

Quercetin and Rutin Reduced the Bioavailability of Cyclosporine from Neoral, an Immunosuppressant, through Activating P-Glycoprotein and CYP 3A4

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ABSTRACT: Quercetin and rutin are popular flavonoids in plant foods, herbs, and dietary supplements. Cyclosporine (CSP), an immunosuppressant with a narrow therapeutic window, is a substrate of P-glycoprotein (P-gp) and cytochrome P-450 3A4 (CYP3A4). This study investigated the effects of quercetin and rutin on CSP pharmacokinetics from Neoral and relevant mechanisms. Rats were orally administered Neoral with and without quercetin or rutin. The blood CSP concentration was assayed by a specific monoclonal fluorescence polarization immunoassay. The results showed that quercetin and rutin significantly decreased the C_{max} of CSP by 67.8 and 63.2% and reduced the AUC_{0-540} by 43.3 and 57.2%, respectively. The *in vitro* studies indicated that the quercetin and rutin induced the functions of P-gp and CYP3A4. In conclusion, quercetin and rutin decreased the bioavailability of CSP through activating P-gp and CYP3A. Transplant patients treated with Neoral should avoid concurrent consumption of quercetin or rutin to minimize the risk of allograft rejection.

KEYWORDS: Bioavailability, cyclosporine, quercetin, rutin, P-gp, CYP 3A4

INTRODUCTION

Flavonoids are a group of natural polyphenols widely distributed in plants. Many epidemiological studies showed that high flavonoid intake lowered the occurrences of coronary heart disease and possibly cancer.¹ In addition, their abilities to modulate cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (P-gp) draw more interests than ever for the roles that they play in drug interactions.^{2,3}

Quercetin (chemical structure shown in Figure 1), an ubiquitous flavonoid, and its glycosides are popular constituents in plant foods and medicinal herbs, such as onion, grapefruit, strawberry, grape, ginkgo, and St. John's wort (SJW). Quercetin has been reported to exert numerous pharmacological activities, such as free radical scavenging,⁴ TNF- α inhibition,⁵ and anticarcinogenic effects.⁶⁻⁹ Rutin (chemical structure shown in Figure 1), a glycoside of quercetin, is more abundant than quercetin in plants and has been used for improving intermittent claudication. Nowadays, commercial products of dietary supplements containing rich rutin and quercetin are easily purchasable in the markets, and the recommended dose was 250–500 mg twice per day. Rutin has been known to be hydrolyzed into quercetin in gut lumen and thought to demonstrate similar bioactivities as quercetin.^{10,11} A previous study reported that quercetin was an inhibitor of CYP 3A4 *in vitro*,¹² while conflicting modulation effects of quercetin on P-gp, either inhibition or stimulation, had been demonstrated in different models.¹²⁻¹⁴

Cyclosporine (CSP) is an important immunosuppressant with a narrow therapeutic window. Clinically, a supratherapeutic CSP blood level would cause adverse effects including nephrotoxicity,

hepatotoxicity, and neurotoxicity. Conversely, a subtherapeutic blood level would cause allograft rejection in transplant patients.¹⁵ The metabolism and transport of CSP were found to be associated with CYP3A4 and P-gp, respectively.^{16,17} Accordingly, any modulator of P-gp or CYP3A4 may alter the pharmacokinetics and pharmacodynamics of CSP.

The original oil-based formulation Sandimmune demonstrated unpredictable absorption of CSP and resulted in an increased frequency of acute and chronic rejection in patients with poor bioavailability. Subsequently, a new microemulsion dosage form Neoral was thus developed to cope with this problem.^{18,19} The Neoral formulation has self-emulsifying properties, which is less dependent on bile salts for absorption than Sandimmune.¹⁹⁻²¹ As compared with Sandimmune, Neoral provides increased bioavailability as evident in an increased area under the curve (AUC), increased peak blood concentration (C_{max}), and decreased time to peak blood concentration (T_{max}).

Although our previous study had reported decreased bioavailability of Sandimmune by coadministration of quercetin in pigs and rats,²² this study continued to access the effects of both quercetin and its glycoside rutin on the pharmacokinetics of CSP from the new dosage form Neoral in rats. Furthermore, *in vitro* models including LS180 cell line and recombinant CYP3A4 isozyme were used to identify the possible mechanisms of interaction.

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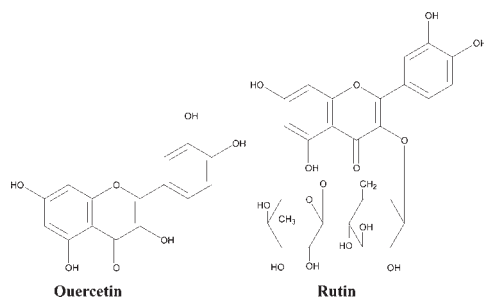


Figure 1. Chemical structures of quercetin and rutin.

MATERIALS AND METHODS

Chemicals and Reagents. CSP (Neoral, 100 mg/mL) was kindly provided by Novartis (Taiwan) Co. Ltd. Rutin hydrate (purity 95%), quercetin (purity 98%), glycofurol, rhodamine 123, sodium dodecyl sulfate (SDS), dimethyl sulfoxide (DMSO), 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyltetrazolium bromide (MTT), Triton X-100, verapamil, and sulfatase (type H-1 from *Helix pomatia*) 2-morpholinoethanesulfonic acid monohydrate (MES monohydrate) were purchased from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), trypsin/ethylenediaminetetraacetic acid (EDTA), nonessential amino acid, Hank's buffered salt solution (HBSS), and Vivid CYP450 screening kits were purchased from Invitrogen (Grand Island, NY). TDx kit was supplied by Abbott Laboratories (Abbott Park, IL). Milli-Q plus water (Millipore, Bedford, MA) was used for all preparations.

Drug Administration and Blood Collection. Eighteen Sprague–Dawley rats weighing 250–350 g were randomly divided into three groups. The rats were fasted for 12 h before dosing, and food was withheld for another 3 h. Water was supplied ad libitum. The CSP solution was prepared by diluting Neoral with deionized water to afford a concentration of 625 $\mu\text{g/mL}$. Quercetin and rutin were dissolved in glycofurol. CSP (1.25 mg/kg) was given orally with and without an oral dose of 50 mg/kg of quercetin and 110 mg/kg of rutin (equimolar with 50 mg/kg of quercetin) in a parallel design. Control rats received an equal volume of glycofurol (1.0 mL/kg) as a blank vehicle. CSP was administered immediately after quercetin and rutin. Blood samples were withdrawn via cardiopuncture at 20, 40, 60, 180, 300, and 540 min after dosing of CSP.

For all treatments described above, the blood samples were collected into small plastic vials containing EDTA and assayed within 24 h. Water was supplemented to rats by feeding with gastric gavage at specific times during the experiment. One week was allowed for washout. This animal study protocol has been approved by China Medical University, Taichung, Taiwan (CMU95-79-N), and all animal experiments adhered to “The Guidebook for the Care and Use of Laboratory Animals” published by the Chinese Society of Animal Science, Taiwan, Republic of China.

Quantitation of Blood CSP Concentration. The CSP concentration in blood was measured by a specific monoclonal fluorescence polarization immunoassay (Abbott). The validation of calibration curve was conducted by testing three controls before the sample assay. Otherwise, a new calibration curve will be constructed if necessary. The calibration range was 0.0–1500.0 ng/mL, and the LLOQ was 25.0 ng/mL.

Cell Line and Culture Conditions. LS 180, the human colon adenocarcinoma cell line, was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (Biological Industries Ltd., Kibbutz Beit Haemek, Israel), 0.1 mM nonessential amino acid, 100 units/mL of penicillin, 100 $\mu\text{g/mL}$ of streptomycin, and 292 $\mu\text{g/mL}$ of glutamine. Cells were grown at 37 °C

in a humidified incubator containing 5% CO_2 . The medium was changed every other day, and cells were subcultured when 80–90% confluency was reached.

Cell Viability Assay. The effects of quercetin, rutin, verapamil, and DMSO on the viability of LS 180 cells were evaluated by MTT assay.²³ Cells were seeded into a 96-well plate. After overnight incubation, the tested agents were added into the wells and incubated for 72 h, and then, 15 μL of MTT (5 mg/mL) was added into each well and incubated for additional 4 h. During this period, MTT was reduced to formazan crystal by live cells. An acid–SDS (10%) solution was added to dissolve the purple crystal at the end of incubation, and the optical density was detected at 570 nm by a microplate reader (BioTex, Highland Park, Winooski, VT).

Effects of Quercetin and Rutin on P-gp Activity. The transport assay of rhodamine 123 was modified from a previous method.²⁴ Briefly, LS 180 cells (1×10^5) were cultured in each well in a 96-well plate. After overnight incubation, the medium was removed and washed three times with ice-cold PBS buffer. Rhodamine 123 in HBSS (1 μM , 100 μL) was added into each well and incubated at 37 °C. After 1 h of incubation, the supernatants were removed and washed three times with ice-cold PBS. Then, quercetin, rutin, verapamil (as a positive control of P-gp inhibitor), and DMSO were added to correspondent wells and incubated at 37 °C. After 4 h of incubation, the medium was removed, and the cells were washed three times with ice-cold PBS. Subsequently, 100 μL of 0.1% Triton X-100 was added to lyse the cells, and the fluorescence was measured with excitation at 485 nm and emission at 528 nm. To quantitate the content of protein in each well, 10 μL of cell lysate was added to 200 μL of diluted protein assay reagent (Bio-Rad, Hercules, CA), and the optical density was measured at 570 nm. The relative intracellular accumulation of rhodamine 123 was calculated by comparison with that of control.

Preparation and Characterization of Serum Metabolites of Rutin. To mimic the molecules interacting with CYP 3A in enterocytes, the serum metabolites of rutin in rats were prepared and characterized. Rutin was orally administered at 250 mg/kg to rats fasted overnight. Blood was collected via cardiopuncture at 30 min after dosing. After coagulation, the serum was vortexed with a 3-fold volume of methanol. After centrifugation at 10000g for 15 min, the supernatant was concentrated in a rotary evaporator under vacuum to dryness. To the residue, an appropriate volume of water was added to afford a solution with a 10-fold serum concentration, which was divided into aliquots and stored at –80 °C for later use.

A portion of the metabolite solution was characterized following a method reported previously.²⁵ Briefly, 200 μL of serum sample was mixed with 100 μL of sulfatase (containing 100 units/mL of sulfatase and 3560 units/mL of β -glucuronidase) and 50 μL of ascorbic acid (200 mg/mL) and incubated at 37 °C for 1 h under anaerobic condition. After hydrolysis, the serum was acidified with 0.1 N HCl and partitioned with ethyl acetate (containing 6,7-dimethoxycoumarin as an internal standard). The ethyl acetate layer was evaporated under N_2 to dryness and reconstituted with an appropriate volume of methanol prior to HPLC analysis. On the other hand, blank serum was vortexed with a 3-fold volume of methanol to prepare deproteinized specimens with 1/8- and 1/4-fold serum concentrations as controls for comparison with corresponding specimens of serum metabolites of rutin.

Effects of Serum Metabolites of Rutin on CYP3A4 Activity. Vivid CYP450 screening kits (Invitrogen, Carlsbad, CA) was used to evaluate the effect of serum metabolites of rutin on the activity of CYP3A. All of the procedures were performed according the manual provided by the manufacturer. Briefly, after serum metabolites of rutin (1/4- and 1/8- fold serum concentration) or deproteinized blank serum specimens were incubated with CYP450 recombinant Baculosomes, glucose-6-phosphate and glucose-6-phosphate dehydrogenase in 96-well black plate at room temperature for 20 min, a specific CYP3A substrate

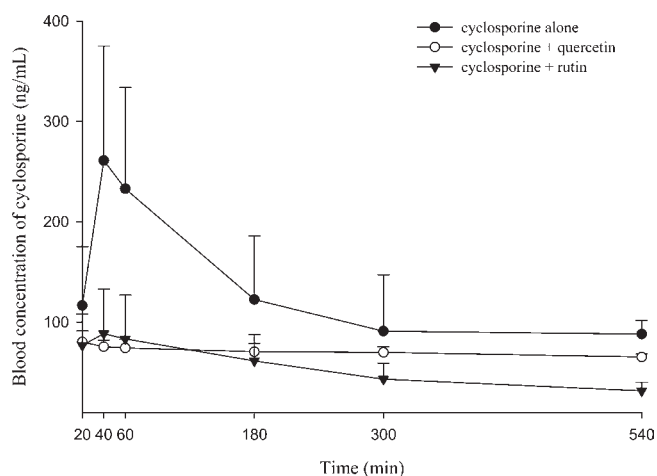


Figure 2. Mean (\pm SD) blood concentration–time profiles of CSP after oral administration of CSP alone (1.25 mg kg^{-1}) (\bullet) and coadministration with quercetin (50 mg kg^{-1}) (\circ) and rutin (110 mg kg^{-1}) (\blacktriangledown) to six rats in each group.

(Vivid BOMR) and NADP^+ were added and incubated at room temperature for another 30 min. At the end of incubation, ketoconazole was added to stop the reaction, and the fluorescence was measured with an excitation at 530 nm and an emission at 590 nm.

Data Analysis. The pharmacokinetic parameters of CSP were calculated using a noncompartment model with the aid of WinNonlin (version 1.1, SCI software, Statistical Consulting, Inc., Apex, NC). The peak blood concentrations (C_{max}) were obtained from experimental observation. The area under the serum concentration–time curve (AUC_{0-t}) was calculated using a trapezoidal rule to the last point. The statistical software SPSS was used for analyzing the differences among treatments by using analysis of variance for three groups and unpaired Student's t test for two groups. Statistical significance level was set at $p < 0.05$.

RESULTS

The blood profiles of CSP in rats administered Neoral with and without quercetin (50 mg/kg) and rutin (110 mg/kg) are shown in Figure 2, and the pharmacokinetic parameters of three treatments are listed in Table 1. The results showed that quercetin and rutin significantly decreased the C_{max} of CSP by 67.8 and 63.2% and reduced the AUC_{0-540} by 43.3 and 57.2%, respectively.

To explore the possible involvement of P-gp in the observed pharmacokinetic interaction, LS 180 was used for the transport assay employing a typical P-gp substrate rhodamine 123. The MTT assay showed that incubation of quercetin ($50 \mu\text{M}$) and rutin ($50 \mu\text{M}$) with LS 180 for 72 h exerted no significant influences on cell viability. In the transport assay, the accumulation of rhodamine 123 in LS 180 cells measured after 4 h of incubation with tested agents is shown in Figure 3. The positive control verapamil at $100 \mu\text{M}$ significantly increased the intracellular accumulation of rhodamine 123 by 54.1%, whereas DMSO at 0.5% (v/v) did not show significant influence. Quercetin at 10 and $50 \mu\text{M}$ significantly decreased the intracellular accumulation of rhodamine 123 by 29.6 and 23.6%, and rutin at 10 and $50 \mu\text{M}$ significantly decreased the intracellular accumulation of rhodamine 123 by 19.5 and 31.8%, respectively.

Characterization of rutin metabolites in the serum specimen showed that the major molecules were quercetin

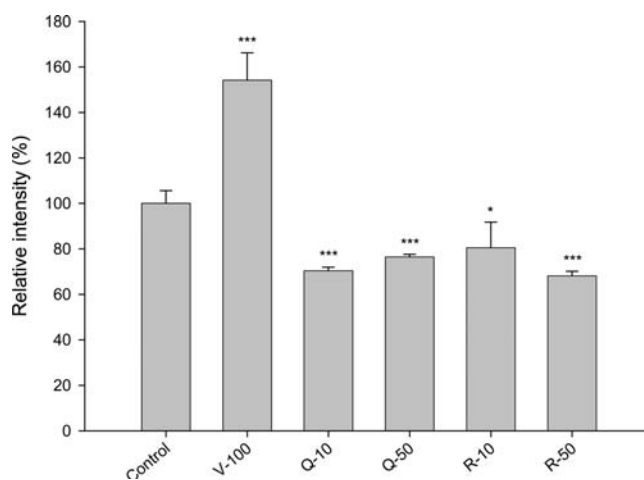


Figure 3. Effects of quercetin (Q, μM), rutin (R, μM), and verapamil (V, $100 \mu\text{M}$) on the accumulation of rhodamine 123 in LS 180 cells. * $p < 0.05$, and *** $p < 0.001$.

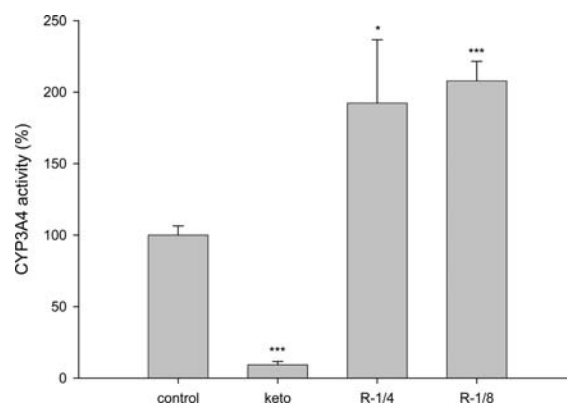


Figure 4. Effects of serum metabolite of rutin (R, 1/4- and 1/8-fold serum concentration) and ketoconazole (Keto, $10 \mu\text{M}$) on CYP3A4 activity. * $p < 0.05$, and *** $p < 0.001$.

glucuronides/sulfates in a concentration of 3.4 nmol/mL . The effects of rutin metabolites at 1/8- and 1/4-fold serum concentrations on CYP3A activity are shown in Figure 4. As a positive control, ketoconazole at $10 \mu\text{M}$ significantly decreased CYP3A activity by 90.8%. Contrary to the effect of ketoconazole, rutin metabolites at 1/8- and 1/4-fold serum concentration significantly increased CYP3A4 activity by 208.0 and 194.0%, respectively, when compared to those of the corresponding concentration of deproteinized blank serum specimen.

DISCUSSION

The use of botanical products as antioxidant supplements is on the rise among the global population in recent decades. Although the safety profile of many botanical products is promising, accumulated evidence showed significant interactions with critical medicines, which can place individual patients at great risk. This study found that the oral bioavailability of CSP from Neoral was significantly decreased by quercetin and rutin, which might result in subtherapeutic blood levels of CSP and expose transplant patients to a non-negligible hidden risk of allograft rejection.

Table 1. Pharmacokinetic Parameters of CSP after Oral Administration of CSP Alone (1.25 mg kg⁻¹) and Coadministration with Quercetin (50 mg kg⁻¹) and Rutin (110 mg kg⁻¹) to Six Rats in Each Group^a

parameters	treatments		
	CSP alone	CSP + quercetin	CSP + rutin
C _{max} (ng mL ⁻¹)	261.5 ± 114.0 a	84.1 ± 6.9 b (-67.8%)	96.3 ± 45.1 b (-63.2%)
AUC ₀₋₅₄₀ (μg min mL ⁻¹)	65.5 ± 25.8 a	37.2 ± 2.2 b (-43.3%)	28.0 ± 11.1 b (-57.2%)
MRT (min)	225.5 ± 17.7	267.3 ± 5.2	224.4 ± 21.1

^aData are expressed as means ± SDs. Means in a row without a common letter differ. $P < 0.05$. C_{max}, peak blood concentration; AUC₀₋₅₄₀, area under the blood concentration–time curve to 540 min; and MRT, mean residence time.

Because of the poor solubility of quercetin and rutin in water, glycofurol was used to prepare the oral dosing solution in this study. We previously found that CSP bioavailability was markedly reduced in second dose administration of Neoral in rats,²² and a protocol of parallel design was thus conducted. The result of this study showing that quercetin markedly decreased the bioavailability of CSP from Neoral was in good agreement with that reported for the oil-based Sandimmune.²⁶ In regard to the rutin–CSP interaction, this is the first report to demonstrate that rutin likewise reduced the bioavailability of CSP. Being a glycoside of quercetin, rutin has been known to be hydrolyzed to quercetin in gut lumen and then presented as quercetin sulfates/glucuronides in the circulation, which was the same as the metabolic fate of quercetin.^{27–29} Therefore, that equimolar doses of rutin and quercetin conferred comparable interaction with CSP can be accounted for by their metabolic relevance.

P-gp has been recognized to play an important role in the barrier function of the intestine and drug–drug interactions.³⁰ To explore the possible involvement of P-gp in these interactions, a transport assay of rhodamine 123 was conducted by using LS 180 cells. The MTT assay of LS 180 showed that quercetin and rutin below 50 μM did not affect the cell viability, indicating that the cells were normal throughout the experiment period. As shown in Figure 2, contrary to verapamil (a positive control of P-gp inhibitor), quercetin and rutin significantly decreased the intracellular accumulation of rhodamine 123, indicating activation of P-gp, which was in agreement with the findings of two previous studies,^{13,31} and could in part explain the decreased blood levels of CSP in rats. On the contrary, quercetin has been reported as an inhibitor of P-gp in numerous studies using breast and pancreatic cell lines,^{12,13,32–34} which was apparently not consistent with our *in vivo* evidence. In regard to these discrepant effects of quercetin on P-gp among *in vitro* studies, either inhibition or stimulation, we contemplate that it might be arisen from the differences of cell models in use. We suspect that a different metabolic capability among cell lines may result in a differential amount of quercetin metabolites after incubation with quercetin for certain duration, which may lead to discrepant effects on P-gp activity. Therefore, cellular metabolism of quercetin in various cell lines requires more future studies.

Pharmacokinetic studies of quercetin and rutin have identified glucuronides/sulfates of quercetin as the major molecules in the circulation.^{25,27} We proposed that the serum metabolite of rutin could mimic the molecules interacting with enteric or hepatic CYP3A, located in the microsomes of cells, after intake of either rutin or quercetin. Therefore, we prepared and characterized the serum metabolite of rutin from rats to evaluate the *in vivo* effects of rutin and quercetin on CYP 3A activity. Our results show that the serum metabolite of rutin, containing mainly quercetin

glucuronides/sulfates, increased CYP 3A activity and clearly implied that the CYP3A-mediated mechanism can explain in part the decreased bioavailability of CSP caused by rutin or quercetin in rats. This novel approach was different from most *in vitro* studies reporting the effects of herbal extract or natural compounds on CYP 3A4 by using their parent forms, which may not represent the true molecules interacting with CYP 3A4 *in vivo*. Herein, our finding is opposite to a previous *in vitro* study reporting that quercetin was an inhibitor of CYP 3A,¹² which apparently had not taken the metabolism of quercetin by gut into consideration and could not explain our *in vivo* evidence. Therefore, we suggest that to mimic the biological system, understanding of presystemic metabolism of natural polyphenols is very important before *in vitro* studies.

In recent decades, many cases of subtherapeutic blood CSP concentration caused by SJW, many of which were life-threatening, have brought about increasing interests in herb–drug interactions.^{28,29,35,36} With regard to the mechanism of interaction, SJW has been shown to increase the metabolism of various drugs, such as CSP, oral contraceptives, and indinavir, through the induction of CYP3A4 activity.^{37,38} We suspect that the antioxidant supplements rutin and quercetin may bring about risks of critical interactions with western medicines as SJW did.

In conclusion, quercetin and rutin significantly reduced the oral bioavailability of CSP through activating P-gp and CYP 3A4. We suggest that transplant patients treated with CSP should avoid concomitant intake of dietary supplements containing quercetin and rutin to minimize the risk of allograft rejection.

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Author Contributions

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ABBREVIATIONS USED

P-gp, P-glycoprotein; CYP3A4, cytochrome P-450 3A4; SJW, St. John's wort

REFERENCES

- (1) Hertog, M. G.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* **1995**, *155* (4), 381–386.
- (2) Brand, W.; Schutte, M. E.; Williamson, G.; van Zanden, J. J.; Cnubben, N. H.; Groten, J. P.; van Bladeren, P. J.; Rietjens, I. M. Flavonoid-mediated inhibition of intestinal ABC transporters may affect the oral bioavailability of drugs, food-borne toxic compounds and bioactive ingredients. *Biomed. Pharmacother.* **2006**, *60* (9), 508–519.
- (3) Moon, Y. J.; Wang, X.; Morris, M. E. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicol. in Vitro* **2006**, *20* (2), 187–210.
- (4) Horvathova, K.; Novotny, L.; Vachalkova, A. The free radical scavenging activity of four flavonoids determined by the comet assay. *Neoplasma* **2003**, *50* (4), 291–295.
- (5) Park, Y. C.; Rimbach, G.; Saliou, C.; Valacchi, G.; Packer, L. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF- α secretion, and NF- κ B-dependent gene expression in RAW 264.7 macrophages. *FEBS Lett.* **2000**, *465* (2–3), 93–97.
- (6) Birt, D. F.; Hendrich, S.; Wang, W. Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol. Ther.* **2001**, *90* (2–3), 157–177.
- (7) Ramos, S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J. Nutr. Biochem.* **2007**, *18* (7), 427–442.
- (8) Murakami, A.; Ashida, H.; Terao, J. Multitargeted cancer prevention by quercetin. *Cancer Lett.* **2008**, *269* (2), 315–325.
- (9) Jeong, J. H.; An, J. Y.; Kwon, Y. T.; Rhee, J. G.; Lee, Y. J. Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. *J. Cell. Biochem.* **2009**, *106* (1), 73–82.
- (10) Baba, S.; Furuta, T.; Fujioka, M.; Goromaru, T. Studies on drug metabolism by use of isotopes XXVII: urinary metabolites of rutin in rats and the role of intestinal microflora in the metabolism of rutin. *J. Pharm. Sci.* **1983**, *72* (10), 1155–1158.
- (11) Kim, D. H.; Jung, E. A.; Sohng, I. S.; Han, J. A.; Kim, T. H.; Han, M. J. Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. *Arch. Pharm. Res.* **1998**, *21* (1), 17–23.
- (12) Ma, S. Quercetin not only inhibits P-glycoprotein efflux activity but also inhibits CYP3A isozymes. *Cancer Chemother. Pharmacol.* **1995**, *36* (5), 448–450.
- (13) Mitsunaga, Y.; Takanaga, H.; Matsuo, H.; Naito, M.; Tsuruo, T.; Ohtani, H.; Sawada, Y. Effect of bioflavonoids on vincristine transport across blood-brain barrier. *Eur. J. Pharmacol.* **2000**, *395* (3), 193–201.
- (14) Di Pietro, A.; Conseil, G.; Perez-Victoria, J. M.; Dayan, G.; Baubichon-Cortay, H.; Trompier, D.; Steinfels, E.; Jault, J. M.; de Wet, H.; Maitrejean, M.; Comte, G.; Boumendjel, A.; Mariotte, A. M.; Dumontet, C.; McIntosh, D. B.; Goffeau, A.; Castans, S.; Gamarro, F.; Barron, D. Modulation by flavonoids of cell multidrug resistance mediated by P-glycoprotein and related ABC transporters. *Cell. Mol. Life Sci.* **2002**, *59* (2), 307–322.
- (15) Burke, J. F., Jr.; Pirsch, J. D.; Ramos, E. L.; Salomon, D. R.; Stablein, D. M.; Van Buren, D. H.; West, J. C. Long-term efficacy and safety of cyclosporine in renal-transplant recipients. *N. Engl. J. Med.* **1994**, *331* (6), 358–363.
- (16) Edwards, D. J.; Fitzsimmons, M. E.; Schuetz, E. G.; Yasuda, K.; Ducharme, M. P.; Warbasse, L. H.; Woster, P. M.; Schuetz, J. D.; Watkins, P. 6',7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: Effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein. *Clin. Pharmacol. Ther.* **1999**, *65* (3), 237–244.
- (17) Gorsalski, K. B.; Acott, P. D.; Fraser, A. D.; Worth, D.; Sinal, C. J. Brain cyclosporin A levels are determined by ontogenic regulation of mdr1a expression. *Drug Metab. Dispos.* **2006**, *34* (2), 288–295.
- (18) Choc, M. G. Bioavailability and pharmacokinetics of cyclosporine formulations: Neoral vs Sandimmune. *Int. J. Dermatol.* **1997**, *36* (Suppl. 1), 1–6.
- (19) Ritschel, W. A. Microemulsion technology in the reformulation of cyclosporine: The reason behind the pharmacokinetic properties of Neoral. *Clin. Transplant.* **1996**, *10* (4), 364–373.
- (20) Min, D. I. Neoral: A microemulsion cyclosporine. *J. Transpl. Coord.* **1996**, *6* (1), 5–8.
- (21) Friman, S.; Backman, L. A new microemulsion formulation of cyclosporin: Pharmacokinetic and clinical features. *Clin. Pharmacokinet.* **1996**, *30* (3), 181–193.
- (22) Hsiu, S. L.; Hou, Y. C.; Wang, Y. H.; Tsao, C. W.; Su, S. F.; Chao, P. D. Quercetin significantly decreased cyclosporin oral bioavailability in pigs and rats. *Life Sci.* **2002**, *72* (3), 227–235.
- (23) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65* (1–2), 55–63.
- (24) Jia, J. X.; Wasan, K. M. Effects of monoglycerides on rhodamine 123 accumulation, estradiol 17 beta-D-glucuronide bidirectional transport and MRP2 protein expression within Caco-2 cells. *J. Pharm. Pharm. Sci.* **2008**, *11* (3), 45–62.
- (25) Yang, C. Y.; Hsiu, S. L.; Wen, K. C.; Lin, S. P.; Tsai, S. Y.; Hou, Y. C.; Chao, P. D. L. Bioavailability and metabolic pharmacokinetics of rutin and quercetin in rats. *J. Food Drug Anal.* **2005**, *13*, 244–250.
- (26) Yang, C. Y.; Chao, P. D.; Hou, Y. C.; Tsai, S. Y.; Wen, K. C.; Hsiu, S. L. Marked decrease of cyclosporin bioavailability caused by coadministration of ginkgo and onion in rats. *Food Chem. Toxicol.* **2006**, *44* (9), 1572–1578.
- (27) Erlund, I.; Kosonen, T.; Alftan, G.; Maenpaa, J.; Perttunen, K.; Kenraali, J.; Parantainen, J.; Aro, A. Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *Eur. J. Clin. Pharmacol.* **2000**, *56* (8), 545–553.
- (28) Karliova, M.; Treichel, U.; Malago, M.; Frilling, A.; Gerken, G.; Broelsch, C. E. Interaction of *Hypericum perforatum* (St. John's wort) with cyclosporin A metabolism in a patient after liver transplantation. *J. Hepatol.* **2000**, *33* (5), 853–855.
- (29) Ruschitzka, F.; Meier, P. J.; Turina, M.; Luscher, T. F.; Noll, G. Acute heart transplant rejection due to Saint John's wort. *Lancet* **2000**, *355* (9203), 548–549.
- (30) Tsuji, A. Transporter-mediated Drug Interactions. *Drug Metab. Pharmacokinet.* **2002**, *17* (4), 253–274.
- (31) Critchfield, J. W.; Welsh, C. J.; Phang, J. M.; Yeh, G. C. Modulation of adriamycin accumulation and efflux by flavonoids in HCT-15 colon cells. Activation of P-glycoprotein as a putative mechanism. *Biochem. Pharmacol.* **1994**, *48* (7), 1437–1445.
- (32) Chieli, E.; Romiti, N.; Cervelli, F.; Tongiani, R. Effects of flavonols on P-glycoprotein activity in cultured rat hepatocytes. *Life Sci.* **1995**, *57* (19), 1741–1751.
- (33) Borska, S.; Sopol, M.; Chmielewska, M.; Zabel, M.; Dziegiel, P. Quercetin as a potential modulator of P-glycoprotein expression and function in cells of human pancreatic carcinoma line resistant to daunorubicin. *Molecules* **2010**, *15* (2), 857–870.
- (34) Shapiro, A. B.; Ling, V. Effect of quercetin on Hoechst 33342 transport by purified and reconstituted P-glycoprotein. *Biochem. Pharmacol.* **1997**, *53* (4), 587–596.
- (35) Piscitelli, S. C.; Burstein, A. H.; Chaitt, D.; Alfaro, R. M.; Falloon, J. Indinavir concentrations and St John's wort. *Lancet* **2000**, *355* (9203), 547–548.
- (36) Henderson, L.; Yue, Q. Y.; Bergquist, C.; Gerden, B.; Arlett, P. St John's wort (*Hypericum perforatum*): Drug interactions and clinical outcomes. *Br. J. Clin. Pharmacol.* **2002**, *54* (4), 349–356.
- (37) Roby, C. A.; Anderson, G. D.; Kantor, E.; Dryer, D. A.; Burstein, A. H. St John's Wort: Effect on CYP3A4 activity. *Clin. Pharmacol. Ther.* **2000**, *67* (5), 451–457.
- (38) Moore, L. B.; Goodwin, B.; Jones, S. A.; Wisely, G. B.; Serabjit-Singh, C. J.; Willson, T. M.; Collins, J. L.; Kliever, S. A. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97* (13), 7500–7502.