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Potential Risk of Mulberry–Drug Interaction: Modulation on P-Glycoprotein and Cytochrome P450 3A

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ABSTRACT: Mulberry is a fruit containing polyphenol antioxidants. Cyclosporine (CSP), a potent immunosuppressant with a narrow therapeutic range, is widely used in transplant patients. This study investigated the effect of co-administration of mulberry on the bioavailability of CSP, a probe drug of P-glycoprotein (P-gp)/cytochrome P450 3A4 (CYP 3A4), in rats and relevant mechanisms. CSP (2.5 mg/kg) was orally administered with and without a single dose or the seventh dose of mulberry (2 g/kg) to rats. The results showed that a single dose of mulberry significantly decreased the area under the curve of concentration (AUC_{0-540}) and the maximum blood concentration (C_{max}) of CSP by 53.2 and 65.8%, respectively. Repeated dosing of mulberry significantly decreased the AUC_{0-540} and C_{max} of CSP by 23.7 and 39.7%, respectively. Mechanism studies indicated that mulberry significantly increased the activities of P-gp and CYP 3A. In conclusion, mulberry significantly reduced the bioavailability of CSP through activating the functions of P-gp and CYP 3A.

KEYWORDS: *Cyclosporine, cytochrome P450 3A, mulberry, P-glycoprotein*

INTRODUCTION

Berries as a group of fruits rich in polyphenols have great health benefits for lowering the risk factors of chronic diseases.[1] Mulberry (fruits of *Morus alba* L.) contains polyphenol antioxidants, including flavonoids, stilbenes, and anthocyanins, such as rutin, quercetin (chemical structures shown in Figure 1), quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, resveratrol, cyanidin 3-*O*-glucoside, and pelargonidin 3-*O*-glucoside,[2,3] which have been shown to exhibit antioxidation,[4] anti-inflammation,[5] hypolipidemic,[6] and neuroprotective effects.[7]

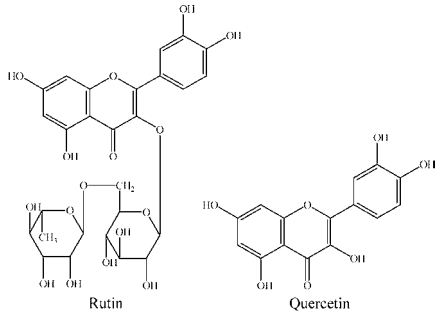

Figure 1. Chemical structures of rutin and quercetin.

Cyclosporine (CSP), a potent immunosuppressive agent with a narrow therapeutic range, is widely used in transplant patients to prevent allograft rejection and also used for the treatments of psoriasis and rheumatoid arthritis.[8] The bioavailability of CSP has been known associated with P-glycoprotein (P-gp), a drug efflux pump, and cytochrome P450 3A4 (CYP 3A4), which are two important determinants for the absorption of oral drugs.[9,10] In this study, CSP was used as a probe drug of P-gp and CYP 3A4.

Previous studies have demonstrated that numerous polyphenols, such as rutin, quercetin, resveratrol, and kaempferol, were modulators of P-gp[11,12] and CYP 3A4.[13−15] However, most studies dealing the modulation effects on P-gp and CYP 3A4 were essentially based on *in vitro* models, and the results were often controversial. For instance, quercetin has been reported as an inhibitor of P-gp;[16] on the contrary, a stimulation effect of quercetin on P-gp has been demonstrated.[11] With regard to *in vivo* evidence, polyphenols, such as rutin, quercetin, and resveratrol, all have been reported to decrease the oral bioavailability of CSP in rats.[17−19] We thus hypothesized that mulberry containing rich polyphenols might result in food–drug interactions with CSP. Therefore, this study investigated the effects of single and repeated dosing of mulberry on CSP pharmacokinetics in rats, and the relevant mechanisms were explored using *in vitro* and *ex vivo* models.

MATERIALS AND METHODS

Chemicals and Reagents. CSP (Neoral, 100 mg/mL) was kindly offered by Novartis (Taiwan) Co., Ltd. Rutin, resveratrol, quercetin, kaempferol, 6,7-dihydroxycoumarin (6,7-DMC), formic acid, sulfatase (type H-1, from *Helix pomatia*), and β-glucuronidase (type B-1, from bovine liver) were purchased from Sigma (St. Louis, MO). Erlotinib (purity of 98%) was obtained from Wuhan Sunrise Technology Development, Inc. (Wujiashan, China). L-(+)-Ascorbic acid, phosphoric acid, and sodium hydroxide solution (1 M) were purchased

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from Riedel-deHaën AG (Seelze, Germany). Ethyl acetate and acetonitrile were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Sodium acetate was purchased from Kohusan Chemical Works, Ltd. (Tokyo, Japan). Methylthiazolyldiphenyltetrazolium bromide (MTT), rhodamine 123, Triton X-100, and verapamil were obtained from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), trypsin/ethylenediaminetetraacetic acid (EDTA), non-essential amino acid (NEAA), Hank's buffered salt solution (HBSS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and Vivid CYP 450 screening kits were obtained from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Biological Industries (Kibbutz, BH, Israel). Milli-Q Plus water (Millipore, Bedford, MA) was used for all processes.

Preparation of the Mulberry Extract. A total of 1 kg of mulberry was heated with 2 L of water to boiling on a gas stove, and boiling was maintained to concentrate the volume until below 1 L. After cooling to room temperature, the marc was filtered with cloth. The extract was added to a sufficient quantity of water to make 1 L, divided into aliquots, and stored at −20 °C for later use. After thawing, the filtrate (300 μL) was added with 700 μL of methanol, and then the mixture was vortexed and centrifuged at 13000*g* for 15 min. The supernatant was diluted with 4-fold methanol. A total of 200 μL was mixed with an equal volume of an internal standard solution (0.5 μg/mL of 6,7-DMC in methanol), and 5 μL of the mixture was subject to high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) analysis.

Characterization of the Mulberry Extract. The HPLC−MS/MS method was used for the characterization of the mulberry extract. The instrumentation included an Accela 1250 pump and autosampler (Thermo Fisher Scientific, Inc., Waltham, MA). A Thermo Hypersil GOLD C18 analytical column (50 × 2.1 mm, 1.9 μm) with a prefilter was used for separation. The mobile phase consisted of 0.01% formic acid (A) with acetonitrile containing 0.01% formic acid (B), and a gradient elution was programmed as follows: A/B of 25:70 (0−1 min) and 0:100 (1.1−4 min). The flow rate was 0.2 mL/min. The column effluent was detected by a HESI-II Probe (atmospheric pressure ionization) TSQ Quantum Access MAX (Thermo Fisher Scientific, Inc., Waltham, MA). Nitrogen was used as sheath gas at 40 arbitrary units, and auxiliary gas was used at 10 arbitrary units. The collision energy was set at 23 V, with spray voltage at 3000 V, capillary temperature at 270 °C, vaporizer temperature at 300 °C, tube lens offset at 118 V, and collision pressure at 1.5 mTorr. The following mass transitions were used for selected reaction monitoring (SRM) analysis: rutin, *m*/*z* 611/303; quercetin, *m*/*z* 303/153; and internal standard, *m*/*z* 207/151. Electrospray ionization−mass spectrometry (ESI−MS) spectra were recorded in the positive-ion mode.

Animal Study. All animal experiments adhered to "The Guidebook for the Care and Use of Laboratory Animals (2002)" published by the Chinese Society of Animal Science, Taiwan, Republic of China. A total of 18 male Sprague−Dawley rats (300−400 g) were supplied by the National Laboratory Animal Center (Taipei, Taiwan) and randomly divided into three groups ($n = 6$). The rats fasted for 12 h before dosing, and food was withheld for another 3 h, with water supplied *ad libitum*. The CSP solution was prepared by diluting Neoral with deionized water to afford a concentration of 1.25 mg/mL. Control rats were orally given CSP [2.5 mg/kg of body weight (bw)] with water (2 mL/kg of bw). A treatment group of rats received oral CSP (2.5 mg/kg of bw) with a single dose of mulberry (2 g per 2 mL/kg of bw). In addition, to reach steady state, another treatment group of rats were given seven doses of mulberry (2 g per 2 mL/kg of bw) twice daily before CSP dosing. The blood samples of rats were withdrawn via cardiac puncture at 20, 40, 60, 180, 300, and 540 min after administration of CSP. All blood samples were collected into small plastic vials containing EDTA and assayed within 24 h. The CSP concentration was measured by a specific monoclonal fluorescence polarization immunoassay using a TDx kit (Abbott, Abbott Park, IL). The assay was calibrated for concentrations ranging from 25.0 to 1500.0 ng/mL. The lower limit of quantitation (LLOQ) of this assay is 25.0 ng/mL.

Cell Line. LS 180, intestinal human colon adenocarcinoma cell line, was obtained from the Bioresource Collection and Research Center (Hsinchu, Republic of China). Cells were routinely grown in DMEM containing 10% FBS, 1% NEAA, 100 units/mL penicillin, and 0.1 mg/mL streptomycin at 37 °C in an atmosphere of 5% CO_2 and 90% relative humidity. For the transport experiments, LS 180 cells were passages 65−75.

Cell Viability Assay. The effects of verapamil (a positive control of P-gp inhibitor), dimethyl sulfoxide (DMSO), and a series of concentrations of the mulberry extract on the viability of LS 180 cells were evaluated by the MTT assay.[20] Cells were seeded in 96-well plates in DMEM culture and allowed to attach overnight. Tested agents were added to the wells and incubated for 72 h; then 15 μL of MTT (5 mg/mL) was added to each well; and the cells were incubated for a further 4 h. During this period, MTT was reduced to formazan crystal by live cells. Acid−sodium dodecyl sulfate (SDS) (10%) solution was added to dissolve the purple crystal at the end of incubation, and the absorbance was recorded at 570 nm by a microplate reader (BioTex, Highland Park, VT).

Effect of Mulberry on P-gp-Mediated Transport. The efflux activity of P-gp was determined by measuring the intracellular accumulation of rhodamine 123, a fluorescent P-gp substrate,[21,22] and CSP in LS 180 cells. The cells in 96-well (1×10^5 cells/well) or 24-well (1×10^6 cells/well) plates were incubated with DMEM at 37 °C overnight and then washed 3 times with ice-cold HBSS. The cells were then incubated with 100 μL of rhodamine (10 μM) or 200 μL of CSP (5 μM) for 1 h. After washing 3 times with ice-cold HBSS, a series of concentrations of mulberry was added to each well and incubated for 4 h and 15 min for the assays of rhodamine and CSP, respectively. HBSS was used as a blank control, and verapamil used as a positive control. The cells were then washed 3 times with ice-cold HBSS and lysed with lysis buffer (0.1% Triton X-100). Rhodamine fluorescence was measured with excitation at 485 nm and emission at 528 nm.

With regard to the quantitation of the intracellular concentration of CSP, the cell lysate was partitioned with an equal volume of ethyl acetate containing erlotinib as an internal standard. The ethyl acetate layer was evaporated under N_2 gas to dryness and reconstituted with 100 μL of methanol, and then 5 μL was subject to HPLC−MS/MS analysis. Chromatographic separation of CSP was achieved using a Phenomenex Luna C18 analytical column (150 × 1.0 mm, 5 μm) with a prefilter. The mobile phase was composed of acetonitrile containing 0.01% formic acid and formic acid (0.01%, v/v) at a ratio of 9:1 and run isocratically. The flow rate was 0.2 mL/min. The collision energy was set at 50 V. The following mass transitions were used for SRM analysis: CSP, *m*/*z* 1203/425; and internal standard, *m*/*z* 394/278. ESI−MS spectra were recorded in the positive-ion mode.

To quantitate the content of protein in each well, 10 μL of cell lysate was added to 200 μL of protein assay reagent (Bio-Rad, Hercules, CA) and the optical density was measured at 570 nm. The relative intracellular accumulation of rhodamine 123 and CSP was calculated by comparing the florescence intensity to that of the control.

Preparation and Characterization of the Serum Metabolites of the Mulberry Extract. To mimic the molecules interacting with CYP 3A in enterocytes, the serum metabolites of mulberry in rats were prepared. Mulberry was orally administered 2 g/kg to rats fasted overnight, and blood was collected via cardiopuncture at 30 min after dosing. Blood was centrifuged at 13000*g* for 15 min. Serum was vortexed with a 4-fold volume of methanol. After centrifugation at 13000*g* for 15 min, the supernatant was concentrated in a rotatory evaporator under vacuum to dryness. To the residue, an appropriate volume of water was added to afford a solution with 10-fold serum concentration, which was divided into aliquots and stored at −80 °C for later use. In addition, blank serum was likewise processed to prepare specimens with 1.0-, 0.5-, and 0.25-fold serum concentrations as controls for comparison to correspondent specimens of serum metabolites of mulberry.

For the characterization of the serum metabolites of the mulberry extract, serum samples were assayed before and after hydrolysis with β-

glucuronidase and sulfatase individually for the quantitation of quercetin and its glucuronides and sulfates. Briefly, 100 μL of serum metabolite was mixed with 50 μL of β-glucuronidase (1000 units/mL), sulfatase (1000 units/mL), or enzyme-free buffer at pH 5 and 25 μL of ascorbic acid (200 mg/mL), incubated 2 and 1 h for the hydrolysis of glucuronides and sulfates, respectively, under anaerobic conditions, and protected from light at 37 °C. After hydrolysis, the mixture was acidified with 10 μL of 1.2 N HCl and partitioned with an equal volume of ethyl acetate containing 6,7-DMC as an internal standard. The ethyl acetate layer was evaporated under N_2 gas to dryness and reconstituted with 100 μL of methanol, and then 5 μL was subject to HPLC−MS/MS analysis.

Effects of the Mulberry Extract and Its Serum Metabolites on CYP 3A4 Activity. To evaluate the effect of the mulberry extract and it serum metabolites on CYP 3A4 activity, an *ex vivo* study using the serum metabolite of mulberry were conducted. The procedure followed the manual of the Vivid CYP 450 screening kit (Invitrogen, Carlsbad, CA). Briefly, after incubation of the mulberry extract (15.0 and 3.8 mg/mL), its serum metabolites (1.0-, 0.5-, and 0.25-fold serum concentration), and a blank serum specimen with CYP 450 recombinant BACULOSOMES, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase in a 96-well black plate at room temperature for 20 min, a specific CYP 3A substrate Vivid BOMR or CSP 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, the fluorescence was measured with excitation at 530 nm and emission at 590 nm, and CSP was quantitated using LC−MS/MS described above.

Statistical Analysis. Pharmacokinetic parameters were calculated by the non-compartment model of WinNonlin (version 1.1 SCI software, Statistical Consulting, Inc., Apex, NC). The peak blood concentrations (C_{max}) were calculated on the basis of experimental measurement. The areas under the curves (AUC_{0-t}) from time zero to last were calculated by the trapezoidal rule. Pharmacokinetic parameters among various treatment groups were compared using one-way analysis of variance (ANOVA) with Scheffe's test. Data from the transport study and CYP 3A4 activity were statistically compared using unpaired Student's *t* test. The statistical significance level was set at $p < 0.05$.

RESULTS

Characterization of the Mulberry Extract and Its Serum Metabolites. Figure 2 shows the HPLC−MS/MS chromatogram of the mulberry extract, where rutin, quercetin, and 6,7-DMC (internal standard) were well-resolved within 5 min. However, other known polyphenol constituents, such as resveratrol and kaempferol, were not detected in the extract. Good linear relationships existed over the concentration ranges of 0.63−10.0 and 0.31−10.0 μg/mL for rutin (R^2 = 0.993) and quercetin (R^2 = 0.999), respectively. Validation of the analysis method showed that the coefficients of variation were below 9% and the relative errors were below 8% for intra- and interday assays. The limits of detection (LODs) of rutin and quercetin were 0.002 and 0.01 μg/mL, respectively. The contents of rutin and quercetin in the mulberry extract were 43.2 and 45.4 μg/mL, respectively; thus, a dose of 2 g per 2 mL/kg of mulberry contained 86.4 μg/kg of rutin and 90.8 μg/kg of quercetin.

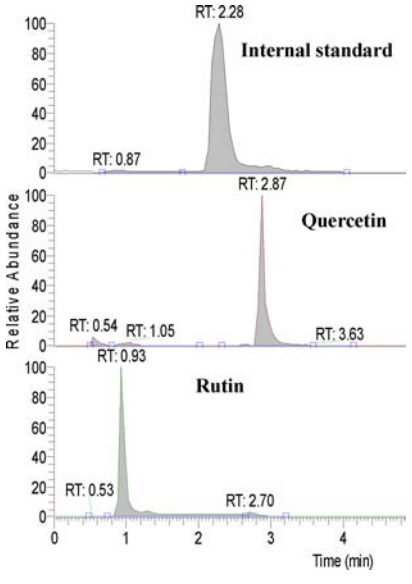

Figure 2. HPLC−MS/MS chromatogram of the mulberry extract: rutin, 6,7-DMC (internal standard), and quercetin.

Characterization of the serum metabolites of the mulberry extract indicated that the concentrations of quercetin glucuronides and quercetin sulfates were 0.7 and 0.9 μg/mL, respectively, whereas the parent form of quercetin was not detected.

Effect of Mulberry on CSP Pharmacokinetics in Rats. The blood CSP concentration−time profiles after oral administration of CSP alone and oral co-administrations with single or multiple doses of mulberry are shown in Figure 3. The

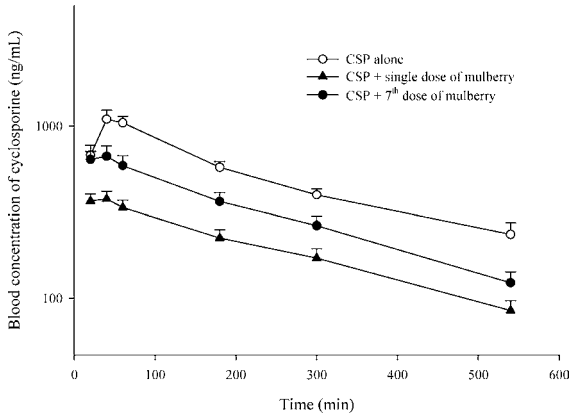

Figure 3. Mean [±standard error (SE)] blood concentration−time profiles of CSP after oral administration of CSP alone (○, 2.5 mg/kg) and co-administration with a single dose (▲) and the seventh dose (●) of mulberry (2 g/kg) to rats (n = 6 in each group).

pharmacokinetic parameters of CSP for various treatments are given in Table 1. The results showed that a single dose of mulberry significantly reduced the AUC_{0-t} and C_{max} of CSP by 53.2 and 65.8%, respectively. After pretreatment with seven doses of mulberry, the AUC_{0-t} and C_{max} of CSP were significantly decreased by 23.7 and 39.7%, respectively.

Effect of Mulberry on P-gp Activity. The LS 180 cell line was used for the transport assay of rhodamine 123 and CSP to clarify the involvement of P-gp in the interaction. The MTT assay showed that incubation of LS 180 with 60 mg/mL mulberry for 72 h exerted no significant influences on cell viability. The intracellular accumulation of rhodamine 123 in the presence and absence of mulberry is shown in Figure 4a. Mulberry at 60, 10, and 2.5 mg/mL significantly decreased the intracellular accumulation of rhodamine 123 by 54, 38, and 35%, respectively. On the contrary, verapamil (100 μM), a positive control of P-gp inhibitor, significantly increased the intracellular accumulation of rhodamine 123 by 32%. The

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Table 1. Mean (±SE) Pharmacokinetic Parameters of CSP in Rats (n = 6 in Each Group) after Giving CSP (2.5 mg/kg) Alone and Co-administration with a Single Dose and the Seventh Dose of Mulberry (2 g/kg) to Rats[a]

treatment parameter	CSP alone	CSP + mulberry single dose	CSP + mulberry seventh dose
C_{max}	1135.3 ± 123.5 a	387.8 ± 38.8 b (−65.8%)	684.0 ± 89.2 b (−39.7%)
$AUC_{0-\infty}$	227.5 ± 25.2 a	106.4 ± 12.0 b (−53.2%)	173.5 ± 22.9 b (−23.7%)
MRT_{0-540}	197.0 ± 5.42	201.1 ± 4.2	192.0 ± 4.7

[a]Data are expressed as the mean ± SE. C_{max} (ng mL^{-1}), peak blood concentration; AUC_{0-540} (μg min mL^{-1}), area under the blood concentration−time curve to 540 min; and MRT (min), mean residence time. Means in a given row without a common letter differ at $p < 0.05$. A mean with a symbol of "a" was significantly different from a mean with a symbol of "b".

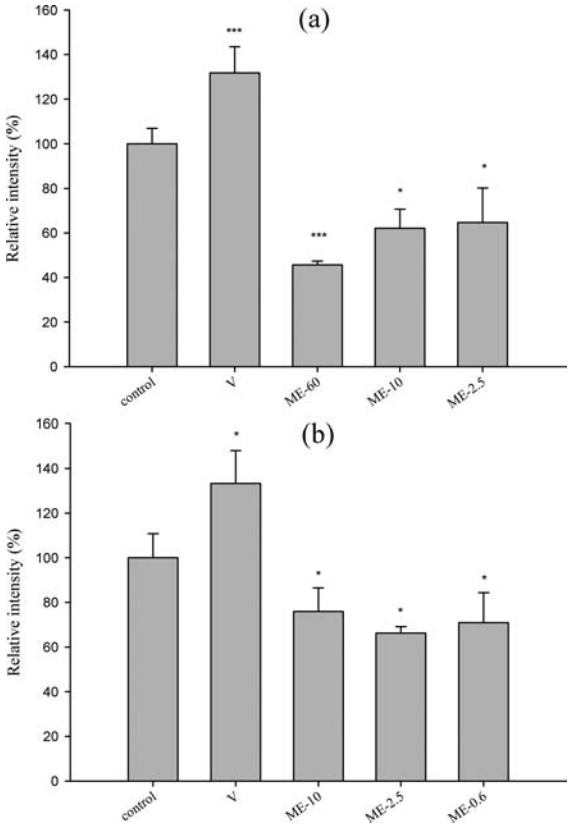

Figure 4. Effect of the mulberry extract (ME, mg/mL) on the intracellular accumulation of (a) rhodamine 123 and (b) CSP in LS 180 cells. V = verapamil (positive control of the P-gp inhibitor). (*) $p < 0.05$ and (***) $p < 0.001$.

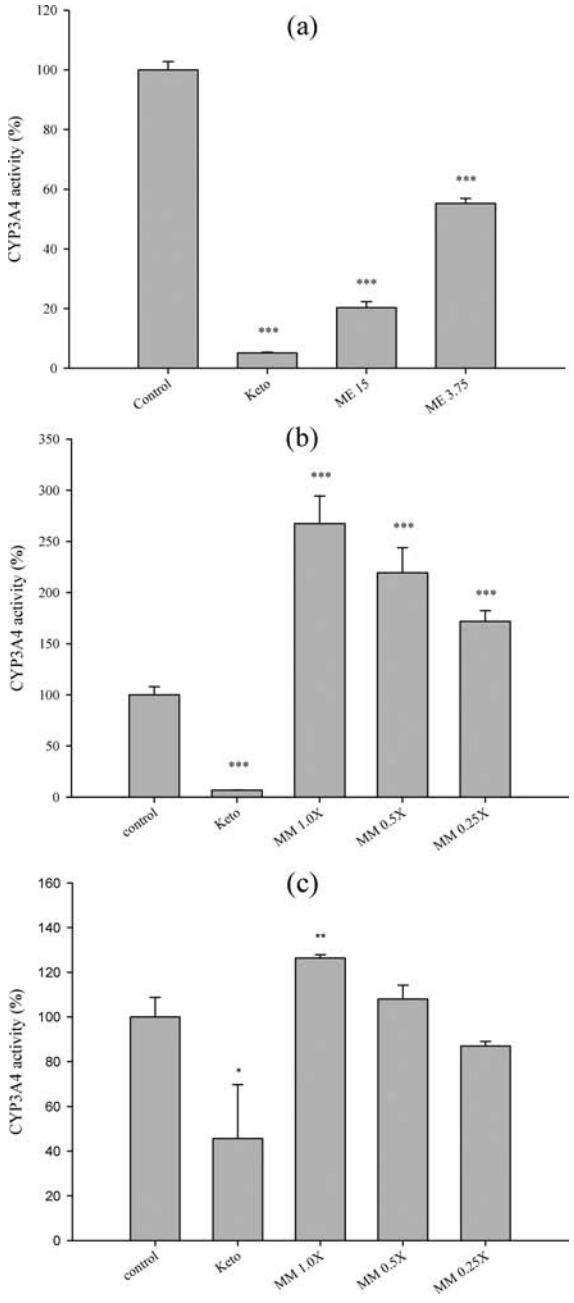

Figure 5. Effects of the mulberry extract (ME, mg/mL) and mulberry serum metabolites (MM, 1.0-, 0.5-, and 0.25-fold serum concentrations) on CYP 3A4 activity (a and b) using Vivid BOMR as the CYP 3A4 substrate and (c) using CSP as the CYP 3A4 substrate. Keto = ketoconazole (positive control of the CYP 3A4 inhibitor). (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

intracellular accumulations of CSP in the presence and absence of the mulberry extract are shown in Figure 4b. Mulberry at 10, 2.5, and 0.6 mg/mL significantly decreased the intracellular concentrations of CSP by 24, 34, and 29%, respectively. In contrast, verapamil (200 μM) significantly increased the intracellular concentration of CSP by 33%.

Effect of Mulberry on CYP 3A4 Activity. The effects of the mulberry extract and its serum metabolites on the activity of CYP 3A4 using Vivid BOMR as a CYP 3A4 substrate are shown in panels a and b of Figure 5, respectively. The mulberry extract at 15.0 and 3.8 mg/mL significantly decreased the CYP 3A4 activity by 80 and 46%, respectively. On the contrary, mulberry metabolites at 1.0-, 0.5-, and 0.25-fold serum concentrations significantly increased CYP 3A4 activity by 167, 119, and 72%, respectively, when compared to those of correspondent concentrations of the blank serum specimen. Moreover, when CSP was monitored as a CYP 3A4 substrate, Figure 5c showed that mulberry metabolites at 1.0-fold serum concentration significantly induced CYP 3A4 activity by 26% when compared to the blank serum specimen.

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DISCUSSION

Our pharmacokinetic study showed that single-dose co-administration of mulberry significantly decreased the peak blood concentration and the systemic exposure of CSP, indicating that the oral bioavailability of CSP was markedly reduced by mulberry. In addition, repeated dosing of mulberry also had significantly decreased the oral bioavailability of CSP but to a lesser extent. Observation on the decline slopes of three blood profiles in Figure 3 revealed that they appeared largely in parallel, implying that the elimination rate of CSP was not affected by single or repeated dosing of mulberry. Accordingly, we can infer that the alteration of CSP pharmacokinetics caused by mulberry should occur at the absorption site rather than the elimination phase. Therefore, it can be speculated that the absorption of CSP at the intestine was hampered by mulberry.

Intestinal P-gp and CYP 3A4 are known to play significant roles in the first-pass effect of CSP.[9] To explore the putative involvement of P-gp in this interaction, a transport assay of rhodamine 123, a fluorescent P-gp substrate, and CSP were conducted in LS 180 cells. As shown in Figure 4, contrary to verapamil (a positive control of the P-gp inhibitor), mulberry significantly decreased the intracellular accumulation of rhodamine 123 and CSP, suggesting that the function of P-gp was activated, which could in part explain the decreased absorption of CSP in rats.

Being a constituent in mulberry and a deglycosylated metabolite of rutin, quercetin has been reported as an inhibitor of P-gp when the breast and cervical cancer cell line models were used,[16,23] which apparently failed to explain our *in vivo* result. Conversely, a study demonstrated that quercetin was an activator of P-gp in the HCT-15 colon cell line model.[11] With regard to these opposite modulation effects of quercetin on P-gp activity among *in vitro* studies, we contemplate that this discrepancy might stem from different cell line models or discordant incubation protocols in use. Different cell lines might have variable metabolic capability and result in a differential amount of quercetin metabolites during incubation, which might exert an opposite effect on P-gp with the parent form of quercetin.[24]

Besides being a substrate of P-gp, CSP is a substrate of CYP 3A4.[10] The involvement of CYP 3A4 in this pharmacokinetic interaction was also investigated in this study. On the basis of increasing the understanding of the pharmacokinetics of polyphenols, we proposed that the serum metabolite of mulberry could mimic the molecules interacting with enteric or hepatic CYP 3A located in the microsomes of cells in rats. Therefore, we had prepared the serum metabolite of mulberry from rats to evaluate the effect of mulberry on the activity of CYP 3A4. As shown in Figure 5b, the serum metabolites of mulberry considerably increased the activity of CYP 3A4 compared to the blank serum specimen, implying that the activation of CYP 3A can in part explain the decreased absorption of CSP caused by mulberry. For the purpose of comparison, the effect of the mulberry extract on the activity of CYP 3A4 was also investigated and shown in Figure 5a. The result revealing that the mulberry extract markedly decreased the activity of CYP 3A4 indicated that the mulberry extract exerted opposite effects with its serum metabolites, which were the virtual molecules interacting with CYP 3A4. Moreover, when CSP was monitored as a substrate of CYP 3A4, the same inductive effect was further confirmed. The inductive effect of mulberry serum metabolites on CSP metabolism appeared weaker than that on Vivid BOMR metabolism, which might be due to higher oxidation reactivity of Vivid BOMR than CSP. Taken together, mulberry may not only enhance the function of P-gp but also induce the activity of CYP 3A, which additively resulted in decreased absorption of CSP.

In our previous studies, we have found that co-administration of polyphenols, such as quercetin and rutin, resulted in a marked decrease of CSP absorption in rats.[17,18] With regard to the underlying mechanism of quercetin−CSP and rutin−CSP interactions, activation of both P-gp and CYP 3A has been identified.[18] These findings suggest that rutin and quercetin may contribute to the mulberry−CSP interaction. Despite the low concentrations of quercetin and rutin in the mulberry extract, the estimated concentration of quercetin in gut lumen was well above the effective concentration (10 μM) reported to activate the function of P-gp.[18]

Repeated dosing of mulberry also significantly lowered the oral bioavailability of CSP, but the magnitude of the interaction seemed weaker than that caused by a single dose, although a significant difference was not reached. It has been reported that quercetin was capable of inhibiting the upregulation of P-gp mRNA and brought about suppression of P-gp protein in multidrug-resistant cell lines,[23,25] which led us to speculate that the expression of P-gp protein may be decreased after repeated dosing of mulberry. Given that the activity of P-gp was induced by a single dose of mulberry, whereas the expression of P-gp protein was suppressed by repeated dosing of mulberry, the effect caused by acute activation of P-gp might be canceled out to some extent by that arisen from inhibition on P-gp expression, which could account for the lower magnitude of inhibition on CSP absorption following repeated dosing of mulberry.

In clinical practice, therapeutic drug monitoring is usually based on the blood level of CSP. Some events of the subtherapeutic blood CSP level, which led to acute rejections of nerve, kidney, and liver in transplant patients, have been reported because patients combined the use of CSP with P-gp or CYP 3A4 inducers, such as St. John's wort.[26] The results of this study indicating mulberry decreased the absorption of CSP would certainly shed light on the way to prevent the occurrence of unexpected allograft rejection. We would further suggest that the concurrent intake of mulberry should be avoided for patients treated with critical medicines that are substrates of P-gp and/or CYP 3A4. In conclusion, mulberry significantly reduced CSP absorption through activating the functions of P-gp and CYP 3A.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AUC, area under the curve of concentration; C_{max}, maximum blood concentration; CSP, cyclosporine; CYP, cytochrome P450; P-gp, P-glycoprotein

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