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Research Paper

The Efflux of Flavonoids Morin, Isorhamnetin-3-*O*-Rutinoside and Diosmetin-7-*O*-β-D-Xylopyranosyl-(1-6)-β-D-Glucopyranoside in the Human Intestinal Cell Line Caco-2

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Purpose. In this study, we chose three of the flavonoids isorhamnetin-3-*O*-rutinoside(IRR) diosmetin-7-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside(DXG) and morin, which showed obvious efflux, to test the hypothesis that a specific efflux transporter is responsible for their transportation.
Methods. The intestinal epithelial membrane transport of the flavonoids were examined using the monolayer of the human Caco-2 cell line grown in Transwells, a common model of intestinal absorption. The flavonoids were measured by high performance liquid chromatography with UV detector.
Result. The efflux of morin, IRR and DXG, across Caco-2 cell monolayers was examined over the concentration range from 2 to 200 μM and showed a saturable process. The depletion of the cellular ATP stores with 5 mM iodoacetamide led to a significant inhibition of the efflux. Fifty micromolar verapamil, a chemical inhibitor of *P*-glycoprotein, had no effect on the transport of the three flavonoids, while the presence of 50 μM MK-571 and 1 mM probenecid, MRP inhibitors, resulted in an obvious reduction in the efflux. Moreover, inhibition of morin transport by MK-571 demonstrated concentration dependence. The transportation of the three flavonoids was compared with apigenin.
Conclusion. These data support a role for MRPs in the intestinal transcellular efflux of morin, IRR, DXG and possibly other hydrophilic flavonoid aglycons and glycosides.

KEY WORDS: caco-2 cells; flavonoid; isorhamnetin-3-*O*-rutinoside; morin; transport.

INTRODUCTION

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. It has been reported that flavonoids exhibit a wide range of biological activities (1), including antibacterial, antiviral (2), anti-inflammatory, antiallergic (1–4), and vasodilatory (5) actions. In addition, flavonoids inhibit lipid peroxidation (LPO) (6,7), platelet aggregation (8–13), capillary permeability, and fragility (14,15), and the activity of enzyme systems including cyclooxygenase and lipoxygenase (13,16). These effects can be traced up to their activity as antioxidant (2,17–19) and metal chelator (20). To be able to determine the level of intake that will maximize the health-promoting effects of this class of compounds, some knowledge in the bioavailability of flavonoids is required.

We have used the human Caco-2 cell model of intestinal absorption (21,22) in an attempt to better understand the extent of and the mechanisms governing flavonoid absorption. With this model, we have previously determined (data not published) the P_{app} values of 33 flavonoids and found that flavonoids pass through the Caco-2 cell monolayer mainly by passive diffusion, while morin and a few glycosides display basolateral to apical efflux. In this study, we chose three of the flavonoids morin, isorhamnetin-3-*O*-rutinoside (IRR) and diosmetin-7-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside (DXG) (Fig. 1), which show efflux most obviously, to test if there is efflux mechanism in the transportation of these flavonoids across the Caco-2 cell monolayer and what the efflux pumps would be. Morin (23–27) is an effective antioxidant and can reduce Lipid peroxidation. It has an antihypertensive effect and also can reduce the incidence of various chemical-induced cancers. IRR and DXG are the glycoside of quercetin and diosmetin the common flavonoid aglycon.

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ABBREVIATIONS: DMEM, Dulbecco's Mocified Eagle's Medium; DXG, diosmetin-7-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside; EC, (−)-epicatechinHBSS, Hank's Balanced Salts Solution; HPLC, high performance liquid chromatography; IRR, isorhamnetin-3-*O*-rutinoside; OATsorganic anion transporters; P_{app}, apparent permeability coefficient; Teer, transepithelial electrical resistance.

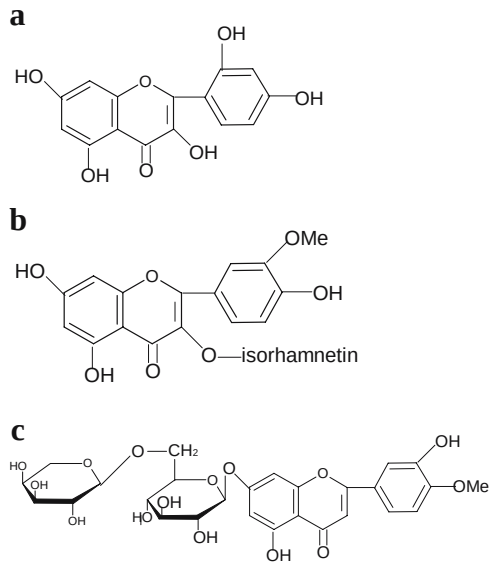

Fig. 1. Chemical structures of the flavonoids (a) morin; (b) IRR; (c) DXG.

MATERIALS AND METHODS

Materials

Hanks' Balanced Salts Solution (HBSS) and other culture media and supplements were obtained from Gibco (Grand Island, NY). Fetal calf serum was from Hyclone (USA). The flavonoids were isolated from corresponding herbs and characterized in our labs. MK571 was obtained from Calbiochem (an Affiliate of Merk KGaA, Darmstadt, Germany); Verapamil, vinblastine, probenecid, iodoacetamide were purchased from Sigma Chemical Co (Deisenhofen, Germany). Other chemicals were of analytical grade. The human intestinal cell line Caco-2 (HTCC No. HTB-37) was obtained from Cell Culture Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. Transwells were purchased from Corning Costar (Cambridge, MA, USA).

Cell Culture

Caco-2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 $g \cdot l^{-1}$D-glucose, 3.7 $g \cdot l^{-1}$ $NaHCO_3$, supplemented with 10% fetal calf serum, 1% non-essential amino acids, 100 U ml^{-1} penicillin and 100 $\mu g \cdot ml^{-1}$ streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. Caco-2 cells were seeded at a density of about 1×10^5 cells/cm^2 on a Transwell insert filter (surface area = 1.1 cm^2, pore size = 3 μm; Corning Costar Co., Cambridge, MA, USA). Cells were left to grow for 21 days to reach confluence and differentiation. At this time, Caco-2 cells were fully differentiated and used for flux experiments.

Standardized Conditions

The integrity of the monolayer were checked by measuring the transepithelial electrical resistance (TEER) with an epithelial voltohmmeter (EVOM, World Precision Instrument, Sarasota, FL) equipped with a chopstick. The measurements were performed before and after transport experiments. The TEER values >500 $\Omega \cdot cm^2$ were required. All cells used in this study were between passages 46 and 61.

Transport Studies

The following experiment were undertaken to measure the flux of the flavonoids. Flux describes the movement of a substance across polarized Caco-2 monolayers either in absorptive (apical→basolateral) or in secretory direction (basolateral→apical). The monolayers were washed twice for 30 min with warm transport medium HBSS (Hanks' Balanced Salt Solution containing 25 mM HEPEs), pH7.4. The flavonoids stock solutions of 10 mM in dimethylsulfoxide (DMSO) were diluted to 200, 100, 50, 25, 20, 15, 10, 5, and 2 μM (the concentration of DMSO were 2~0.5%) with the transport buffer. Transport medium containing flavonoids was added to the apical side (0.5 ml) or basolateral (1.5 ml) side of the inserts, while the receiving chamber contained the corresponding volume of transport medium. After shaking at 55 rpm for 1-h at 37°C in a shaking water bath, samples were collected from both sides of the cell monolayer and immediately frozen, lyophilized and preserved below −20°C before analysis by HPLC.

Transport in the Presence of Iodoacetamide

In order to deplete cellular ATP stores during the transport experiments and thus to inhibit ATP-dependent transport the alkylating reagent, iodoacetamide was applied. A stock solution was prepared in DMSO and added to the transport medium HBSS, which was given to both the apical and the basolateral chambers of the Transwell-system to achieve a final concentration of 5 mM and the flavonoids (50 μM; the concentration of DMSO were 0.5%) was added to one side. Transport experiments were undertaken as described above.

Inhibition of Transport

These experiments were carried out as described above in transport studies with some modification. MK-571 and probenecid were used at 50 μM and 1 mM, respectively, as selective inhibitors of MRP1/MRP2 (28,29), while verapamil was used at 50 μM as selective inhibitor of *P*-glycoprotein (30,31). The cells were pre-incubated with the inhibitors for 30 min. After the pre-incubation, the inhibitors were added to both sides of the membrane and the flavonoids (50 μM; the concentration of DMSO were 0.5%) were added to one side. Flavonoids transport experiments were then undertaken as described above.

HPLC Analysis of Samples

To determine the corresponding flavonoinds, the lyophilized samples were dissolved in 200 μl methanol, centrifuged at 16000 rpm for 10 min. Aliquot of 20 μl of the resulting supernatant solution was used for assay by a reversed-phase HPLC system (Diamonsil C_{18} column, 250 × 4.6 mm I.D.,

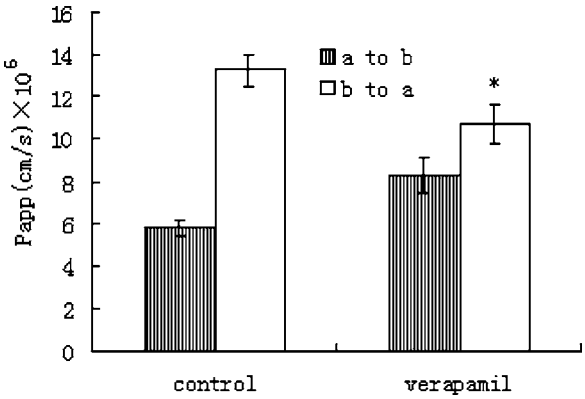

Fig. 2. 20 μM vinblastin transport (60 min) in Caco-2 cells in the absence (control) or presence of 50 μM verapamil in pH 7.4 HBSS. a to b: transport of vinblastin from apical to basolateral; b to a: transport of vinblastin from basolateral to apical. Presented values are means ± SD (n = 3 – 6). *P < 0.05 lower than control.

5 μM). Elute peak areas were monitored with an UV detector at the highest absorption of corresponding flavonoids among 200 to 400 nm (morin: 350 nm; IRR: 325 nm; DXG: 339 nm; apigenin:338 nm). The mobile phase consisted of A (methanol) / B (0.15% potassium dihydrogen phosphate + 0.01 mM phosphoric acid) (80/20~38/62). Flow rate was 1 ml·min^{-1}. Quantification was carried out by peak area measurements in comparison with standard curves for each corresponding flavonoids.

RESULTS

Validation of the Caco-2 Cell System

To monitor the Caco-2 cell system, the polarized transport of a well-known ABC-transporter compound, vinblastine (32), and the inhibition of its directed transepithelial flux by verapamil (50 μM) was measured. Vinblastine was transported from the basolateral chamber to the apical chamber with P_{app} value of 1.32 ± 0.07 × 10^{-5} cm/s, whereas the flux from apical to the basolateral chamber was 5.82 ± 0.4 × 10^{-6} cm/s. Verapamil (50 μM) inhibited the transport from the basolateral chamber to the apical chamber with P_{app} value of 1.07 ± 0.09×10^{-5} cm/s, whereas the flux from apical to the basolateral chamber was 8.26 ± 0.86 × 10^{-6} cm/s (Fig. 2).

Transcelllular Efflux of Flavonoids

The efflux of the three flavonoids, morin, IRR and DXG, across Caco-2 cell monolayers was examined for concentration dependence. Transport of the three flavonoids were in the

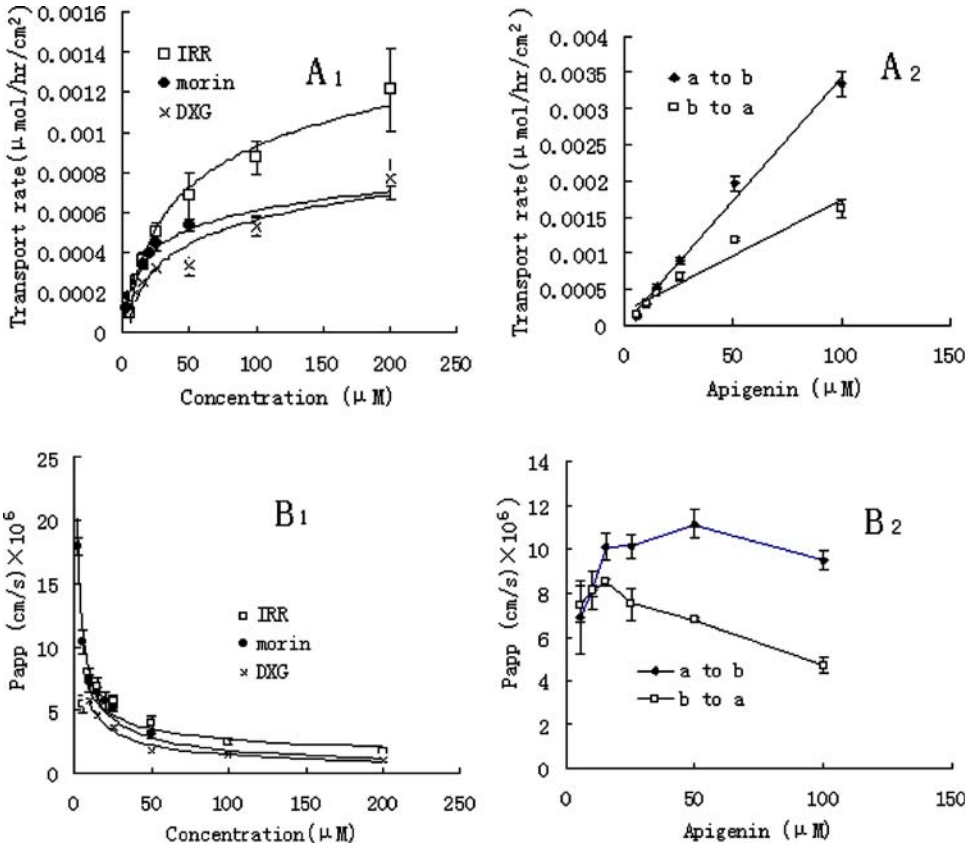

Fig. 3. Concentration-dependent transport of IRR, morin, DXG and apigenin. Samples were collected for analysis after 1 h. (A) Transport rate of the four flavonoids. (B) The apparent permeability coefficient (P_{app}) of the four flavonoids. (A_1), (B_1): transport of IRR, morin and DXG from basolateral to apical; (A_2), (B_2): transport of apigenin. a to b: transport of apigenin from apical to basolateral (r^2 = 0.99); b to a: transport of apigenin from basolateral to apical (r^2 = 0.94). Presented values are means ± SD (n = 3 – 6).

basolateral-to-apical direction over the concentration range from 2 to 200 μM. It can be seen that the rates of membrane permeation of the flavonoids increased linearly with increasing concentration of flavonoids up to 25 μM followed by a more gradual further increase, approaching a plateau (Fig. $3A_1$). The apparent permeability coefficient (P_{app}) decreased significantly with increased concentration over the range examined, also consistent with a saturable secretory mechanism (Fig. $3B_1$). The solubility of the three flavonoids was determined to be >200 μM and, thus, did not influence the results shown in Fig. 3. The rate of membrane permeation of apigenin, which is known in our previous study to be transported across the cell monolayer by passive diffusion, increased almost linearly with the concentration up to an upper range of the concentration test (100 μM) (the solubility of apigenin was below 150 μM) (Fig. $3A_2$), while the P_{app} value was found to be concentration independent (Fig. $3B_2$).

Inhibition of the Active Transport by ATP Depletion

The depletion of the cellular ATP stores led to a significant inhibition of efflux (Fig. 4a–c). A flavonoid concentration of 50 μM (the concentration of DMSO were 0.5%) was applied to either the apical or the basolateral chambers and treated with iodoacetamide at the same time in both side for 1 hr. The P_{app} values from basolateral to apical were decreased dramatically compared with that of the control cells (Fig. 4a–c) and the reduction in apparent permeability were $3.48 \pm 0.35\times10^{-6}$ cm/s, $3.23 \pm 0.03\times10^{-6}$ cm/s, $0.61 \pm 0.02\times10^{-6}$ cm/s, for morin, IRR and DXG, respectively, in the presence of 5 mM iodoacetamide.

The P_{app} values of apigenin from basolateral to apical in the presence of iodoacetamide was same as that of the control cells (Fig. 4d).

Transport Inhibition by Verapamil, Probenecid, MK571

The chemical inhibition of the ABC-transport protein, *P*-glycoprotein with 50 μM verapamil had no effect on the transport of the three flavonoids (Fig. 4a–c) Under these experiment conditions, it appears that this transport protein does not take part in the polarized net transport.

The presence of 50 μM MK-571, a MRP inhibitor, resulted in an obvious reduction in apparent permeability (Fig. 4a–c) of morin, IRR, DXG and the presence of 1 mM probenecid, a MRP1 and OATs (organic anion transporters) inhibitor brought about decrease of P_{app} values in morin and IRR during transportation (Fig. 4a, b). This finding in combination with the detection of MRP1 and MRP2 (33,34) in Caco-2 cells implicated a role for MRP1and MRP2 in the transcellular efflux of the three flavonoids. The P_{app} values of apigenin transport from basolateral to apical in the presence MK-571 was same as that of the control. (Fig. 4d).

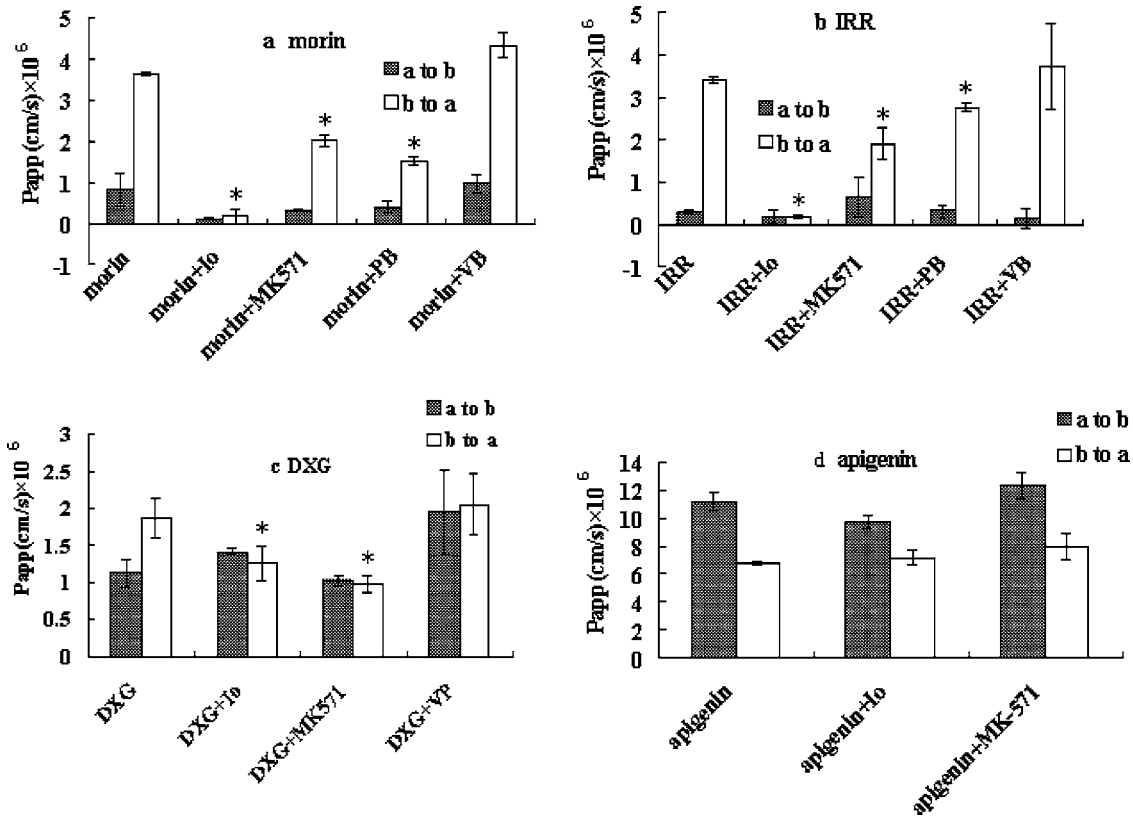

Fig. 4. Transport of flavonoids in Caco-2 in the presence of transport inhibitors. (a) 50 μM morin; (b) 50 μM isorhamnetin-3-*O*-rutinoside (*IRR*); (c) 50 μM diosmetin-7-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside (*DXG*); (d) 50 μM apigenin. a to b: transport of the flavonoids from apical to basolateral; b to a: transport of the flavonoids from basolateral to apical. *Io*: 5 mM iodoacetamide; *MK-571*: 50 μM MK-571; *PB*: 1 mM probenecid; *VP*: 50 μM verapamil. Presented values are means ± SD ($n = 3 – 6$). *$P < 0.05$ lower than control.

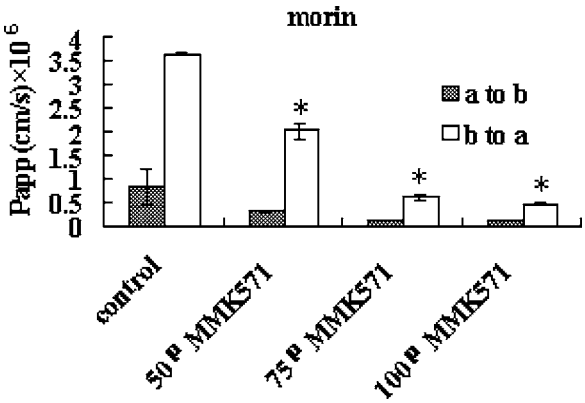

Fig. 5. Concentration-dependent inhibition of morin transport by MK-571. a to b: transport of morin from apical to basolateral; b to a transport of morin from basolateral to apical. Presented values are means ± SD (n = 3–6). *P < 0.05 lower than control.

To determine the potential role of MRP in the transport of the flavonoids, different concentration of the MRP transport inhibitor MK-571 were used. As shown in Fig. 5, inhibition of morin transported by MK-571 demonstrated concentration dependence. With 100 μM MK-571 the inhibition was 87% compared with control.

DISCUSSION

It is essential to know the bioavailability of flavonoids when attempting to extrapolate from *in vivo* to the *in vitro* situation. We have determined the permeability of 33 different flavonoids (data not published) using the human Caco-2 cell monolayers, a well-accepted model of human intestinal absorption (21,35). Most Flavonoids showed very similar P_{app} values in both directions, either from the apical chambers to the basolateral side (a to b) or from the basolateral chambers to the apical side (b to a). For these flavonoids, a passive diffusion seems to be a reasonable explanation. While incubation of Caco-2 cells with the more hydrophilic morin and a few glycosides resulted in an apical-directed transport towards the upper chambers of the Transwell-system, they are effluxed. With respect to Caco-2 cell polarization, the net transportation fluxes were directed to the luminal side; simple diffusion would not result in a detectable polarization.

In the present experiments, morin, isorhamnetin-3-*O*-rutinoside (IRR) and diosmetin-7-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside (DXG) exhibiting obvious efflux were studied for their transportation mechanism. It can be seen that the rates of membrane permeation of the three flavonoids in the basolateral-to-apical direction increased linearly with increasing concentration of the flavonoids up to 25 μM followed by a gradual further increase, approaching plateau (Fig. 3A_1). The apparent permeability coefficient (P_{app}) decreased significantly with increased concentration over the range examined (Fig. 3B_1). Saturation was observed at the concentration range from 2 to 200 μM, which is consistent with a saturable secretory mechanism (Fig. 3A_1, B_1). For comparison, a lipophilic flavonoid apigenin was tested for caco-2 monolayer permeation. The apical-to-basolateral permeability of apigenin is larger than that in the reversed direction. The transportation rate in apical-to-basal direction increased linearly with the concentration (Fig. 3 $A_2 r^2$ = 0.99). Although a curved concentration dependency was observed for the velocity-concentration plot of the basal-to-apical transport of apigenin, the permeation rate chiefly bared a linearly correlation (r^2 = 0.94) with the concentration. In addition, the P_{app} value of apigenin was found not to be concentration dependent (Fig. 3B_2) and inhibition of active transportation by iodoacetamide or MK-571 did not result in significant changes in permeability (Fig. 4d). These results suggest that apigenin essentially pass through the caco-2 monolayer by passive diffusion, however, there might also be efflux mechanisms for apigenin, which could be concealed by the high degree of passive diffusion and need further investigation. Nevertheless, the results suggest that an efflux pump localized on the apical membrane, with a high specificity for morin, IRR and DXG compared with apigenin, must be responsible for these observations.

Caco-2 cells express ABC-transport proteins, which work as multispecific efflux pumps in similar manner as in the *in vivo* situation (34,36–39). The validation of our cell model showed that vinblastine had been transported to the apical chamber with comparable transportation flux rates as previously reported by Hunter *et al.* (32). Fifty micromolar of verapamil inhibited the transport of vinblastine significantly and this is also similar to previous studies using Caco-2 cells (32) (Fig. 2).

Bartels *et al.* (40) used the alkylating reagent iodoacetamide to show the energy dependence of the polarized transportation rates of daunomycin, a *P*-glycoprotein substrate, in Caco-2 cells. When monolayers were treated with 5 mM iodoacetamide (to inhibit energy metabolism and deplete cellular ATP stores), the basolateral to apical fluxes of daunomycin were decreased to the same rates as the flux to the opposite direction. The result was a complete inhibition of an apical-directed net secretion, revealing that this flux was energy dependent. In the present work, a comparable effect has been found in transport studies with morin, IRR and DXG. It is worthwhile to note that we used a near saturation concentration of substrates in the studies in order to maximize the inhibitory effects of inhibitors, however, the contribution of transporters herein and in discussion below may be underestimated due to increased diffusive permeation. Nonetheless, it was observed that iodoacetamide led to a depeletion of ATP in the enterocytes as well as to a clear inhibition of the apically directed transportation of the three compounds (Fig. 4). Therefore, the apically directed transport of the three compounds was energy dependent.

Caco-2 cells express at least three typical members of the ABC-transport protein familily: *P*-glycoprotein, MRP1 and 2 (34,36,38,41) and have been used extensively with the Transwell-system to study the effects of these specific proteins (36,38). For example, different *P*-glycoprotein substrates such as vinblastine sulfate (32) and daunomycin (40) showed apically directed net flux rates. In similar studies, the calcium channel blocker verapamil inhibited the transport of these substrates to the luminal side because it is a specific inhibitor of *P*-glycoprotein (42). As shown in Fig. 4, the incubation of Caco-2 cells with 50 μM verapamil did not have any effect on the polarized transportation of the morin, IRR and DXG,

although it inhibited the transport of the *P*-glycoprotein substrate, vinblastine. This suggests that under these experimental conditions *P*-glycoprotein may not be involved in the net transportation rates described so far.

Previous studies have also shown the presence of MRP in the Caco-2 cell lines. Versantvoort *et al.* (43) reported that the efflux of the organic anion calcein, a substrate for MRP-mediated transport, from Caco-2 cells was approximately four-fold higher to the apical than to the basolateral compartment and that the apical efflux was susceptible for inhibition with probenecid, an inhibitor of MRP-mediated transport. They demonstrated the presence of MRP-mediated efflux system(s) in the apical and basolateral membranes of intestinal epithelial cells. Vaidyanathan and Walle reported (44) that they studied the intestinal epithelial membrane transport of (−)-epicatechin (EC), an important flavonoid, using the monolayer of the human Caco-2 cell line grown in transwells and they found there was a 50% reduction in the efflux of EC in the presence of 50 μM MK-571. Most important, the presence of 50 μM MK-571 resulted in clearly measurable apical to basolateral absorptiot of EC. They inferred that MRP2 transporter expressed in the apical membrane of Caco-2 cells was responsible for EC efflux. In Walle's lab (45) they have shown evidence that genistin, a flavonoid glucoside, is a substrate for MRP2. Walgren *et al.* (38) observed the efflux of quercetin 4′-β-glucoside, another flavoniod glucoside, across Caco-2, a saturable process, was competitively inhibited by MK-571 and the MRP2 but MRP1 localized to the apical membrane by immunofluorescent localization. They suggested a role for MRP2 in the intestional transeclular efflux of quercetin 4′-β-glucoside. In this study the efflux of the three flavonoids, morin, IRR and DXG were competitively inhibited by MK-571 and the efflux of morin and IRR were inhibited by probenecid. Moreover, MK-571 showed a dose-dependent effect to inhibit the morin efflux (Fig. 5). This observation in combination with the identification of MRP in Caco-2 cell monolayers (38) supports a role for MRP in the efflux of the morin, IRR and DXG across the apical menbranes of enterocytes. Since permeability for the three flavinoids tends to be low and it is very hard to monitor the time process of transport. All these conclusions are based on that there weren't time lag before the appearance of flavonoid to the opposite side of monolayers.

The spectrum of agents recognized as substrates by MRP1 and MRP2 is similar, consisting primarily of organic anions, and is principally composed of glutatione and glucuronic acid conjugates of lipophilic compounds (46). It is therefore interesting that these neutral uncharged flavonoids are substrate for MRP and it suggests that a large new class of agents may be substrates for MRP-mediated transport. In our previous study we have found that the hydrophilic flavonoid aglycon taxifolin, eriodictyol and flavonoid glycoside isovitexin baicalin, luteolin-7-*O*-glucoside, diosmetin-7-*O*-β-glucopyranoside, isoquecitrin, quercitrin, daidzin all showed efflux in varying degrees. These flavonoids may also be substrates of MRP. Why so much neutral flavonoids are substrates of MRP requires further studies and it may be important to further understand the bioavailability and bioactivity of flavonoids *in vivo* and to recognize the substrate spectrum of MRP.

It was reported that morin inhibited *P*-gp mediated cellular efflux (47) as well as MRP1-mediated drug transport (48). Although in both cases, direct binding of morin to the efflux transporters are suggested, the differences and mechanisms of actions of morin on two proteins are not clear. The present study may provide some clues for this question. Since morin is shown to exhibit obvious efflux affected by MRP1 inhibitors, it is quite possible that morin may be an inhibitory substrate of MRP1, thus decrease the efflux by competitively binding with the MRP1 substrates site; on the other hand, morin could only be a non-competitive inhibitor for *P*-gp. Nevertheless, further investigations should be carried out to clarify the mechanisms by which morin inhibits the efflux transporters.

Mizuno *et al.* (49) classified the effect of *P*-gp inhibitors on the trasncellular transport in Caco-2 cells into three categories based on the kinetic consideration. In the first type, the basal-to-apical flux scarcely changes and the apical-to-basal flux increases markedly in the presence of a *P*-gp inhibitor. In the second case, fluxes from both directions are changed but the degree of change in the apical-to-basal flux is greater than that in the basal-to-apical flux in the presence of a *P*-gp inhibitor. In the third case, both fluxes are changed but the degree of change in the basal-to-apical flux is greater than that in the apical-to-basal flux in the presence of a *P*-gp inhibitor. The present results could be attributed into the third case, both fluxes are changed but the degree of change in the basal-to-apical flux is greater than that in the apical-to-basal flux in the presence of a MRP inhibitor.

Inhibition of apical efflux could increase the apical-to-basal transport. The apical-to-basal transport of the flavonoids was not affected by the ATP-depletion and other inhibitors. The reason for this phenomenon might be: (a) to maintain the integrity of the cell monolayer, inhibitors used were at a safe concentrations, which may not be sufficient; (b) the efflux transporters on the apical membrane, besides MRP, may also play a role in the efflux of the flavonoids; (c) the inhibitors did not increase, rather decreased, apical-to-basal transport of flavonoids, suggesting that certain transporters might be involved in the uptake process which can be inhibited by MK-571. This possibility should be investigated in the future; (d) the inhibitors may change the mobility of the cytoplasmic membrane of the monolayer and thus effect the passive diffusion of the flavonoids.

Besides transport across the intestinal in intact form, many reports on flavonoid bioavailability often describe the formation of various cellular metabolites of flavonoids especially a number of dietary flavonoids. In our preliminary study, we found that apart from their stability in transport medium as well as during the sample disposition process, the total amount of morin and DXG did not changed obviously before and after the transport experiment across the Caco-2 cell monolayer, while the amount of IRR changed a little. *In vivo* metabolism of the three compounds may be another important role effect the bioavailability besides the absorption of the intact compounds. The product and the quantity of the metabolized forms should be tested further in order to clarify the importance of metabolition in flavonoids absorption.

In summary, this study demonstrates that MRP both limits the absorption of and mediates the efflux of morin,

isorhamnetin-3-*O*-rutinoside and diosmetin-7-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside across cell monolayer.

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