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Effect of Itraconazole on the Pharmacokinetics of Everolimus Administered by Different Routes in Rats

Akira Yokomasu, Ikuko Yano, Eriko Sato, Satohiro Masuda, Toshiya Katsura and Ken-ichi Inui*
Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

ABSTRACT: The effect of itraconazole on the pharmacokinetics of everolimus was investigated in rats. Ten minutes after an intravenous or intraintestinal administration of itraconazole, everolimus was delivered intravenously (0.2 mg/kg) or intraintestinally (0.5 mg/kg). Blood concentrations of everolimus were measured up to 240 min, and pharmacokinetic parameters were calculated. Intraintestinally administered itraconazole (20 mg/kg) significantly increased the area under the concentration–time curve (*AUC*) of intraintestinally administered everolimus about 4.5-fold, but even at 50 mg/kg did not affect the *AUC* of intravenously administered everolimus. However, intravenously administered itraconazole (50 mg/kg) increased the *AUC* of both intraintestinally and intravenously administered everolimus approximately 2-fold. Using a value for hepatic blood flow from the literature (50 ml/min/kg), the apparent intestinal and hepatic extraction of everolimus without itraconazole was calculated as about 80% and 13%, respectively. Intraintestinally administered itraconazole (20 mg/kg) changed the apparent intestinal extraction by 0.26-fold from 0.829 to 0.215, but the hepatic availability of everolimus was almost unchanged after the intravenous or intraintestinal administration of itraconazole even at a dose of 50 mg/kg from 0.871 to 0.923 or 0.867, respectively. In conclusion, intraintestinally administered itraconazole dramatically increased the *AUC* of everolimus delivered intraintestinally by inhibiting the intestinal first-pass extraction of this drug. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: everolimus; itraconazole; interaction; pharmacokinetics; first-pass extract

Introduction

Sirolimus, a prototype inhibitor of the mammalian target of rapamycin (mTOR), was approved by the Food and Drug Administration (FDA) as a new class of immunosuppressant in 1999. To improve the pharmacokinetic properties of sirolimus, new mTOR inhibitors which differ little in terms of pharmacodynamic effects were developed [1]. Everolimus, a derivative of sirolimus, exhibits greater polarity than sirolimus, has a slightly improved bioavailability, and is expected to achieve a steady-state more easily because of a shorter half-life [2]. A calcineurin inhibitor (e.g. cyclosporine or tacrolimus) is often used as a main immunosuppressant after organ transplantations, but causes complications such as renal dysfunction [3]. Since the available evidence indicates that everolimus is less associated with renal toxicity [4], everolimus is expected to be safe in the point of renal function and effective for immunosuppressive therapy with reduced doses of calcineurin inhibitors [5–7]. Everolimus is absorbed rapidly but its pharmacokinetics vary, probably due to the different activities of the hepatic and intestinal cytochrome P450 (CYP) 3A subfamily and drug efflux pump P-glycoprotein (Pgp) [2,8,9].

*Correspondence to: Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-8507, Japan.
E-mail: inui@kuhp.kyoto-u.ac.jp

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Therefore, therapeutic drug monitoring (TDM) is recommended to avoid the side effects of everolimus (e.g. leucopenia and hyperlipemia) due to its narrow therapeutic window [1,4].

Owing to immunosuppressive therapy after organ transplantations, patients are at an increased risk of developing a variety of infections, including fungal infections. For this reason, antifungal agents are usually used for prophylaxis or therapy. Azole antifungals such as ketoconazole and itraconazole are known to inhibit strongly both CYP3A-mediated metabolism and Pgp-mediated transport [10,11], and pharmacokinetic interactions are expected to be clinically significant in treatment with everolimus. Kovarik *et al*. [11] reported that during the coadministration of ketoconazole, the maximum concentration of everolimus increased 3.9-fold, and its area under the concentration–time curve (*AUC*) increased 15-fold in healthy subjects. Although pharmacokinetic interactions between itraconazole and tacrolimus or cyclosporine, which are substrates of CYP3A and Pgp, have been extensively reported [10], limited information is available on interactions between everolimus and intraconazole. Kovarik *et al*. [12] reported in their population pharmacokinetic evaluation of everolimus that one patient receiving itraconazole had a 74% reduction in clearance compared with the population average in renal transplant patients. Since itraconazole is available as oral and intravenous formulations, it is of interest to examine how differently the administered routes of itraconazole affect the pharmacokinetics of everolimus. This study investigated the effect of itraconazole on the pharmacokinetics of everolimus delivered via different routes, and quantitatively estimated the intestinal and hepatic extraction of everolimus in rats with or without itraconazole.

Materials and Methods

Materials

Everolimus was kindly provided by Novartis Pharma AG (Basel, Switzerland). It was formulated as a microemulsion preconcentrate for oral application and a concentrate for injection. 32-Desmethoxyrapamycin was obtained from Wyeth (Madison, NJ). Itraconazole (Itrizole injection 1% and Itrizole oral solution 1%) was obtained from Janssen Pharmaceutical K.K. (Tokyo, Japan). All other chemicals used were of the highest purity available.

Animals

Male Wistar/ST rats weighing 220–260 g (8 weeks old) were used for the experiments *in vivo*. Before the experiment, the animals were fasted overnight but given free access to water. Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), with supplemental doses administered as required. Body temperature was maintained with heating lamps. The experiments with animals were performed in accordance with the Guidelines for Animal Experiments of Kyoto University.

Pharmacokinetic analysis in rats

In each experiment, the femoral artery was cannulated with a polyethylene tube (PE-50; BD Biosciences, San Jose, CA) filled with heparinized saline (50 U/ml) for blood sampling. In the experiments for intravenous administration, the femoral vein was cannulated, and everolimus (0.2 mg/kg) and/or itraconazole were administered via the femoral vein. In the experiments for intraintestinal administration, the abdominal cavity was opened via a midline incision, and the upper site of the duodenum was exposed to administer everolimus and/or itraconazole. To examine the effect of the intraintestinal or intravenous administration of itraconazole (20 or 50 mg/kg), itraconazole or saline (control) was administered 10 min before everolimus. A 10 min delay between administration of itraconazole and everolimus was fixed in order to avoid pharmaceutical interactions of both drugs in the intestine. Blood samples were collected at 5, 15, 30, 60, 120, 180 and 240 min after the start of the administration of everolimus. Samples were placed into EDTA anticoagulant tubes.

Analytical methods

Whole blood samples (150 µl) were transferred to 13 ml tubes and spiked with an internal standard (10 µl of 300 ng/ml of 32-desmethoxyrapamycin in blood). Concentrations of everolimus were

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determined using high performance liquid chromatography with tandem mass spectrometry (LC/MS/MS) [9]. The lower limit of quantification was 0.5 ng/ml.

Pharmacokinetic analysis

Pharmacokinetic parameters, *AUC*, apparent clearance (CL/F) after intraintestinal administration, half-life ($T_{1/2}$), total body clearance (CL) and volume of distribution (V_d), were calculated by a non-compartment analysis using the program WinNonlin version 4.0.1 (Pharsight Co. Mountain View, CA). Maximum blood concentration (C_{max}) and time of maximum blood concentration (T_{max}) were obtained from the concentration–time curve of everolimus. Hepatic extraction was calculated with Equation (1), using a hepatic blood flow rate (Q, 50 ml/min/kg) from the literature [13]. The bioavailability (F) of everolimus administered intraintestinally was calculated from the ratio of dose-normalized *AUC* after the intraintestinal and intravenous administration according to Equation (2). Hepatic availability (F_h) was calculated using Equation (3). Apparent intestinal availability ($F_a{*}F_g$) and apparent intestinal extraction (E'_g) were calculated with Equations (4) and (5), respectively:

$$E_h = CL/Q \tag{1}$$

$$F = \frac{AUC_{i.i.}/\mathrm{Dose}_{i.i.}}{AUC_{i.v}/\mathrm{Dose}_{i.v.}} \tag{2}$$

$$F_h = 1 - E_h \tag{3}$$

$$F_a * F_g = F/F_h \tag{4}$$

$$E'_g = 1 - F_a * F_g \tag{5}$$

where $AUC_{i.i.}$ and $AUC_{i.v.}$ represent the *AUC* after intraintestinal and intravenous administration, respectively. $\mathrm{Dose}_{i.i.}$ and $\mathrm{Dose}_{i.v.}$ represent the dose of everolimus after the intraintestinal and intravenous administration, respectively. F_a and F_g represent the fraction of absorption and intestinal availability, respectively.

Statistical analysis

Values are expressed as the mean ± standard error of the mean (SE) for n experiments except for T_{max} which is shown as the median (min-max). Comparisons of mean blood concentrations among groups were performed with the repeated measures ANOVA. The statistical significance of differences in mean pharmacokinetic parameters between the two groups was analysed with a non-paired t-test provided that the variances were similar. If this was not the case, the Mann-Whitney test was applied. Multiple comparisons were performed with the Dunnett test following the ANOVA. The statistical analysis of the distribution of T_{max} was performed using the Mann-Whitney test. A difference was considered significant at $p < 0.05$.

Results

When both everolimus (0.5 mg/kg) and itraconazole (20 mg/kg) were administered intraintestinally, the blood concentration of everolimus was significantly increased compared with the control (Fig. 1A). As shown in Table 1A, the *AUC* was significantly increased about 4.5 times by itraconazole. The T_{max} tended to be delayed and the C_{max} was significantly increased about 3-fold, while the $T_{1/2}$ of everolimus did not differ between the control and itraconazole groups. The effect of intraintestinal itraconazole (50 mg/kg) on the pharmacokinetics after the intraintestinal administration of everolimus was not examined because of the pharmaceutical interaction of both drugs in the intestine. The uneven number of animals in Figure 1 was due to technical complications.

In a separate experiment, everolimus (0.5 mg/kg) was administered intraintestinally 10 min after the intravenous administration of itraconazole (50 mg/kg). The concentrations of everolimus were increased significantly (Fig. 1B), and the *AUC* was increased significantly about 2-fold, while the C_{max} and $T_{1/2}$ did not differ significantly between the two groups (Table 1A).

The intravenous administration of itraconazole (50 mg/kg) tended to increase the *AUC* and $T_{1/2}$ of everolimus delivered intravenously (Fig. 1C, Table 1B). After the intraintestinal administration of itraconazole (50 mg/kg), none of the pharmacokinetic parameters, *AUC*, *CL*, V_d or $T_{1/2}$, of everolimus administered intravenously was affected (Table 1B).

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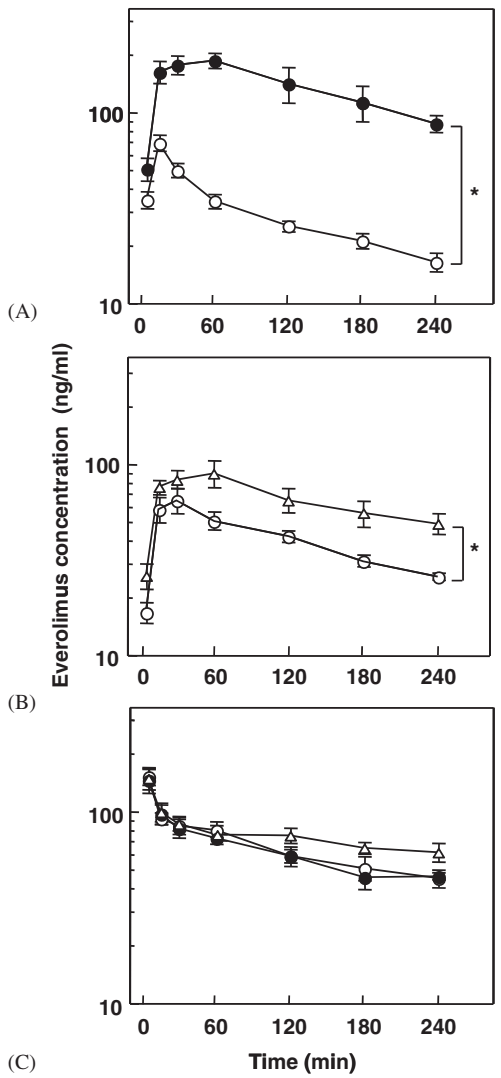

Figure 1. Effect of itraconazole on the blood concentrations of everolimus in rats. In panel A, everolimus was administered intraintestinally (0.5 mg/kg) 10 min after the intraintestinal administration of itraconazole (20 mg/kg: closed circles, $n = 4$) or saline (control: open circles, $n = 4$). In panel B, everolimus was administered intraintestinally (0.5 mg/kg) 10 min after the intravenous administration of itraconazole (50 mg/kg: open triangles, $n = 4$) or saline (control: open circles, $n = 5$). In panel C, everolimus was administered intravenously (0.2 mg/kg) 10 min after the intraintestinal (50 mg/kg: closed circles, $n = 3$) or intravenous administration of itraconazole (50 mg/kg: open triangles, $n = 4$) or saline (control: open circles, $n = 4$). $^{*}p < 0.05$ compared with the control by the repeated measures ANOVA

On the basis of these pharmacokinetic parameters, the hepatic and intestinal extraction of everolimus were calculated. The F of everolimus

Table 1. Pharmacokinetic parameters of everolimus with or without itraconazole

	AUC (mg h/l)	T_{max}[a] (min)	C_{max} (ng/ml)	*CL*/*F* (l/h/kg)	$T_{1/2}$ (h)
(A) Intraintestinal administration of everolimus (0.5 mg/kg)					
Control A ($n = 4$)	0.200 ± 0.025	15 (15–15)	69.0 ± 6.4	2.59 ± 0.27	3.38 ± 0.52
Itraconazole (i.i.) 20 mg/kg ($n = 4$)	0.891 ± 0.095[b]	60 (30–120)	201 ± 19[b]	0.586 ± 0.077[b]	2.84 ± 0.54
Control B ($n = 5$)	0.289 ± 0.026	30 (15–30)	65.2 ± 9.7	1.78 ± 0.14	3.50 ± 0.63
Itraconazole (i.v.) 50 mg/kg ($n = 4$)	0.548 ± 0.092[b]	60 (15–60)	90.5 ± 10.5	1.01 ± 0.20[b]	4.18 ± 0.70
	AUC (mg h/l)	*CL* (l/h/kg)	V_d (l/kg)	$T_{1/2}$ (h)	
(B) Intravenous administration of everolimus (0.2 mg/kg)					
Control ($n = 4$)	0.542 ± 0.072	0.386 ± 0.042	2.26 ± 0.30	4.19 ± 0.61	
Itraconazole (i.i.) 50 mg/kg ($n = 3$)	0.505 ± 0.027	0.399 ± 0.021	2.14 ± 0.18	3.78 ± 0.48	
Itraconazole (i.v.) 50 mg/kg ($n = 4$)	0.993 ± 0.194	0.232 ± 0.055	2.20 ± 0.16	7.53 ± 1.63	

(A) Everolimus (0.5 mg/kg) was administered intraintestinally 10 min after the intraintestinal (20 mg/kg) or intravenous (50 mg/kg) administration of itraconazole or saline (control A and B, respectively). (B) Everolimus (0.2 mg/kg) was administered intravenously after intraintestinal or intravenous administration of itraconazole (50 mg/kg).

[a]Median (min-max).

[b]$p < 0.05$ compared with the respective control.

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Table 2. Calculated parameters of bioavailability (F), apparent intestinal and hepatic extraction (E'_g, E_h), apparent intestinal availability (F_a*F_g) and hepatic availability (F_h) for everolimus

	F (fold)[b]	F_a*F_g (fold)	E'_g (fold)[b]
(A) Intraintestinal administration of everolimus (0.5 mg/kg)			
Control A	0.149	0.171	0.829
Itraconazole (i.i.) (20 mg/kg)	0.681 (× 4.57)	0.785[a] (× 4.59)	0.215 (× 0.259)
Control B	0.217	0.249	0.751
Itraconazole (i.v.) (50 mg/kg)	0.230 (× 1.06)	0.249 (× 1.00)	0.751 (× 1.00)
	E_h (fold)	F_h (fold)	
(B) Intravenous administration of everolimus (0.2 mg/kg)			
Control	0.129	0.871	
Itraconazole (i.i.) (50 mg/kg)	0.133 (× 1.03)	0.867 (× 0.995)	
Itraconazole (i.v.) (50 mg/kg)	0.077 (× 0.597)	0.923 (× 1.06)	

[a]This value was calculated using the F_h value in the case of itraconazole (i.i., 50 mg/kg).
[b]This value shows the ratio compared with each control.

was 0.149 or 0.217, the E_h was 0.129, and the E'_g was 0.829 or 0.751 without itraconazole (Table 2). After the intraintestinal administration of itraconazole (20 mg/ml), the E'_g of everolimus was significantly decreased from 0.829 to 0.215, accompanied by an increased in the F value to 0.681, while the E_h of everolimus was little changed even at a dose of 50 mg/kg. The E_h of everolimus decreased from 0.129 to 0.077 after the intravenous administration of itraconazole (50 mg/kg). However, the decrease little affected the F_h or F of everolimus.

Discussion

Everolimus is a substrate of CYP3A and Pgp, with a bioavailability that is relatively limited [2,8]. Itraconazole is an inhibitor of both CYP3A and Pgp, and is administered orally and intravenously in the clinical setting. Since CYP3A is expressed in the gastrointestinal tract wall and the liver, inhibition of CYP3A-mediated metabolism by itraconazole can occur at two sites. In addition, the dual cooperative functions of CYP3A and Pgp in the intestine may affect the interaction between itraconazole and everolimus. Therefore, it is of interest to evaluate quantitatively the influence of itraconazole on the pharmacokinetics of everolimus after the intraintestinal or intravenous administration of each drug in rats.

The *AUC* of everolimus administered intraintestinally was increased significantly about 4.5-fold by an intraintestinal administration of itraconazole (20 mg/kg) and about 2-fold by an intravenous administration (50 mg/kg). From these results, intraintestinally administered itraconazole more strongly affected the blood concentrations of everolimus delivered intraintestinally than did intravenously administered itraconazole. The effect of the intravenous or intraintestinal administration of itraconazole (50 mg/kg) on the pharmacokinetics of everolimus delivered intravenously was also examined, and the hepatic extraction of everolimus calculated using a rate of hepatic blood flow of 50 ml/min/kg [13]. The apparent intestinal extraction of everolimus was decreased 0.25-fold from 0.829 to 0.215 after the intestinal administration of itraconazole (20 mg/kg), but hepatic extraction was not affected by the intestinal administration of itraconazole even at a dose of 50 mg/kg from 0.129 to 0.133. However, the hepatic extraction of everolimus was decreased 0.6-fold from 0.129 to 0.077 after the intravenous administration of itraconazole (50 mg/kg). Therefore, although itraconazole can inhibit both the intestinal and hepatic extraction of everolimus, intraintestinally administered itraconazole markedly increased the blood concentration of everolimus delivered intraintestinally due to the inhibition of intestinal first-pass metabolism. This may be because a higher concentration of itraconazole is expected in the intestinal epithelial cells than in the hepatic cells after the intraintestinal administration and/or because itraconazole could inhibit the cooperative actions of CYP3A and Pgp in the intestine. That the calculated apparent intestinal

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extraction of everolimus was not affected by the intravenously administered itraconazole supports the use of the hepatic blood flow rate in the literature, since intravenously administered itraconazole does not exist in the intestine.

The blood concentrations of everolimus delivered intravenously were not affected by the intraintestinal administration of itraconazole (50 mg/kg), but were slightly increased by the intravenous administration of itraconazole (50 mg/kg) (Fig. 1C, Table 1). The bioavailability of itraconazole after the administration of an oral solution at a dose of 10 mg/kg was reported as 34.9% in rats due to a considerable intestinal first-pass effect [14]. Therefore, in the present study, the itraconazole concentration in the liver may have been lower after the intraintestinal administration than intravenous administration due to the intestinal first-pass effect, and intraintestinally administered itraconazole did not inhibit the CYP3A4-mediated metabolism of everolimus in the liver. In this study, the plasma concentration of itraconazole (50 mg/kg) at 240 min after the administration of everolimus was preliminarily measured by high performance liquid chromatography. The concentration of itraconazole and its active metabolite hydroxyitraconazole was, respectively, approximately 1500 and 700 ng/ml after the intestinal administration, and approximately 4500 and 1700 ng/ml after the intravenous administration. The trough concentration of itraconazole determined after 3 days of treatment with 200 mg twice a day is recommended as 500–2000 ng/ml for fungal treatment [15], which is similar to the range of concentration in our study.

Kovarik *et al*. [11] reported that the *AUC* of everolimus was increased by 15-fold after the coadministration of ketoconazole (200 mg) twice daily for a total of 8 days in healthy subjects. Ketoconazole is a potent inhibitor of CYP3A-mediated metabolism, as well as an inhibitor of P-gp-mediated transport [10]. Assuming that the bioavailability of everolimus in humans is similar to that in rats (approximately 20%), it is considered that ketoconazole inhibited both the first-pass metabolism and the systemic clearance of everolimus. It was reported that the inhibitory effect of ketoconazole on the metabolism of midazolam, a typical CYP3A substrate, was stronger than that of itraconazole in human intestinal and liver microsomes [16]. Therefore, the oral coadministration of itraconazole might moderately increase the *AUC* of everolimus delivered orally in humans as well as in rats (5-fold), but would not have as great an effect as ketoconazole (15-fold).

Several reports have been published concerning potential drug interaction between cyclosporine and itraconazole in transplant patients. Increases in cyclosporine blood concentrations were reported to range from 40% to 226% [17]. Similarly, several reports demonstrated drug interaction between tacrolimus and itraconazole, with increases in tacrolimus blood concentrations of 2- to 6.6-fold [17]. During concomitant therapy with an oral preparation of itraconazole, doses of cyclosporine and tacrolimus should be reduced 50–60%, and blood concentrations of the immunosuppressants should be monitored at the start of treatment [10]. Leather *et al.* [17] conducted an open-label, prospective evaluation of the pharmacokinetic drug interaction between intravenous itraconazole and intravenous cyclosporine or intravenous tacrolimus in 17 allogeneic hematopoietic stem cell transplant recipients. They suggested dose reductions of tacrolimus and cyclosporine in the range of 50% to 100%, which is comparable to the magnitude of the interaction seen with oral itraconazole and oral cyclosporine or tacrolimus. Although one should take species differences in the pharmacokinetic properties of these drugs into consideration, the magnitude of the interaction between oral itraconazole and oral everolimus might be greater than that between itraconazole and cyclosporine or tacrolimus.

The concentrations of tacrolimus and cyclosporine delivered intravenously were significantly increased after a switch from the intravenous to oral administration of fluconazole in clinical cases [18]. Therefore, the pharmacokinetic properties of inhibitory drugs for CYP3A should be considered when switching the route of administration. We also emphasize that since this is a single dose experiment using everolimus and itraconazole, extrapolation to any clinical scenario is limited since these drugs are used as multiple dose regimens.

In the present study, it was quantitatively demonstrated that significant pharmacokinetic interaction occurred between everolimus and

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itraconazole when the two drugs were administered intraintestinally, because of the high intestinal extraction. Since numerous drugs used clinically, including calcineurin inhibitors, are metabolized or eliminated by CYP3A4 or Pgp, intestinal and hepatic extraction ratios and the route of administration are important to predictions of the degree of drug–drug interaction. The findings presented here provide useful basic information about the pharmacokinetics of everolimus as well as a model of drug–drug interaction for achieving the optimal usage of pharmaceutical products.

Conclusion

The interaction of itraconazole with everolimus was most evident when both drugs were administered intraintestinally, because of inhibition of the intestinal first-pass metabolism of everolimus.

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