# EFFECT OF DEXAMETHASONE ON THE INTESTINAL FIRST-PASS METABOLISM OF INDINAVIR IN RATS: EVIDENCE OF CYTOCHROME P-450 A AND P-GLYCOPROTEIN INDUCTION

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## ABSTRACT:

Indinavir, a potent and specific inhibitor of HIV protease, is a known substrate of cytochrome P-450 (CYP) 3A and p-glycoprotein. The purpose of this study is to investigate and compare the inducing effect of dexamethasone (DEX) on CYP3A and p-glycoprotein in the hepatic and intestinal first-pass metabolism of indinavir in rats. Pretreatment of rats with DEX had little effect on the pharmacokinetics (Cl and $T_{1/2}$) after i.v. administration of indinavir, whereas DEX markedly altered the peak concentration ($C_{max}$) and bioavailability of indinavir after oral dosing. The $C_{max}$ decreased from 2.8 $\mu$M in control rats to 0.28 $\mu$M in DEX-treated rats, and bioavailability decreased from 28 to 12.4%. The decreased bioavailability after DEX pretreatment was due mainly to an increase in first-pass metabolism. Intestinal first-pass metabolism ($E_G$) increased from 6% in control rats to 34% in DEX-treated rats, and

hepatic first-pass metabolism ($E_H$) increased from 65 to 82%. Analysis of in vitro kinetic data revealed that the increased intestinal and hepatic metabolism by DEX was attributed to an increase in the $V_{max}$, as a result of CYP3A induction, without a significant change in the $K_m$ values. DEX pretreatment also induced p-glycoprotein in the intestine and liver of rats. p-Glycoprotein appeared to increase the intestinal metabolism of indinavir whereas it had little effect on the hepatic metabolism of indinavir. Although it has been suggested that the role of intestinal metabolism for some drugs is quantitatively greater than that of hepatic metabolism in the overall first-pass metabolism, the contribution of intestinal metabolism to the overall first-pass metabolism of indinavir in rats is not quantitatively as important as the hepatic metabolism, regardless of DEX induction.

Although the small intestine is regarded as an absorptive organ in the uptake of orally administered drugs, it also has the ability to metabolize drugs by numerous pathways involving both phase I and II reactions (Renwick and George, 1989; Ilett et al., 1990; Krishna and Klotz, 1994). Almost all of the drug-metabolizing enzymes present in the liver are found in the small intestine, despite the fact that the enzyme levels generally are much lower in the small intestine than in the liver (Lin et al., 1999).

Anatomically, the small intestine has a serial relationship with the liver and is the anterior organ. Thus, the amount of an orally administered drug that reaches the systemic circulation can be reduced by both intestinal and hepatic metabolism. Although it is widely believed that the liver is the major site of first-pass metabolism, recent studies have indicated that the small intestine contributes significantly to the overall first-pass metabolism of many drugs. In some cases, it has even been suggested that the role of intestinal metabolism is quantitatively greater than that of hepatic metabolism in the overall first-pass effect (Wu et al., 1995; Paine et al., 1996; Holtbecker et al., 1996; Fromm et al., 1996). These reports, therefore, raise an important question of whether intestinal metabolism truly plays such an important role in the first-pass effect.

Cytochrome P-450 (CYP)[1] 3A is the dominant CYP in the human small intestine and accounts for the majority of total microsomal P-450 found in the mucosal epithelium of the small intestine (Kolars et al., 1994). CYP3A is also a major CYP in the rat small intestine (Watkins et al., 1987). Recently, the potential role of p-glycoprotein (P-gp), an efflux transporter that is located on the apical brush membrane of the epithelium of the small intestine, in limiting drug absorption has been increasingly appreciated (Benet et al., 1996; Watkins, 1997). It has been proposed that the CYP3A system and P-gp may functionally work together in reducing oral bioavailability of drugs. Literature surveys revealed a striking overlap between substrates for CYP3A4 and P-gp (Wacher et al., 1995). In addition to similarity in substrate specificity, CYP3A and P-gp appeared to be induced by the similar inducer. Salphati and Benet (1998) have shown that both CYP3A and P-gp in rat livers were induced significantly by dexamethasone (DEX).

Indinavir, a potent HIV protease inhibitor that is widely used for the treatment of AIDS, has been demonstrated to be a substrate of the CYP3A system and P-gp in rats and humans and in vitro system (Lin

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[1] Abbreviations used are: CYP, cytochrome P-450; DEX, dexamethasone; Cl, plasma clearance; $E_G$, intestinal first-pass metabolism (extraction ratio); $E_H$, hepatic first-pass metabolism (extraction ratio); P-gp, p-glycoprotein; $[AUMC]_{0-\infty}$, total area under the first moment of the drug concentration curve from zero to infinity; $F$, bioavailability; $Q_H$, hepatic blood flow; $Q_G$, intestinal mucosal blood flow; $Cl_{int,H}$, hepatic intrinsic clearance; $Cl_{int,G}$, intestinal intrinsic clearance; $f_u$, unbound fraction of indinavir in plasma; 3-MC, 3-methylcolanthrene.

et al., 1996; Chiba et al., 1996; Kim et al., 1998). The purpose of this study is to investigate and compare the inducing effect of DEX on CYP3A and P-gp on the hepatic and intestinal first-pass metabolism of indinavir in rats.

## Experimental Procedures

**Materials.** Indinavir and radiolabeled indinavir were synthesized at Merck Research Laboratories. The labeled drug was prepared with $^{14}C$ in the position of the pentanamide. [$^{14}C$]Indinavir was at least 98% pure by HPLC. The specific activity was 32.9 $\mu$Ci/mg for [$^{14}C$]indinavir. L-703,943, *N-tert*-butyl-decahydro-2[(*R*)-hydroxy-4-phenyl-3(*s*)-[3(*s*)-1,1-dioxotetrahydrothien-3-yloxycarbon-ylamino]-butyl]-(4*as*,8*as*) isoquinoline-3(*s*)-carboxyamide, was used as an internal standard for the HPLC assay. Antirat CYP3A2 antibody and preimmune IgG were obtained from Gentest Corporation (Woburn, MA). Monoclonal antibody C219 was purchased from Centocor (Malvern, PA). DEX, testosterone, and horseradish peroxidase were obtained from Sigma (St. Louis, MO). All other reagents were of analytical grade.

**Animals.** Male Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing 250 to 350 g were used for in vitro and in vivo studies. The animals were housed according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and maintained under a 12-h light/dark cycle with access to water. Animals were fed standard laboratory chow ad libitum (Purina Mills, St. Louis, MO). DEX-treated rats received a daily oral dose of DEX at 40 mg/kg/day for 3 days. DEX was dissolved in corn oil and the final concentration was 12 mg/ml. Control rats received oral administration of the vehicle. All animal studies were approved by the Animal Care Committee (IACUC).

**In Vivo Kinetic Studies.** For kinetic studies, all rats had an indwelling cannula (silicone rubber/polyethylene) implanted in the right jugular vein for blood sampling. The surgery was performed under light pentobarbital anesthesia (40 mg/kg i.p.) 1 day before the experiment. During the study, all animals were housed individually in plastic metabolism cages and were unrestrained throughout the experiment.

For i.v. study, DEX-treated ($n = 4$) and control ($n = 4$) rats received a single dose of indinavir at 10 mg/kg. The drug was administered as a solution of dimethyl sulfoxide via the cannula in the right jugular vein. The volume of dimethyl sulfoxide administered was 0.5 ml/kg. Blood samples were collected periodically at appropriate time intervals. Plasma samples were obtained by immediate centrifugation of blood samples and were kept frozen ($-20°C$) until assayed by HPLC. For oral study, DEX-treated and control rats received an oral dose (20 mg/kg) of indinavir as a solution in 0.05 M citric acid after an overnight fast. Blood samples were collected at a predetermined time. The plasma samples were kept frozen ($-20°C$) until assayed by HPLC.

The extent of hepatic first-pass effect of indinavir was determined by comparing the drug concentrations in the systemic circulation during portal vein and femoral vein infusion. Cannulation of the portal and femoral veins was performed 2 h before dosing, under nembutal anesthesia (35 mg/kg i.p.). Indinavir was infused at a constant rate of 12 $\mu$g/min. Blood samples were collected at 15, 30, 45, and 60 min after the infusion. The extent of intestinal first-pass metabolism was determined with the in situ isolated intestine loop preparations. The detailed surgical procedures were described elsewhere (Lin et al., 1996). Briefly, rats were anesthetized with pentobarbital (40 mg/kg i.p.) and a cannula was inserted into the left jugular vein. Implantation of the mesenteric venous cannula and preparation of the isolated intestinal loop via ligation of the top and bottom portion of the proximal jejunum of 15 to 20 cm were then performed. All mesenteric venous blood from the loop was collected via mesenteric venous cannula continuously for 60 min. Replacement rat blood (fresh) was infused simultaneously via the jugular vein cannulation at approximately the same rate that blood drained from the mesenteric venous cannula (0.1–0.15 ml/min). Blood samples were collected every 5 min up to 60 min after intrajejunum injection of [$^{14}C$]indinavir. The plasma samples were analyzed for unchanged drug and radioactivity.

**In Vitro Metabolism Studies.** Hepatic and intestinal microsomes were prepared freshly from DEX-treated and control rats. All animals were sacrificed 24 h after the last treatment. The liver and intestine were excised quickly from the animals and perfused with ice-cold 1.15% KCl. Hepatic and intestinal microsomes were prepared. The metabolism of indinavir and testosterone was measured in a system consisting of an NADPH-generating system and hepatic (or intestinal) microsomes according to the method described elsewhere (Chiba et al., 1997).

**Measurement of Hepatic and Intestinal P-gp and CYP3A.** Liver and intestines were excised from control and DEX-treated rats 24 h after the last treatment and placed immediately in ice-cold buffers before membrane preparation. Liver canalicular membrane and intestinal brush-border membrane vesicles were prepared from the liver and intestine according to the methods described elsewhere (Kessler et al., 1978; Kobayashi et al., 1990). Hepatic and intestinal microsomes were prepared from liver and intestine obtained from the same animal according to the method described previously (Chiba et al., 1997).

SDS electrophoresis was conducted in 6 and 10% Tris-glycine polyacryl-amide gels for P-gp membrane (brush-border and canalicular) vesicle samples and P-450 microsomal samples, respectively, according to the procedure of Laemmli (1970). Before electrophoresis, all samples were solubilized in a solution of SDS and $\beta$-mercaptoethanol. Brush-border and canalicular membrane samples were then left standing at room temperature for 20 min, whereas microsomal samples were heated at 100°C for 3 min. The amounts of protein loaded onto wells were 20, 10, and 5 $\mu$g for intestinal brush-border membrane, liver canalicular membrane, and microsomal (intestine and liver) samples, respectively. Gels were run for 1.5 h at 125 V. Proteins were transferred for 2 h at 30 V onto nitrocellulose membranes after SDS electrophoresis (Towbin et al., 1979). After blocking the nitrocellulose membranes with 5% milk, P-gp was probed using C219 mouse monoclonal antibody at a concentration of 3 $\mu$g/ml for 16 h at 4°C, whereas CYP3A2 was probed using anti-CYP3A2 goat antiserum at a dilution of 1:600 for 1 h at room temperature. The membranes were washed and then incubated with anti-mouse or anti-goat antibody raised in rabbit diluted 1:1000. After washing the membranes, goat anti-rabbit anti-body, conjugated with a horseradish peroxidase diluted 1:1000, was applied. Membranes were washed again, and bands were developed using 3′,3-diaminobenzidine as substrate. The densities were measured by a chromato-scanner (BioRad GS670; Bio-Rad, Richmond, CA) to determine the relative intensities. A CYP3A2 standard supplied with the antibodies was used as a control marker while a molecular marker (Novex, San Diego, CA) was used in lieu of P-gp standard, which was unavailable at the time of the study.

**Analytical Procedures.** The concentrations of indinavir in plasma were determined by HPLC (Chen et al., 1995). To 0.2 ml of plasma was added 125 ng of the internal standard, L-707,943 and 5 ml of diethyl ether. After shaking the sample for 15 min and centrifuging, the organic layer was transferred to a clean tube where it was evaporated to dryness under $N_2$. The residue was dissolved in 250 $\mu$l of mobile phase and 200 $\mu$l was injected onto a Zorbax RX-C8 (4.6 mm $\times$ 25 cm) analytical column. The flow rate of the mobile phase, acetonitrile/phosphoric acid (15 mM) (24:76, v/v, adjusted to pH 3.2 with triethylamine), was 1.5 ml/min. The column effluent was monitored by UV absorption at 220 nm and the limit of detection was 50 nM for a 0.2-ml sample. The accuracy of quality control samples (50 and 5000 nM) ranged from 92 to 104%, with an intraday precision ranging between 0.8 and 5.5%.

**Pharmacokinetic Analysis.** The plasma clearance (Cl) of indinavir was calculated as the i.v. dose divided by the $AUC_{0-\infty}$. Half-life was estimated from the slope of the terminal phase of the log plasma concentration-time points fitted by the method of least squares. The $V_{dss}$ of the drug was determined as follows:

$$V_{dss} = \frac{dose_{iv} \times [AUMC]_0^\infty}{(AUC_0^\infty)^2} \tag{1}$$

where $[AUMC]_{0-\infty}$ is the total area under the first moment of the drug concentration curve from zero to infinity. The AUC and AUMC values were calculated by the Lagran numerical integration program (Rocci and Jusko, 1983). The residual areas were determined by the slope and the drug concentrations at the last time point. The bioavailability ($F$) was estimated by comparing the AUC after oral and i.v. administration as follows:

$$F(\%) = \left(\frac{AUC_{po}}{AUC_{iv}} \times \frac{dose_{iv}}{dose_{po}}\right) \times 100 \tag{2}$$

**Statistical Analysis.** Statistical analysis was performed by ANOVA test (Tallarida and Murray, 1987). *P* values less than .05 were considered to be significant.

TABLE 1

*Pharmacokinetics of indinavir in control and DEX-treated rats after i.v. administration of 10 mg/kg (mean ± S.D., n = 4)*

| Treatment | Cl | $V_{dss}$ | $T_{1/2}$ |
|---|---|---|---|
| | *ml/min/kg* | *liter/kg* | *min* |
| Control | 100 ± 21.1 | 2.2 ± 0.46 | 25.1 ± 11.3 |
| DEX | 135 ± 36.1 | 2.5 ± 1.0 | 16.2 ± 8.6 |
| Statistical analysis | N.S.[a] | N.S.[a] | N.S.[a] |

[a] Not significant.

TABLE 2

*Absorption kinetics of indinavir in control and DEX-treated rats after oral administration of 20 mg/kg (mean ± S.D., n = 4–5)*

| Treatment | $C_{max}$[a] | $T_{max}$[b] | AUC | $F$[c] |
|---|---|---|---|---|
| | *μM* | *min* | *μM · h* | *%* |
| Control | 2.8 ± 1.0 | 30 ± 0.0 | 1.85 ± 0.59 | 28.0 |
| DEX | 0.28 ± 0.06 | 66 ± 21 | 0.61 ± 0.33 | 12.4 |
| Statistical analysis | *p < .001* | *p < .05* | *p < .001* | |

[a] Peak concentration.

[b] Time to reach peak concentration.

[c] Bioavailability ($F$) was estimated from the mean AUCs after i.v. and p.o. administration, correcting for the dose.

**Scale-up of In Vitro $V_{max}/K_m$.** To scale up the $V_{max}/K_m$ values (μl/min/mg microsomal protein) to the intrinsic clearance (ml/min/kg body weight) requires the knowledge of microsomal protein yield per gram tissues (mg/g liver or intestine) as well as the liver and intestine weight (g/kg body weight). The intestinal and hepatic microsomal protein yields have been reported to be about 3 and 50 mg/g tissue, respectively (Borm et al., 1982; Houston, 1994). The liver (45 g/kg body weight) and intestine (45 g/kg body weight) weights were taken from the literature (Boxenbaum, 1980; Gerlowski and Jain, 1983).

The unbound fraction of indinavir in the microsomal reaction mixture was measured to correct the $K_m$ values for unbound drug concentrations. Indinavir was added to the reaction mixture to yield a range of drug concentrations (0.5–50 μM). After incubation of the reaction mixture without NADPH at 37°C for 10 min, the unbound fraction was measured using a Centrifree device (Amicon Corp., Danvers, MA) as described elsewhere (Chiba et al., 1997).

The intestinal ($E_G$) and hepatic ($E_H$) first-pass metabolism (extraction ratio) was determined as follows (Gillette and Pang, 1977; Klippert et al., 1982; Wilkinson, 1987):

$$E_H = \frac{f_u \cdot Cl_{int,H}}{Q_H + f_u \cdot Cl_{int,H}} \quad (3)$$

$$E_G = \frac{f_u \cdot Cl_{int,G}}{Q_G + f_u \cdot Cl_{int,G}} \quad (4)$$

where $Q_H$ and $Q_G$ are hepatic blood flow and intestinal mucosal blood flow, respectively. $Cl_{int,H}$ represents hepatic intrinsic clearance and $Cl_{int,G}$ is intestinal intrinsic clearance, and $f_u$ is the unbound fraction of indinavir in plasma. The hepatic blood flow and mucosal blood flow have been reported to be 65 ml/min/kg (Boxenbaum, 1980) and 15 ml/min/kg (Klippert et al., 1982), respectively.

## Results

**Intestinal and Hepatic First-Pass Metabolism.** After i.v. administration (10 mg/kg), indinavir was cleared rapidly in rats with a Cl of 100 ml/min/kg and $T_{1/2}$ of 25 min (Table 1). Pretreatment of rats with DEX (40 mg/kg/day p.o. for 3 days) had little effect on the pharmacokinetic parameters of indinavir after i.v. administration. The Cl and $T_{1/2}$ of indinavir in DEX-treated rats were 130 ml/min/kg and 16 min, respectively (Table 1). There were no statistically significant differences in the pharmacokinetic parameters. In contrast, DEX pretreatment markedly altered absorption kinetics of indinavir after oral dosing (20 mg/kg). The peak concentration ($C_{max}$) of indinavir decreased from 2.8 μM in control rats to 0.28 μM in DEX-treated rats and bioavailability decreased from 28 to 12.4% (Table 2). The decreased bioavailability after DEX pretreatment was most likely due to an increase in first-pass metabolism.

To estimate the relative contribution of intestinal and hepatic metabolism to the overall first-pass effect of indinavir, in vivo intestinal and hepatic first-pass metabolism was measured in control and DEX-treated rats. Using the in situ intestinal loop technique, the intestinal first-pass metabolism was determined by comparing the amount of total radioactivity and unchanged drug in mesenteric blood. When a high dose of [$^{14}$C]indinavir (10 mg/kg; 3 mg/1.5 ml) was introduced directly into the jejunum, approximately 94% of radioactivity col-

lected in the mesenteric blood was found to be unchanged drug in control rats and 66% in DEX-treated rats (Fig. 1). Because the entire drug absorbed was collected in the mesenteric blood, the differences in the amount between total radioactivity and the unchanged drug reflect $E_G$ during absorption. Thus, the intestinal first-pass metabolism was estimated to be 6% for control rats and 34% for the DEX-treated rats. When a small dose of $^{14}$C-indinavir (0.1 mg/kg; 0.03 mg/1.5 ml) was given, the intestinal first-pass metabolism was found to be 23% for control (untreated) rats (Fig. 2). The intestinal first-pass metabolism was much greater at low dose (0.1 mg/kg) than high dose (10 mg/kg) (23 versus 6%). These results clearly suggest that the intestinal first-pass metabolism is dose-dependent.

The hepatic first-pass metabolism was estimated by comparing the steady-state concentrations of indinavir in the systemic circulation during portal and femoral vein infusion of the drug. When indinavir was infused at a constant rate of 12 μg/min, the plasma concentrations of indinavir were significantly lower during portal vein infusion than those during femoral vein infusion in both control and DEX-treated rats (Fig. 3). The hepatic first-pass metabolism of indinavir was calculated to be 65% in control rats and 82% in DEX-treated rats. Collectively, these results indicated that the relative contribution of the small intestinal to the overall first-pass metabolism of indinavir in rats is not quantitatively as important as the liver, regardless of induction.

**In Vitro Intestinal and Hepatic Metabolism.** An in vitro kinetic study was conducted to assess the effect of DEX on the intestinal and hepatic metabolism of indinavir using rat intestinal and hepatic microsomes. As shown in Fig. 4, pretreatment with DEX resulted in a 10-fold increase in hepatic metabolism of indinavir and only a 2.5-fold increase in intestinal metabolism at a concentration of 5 μM. Analysis of the in vitro kinetic data revealed that the increased intestinal and hepatic metabolism induced by DEX was due mainly to an increase in the maximum velocity ($V_{max}$), without a significant change in $K_m$ values (Table 3). The metabolic clearance ($V_{max}/K_m$) was increased 8.5-fold in the liver and 3-fold in the intestine after DEX treatment. These results suggest that DEX had a differential effect on the intestinal and hepatic metabolism of indinavir. Similarly, the differential effect of DEX on the intestinal and hepatic metabolism of testosterone, a marker substrate of CYP3A, was observed. As shown in Table 4, CYP3A-mediated testosterone 2β-hydroxylation and 6β-hydroxylation were increased 7- to 11-fold in the liver after DEX pretreatment, whereas there was only a 3- to 4-fold increase in the intestine after DEX induction. For both indinavir and testosterone, intestinal metabolism was much lower than hepatic metabolism, on the basis of milligrams of microsomal protein (Tables 3 and 4).

**Western Blot Analysis of CYP3A and P-gp.** To determine whether the increased intestinal and hepatic metabolism is due to the enzyme induction, the expression of CYP3A was investigated by Western blot analysis using a rabbit polyclonal antibody that cross-
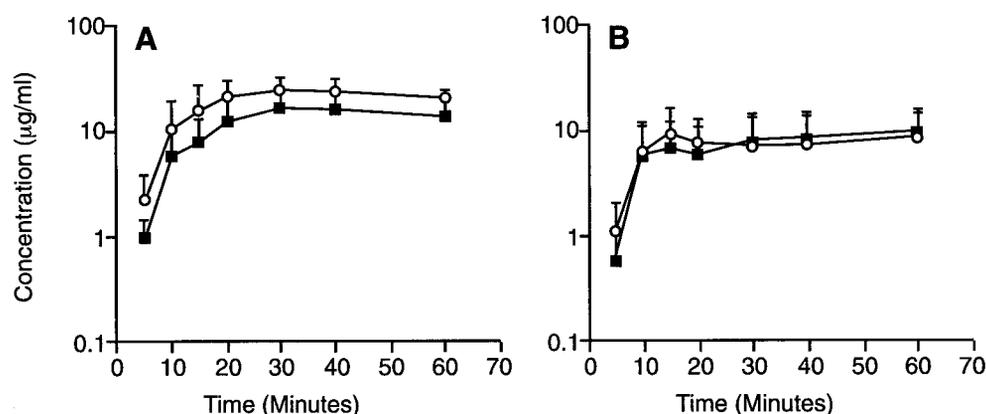
FIG. 1. *Mean concentration of radioactive equivalents (■) and unchanged indinavir (○) in mesenteric blood of DEX-treated rats (A) and control rats (B) and after an intrajejunal injection of a high dose of [$^{14}$C]indinavir (~10 mg/kg; 3 mg/1.4 ml; mean ± S.D., n = 4).*
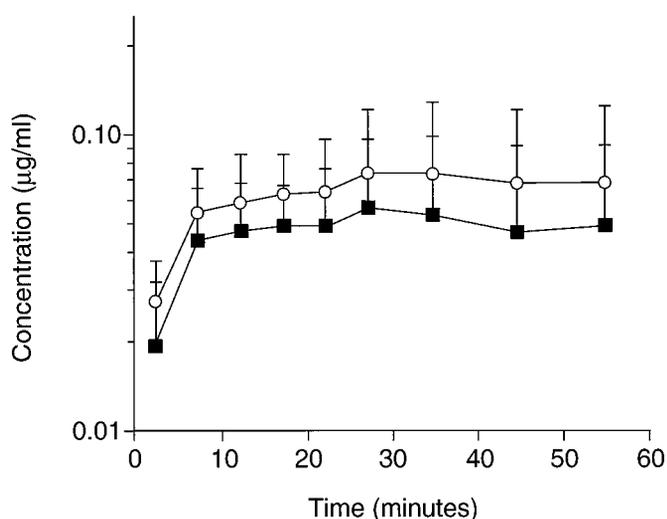


FIG. 2. *Mean concentration of radioactive equivalents (○) and unchanged indinavir (■) in mesenteric blood of control (untreated) rats after an intrajejunal injection of a low dose of [$^{14}$C]indinavir (~0.1 mg/kg; 0.03 mg/1.5 ml; mean ± S.D., n = 4).*

reacts with CYP3A1 and CYP3A2. DEX pretreatment increased CYP3A levels in intestinal and hepatic microsomes by approximately 5- and 7-fold, respectively (Fig. 5), suggesting a profound intestinal and hepatic enzyme induction.

To study whether DEX pretreatment induces the expression of P-gp, the levels of P-gp expression in the intestine and liver also were studied by Western blot analysis using the monoclonal antibody C219. As shown in Fig. 6, the Western blot analysis revealed a band of 140 to 150 KDa, corresponding to P-gp. DEX pretreatment appeared to increase both the intestinal and hepatic P-gp by approximately 2- to 3-fold (Fig. 6).

**Prediction of In Vivo Intestinal and Hepatic First-Pass Metabolism.** Using the in vitro $V_{max}/K_m$ data, the intestinal and hepatic clearance and first-pass metabolism were predicted and are summarized in Table 5. The in vitro $E_H$ of indinavir was predicted to be 45% in control rats and 88% in DEX-treated rats, respectively. These in vitro predicted values were in good agreement with the observed in vivo $E_H$ both in control and DEX-treated rats. However, there was a marked discrepancy between the in vitro predicted and in vivo observed $E_G$. The predicted in vitro $E_G$ values were 0.98% in control rats and 3.6% in DEX-treated rats, whereas the observed $E_G$ values were 6 and 34% in control and DEX-treated rats, respectively. The pre-

dicted $E_G$ was much lower than the observed in vivo $E_G$ by approximately 6-fold in control rats and 10-fold in DEX-treated rats.

## Discussion

In the present study, we used in vitro and in vivo approaches to estimate the intestinal and hepatic metabolism of indinavir in rats. Clearly, both in vitro and in vivo data demonstrated that the intestinal metabolism was quantitatively much less significant than the hepatic metabolism (Fig. 1 versus Fig. 3; Table 5). With the limited amount of drug-metabolizing enzymes, particularly CYP enzymes in the intestine (Lin et al., 1999), it is not surprising that the intestinal metabolism of indinavir is minimal. Consistent with indinavir metabolism, intestinal metabolism of testosterone also was quantitatively much less important than the hepatic metabolism (Table 4).

The major metabolic pathways of indinavir in rats have been identified as pyridine *N*-oxidation, parahydroxylation of the phenylmethyl group, 3'-hydroxylation of the indan and *N*-depyridomethylation (Lin et al., 1996). The metabolite profile of indinavir has been demonstrated to be qualitatively similar in rat intestine and liver (Chiba et al., 1997). All metabolites observed in rat liver microsomes also were formed in rat intestinal microsomes. Inhibition studies with anti-rat CYP3A1 antibody and ketoconazole further suggested that both hepatic and intestinal metabolism of indinavir in rats are catalyzed by the same CYP3A subfamily (Chiba et al., 1997). Immunoblotting analysis revealed that the liver had a higher enzyme level of CYP3A than the intestine by a factor of 5- to 10-fold either before or after DEX induction (Fig. 5). Consistent with the enzyme level, the functional activity of CYP3A measured by indinavir metabolism also was much higher in the liver than in the intestine before and after inducer treatment (Table 3). These results strongly support the notion that the limited intestinal metabolism of indinavir was due mainly to the low level of CYP3A enzyme expression.

When the in vitro $V_{max}/K_m$ values ($\mu$l/min/mg microsomal protein, Table 3) were scaled up to intrinsic clearance of whole body (ml/min/kg body weight, Table 5), the differences in metabolizing capacity between the liver and intestine became even more significant. The differences in the $V_{max}/K_m$ were approximately 20- to 50-fold between the liver and intestine (Table 3), whereas the differences in the intrinsic clearance were approximately 300- to 700-fold (Table 5). This is due to the large difference in the yield of microsomal protein between these two organs; the yield of hepatic microsomes has been reported to be about 50 mg/g liver (Houston, 1994) whereas only 3 mg/g for intestine (Borm et al., 1982). The reason for the low yield of intestinal microsomes is the location of the intestinal CYP mainly in
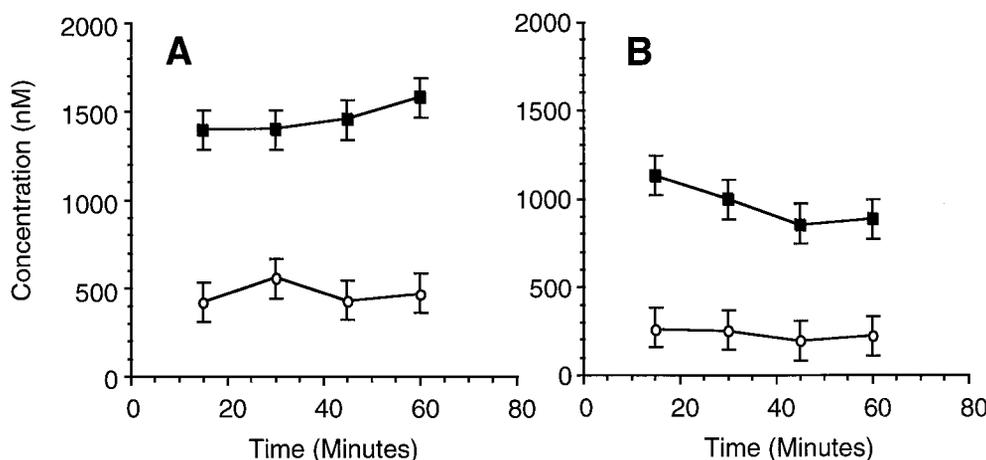
FIG. 3. *Mean concentrations of indinavir in systemic circulation of control (A) and DEX-treated (B) rats during portal (○) and femoral (■) vein infusion at a constant rate of 12 μg/min (mean ± S.D., n = 4).*
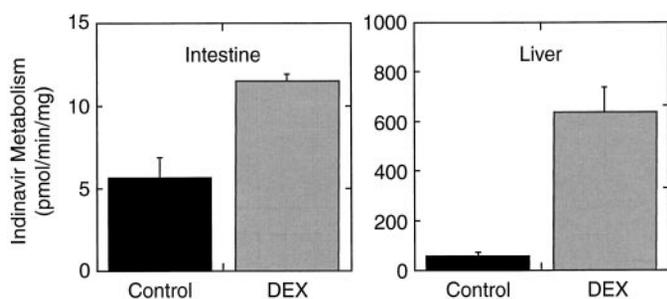


FIG. 4. *Effect of DEX on the intestinal and hepatic metabolism of indinavir (5 μM) in rats (mean ± S.D., n = 4).*

DEX was given orally 40 mg/kg for 3 days.

TABLE 3

*Effects of DEX on the intestinal and hepatic metabolism of indinavir in rats (mean ± S.D., n = 3–4)*

| Microsomes | Treatment | $K_m$ | $V_{max}$ | $V_{max}/K_m$ |
|---|---|---|---|---|
| | | μM | pmol/min/mg microsomal protein | μl/min/mg microsomal protein |
| Liver | Control | 2.12 ± 1.30 | 100 ± 44 | 59.0 ± 26.8 |
| | DEX-treated | 2.64 ± 0.85 | 1325 ± 449[a] | 500 ± 33[a] |
| Intestine | Control | 4.78 ± 2.74 | 12.8 ± 5.0 | 2.94 ± 0.86 |
| | DEX-treated | 3.22 ± 1.56 | 29.8 ± 4.6[b] | 9.25 ± 3.4[b] |

[a] Significantly different between control and DEX-treated rats at $p < .001$.
[b] Significantly different between control and DEX-treated rats at $p < .01$.

the villus tip cells, which account for only a very small fraction of total intestinal cell population (Kaminsky and Fasco, 1992).

Using the intrinsic clearance values ($Cl_{int,H}$ and $Cl_{int,G}$) from in vitro data, the $E_G$ of indinavir was estimated to be approximately 1 and 4% for control and DEX-treated rats, respectively. The corresponding values for $E_H$ were 45 and 87% (Table 5). As shown in Table 5, the predicted $E_H$ obtained from in vitro data was in reasonably good agreement with the observed in vivo $E_H$ both before and after induction, whereas a significant discrepancy between the in vitro and in vivo $E_G$ was observed. The predicted in vitro $E_G$ was much lower than that determined in vivo by approximately 6-fold in control rats and 10-fold in DEX-treated rats (Table 5).

One possible explanation for the discrepancy is the involvement of P-gp in the intestinal first-pass metabolism of indinavir. P-gp, located in the apical brush-border membrane of enterocytes of the small intestine, can act as an efflux transporter that extrudes drug from

TABLE 4

*Effect of DEX on the intestinal and hepatic metabolism of testosterone (500 μM) in rats (mean ± S.D., n = 3)*

| Microsomes | Treatment | Testosterone | |
|---|---|---|---|
| | | 2β-Hydroxylation | 6β-Hydroxylation |
| | | pmol/min/mg protein | |
| Liver | Control | 64.0 ± 13.7 | 722 ± 132 |
| | DEX-treated | 736 ± 123[a] | 5011 ± 454[a] |
| Intestine | Control | 0.667 ± 0.104 | 16.3 ± 0.6 |
| | DEX-treated | 2.40 ± 0.78[a] | 50.9 ± 3.3[a] |

[a] Significantly different between control and DEX-treated rats at $p < .001$.

inside the enterocytes into the intestinal lumen as the drug is being absorbed across the epithelial cells. A portion of the extruded drugs can be reabsorbed into the enterocytes. Consequently, P-gp increases the exposure of drugs to drug-metabolizing enzymes and hence enhances intestinal metabolism of drugs by prolonging the intracellular residence time through the repetitive processes of extrusion and reabsorption (Benet et al., 1996). Indinavir has been shown to be a substrate of P-gp (Kim et al., 1998). Thus, it is possible that the effect of P-gp may be to increase the intestinal metabolism of indinavir by prolonging the intracellular residence time of the drug during absorption. Recently, the effect of P-gp on intestinal metabolism of indinavir in Caco-2 cells has been investigated in our laboratory. Indinavir was metabolized to a similar extent when the drug was administered to the apical side of cells than to the basolateral side. However, when the amount of metabolites formed was normalized by the amount of indinavir transported, the intestinal metabolism of indinavir was much greater from the apical side than from the basolateral side (J. Hochman, M.C., M.Y. and J.H.L., unpublished data). Similar results have also been reported for other P-gp substrates, cyclosporine A, and terfenadine (Gan et al., 1996; Raeissi et al., 1999). The intestinal metabolism of these two drugs was greater when they were added to the apical side of Caco-2 cells than to the basolateral side. The possible involvement of P-gp in the intestinal metabolism of indinavir was further supported by the observations that the rate of indinavir absorption was reduced, as indicated by the prolongation of the time to reach peak concentration ($T_{max}$) in DEX-treated rats (Table 2), and that the magnitude of the discrepancy between in vitro and in vivo intestinal metabolism ($E_G$) was larger in DEX-treated rats (10-fold) than that in control rats (6-fold) (Table 5).

Another possibility for the discrepancy may lie on the kinetic model used for the prediction of $E_G$ (eq. 4). Although the well-stirred model
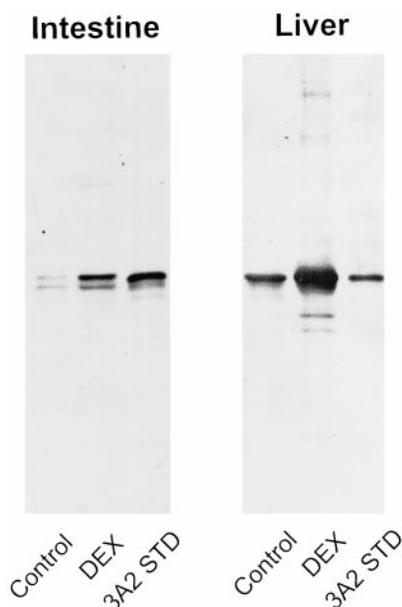
FIG. 5. *Immunoblot analysis of CYP3A in intestine and liver from control and DEX-treated rats.*

Aliquots of intestinal and hepatic microsomal protein (5 $\mu$g) were separated by gel electrophoresis and immunoblots were determined by antibody anti-CYP3A, as described in *Experimental Procedures*. CYP3A2 was used as standard for comparison.
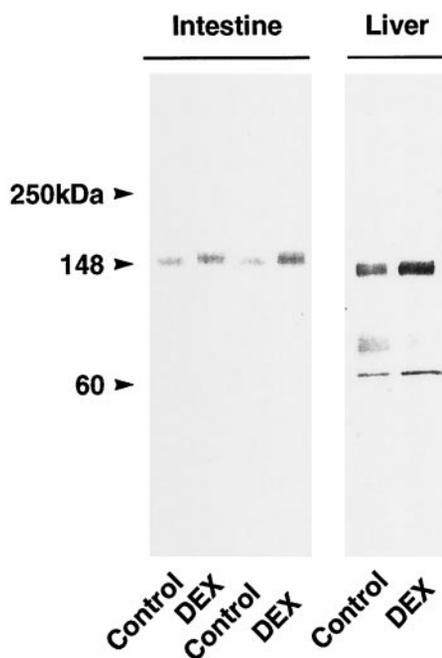


FIG. 6. *Immunoblot analysis of P-gp in intestine and liver from control and DEX-treated rats.*

Aliquots of solubilized membrane fractions (containing 20 $\mu$g for intestinal brush-border membrane, 10 $\mu$g for hepatic canalicular membrane) were determined by gel electrophoresis using C219 antibody, as described in *Experimental Procedures*.

has been used widely in the prediction of intestinal metabolism (Klippert et al., 1982; Paine et al., 1996; Thummel et al., 1997), the validity of the model with respect to intestinal metabolism has not yet been tested carefully. A controversy exists in whether protein binding ($f_u$) should be taken into consideration in the prediction of $E_G$ (eq. 4). From the literature, the model appears to predict the $E_G$ reasonably well for low protein binding drugs, but not for high protein binding drugs. Using the intestinal intrinsic clearance ($V_{max}/K_m$) obtained from rat mucosal cells and a literature value for mucosal blood flow, Klippert et al. (1982) successfully predicted the intestinal first-pass metabolism of phenacetin, a low protein binding drug, in control and 3-methylcolanthrene (3-MC)-treated rats. Based on in vitro data, the $E_G$ was estimated to be in the range of 0.3 to 0.5 for 3-MC-treated rats, a value that was in good agreement with the measured in vivo intestinal extraction ratio of 0.5. However, the intestinal well-stirred model did not accurately predict the $E_G$ of midazolam in humans, a high protein binding drug (more than 96% bound to human plasma proteins). The $E_G$ for midazolam in humans was estimated to be only 0.06 using in vitro $V_{max}/K_m$ data, when protein binding ($f_u = 0.04$) was taken into account (Thummel et al., 1997). The in vitro predicted $E_G$ was much lower than the in vivo $E_G$ of 0.43 determined in liver transplant patients during the anhepatic phase (Paine et al., 1996). A better estimate of the in vitro $E_G$ (0.35) of midazolam was obtained when protein binding was not taken into consideration. These results led to speculation by Thummel et al. (1997) that protein binding is not an important factor in intestinal first-pass metabolism. Whether or not plasma protein binding influences the vectoral movement of drug from the intestinal lumen and the extent of intestinal first-pass metabolism remains to be carefully examined. It should be noted that indinavir is a low protein binding drug with an unbound fraction of 40 to 60% in plasma proteins of animals and humans (Lin et al., 1996).

Both CYP3A and P-gp are known to be inducible. Recently, Salphati and Benet (1998) have reported that DEX treatment resulted in a significant induction of both CYP3A enzyme ($\sim$6-fold) and P-gp ($\sim$5-fold) in rat liver after i.p. dosing of DEX at 100 mg/kg/day for 4 days. Consistent with their observations, in the present study we have demonstrated that DEX pretreatment caused a significant induction of CYP3A enzyme and P-gp in both the small intestine and liver of rats after oral administration of DEX at 40 mg/kg/day for 3 days (Figs. 4 and 5). Coinduction of CYP1A enzymes and P-gp in rat liver by the administration of 2-acetylaminofluorene also has been reported by Gant et al. (1991). Because CYPs and P-gp work together in minimizing the exposure of the body to xenobiotics, the concurrent induction of CYPs and P-gp may reflect the evolution of Mother Nature in designing defense systems to protect the body against toxic xenobiotics.

Unlike intestinal P-gp, which is located in the apical brush-border membrane of enterocytes, hepatic P-gp is located in the canalicular membrane of hepatocytes. The location of hepatic P-gp is not contributory to prolonging of the intracellular residence time of drugs, when the drugs are rapidly and extensively metabolized. Thus, hepatic P-gp may have little effect on hepatic metabolism of high-clearance drugs. Although DEX pretreatment increased hepatic P-gp by approximately 3-fold (Fig. 6), the biliary excretion of indinavir and its metabolites in DEX-treated rats was essentially identical with that in control rats after i.v. dosing (data not shown). Approximately 60% of the dose was recovered in the bile 24 h after an i.v. administration of 10 mg/kg $^{14}$C-indinavir. Only a small fraction (<5%) was recovered as unchanged drug and the metabolite profile was similar, both qualitatively and quantitatively, between control and DEX-treated rats. The lack of significant effect of hepatic P-gp on hepatic metabolism was supported further by the good agreement between the in vitro predicted $E_H$ and in vivo $E_H$ (Table 5).

In vitro studies have shown that indinavir distributed in the erythrocytes equilibrated rapidly with that in plasma and the ratio of indinavir concentration in rat blood to that in rat plasma ($C_{blood}/C_{plasma}$) was about 1.0 (Lin et al., 1996). Accordingly, the blood clearance of indinavir was equivalent to the Cl. The clearance values

TABLE 5

*Prediction of intestinal and hepatic first-pass metabolism of indinavir in control and DEX-treated rats (mean $\pm$ S.D., n = 4)*

| Rat | Intestine | | | Liver | | |
|---|---|---|---|---|---|---|
| | Intrinsic clearance[a] | Predicted $E_G$ | Observed $E_G$[b] | Intrinsic clearance[a] | Predicted $E_H$ | Observed $E_H$[c] |
| | *ml/min/kg* | *%* | | *ml/min/kg* | *%* | |
| Control | $0.50 \pm 0.14$ | $0.98 \pm 0.28$ | $6.0 \pm 3.0$ | $166 \pm 75$ | $45 \pm 10$ | $65 \pm 19$ |
| DEX-treated | $1.87 \pm 1.0$ | $3.6 \pm 2.0$ | $34 \pm 13$ | $1403 \pm 93$ | $87 \pm 8$ | $82 \pm 18$ |

[a] The intrinsic clearance values were scaled up from the in vitro $V_{max}/K_m$ values with the knowledge of the yield of microsomal protein and organ weight.
[b] The observed $E_G$ was determined using the in situ intestinal loop technique.
[c] The observed $E_H$ was determined by comparing the drug concentrations in the systemic circulation during portal and femoral vein infusion of indinavir.

of indinavir in control (100 ml/min/kg) and DEX-treated rats (130 ml/min/kg) were greater than the reported rat hepatic blood flow (70 ml/min/kg), suggesting that extrahepatic metabolism and excretion contributed significantly to the elimination clearance of indinavir in rats. It is evident that intestinal metabolism alone cannot account for the entire extrahepatic metabolism. There must be other sites of extrahepatic metabolism that remain to be studied.

In summary, pretreatment of rats with DEX resulted in a significant induction of CYP3A and P-gp in both the intestine and liver of rats. Because of its location, P-gp appeared to increase intestinal metabolism of indinavir, whereas glycoprotein had little effect on hepatic metabolism of indinavir. Although it has been suggested that the role of human intestinal metabolism for some drugs is quantitatively greater than that of hepatic metabolism in the overall first-pass metabolism, the contribution of intestinal metabolism to the overall first-pass metabolism of indinavir in rats is not quantitatively as important as the hepatic metabolism, regardless of DEX induction.

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