Enhanced Oral Bioavailability of Paclitaxel by Coadministration of the P-Glycoprotein Inhibitor KR30031

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Purpose. In an attempt to improve the oral bioavailability of paclitaxel, a novel P-glycoprotein inhibitor, KR30031, which is verapamil analog with fewer cardiovascular effects, was coadministered with paclitaxel, and to elucidate other possible causes of the low oral bioavailability of paclitaxel, an inhibitor of hepatic metabolism, ketoconazole, was also coadministered with paclitaxel.

Methods. *In vivo* oral absorption was tested in rats, and an *in vitro* study was also performed with a Caco-2 cell monolayer to identify the extent of P-glycoprotein inhibition.

Results. After coadministration of paclitaxel with ketoconazole, KR-30031, or KR-30031 and ketoconazole, bioavailability was increased about 1.6-, 7.5-, or 8.9-fold as compared with control, respectively. These results show that P-glycoprotein plays a major role in the oral bioavailability of paclitaxel. The effect of ketoconazole on oral bioavailability of paclitaxel was limited relative to the P-glycoprotein inhibition effect of KR-30031. *In vitro* study of Caco-2 cell transport showed that paclitaxel permeation was significantly higher when the drug was given from the basolateral side as compared to the permeation from the apical side, indicating the involvement of an enzyme reaction in the active efflux mechanism. Apical-to-basolateral transport of paclitaxel was increased in the presence of KR-30031. The ability of KR-30031 to reduce this efflux transport is equal to that of verapamil, a well-known P-glycoprotein inhibitor.

Conclusions. Our findings suggest that about 54% of a paclitaxel oral dose is extruded to the gut lumen by P-glycoprotein. Thus, the bioavailability of paclitaxel could be enhanced by coadministration of a P-glycoprotein inhibitor, KR-30031.

KEY WORDS: Paclitaxel; P-glycoprotein inhibitor; KR-30031; P-glycoprotein-mediated efflux pump; CYP3A inhibitor; poor oral bioavailability.

INTRODUCTION

Paclitaxel, a member of a new class of antimicrotubule anticancer drugs, is slightly soluble in water (1), and this has created significant problems in developing suitable injectable and infusion formulations useful for anticancer chemotherapy. The currently marketed form (Taxol®) of paclitaxel for intravenous infusion has been formulated utilizing Cremophor EL™ (polyethoxylated castor oil derivatives, BASF) and dehydrated alcohol (1:1).

The ethanol–Cremophor vehicle required to solubilize the paclitaxel in Taxol® is toxic. Cremophor EL™ itself is toxic and produces vasodilation, labored breathing, lethargy, and hypotension when administered intravenously (2). Hypersensitivity reactions mediated by endogenous histamine release are most prevalent with intravenous bolus administration and shorter infusion schedules (3,4).

In an attempt to develop safer clinical formulations, many studies have been directed to new oral formulations. Paclitaxel, however, is very poorly absorbed when administered orally (5–7). Several investigators (8–10) reported that poor bioavailability of paclitaxel could result from metabolism by enzymes or countertransport processes by P-glycoprotein in the gut wall. It has been speculated that, in some cases (10–13), the poor absorption of drugs after oral administration results from the activity of a multidrug transporter, a membrane-bound P-glycoprotein, which functions as an energy-dependent transporter or efflux pump to decrease intracellular accumulation of drugs by extruding xenobiotics from the cell. It is believed that the P-glycoprotein efflux pump prevents certain pharmaceutic compounds from transversing the mucosal cells of the small intestine and, therefore, from being absorbed into the systemic circulation. A number of pharmacologic agents including cyclosporine, verapamil, tamoxifen, quinidine, and phenothiazines have been shown to inhibit P-glycoprotein (11–13).

Verapamil and cyclosporine are the most extensively characterized inhibitors of P-glycoprotein and were the first multidrug resistance (MDR)-reversal agents that reached clinical trial (11,14,15). However, the usefulness of these drugs is limited because the plasma concentrations required to reverse MDR could result in cardiac toxicity such as hypotension, congestive heart failure, and heart block (16). Accordingly, considerable efforts have been directed toward the development of compounds that can inhibit P-glycoprotein without undesired toxicologic effects (17). Thus, the purpose of present study was to investigate the possibility of employing KR-30031, a verapamil analog with a P-glycoprotein-inhibiting effect but without cardiovascular adverse effects.

In addition to a multidrug efflux pump, phase I metabolism by intestinal cytochrome P450s (CYPs) is now becoming recognized as a significant factor in oral drug bioavailability (18,19). Phase I metabolism by intestinal CYPs has, until recently, been considered a relatively minor determinant of oral drug bioavailability because concentrations of individual CYPs, normalized for the entire intestine, are estimated to be approximately 1/200 to 1/20 of those found in the liver. This traditional view of intestinal metabolism has recently been reexamined in light of the findings that enzymes of the CYP3A subfamily, which have been considered to be the major phase I drug-metabolizing enzymes in humans, are expressed at high levels in the mature villus tip enterocytes of the small intestine. Although CYP3A constitutes only 30% of total human hepatic CYP, it accounts for approximately 70% of CYP in human enterocytes. It is worth noting that although the small intestinal villi receive a much lower blood flow than the liver does, the concentration of CYP3A in the villus tip enterocytes equals or exceeds the concentrations in the liver facilitating substantial first-pass metabolism.

In a recent publication, Sparreboom *et al.* (10) have shown that absorption of orally ingested paclitaxel was increased in mice homozygous for a disruption of the *mdr1a* gene in comparison with normal mice, demonstrating that P-glycoprotein played a major role in reducing the bioavailability of this agent. In addition, Asperen *et al.* (17) have

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shown that the oral bioavailability of paclitaxel in mice is substantially increased by the concomitant administration of a P-glycoprotein inhibitor such as the nonimmunosuppressive cyclosporine analog SDZ PSC 833. Until recently, there has been no paper that clearly described the quantitative profile of P-glycoprotein inhibition or extensive presystemic metabolism in the published studies.

In this study, we investigated how much the oral absorption of paclitaxel is affected by P-glycoprotein inhibition and extensive presystemic metabolism.

MATERIALS AND METHODS

Materials

Paclitaxel (98.3% as anhydrous base) and internal standard (13-{(2′*R*, 3′*S*)-3′-[(tert-butoxycarbonyl)amino]-2′-hydroxy-4′-methylpentanoyl}-9(*S*)-dihydro-14(*S*)-hydroxy-10-deacetyl baccatin III (HM50054), a paclitaxel derivative) were synthesized at Hanmi Pharm. Central R&D Center (Seoul, Korea). KR-30031 was synthesized at Bio-organic Division of Korea Research Institute of Chemical Technology (KRICT, Taejon, Korea) (Fig. 1). The following agents were kindly contributed by their suppliers: Cremophor ELP™ (Purified Cremophor EL, BASF, Germany), dimethylisosorbide (ICI Surfactants, GB), and Tween® 80 (ICI Surfactants, GB). All chemicals used were of reagent grade, and other solvents were of HPLC grade.

Deionized water was purchased from Merck (Lichrosolv® water, Germany). Male Sprague–Dawley rats (4–6 weeks old, 240 g) fed on a standard laboratory diet and allowed tap water *ad libitum* were used. Rats were kept at a temperature of 20–27°C, and the relative humidity of 55 ± 10% throughout the study.

Preparation of Paclitaxel Microemulsion Formulation

Paclitaxel was dissolved in vehicle consisting of dimethylisosorbide, Tween® 80, and *dl*-α-tocopheryl acetate at a concentration of 6 mg/mL. This formulation may include a P-glycoprotein inhibitor or CYP3A inhibitor as an absorption-enhancing agent for paclitaxel.

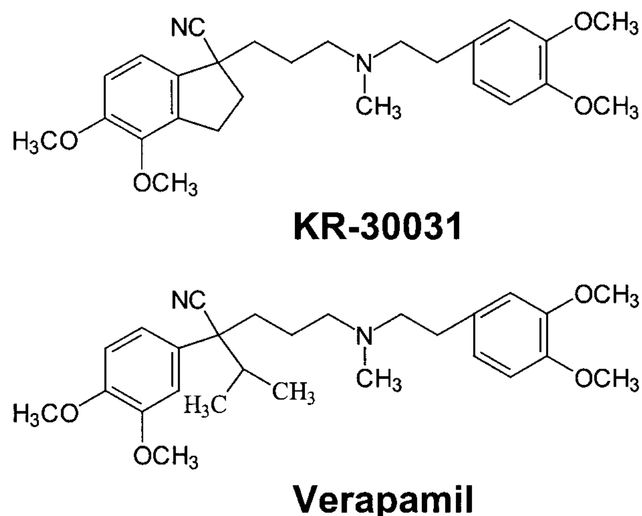

Fig. 1. Chemical structures of KR-30031 and verapamil.

Animal Experiments

All animal experiments were performed according to the Chungnam National University Guidelines for experimental animal care.

Paclitaxel was given orally at a dose of 25 mg/kg by injection with a blunt needle via the esophagus into the stomach. A rapid intravenous bolus injection was also done through a femoral vein at a dose of 1.33 mg/kg. Animals were anesthetized with diethyl ether and fixed in a supine position. Blood samples for oral administration were collected after 2, 4, 6, 8, 15, and 24 h by heart puncture using a 5 mL syringe filled with 100 IU of heparin as anti-coagulant.

For intravenous bolus experiments, blood samples were collected at 0.033, 0.083, 0.167, 0.333, 0.5, 1, and 1.5 h after dosing through a femoral artery cannulated with PE-50 polyethylene tubing. Urine was collected in a metabolic cage for intravenous bolus injections over the interval of 0–24 h after dose. Blood samples were centrifuged in an Eppendorf microvial for 5 min at 8,000 *g*. The supernatant plasma fraction was transferred to a clean vial and stored at –20°C until analysis. Five animals were used per sampling time point.

Analysis of Paclitaxel

An aliquot (200 μL) of plasma sample was mixed with 400 μL of internal standard HM50054 solution (paclitaxel derivative, 100 ng/mL in acetonitrile). After vortex mixing, the mixture was centrifuged for 5 min at 1,000 *g*, and 50 μL of supernatant was analyzed with a semimicro HPLC system using column switching. The limit of quantification (LOQ) for the assay was 0.5 ng/mL, based on the signal-to-noise ratio of 3.0. A semimicro HPLC system (SI-1 model) with two pumps, degasser, UV detector, column-switching valve, column oven, and autosampler was purchased from Shiseido (Japan). Samples were filtered through 0.22-μm PVDF membrane filters (Millipore No. SLGV013NL, USA).

The column-switching system was composed of an analytic column (Capcell Pak C_{18} UG120, 5 μm, 1.5 × 250 mm, Shiseido), a precolumn (Capcell Pak MF Ph-1, 4.6 × 10 mm, Shiseido), and a concentration column (Capcell Pak C_{18} UG120, 5 μm, 2.0 × 35 mm). Mobile phases for paclitaxel were 20% acetonitrile for precolumn and 55% acetonitrile for analytical column. The UV detector was set at 227 nm with 0.001 AUFS.

Caco-2 Cell Culture

Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were grown in 75 cm^2 tissue culture flasks at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity using Eagle's minimal essential medium supplemented with 10% fetal bovine serum, nonessential amino acids (1% v/v), penicillin (100 U/mL) and streptomycin (0.1 mg/mL). Cells were passaged at 80–90% confluency using phosphate-buffered saline containing 0.02% EDTA and 0.05% trypsin (20). For transport studies, the Caco-2 cells were seeded in Transwell with 1-cm^2 permeable polycarbonate inserts (0.4 μm pore size; Coring Costar Corp., Cambridge, MA) in 12-well plates (9). Medium was changed every other day until day 14 and then daily until they were used for experiments, 20–30 days after seeding (20). The integrity of the cell monolayers was measured by transepithelial electrical

resistance using an EVOM voltohmmeter (World Precision Instruments Inc., Sarasota, FL). Cells between passages 40 and 50 were used throughout.

Transport of Paclitaxel in Caco-2 Cell Monolayer

Before experiments, culture medium was removed, and the filter-grown Caco-2 cells were washed three times with warm HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-buffered Hanks' balanced salt solution (HBSS, pH 7.4) (20). After each wash, the plates were returned to the incubator for 30 min. Transepithelial electrical resistance was measured after the last wash. The buffer was then replaced with fresh HBSS/HEPES buffer on one side of the cell layer and paclitaxel microemulsion (5 μM as paclitaxel) in HBSS/HEPES buffer on the other side. For the absorption enhancement study, KR-30031 was added to the microemulsion at various concentrations. The volumes of apical side of the cell layer (insert) and the basolateral side (well) were 0.5 mL and 1.5 mL, respectively. In each experiment, three inserts were used for each treatment. Samples from the donor and receiver compartments were taken at every 30 min, and the drug transport was quantified by HPLC. Paclitaxel transport was expressed as a P_{app}, expressed in centimeters per second (20).

In transport inhibition experiments, verapamil hydrochloride (25 μM) and a new verapamil analog (KR-30031, 25 μM) were added to the buffer on the apical side. The paclitaxel concentration used in these experiments was 5 μM.

Data Analysis

The apparent permeability coefficients (P_{app}) in Caco-2 cell transport (9), expressed in centimeters per second, were calculated from Eq. (1).

$$P_{app} = \frac{\Delta Q}{\Delta t} \cdot \frac{1}{C_o \cdot A} \tag{1}$$

where $\Delta Q/\Delta t$ is the permeability rate (mol/s), C_o is the initial concentration on the apical side of cell monolayer (mol/mL), and A is the surface area of the porous membrane (cm^2).

Values for area under the paclitaxel concentration–time curve (AUC) were calculated by trapezoidal rule. AUC values were extrapolated from the 24-h time point with use of the elimination rate constant. Intravenous paclitaxel clearance (CL_{iv}) and observed oral bioavailability (F_{mean}) were calculated by noncompartmental methods as follows:

$$CL_{iv} = \frac{Dose}{AUC_{iv}} \tag{2}$$

$$F_{mean} = \frac{AUC_{oral}}{AUC_{iv}} \cdot \frac{Dose_{iv}}{Dose_{oral}} \tag{3}$$

The hepatic extraction ratio ($ER_h = CL/Q_h$) was calculated from intravenous data with hepatic plasma flow (Q_h) taken as 686 mL/h for a 240-g rat (21).

Differences in pharmacokinetic parameters were analyzed by the paired Student's t test.

RESULTS AND DISCUSSION

Caco-2 Cell Transport

Paclitaxel was loaded on either the apical or basolateral side of Caco-2 cell monolayers, and the fluxes of the drug across Caco-2 cell monolayers are shown in Fig. 2. The efflux from the basolateral side to the apical side (B→A) was about 12-fold greater than the flux from the apical side to the basolateral side (A→B), indicating the presence of an active efflux mechanism. Flux in A→B was about 9.1% of B→A efflux. The observed B→A efflux would be the result of passive diffusion plus active transport; the A→B flux would be the result of passive diffusion minus active transport. The active transport rate could be calculated as (basolateral transport – apical transport)/2 (9).

A series of inhibition experiments were carried out to elucidate the nature of the efflux from the basolateral to apical side. In these experiments, a 25 μM concentration of KR-30031, as the P-glycoprotein inhibitor, increased the apical-to-basolateral flux about twofold ($p = 0.007$). In similar experiments, a 25 μM concentration of verapamil, a calcium channel blocker, as the P-glycoprotein inhibitor increased the apical-to-basolateral flux about twofold ($p = 0.001$).

The ability of KR-30031 to reduce this active transport is equipotent with verapamil, a well-known P-glycoprotein inhibitor ($p > 0.05$). In the previous report, Choi *et al.* showed that KR-30031 was equipotent with verapamil in inhibiting the effects of multidrug resistance (MDR). Importantly, KR-30031 was 25- to 70-fold less potent than verapamil as a vasorelaxant and in decreasing left ventricular pressure (LVP) in guinea pig heart (16), suggesting that it could have few cardiovascular adverse effects. In other words, KR-30031 is a potent inhibitor of P-glycoprotein with reduced cardiovascular activity and could potentially be useful as a bioavailability-enhancing agent in combination with anticancer drugs.

The apparent permeability coefficients increased dose-dependently in concentration ratio of KR-30031 to paclitaxel up to 1:1. Above 1:1 concentration ratios, the P_{app} was shown to be saturable (Fig. 3), indicating the involvement of an enzyme reaction in the active efflux mechanism. The optimal

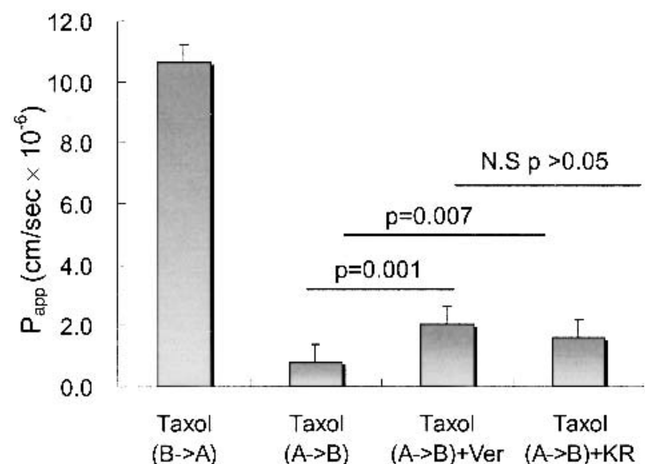

Fig. 2. Effect of verapamil and KR-30031 on paclitaxel apical (A) to basolateral (B) transport. Paclitaxel concentration was 5 μM. The concentrations of verapamil and KR-30031 were 25 μM. Each point is the mean value (±S.D.) of three experiments.

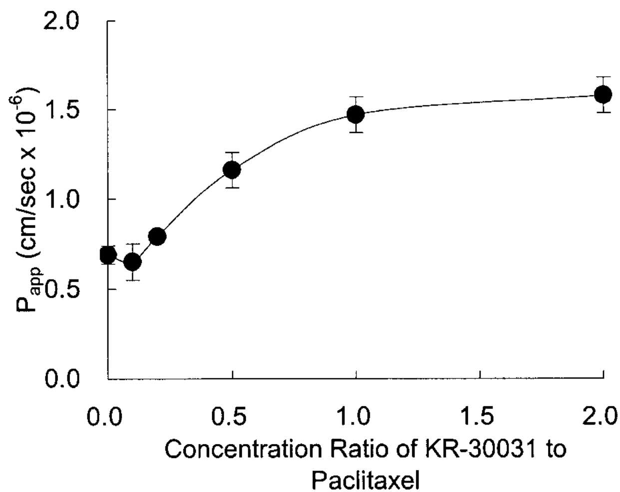

Fig. 3. Dose dependence of KR-300031 on apparent permeability (P_{app}) of paclitaxel apical-to-basal flux (absorption) in Caco-2 cell monolayer. Paclitaxel concentration was 5 μM. KR-30031 was added at various concentration ratios (1:0, 1:0.1, 1:0.2, 1:0.5, 1:1, 1:2).

dose of the enhancing agent (KR-30031) coadministered with paclitaxel for P-glycoprotein inhibition is about a 1:1 concentration ratio in the *in-vitro* Caco-2 cell transport experiment.

In Vivo Animal Studies

To improve the poor oral bioavailability of paclitaxel, an oral absorption enhancer was coadministered with paclitaxel. The absorption enhancers used in this study are KR-30031, a P-glycoprotein inhibitor, and ketoconazole (22,23), a CYP3A inhibitor.

If P-glycoprotein in the intestinal mucosa of rats is limiting the bioavailability of paclitaxel, the drug plasma level should be higher when it is coadministered with KR-30031 than when it is given alone. As shown in Fig. 4, administration of 25 mg/kg paclitaxel in combination with KR-30031 (20 mg/kg) resulted in a marked increased in *AUC* from 448.3 ± 64.71 (control) to 3,367 ± 341.2 ng·h/mL, an approximately 7.5-fold increase in *AUC*. Coadministration of paclitaxel with verapamil (20 mg/kg) produced an *AUC* of 2,231 ± 712.6 ng·h/mL, which was significantly lower than that (3,367 ± 341.2 ng·h/mL) obtained with KR-30031 (20 mg/kg) ($p < 0.05$) (Fig. 4).

*AUC*s of paclitaxel increase dose-dependently in the KR-30031 dose range up to 20 mg/kg. Above 20 mg/kg, the *AUC* was shown to be clearly saturable (Fig. 5), indicating the involvement of an enzyme reaction in the active efflux mechanism. The dosage range of KR-30031 coadministered with paclitaxel for P-glycoprotein inhibition is about 5–30 mg/kg in rats, optimally about 20 mg/kg. Our results show that P-glycoprotein in the intestine inhibits the uptake of orally administered paclitaxel and suggest that KR30031 enhances paclitaxel absorption by inhibiting P-glycoprotein.

Paclitaxel was reported to be metabolized by cytochrome P450 3A (CYP3A) in both the liver and epithelial cells of small intestine (8). Because of this, the effect of ketoconazole, a competitive CYP3A inhibitor, on the metabolism of paclitaxel was investigated (22). Plasma concentration–time curves for paclitaxel administered alone orally and coadministered with ketoconazole are shown in Fig. 6, and the pharmacokinetic parameters are given in Table I. The *AUC* of paclitaxel

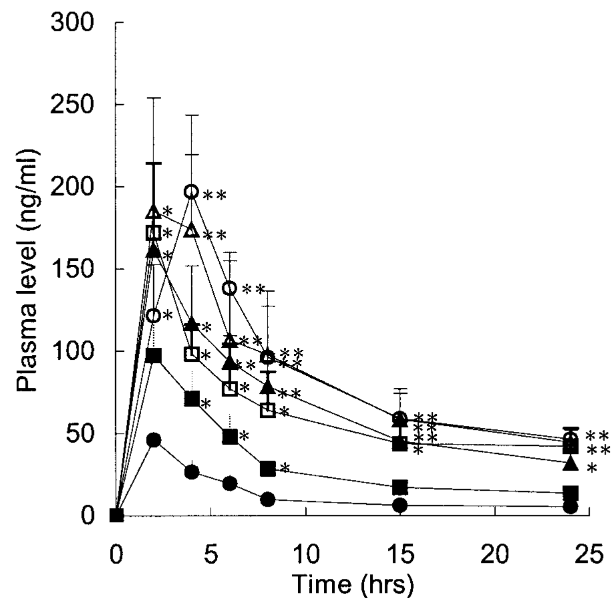

Fig. 4. Plasma concentration–time curves of paclitaxel in the rats after oral administration of paclitaxel (25 mg/kg) alone (control) and in combination with KR-30031or verapamil, Data are shown as the mean concentration, and error bars represent the S.E.(n = 5). Key: ●, control ; ■, +KR-30031, 5 mg/kg; ▲, +KR-30031, 10 mg/kg; ○, +KR-30031, 20 mg/kg; △, +KR-30031, 30 mg/kg; □, +verapamil, 20 mg/kg. *Significantly different compared with control group ($p < 0.05$). **Significantly different compared with control group ($p < 0.01$).

was significantly increased following combined treatment with ketoconazole (25 mg/kg) as compared to a control group (710.4 ± 82.30 ng·h/mL vs. 448.3 ± 64.71 ng·h/mL) ($p < 0.05$); C_{max}, however, was not significantly different ($p > 0.05$). Therefore, it is hypothesized that the low oral bioavailability of paclitaxel may be more dependent on the P-glycoprotein efflux pump in the intestinal mucosa than on intestinal metabolism by CYP3A.

To examine the additional effect of ketoconazole on bioavailability of paclitaxel, the drug and KR-30031 were coad-

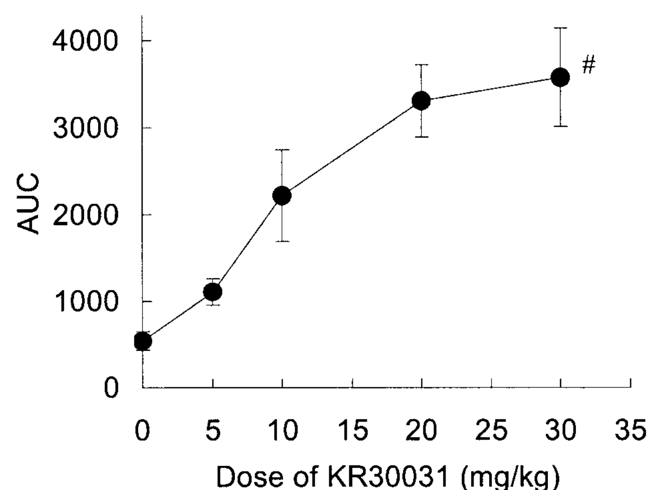

Fig. 5. Effect of KR-30031 dose on the AUC of paclitaxel. #Not significantly different compared with 20 mg/kg of KR-30031 ($p > 0.05$).

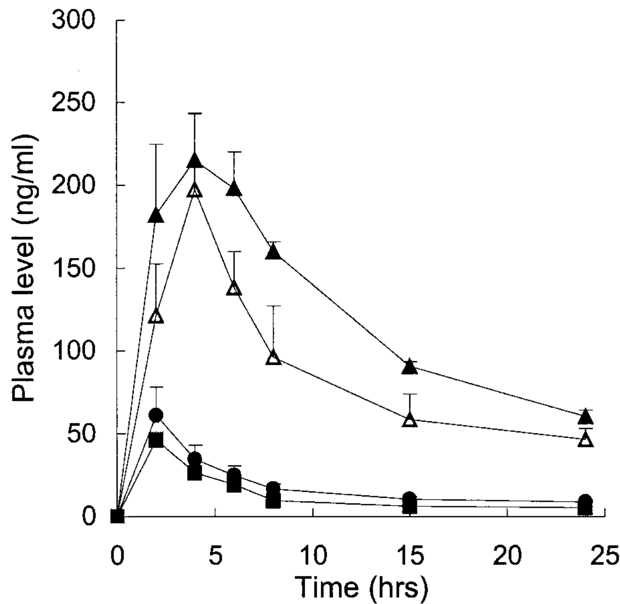

Fig. 6. Plasma concentration–time curves of paclitaxel in rats after oral administration of paclitaxel (25 mg/kg) alone (control), in combination with ketoconazole (25 mg/kg) or KR-30031 (20 mg/kg), and in combination with both KR-30031 (20 mg/kg) and ketoconazole (25 mg/kg). Data are shown as the mean concentration, and *error bars* represent the S.E. (n = 5). Key: ■, control; ●, +ketoconazole, 25 mg/kg; △, +KR-30031, 20 mg/kg; ▲, +KR-30031, 20 mg/kg, +ketoconazole, 25 mg/kg.

ministered with ketoconazole (Fig. 6), and the pharmacokinetic parameters of paclitaxel were calculated and are given in Table I. The AUC (4,001 ± 200.4 ng·h/mL) of paclitaxel in combination with ketoconazole and KR-30031 was significantly higher (3,367 ± 341.2 ng·h/mL) than that with coadministration of KR-30031 alone ($p < 0.05$), but the C_{max} value was not significantly different between them ($p > 0.05$). Based on these results, it might be concluded that the bioavailability of paclitaxel coadministered with a P-glycoprotein inhibitor such as KR-30031 could be increased to some extent by inhibition of hepatic metabolism with CYP3A inhibitor such as ketoconazole.

Plasma concentration–time courses for intravenous paclitaxel before and after ketoconazole and KR-30031 treatment are shown in Fig. 7. Pharmacokinetic parameters calculated from these data are given in Table I. The effect of ketoconazole and KR-30031 coadministered with paclitaxel on the hepatic and intestinal metabolism of paclitaxel can be differentiated by treating the observed oral bioavailability (F_{oral}) as the product of the fraction absorbed intact from the intestinal lumen (F_{abs}) multiplied by the fraction reaching the portal blood unmetabolized by the intestine (F_G) multiplied by the fraction passing through the liver unmetabolized (F_H). Oral bioavailability (F_{oral}) is given in Eq. (4) (19,24).

$$F_{oral} = F_{abs} \cdot F_G \cdot F_H \tag{4}$$

Gut and hepatic availability may be defined as 1 minus the extraction ratio (ER) at each site.

$$F_{oral} = F_{abs} \cdot (1 - ER_G) \cdot (1 - ER_H) \tag{5}$$

When a drug is given intravenously, it is possible to calculate the hepatic extraction ratio, as given in Eq. (6), from the ratio of the hepatic clearance (CL_{H}) to the hepatic blood flow (Q_{H}).

$$ER_H = CL_H/Q_H \tag{6}$$

For a drug administered intravenously, it is possible to estimate the hepatic inhibition ratio (X_{H}) from the extraction ratios determined postketoconazole (Ket) and preketoconazole (control) dosing as given in Eq. (7). The oral bioavailability during the inhibition period may be estimated by Eq. (8), where X_{G} represents the gut inhibition ratio.

$$X_{\mathrm{H}} = \frac{ER_{\mathrm{H}}^{\mathrm{Ket}}}{\mathrm{ER}_{\mathrm{H}}} \tag{7}$$

$$F_{\mathrm{oral}} = F_{\mathrm{abs}}^{\mathrm{Ket}} \cdot (1 - X_{\mathrm{G}}ER_{\mathrm{G}}) \cdot (1 - X_{\mathrm{H}}\mathrm{ER}_{\mathrm{H}}) \tag{8}$$

As shown in Table I, values for F_{oral}, ER_{H}, and X_{H} can be determined directly from the data in Table I.

The total clearance of a drug is equal to the hepatic clearance when the liver is the sole organ responsible for elimination. Another way of looking at this relationship is that clearance terms are additive. Therefore:

$$CL_{\mathrm{total}} = CL_{\mathrm{H}} + CL_{\mathrm{R}} + CL_{\mathrm{other\ organs}} \tag{9}$$

and CL_{total} is equal to CL_{H} plus CL_{R} when $CL_{\mathrm{other\ organs}}$ are negligible.

The time-averaged renal clearance (CL_{R}) was calculated by dividing the X_U^∞ (the total amount of drug excreted intact in urine) by the AUC^∞ after i.v. bolus injection. The renal clearance of paclitaxel after i.v. bolus injection is 1.9 L/h/kg (X_U^∞ = 229 μg for rats weighing 240 g).

The hepatic bioavailability (F_{H}) was calculated utilizing Eq. 6 assuming that substracting the renal clearance from the total clearance gives the hepatic clearance after an intrave-

Table I. Mean (±S.E.) Pharmacokinetic Parameters following Oral (25 mg/kg) and Intravenous (1.33 mg/kg) Administration of Paclitaxel in Five Rats

	Parameters						
Administration	AUC_{iv} (ng · h/mL)	AUC_{oral} (ng · h/mL)	CL_{iv} (L/h/kg)	F_{oral}[a] (%)	F_{H} (%)	X_{H}	BA[b] (%)
Paclitaxel alone	520.1 ± 63.15	448.3 ± 64.71	2.56 ± 0.35	4.6	77	—	4.6
Combination with ketoconazole	592.8 ± 19.86	710.4 ± 82.30	2.24 ± 0.07	6.4	88	0.45	7.3
Combination with KR-30031	556.9 ± 14.22	3367 ± 341.2	2.40 ± 0.06	32.2	—	—	34.4
Combination with ketoconazole and KR-30031	624.8 ± 25.53	4001 ± 200.4	2.14 ± 0.09	34.1	—	—	40.9

[a] F_{oral} is the value of $AUC_{\mathrm{oral}}/AUC_{\mathrm{iv}}$ in each administration group expressed as mean.
[b] BA (bioavailability) is the value of (AUC_{oral} in each group)/(AUC_{iv} in the administration group of paclitaxel alone) expressed as mean.

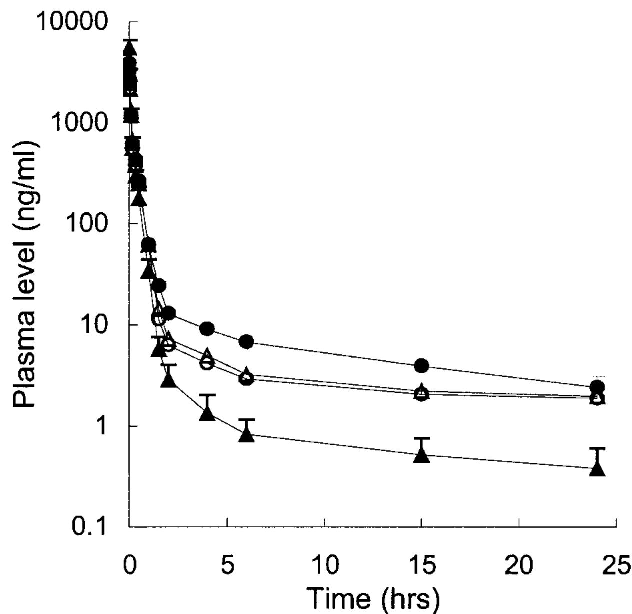

Fig. 7. Plasma concentration–time curves following intravenous bolus administration of paclitaxel into the rats (dose = 1.33 mg/kg). Data are shown as mean concentrations, and *error bars* represent the S.E. (n = 5). Key: ▲, paclitaxel alone; ○, combination with KR-30031; △, combination with ketoconazole; ●, combination with KR-30031 and ketoconazole.

nous dose and that hepatic plasma flow is 686 mL/h for rats weighing 240 g (21).

It is apparent from Eqs. (5) and (8) above that three unknown parameters exist: the fraction of oral dose absorbed (assuming that ketoconazole has no effect on absorption) (22); the extraction ratio in the gut; and the effect of ketoconazole on the gut extraction (X_G).

The inhibitory effect of ketoconazole on hepatic extraction can be calculated using Eq. (7) as given in Table I, assuming that the inhibition of metabolic enzymes in the gut is identical to that in the liver, i.e., $X_G = X_H$, thus allowing solution of F_{abs} and ER_G from Eqs. (5) and (8). Substituting data in Table I into Eqs. (5) and (8) comes up with 8.2% for F_{abs} and 0.277 for a gut metabolic extraction ratio (ER_G).

The unabsorbed ratio after coadministeration of KR-30031 was calculated using 0.723 for F_G and 0.770 for F_H assuming that the P-glycoprotein is inhibited completely by KR-30031. Substituting the value (F_{oral} *0.344*) after coadministration of KR-30031 in Table I into Eq. (10) yields a value of 0.38 for the total unabsorbed ratio.

$$F_{oral} = (1 - \textit{total unabsorbed ratio}) \cdot F_G \cdot F_H \quad (10)$$

In addition, substituting the value (F_{abs} *0.082*) from the control into Eq. (11) yields a value of 0.54 for the efflux ratio by P-glycoprotein in the intestinal mucosa.

$$\begin{aligned} F_{abs}(0.082) &= 1 - \textit{total unabsorbed ratio} \\ &= 1 - (\textit{unabsorbed ratio} + \textit{efflux ratio}) \quad (11) \end{aligned}$$

These results suggest that (a) the administration of paclitaxel alone produces poor bioavailability, and (b) about 54% of paclitaxel absorbed orally is pumped out of the gut lumen by P-glycoprotein.

A schematic diagram depicting the various processes leading to the low bioavailability following oral administration of paclitaxel is shown in Fig. 8. The high oral bioavailability of paclitaxel in this study confirmed that the P-glycoprotein inhibitor KR-30031 was absorbed to cause F_{oral} to increase from about 4.6% to about 34%. In addition, the inhibition of metabolism in the liver was observed to cause F_{oral} to increase from about 34% to about 41%.

Because such a large number of drugs are substrates for CYP3A and/or P- glycoprotein, which both reside in the human intestine, it is very likely that decreased bioavailability for a number of compounds could result from metabolism and/or countertransport of absorbed drug back into the intestinal lumen. Selective inhibition of these processes in the gut to increase drug bioavailability while decreasing variability is an attractive approach and has excellent prospects for successful drug delivery.

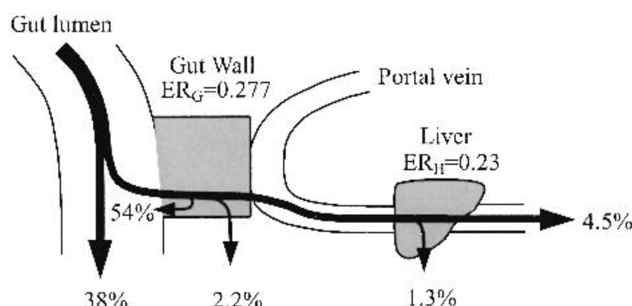

Fig. 8. Schematic diagram depicting the various processes explaining the bioavailability following an oral dose of paclitaxel in the control (paclitaxel only). The values at the bottom of each figure indicate the average fraction of the dose lost in each of the processes.

CONCLUSIONS

The low oral bioavailability of paclitaxel in this study confirmed that about 54% of a paclitaxel oral dose is extruded to the gut lumen by P-glycoprotein. This result shows a strikingly increased oral bioavailability of paclitaxel in the group treated in combination with KR-30031 compared with the control group. Treatment with KR-30031 increased the bioavailability (= $(AUC_{oral} / AUC_{iv}) \cdot (Dose_{iv} / Dose_{oral}) \times$ 100%) from 4.6% to 34.4%.

In conclusion, the P-glycoprotein inhibitor KR-30031 could significantly enhance the oral absorption of paclitaxel. Selective inhibition of this process in the gut to increase drug bioavailability is an attractive method for effective delivery.

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