Journal of Food and Drug Analysis, Vol. 15, No. 3, 2007, Pages 265-270 藥物食品分析 第十五卷 第三期

Effects of Coadministrations of Unripe Fruits and Ripe Peels of *Citrus aurantium* on Cyclosporine Pharmacokinetics in Rats

MENG-JU WANG[1], YU-CHI HOU[2], SHANG-YUAN TSAI[2], SU-LAN HSIU[2], KUO-CHING WEN[3] AND PEI-DAWN LEE CHAO[2]*

[1.] Institute of Chinese Pharmaceutical Sciences, [2.] School of Pharmacy, [3.] School of Cosmeceutics, China Medical University, Taichung, Taiwan, R.O.C.

(Received: September 13, 2006; Accepted: December 12, 2006)

ABSTRACT

The unripe fruits (UF) and ripe peels (RP) of *Citrus aurantium* are two widely used Chinese herbs. Cyclosporine, an important immunosuppressant with narrow therapeutic window, is clinically subject to relevant interaction with *Citrus* herbs as with grapefruit juice. This study investigated the effects of coadministration of UF and RP on cyclosporine pharmacokinetics in rats. Sprague-Dawley rats received cyclosporine (2.5 mg/kg) orally with and without the UF and RP decoctions individually. Blood samples taken via cardiopuncture were assayed for cyclosporine by a specific monoclonal fluorescence polarization immunoassay. After coadministration orally with UF decoction, the C_{max} and AUC_{0-t} of cyclosporine were significantly decreased by 72.8% and 55.6%, respectively. However, when RP was coadministered orally, no conspicuous alteration of cyclosporine pharmacokinetics was observed. It can be concluded that UF, but not RP decoction, significantly decreased the bioavailability of oral cyclosporine. We suggest that coadministration of *Citrus* herbs with cyclosporine is better avoided to ensure the efficacy and safety of cyclosporin medication.

Key words: cyclosporine, *Citrus aurantium*, interaction, pharmacokinetics, decoction

INTRODUCTION

The unripe fruits (UF) and ripe peels (RP) of *Citrus aurantium*, a sour orange, are widely used in clinical Chinese medicine for different therapeutic purposes. UF are collected in May-June and clinically used in the treatment of food retention, constipation with abdominal pain, distention in the chest and epigastrium[1]. The chemical constituents of UF include naringin, naringenin, hesperidin, neohesperidin, β-carotene, l-carotene, citraurin, synephrine, N-methyltyramine, auraptenol, rhoifolin, lonicerin, poncirin, limonin and 5-O-desmethylnobiletin[2,3]. RP are collected in July and used for treating pain in the epigastrium or abdomen and poor appetite due to stagnation of spleen and stomach[1]. RP contains naringin, naringenin, hesperidin, neohesperidin, β-carotene, l-carotene, citraurin, synephrine, N-methyltyramine, auraptenol, aurantiamaric acid and aurantiamarin[2,3].

Chemotaxonomically, *Citrus aurantium* belongs to the same genus as grapefruit and shares many common constituents including naringin, naringenin and hesperidin[3]. UF and RP decoctions may clinically be subject to relevant interaction with western medicines like cyclosporine as grapefruit juice does[4].

Cyclosporine, a cyclic peptide, is a potent immunosuppressant with narrow therapeutic window. Supratherapeutic level of cyclosporine may lead to adverse effects including nephrotoxicity, thrombotic-microangiopathy and hypertension[5,6] whereas subtherapeutic level causes acute rejections of heart, kidney and liver in transplant patients[7].

Cytochrome 3A4 (CYP3A4) and P-glycoprotein (P-gp) are associated with the first pass extraction of cyclosporine[8]. Lines of evidence from previous studies suggested that grapefruit juice inhibited the metabolism of cyclosporine by CYP3A4 at the site of intestinal absorption and caused the increased exposure of cyclosporine[9]. Therefore, this study is aimed to investigate the effects of co-administration of UF and RP decoctions on the absorption and disposition of cyclosporine in rats.

MATERIALS AND METHODS

I. *Chemicals*

Cyclosporine (Neoral®, 100 mg/mL) was a gift kindly provided by Novartis Co. Ltd. (Taiwan). UF and

* Author for correspondence. Tel: +886-4-22053366 ext. 1905; Fax: +886-4- 22031028; E-mail: pdlee@mail.cmu.edu.tw

RP were purchased from a traditional Chinese pharmacy in Taichung and identified by microscopic examination. The specimens were deposited at the Institute of Chinese Pharmaceutical Sciences. TDx kit was supplied by Abbott Laboratories (Abbott Park, IL, USA). Rhodamine 123 was purchased from Aldrich Chemical Company (Milwakee, WI, USA). Milli-Q plus water (Millipore, Bedford, MA, USA) was used for all preparations. Vacutainer tube containing EDTA was purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

II. *Preparation of Decoctions*

To prepare decoctions, 25 g of UF and RP were each added 500 mL of water, and then heated on a gas stove. After boiling, the mixture was heated gently until the volume of decoction was reduced to less than 100 mL. The mixture was filtered while hot and sufficient water was added to make 100 mL to afford decoctions containing 0.25 g of crude drugs in each mL.

III. *Instrumentation*

The high-performance liquid chromatography (HPLC) apparatus included a pump (LC-10AS, Shimadzu, Japan) and an UV/VIS detector (SPD-10A, Shimadzu, Japan). The RP-18e column (Apollo®, 5 μm, 250×4.6 mm) was equipped with a guard column (LiChrospher 100, 5 μm).

IV. *Fingerprinting and Quantitation of Naringin in Decoctions of UF and RP*

The decoction (3 mL) was added with methanol (7 mL). The mixture was then vortexed for 30 sec and centrifuged at 9860 rpm for 15 min. In order to obtain more detailed fingerprints, the wavelength of UV detector was set at 220 nm and the flow rate was 1.0 mL/min with a gradient elution program as follows: acetonitrile/water: 20/80 (0 min), 80/20 (60 min). As for the quantitation of naringin in both decoctions, the wavelength of was set at 280 nm and an isocratic elution with acetonitrile/water (22/78) was used[4].

V. *Animals and Drug Administration*

Following an overnight fast, male Sprague-Dawley rats (n = 7) were given oral cyclosporine orally at a dose of 2.5 mg/kg with and without the UF or RP (2 g/kg) decoctions, in a crossover design, respectively. Drug administrations were carried out via gastric gavage. Two-week was allowed for washout between two treatments.

Another group of male rats (n = 8) were fasted overnight before intravenous bolus of 0.8 mg/kg cyclosporine with and without an oral dose of UF decoction in a crossover design. Cyclosporine was given via tail vein. UF decoction was orally administered via gastric gavage immediately after the bolus of cyclosporine. The control rats received an equal volume of water orally.

VI. *Blood Collection*

Blood samples (0.3 mL) were withdrawn via cardiopuncture at 20, 40, 60, 180, 300, 540, 1440 and 2880 min after the oral dose of cyclosporine. For iv bolus, blood samples were collected at 5, 10, 20, 40, 60, 180, 300 and 540 min. Blood samples were collected in vacutainer tubes containing EDTA, and stored at 4°C and then analyzed within 24 hr. Water was supplied with gastric gavage at 3-hr intervals during the experiment. The animal study adhered to "The Guidebook for the Care and Use of Laboratory Animals (2002)" (Published by The Chinese Society for the Laboratory Animal Science, Taiwan, ROC).

VII. *Quantitation of Cyclosporine in Blood*

Blood cyclosporine concentration was assayed by a specific monoclonal fluorescence polarization immunoassay method (FPIA). The assay was calibrated for concentrations from 25.0 to 1500.0 ng/mL.

VIII. *Data Analysis*

Noncompartment model of WINNONLIN (version 1.1, SCI software, Statistical Consulting Inc., Apex, NC, USA) was used for the computation of pharmacokinetic parameters of cyclosporine. The area under the serum concentration - time curve (AUC_{0-t}) was calculated by the trapezoidal rule to the last point. Pharmacokinetic parameters among various treatment groups were compared using paired Student's t-test or one way ANOVA with Sheffe's test, taking $p < 0.05$ as significant.

IX. *Everted Rat Gut Sac Study*

The effects of UF and RP decoctions on P-gp function were evaluated through everted gut sac study. Nine Sprague-Dawley rats were sacrificed and the jejunum and ileum were isolated. After flushing with ice-cold saline, each segment was everted and both ends were ligated tightly to prepare a 25-cm long gut sac. It was then immersed in 50 mL of medium TC 199 prewarmed at 37°C and preoxygenated with a mixture gas of 95% O_2/5% CO_2. After incubating under the same conditions for 20 min, 3 mL of rhodamine 123 solution (20.0 mg/mL in medium 199) was introduced into the everted sac (serosal side). The transport of rhodamine 123 solution from the serosal to mucosal surfaces across the intestine was then measured fluorometrically (Luminescence Spectrometer LS-50B, Perkin Elmer, USA) as a control sampled every 20 min from the mucosal medium until 100 min. UF and RP decoctions were added to medium TC 199 to give designated final concentrations of 5 and 10 mg/mL. The

Journal of Food and Drug Analysis, Vol. 15, No. 3, 2007

transport of rhodamine 123 was measured as described for the control.

RESULTS

HPLC fingerprints of UF and RP decoctions are shown in Figure 1, indicating that their constituents are profoundly different. The contents of major constituent naringin in UF and RP decoctions were 0.66 and 1.12 mg/mL, respectively. Figure 2 depicts the blood cyclosporine profiles after oral dosing of cyclosporine alone (2.5 mg/kg) and coadministrations with UF and RP decoctions (2 g/kg). The pharmacokinetic parameters of cyclosporine after various treatments are listed in Table 1. After coadminstration with UF, the C_{max} and AUC_{0-t} of cyclosporine were significantly decreased by 72.8% and 55.6%, respectively. In contrast, no significant alteration of cyclosporine pharmacokinetics was observed after coadministration with RP decoction.

Figure 3 depicts the blood cyclosporine profiles after intravenous bolus of cyclosporine alone (0.8 mg/kg) and coadministration with UF decoction (2 g/kg). No significant change of cyclosporine pharmacokinetics was observed between two treatments.

The effects of UF and RP decoctions on the efflux of rhodamine 123 in jejunum and ileum are shown in Figures 4 and 5, respectively. The results indicated that RP decoction significantly inhibited the efflux of rhodamine 123 from serosal side to mucosal side in jejunum, whereas UF decoction exerted no influence on P-gp function.

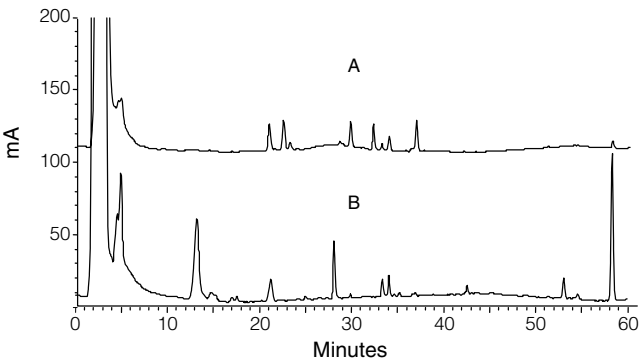

Figure 1. HPLC fingerprints of (A) RP and (B) UF decoctions detected at 220 nm.

Table 1. Pharmacokinetic parameters of cyclosporine in seven rats given cyclosporine alone (2.5 mg/kg, Cy) and coadministered with UF and RP decoction (2 g/kg, Cy+UF and Cy+RP) in a crossover design

Parameters \ Treatments	Cy	Cy+UF	Cy+RP
AUC_{0-2880}	411.0 ± 23.0[a]	182.4 ± 21.1[b]	381.4 ± 49.5[a]
C_{max}	777.7 ± 29.7[a]	211.3 ± 40.8[b]	675.2 ± 62.6[a]
T_{max}	57.1 ± 2.9	105.7 ± 26.5	42.9 ± 5.2
MRT	719.9 ± 16.1	975.7 ± 55.3	733.8 ± 38.8

Data expressed as mean ± S.E. Means in a row without a common superscript differ, $p < 0.05$.
AUC_{0-2880} (mg · min · mL^{-1}): area under concentration-time curve to the last point.
C_{max} (ng · mL^{-1}): the peak or maximum concentration.
T_{max} (min): the time of peak concentration.
MRT (min): mean residence time.
(For continuous variables, means in a row with superscripts without a common letter differ, $p < 0.05$.)

DISCUSSION

This study employed a rat model to evaluate the effects of coadministration of two *Citrus* herbal decoc-

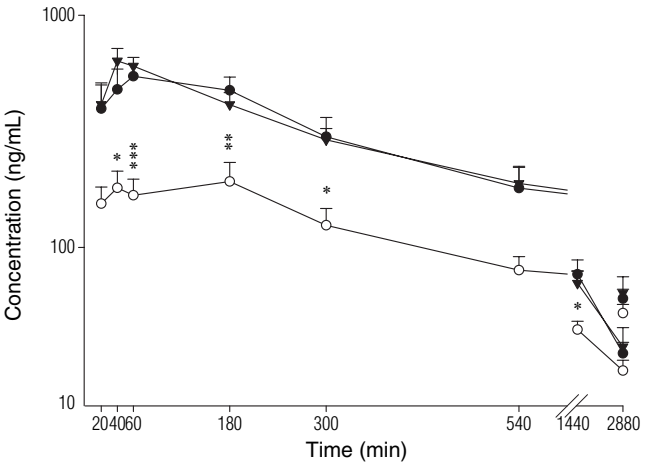

Figure 2. Mean (± S.E.) blood concentration-time profiles of cyclosporine after oral administration of cyclosporine alone (2.5 mg/kg) (●) and coadministration with UF (○) and RP (▼) to eight rats in a crossover design. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared with cyclosporine alone using one way ANOVA with Sheffe's test.

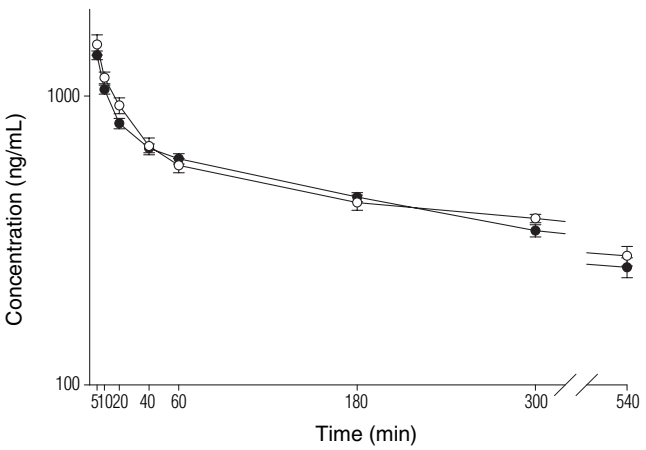

Figure 3. Mean (± S.E.) blood concentration-time profiles of cyclosporine after intravenous bolus of cyclosporine alone (●) and co-administration with UF (○).

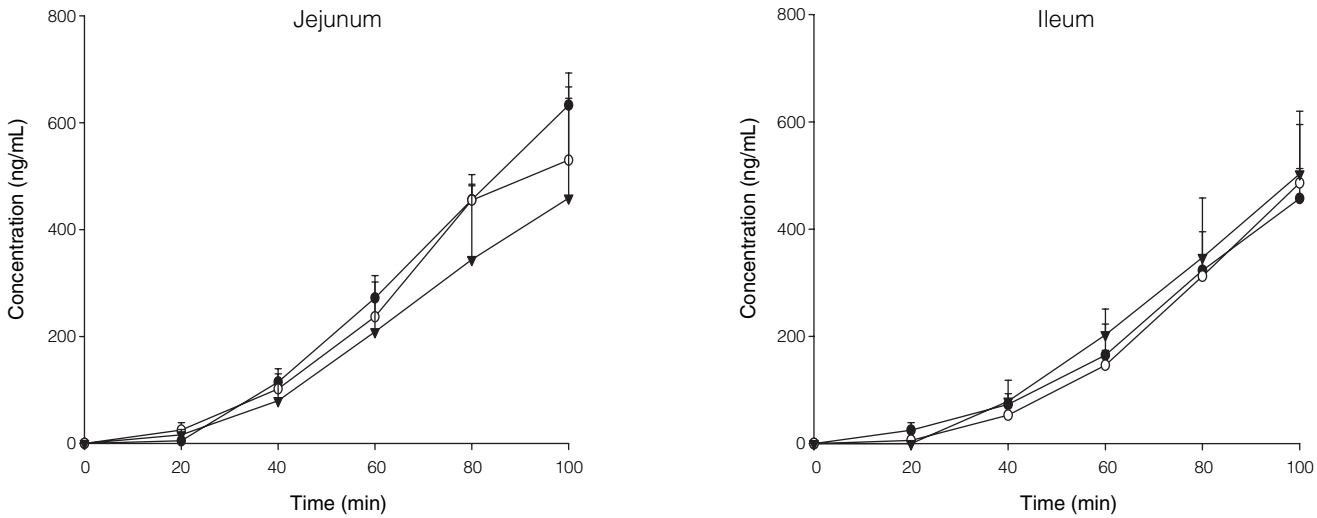

Figure 4. Average transport of rhodamine 123 (ng/mL) across jejunum and ileum in the absence (●) and the presence of UF decoction at concentrations of 5 mg/mL (○) and 10 mg/mL (▼).

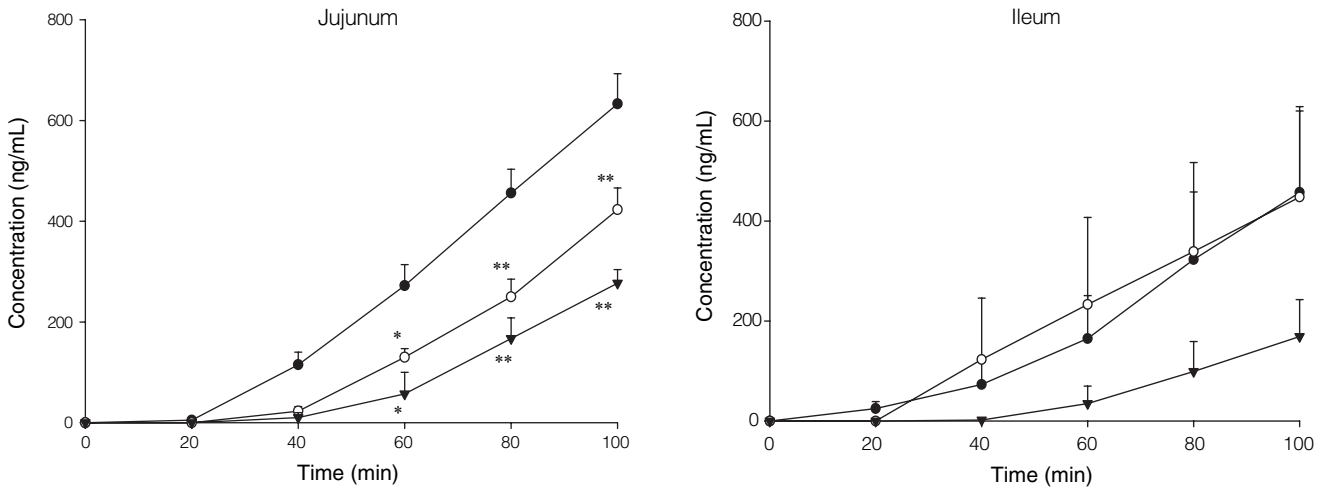

Figure 5. Average transport of rhodamine 123 (ng/mL) across jejunum and ileum in the absence (●) and the presence of RP decoction at concentrations of 5 mg/mL (○) and 10 mg/mL (▼). *$p < 0.05$; **$p < 0.01$ compared with control alone using one way ANOVA with Sheffe's test.

tions on cyclosporine pharmacokinetics. This rat model has been validated through an interaction between St. John's wort and cyclosporine in a previous study[10]. The results showed that UF, but not RP decoction significantly decreased the oral bioavailability of cyclosporine. Our previous study reported that RP caused significant increase of cyclosporine oral bioavailability and resulted in acute intoxication of pigs[4]. Such discrepancy of RP - cyclosporine interaction between pigs and rats might be attributed to species difference or due to different dose used. As UF - cyclosporine interaction in pigs has not been reported, no comparison can be made between these two animals.

Although UF and RP are from the same plant, their influences on cyclosporine pharmacokinetics were found markedly different. The possible reason for these discrepant effects might be attributed to the changing constituents at different stage of fruit ripeness. For instance, lonicerin, rhoifolin and 5-*O*-desmethylnobiletin were only reported in UF but not in RP, indicating that these constituents might disappear upon maturation. Besides, aurantiamaric acid and aurantiamarin exist in RP, but not in UF. Therefore, the HPLC fingerprints of UF and RP decoctions showed conspicuous difference. However, the causative components in UF decoction to dramatically lower the cyclosporine bioavailability remain unknown and await further studies.

Grapefruit juice is well known for its effect in elevating the exposure of cyclosporine[9], whereas the UF decoction showed an opposite effect in this study. Grapefruit juice contains naringin, naringenin, quercetin, bergamottin, 6',7'-dihydroxybergamottin, hesperi-

din, rutin, apigenin, campherol and kaempferol, among which naringin, the major constituent, has been proved not the causative agent for grapefruit juice - cyclosporine interaction[11]. The lack of influence on cyclosporine pharmacokinetics by naringin was also confirmed by this rat model in our laboratory (data not shown). On the other hand, two minor constituents, bergamottin and 6,7-dihydroxybergamottin, instead were professed to be the possible causes for such kind of interaction[12,13]. As indicated in the results from the inhibition on CYP3A4 - mediated metabolism in *in vitro* experiments[12,13], these two constituents might be responsible for the modulation of intestinal CYP3A4 caused by grapefruit juice consumption[14,15].

Recently, a study with furanocoumarin - free grapefruit juice proved that furanocoumarins are the mediators of the grapefruit juice - felodipine interaction, indicating that furanocoumarins are the active ingredients for enhancing the systemic exposure of felodipine and probably other CYP3A4 substrates that undergo extensive intestinal first-pass metabolism[16]. Probably due to the absence of bergamottin and 6,7-dihydroxybergamottin, UF and RP did not result in any elevation of cyclosporine exposure as grapefruit juice did. In our study, after the removal of lipophilic fraction upon partitioning with ethyl acetate, the remaining aqueous fraction of RP decoction significantly decreased the blood level of cyclosporine, as the corresponding UF decoction did (data not shown), indicating that the discrepancy between RP and UF decoctions on cyclosporine exposure should be attributed to the lipophilic components. It can also be proposed that the ethyl acetate fraction of RP seemed to compensate the effect of its aqueous layer. Which constituents in the aqueous fractions of UF and RP decoction actually caused the marked decrease of cyclosporine exposure is still on the agenda of future research.

In order to illuminate the possible mechanism, the effect of coadminisatration of UF decoction on the pharmacokinetics of intravenous cyclosporine was also investigated. However, no significant influence on cyclosporine pharmacokinetics was found, implying that the interaction between UF decoction and oral cyclosporine should occur at the absorption site. The fate of cyclosporine at the absorption site is affected by two possible mechanisms, being pumped out by P-gp in intestine surface and subsequently metabolized of the residual by CYP3A4 in intestine[17]. Therefore, the agents that exert any influence on the functions of P-gp and/or CYP3A4 will affect the absorption and the disposition of cyclosporine. This study measured the effect of UF and RP on the function of intestinal P-gp using everted rat gut sac to explore the possible involvement of P-gp in the interaction. Our results demonstrated that UF did not affect the function of intestinal P-gp, suggesting that the decreased bioavailability of cyclosporine was not associated with the function of intestinal P-gp. In contrast, RP significantly inhibited the function of intestinal P-gp and this might explain the much higher bioavailability of cyclosporine upon coadministration with RP relative to UF.

Regarding to the effect on CYP3A4, a previous study reported that UF and RP decoctions exerted very weak or even no inhibition on the CYP3A activity of human liver microsomes in testosterone 6-hydroxylation[18]. Based on previous pharmacokinetics studies on traditional Chinese medicine, we found that it was indispensable for various water soluble polyphenol glycosides to be biotransformed to lipophilic derivatives before absorption, and the aglycones were further metabolized to conjugated metabolites[19,20]. Since the metabolism of the constituents in *Citrus* herbs has not been taken into consideration, it is therefore not appropriate to extrapolate the *in vitro* effect on CYP3A for explaining the *in vivo* interactions. Therefore, the mechanism of interaction between *Citrus* herbs and cyclosporine is still unclear and more efforts should be devoted to the relevant inquiries.

In conclusion, the coadministration of UF, but not RP decoction markedly decreased the oral bioavailability of cyclosporine in rats. Together with our previous results from studies of cyclosporine pharmacokinetics in pigs, it seems prudent for organ allograft recipients to avoid the concurrent use of *Citrus* herbs to ensure the efficacy and safety of cyclosporine.

ACKNOWLEDGEMENTS

The work was in part supported by the National Science Council, ROC (NSC 92-2320-B-039-016) and the China Medical University, Taichung, Taiwan, ROC (CMU92-P-02, CMU95-087).

REFERENCES

1. Huang, B. and Wang, Y. 1993. Thousand Formulas and Thousand Herbs of Traditional Chinese Medicine. pp. 237-241. Heilongjiang Education Press. Harbin, China.
2. Wang, Y. S. and Luo, W. Y. 1989. Studies on quantitative determination of total flavonoid in qingpi, zhiqiao and zhishi by TLC-densitometric methods. Zhongguo Zhong Yao Za Zhi 14: 230-232, 255.
3. Pellati, F., Benvenuti, S. and Melegari, M. 2004. High-performance liquid chromatography methods for the analysis of adrenergic amines and flavanones in *Citrus aurantium* L. var. amara. Phytochem. Anal. 15: 220-225.
4. Hou, Y. C., Hsiu, S. L., Tsao, C. W., Wang, Y. H. and Chao, P. D. 2000. Acute intoxication of cyclosporine caused by coadministration of decoctions of the fruits of *Citrus aurantium* and the pericarps of *Citrus grandis*. Planta Med. 66: 653-655.
5. Calne, R. Y., White, D. J. G., Thiru, S., Evans, D. B., McMaster, P., Dunn, D. C., Thiru, S. and Craddock,

G. N. 1978. Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet 2: 1323-1327.

6. Lapointe, M., Baillie, G. M., Bhaskar, S. S., Richardson, M. S., Self, S. E., Baliga, P. K. and Rajagopalan, P. R. 1999. Cyclosporine-induced hemolytic uremic syndrome and hemorrhagic colitis following renal transplantation. Clin. Transplant 13: 526-530.
7. Barone, G. W., Gurley, B. J., Ketel, B. L. and Abul-Ezz, S. R. 2001. Herbal supplements: a potential for drug interactions in transplant recipients. Transplantation 71: 239-241.
8. Lown, K. S., Mayo, R. R., Leichtman, A. B., Hsiao, H. L., Turgeon, D. K. and Schmiedlin, R. P. 1997. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin. Pharmacol. Ther. 62: 248-260.
9. Harris, R. Z., Jang, G. R. and Tsunoda, S. 2003. Dietary effects on drug metabolism and transport. Clin. Pharmacokinet. 42: 1071-1088.
10. Yang, C. Y., Wang, Y. H., Hou, Y. C., Hsiu, S. L. and Chao, P. D. 2003. St. John's Wort-Cyclosporin Interaction in Rats and Pigs. Mid Taiwan J. Med. 8: 127-133.
11. Bailey, D. G., Kreeft, J. H., Munoz, C., Freeman, D. J. and Bend, J. R. 1998. Grapefruit juice- felodipine interaction: effect of naringin and 6',7'- dihydroxybergamottin in humans. Clin. Pharmacol. Ther. 64: 248-256.
12. Edwards, D. J., Bellevue, F. H. and Woster, P. M. 1996. Identification of 6',7'- dehydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. Drug Metab. Dispos. 24: 1287-1290.
13. Ho, P. C., Saville, D. J. and Wanwimolruk, S. 2001. Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. J. Pharm. Pharm. Sci. 4: 217-227.
14. Guo, L. Q., Fukuda, K., Ohta, T. and Yamazoe, Y. 2000. Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. Drug Metab. Dispos. 28: 766-771.
15. Tassaneeyakul, W., Guo, L. Q., Fukuda, K., Ohta, T. and Yamazoe, Y. 2000. Inhibition selectivity of grapefruit juice components on human cytochromes P450. Arch. Biochem. Biophys. 378: 356-363.
16. Paine, M. F., Widmer, W. W., Hart, H. L., Pusek, S. N., Beavers, K. L., Criss, A. B., Brown, S. S., Thomas, B. F. and Watkins, P. B. 2006. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. Am. J. Clin. Nutr. 83: 1097-1105.
17. Saeki, T., Ueda, K., Tanigawara, Y., Hori, R. and Komano, T. 1993. Human P-glycoprotein transports cyclosporin A and FK506. J. Biol. Chem. 268: 6077-6080.
18. Guo, L. Q., Taniguchi, M., Chen, Q. Y., Baba, K. and Yamazoe, Y. 2001. Inhibitory potential of herbal medicines on human cytochrome P450-mediated oxidation: properties of umbelliferous or *Citrus* crude drugs and their relative prescriptions. Jpn. J. Pharmacol. 85: 399-408.
19. Lai, M. Y., Hou, Y. C., Hsiu, S. L., Chen, C. C. and Chao, P. D. L. 2002. Relative flavone bioavailability of Scutellariae Radix between traditional decoction and commercial powder preparation in humans. J. Food Drug Anal. 10: 75-80.
20. Chiang, H. M., Yeh, Y. R., Chao, P. D. L., Hsiu, S. L., Hou, Y. C., Chi, Y. C., Wen, K. C. 2005. Metabolic pharmacokinetics of isoflavones in the roots of *Pueraria lobata in* rats. Mid Taiwan J. Med. 10: 57-64.