EFFECT OF THE GRAPEFRUIT FLAVONOID NARINGIN ON PHARMACOKINETICS OF QUININE IN RATS

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SUMMARY

The effect of the grapefruit flavonoid naringin, an inhibitor of CYP3A4, on the pharmacokinetics of quinine in rats after oral or intravenous (i.v.) dosing of quinine was investigated. Female Wistar rats (wt 190-220 g) were used in two separate studies, i.e. oral and i.v. administration of quinine. The animals were divided into two groups, one served as control and the other group was pretreated with 25 mg/kg naringin once a day for 7 consecutive days before the pharmacokinetic study. On the study day, quinine (25 mg/kg) was administered to the rats by either the oral or i.v. route. Blood samples were collected at different times, up to 6 h after quinine administration. Plasma quinine concentration was assayed by HPLC. Pretreatment with naringin did not cause any significant change in the pharmacokinetics of quinine after the i.v. dose. However pretreatment with naringin led to a 208% increase in peak plasma concentration (C_{max}), a 93% increase in time to reach C_{max} (t_{max}), and a 152% increase in the area under the plasma concentration-time curve (AUC) of quinine after oral administration. Consequently, the oral bioavailability of quinine was significantly increased ($p < 0.05$) from

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17% (control) to 42% after pretreatment with naringin. There was no significant difference in the elimination half-life ($t_{½}\beta$) of quinine between the two groups. These results suggest that pretreatment with the grapefruit flavonoid naringin is associated with increased oral bioavailability of quinine in rats.

KEY WORDS

quinine, naringin, CYP3A, food-drug interaction, pharmacokinetics

INTRODUCTION

Quinine is an important drug for the treatment of severe and complicated malaria /1/. It is extensively metabolised in the liver and, after an oral dose, less than 10% of the drug is excreted unchanged in the urine /2,3/. In humans, seven metabolites have been identified in urine, with 3-hydroxyquinine considered to be the major metabolite /3/. *In vitro* human and rat hepatic microsomal studies have shown that quinine is metabolised by CYP3A with 3-hydroxyquinine appearing to be the major metabolite /4-7/.

Grapefruit juice has been shown to cause significant increases in the plasma concentrations of many clinically important therapeutic agents, such as the dihydropyridine calcium channel antagonists, cyclosporine, and midazolam /8-12/. These interactions are believed to be due to inhibition of intestinal CYP3A4 by flavonoids and/or other chemicals in grapefruit juice, although the mechanism of this inhibition has not been fully characterised /13,14/. Some flavonoids, such as naringin, naringenin, quercetin, kaempferol, hesperetin and apigenin, were reported to be responsible for the inhibition described /13,15/.

The most prevalent bioflavonoid in grapefruit juice is naringin, which is not found in orange juice /16/. Naringin appears to be a weak inhibitor of microsomal felodipine oxidation /17/. However, its aglycone, naringenin, which is not normally found in grapefruit juice, seems to be a much more potent enzyme inhibitor of CYP3A4 that metabolises most calcium antagonists /17,18/. Consistent with this, preliminary studies using microsomes prepared from human and rat livers have shown that both naringin and naringenin inhibit the

metabolism of quinine to form 3-hydroxyquinine. Since quinine is metabolised by CYP3A and naringin can inhibit the CYP3A subfamily, theoretically an interaction between quinine and naringin is likely to occur. Therefore, the present study was conducted to determine the effects of naringin on the pharmacokinetics of quinine using the rat as an animal model.

MATERIALS AND METHODS

Chemicals

Naringin, quinine hydrochloride, and an internal standard (cinchocaine) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Polyethylene glycol 400 and sodium dodecyl sulphate were obtained from BDH Chemicals Ltd. (Poole, England). All other chemicals used were obtained from usual commercial sources and were of analytical grade.

Animal protocol

Inbred, female Wistar rats, 190-220 g, were housed in groups of 2-4 animals, in plastic cages with pinewood shavings as bedding. The animals were fed a commercial rodent diet (F49, Reliance Stock Food Co., Dunedin, New Zealand) and were acclimatised for five days before the experiment. During the pharmacokinetic experiments, rats were housed separately in normal or metabolism cages. Food, but not water, was withdrawn the night before drug administration. The animals were provided with food and water during the experimental period. All animals were cannulated with Silastic tubing in the right jugular vein under light ether anaesthesia 24 h before the pharmacokinetic experiment. The study was approved by the Committee of Ethics in the Care and Use of Laboratory Animals, University of Otago, Dunedin, New Zealand.

Experimental design

The rats were used in two separate studies, i.e. oral and intravenous (i.v.) administration of quinine. In each study, the pharmacokinetic experiments were performed with two groups of rats (n = 6-9 for each group). One group served as a control and received no naringin

pretreatment. Instead, they were given vehicle solvent (polyethylene glycol 400 [PEG 400]) by gavage in a volume of 0.2 ml/100 g body weight, once a day for 7 days before drug administration. Another group of rats was pretreated with 25 mg/kg naringin (12.5 mg/ml in PEG 400) administered directly into the stomach using a thin plastic tube attached to a syringe. The animals received naringin once a day for 7 consecutive days before the pharmacokinetic experiments.

Pharmacokinetic studies and drug administration

Quinine solutions were freshly prepared by dissolving the drug in normal saline. On the study day, animals were placed individually in metabolism cages. Quinine, in a volume of 0.2 ml/100 g (25 mg/kg), was administered to the rats either by the oral or i.v. route. Blood samples (0.2 ml) were collected at 0, 0.17, 0.33, 0.5, 1, 2, 3, 4, and 6 h after the injection of quinine. The same volume of saline (0.9% NaCl) was used as fluid replacement at the end of each blood sampling. Blood samples were collected in heparinised tubes. Plasma was separated after centrifugation and stored at -20°C pending analysis.

Drug analysis

Quinine concentration in plasma samples was determined by a specific HPLC method as reported previously /19/. This procedure is selective for quinine and has a detection limit of 18 ng/ml. The intra- and inter-assay variability was low with a coefficient of variation of less than 10%.

Data analyses

The pharmacokinetics of quinine were analysed by iterative weighted non-linear least-squares regression analysis. The computer program utilised was MINIM (courtesy of Dr R.D. Purves, Department of Pharmacology, University of Otago, New Zealand). The i.v. and oral data were fitted using one- or two-compartment pharmacokinetic models. The appropriate model was chosen as the one which gave the minimum value according to Akaike's information criterion /20/. The plasma drug concentration-time curves after i.v. drug administration were found to be adequately fitted to a standard two-compartment open model /21/ described by the following equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C is the plasma drug concentration, A and B are mathematical coefficients, α is the hybrid rate constant for the distribution phase, and β is the hybrid rate constant for the terminal elimination phase. Drug elimination half-life ($t_{1/2}\beta$) at the terminal phase was calculated as ($t_{1/2}\beta$) = $0.693/\beta$. The area under the plasma concentration-time curve (AUC) was estimated using the linear trapezoidal rule, with extrapolation from the last measured concentration to infinity determined by use of the terminal elimination rate constant. The total plasma clearance after intravenous (CL) and oral (CL/F) administration was estimated by dividing the administered dose by AUC (CL = dose/AUC). The volume of distribution (V_d = dose/(AUC·β)) was also calculated for i.v. data. Absolute bioavailability (F) was calculated as: $F = AUC_{po} \times Dose_{iv} / AUC_{iv} \times Dose_{po}$. The peak concentration (C_{max}) and time to reach C_{max} (t_{max}) were obtained directly from the concentration-time profile.

Statistical analysis

Results are reported as mean ± S.D. Student's unpaired t-test was used to assess statistical differences in pharmacokinetic parameters between the two groups. Differences were considered significant if $p < 0.05$.

RESULTS

There was no endogenous peak found to interfere with quinine and the internal standard in both human blank plasma and blank plasma from rats (i.e. prior to quinine dose). Therefore, plasma quinine standard solutions for the calibration curve were prepared using human blank plasma. The plasma taken from rats pretreated with naringin (80 mg/kg) also showed no interference with the quinine assay.

The standard curve of quinine in plasma was linear over the concentration range 0.05-10 μg/ml. This range of concentrations covered the plasma quinine concentrations observed in the samples from this study. The standard curve for urinary quinine was linear over the concentration range of 0.3-18.0 μg/ml.

There was no significant difference in the body weights on the study day between control and naringin pretreated rats (Table 1). Plasma quinine concentration-time profiles in the control and naringin pretreated rats after oral and i.v. administration are illustrated in Figure 1. The profiles of plasma quinine concentration after i.v. dose were almost superimposed between the control and naringin-pretreated groups. After oral administration of quinine, the plasma quinine concentrations in the naringin-pretreated group were significantly higher than the corresponding concentrations observed in the control group. Derived pharmacokinetic parameters of quinine are summarised in Table 1. There were no significant differences between the two groups in quinine pharmacokinetic parameters, i.e., AUC, CL, V_d and $t_{½}$, after intravenous administration (Table 1). After oral administration, however, pretreatment with naringin led to a 208% increase in peak plasma concentration (C_{max}), a 93% increase in time to reach C_{max} (t_{max}) and a 152% increase in the area under the plasma

TABLE 1

Pharmacokinetic parameters of quinine after i.v. and oral administration of quinine in two separate studies

	i.v. Quinine		Oral Quinine	
	Control (n = 8)	**Naringin** (n = 9)	**Control** (n = 6)	**Naringin** (n = 7)
Body Wt. (g)	203.8 ± 13.8	205 ± 11	202 ± 10	215 ± 15
C_{max} (μg/ml)	–	–	0.2 ± 0.1	0.7 ± 0.4*
t_{max} (min)	–	–	35.0 ± 12.2	57.8 ± 30.5*
$t_{½}\beta$ (h)	1.2 ± 0.4	1.2 ± 0.9	1.3 ± 1.1	1.3 ± 0.7
AUC (mg·h/l)	4.5 ± 1.0	4.5 ± 1.8	0.8 ± 0.4	1.9 ± 0.6*
F (%)	100	100	17.1	42.0
CL (l/h/kg)	5.9 ± 1.5	6.6 ± 3.0	–	–
CL/F (l/h/kg)	–	–	44.0 ± 25.9	14.1 ± 4.6*
V_d (l/kg)	10.1 ± 4.0	11.1 ± 10.9	–	–

Values are mean ± S.D. * $P < 0.05$.

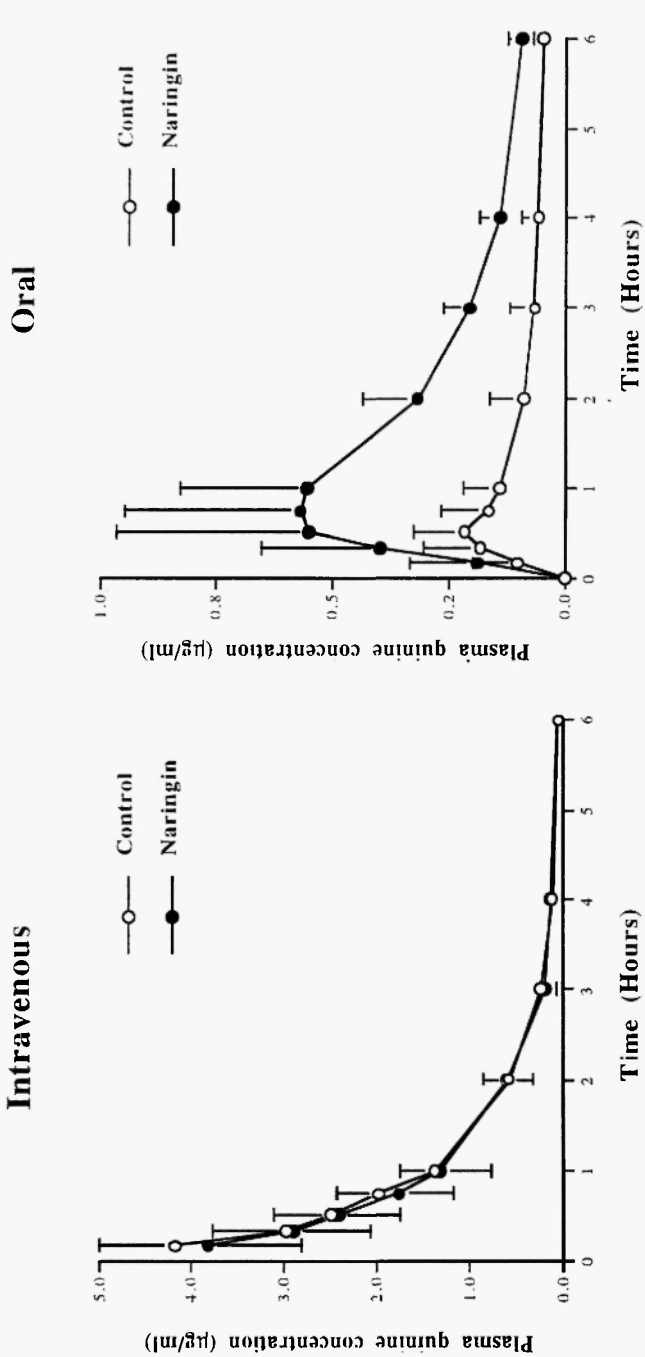

Fig. 1: Plasma concentration-time profiles of quinine after intravenous or oral administration of quinine in control rats pretreated with vehicle solvent (PEG400) and naringin pretreated rats. Pharmacokinetic parameters derived from these data are given in Table 1. Results are presented as mean and S.D. bar.

concentration-time curve (AUC) of quinine (Table 1). Consequently, the oral bioavailability of quinine was increased from 17% (control) to 42% (naringin) after the pretreatment with naringin. There was no significant difference in the elimination half-life ($t_{½}\beta$) of quinine after intravenous or oral administration.

DISCUSSION

Grapefruit juice has been reported to increase oral bioavailability of cyclosporine, midazolam, triazolam, and several calcium antagonists such as felodipine, nifedipine, nitrendipine and nisoldipine /14/. All these drugs are primarily metabolised by human liver CYP3A4. Grapefruit juice contains high concentrations of flavonoids, such as naringin, quercetin and kaempferol /16/, which have been shown to inhibit CYP3A activity *in vitro* /18,22/. The most prevalent bioflavonoid in grapefruit juice is naringin, the glycoside of naringenin, which is not found in orange juice /15/.

It was demonstrated that the major metabolite of quinine in human and rat livers is 3-hydroxyquinine and that quinine 3-hydroxylation is catalysed by CYP3A /4-7/. Data from preliminary experiments carried out using *in vitro* human and rat liver microsomal preparations have shown that both naringin and naringenin inhibit the metabolism of quinine catalysed by CYP3A. Naringin appears to be a much weaker inhibitor than naringenin.

In the present study, the effect of naringin on the pharmacokinetics of quinine after oral and intravenous administration in rats was examined. The calculated parameters during control conditions are similar to values reported by other investigators /23,24/. The clearance (CL) of quinine after intravenous administration in rats has been reported to be 6.9 l/h/kg /23/ which is comparable to 5.8 l/h/kg observed in this investigation. Oral bioavailability of quinine in rats was 17% in the present study, somewhat lower than that (F = 30%) reported by Genne *et al.* /24/.

The results have shown that naringin did not cause any significant change in the pharmacokinetics of quinine after an intravenous dose (Fig. 1, Table 1). However, after oral administration, the AUC and C_{max} of oral quinine were significantly higher in the group of rats pretreated with naringin than in the controls. Oral bioavailability of quinine (Table 1) was markedly increased from 17% (control) to 42%

(naringin) after pretreatment with naringin. Lack of effect of naringin on quinine pharmacokinetics after the i.v. dose, and the increased AUC and C_{max} after an oral dose with no alteration of the half-life, indicate that the oral bioavailability of quinine was increased as the result of the augmented systemic availability of quinine by naringin. This suggests that the primary site of CYP3A inhibition by naringin or naringenin in the rat is likely to be in the intestinal wall, rather than in the liver. It is also possible that the increased oral bioavailability of quinine in the absence of clearance changes could be a result of inhibition of hepatic first pass metabolism. Quinine has been shown to be a high clearance drug in rats with hepatic extraction rate of 56% /25/. Decreases in hepatic enzymatic capacity (i.e. due to a reduction in enzyme expression) for high extraction drugs result in a reduction in their oral clearance (CL/F) and increased oral bioavailability, but not systemic clearance.

The results from this study in rats are different from those observed in healthy human volunteers. It has been shown that grapefruit juice has no effect on quinine pharmacokinetics in humans co-administrated grapefruit juice /26/. One of the explanations is that quinine is a low clearance drug with a relatively high (90%) oral bioavailability in humans, but not in rats which were reported to have a low bioavailability (30%) /24/. This explanation is supported by the fact that those drugs interacting with grapefruit juice have a low oral bioavailability; for example, the bioavailability for felodipine, nifedipine, nisoldipine, midazolam and cyclosporine is approximately 15%, 50%, 8%, 24% and 40%, respectively /14,27/. The difference in results between rats and humans emphasizes species differences in drug metabolism, and data extrapolation from animal studies to humans should be viewed with caution.

There is increasing interest in the potential role and importance of P-glycoprotein, a transmembrane protein involved in drug efflux from the enterocyte back into the intestinal lumen. Any inhibition of P-glycoprotein activity may increase the extent of drug absorption from the intestinal lumen into enterocytes and would result in increased systemic availability of the drug. Quinine is considered to be both a CYP3A- and P-glycoprotein substrate. Another possible explanation of these results is that an increase in quinine oral bioavailability may result from naringin-induced inhibition of P-glycoprotein rather than CYP3A inhibition. However, Soldner *et al.* /28/ reported that

grapefruit juice significantly activates P-glycoprotein-mediated efflux of drugs that are substrates of P-glycoprotein, potentially partially counteracting the CYP3A-inhibitory effect of grapefruit juice.

The components of grapefruit juice that are responsible for clinical drug interaction have yet to be fully determined. Naringin has been the primary suspect because it is the main bitter component with concentrations up to and exceeding 500 mg/l /29/. Some *in vitro* studies such as with nifedipine have indicated that naringin is a less potent inhibitor of CYP3A than many other flavonoids, although its aglycone, naringenin, which is formed *in vivo* is considerably more active /17,30/. However, there were no significant changes in the pharmacokinetics of felodipine or nisoldipine in humans when naringin was co-administered /30/. It should be noted that most *in vitro* studies were performed using hepatic microsomes rather than intestinal microsomes. The major isoenzymes of CYP3A are found in both liver and intestine /31-33/. CYP3A accounts for nearly 30% of the total P450 content in human liver and for as much as 70% in the gut wall /34/. There is little information about the extent to which the activity and substrate specificity of small intestinal CYPs parallels that of the corresponding enzymes found in the liver, or whether liver and gut enzymes are under different regulation /32,35/. Waziers *et al.* /36/ reported that CYP3A4 in gut wall represented a total of 8.8% of liver-specific content in human. Vernet and Siess /37/ directly compared the effects of flavonoids on rat microsomal enzymes from both liver and intestine. They found that naringenin and hesperitin had qualitatively similar effects on ethoxycoumarin deethylase in the intestinal wall and in the hepatocyte.

In conclusion, the present study has shown that pretreatment with the grapefruit flavonoid naringin is associated with increased oral bioavailability of quinine in rats. These findings also provide additional evidence to support the hypothesis that drugs with low bioavailability due to presystemic metabolism mediated by CYP3A appear to be most affected by grapefruit juice.

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REFERENCES

1. White NJ. The treatment of malaria. N Engl J Med 1996; 335: 800-806.
2. White NJ, Chanthavanich P, Kirshna S, Bunch C, Silamut K. Quinine disposition kinetics. Br J Clin Pharmacol 1983; 16: 399-403.
3. Wanwimolruk S, Wong SM, Zhang H, Coville PF. Metabolism of quinine in man: 1. Identification of a major metabolite, and effect of smoking and rifampicin pretreatment. J Pharm Pharmacol 1995; 47: 957-963.
4. Wanwimolruk S, Wong S, Coville PF. In vitro hepatic microsomal metabolism of quinine: identification of P450 enzymes responsible for quinine metabolism. Can J Physiol Pharmacol 1994; 72 (Suppl 1): 294.
5. Zhang H, Wanwimolruk S, Coville PF. In vitro hepatic metabolism of quinine in rats. Proc Aust Soc Clin Exp Pharmacol Toxicol 1995; 2: 120.
6. Zhang H, Coville P, Walker R, Miners J, Birkett D, Wanwimolruk S. Evidence for involvement of human CYP3A in the 3-hydroxylation of quinine. Br J Clin Pharmacol 1997; 43: 245-252.
7. Zhao XJ, Ishizaki T. The in vitro hepatic metabolism of quinine in mice, rats and dogs: comparison with human liver microsomes. J Pharmacol Exp Ther 1997; 283: 1168-1176.
8. Bailey DG, Spence JD, Munoz C, Arnold JMO. Interaction of citrus juices with felodipine and nifedipine. Lancet 1991; 337: 268-269.
9. Ducharme MP, Provenzano R, Dehoorne-Smith M, Edwards DJ. Trough concentrations of cyclosporine following administration with grapefruit juice. Br J Clin Pharmacol 1993; 36: 457-459.
10. Yee GD, Stanley DL, Pessa L, Dalla Costa T, Beltz SE, Ruiz J, Lowenthal DT. Effect of grapefruit juice on blood cyclosporine concentration. Lancet 1995; 345: 955-956.
11. Ha HR, Chen J, Leuenberger PM, Freiburghaus AU, Follath F. In vitro inhibition of midazolam and quinidine metabolism by flavonoids. Eur J Clin Pharmacol 1995; 48: 367-371.
12. Kupferschmidt H, Ha H, Ziegler W, Meier P, Krahenbuhl S. Interaction between grapefruit juice and midazolam in humans. Clin Pharmacol and Ther 1995; 58: 20-28.
13. Ameer B, Weintraub RA. Drug interaction with grapefruit juice. Clin Pharmacokinet 1997; 33: 103-121.
14. Bailey DG, Arnold JMO, Spence JD. Grapefruit juice-drug interactions. Br J Clin Pharmacol 1998; 46: 101-110.
15. Bailey DG, Arnold JMO, Spence JD. Grapefruit juice and drugs: How significant is the interaction? Clin Pharmacokinet 1994; 26: 91-98.
16. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev Nutr Diet 1976; 24: 117-191.
17. Guengerich FP, Kim DH. In vitro inhibition of dihydropyridine oxidation and aflatoxin B_1 activition in human liver microsomes by naringenin and other flavonoids. Carcinogenesis 1990; 11: 2275-2279.

18. Miniscalco A, Lundahl J, Regardh CG, Edgar B, Eriksson UG. Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. J Pharmacol Exp Ther 1992; 261: 1195-1199.
19. Zoest AR, Wanwimolruk S, Hung CT. Simple high-performance liquid chromatographic method for the analysis of quinine in human plasma without extraction. J Liq Chromatogr 1990; 13: 3481-3491.
20. Yamaoka K, Nakagawa T, Uno T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharm 1978; 6: 165-175.
21. Gibaldi M, Perrier D. Pharmacokinetics. New York: Marcel Dekker, 1982.
22. Guengerich FP. Enzymatic oxidation of xenobiotic chemicals. CRC Crit Rev Biochem Mol Biol 1990; 25: 97-153.
23. Wanwimolruk S, Nyika S, Kepple M, Ferry DG, Clark CR. Effects of capsaicin on the pharmacokinetics of antipyrine, theophylline and quinine in rats. J Pharm Pharmacol 1993; 45: 618-621.
24. Genne P, Duchamp O, Solary E, Magnette J, Belon JP, Chauffert B. Cinchonine per os: efficient circumvention of P-glycoprotein-mediated multidrug resistance. Anti-Cancer Drug Design 1995; 10: 103-118.
25. Coleman MD, Timony G, Fleckenstein L. The disposition of quinine in the rat isolated perfused liver: effect of dose size. J Pharm Pharmacol 1990; 42: 26-29.
26. Ho PC, Chalcroft SC, Coville PF, Wanwimolruk S. Grapefruit juice has no effect on quinine pharmacokinetics. Eur J Clin Pharmacol 1999; 55: 393-398.
27. Jack DB. Handbook of clinical pharmacokinetic data. London: Macmillan, 1992.
28. Soldner A, Christians U, Susanto M, Wacher VJ, Silverman JA, Benet LZ. Grapefruit juice activates P-glycoprotein-mediated drug transport. Pharmaceut Res 1999; 16: 478-85.
29. Rousseff RL, Martin SF, Youtsey CO. Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. J Agric Food Chem 1987; 35: 1027-1030.
30. Bailey DG, Arnold JMO, Strong HA, Munoz C, Spence JD. Effects of grapefruit juice and naringin on nisoldipine pharmacokinetics. Clin Pharmacol Ther 1993; 54: 589-594.
31. Kolars JC, Lown KS, Schmiedlin-Ren P, et al. CYP3A gene expression in human gut epithelium. Pharmacogenetics 1994; 4: 247-259.
32. Lown KS, Kobars JC, Thummel KE, et al. Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel: lack of prediction by the erythromycin breath test. Drug Metab Dispos 1994; 22: 947-955.
33. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994; 270: 414-423.
34. Slaughter RL, Edwards DJ. Recent advances: the cytochrome P450 enzymes. Ann Pharmacother 1995; 29: 619-624.

35. Lampen ACU, Guengerich F, et al. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variablility. Drug Metab Dispos 1995; 23: 1315-1324.
36. Waziers D, Cugnenc PH, Yang CS, Leroux JP, Beaune PH. Cytochrome P450 isoenzymes, epoxide hydrolase and glutathione transferases in rats and human hepatic and extrahepatic tissues. J Pharmacol Exp Ther 1989; 253: 387-393.
37. Vernet A, Siess MH. Comparison of the effects of various flavonoids on ethoxycoumarin deethylase activity of rat intestinal and hepatic microsomes. Food Chem Toxicol 1986; 24: 857-861.