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Hepatic and Intestinal Metabolism of Indinavir, an HIV Protease Inhibitor, in Rat and Human Microsomes

MAJOR ROLE OF CYP3A

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ABSTRACT. The metabolism of indinavir, a human immune deficiency virus (HIV) protease inhibitor, has been characterized extensively in rats and humans. All oxidative metabolites found *in vivo* were formed when indinavir was incubated with NADPH-fortified hepatic and intestinal microsomes obtained from rats and humans. *In vitro* kinetic studies revealed that V_{max}/K_m values (μL/min/mg protein) in rat and human liver microsomes were approximately 8- and 2-fold greater than those in the intestinal microsomes of the corresponding species (55.8 and 6.7 for the liver and intestine, respectively, in rats; 16.5 and 7.7 for the liver and intestine, respectively, in humans). However, when V_{max}/K_m was scaled up to intrinsic clearance (mL/min/kg body weight), hepatic intrinsic clearance was much greater than the intestinal clearance by 50- to 200-fold. These results suggest that the liver plays a much greater role in first-pass metabolism of indinavir than the intestine in both species. Consistently, ketoconazole, a selective inhibitor for CYP3A, and an anti-rat CYP3A1 antibody strongly inhibited hepatic and intestinal metabolism of indinavir in both rats and humans, suggesting the involvement of CYP3A isoforms in both organs. Oral treatment of rats with dexamethasone (50 mg/kg/day for 4 days), a potent CYP3A inducer, increased both hepatic and intestinal metabolism of indinavir by a factor of 7 and 3, respectively. Furthermore, indinavir selectively inhibited 6β-hydroxylase activity of testosterone, a CYP3A marker activity, in rat and human liver microsomes; the interactions between testosterone and indinavir were competitive with K_i values of < 1.0 μM. BIOCHEM PHARMACOL 53;8:1187–1195, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. oxidation; CYP3A2; CYP3A4; HIV protease inhibitor; intestinal metabolism

Many factors can influence the oral bioavailability of drugs. One of the most important factors is first-pass metabolism. Because of a unique anatomical arrangement of the intestine and liver, oral drugs have to pass through the intestine first and then the liver before reaching systemic circulation. For this reason, there is an increasing interest in studying intestinal and hepatic first-pass metabolism [1, 2]. It has been demonstrated that the expression of P450s† in the intestine is isoform-selective in rats and humans [2, 3],and P450 isoforms in the CYP3A subfamily are present in the intestine of both species [2–4]. Intestinal first-pass metabolism mediated by CYP3A has been shown to be clinically relevant with several drugs, such as cyclosporin A [5] and midazolam [6].

Indinavir (L-735,524,MK-0639,CRIXIVAN®),*N*-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4(S)-hydroxy-5-(1-(4-(3-pyridylmethyl)-2(S)-*N'*-(*t*-butylcarboxamido)piperazinyl))pentanamide, is a potent (K_i = 0.41 nM) and highly selective inhibitor for HIV protease [7]. Pharmacokinetic studies revealed that oxidative metabolism is the major route of elimination for indinavir in rats and humans and the contribution of conjugation to the elimination is minimal (< 0.5% of dose) [8, 9]. Our previous *in vivo* studies in rats have shown that indinavir is subject to a substantial hepatic first-pass effect, whereas an intestinal first-pass effect is minimal [9]. The purpose of this study was to investigate and compare the hepatic and intestinal metabolism of indinavir in rats and humans using liver and intestinal microsomes. In addition, attempts were made to predict hepatic and intestinal first-pass extraction ratios using *in vitro* metabolic data (V_{max}/K_m).

MATERIALS AND METHODS

Chemicals

Indinavir (MK-0639) and its carbon-14 form were synthesized at Merck Research Laboratories (West Point, PA). The carbon-14 label was incorporated at the carbonyl position (Figure 1) with a specific activity of 33.08 mCi/mg.

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† *Abbreviations:* HIV, human immune deficiency virus; and P450, microsomal cytochrome P450.

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The drug occasionally was purified according to the HPLC method described below. Dexamethasone, phenobarbital, testosterone, and diethyldithiocarbamate were purchased from the Sigma Chemical Co. (St. Louis, MO). Ketoconazole was obtained from Research Diagnostics Inc. (Flanders, NJ). Furafylline, sulfaphenazole and *S*-mephenytoin were purchased from the Gentest Corp. (Woburn, MA). Tolbutamide was obtained from Research Biochemical International (Natick, MA). Hydroxylated metabolites of testosterone at the 2α-, 2β-, 6β-, 7α-, 16α-, and 16β-positions were obtained from Steraloids (Wilton, NH). All other reagents were of analytical grade.

Liver and Intestinal (Jejunum) Microsomes from Human and Anti-Rat CYP3A1 Rabbit Polyclonal Antibody

Microsomal fractions of human jejunum and liver were obtained from the Keystone Skin Bank (Exton, PA). The original liver microsome codes (age, sex) were HHM-0057 (15, M); 0059 (50, F); 0065 (46, F); 0071 (60, F); 0079 (40, M); and 0095 (63, F), where M and F represent male and female, respectively. Similarly, the original intestinal microsome codes (age, sex) were HJM-0001 (26, M); 0003 (41, M); and 0006 (21, M). All microsomes were obtained from Caucasian donors. Microsomes were used as supplied.

Rabbit polyclonal antibody prepared against rat CYP3A1 and pre-immune control IgG were obtained from Human Biologics Inc. (Phoenix, AZ). The immunoinhibitory potency for CYP3A1/2- and 3A4-catalyzed activities in rat and human liver microsomes, respectively, was checked by measuring testosterone 2β- and 6β-hydroxylation activities in the presence of different amounts of antibody. The preliminary studies revealed that the anti-rat CYP3A1 antibody showed a strong immunoinhibitory effect on testosterone 2β- and 6β-hydroxylation in both rat and human liver microsomes (data not shown).

Animals and Microsomal Preparation

Male adult (12-week-old) rats of the Sprague–Dawley strain were purchased from Harlan Industries (Indianapolis, IN). For induction studies, rats were pretreated with an oral dose of phenobarbital (80 mg/kg/day for 4 days) or dexamethasone (50 mg/kg/day for 4 days). All rats were killed 24 hr after the last treatment. The liver and intestine were excised quickly from the same animal and perfused with ice-cold 1.15% KCl (w/v). Hepatic microsomes were prepared by differential ultracentrifugation [10]. Microsomal final pellets were resuspended in 0.15 M Tris–HCl buffer (pH 7.4) and stored at −70° until used. Intestinal microsomal fractions were prepared from the upper segment (e.g. duodenum + upper jejunum) of control and pretreated rats according to a technique that yielded a high specific content of total CO-binding protein [11]. Microsomal protein was measured by the method of Lowry *et al.* [12], with bovine serum albumin as the standard.

Indinavir Metabolism Assay

The oxidative metabolism of indinavir was measured in a system consisting of an NADPH-generating system and microsomes according to the method specified below.

Preliminary experiments indicated that the total metabolism of indinavir was linear against incubation time for up to 25 min at 4 mg/mL protein in human and rat preparations, except for dexamethasone-induced rat liver where linearity was found up to 10 min at 0.5 mg/mL protein. The incubation mixture (final volume of 100 μL in 0.15 M Tris–HCl buffer, pH 7.4) consisted of an NADPH-generating system [20 mM glucose-6-phosphate (G6P), 0.4 I.U. G6P dehydrogenase (G6PDH), 20 mM $MgCl_2$], various concentrations of [^{14}C]indinavir (0.2 to 5 or 10 μM), and 4 mg/mL microsomes. For the competitive inhibition studies with quinine, quinidine, S-mephenytoin and ketoconazole, an appropriate concentration of inhibitor (up to 100 μM) also was included in the reaction mixture. After a 5-min preincubation at 37°, the reaction was initiated by the addition of 10 μL of 50 mM NADPH. For the inhibition study with furafylline and diethyldithiocarbamate, both mechanism-based inhibitors, a different concentration of inhibitor (up to 100 μM) was preincubated with NADPH-fortified microsomes for 20 min at 37° prior to the addition of substrate. After the reaction started, the mixture was incubated for 5 or 20 min. The reaction was terminated by the addition of 300 μL of ice-cold acetonitrile. The resultant mixture was mixed vigorously and centrifuged at 14,000 *g* for 4 min to precipitate protein. An aliquot of the supernatant (380 μL) was transferred to a clean test tube and evaporated under nitrogen. The sample was reconstituted with 300 μL H_2O for injection onto the HPLC system described below. The recovery of total counts after the protein-precipitation procedure was found to be greater than 90%.

The designated amount of polyclonal anti-rat CYP3A1 rabbit antibody or pre-immune rabbit IgG was incubated with microsomes for 30 min at room temperature prior to adding the reaction mixture. The incubation mixture consisted of the same composition as described above, with the exception of the final microsomal concentration which was adjusted to 0.5 mg/mL. After the start of the reaction, the incubation was carried out for 40 min. The metabolism of indinavir was measured by the same protocol as described above.

Indinavir and its metabolites were measured by an HPLC system (Waters 600E System/712 WISP Autosampler, Milford, MA) equipped with a reverse-phase column (Supelcosil LC-18, 15 cm × 4.6 mm, 3 μm). Mobile phase A consisted of 10 mM phosphate buffer (pH 5.2) and 5 mM hexyltriethylammonium phosphate. Mobile phase B consisted of acetonitrile. The following gradient system was used: 25% B to 30% B linearly over the first 40 min, isocratic 30% B for 15 min, and then 30% to 25% B for 5 min. The flow rate was 1 mL/min. Each biological sample was treated with a mixture of synthetic indinavir metabolites,

and elution from the column was monitored at 210 nm. The column eluent was collected by a fraction collector as follows: 1-min samples were collected for 9 min, 30-sec samples were collected between 9 and 31 min, and 2-min samples were collected up to 55 min. The ^{14}C-radioactivity in the collected samples was measured with a liquid scintillation spectrophotometer (model LS-5000CE, Beckman Instruments, Fullerton, CA). The chromatographic peaks were assigned (based on the retention times of the corresponding standards) as follows (see Fig. 1): 2′,3′-*trans*-dihydroxyindanylpyridine *N*-oxide (M2), 2′,3′-*trans*-dihydroxyindan (M3), pyridine *N*-oxide (M4a) and *p*-hydroxyphenylmethyl (M4b) analogs of indinavir, *N*-[2(S),3(S)-dihydroxyl-1(S)-indanyl]-5-[2(S)-(1,1-dimethylethylaminocarbonyl) piperizin-1-yl]-4(S)-(hydroxy-2(*R*)-phenylmethylpentanamide (M5), and *N*-[2(*R*)-hydroxy-1(S)-indanyl]-5-[2(S)-(1,1-dimethylethylaminocarbonyl)piperizin-1-yl]-4(S)-hydroxy-2(*R*)-phenylmethyl pentanamide (M6). The recovery after HPLC analysis was calculated to be more than 90%, as determined by comparing the injected counts with the integrated counts in the eluted samples.

Testosterone Hydroxylase Assay

P450-isoform specific hydroxylase assays of testosterone were performed using HPLC according to the following method specified below.

As incubation mixture (final volume of 250 μL in 0.15 M Tris–HCl buffer, pH 7.4), containing an NADPH-generating system (G6P, 20 mM; G6PDH, 4 IU/mL; $MgCl_2$, 20 mM), 1 mg/mL microsomes, testosterone (100 μM for the competitive inhibition study by indinavir; 12.5, 25, 50, and 100 μM for examining the type of interaction between testosterone and indinavir), and various concentrations of indinavir, was preincubated for 5 min at 37°. Metabolism was initiated by the addition of NADPH at a final concentration of 1 mM. The reaction was conducted at 37° for 20 min and stopped by the addition of ice-cold ethyl acetate (1.0 mL). Then an internal standard (50 μL of 20 μM cortisone) was added to each sample, followed by an additional 1.5 mL of ethyl acetate to extract metabolites. The resulting mixture was vortex-mixed, and the ethyl acetate layer was separated by centrifugation, followed by evaporation to dryness under nitrogen. The residue was reconstituted in 150 μL of 20% methanol in water.

An HPLC assay was performed on a Supelco LC-18 column (5 μm, 4.6 mm × 15 cm) with a Spectra-Physics HPLC system (Fremont, CA). The HPLC method involved isocratic elution for 3.5 min with an 83:17 (v/v) mixture of 30% methanol in water (mobile phase A) and 10% acetonitrile in methanol (mobile phase B) at a flow rate of 1.0 mL/min. Both mobile phases were adjusted to pH 4.5 with glacial acetic acid. Then a linear gradient was run until 10 min, when the proportion of mobile phase A was decreased from 83 to 70%. Mobile phase A remained at 70% until 25 min, after which it was further decreased to 50% at 30 min. Then mobile phase A was returned to 83% at 35 min. The metabolites were monitored at 240 nm.

Scale up of in vitro V_{max}/K_m

To extrapolate the *in vitro* V_{max}/K_m (μL/min/mg protein) to the *in vivo* intrinsic clearance for predicting first-pass elimination in the liver and intestine, we used the following parameters and equations. The *in vitro* value was extrapolated to the *in vivo* intrinsic clearance ($CL_{int,\ in\ vivo}$, mL/min/kg body weight) according to the equation:

$$CL_{int,\ \text{in vivo}} = (V_{max}/K_m) \cdot M \cdot OW/f_u \quad (1)$$

where M, *OW*, and f_u represent the microsomal yield (mg/g tissue), liver or intestine weight (g/kg body weight), and the unbound fraction of indinavir in the microsomal reaction mixture, respectively. The microsomal protein yield (M) has been reported to be about 3 and 50 mg/g organ for the intestine [13] and liver [14], respectively, and we assumed the same protein contents between human and rat. The liver weight was estimated from the allometric relationship: liver weight = 0.037 $W^{0.85}$ [where W is the body weight (kg)] [15]. The liver weight (normalized by kg body weight) used in the calculation was 45 and 20 g/kg for rats and humans, respectively. The intestinal weights for rats and humans were taken from literature [16]: 45 and 30 g/kg for rats and humans, respectively. To correct the K_m value for unbound drug concentration, we measured the unbound fraction of indinavir in the microsomal reaction mixture (f_u). Indinavir was added to the reaction mixture described above to yield a final concentration of 5 μM. Following incubation of the reaction mixture without NADPH at 37° for 20 min, an aliquot of the mixture (1 mL) was transferred to a Centrifree tube (Amicon Co., Danvers, MA) and centrifuged at 1500 *g* for 15 min at 37°. The unbound fraction was estimated directly from the ratio of drug concentration in the ultrafiltrate to that in the original reaction mixture sample. No significant difference was observed in the unbound fraction between the liver and intestine in either species.

The hepatic clearance (CL_H) and the hepatic extraction ratio (*E*) were determined according to the "well-stirred" model as follows [17]:

$$CL_H = Q \cdot E = \frac{Q \cdot f_B \cdot CL_{int,\ \text{in vivo}}}{Q + f_B \cdot CL_{int,\ \text{in vivo}}} \quad (2)$$

where *Q* is the hepatic blood flow and f_B is the unbound fraction of indinavir in the blood. The hepatic blood flow rates used in the calculation of CL_H and *E* are 65 mL/min/kg for rats and 20 mL/min/kg for humans [15]. The unbound fraction of indinavir in the blood for rats and humans was

taken from the literature [9]. For the intestine, the same equation was used to predict clearance and first-pass elimination. The mucosal blood flow rate was used in the equation instead of the total intestinal blood flow rate as proposed by Klippert *et al.* [18]: the mucosal blood flow rate for rats was taken from the literature (15 mL/min/kg [19]). The same ratio of mucosal to hepatic blood flow rate as used for rats was employed to calculate the human mucosal blood flow rate [i.e. 20 × (15/65) = 4.6 mL/min/kg].

RESULTS

Hepatic and Intestinal Metabolism of Indinavir

In a clinical study, seven prominent metabolites were isolated from human urine and characterized by NMR, MS, and/or chromatographic comparisons with authentic standards [20]. The major metabolic pathways were identified in human as (a) glucuronidation at the pyridine nitrogen to yield a quaternized ammonium conjugate (M1), (b) pyridine N-oxidation (M2 and M4a), (c) *para*-hydroxylation of the phenylmethyl group (M4b), (d) 3′-hydroxylation of the indan moiety (M2, M3, and M5), and (e) N-depyridomethylation (M5 and M6) (Fig. 1). All oxidative metabolites observed *in vivo* also were formed in NADPH-fortified liver and intestinal microsomes. N-Dealkylation was quantitatively the most important biotransformation pathway for indinavir; more than 50% of the drug was converted to N-dealkylated metabolites, M5 and M6 (data not shown). Because of the formation of the secondary oxidative metabolites (M2, M5, and M6) during incubation, kinetic studies were conducted to estimate apparent K_m and V_{max} values for the formation of total metabolites (M2 + M3 + M4a + M4b + M5 + M6). The apparent K_m and V_{max} values are summarized in Table 1. The K_m values in the liver microsomes of both rats and humans were approximately 2-fold smaller than those obtained from the intestine of the corresponding species (Table 1). The low K_m values, ranging from 1.0 to 2.5 μM, suggest a high affinity of indinavir for the metabolizing enzymes. When the enzyme activities were expressed per milligram of microsomal protein, the average of V_{max} values obtained from rat liver microsomes was approximately 5-fold larger than that from the intestinal microsomes, whereas there was no significant difference in V_{max} between the liver and intestine in humans. The values for V_{max}/K_m of both species were 2- to 8-fold higher in the liver than in the intestine (Table 1).

Identification of P450 *Isoform(s) Responsible for Hepatic and Intestinal Indinavir Metabolism*

In our previous study with human liver microsomes [21], we demonstrated that all oxidative metabolic pathways of indinavir were mediated by CYP3A4. This conclusion was based on the results of five *in vitro* approaches proposed by

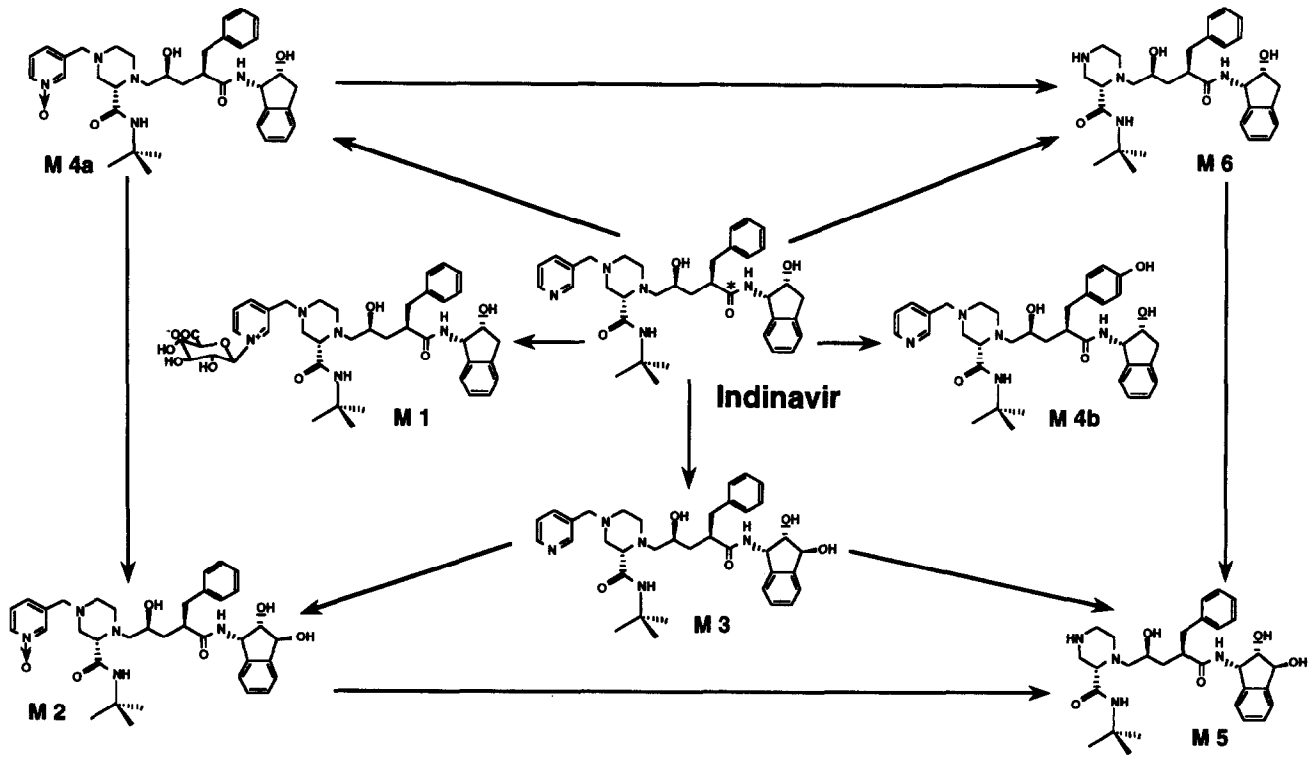

FIG. 1. Proposed metabolic pathway of indinavir in humans. Metabolite structures have been identified in human urine and feces samples [20].

TABLE 1. Kinetics of liver and intestine microsomal (total) metabolism of indinavir in rat and human*

Species	Organ	K_m (μM)	V_{max} (pmol/min/mg)	V_{max}/K_m (μL/min/mg)
Rat†	Liver	1.03 (0.07)	57.4 (9.9)	55.8 (7.9)
	Intestine	1.68 (0.06)	11.3 (1.9)	6.74 (1.21)
Human	Liver‡	1.30 (0.25)	22.9 (20.5)	16.5 (13.3)
	Intestine§	2.51 (0.77)	17.4 (3.4)	7.74 (4.03)

* Total metabolic rate at each substrate concentration was generated by summing formation rates. The obtained Michaelis–Menten-type saturable curve then was analyzed for K_m and V_{max} values.

† Data represent the average of results obtained from three preparations. Numbers in parentheses represent the SD.

‡ Data represent the average of results obtained from six different human liver microsomes. Numbers in parentheses represent the SD.

§ Data represent the average of results obtained from three different human intestine (jejunum) microsomes. Numbers in parentheses represent the SD.

Guengerich and Shimada [22], namely: (1) chemical inhibition, (2) immunochemical inhibition, (3) metabolism by cDNA-expressed human P450 enzymes, (4) correlation analysis, and (5) competitive inhibition of marker activities. To determine which P450 isoform(s) is responsible for the oxidative metabolism of indinavir in rat hepatic and intestinal microsomes and human intestinal microsomes, we examined the effect of ketoconazole and an anti-rat CYP3A1 antibody on indinavir metabolism. For comparison, the effects of ketoconazole and an anti-rat CYP3A1 antibody on indinavir metabolism in human hepatic microsomes also were studied.

Ketoconazole, a CYP3A-selective inhibitor, strongly inhibited indinavir metabolism in both hepatic and intestinal microsomes of rats and humans in a concentration-dependent manner (Fig. 2). Other chemical inhibitors (i.e. furafylline, quinidine, sulfaphenazole, diethyldithiocarbamate, and S-mephenytoin for human microsomes; quinine and furafylline for rat microsomes) had little effect on either hepatic and intestinal metabolism of indinavir (data not shown). These results suggest the possible involvement of CYP3A isoforms in both liver and intestinal microsomes of rats and humans. Consistent with this, an anti-rat CYP3A1 antibody, which shows a cross-reactive inhibition

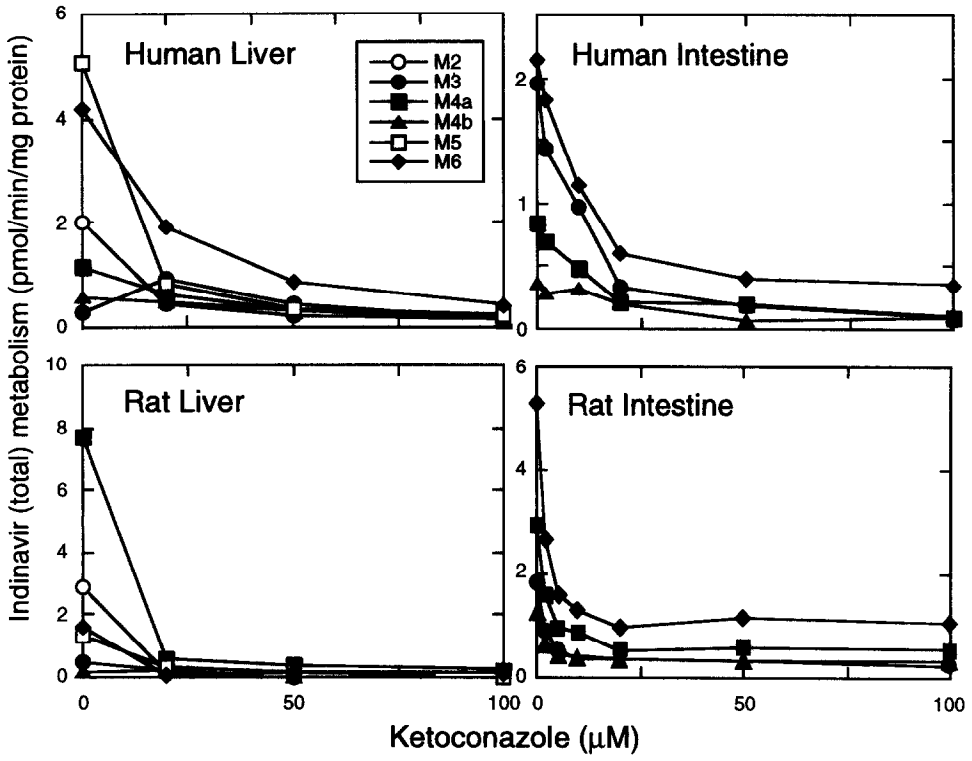

FIG. 2. Effect of ketoconazole on indinavir metabolism in human and rat microsomes. Secondary metabolites M2 and M5 were not clearly formed in the intestinal microsomes under these conditions. Microsomal fraction pooled from three individuals was used.

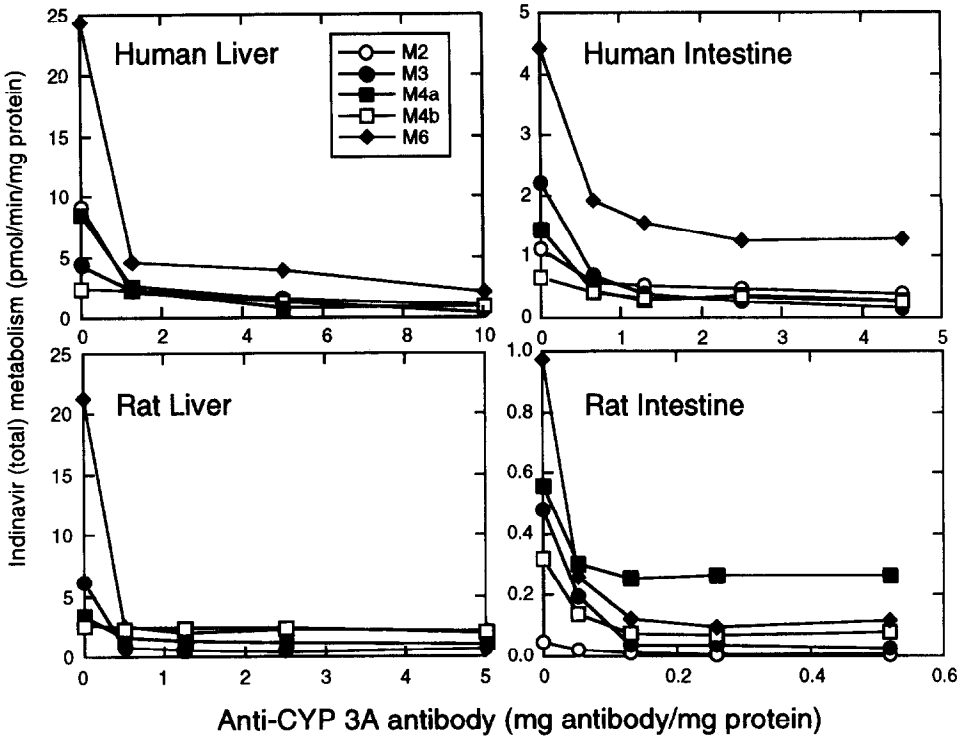

FIG. 3. Effect of anti-CYP3A1 antibody on indinavir metabolism in human and rat microsomes. Pre-immune IgG had little effect on indinavir metabolism (data not shown). Microsomal fraction pooled from three individuals was used.

of human CYP3A4-dependent testosterone 6β-hydroxylation, markedly inhibited all hepatic and intestinal microsomal oxidations of indinavir in both species (Fig. 3). Furthermore, oral treatment of rats with dexamethasone, a potent CYP3A inducer, increased the formation of all metabolites of indinavir in both rat liver and intestinal microsomes by a factor of 7 and 3, respectively (Fig. 4). Phenobarbital induced hepatic metabolism of indinavir ap-

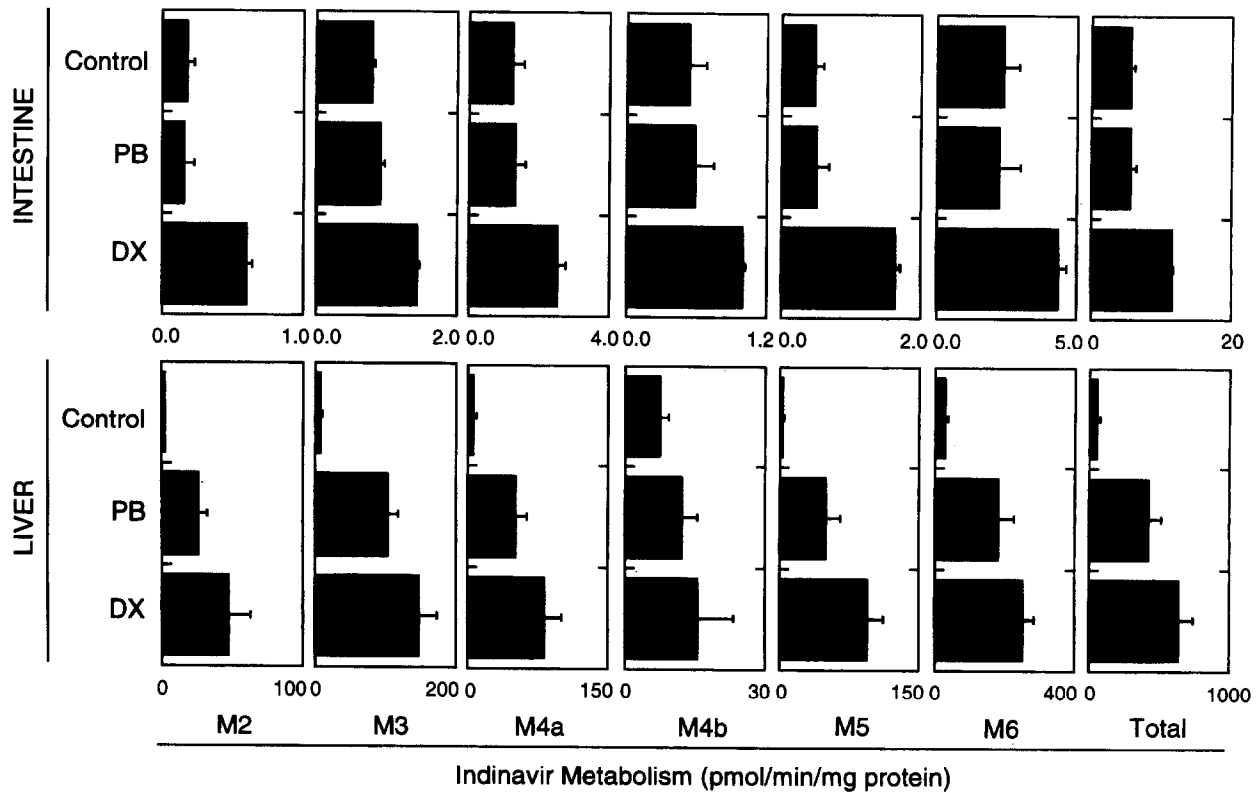

FIG. 4. Indinavir metabolism in induced rat liver and intestinal microsomes. Microsomes were obtained from rats pretreated with DX (50 mg/kg/day dexamethasone p.o. for 4 days) or PB (80 mg/kg/day phenobarbital p.o. for 4 days). Control microsomes were obtained from untreated rats. Data represent the means ± SD of three different preparations.

proximately 2-fold, but had little effect on intestinal indinavir metabolism. These results supported the fact that a CYP3A isoform(s) is responsible for indinavir metabolism in rats.

Competitive inhibition studies of CYP3A-selective marker activity further confirmed that the major oxidative metabolic pathways were catalyzed mainly by a CYP3A isoform(s) in rat and human liver microsomes. Indinavir strongly inhibited testosterone 2β-/6β-hydroxylase activities in both rat and human liver microsomes in a concentration-dependent manner (Fig. 5). Kinetic analysis (Lineweaver–Burk plots, Fig. 6A; Dixon plots, Fig. 6B) revealed that indinavir is a potent competitive inhibitor on CYP3A isoforms in rat liver microsomes, with a K_i value of approximately 0.7 μM. Similarly, the competitive inhibition of indinavir on 6β-hydroxylase activity also was observed in human liver microsomes, with a K_i value of 0.5 μM. The inhibition constant values were comparable to those of the apparent K_m values for total indinavir metabolism in rat and human liver microsomes (~1.0 μM) (Table 1).

DISCUSSION

In both rats and humans, indinavir was metabolized much more rapidly by liver microsomes than by intestinal microsomes. The V_{max}/K_m values (μL/min/mg microsomal protein) obtained from hepatic microsomes were 8-fold higher than those from intestinal microsomes for the rat, and 2-fold higher for human (Table 1). When the *in vitro* V_{max}/K_m values were scaled up to *in vivo* intrinsic clearance (mL/min/kg body weight), the differences in metabolizing capacity between the liver and intestine became more significant (i.e. 200-fold for the rat and 50-fold for the human) due to the big difference in the total content of microsomal protein between the two tissues; microsomal yield in the liver has been reported to be ~50 mg/g liver [14], while only 3 mg/g organ for the intestine [13].

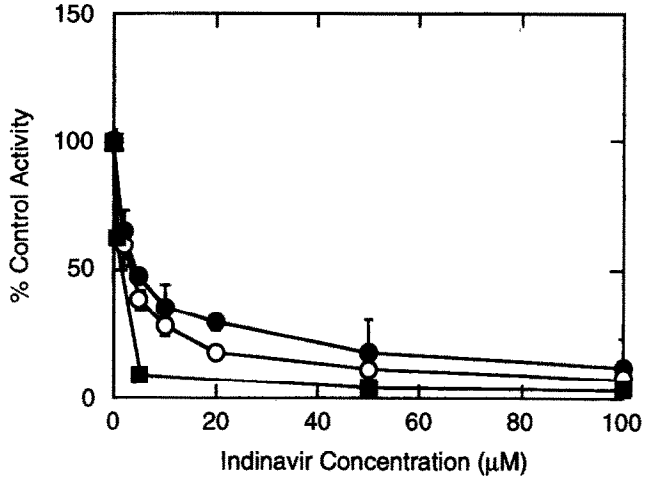

FIG. 5. Effect of indinavir on testosterone hydroxylase activities in rat and human liver microsomes. Hydroxylase activities of testosterone at the 2β- (●) and 6β- (○) positions in rat liver microsomes and the 6β-position in human liver microsomes (■) were measured. Data represent means ± SD of three different preparations for rats. Metabolic activities at 0 μM indinavir were: 0.017 ± 0.002 nmol/min/mg protein for 2β-hydroxylase in rats (N = 3, mean ± SD); 0.222 ± 0.018 nmol/min/mg protein for 6β-hydroxylase in rats (N = 3, mean ± SD); and 0.105 nmol/min/mg protein for 6β-hydroxylase in human (N = 1).

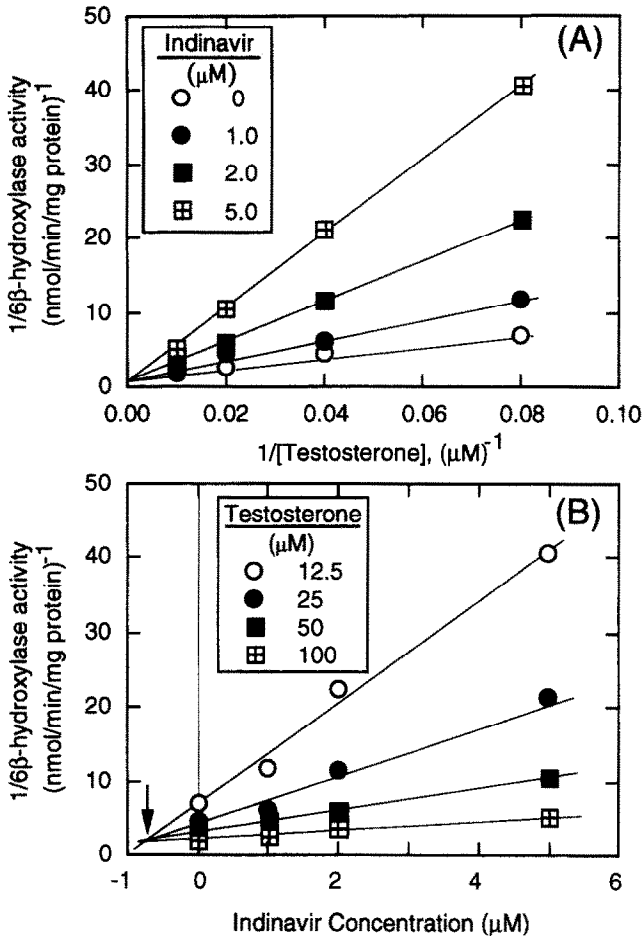

FIG. 6. Inhibition kinetics of testosterone 6β-hydroxylase activity by indinavir in rat liver microsomes. Lineweaver–Burk plots (A) revealed a competitive interaction between indinavir metabolism and testosterone 6β-hydroxylation. The inhibition constant was graphically estimated to be 0.7 μM based on the Dixon plots (B).

Hepatic and intestinal first-pass effects have been predicted successfully based on *in vitro* metabolic data [18]. With the same approach, we estimated the hepatic and intestinal first-pass extraction ratio using *in vitro* V_{max}/K_m data. The estimations were carried out using the well-stirred model, which incorporates the mucosal blood flow rate instead of the total intestinal flow rate. In rats, hepatic and intestinal first-pass effects were estimated to be 42 and 2%, respectively (Table 2). These values agreed reasonably well with the *in vivo* observations in rats [9]. The hepatic first-pass metabolism of indinavir in rats was estimated to be 67% when drug concentrations in the systemic blood during portal and femoral vein infusion were compared under the steady-state condition. On the other hand, the intesti-

TABLE 2. Prediction of hepatic and intestinal first-pass effects of indinavir based on *in vitro* kinetic parameters

Species	Organ	V_{max}/K_m* (μL/min/mg)	$CL_{int,\ in\ vivo}$† (mL/min/kg)	Blood flow rate‡ (mL/min/kg)	f_B§	CL_{organ}‖ (mL/min/kg)	E_{organ}¶ (%)
Rat	Liver	69.7	159	65	0.30	27.5	42.3
	Intestine	8.41	0.808	15	0.30	0.326	2.17
Human	Liver	18.6	18.2	20	0.39	5.23	26.1
	Intestine	8.71	0.373	4.6	0.39	0.287	6.23

* Values were calculated from: $(V_{max}/K_m)/f_u$, where V_{max}/K_m was taken from Table 1 and f_u is an unbound fraction of indinavir in the microsomal reaction mixture (0.801 for rat microsomes, 0.889 for human microsomes).

† $CL_{int,\ in\ vivo} = (V_{max}/K_m) \cdot$ (microsomal content, mg/g organ) · (organ weight, g/kg body weight). Microsomal contents were assumed to be 3 and 50 mg/g organ for intestine and liver, respectively [13, 14]. Organ weights for rat and human liver were 45 and 20 g/kg, respectively; the values for rat and human intestine were 45 and 30 g/kg, respectively (see Materials and Methods).

‡ Liver blood flow rates were taken from the literature [15]. Mucosal blood flow was used instead of total intestinal blood flow to predict a flow rate for the "metabolizing compartment" in the intestine as proposed by Klippert *et al.* [18]. Human mucosal blood flow rate was calculated by using the blood flow rate ratio of intestine to liver of rats [i.e. 20 × (15/65) = 4.6 mL/min/kg].

§ Taken from Ref. 9.

‖ $CL_{organ} = \dfrac{Q \cdot f_B \cdot CL_{int,\ in\ vivo}}{Q + f_B \cdot CL_{int,\ in\ vivo}}$ where Q represents organ blood flow rate.

¶ $E_{organ} = \dfrac{f_B \cdot CL_{int,\ in\ vivo}}{Q + f_B \cdot CL_{int,\ in\ vivo}}$

nal first-pass elimination was minimal (< 10%). More than 90% of the radioactivity collected in the mesenteric blood from the *in situ* isolated intestinal loop preparation was unchanged drug. With the same *in vitro* approach, we predicted that the hepatic first-pass metabolism of indinavir was 26%, while the contribution of the intestine was limited (E = 6%) in human (Table 2).

In summary, *in vitro* kinetic studies revealed that the liver plays a much greater role in the first-pass metabolism of indinavir than the intestine after oral administration. Using *in vitro* metabolic data, hepatic and intestinal first-pass metabolism was predicted reasonably well in rats. Based on *in vitro* intrinsic clearance of the human hepatic and intestinal microsomes, a significant first-pass metabolism was predicted in the liver, while the metabolic elimination in the intestine would be limited.

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