Arch Pharm Res Vol 29, No 1, 102-107, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Effect of Naringin Pretreatment on Bioavailability of Verapamil in Rabbits

Cheul-Ho Yeum and Jun-Shik Choi[1]

College of Medicine and [1]College of Pharmacy, Chosun University, Gwangju 501-759, Korea

(Received August 24, 2005)

The aim of present study is to investigate the effect of naringin on the pharmacokinetics of verapamil and its major metabolite, norverapamil in rabbits. The pharmacokinetic parameters of verapamil and norverapamil were determined after administering verapamil (9 mg/kg) orally to rabbits in the pretreated with naringin (1.5, 7.5, and 15 mg/kg). Naringin pretreatment significantly altered the pharmacokinetic parameters of verapamil. Compared with the control group (given verapamil alone), the K_a, C_{max} and AUC of verapamil were significantly ($p<0.05$ or $p<0.01$) increased in the pretreatment of naringin, However there were no significant change in T_{max} and $t_{1/2}$ of verapamil. Consequently, pretreatment of naringin significantly ($p<0.05$, $p<0.01$) increased the AB% of verapamil significantly in a dose dependent manner ($p<0.05$ or $p<0.01$), and elevated the RB% of verapamil by 1.26- to 1.69-fold. the MR of verapamil were significantly ($p<0.05$) increased in the pretreatment of naringin, implying that pretreatment of naringin may effectively inhibit the CYP3A4-mediated metabolism of verapamil. In conclusion, pretreatment of naringin enhanced the oral bioavailability of verapamil. Based on these results, the verapamil dosage should be adjusted when given with naringin or a naringin-containing dietary supplement.

Key words: Naringin, Verapamil, CYP3A4, Pharmacokinetics, Rabbit

INTRODUCTION

Verapamil, a calcium channel-blocker that is widely used as an antiarrhythmic agent to control supraventricular tachyarrhythmias. Due to its potent vasodilating and negative inotropic properties of verapamil is useful for the treatment of hypertension, ischaemic heart disease, and hypertrophic cardiomyopathy (Fleckenstein *et al.*, 1977; Gould *et al.*, 1982; Lewis *et al.*, 1978). Verapamil is rapidly absorbed after oral administration and widely distributed throughout the body. Orally administered verapamil undergoes the extensive first-pass hepatic metabolism through the portal circulation, resulting in a low bioavailability (10-20%) (Schomerus *et al.*, 1976). The oxidative metabolic pathways of verapamil and the contribution of the different cytochrome P450 enzymes involved have been studied extensively (Buse *et al.*, 1995; Eichelbaum *et al.*, 1979; Kroemer *et al.*, 1993). The primary metabolic pathways of verapamil include N-demethylation and N-dealkylation. CYP3A4 is mainly responsible for the N-demethylation of verapamil, while the N-dealkylated metabolite is formed by CYP1A2. Norverapamil, a major metabolite, is a N-demethylated metabolite of verapamil and appears to have approximately 20% of the coronary vasodilator activity of the parent compound in dogs (Eichelbaum *et al.*, 1984). Verapamil is also known to be a substrate and inhibitor of the P-glycoprotein (P-gp) (Doppenschmitt *et al.*, 1999). CYP3A4 and P-gp might be the main factors lowering bioavailability of verapamil in the intestine, since both of them located in the intestinal membrane to resist the systemic exposure of their substrates (Benet *et al.*, 2003; Cummins *et al.*, 2002).

Naringin is a naturally occurred flavonoid and mainly presented as glycosides components of the daily diet, such as grapefruit juice, apples, onions, and tea (Cody *et al.*, 1986, 1988; Dixon *et al.*, 1999). Naringin displays a variety of biological actions as anti-oxidation, antiulcer, antiallergic and anticancer, etc (Davis *et al.*, 2000; Nijveldt *et al.*, 2001; Takahama *et al.*, 1985). Naringin has been reported as a CYP3A4 inhibitor (Doostdar *et al.*, 2000; Hodek *et al.*, 2002; Dupuy *et al.*, 2003) as well as a P-gp modulator (Dupuy *et al.*, 2003; Bardelmeijer *et al.*, 2000).

Correspondence to: Jun-Shik Choi, College of Pharmacy, Chosun University, Susuk-dong, Dong-gu, Gwangju 501-759, Korea
Tel.: 82-62-230-6365, Fax: 82-62-222-5414.
E-mail: jsachoi@chosun.ac.kr

Naringin is rapidly transformed into naringenin by the bacteria producing alpha-rhamnosidase and beta-glucosidase in human intestinal microflora (Kim *et al.*, 1998). It is reported that oral bioavailability of quinine was significantly increased from 17% to 42% after pretreatment with naringin which inhibited CYP3A4 in rats (Zhang *et al.*, 2000) and *in vitro* test (Ho *et al.*, 2001). Both naringin and naringenin reduced apical efflux of vinblastine, a substrate of P-gp in Caco-2 cells (Takanaga *et al.*, 1998). Naringin and naringenin also inhibit CYP3A4-mediated metabolism and P-gp transport of saquinavir in Caco-2 cell (Eagling *et al.*, 1999). Therefore, naringin might act as the inhibitor of CYP3A4 and P-gp. Considering that CYP3A4-mediated metabolism and P-gp mediated transport are the major factors limiting the oral bioavailability of verapamil, the concomitant use of potent inhibitors for both CYP3A4 and P-gp could be effective to alter the bioavailability of verapamil. Therefore, the aim of present study is to investigate the effect of naringin on the bioavailability & pharmacokinetics of verapamil and its major metabolite, norverapamil, after oral administration of verapamil pretreated with naringin to rabbits.

MATERIALS AND METHODS

Materials

Verapamil, norverapamil, naringin, and propranolol were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetonitrile, triethylamine and diethylether were purchased from Merck Co. (Darmstadt, Germany). Phosphoric acid was purchased from the Junsei Co. (Tokyo, Japan). All other chemicals were reagent grade and all solvents were HPLC grade.

Animal studies

White male New Zealand rabbit was chosen as the animal model in this study because the pharmacokinetics of verapamil have been well characterizend in rabbits (Giacomini *et al.*, 1985; Dey *et al.*, 2003), and recent studies have shown that rabbit MDR1 and human MDR1 share a high sequence homology (89%) (Mori *et al.*, 2001). All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (U.S.A.) and the animal care committee of Chosun University approved experimental protocols. The animals were kept in these facilities for at least one week prior to the experiments. White male New Zealand rabbits weighing 2.0-2.4 kg were fasted for at least 24 h prior to the experiment and were given water ad libitum. Rabbits were given orally 9 mg/kg of verapamil in a single dose (control group) with or without pretreatment of naringin (1.5 mg/kg, 7.5 mg/kg or 15 mg/kg) 30 min prior to verapamil administration (pretreated group). 2 mg/kg of verapamil was administered intravenously to rabbits *via* an ear vein (iv group). Blood samples were withdrawn from the femoral vein at 0, 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, and 24 h of time point after verapamil administration. Blood samples were centrifuged at 3,000 rpm for 10 min and the plasma (0.5 mL) was transferred and stored at -40°C until analyzed by HPLC.

HPLC assay

The plasma concentrations of verapamil and norverapamil were determined by a HPLC assay using the method reported by Krecis-Shepard *et al.*with some modification. Briefly, 0.1 mL of propranolol HCl (400 ng/mL) as the internal standard, 50 μL of a 2 N sodium hydroxide solution and 6 mL of diethyl ether were added to 0.5 mL of the plasma samples. The mixture was then stirred for 3 min and centrifuged at 5,000 rpm for 10 min. 5 mL of the organic layer were transferred to a clean test tube and evaporated at 35°C under a stream of nitrogen. The residue was dissolved in 250 μL of the mobile phase, centrifuged at 5,000 rpm for 5 min and then 50 μL of the supernatant was injected into an HPLC system.

The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a fluorescence detector (Model RF-10A), a system controller (Model SCL-10A), degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The fluorescence detector was set at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. The stationary phase was a Kromasil KR 100-5C8 column (5 μm, 4.6×250 mm, EKA chemicals, Sweden) and the mobile phase was acetonitrile: 0.05 M KH_2PO_4 with 0.05% triethylamine (30:70, v/v). The pH of the buffer was adjusted to 4.0 with 20% phosphoric acid. The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter. Chromatograms of rabbit's blank plasma and the plasma spiked with verapamil, norverapamil and internal standard propranolol were shown in Fig. 1. The retention times at a flow rate of 1.5 mL/min were as follows: internal standard, 4.5 min, norverapamil, 12.2 min, and verapamil, 13.4 min. The calibration curve was obtained from the standard samples at the following concentration; 2, 10, 20, 50, 100, 200, and 400 ng/mL for norverapamil and 5, 15, 50, 100, 400, and 2000 ng/mL for verapamil. The following regression were obtained; y=0.023x-0.088 (r=0.999) for verapamil, y=0.035x -0.149 (r=0.999) for norverapamil.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed by the LAGRAN method using the LARGAN computer program (Rocci *et al.*, 1983) The area under the plasma concentration-time curve from time zero to infinity

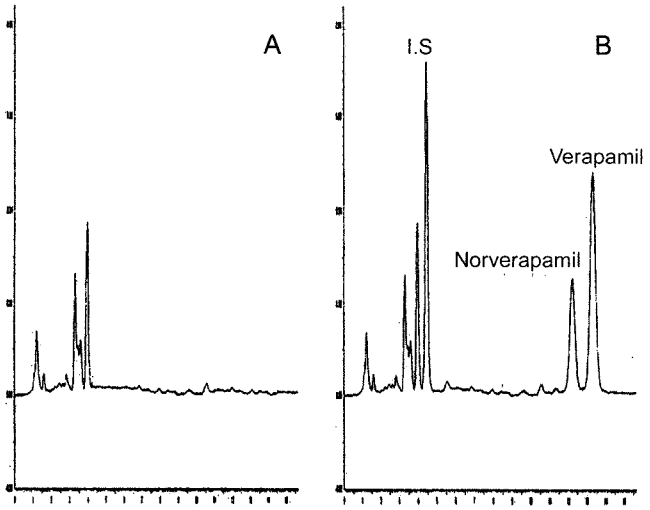

Fig. 1. Chromatograms of blank plasma (A) and blank plasma spiked with verapamil (retention time of 13.4 min), norverapamil (retention time of 12.2 min), and an internal standard (IS: retention time of 4.5 min) (B).

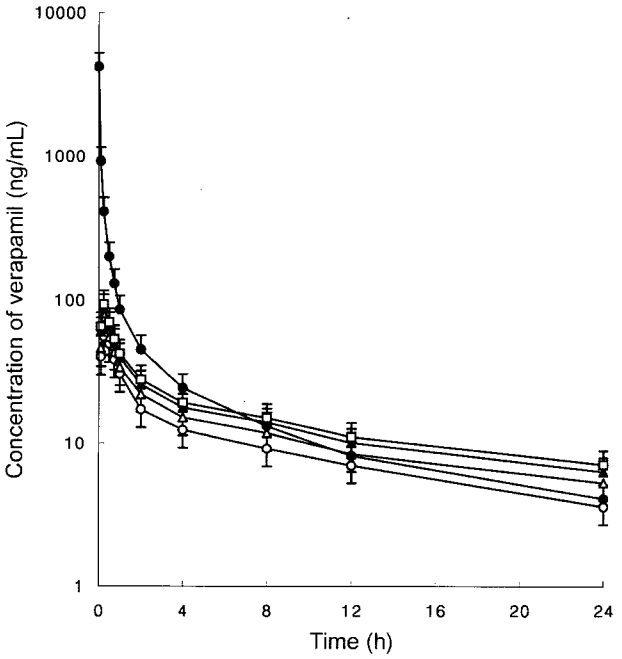

Fig. 2. Mean plasma concentration-time profiles of verapamil after intravenous (2 mg/kg) or oral administration of verapamil (9 mg/kg) with or without naringin in rabbits. Bars represent the standard deviation (n = 6). (○) Control (verapamil alone), (△) Pretreated with naringin 1.5 mg/kg, (▲) Pretreated with naringin 7.5 mg/kg, (□) Pretreated with naringin 15 mg/kg, (●) Verapamil IV 2 mg/kg.

(AUC) was computed using the LAGRAN method in order to reduce the errors associated with using the trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were determined by obtained from the experimental data. The elimination rate constant (K_{el}) was estimated from the slope of the regression line of best fit, and the half-life ($T_{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability (AB%) of verapamil was calculated by $(AUC_{oral}/AUC_{i.v.})(Dose_{i.v.}/Dose_{oral})\times100$, and the relative bioavailability (RB%) of verapamil was estimated by $(AUC_{pretreated}/AUC_{control})\times100$. The metabolite-parent AUC ratio (MR) was estimated by $AUC_{norverapamil}/AUC_{verapamil}$.

Statistical analysis

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A $p<0.05$ was considered statistically significant.

RESULTS

The mean plasma concentration-time profiles of verapamil pretreated with naringin were characterized in rabbits (Fig. 2). The mean pharmacokinetic parameters of

Table I. Mean pharmacokinetic parameters of verapamil after an intravenous (2 mg/kg) or oral administration of verapamil (9 mg/kg) pretreated with naringin to rabbits

Parameters	Verapamil control	Naringin pretreatment			IV 2 mg/kg
		1.5 mg/kg	7.5 mg/kg	15 mg/kg	
AUC (ng/mL·h)	291 ±75.7	370 ±96.1*	445 ±1025**	494 ±142**	731 ±190.1
C_{max} (ng/mL)	55.2 ±14.4	66.5 ±17.3	86.9 ± 53.3**	92.6 ± 54.5**	
T_{max} (h)	0.25± 0.07	0.25± 0.08	0.25± 0.06	0.25± 0.08	
K_a (h^{-1})	3.8 ± 0.99	5.1 ± 1.30	6.1 ± 1.59*	6.8 ± 1.72*	
$t_{1/2}$ (h)	12.0 ± 3.12	13.4 ± 3.46	13.6 ± 3.51	13.9 ± 3.61	8.4± 2.2
AB (%)	8.8 ± 2.3	11.2 ± 2.8*	13.5 ± 3.2**	15.0 ± 3.4**	100
RB (%)	100	126	153	169	

Mean±S.D. (n = 6), *p<0.05, **p<0.01, significant difference compared to control.

K_a: absorption rate constant, AUC: area under the plasma concentration-time curve from 0 h to infinity, C_{max}: peak concentration, T_{max}: time to reach peak concentration, $t_{1/2}$: terminal half-life, AB (%): absolute bioavailability, RB (%): relative bioavailability, compared $AUC_{pretreated}$ to AUC_{contro}.

verapamil were also summarized in Table I. As shown in Table I, Pretreatment of naringin significantly altered the pharmacokinetics of verapamil ($p<0.05$ or $p<0.01$). Compared to the control group. The K_a, C_{max} and AUC of verapamil were significantly ($p<0.05$ or $p<0.01$) increased in the rabbits pretreated with naringin, while there was no significant change in T_{max} and $t_{1/2}$. Consequently, Naringin pretreatment significantly ($p<0.05$ or $p<0.01$) increased the AB% of verapamil and elevated the RB% of verapamil by 1.26- to 1.69- fold. The bioavailability of verapamil was significantly enhanced by increasing the dose of naringin from 1.5 mg/kg to 15 mg/kg, implying that the inhibition effect of naringin was dose-dependent within the dose range from 1.5 to 15 mg/kg.

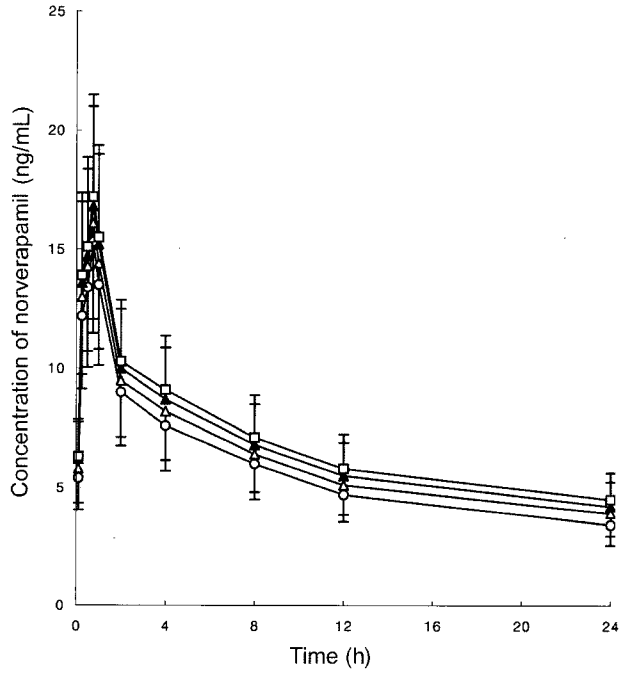

Fig. 3. Mean plasma concentration-time profiles of norverapamil after oral administration of verapamil (9 mg/kg) with or without naringin to rabbits. Bars represent the standard deviation (n = 6). (○) Control (verapamil alone), (△) Pretreated with naringin 1.5 mg/kg, (▲) Pretreated with naringin 7.5 mg/kg, (□) Pretreated with naringin 15 mg/kg.

Table II. Mean pharmacokinetic parameters of norverapamil, a major metabolite of verapamil after oral administration of verapamil (9 mg/kg) pretreated with naringin to rabbits

Parameters	Verapamil control	Naringin pretreatment		
		1.5 mg/kg	7.5 mg/kg	15 mg/kg
AUC (ng/mL·h)	223 ±59.3	247 ±62.7	268 ±69.7	289 ±75.1
C_{max} (ng/mL)	15.3 ± 3.98	16.1 ± 4.19	16.8 ± 4.37	17.2 ± 4.47
T_{max} (h)	0.75± 0.20	0.75± 0.20	0.75± 0.20	0.75± 0.20
$t_{1/2}$ (h)	18.9 ± 4.94	19.6 ± 5.02	20.0 ± 5.2	20.8 ± 5.36
MR	0.78± 0.21	0.66± 0.20	0.60± 0.18*	0.58± 0.17*

Mean±S.D. (n = 6), *$p<0.05$, significant difference compared to control. AUC: area under the plasma concentration-time curve from 0 h to infinity, C_{max}: peak concentration, T_{max}: time to reach peak concentration, $t_{1/2}$: terminal half-life, MR: metabolite ratio, compared $AUC_{norverapamil}$ to $AUC_{verapamil}$

The pharmacokinetic profiles of norverapamil, a major metabolite of verapamil, were also evaluated after oral administration of verapamil pretreated with naringin (Fig. 3). As summarized in Table II, the C_{max} and AUC of norverapamil were not significantly altered between control and pretreatment groups. However, MR in the rabbits pretreated with naringin were significantly ($p<0.05$ or $p<0.01$) decreased compared to the control group, implying that pretreatment of naringin may be effective to inhibit the CYP3A4-mediated metabolism of verapamil.

DISCUSSION

Intestinal phase I metabolism and active efflux of absorbed drug have been recognized as major determinants of bioavailability of many drugs (Wacher *et al.*, 1996; Kumar *et al.*, 1994; Rahman *et al.*, 1994). Both CYP3A4, the major phase I drug metabolizing enzyme in humans, and the multidrug efflux pump, P-gp, are present at high levels in the small intestine, the primary site of absorption for orally administered drugs. Moreover, those proteins demonstrate a broad overlap in substrate and inhibitor specificities, suggesting that they act as a concerted barrier to drug absorption (Benet *et al.*, 2003; Cummins *et al.*, 2002). Therefore, dual inhibitors against both CYP3A4 and P-gp should have a great impact on the bioavailability of many drugs where these mechanisms are the major barrier to the systemic availability.

Present study evaluated the influence of naringin, a naturally occurred flavonoid, on the pharmacokinetics of verapamil in rabbits with respect to a potential drug interaction between naringin and verapamil via the dual inhibition of CYP3A4 and P-gp.

The K_a, C_{max} and AUC of verapamil were significantly ($p<0.05$ or $p<0.01$) increased in the rabbits by pretreatment of naringin. Consequently, AB% and RB% values of verapamil in the rabbits pretreated with naringin were higher than those from the control group. Based on our previous study (Kim and Choi, 2005), although naringin (7.5 mg/kg) coadminstration significantly increased the AUC and C_{max} of verapamil, but the AB% of verapamil is lower (12.6%) than the same dose of naringin pretreatment (13.5%). Naringin should be given with a certain lead-time prior to verapamil administration to ensure inhibition effect of naringin on the CYP3A4 and P-gp. This may be explained by a relatively slower absorption of naringin than verapamil (Manach *et al.*, 1997; Ader *et al.*, 2000; Meng *et al.*, 2004).

The pharmacokinetic profiles of norverapamil, a major metabolite of verapamil were also evaluated after oral administration of verapamil with naringin pretreatment (Table II). The C_{max} and AUC of norverapamil were not significantly different between control and naringin pretreatment groups. However, MR in the rabbits pretreated with naringin were significantly ($p<0.05$ or $p<0.01$) decreased compared to the control implying that naringin pretreatment could be effective to inhibit the CYP3A4-mediated metabolism of verapamil in the liver and intestine. Collectively, pharmacokinetics of verapamil could be altered by the pretreatment of naringin, a dual inhibitor of CYP3A4 and P-gp. Clinical importance of these findings should be further investigated in clinical trials.

CONCLUSION

Naringin pretreatment significantly enhanced the oral bioavailability of verapamil. Therefore, if these results obtained from an animal model is confirmed in clinical trials, the adjustment of verapamil dosage should be considered for potential drug interaction when given with naringin or a naringin-containing dietary supplement.

REFERENCES

Ader, P., Wessmann, A., and Wolffram, S., Bioavailability and metabolism of the flavonol quercetin in the pig. *Free Radical Biol. Med.*, 28, 1056-1067 (2000).

Bardelmeijer, H. A., Beijnen, J. H., Brouwer, K. R., Rosing, H., Nooijen, W. J., Schellens, J. H., and van Tellingen, O., Increased oral bioavailability of paclitaxel by GF120918 in mice through selective modulation of P-glycoprotein. *Clin. Cancer Res.*, 6, 4416-4421 (2000).

Benet, L. Z., Cummins, C. L., and Wu, C. Y., Transporter-enzyme interactions: implications for predicting drug-drug interactions from *in vitro* data. *Curr. Drug Metab.*, 4, 393-398 (2003).

Buse, D., Cosme, J. P., Beaune, H. K., and Kroemer, M., Eichelbaum, Cytochromes of the P450 2C subfamily are the major enzymes imvolved in the *O*-demethylation of verapamil in humans. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 353, 116-121 (1995).

Cody, V., Plant Flavoniods in Biology and Medicine. *Prog. Clin. Biol. Res.*, 213, 233-239 (1986).

Cody, V., Plant Flavonoids in Biology and Medicine, part II. *Prog. Clin. Biol. Res.*, 280, 111-120 (1988).

Cummins, C. L., Jacobsen, W., and Benet, L. Z., Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. *J. Pharmacol. Exp. Ther.*, 300, 1036-1045 (2002).

Davis, J., Williams, L. S., Hill, D., and Lowenthal, D. T., Effects of fosinopril or sustained-release verapamil on blood pressure and serum techolamine concentrations in elderly hypertensive men. *Am. J. Ther.*, 7, 3-9 (2000).

Dey, C. S., Varma, M. V., Ashokraj, Y., and Panchagnula, R., P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. *Pharmacol. Res.*, 48, 347-359 (2003).

Dixon, R. A. and Steele, C., Flavonoids and isoflavonoids-gold mine for metabolic engineering. *Trends Plant Sci.*, 4, 394-400 (1999).

Doppenschmitt, S., Spahn-Langguth, H., Regardh, C. G., and Langguth, P., Role of P-glycoprotein-mediated secretion in absorptive drug permeability: An approach using passive membrane permeability and affinity to P-glycoprotein. *J. Pharm. Sci.*, 88, 1067-1072 (1999).

Doostdar, H., Burke, M. D., and Mayer, R. T., Bioflavoniods: selective substrates and inhibitors for cytochrome P450 CYP1A and CYP1B1. *Toxicology,* 144, 31-38 (2000).

Dupuy, J., Larrieu, G., Sutra, J. F., Lespine, A., and Alvinerie, M., Enhancement of moxidectin bioavailability in lamb by a natural flavonoid. *Vet. Parasitol.*, 112, 337-347 (2003).

Eagling, V. A., Profit, L., and Back, D. J., Inhibition of the CYP3A4-mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by grapefruit juice components. *Br. J. Clin. Pharmacol.*, 48, 543-552 (1999).

Eichelbaum, M., Mikus, G., and Vogelgesang, B., Pharmacokinetics of (+)-,(-)- and (±)-verapamil after intravenous administration. *Brit. J. Clin. Pharmacol.*, 17, 453-458 (1984).

Eichelbaum, M., Remberg, E. G., Schomerus, M., and Dengler, H. J., The metabolism of D,L(14C) verapamil in man. *Drug Metab. Dispos.*, 7, 145-148 (1979).

Fleckenstein, A., Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Ann. Rev. Pharmacol. Toxicol.*, 17, 149-166 (1977).

Giacomini, J. C., Nelson, W. L., Theodore, L., Wong, F. M., Rood, D., and Giacomini, M., The pharmacokinetics and pharmacodynamics of d- and dl-verapamil in rabbits. *J. Cardiovasc Pharmacol.*, 7, 469-475 (1985).

Gould, B. A., Mann, S., Kieso, H., Bala Subramanian, V., and Raftery, E. B., The 24-hour ambulatory blood pressure profile with verapamil. *Circulation*, 65, 22-27 (1982).

Ho, P. C., Saville, D. J., and Wanwimolruk, S., Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. *J. Pharm. Pharm. Sci.*, 4, 217-227 (2001).

Hodek, P., Trefil, P., and Stiborova, M., Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. *Chem. Biol. Interact.*, 139, 1-21 (2002).

Kim, H. J. and Choi, J. S., Effects of naringin on the pharmacokinetics of verapamil and one of its metabolites, narverapamil, in rabbits. *Biopharm. Drug Dispos.*, 26, 295-300 (2005).

Kim, D. H., Jung, E. A., Shong, I. S., and Han, J. A., Intestinal bacterial metabolism of flavonoids and its relation to some

biological activities. *Arch. Pharm. Res.*, 21, 17-23 (1998).

Krecic-Shepard, M. E., Barmas, C. R., and Schwartz, J. B., Faster clearance of sustained release verapamil in men versus women: Continnuing observations on sex specific differences after oral administration of verapamil. *Clin. Pharmacol. Ther.*, 68, 286-292 (2000).

Kroemer, H. K., Gautier, J. C., Beaune, P., Henderson, C., Wolf, C. R., and Eichelbaum, M., Identification of P450 enzymes involved in metabolism of verapamil in humans. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 348, 332-337 (1993).

Kumar, G. N., Walle, U. K., and Walle, T., Cytochome P450 3A-mediated human liver microsomal taxol 6á-hydroxylation. *J. Pharmacol. Exp. Ther.*, 268, 1160-1165 (1994).

Lewis, G. R., Morley, K. D., Lewis, B. M., and Bones, P. J., The treatment of hypertension with verapamil. *NZ Medical J.*, 87, 351-354 (1978).

Manach, C., Morand, C., Demigne, C., Texier, O., Regerat, F., and Remesy, C., Bioavailability of rutin and quercetin in rats. *FEBS Lett.*, 409, 12-16 (1997).

Meng, X., Maliakal, P., Lu, H., Lee, M. J., and Yang, C. S., Urinary and plasma levels of resveratrol and quercetin in humans, mice, and rats after ingestion of pure compounds and grape juice. *J. Agric. Food Chem.*, 52, 935-942 (2004).

Mori, Y., Hanada, K., Mori, T., Tsukahara, Y., Hashiguchi, M., and Ogata, H., Stereoselective pharmacokinetics and pharmacodynamics of verapamil and norverapamil in rabbits. *Biol. Pharm. Bull.*, 24, 806-810 (2001).

Nijveldt, R. J., van Nood, E., van Hoorn, D. E. C., Boelens, P. G., van Norren, K., and van Leeuwen, P. A. M., Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.*, 74, 418-425 (2001).

Rahman, A., Korzekwa, K. R., Grogan, J., Gonzalezs, F. J., Harris, J. W., Selective biotransformation of taxol to 6α-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res.*, 54, 5543-5546 (1994).

Rocci, M. L. and Jusko, W. J., LAGRAN program for area and moments in pharmacokinetic analysis. *Comp. Prog. In. Biomed.*, 16, 203-209 (1983).

Schomerus, M., Spiegelhaider, B., Stieren, B., and Eichelbaum, M., Physiologic disposition of verapamil in man. *Cardiovasc. Res.*, 10, 605-612 (1976).

Takanaga, H. A., Ohnishi, H., and Matsuo, Y., Sawada, Inhibition of vinblastine efflux mediated by P-glycoprotein by grapefruit juice components in caco-2 cells. *Biol. Pharm. Bull.*, 21, 1062-1066 (1998).

Takahama, U., Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. *Phytochemistry*, 24, 1443 -1446 (1985).

Wacher, V. J., Salphati, L., and Benet, L. Z., Active secretion and enterocytic drug metabolism barriers to drug absorption. *Adv. Drug Deliver. Rev.*, 20, 99-112 (1996).

Zhang, H., Wong, C. W., Coville, P. G., and Wanwimolruk, S., Effect of the grapefruit flavonoid naringin on pharmacokinetics of quinine in rats. *Drug Metabol. Drug Interact.*, 17, 351-363 (2000).