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MARKED DECREASE OF CYCLOSPORINE ABSORPTION CAUSED BY COADMINISTRATION OF GINKGO AND ONION IN RATS

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Introduction: Cyclosporine is an immunosuppressive agent with narrow therapeutic range and was found to be substrate of CYP3A4 and P-glycoprotein (Pgp). Ginkgo and onion contain quercetin and its glycosides which were reported to alter the activities of CYP3A4 and Pgp. For organ transplant patients treated with cyclosporine, ginkgo and onion might affect the therapeutic outcome.

Methods: Rats were given Neoral® (cyclosporine) alone or coadministered with quercetin, ginkgo and onion respectively. The blood samples were withdrawn via cardiopuncture. The blood concentration of cyclosporine was determined by a specific monoclonal FPIA method. The *in vitro* everted intestine sac study was conducted to explore the mechanism of interaction.

Results: Cyclosporine absorption was significantly decreased by the coadministrations of quercetin, ginkgo and onion, respectively, by 43 %, 51 % and 82 %, respectively. The mechanism for this interaction was not explainable by their *in vitro* effects on Pgp.

Conclusion: Herb-food-drug interaction is a hidden risk for patients. When patients are prescribed Neoral®, concurrent use of quercetin glycoside-containing dietary supplements and herbs should be taken into consideration by clinicians.

Flavonoids have attracted increasing attention in recent years because of their various beneficial bioactivities including antioxidation (1), free radical scavenging (2), anticancer (3), antivirus (4) etc. and furthermore, their additional abilities to modulate both CYP 3A4 and P-glycoprotein (Pgp), the product of *mdr* (multidrug - resistance) genes (5-8). CYP3A4 is mainly present in intestine and liver. The significant role of CYP 3A4 for drug– drug interactions was well recognized. Pgp is expressed in various normal human tissues such as small intestine, kidney, liver and capillary endothelial cells of brain and testes (9-11), and its significant roles for chemoprevention of organisms and drug - drug interaction had been proposed (12-14). Quercetin was shown to be a potent inhibitor of CYP3A4 in *in vitro* studies (15). In our laboratory, an *in vitro* everted intestinal sac study indicated that quercetin inhibited the function of P-gp in a dose – dependent manner as shown in Fig. 1.

Quercetin, the most popular flavonoid in food and herbs, is present mostly as glycoside in nature. Ginkgo, an herbal product of the leaves of *Ginkgo biloba* widely used for treating neurodegeneration and cardiovascular disease as well as a dietary supplement worldwide, contains quercetin glycoside which was considered as one of the active components for standardization. Onion, a common food and also a dietary supplement in Europe, contain 0.03% of quercetin glycoside. The fate of flavonoid glycosides was better understood in recent decade that quercetin glycosides were

hydrolyzed by enterobacteria in gastrointestinal tract and transformed into quercetin which was absorbable into circulation.

Cyclosporin is a widely used immunosuppressant with a narrow therapeutic range. Cyclosporin is a substrate of both CYP 3A4 (16) and Pgp (17). In a recent report, inhibition of P-glycoprotein was proposed to be a more important mechanism for enhanced cyclosporin absorption than inhibition of CYP 3A4 (18). In this study, we attempted to investigate the *in vivo* effect of quercetin, ginkgo and onion on the pharmacokinatics of cyclosporin in rats

Materials and Methods

Chemicals

Cyclosporin (Sandimmune Neoral®, 100 mg/ml) was kindly offered by Novartis (Taiwan) Co. Ltd.. Rhodamine 123 was purchased from Aldrich (Milw. WI, U. S. A.). Glycofurol and medium 199 were supplied by Sigma (St. Louis, MI, U. S. A.) Milli-Q plus Water (Millipore, Bedford, MA, U. S. A.) was used for all preparation.

Everted intestine sac study

Female Sprague-Dawley rats were sacrificed. The jejunum (30 cm long from stomach) and ileum (30 cm long from the ileocecum) were isolated. After flushing with ice-cold saline, each segment was everted and both ends were ligated tightly to

prepare a 25 cm long everted sac. Then the sac was immersed into 50 ml medium 199 prewarmed at 37 ^{0}C and preoxygenated with 95% O_2/5% CO_2. After incubating for 20 min, 3 ml rhodamine 123 solution (20 μg/ml in medium 199) was introduced into the everted sac (serosal side). Under bubbling with the 95% O_2/5% CO_2 mixture gas, the transport of rhodamine 123 solution from serosal to mucosal surfaces across the intestine was measured by sampling the mucosal medium every 20 min until 100 min. On the other hand, quercetin or rutin was dissolved with glycofurol and added to the mucosal medium in order to give designated final concentrations of 200 and 400 μM. The transport of rhodamine 123 in the absence (control) or presence of the modulator was measured fluorometrically using Luminescence Spectrometer LS 50B (Perkin Elmer, U. S. A.).

Drug administration and blood collection

Female Sprague-Dawley rats (n = 6-12) weighing 200 ~ 300 g were fasted for 12 hr before drug administration. Half of the were given 1.25 mg/kg cyclosporin which had been prepared by diluting Neoral® with deionized water, whereas the other half rats were administered a single dose of 50 mg/kg quercetin, 2 g/kg ginkgo leaf (as water decoction) and 2 ml/rat onion juice 30 sec before cyclosporin via gastric gavage. The rats were randomly assigned to receive monotherapy and combined therapy in a parallel design for quercetin treatment, whereas crossover design for ginkgo or onion

treatment. Blood samples (0.3 ml) were withdrawn via cardiopuncture at 0, 20, 40 min, 1, 3, 5, 7 and 9 hr after drug administration. The blood was collected into small plastic vials containing EDTA and assayed within one week.

Quantitation of blood cyclosporin concentrations

Cyclosporin concentration in blood was measured by using a specific monoclonal fluorescence polarization immunoassay (Abbott, Abbott Park, Ill, USA). The assay was calibrated for concentrations from 25.0 to 1500.0 ng/ml.

Data analysis

Pharmacokinetic parameters of cyclosporin were calculated by using noncompartment model of WINNONLIN Version 1.1, SCI software, Statistical Consulting, Inc., Apex, NC) Unpaired and paired Student's *t*-tests were used for quercetin treatment and ginkgo or onion treatment, respectively, taking $p < 0.05$ as significant.

Results and Discussion

Fig. 1 shows the effect of quercetin on the efflux transport of rhodamin 123 from the serosal side to the mucosal side. The result indicated that quercetin significantly inhibited the function of intestinal Pgp in a dose - dependant manner for both jejunum and ileum. This prompted us to investigate the effect of quercetin,

ginkgo and onion on the absorption and disposition of cyclosporin. Fig. 2, 3 and 4 depict the blood profiles of cyclosporin after administration ofcyclosporin alone and coadministration with a single dose of quercetin, ginkgo and onion, respectively. The pharmacokinetic parameters of cyclosporin for monotherapy and combined therapy are given in Table 1, 2 and 3. Our results showed that quercetin, ginkgo and onion significantly decreased the AUC of cyclosporin by 43 %, 51 % and 82 %, respectively, indicating that the bioavailability of cyclosporin was markedly decreased. These results could not be explainable by the inhibition of intestinal Pgp by quercetin as observed from the*in vitro* studies. Modulation of Pgp and/or CYP 3A4 is an important mechanism of drug interaction. However, the fate of a drug and metabolites frequently is not only determined by Pgp and/or CYP 3A4, but also by other metabolic enzymes and possibly by other transporters e.g. MRP (multidrug resistance protein) (14).

Several phamacokinetic studies of quercetin glycosides reported that it is indispensable to be hydrolyzed into quercetin before absorption. Quercetin was then rapidly metabolized into its glucuronides/sulfates by the enterocytes and hepatocytes and circulating in the bloodstream as these conjugated metabolites. Therefore, the glucuronides/sulfates of quercetin derived *in vivo* from glycosides might play a more important role in drug interaction than the parent glycoside or the aglycone. The

significant role of the glucuronidated/sulfated metabolites of flavonoids for drug-drug interaction is worthy of investigation. The direct action of the glucuronides/sulfates on Pgp and CYP 3A4 need to be further studied.

The pharmacokinetic interaction study was carried out in a crossover design for ginkgo and onion, whereas for quercetin a parallel design was conducted because glycofurol, the solvent used to dissolve quercetin, lowered even lower the blood cyclosporin level at the second treatment after one week wash – out. The data analysis of quercetin study was thus calculated based on the comparisons between two parallel groups. It is suggested that when using organic solvent to dissolve the precipitant drug in a drug - cyclosporin (Sandimmun Neoral®) study, a parallel design is preferable to crossover design.

In recent years, many transplant recipients were reported to show subtherapeutic cyclosporin concentrations after they started self-medicating with St. John's Wort (*Hypericum perforatum*) (19, 20). St. John's Wort, containing quercetin and its glycosides, was likely to act as potent inducer of hepatic enzymes. Most *in vitro* studies have agreed that St. John's Wort doubles the metabolic activity of CYP 3A4. Other cytochrome P-450 isoenzymes as well as Pgp, may be affected by St. John's Wort (21). It is proposed that herbs represent a potential and possibly an overlooked cause for drug interaction in transplant recipients.

In summary, ginkgo and onion markedly decreased the oral bioavailability of cyclosporin. Because cyclosporin is a substrate of both Pgp and CYP 3A4, we suggest that the coadministration of quercetin or quercetin containing herb or food with cyclosporin or other medications whose absorption and metabolism are mediated by Pgp and/or CYP 3A4 should require close monitoring. Healthcare providers should be cautious of the hidden risk of these herb – drug interactions.

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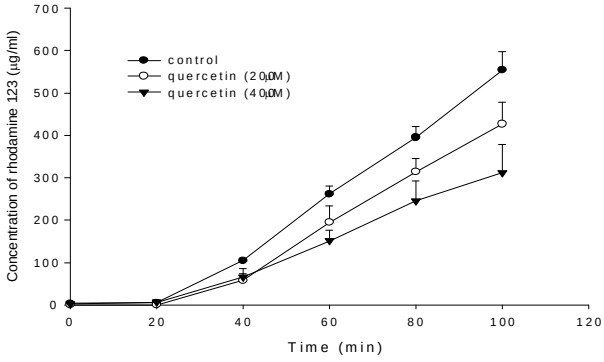
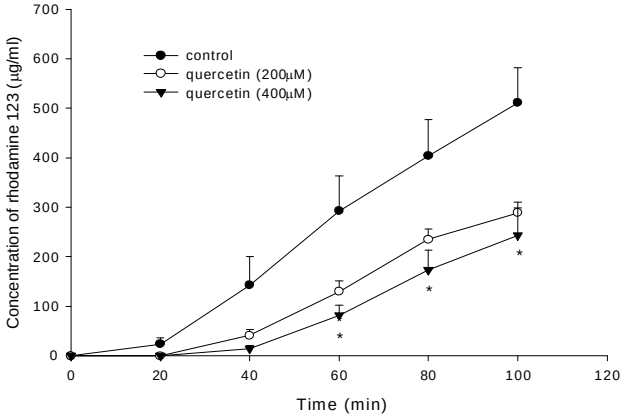


(a)



(b)

Fig.. 1. Mean (± S.E.) transport of rhodamine 123 (μg/ml) across (a) jejunum and (b) ileum in the absence (●) or presence of 200 μM (○) and 400 μM (▼) quercetin ; (n=3, * $p < 0.05$).

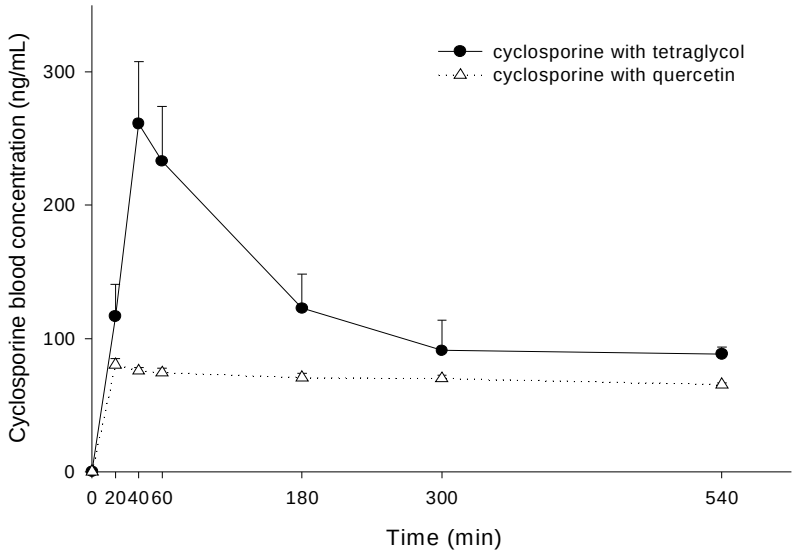


Fig. 2 Mean (±S.E.) blood concentration-time profiles of cyclosporine after oral administration of cyclosporine with tetraglycol in 6 rats (●) and coadministration with quercetin (50 mg/kg) in another 6 rats (△),

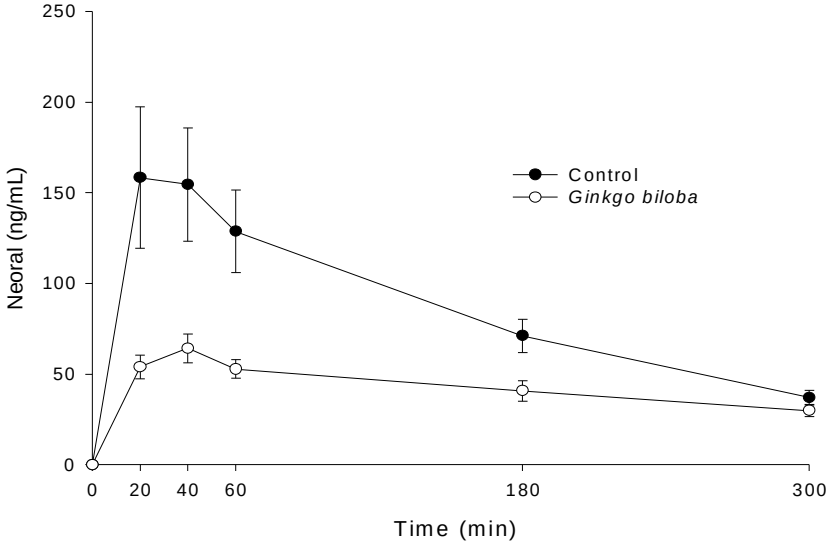


Fig. 3. Mean (± s. e.) blood concentration– time profiles of cyclosporin after administation of cyclosporin alone (●) and coadministration with ginkgo (○) in 6 rats.

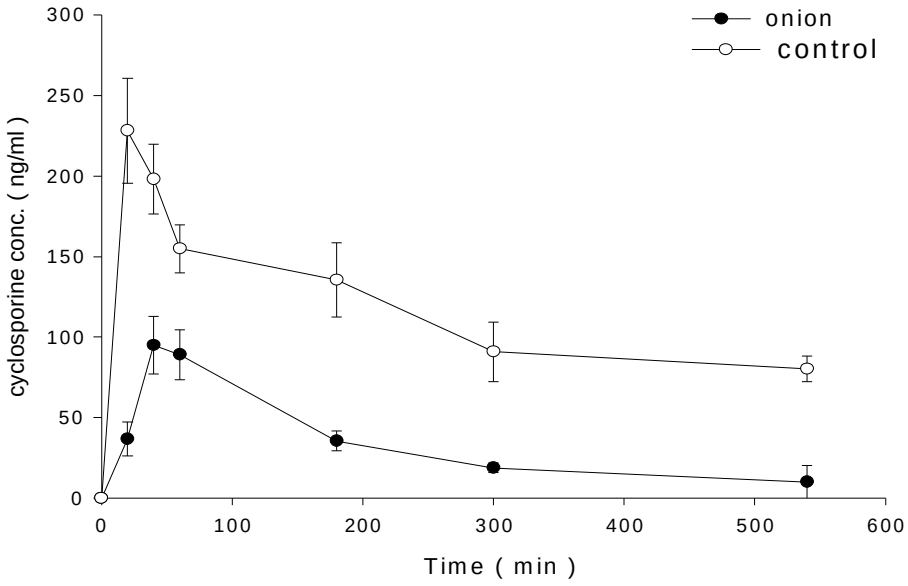


Fig. 4. Mean (± s. e.) blood concentration – time profiles of cyclosporin after administation of cyclosporin alone (○) and coadministration with onion (●) in 7 rats.

Table 1. Comparison of pharmacokinetic parameters of cyclosporine in rats between receiving 1.25mg/ kg cyclosporine alone and coadministration with 50 mg/kg quercetin.

Parameters	With quercetin Mean ± S.E.	Cyclosporin alone Mean ± S.E.	Difference (%)
$AUC_{0\text{-}5h}$(ng·min·mL^{-1})	3.72 (± 0.09)E4	6.55 (± 1.05)E4	-43.3*
T_{max}(min)	30.0 ± 6.8	43.3 ± 3.3	-30.7
C_{max}(ng·mL^{-1})	84.1 ± 2.8	261.5 ± 46.6	-67.8**
MRT(min)	267.3 ± 2.1	225.5 ± 7.2	18.5***

*p < 0.05, **p < 0.01, ***p < 0.001

Table 2. Comparison of pharmacokinetic parameters of cyclosporine in 6 rats between receiving cyclosporine alone and coadministration with decoction of *Gnkgo biloba*.

Parameters	Cyclosporin Alone Mean ± S.E.	With Ginkgo Mean ± S.E.	Difference (%)
$AUC_{0\text{-}5h}$(ng·min·mL^{-1})	26024.4 ± 4011.3	12737.0± 1306.4	-51.1*
T_{max}(min)	30.0 ± 4.5	36.7± 3.3	22.2
C_{max}(ng·mL^{-1})	169.4 ± 35.6	65.2± 7.9	-61.5*
MRT(min)	114±64.6	134.1 ± 3.5	17.0*

*p < 0.05

Table 3. Comparision of pharmacokinetic parameters of cyclosporine in 7 rats etween receiving cyclosporin alone and coadministration with onion juice

Parameter	Cyclosporin Alone Mean±S.E	With Onion Mean±S.E	Difference (%)
$AUC_{0\text{-}9h}$（ng.min.mL^{-1}）	89594.2±19343.9	16068.2±3758.7	-82.1**
T_{max}	25.7±3.7	48.6±4.0	88.9
C_{max}（ng.mL^{-1}）	331.1±69.0	98.4±16.5	-70.3**

MRT（min）	226.3±5.3	130.9±18.5	-42.2