Quantitative analysis of intestinal absorption and metabolism of benazepril in rat intestinal single-pass perfusion experiment

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Recently, we tried to develop a prediction method of intestinal hydrolysis during in vivo absorption of prodrug, using the *in vitro-in situ* correlation between intrinsic clearance for hydrolysis (CLint) of prodrug and its hydrolysis rate at steady-state of intestinal absorption in rat intestinal single-pass perfusion experiment. To confirm the relation, we need some data for prodrugs slowly hydrolyzed in intestine. We selected Benazepril that is conjugated ethanol to benazeprilat to improve oral bioavailability. The aim of this study is to evaluate the absorption behavior of Benazepril in mucosal membrane transport. *In vitro* hydrolysis of Benazepril in 9000g supernatant (S9) fraction of rat jejunal mucosa resulted in Km (754±116 µM) and Vmax (2.7±0.2 µL/min/mg protein) from Michaelis–Menten equation. CLint estimated by Vmax/Km was 3.6±0.5 (µL/min/mg). Inhibition experiment by paraoxon, DFP, PCMB and BNPP indicated carboxylesterase as a major enzyme for hydrolysis of Benazepril. *In situ single-pass* perfusion was conducted by simultaneous perfusion of rat jejunum (10 cm) and mesenteric vein by MES buffer (pH 6.5) including 100µM Benazepril and KHBB buffer (pH 7.4) including BSA (3%), respectively. Steady-state was reached at about 90 minutes after perfusion. Permeability coefficient (6.52±1.39 x 10⁻⁵ cm/sec) suggested 100 % intestinal absorption of benazepril. The metabolism clearance and absorption clearance of Benazepril were calculated as 4.5±0.35 (µL/min), 1.2±0.10 (µL/min), respectively. Consequently, 79% of Benazepril taken up into mucosal cells was hydrolyzed to Benazeprilat, and only 37% of that was absorbed into the vein, because Benazeprilat was transported four times faster in intestinal lumen than in vein.