ORIGINAL ARTICLE

Application of Rat *In Situ* Single-pass Intestinal Perfusion in the Evaluation of Presystemic Extraction of Indinavir Under Different Perfusion Rates

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Background/Purpose: First-pass effect has been an important concern for oral pharmaceuticals. An *in vivo* system was developed for measuring different concentrations of pharmaceuticals in the portal vein and hepatic vein (via the inferior vena cava) for delineating presystemic metabolism under different perfusion rates by using indinavir as an exemplary agent.

Methods: An *in situ* single-pass intestinal perfusion technique was modified from previous studies to concomitantly obtain portal and hepatic venous bloods. Portal and hepatic venous samples were simultaneously taken from rats at appropriate time points using the perfusion model of 1 mg/mL indinavir at flow rates of 0.05, 0.1, 0.5 and 1.0 mL/min. The indinavir concentrations were assayed by binary-gradient high-pressure liquid chromatography with UV detection.

Results: The mean indinavir concentrations in portal vein concentration–time profiles at different perfusion times under various flow rates were all higher than those obtained for hepatic veins. At flow rates of 0.5 and 1.0 mL/min, in particular, the area under the curve (AUC) and maximal concentration (C_{max}) of indinavir absorption were significantly different between portal veins and hepatic veins ($p < 0.05$), indicating considerable hepatic involvement in the presystemic extraction of indinavir. The system also has potential for use when estimating the hepatic extraction ratio (E_H) and hepatic clearance (Cl_H).

Conclusion: This *in vivo* approach could provide another useful tool for improving our basic understanding of the absorption kinetics and hepatic metabolism of pharmaceuticals under development and facilitating the clinical application of such. [*J Formos Med Assoc* 2008;107(1):37–45]

Key Words: drug absorption, indinavir, intestinal perfusion

The oral bioavailability of pharmacologically active drugs is often limited by first-pass biotransformation.[1–5] Being able to quantify the magnitude of the contributions made by the intestines and by the liver to first-pass metabolism is certainly of academic and clinical importance. The differentiation of intestinal and hepatic impacts may facilitate delineating the basic mechanisms underlying absorption, developing suitable oral drug-delivery formulations, predicting inter- and intra-individual variability, and optimizing drug therapy. Quantification of the respective magnitudes of gut and hepatic drug-eliminating effects has been attempted by using cyclosporine[6]

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and midazolam[7] as probe drugs in humans. However, the results of these trials are questionable, as they involved indirect measurements, which either assessed discrete intestinal and hepatic extraction by comparing the pharmacokinetic disparity between oral and intravenous administration routes, or which were obtained by performing supplementary *in vitro* experiments and using a theoretical paradigm.

Direct measurements of compound absorption in humans are not always feasible for obvious reasons.[8] Many researchers therefore use rats for their preclinical oral absorption studies. The *in situ* single-pass perfusion animal model, which is used to measure the disappearance of test substances from the perfused intestinal segment, has long been used for studies of drug absorption.[9–11] Assessments of intestinal membrane permeability have shown that this model provides predictive values for *in vitro* absorption of drugs in humans.[8,12,13] Nevertheless, the amount of the drug that disappears from the intestinal lumen may not exactly reflect the amount that emerges in the systemic blood because drug retention, absorption and metabolism are likely to take place before it reaches the systemic circulation. Hence, it is essential that serial blood drainage from mesenteric veins, portal vein or other systemic blood vessels be incorporated into the perfusion model in order to measure true bioavailability subsequent to drug passage through the intestines and liver.

The primary objectives of the present study, therefore, were to: (1) develop an *in vivo* system for the simultaneous quantitative sampling of portal and hepatic venous blood using an *in situ* single-pass intestinal perfusion technique; and (2) investigate the effects of perfusion flow rates on indinavir absorption and presystemic metabolism.

Methods

Materials

Indinavir sulfate 400 mg capsules (MK-639, L-735,524, Crixivan®) for intestinal perfusion were obtained from Merck & Co., Inc. (Elkton, VA, USA). The reference standard of indinavir sulfate was kindly supplied by Merck Research Laboratories (Rahway, NJ, USA). Carboxymethyl cellulose sodium salt (CMC-Na) and sodium pentobarbital were purchased from Sigma (St. Louis, MO, USA). All other reagents, including high-performance liquid chromatography (HPLC)-grade acetonitrile, *n*-hexane, methanol and methyl *tert*-butyl ether (MTBE), were obtained from Merck (Darmstadt, Germany). Highly purified water produced by a Millipore Direct-Q5 system (Billerica, MA, USA) was used for all preparations.

Preparation of standard and perfusion solutions

The stock solution (200 μg/mL) of indinavir sulfate reference standard was prepared by dissolving an accurately weighed amount of the drug in methanol. This was then further diluted in normal saline to produce various standard working solutions. The intestinal perfusion solution was prepared by suspending 400 mg of indinavir in a 0.5% CMC-Na dispersion and then diluting this with normal saline to achieve a final indinavir concentration of 1 mg/mL.

Animals

Male Wistar rats, weighing 366.4 ± 13.5 g (mean ± SD), were obtained from the Laboratory Animal Center of the College of Medicine at the National Taiwan University, Taipei, Taiwan. The animals had free access to water and a standard laboratory rodent diet (#5001; Purina Mills, Richmond, IN, USA) and were housed under conditions of controlled temperature (20–22°C) and lighting (12 hours light/dark cycle, light on at 08:00 hours). The Institutional Animal Care and Use Committee of the College of Medicine, National Taiwan University, reviewed and approved the experimental protocol.

In situ single-pass intestinal perfusion

The perfusion procedure (Figure 1) was adapted from Yu et al with minor modifications as described herein.[14] Rats, having fasted for 24 hours with free access to water prior to the perfusion

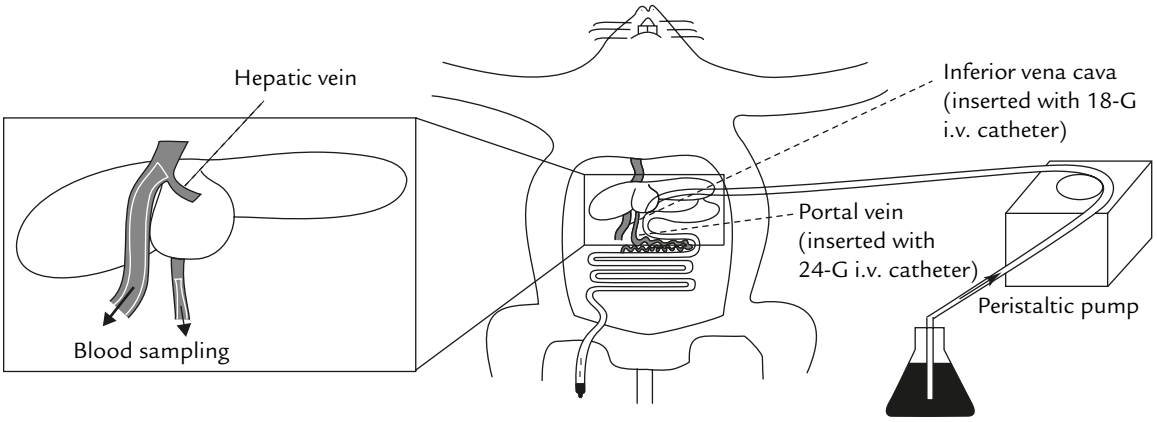

Figure 1. Illustration of the experimental set-up for *in situ* single-pass intestinal perfusion with cannulations for portal and hepatic (via inferior vena cava) venous collections. A 24-gauge i.v. catheter was inserted into the portal vein, whereas the tip of an 18-gauge i.v. catheter was implanted and secured into the junction of the inferior vena cava and hepatic vein for blood collection. The arrows indicate the flow directions of either intestinal perfusate or blood sampling. Please refer to the *Methods* section for details of the surgical and experimental procedures.

study, were anesthetized with 3.5% pentobarbital solution (1 mL/kg, i.p.). After a gentle midline incision, the small intestine segment (which was, on average, 72 cm in length) was selected for inlet (proximal end of duodenum) and outlet (distal end of ileum) cannulation with silicone tubes.

First, the intestinal lumen was cleaned by isotonic saline (37°C) perfusion via the inlet until the effluent from the outlet was judged to be free of feces and clear. The inlet tubing was then filled with warmed (37°C) indinavir perfusion solution, which was pumped at flow rates of either 0.05, 0.1, 0.5 or 1 mL/min using a peristaltic pump (Minipuls 3, Gilson, Villiers le Bel, France). The initial 10-minute drug-perfusion period was considered to be the lag time phase. The collection of venous blood was not initiated until the end of the lag time period. During the perfusion experiment, the exposed intestines were covered with a gauze that had been moistened by frequent applications of warm (37°C) saline, and kept warm by a small lamp placed over the area. The small intestine remained viable throughout the experimental period.

Pharmacokinetic studies and sample preparation

The blood-drawn design adapted from Kukan's liver perfusion procedure is illustrated in Figure 1.[15] The surgical procedure for intravenous (i.v.) catheter implantation, for use when sampling portal and hepatic venous blood, was completed before the initiation of the perfusion study. A 24-gauge i.v. catheter (Surflo, Terumo Corp., Tokyo, Japan) was inserted into the portal vein, whereas an 18-gauge i.v. catheter (Jelco, Ethicon Endo-Surgery, Cincinnati, OH, USA) was inserted into, and secured at, the junction of the hepatic vein and the inferior vena cava (IVC). The tip of the 18-G i.v. catheter was inserted into the IVC from just beneath the liver and gently guided proximally to the hepatic vein junction. The length of the 18-G i.v. catheter embedded in the vessel was 2.3 cm. At the end of the lag time period, 0.4-mL aliquots of blood were withdrawn via alternate syringes from both catheters over a total time period of 2 hours, at the following times: 0, 5, 15, 30, 60, 90 and 120 minutes. Plasma samples were obtained by centrifuging the blood samples at 9000*g* for 15 minutes at 4°C, and were immediately frozen in a deep freezer at –80°C until analysis was carried out.

The plasma specimens were further extracted using procedures modified from reported methods.[16,17] The following were added to 10-mL glass tubes: 150 μL of plasma sample, 150 μL of working internal standard solution (1 μg/mL propylparaben in methanol), 500 μL of 10% NH_4OH and 5 mL of MTBE. The tube was closed with

a Teflon-seated plastic cap. After shaking vigorously for 5 minutes, the samples were subsequently centrifuged at 2100 rpm for 10 minutes at 4°C. The upper organic layer was transferred to a clean glass tube and evaporated to dryness under clean air at room temperature. The residue was dissolved in 500 μL of the starting mobile phase (50 mM phosphate buffer, pH 4.8/acetonitrile: 68/32%) and 3 mL of *n*-hexane. The mixture was then vortexed for 5 minutes and then centrifuged (4°C, 12000*g*, 20 minutes). The upper organic layer was discarded and the eluent was used for HPLC analysis of indinavir.

Liquid chromatography

Indinavir in rat plasma was assayed using a Shimadzu HPLC system (Kyoto, Japan) equipped with two solvent delivery pumps (LC-10AD), a system controller (SCL-10A*VP*), an automatic injector (SIL-10A), a UV-VIS detector (SPD-10AV) and an EverSeiko Gastorr GT-102 degasser (Tokyo, Japan). Instrument control and data analysis were carried out using Shimadzu CLASS-VP software (version 6.12) run through Windows 2000. The chromatographic conditions were adapted from Hugen et al and Yamada et al.[16,17] The column used was a Phenomenex LUNA C_{18}(2) (150 × 4.6 mm, 5 μm particle size [Torrance, CA, USA]), with a small guard column containing the same material (Phenomenex SecurityGuard, 4.0 × 3.0 mm).

Chromatographic analysis was performed at room temperature with gradient elution at a wavelength of 215 nm. Mobile phase A was acetonitrile; phase B was 50 mM Na_2HPO_4 adjusted to pH 4.8 with 85% H_3PO_4. The gradient conditions were as follows: 0–11.5 minutes, 68% B; 11.5–13.5 minutes, 68–57% B; 13.5–32 minutes, 57% B; 32–34 minutes, 57–68% B; 34–40 minutes, 68% B. The injection volume was 50 μL. The total run time of the analysis was 40 minutes at a flow rate of 1.5 mL/min.

Pharmacokinetic analysis

Pharmacokinetic calculations were performed on each individual animal's data using the pharmacokinetic calculation software WinNonlin Enterprise version 4.1 (Pharsight Corp., Mountain View, CA, USA) by a non-compartmental method. The area under the curve (AUC) of the plasma concentration–time curve after oral administration from time zero to 2 hours (AUC_{0-2h}) was calculated using the linear trapezoidal rule. The maximal concentration (C_{max}) and the time to achieve C_{max} (T_{max}) were observed. The absolute bioavailability (F) is defined as a product of F_G (fraction of unmetabolized drug absorbed and passed through the gut into the portal blood) and F_H (the hepatic first-pass availability). The hepatic extraction ratio (E_H) and hepatic clearance (Cl_H) were determined as follows:

$$E_H = (C_{in} - C_{out})/C_{in}$$
$$Cl_H = Q_H \cdot E_H = Q_H \cdot [(C_{in} - C_{out})/C_{in}]$$

The concentrations of indinavir in portal and hepatic venous blood were designated as C_{in} and C_{out}, respectively. The hepatic blood flow (Q_H) used when calculating Cl_H was 65 mL/min/kg.[18,19]

Statistical analysis

The statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). The paired *t* test was used to examine statistical differences between portal and hepatic venous blood concentrations, and one-way ANOVA with *post hoc* analysis to compare the various perfusion flow rates of groups. All values are expressed as mean ± standard deviation. Means were assumed to be statistically significant when $p < 0.05$.

Results

Figure 2 shows the rat portal and hepatic (systemic) plasma concentration–time profiles of indinavir, obtained through *in situ* single-pass intestinal perfusion at 1 mg/mL under four different flow rates (0.05, 0.1, 0.5 and 1.0 mL/min). Mean indinavir concentrations were all higher in portal veins than they were in hepatic veins at different time points at various flow rates. At the 1.0 mL/min flow rate in particular, the indinavir

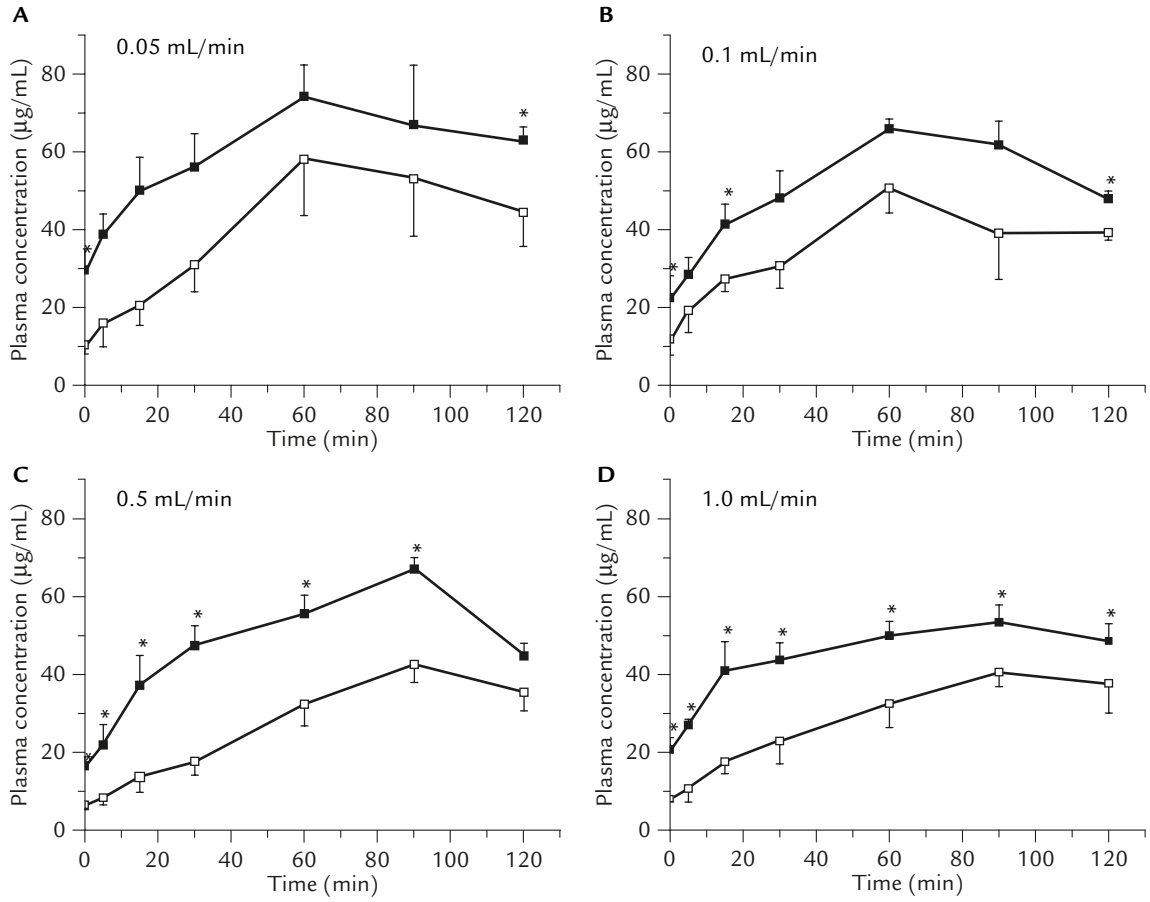

Figure 2. Portal (■) and hepatic (□, at the junction of hepatic vein and inferior vena cava) plasma concentration–time profiles after *in situ* single-pass intestinal perfusion of indinavir (1 mg/min) to anesthetized rats at various flow rates. Venous blood collection was not started during the initial 10-minute drug-perfusion period (lag time). *Concentrations between portal and hepatic (systemic) veins were significantly different ($p < 0.05$, paired t test). Results are given as mean ± standard deviation ($n = 3$).

concentrations of the two venous groups were significantly different ($p < 0.05$) at all time points studied, indicating considerable presystemic metabolism of indinavir in the liver. The basal plasma concentration recorded at time zero was due to an initial 10-minute lag time period before venous blood collections began.

Additionally, the extent (AUC_{0-2h}) and maximal concentration (C_{max}) of indinavir absorption between the portal and hepatic venous groups were significantly different at the flow rates of 0.5 and 1.0 mL/min (0.5 mL/min: $AUC_{0-2h} = 6077.8 \pm 413.6$ *vs.* 3419.6 ± 272.6 μg/min/mL, $C_{max} = 66.9 \pm 3.0$ *vs.* 42.7 ± 4.3 μg/mL; 1.0 mL/min: $AUC_{0-2h} = 5570.4 \pm 388.3$ *vs.* 3587.7 ± 590.0 μg/min/mL, $C_{max} = 53.3 \pm 4.5$ *vs.* 40.6 ± 3.8 μg/mL; $p < 0.05$; Table 1). Further comparative analyses of AUC_{0-2h}, C_{max} and T_{max} at various flow rates within the same venous groups were also performed (Table 1). There were no statistical differences within the hepatic group. However, comparisons of the AUC_{0-2h} at flow rates 0.05 mL/min and 1.00 mL/min within the portal group did reach statistical difference (7437.2 ± 975.5 *vs.* 5570.4 ± 388.3 μg/min/mL, $p < 0.05$).

Within both portal and hepatic groups, the absorption process seemed to be deferred at higher flow rates because a lower C_{max} (Figure 3A) and a longer T_{max} (Figure 3B) were observed. In addition, the magnitude of AUC_{0-2h} (Figure 3C), which was evaluated using the linear trapezoidal method, and absolute bioavailability (Table 1) were also apparently correlated inversely with perfusion flow rate—that is, a higher amount of

Table 1. Pharmacokinetic parameters of indinavir after *in situ* single-pass intestinal perfusion of indinavir (1 mg/min) to anesthetized rats at various flow rates

Flow rate (mL/min)	Portal venous blood			Hepatic venous blood			Availability		
	$AUC_{0-2h,P}$ (μg/min/mL)	$C_{max,P}$ (μg/mL)	$T_{max,P}$ (min)	$AUC_{0-2h,H}$ (μg/min/mL)	$C_{max,H}$ (μg/mL)	$T_{max,H}$ (min)	F_G*	F_H†	F‡ (= $F_G \times F_H$)
0.05	7437.2 ± 975.5§	74.9 ± 8.9	70.0 ± 17.3	5099.8 ± 1,229.2	58.6 ± 14.3	70.0 ± 17.3	0.062	0.686	0.043
0.10	6420.9 ± 390.7	66.0 ± 2.6	70.0 ± 17.3	4487.9 ± 772.7	50.7 ± 6.4	60.0 ± 0.0	0.053	0.699	0.037
0.50	6077.8 ± 413.6¶	66.9 ± 3.0‖	90.0 ± 0.0	3419.6 ± 272.6¶	42.7 ± 4.3‖	80.0 ± 17.3	0.051	0.563	0.029
1.00	5570.4 ± 388.3§¶	53.3 ± 4.5‖	90.0 ± 0.0	3587.7 ± 590.0¶	40.6 ± 3.8‖	100.0 ± 17.3	0.046	0.644	0.030

**F_G = fraction of drug absorbed and passed through the gut into the portal blood unmetabolized, calculated by $AUC_{0-2h,P}/AUC_{GI}$, where AUC_{GI} is the product of indinavir concentration (1000 μg/mL) and perfusion time (120 min); †F_H = the hepatic first-pass availability, calculated by $AUC_{0-2h,P}/AUC_{0-2h,H}$; ‡F = absolute bioavailability, the product of F_G and F_H, results are mean ± standard deviation of three rats per group; §$p < 0.05$, comparison of $AUC_{0-2h,P}$ between flow rates of 0.05 and 1.00 mL/min within the portal venous group; ‖$p < 0.05$, comparison of C_{max} between portal and hepatic groups under the same flow rates—that is, 0.50 and 1.00 mL/min, respectively; ¶$p < 0.05$, comparison of AUC_{0-2h} between portal and hepatic groups under the same flow rates—that is, 0.50 and 1.00 mL/min, respectively.*

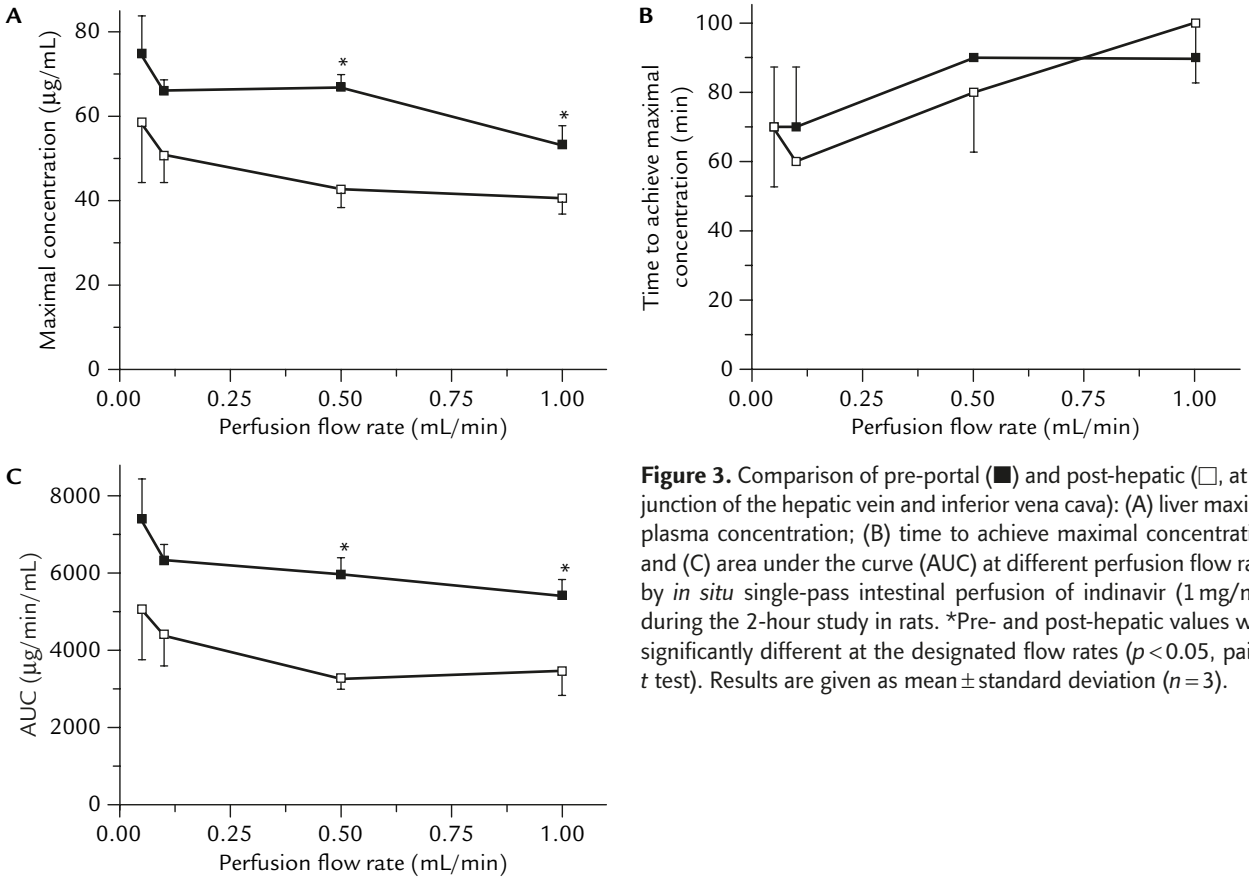

Figure 3. Comparison of pre-portal (■) and post-hepatic (□, at the junction of the hepatic vein and inferior vena cava): (A) liver maximal plasma concentration; (B) time to achieve maximal concentration; and (C) area under the curve (AUC) at different perfusion flow rates by *in situ* single-pass intestinal perfusion of indinavir (1 mg/min) during the 2-hour study in rats. *Pre- and post-hepatic values were significantly different at the designated flow rates ($p < 0.05$, paired *t* test). Results are given as mean ± standard deviation ($n = 3$).

absorption at lower rates. These data imply that the indinavir intestinal absorption process is flow-rate-dependent and that a considerable presystemic metabolization exists.

To quantify the extent of this presystemic metabolization, E_H and Cl_H at various flow rates were assessed (Table 2). Basically, both E_H and Cl_H gradually decreased with time, perhaps due to intestinal viability, circulatory integrity, and enzyme saturation under conditions of continuous perfusion. The statistical comparison between various flow rates of E_H and Cl_H at respective perfusion times revealed no difference except at 15 minutes after perfusion, at which E_H and Cl_H

Table 2. Hepatic extraction ratio (E_H) and clearance (Cl_H) of indinavir (1 mg/min) after *in situ* single-pass intestinal perfusion in rats at various flow rates*

Flow rate (mL/min)	0 min	5 min	15 min	30 min	60 min	90 min	120 min	Average
Hepatic extraction ratio (E_H)								
0.05	0.64 ± 0.09	0.57 ± 0.19	0.58 ± 0.14	0.43 ± 0.20	0.22 ± 0.16	0.32 ± 0.15	0.30 ± 0.10	0.44 ± 0.20
0.10	0.47 ± 0.11	0.31 ± 0.21	0.34 ± 0.07†	0.36 ± 0.13	0.23 ± 0.12	0.36 ± 0.23	0.18 ± 0.06	0.32 ± 0.15
0.50	0.62 ± 0.07	0.61 ± 0.07	0.63 ± 0.07†	0.63 ± 0.06	0.42 ± 0.10	0.37 ± 0.04	0.21 ± 0.11	0.50 ± 0.17
1.00	0.61 ± 0.07	0.60 ± 0.13	0.57 ± 0.09	0.48 ± 0.13	0.35 ± 0.08	0.24 ± 0.01	0.23 ± 0.09	0.44 ± 0.18
Clearance (Cl_H)								
0.05	15.4 ± 2.6	13.9 ± 5.1	13.9 ± 3.9	10.3 ± 4.8	5.3 ± 4.0	7.6 ± 4.1	7.2 ± 2.7	10.7 ± 5.0
0.10	11.5 ± 2.6	7.7 ± 5.0	8.3 ± 1.7†	8.6 ± 3.1	5.6 ± 2.8	8.7 ± 5.6	4.4 ± 1.5	7.8 ± 3.6
0.50	14.9 ± 1.4	14.8 ± 1.7	15.2 ± 1.5†	15.2 ± 1.1	10.1 ± 2.5	8.8 ± 0.9	5.0 ± 2.7	12.0 ± 4.2
1.00	14.7 ± 2.0	14.7 ± 3.5	13.7 ± 2.6	11.6 ± 3.4	8.6 ± 2.1	5.8 ± 0.3	5.6 ± 2.4	10.7 ± 4.4

**Results are presented as mean ± standard deviation of three rats per group; †$p < 0.05$, comparison between flow rates of 0.10 and 0.50 mL/min.*

between 0.10 and 0.50 mL/min were found to be statistically different (E_H: 0.34 ± 0.07 *vs.* 0.63 ± 0.07, $p < 0.05$; Cl_H: 8.3 ± 1.7 *vs.* 15.2 ± 1.5, $p < 0.05$; Table 2).

Discussion

An *in vivo* intestinal absorption animal model may provide intact blood circulation and neural connections for solute uptake and waste product disposal with reasonable tissue viability. Barr and Riegelman pioneered the mesenteric venous blood collection technique for the analysis of salicylamide absorption across rabbit intestines using both perfused-loop and closed-loop methods.[10] Likewise, another closed-loop method involving *in situ* rat intestinal techniques has also been modified and combined with blood sampling, via the jejunal vein, in order to study carbenoxolone absorption characteristics.[20,21]

To further determine the first-pass metabolism of pharmaceuticals traversing the liver as well as the intestines, Doluisio's closed-loop model[20] has been modified to include either sequential portal-jugular or portal-femoral dual venous drainage, or to incorporate an additional rat intraportal infusion technique with femoral artery sampling.[22–24] Examples of such pharmaceuticals include haloperidol, fenoctimine, tolmetin, tolmetin glycine amide and tacrolimus in rats, and GTS-21 in beagles. Despite these advances, however, Doluisio's closed-loop model has been reported to be, in comparison with the single-pass perfusion model, inherently less reliable in terms of its ability to determine the absorption kinetics of theophylline.[11]

The experimental system of this study not only used the more stable *in situ* single-pass intestinal perfusion technique, but also designed and integrated a more juxtahepatic approach to obtain portal and hepatic (systemic) venous blood simultaneously. It is well known that drug-metabolizing enzymes such as cytochrome P_{450} may localize in organs other than the intestines and the liver—for example, in the lung and cardiovascular systems.[25–28] As a result, direct blood sampling from or via the hepatic vein may be superior to that from jugular or femoral blood vessels in order to obtain unambiguous data that can be used to assess first-pass extraction through the liver. In this way, the confounding data that occurs as a result of the extrahepatic systemic metabolism that occurs during the movement of a drug between the hepatic vein and commonly used blood collection sites, such as the jugular vein, femoral vein or femoral artery, can be avoided.

Indinavir is an HIV protease inhibitor that is metabolized by both the intestines and liver.[29,30] Although pharmacokinetic studies of indinavir

in rats have been published elsewhere,[19,31–34] our study model provides a means for directly analyzing presystemic metabolism by comparing differences between portal and hepatic concentrations at various perfusion flow rates. Our data demonstrated significant venous concentration differences between portal and hepatic veins, especially at 0.5 and 1.0 mL/min (see Figures 2 and 3), indicating considerable presystemic extraction of indinavir in the liver.

Our experimental design could also be used to study how different perfusion rates influence the pharmacokinetic profiles of indinavir. The flow rates used in the study were in the range of 0.05 mL/min to 1.0 mL/min, encompassing the reported optimal flow rate for minimizing variability in *in situ* absorption studies of iopanic acid.[35] Our results implied that the absorption pace and the amount absorbed were inversely correlated with flow rates—that is, a faster speed and a greater amount of absorption at lower flow rates (Figures 2 and 3, Table 1). Previous literature has specified that a rapid intestinal transit tends to result in a decrease in steroid absorption due to insufficient physical contact between the drug in question and the intestinal mucosa.[36] Our data provide the first evidence to show that indinavir absorption is also affected by luminal fluid rate. This observation may imply that it might be appropriate for preclinical pharmaceutical researchers to adjust the flow rates of luminal administration in order to identify optimal absorption kinetics.

However, the model developed here may underestimate the true value of C_{out}, due to a lack of practical rat liver physiology values. This will lead to E_H overestimation because it is well known that the human liver receives blood via both the portal vein (roughly 78% of its supply) and the hepatic artery.[37] It must also be noted that our study approach does not take into account drug dissolution, stomach emptying and biliary secretion. These factors should be taken into consideration in future research.

In summary, an *in vivo* system for the analysis of portal–hepatic concentration differences was developed by using *in situ* single-pass intestinal perfusion combined with direct sampling of portal and hepatic venous blood. The system offers means to evaluate, as a function of drug perfusion flow rate, the respective plasma concentration–time profiles of indinavir after absorption from rat intestine. Integration of corroborative evidence from several different experimental systems is unwaveringly essential in ensuring that animal absorption data are of predictive value to humans.[38] We hope that the near-physiologic *in vivo* approach described in this study will be another useful tool for improving the basic understanding and clinical applications of the absorption kinetics and presystemic metabolism (including hepatic involvement) of pharmaceuticals under development.

Acknowledgments

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