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Dose-dependent Intestinal and Hepatic First-pass Metabolism of Midazolam, a Cytochrome P450 3A Substrate with Differently Modulated Enzyme Activity in Rats

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Abstract

The dose-dependent first-pass metabolism of midazolam, a cytochrome P450 (CYP) 3A substrate, was separately estimated in the intestine and liver after administration into a jejunal loop of rats with differently modulated enzyme activity. Modulation of CYP3A enzyme activity of Sprague-Dawley rats was performed by pretreating the rats with inducers such as dexamethasone or by co-administering ketoconazole (an inhibitor) with midazolam.

Bioavailabilities of midazolam administered into the jejunal loop at a dose of 10 μmol were 12% in untreated (control) rats, and 2% in dexamethasone-pretreated rats. Co-administered ketoconazole (2 μmol) significantly increased the bioavailability to 53% and 7%, respectively, in these rats. The intestinal first-pass metabolism of midazolam administered into the jejunal loop at a dose of 50 nmol in untreated and dexamethasone-pretreated rats, estimated by the mesenteric blood-collecting method in-situ, was 25% and 49% of absorbed amount, respectively. The intestinal first-pass metabolism of midazolam was reduced when ketoconazole (0·5 μmol) was co-administered or when the dose of midazolam was increased to 0·5 μmmol in these rats. Assuming that the contribution of intestinal first-pass metabolism could be negligible when midazolam was administered at a much higher dose of 10 μmol, the estimated hepatic first-pass metabolism of midazolam at a dose of 10 μmol in untreated rats, dexamethasone-pretreated rats, untreated rats given ketoconazole, and dexamethasone-pretreated rats given ketoconazole was, respectively, 86, 97, 46, and 92% of the amounts absorbed.

In conclusion, the dose-dependent intestinal first-pass metabolism and the hepatic first-pass metabolism of midazolam in rats with differently modulated CYP3A activities was quantitatively estimated by in-vivo and in-situ absorption studies.

Many clinically important pharmaceuticals, e.g. some immunosuppressive agents, antibiotics, calcium-channel blockers and anticancer drugs are known to be metabolized by cytochrome P450 (CYP) 3A subfamilies in the gastrointestinal enterocytes and hepatocytes in man (Porter & Coon 1991; Guengerich 1992; Lown et al 1994). It is also known that the activities of CYP3A enzymes are relatively easily modulated by inducers such as dexamethasone, clotrimazole and phenobarbital, or by inhibitors such as ketoconazole, itoraconazole and cimetidine (Kolars et al 1992; Olkkola et al 1994; Wrighton & Ring 1994; Lee & Werlin 1995; Mahnke et al 1996).

Midazolam, a short-acting 1,4-benzodiazepine, is a typical CYP3A substrate; it is metabolized to 1′-hydroxy- and 4-hydroxymidazolam by CYP3A4 in man and by CYP3A2 in rats (Kronbach et al 1989; Gorski et al 1994; Ghosal et al 1996; Paine et al 1996; Thummel et al 1996). The literature cited also reports such first-pass metabolism and drug–drug interaction of midazolam in man owing to the

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modulated CYP3A activity. The relative contribution of intestinal and hepatic first-pass metabolism under conditions of differently modulated enzyme activity is not well understood. In this study we have estimated the first-pass metabolism of midazolam separately in the intestine and liver after administration into a jejunal loop in rats with differently modulated enzyme activity.

Materials and Methods

Materials

Midazolam and ketoconazole were kindly supplied by Nippon Roche (Tokyo, Japan) and Janssen Pharmaceutica (Beerse, Belgium), respectively. Other reagents used were of the highest grade available.

Animal studies

Experiments were performed on male Sprague-Dawley rats, 240 – 280 g. Pretreatment of rats with inducers to modify enzyme activity, including that of CYP3A subfamilies, entailed intraperitoneal injection of 50 mg dexamethasone daily for 2 days, 30 mg clotrimazole daily for 3 days, 30 mg phenobarbital daily for 3 days, or 10 mg pregnenolone-16α-carbonitrile daily for 3 days. Untreated (control) rats received an equal volume of the vehicle alone (saline for phenobarbital, 2% Tween 20 for the others). The rats were fasted overnight and subjected to in-vivo or in-situ studies 24 h after the last dose. Rats were anaesthetized with pentobarbital (30 mg kg^{-1}) by intraperitoneal injection, and were affixed supine on a surface kept at 37°C to maintain their body temperature at 36°C (approx.) A femoral artery was cannulated (polyethylene tubing, PE-50) for blood sampling and a femoral vein for intravenous administration. Experiments with animals were performed in accordance with the Guide for Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Sciences, Hiroshima University School of Medicine.

Intravenous administration of midazolam

Midazolam was dissolved in a mixture of 25% ethanol, 25% propylene glycol and 50% water at concentrations of 5, 10 or 20 μmol mL^{-1}. The drug solution was administered intravenously through a femoral vein via the cannula at a dosing volume of 1 mL kg^{-1} in untreated rats. Blood samples (approx. 0·25 mL) were taken at designated time intervals from a femoral artery via the cannula.

Intrajejunal administration of midazolam

After flushing of the small intestine with saline (5 mL; prewarmed to 37°C) two sites of the jejunum were ligated to form a 10-cm loop. Midazolam (10 μmol) with or without ketoconazole (2 μmol) was dissolved in a mixture (1 mL) of 25% ethanol, 25% propylene glycol, and 50% water. The solution (1 mL) was administered into the loop and blood samples (0·25 mL) were collected from a femoral artery via the cannula at designated time intervals.

In-situ estimation of intestinal first-pass metabolism of midazolam

After flushing of the small intestine with saline (5 mL), a 10-cm jejunal loop was prepared as described above. In-situ collection of mesenteric blood was performed as reported elsewhere (Watanabe et al 1997). Briefly, the mesenteric vein derived from the jejunal loop was cannulated with polyethylene tubing (PE-50), through which all mesenteric venous blood was collected into an heparinized tube. Lost blood was substituted by infusing fresh blood, obtained from healthy rats, at a flow rate of 0·38 mL min^{-1} via a cannula in a femoral vein. Midazolam (50 nmol) with or without ketoconazole (0·5 μmol) was dissolved in a mixture (1 mL) of 25% ethanol, 25% propylene glycol and 50% water, and the solution (1 mL) was administered into the loop. A higher dose of midazolam, 0·5 μmol, was also employed. After administration of midazolam, whole mesenteric blood was serially collected at 3-, 5- or 15-min intervals. Luminal fluid in the loop was collected 60 min after midazolam administration by washing the lumen with ice-cold saline (20 mL), and the loop of intestine was also isolated to determine the amounts of midazolam remaining in the tissues.

In a separate experiment, midazolam (0·5 μmol) was administered with ketoconazole (0·5 μmol) into the loop immediately after the rat had been killed by injection of an extra amount of pentobarbital, to estimate the recovery percentage of midazolam of dose from the loop. The luminal fluid in the loop and the intestine were obtained in the same manner as described above immediately after administration. The amount of midazolam recovered was 90·6 ± 2·3% of the dose. This value was used for correction of midazolam recovery from the loop

(luminal fluid, intestinal tissues) in the experiments described above.

Analysis

Concentrations of midazolam in plasma, blood, intestinal fluid and intestinal homogenates were analysed by high-performance liquid chromatography on a reversed-phase TSK gel ODS-80TM column (Tosoh, Tokyo, Japan) and with UV detection at 229 nm. Briefly, blood samples were haemolysed in a fivefold volume of water. Isolated intestine was homogenized in 20 mL water by means of a glass-Teflon Potter homogenizer (1000 rev min^{-1}, 10 strokes), and was centrifuged to furnish the supernatant. These and other biological samples were mixed with an equal volume of saturated disodium phosphate. Midazolam was extracted with ethyl acetate (6 mL). The organic solvent was evaporated under reduced pressure and the residue was dissolved in methanol (100 μL). The mobile phase was a 60 : 29 : 10 : 1 (v/v) methanol – water – 0·1 M phosphate buffer (pH 7·4) – acetonitrile.

Results and Discussion

Midazolam bioavailability under differently modulated CYP3A activity

The increase in CYP3A proteins and CYP-related messenger RNA concentrations in hepatocytes and small bowel enterocytes after pretreatment with inducers in rats has been reported by several investigators (Debri et al 1995; Lee & Werlin 1995). In the current study we initially estimated the effect of pretreatment with different inducers on the bioavailability of midazolam administered into the rat jejunal loop in-vivo. For estimation of midazolam bioavailability, intravenous administration of midazolam was also performed in the dosing range 5 – 20 μmol kg^{-1} in untreated rats. Midazolam disappeared biexponentially from plasma, and the area under the plasma concentration-time curve (AUC) increased in proportion to the dose, with a constant total plasma clearance of 82·7 ± 4·9 mL $min\,kg^{-1}$. These linear kinetics of midazolam in the central circulation indicate that the total plasma clearance of midazolam is essentially blood-flow-limited because of its high hepatic clearance. The apparent bioavailability of midazolam, estimated as the ratio of AUC_{0-120} after intrajejunal administration to $AUC_{0-\infty}$ after intravenous administration, was 11·5% in untreated rats (Table 1). The bioavailability of midazolam in rats pretreated with different inducers decreased substantially compared with corresponding untreated rats (pretreatment with vehicle alone) — clotrimazole pretreatment, 44% reduction; phenobarbital, 75% reduction; pregnenolone-16α-carbonitrile, 81% reduction; dexamethasone, 82% reduction. The decrease in midazolam bioavailability as a result of this pretreatment could be a result of the induction of CYP3A enzymes, because midazolam is known to be metabolized mainly by CYP3A in rats (Ghosal et al 1996).

The effect of ketoconazole, a high affinity noncompetitive inhibitor of CYP3A (Wrighton & Ring 1994), on midazolam bioavailability was further investigated in untreated and dexamethasone-pretreated rats. As shown in Figure 1, co-administration of ketoconazole significantly increased plasma concentrations of midazolam. The estimated bioavailabilities of midazolam increased to 52·6% from 11·5% in untreated rats and to 7·3% from 2·1% in dexamethasone-pretreated rats as a result of the co-administration of ketoconazole. The increased oral bioavailability of midazolam elicited by ketoconazole could be because of the suppression of mid-

Table 1. The first-pass effect of midazolam after intrajejunal administration to differently treated rats.

Treatment	Bioavailability (%) (10 μmol)	Total recovery (%)		Intestinal first-pass metabolism (%)		Hepatic first-pass metabolism (10 μmol)
		(0·05 μmol)	(0·5 μmol)	(0·05 μmol)	(0·5 μmol)	
Untreated	11·5 ± 1·8	76·7 ± 6·8	87·3 ± 4·3	24·6 ± 7·2	16·0 ± 2·0	86·0 ± 2·3
Dexamethasone-pretreated rats	2·1 ± 0·4*	53·9 ± 7·8*	83·4 ± 1·4†	49·0 ± 6·6*	23·1 ± 3·1†	97·3 ± 0·4*
+ Ketoconasole in untreated rats	52·6 ± 8·8*	90·1 ± 7·2	ND‡	10·3 ± 7·7	ND	45·5 ± 8·8*
+ Ketoconasole in dexamethasone-pretreated rats	7·3 ± 2·7	85·1 ± 8·1	ND	15·0 ± 8·1	ND	91·8 ± 2·9

The value in parenthesis represents the dose of midazolam. Rats were pretreated with dexamethasone (50 mg day^{-1} for 2 days). Ketoconazole was co-administered with midazolam at a dose of 0·5 μmol. Each value is the mean ± standard error of results from 3 – 6 trials. ‡Not determined. *$P < 0·05$, significantly different from result from untreated control. †$P < 0·05$, significantly different from result after administration of low dose (0·05 μmol). ND = not determined.

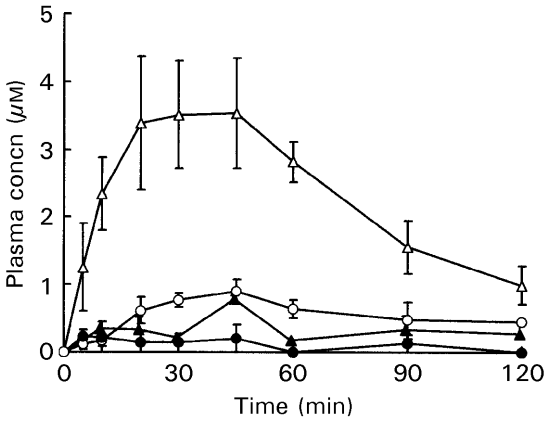

Figure 1. Plasma concentrations of midazolam after intrajejunal administration to untreated control rats (○), dexamethasone-pretreated rats (●), untreated rats given ketoconazole (△), and dexamethasone-pretreated rats given ketoconazole (▲). Doses of midazolam and ketoconazole were 10 μmol and 2 μmol, respectively. Pretreatment of rats with dexamethasone was conducted with a dose of 50 mg daily for 2 days; untreated rats received equal volume of vehicle (2% Tween 20) alone. Each value is the mean ± standard error of results from three to five trials.

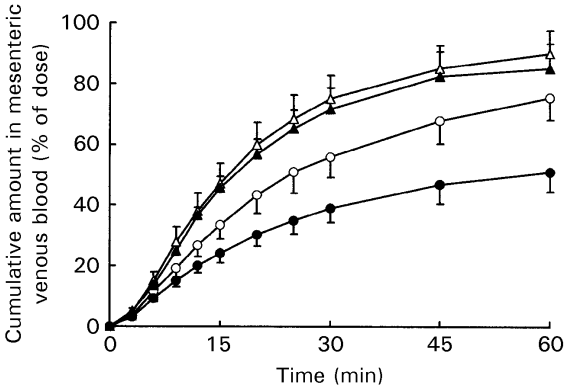

Figure 2. Cumulative amounts of midazolam recovered in mesenteric venous blood after intrajejunal administration to untreated control rats (○), dexamethasone-pretreated rats (●), untreated rats given ketoconazole (△), and dexamethasone-pretreated rats given ketoconazole (▲). Doses of midazolam and ketoconazole were 50 nmol and 0·5 μmol, respectively. Pretreatment of rats with dexamethasone was conducted with a dose of 50 mg daily for 2 days, and untreated rats received an equal volume of vehicle (2% Tween 20) alone. Each value is the mean ± standard error of results from five or six trials.

azolam metabolism in the intestine or the liver, or both.

Dose-dependent intestinal first-pass metabolism of midazolam

The intestinal first-pass metabolism of midazolam administered into the jejunal loop in rats with differently modulated CYP3A activity was evaluated by measuring the amount of midazolam recovered from the whole mesenteric venous blood derived from the loop and the amount remaining in the loop (luminal fluid and intestinal tissues) 60 min after administration. The effect of midazolam dose on intestinal first-pass metabolism was also examined in untreated and dexamethasone-pretreated rats.

As shown in Figure 2, the recovery of midazolam given at a low dose (50 nmol) from mesenteric venous blood was significantly lower for dexamethasone-pretreated rats than for untreated rats. The amount of midazolam remaining in the loop was always <10% of the dosed amount. Coadministration of ketoconazole increased the recovery of midazolam from the mesenteric venous blood of both untreated and dexamethasone-pretreated rats by almost the same extent. This suggested that ketoconazole suppressed the intestinal first-pass metabolism of midazolam in these rats. The percentage total recovery of midazolam from mesenteric venous blood, luminal fluid and intestinal tissues was scattered for differently treated rats, and the recovery of midazolam from dexamethasone-pretreated rats was significantly lower than from other rats (Table 1). When the midazolam dose was increased to 0·5 μmol, the total recovery of midazolam was increased for both untreated and dexamethasone-pretreated rats. The un-recovered amount of midazolam was supposed to be the amount metabolized by first-pass metabolism in the intestine, and the intestinal first-pass metabolism (ER_G) of midazolam was estimated by use of the equation $ER_G = 1 -$ (amount recovered from mesenteric venous blood)/(dosed amount −amount remaining in the loop). In this equation, the difference between the dose of midazolam and the amount recovered from the loop was regarded as the absorbed amount. The estimated ER_G values are listed in Table 1.

The decrease in midazolam ER_G values at higher doses could be because of saturation of intestinal CYP3A-mediated metabolism, indicating that the intestinal first-pass metabolism of midazolam in rats is variable depending on the dose or on the rate of absorption of midazolam. Also, it might be suggested that the contribution of intestinal first-pass metabolism to bioavailability could be ruled out when midazolam was administered to these rats at a much higher dose, e.g. 10 μmol. It has been reported that the oral bioavailability of midazolam in man is less than 50% of the dose; the remainder is eliminated by both gastrointestinal and hepatic CYP3A-mediated metabolism, and the extraction ratios at both sites are comparable (Paine et al 1996; Thummel et al 1996). These results might indicate that intestinal and hepatic first-pass meta-

bolism of midazolam in man are 30% (approx.) As shown in Table 1, a similar extent of intestinal first-pass metabolism was observed after a low (0·05 μmol) dose of midazolam in untreated rats and after a higher dose (0·5 μmol) in dexamethasone-pretreated rats, although the actual concentration of midazolam in the intestinal lumen is not known in the above studies with man. However, current results suggest that rats, specifically those with enhanced CYP3A activity as a result of dexamethasone pretreatment, might be useful for prediction of drug–drug interactions of CYP3A substrates in the intestine in man, if an appropriate dose of the substrate was adopted in rats.

Estimation of hepatic first-pass metabolism of midazolam

The bioavailability (BA) is the product of the absorption ratio (F_A), intestinal availability (F_G), hepatic availability (F_H) and pulmonary availability (F_L), that is, $BA = F_A \times F_G \times F_H \times F_L$. F_G is also expressed as $1 - ER_G$, and F_H is also expressed as $1 - ER_H$ (where ER_H is the hepatic first-pass metabolism). If pulmonary first-pass metabolism can be neglected, ER_H is estimated as $1 - BA \times (F_A \times F_G)^{-1}$. To estimate its bioavailability midazolam was administered at a dose of 10 μmol, as shown in Figure 1, it was not detected in plasma when given as a low dose. In these circumstances, F_G of midazolam could be regarded as unity as discussed above. The estimated ER_H values of midazolam in differently treated rats are listed in Table 1. It was found that midazolam is extensively metabolized in the liver and the low oral bioavailability is mainly because of extensive hepatic first-pass metabolism, because of the saturation of intestinal first-pass metabolism when the dose of midazolam is high. However, the inhibitory effect of ketoconazole on hepatic first-pass metabolism of midazolam was relatively weak under these experimental conditions. The amounts of absorbed midazolam lost as a result of hepatic first-pass metabolism even after co-administration of ketoconazole were (approx.) 46% and 92%, respectively, in untreated and dexamethasone-pretreated rats. Many factors such as the oral bioavailability of ketoconazole itself, dilution of ketoconazole after absorption into the portal vein and restriction of hepatic distribution as a result of protein binding might be related to the relatively weak inhibitory effect of ketoconazole on hepatic first-pass metabolism of midazolam. We recently determined the hepatic extraction ratios of midazolam at a steady-state plasma concentration in differently treated rats; the observed hepatic extraction ratios of midazolam were 0·864 in untreated rats and 0·984 in dexamethasone-pretreated rats (unpublished results). Co-infusion of ketoconazole at a rate of 20 nmol min^{-1} reduced the hepatic extraction ratio to 0·697 in untreated rats and 0·813 in dexamethasone-pretreated rats. The estimated ER_H values of midazolam given intrajejunally in untreated and dexamethasone-pretreated rats in the current study were in good agreement with hepatic extraction ratios determined under conditions of steady-state plasma concentration, even though the contribution of pulmonary first-pass metabolism was neglected irrespective of the presence of CYP3A1 in rat lung (Debri et al 1995; Ghosal et al 1996). This might suggest that the contribution of pulmonary first-pass metabolism of midazolam is low, or non-existent, even in dexamethasone-pretreated rats.

In conclusion, the dose-dependent intestinal and hepatic first-pass metabolism of midazolam under conditions of differently modulated CYP3A activity in rats has been quantitatively estimated by in-vivo and in-situ absorption studies.

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