hits'.

Low aqueous solubility presents a number of challenges. The most obvious of these is poor solubility in the fluids within the gastrointestinal tract and therefore low and variable absorption. In turn this leads to low and unpredictable exposure and activity. This ultimately has ramifications in designing a robust commercial product, but also has significant implications earlier in discovery where low solubility may limit the exposure required to support early proof of concept activity data and toxicity studies.

One solution to the identification of drug candidates with low solubility is structural modification in an attempt to reduce hydrophobicity. In many cases, however, changes to chemical structure lead to parallel decreases in activity and the preferred development candidate remains highly lipophilic and poorly water soluble.

This short course aims to provide a brief review of the issues associated with low water solubility and the approaches that can be taken to minimize and overcome these problems. Subsequently, expert presenters will describe in more detail four common approaches that can be taken to try to promote exposure after oral administration of a poorly water soluble drug. These include alternate salt and crystal forms, solid dispersions, lipid based formulations and cyclodextrins. Two short talks will also be chosen for this session from the submitted presentations.

Afternoon Short Course 1 Timetable

Time	Lecture and Presenter
1:30 PM	**Introduction** *Professor Chris Porter, Monash University, Australia*
1:45 PM	**Altering solid state properties to enhance the dissolution of poorly water soluble drug molecules** *Professor Robin Bogner, University of Connecticut, USA*
2:15 PM	**Using solid dispersions to enhance the bioavailability of poorly water soluble drugs** *Professor Thomas Rades, University of Copenhagen, Denmark*
2:45 PM	**Student Podium: Comparison of biorelevant media and USP compendial media on solution-mediated phase transformation of an amorphous drug during dissolution** *Mrs Mary Kleppe, University of Connecticut, USA*
3:05 PM	**Afternoon tea**
3:30 PM	**Student Podium: Vehicle elicited improvement of intestinal absorption of a phytopharmaceutical compound in the Caco-2 model via increased stability and reduced metabolism** *Ms Ursula Thormann, University of Basel, Switzerland*
3:50 PM	**Enhanced drug solubilisation via lipid based drug delivery systems** *Professor Colin Pouton, Monash University, Australia*
4:20 PM	**Cyclodextrins as a mechanism of solubility and bioavailability enhancement** *Professor Valentino Stella, University of Kansas, USA*
4:50 PM	**General Discussion** *Professor Chris Porter, Monash University, Australia*

Afternoon Short Course 2

FORMULATION AND STABILITY OF PROTEINS, ANTIBODIES AND VACCINES

Course Chair: Professor Christian Schöneich, University of Kansas, USA
Location: Lecture Theatre 2

The development of therapeutic proteins and vaccines is an important area of research and development in the biopharmaceutical industry with currently > 40 antibodies/vaccines in various clinical trials and a pipeline encompassing ca. 350 antibody candidates. Key to the successful formulation of therapeutic proteins and vaccines is to overcome problems related to physical and chemical degradation. Protein degradation can not only lead to a loss of potency but also to changes in bioavailability and, potentially, to immunogenicity. This short course will deal with key mechanisms involved in the physical and chemical degradation of proteins, the potential consequences, and formulation strategies to overcome these stability problems. Each section will introduce well-accepted mechanisms and paradigms, and then cover new results in the respective focus areas in order to acquaint the student with key developments, which may lead to new paradigms important to create stable formulations of therapeutic proteins and vaccines.

Afternoon Short Course 2 Timetable

Time	Lecture and Presenter
1:30 PM	**Chemical stability of proteins: hydrolysis** *Professor Teruna Siahaan, University of Kansas, USA*
1:50 PM	**Chemical stability of proteins: oxidation, photolysis, fragmentation and chemical aggregation** *Professor Christian Schöneich, University of Kansas, USA*
2:10 PM	**Physical stability of proteins: conformational analysis upon variation of formulation conditions** *Associate Professor Jennifer Laurence, University of Kansas, USA*
2:30 PM	**General discussion and questions**
2:40 PM	**Student Podium: Chemical modifications in aggregates of recombinant human insulin induced by metal-catalyzed oxidation: covalent cross-linking via Michael addition to tyrosine oxidation products** *Mr Riccardo Torosantucci, Leiden University, Netherlands*
3:00 PM	**Afternoon tea**
3:30 PM	**Student Podium: Development of lipopeptide vaccine candidate against group a streptococcus** *Miss Saranya Chandrudu, University of Queensland, Australia*
3:50 PM	**Protein formulation and immunogenicity** *Professor Wim Jiskoot, Leiden University, Netherlands*
4:10 PM	**Stability and immunogenicity of glycoproteins** *Associate Professor Thomas Tolbert, University of Kansas, USA*
4:30 PM	**Formulation and stability of vaccines** *Professor David Volkin, University of Kansas, USA*
4:50 PM	**General discusion and questions**

Afternoon Short Course 3

IMAGING MODALITIES FOR ASSESSING DRUG DELIVERY AND DISPOSITION

Course Chairs: Dr Erica Sloan, Monash University, Australia and Professor Jashvant Unadkat, University of Washington, USA
Location: Lecture Theatre 3

In vivo imaging modalities have revolutionized pharmaceutical research by allowing longitudinal assessment of drug delivery and disposition in vivo. **T**he ability to track drug-target interactions in real time also allows analyses of biological responses to pharmaceutical intervention. In vivo imaging modalities include optical imaging, CT, PET, MRI and advanced in vivo microscopy. These technologies allow investigation of molecular and anatomical drug interactions as well as assessment of metabolism and physiology. This short course will describe imaging modalities and discuss their use in cutting-edge pharmaceutical research. We will cover assessment of pharmacodynamics, evaluation of drug response and optimal dosage, and investigation of drug toxicity and host response to treatment. Talks will also describe the use of multiple imaging modalities to increase the depth of analysis of pharmaceutical processes and biological responses.

Afternoon Short Course 3 Timetable

Time	Lecture and Presenter
1:30 PM	**Introduction** *Dr Erica Sloan, Monash University, Australia and Professor Jashvant Unadkat, University of Washington, USA*
1:35 PM	**Theranostic nanoparticles for imaging and drug delivery** *Professor Andrew Mackay, University of Southern California, USA*
2:00 PM	**In vivo bioluminescence to target stressed tumours** *Mr Ming Chai, Monash University, Australia*
2:25 PM	**Use of PET to image GI drug absorption** *Professor Shinji Yamashita, Setsunan University, Japan*
2:50 PM	**Student Podium: Magnetic targeting of magnetic-fluid-loaded liposomes (MFLs) to brain for MRI diagnosis and treatment of glioblastoma** *Miss Helene Marie, Paris-Sud, France*
3:10 PM	**Afternoon tea**
3:30 PM	**Student Podium: Theranostic nanoparticles for the diagnosis and treatment of HCC** *Dr Yongjun Liu, Shandong University, China*
3:50 PM	**Multi-modal imaging to develop new therapeutic strategies for osteosarcoma** *Dr Carl Walkley, St. Vincent's Institute of Medical Research, Australia*
4:15 PM	**PET imaging P-glycoprotein function and inhibition at the human BBB** *Professor Jashvant Unadkat, University of Washington, USA*
4:40 PM	**Concluding remarks** *Dr Erica Sloan, Monash University, Australia and Professor Jashvant Unadkat, University of Washington, USA*

Afternoon Short Course 4

TRANSPORTERS AS MEDIATORS OF DRUG DISPOSITION IN HEALTH AND DISEASE

Course Chair: Professor Kim Brouwer, University of North Carolina, USA and Professor Peter Swaan, University of Maryland, USA
Location: Lecture Theatre 5

Transport proteins play an important role in the absorption, distribution and elimination of many endogenous and exogenous compounds, including drugs and metabolites. Transporters located in the gastrointestinal tract, liver, kidney, and blood-brain barrier can have a major influence on the pharmacokinetics and pharmacodynamics of drug substrates. Characterization of transport proteins at the molecular level, including an understanding of how alterations associated with specific disease states impact the regulation and function of these proteins, is a rapidly evolving field. Many in vitro, in silico, and in vivo methods can be utilized to study the function and regulation of transport proteins, to identify interactions in substrate transport, and to predict the consequences of disease-associated alterations in transport on drug disposition, efficacy and/or toxicity. This short course will provide an update on the prediction of drug-transport interactions, as well as the regulation of transport proteins at the transcriptional level. The role of bile acid transporters in drug absorption and disposition in health and disease will be reviewed. The impact of two disease states, liver disease and neurological disorders, on transporter expression and function will be highlighted. How these specific diseases alter transporter-mediated hepatobiliary disposition of drugs and endogenous substrates, and influence drug disposition at the blood-brain barrier will be discussed. In addition to providing an update on our current knowledge of transport proteins as mediators of drug disposition, future directions of research in this important field will be highlighted.

Afternoon Short Course 4 Timetable

Time	Lecture and Presenter
1:30 PM	**Overview of transporters and importance in drug disposition** *Professor Kim Brouwer, University of North Carolina, USA*
1:35 PM	**Drug-transport interactions: kinetic concepts and clinical studies to demonstrate relevance of in vitro model predictions** *Associate Professor Hiroyuki Kusuhara, University of Tokyo, Japan*
2:05 PM	**Transcriptional regulation of transporters** *Professor Paavo Honkakoski, University of Kuopio, Finland*
2:35 PM	**Student Podium: A role of prostaglandin transporter in regulation of intercellular PGE2 levels under inflammatory conditions** *Mr Taku Kasai, Kanazawa University, Japan*
2:55 PM	**Afternoon tea**
3:30 PM	**Student Podium: Functional characterization of organic cation transport in HEK293 cells** *Mr Kazumasa Kobayashi, Tokyo University, Japan*
3:50 PM	**Role of bile acid transporters in drug absorption and disposition** *Professor Peter Swaan, University of Maryland, USA*
4:15 PM	**Altered hepatic transport of drugs and endogenous substrates in liver disease** *Professor Kim Brouwer, University of North Carolina, USA*
4:40 PM	**The impact of neurological diseases on expression and function of drug transporters at the blood-brain barrier** *Dr Joseph Nicolazzo, Monash University, Australia*

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General Information

Registration and welcome desk

The Registration & Customer Service Desk for GPEN 2012 will be located at the Faculty of Pharmacy and Pharmaceutical Sciences (Monash University) reception, ground floor of Sissons Building between 3:00 pm and 9:00 pm, Wednesday 28th November 2012 and between 8:00 am and 6:00 pm, Thursday 29th November – Saturday 1st December 2012.

Transportation

Travelling to the Parkville campus

By tram: the campus is serviced by tram route 19 (stop 16, Walker Street) which travels along Elizabeth Street and Royal Parade. A tram trip to the campus from Melbourne CBD takes approximately 15 minutes.

By train: catch the Upfield line train to Royal Park Railway Station. The Parkville campus is a short walk from Royal Park train station.

By car: the campus is located on Royal Parade in Parkville, 3 km north of Melbourne city centre. A number of metered parking is available around, however there is no parking on campus.

The GPEN 2012 Organising Committee have provided all delegates with a Myki™ card, containing $20 travel credit, for use on public transport services. We recommend using public transport (tram) to travel to the conference each morning to avoid traffic congestion.

Bus transportation provided by GPEN

After conference events, a free shuttle bus transport service will be provided from Monash University Parkville Campus, RACV club or Mercat to designated conference hotels. The estimated depature times are listed below.

Date	Event	First Bus Depature Time
Wednesday 28th Nov	Welcome Reception	9:00 PM
Thursday 29th Nov	BBQ Dinner	8:45 PM
Friday 30th Nov	Conference Banquet	10:30 PM
Saturday 1st Dec	Social Event	10.00 PM
Sunday 2nd Dec	Day Trip	Dep: 9:15 AM Return: 5:00 PM

Medical information

If you are feeling unwell or injure yourself during the conference please contact security, reception or your friendly GPEN committee members and medical help will be arranged. If you become ill outside of the conference, various medical clinics are avaliable around the Melbourne CBD. Alternatively, contact emergency services directly by dialing 000.

Internet access

Wifi internet access will be available on campus for the duration of the meeting. The Wifi user account name is **ext-ddagpen**. The Wifi password will be communicated to delegates at the Welcome Reception. If you are unable to attend the welcome reception and require the Wifi password please ask one of the organising committee members.

Faculty computers are also be available for use during breaks. For more information, please see the Registration & Customer Service desk upon arrival.

Laptop lockers and chargers

Free laptop lockers will be available for delegates to store and charge their laptops. For more information, please see the Registration & Customer Service desk upon arrival.

Conference Hotel Information

Vibe Hotel
441 Royal Parade
Parkville VIC 3052

Tel: +61 3 9380 9222
Fax: +61 3 9387 6846
Website: http://www.vibehotels.com.au/

Novotel Melbourne
270 Collins Street
Melbourne VIC 3000

Tel: +61 3 9667 5800
Fax: +61 3 9667 5805
Website: http://www.novotelmelbourne.com.au/

IBIS Hotel on Bourke
600 Little Bourke Street
Melbourne 3000

Tel: +61 3 9672 0000
Fax: +61 3 9672 0123
Website: http://www.ibishotel.com/gb/home/index.shtml

IBIS Hotel on Therry
15-21 Therry Street
Melbourne VIC 3000

Tel: +61 3 9666 0000
Fax: +61 3 9666 0052
Website: http://www.ibishotel.com/gb/home/index.shtml

Jasper Hotel
489 Elizabeth Street
Melbourne 3000

Tel: +61 3 8327 2777
Fax: +61 3 9329 1469
Website: http://www.jasperhotel.com.au/

Campus Map

Monash University Parkville campus

Faculty of Pharmacy and Pharmaceutical Sciences

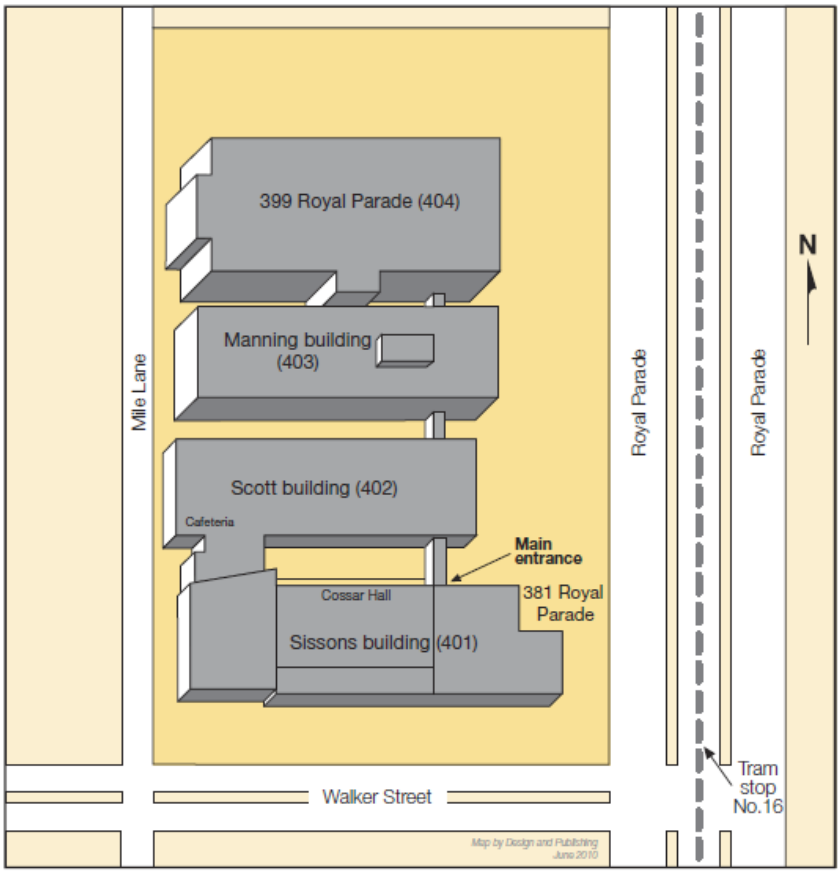
Building 401
- G Reception
- G Student services
- G Cossar Hall
- G Sissons meeting room
- 1 Board room
- 1 Lecture theatre 5
- 1&2 Tutorial rooms
- 1&2 Lecture theatres 1, 2, 3

Building 402
- G Cafeteria
- G Security office
- G Toilets
- G Facilities and services
- 1 Virtual practice environment 1 and 2
- 1 Professional practice suites 1 and 2
- 1 Centre for Medicine Use and Safety

Building 403
- G Library, CL Butchers Pharmacy Library
- 4 Lecture theatre 4

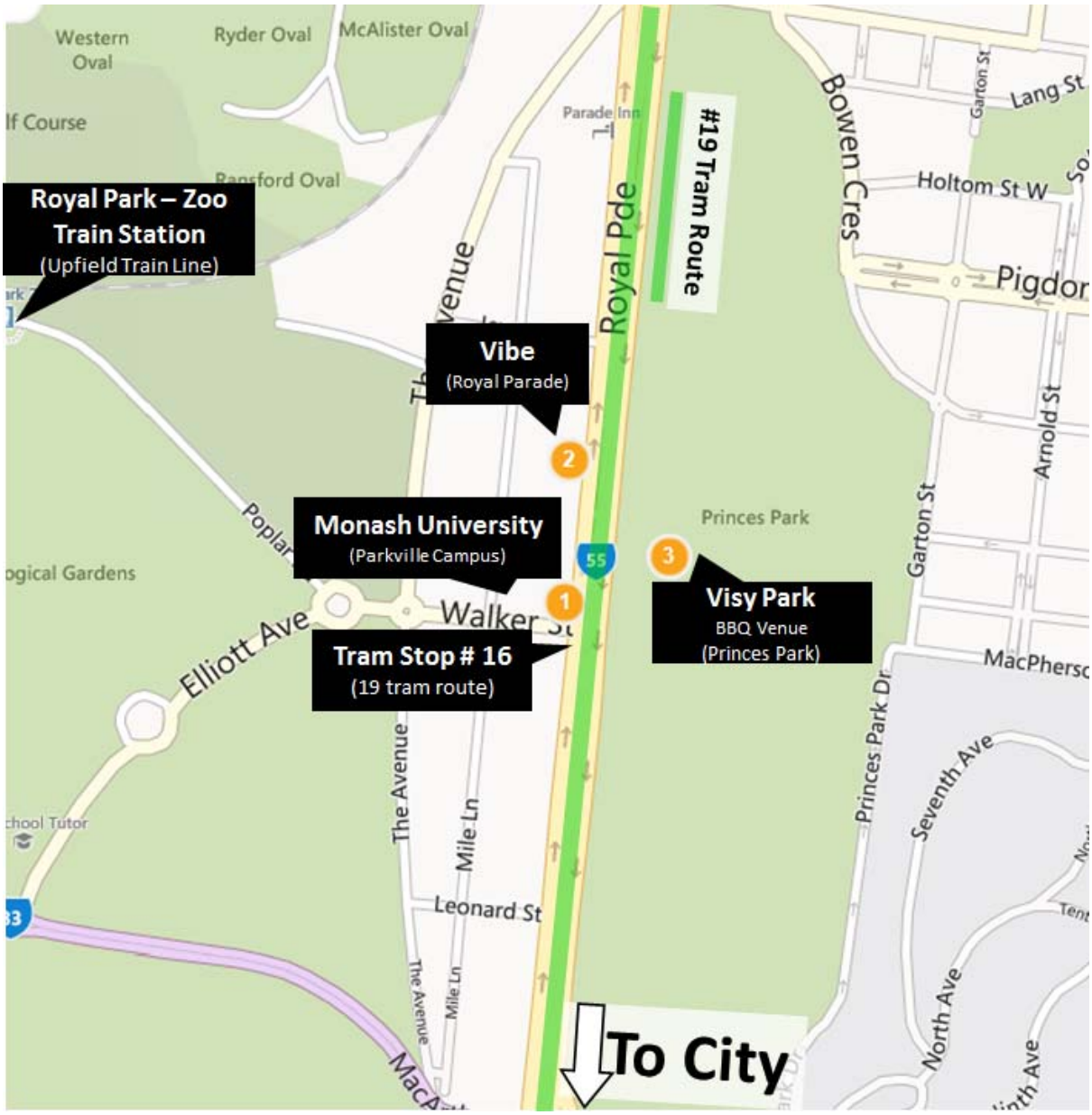
Building 404
- G Office of the Dean
- G Monash Institute of Pharmaceutical Sciences
- G IT services
- G Victorian College of Pharmacy Foundation
- 2 Centre for Drug Candidate Optimisation

Also accommodated at the Parkville campus:
Pharmaceutical Society of Australia (building 401, level 1)



Melbourne CBD Map

Parkville Map



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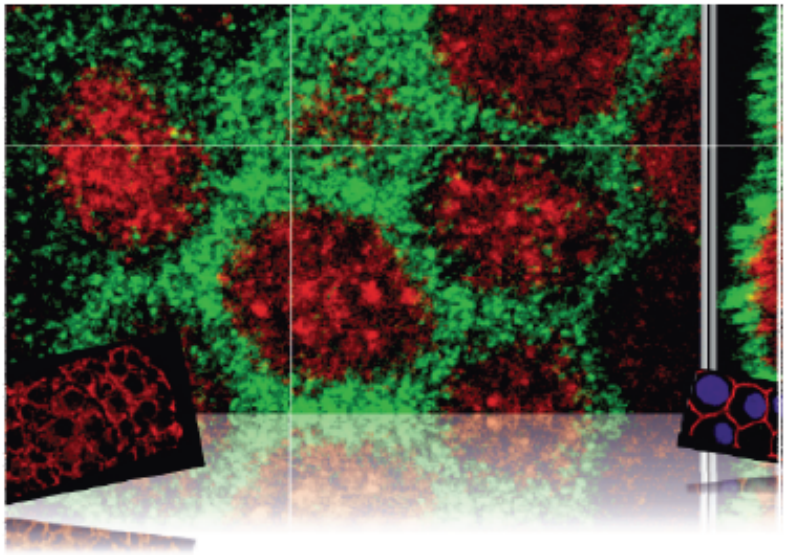
GPEN 2014

Welcome to GPEN 2014 !



GPEN 2014 in Helsinki will be jointly organized by

UNIVERSITY OF HELSINKI and
UNIVERSITY OF EASTERN FINLAND

Enjoy top pharmaceutical science and education, networking and beautiful Finland.

More information will follow.

Notes