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The effect of quercetin phase II metabolism on its MRP1 and MRP2 inhibiting potential

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ABSTRACT

The present study characterises the effect of phase II metabolism, especially methylation and glucuronidation, of the model flavonoid quercetin on its capacity to inhibit human MRP1 and MRP2 activity in *Sf*9 inside-out vesicles. The results obtained reveal that 3′-*O*-methylation does not affect the MRP inhibitory potential of quercetin. However, 4′-*O*-methylation appeared to reduce the potential to inhibit both MRP1 and MRP2. In contrast, glucuronidation in general, and especially glucuronidation at the 7-hydroxylmoiety, resulting in 7-*O*-glucuronosyl quercetin, significantly increased the potential of quercetin to inhibit MRP1 and MRP2 mediated calcein transport with inhibition of MRP1 being generally more effective than that of MRP2. Overall, the results of this study reveal that the major phase II metabolites of quercetin are equally potent or even better inhibitors of human MRP1 and MRP2 than quercetin itself. This finding indicates that phase II metabolism of quercetin could enhance the potential use of quercetin- or flavonoids in general—as an inhibitor to overcome MRP-mediated multidrug resistance.

1. Introduction

Multidrug resistance may hamper the efficacy of cytostatic drugs in cancer treatment [1]. One of the mechanisms involved in cellular multidrug resistance is upregulation of efflux proteins like *p*-glycoprotein and members of the family of multidrug resistance proteins (MRPs) [2]. MRP1 (ABCC1) has a broad substrate specificity and among its substrates are glutathione S-conjugates, glucuronide conjugates, sulphate conjugates, anticancer drugs and organic anions [3–6]. MRP2, the major canalicular multispecific organic anion transporter, is closely related to MRP1 and also has a broad substrate specificity [2,7]. One strategy to overcome transporter-mediated multidrug resistance relies on the identification of compounds that can act as inhibitors of these transporters. Flavonoids are an example of promising agents to revert MRP-mediated multidrug resistance [8–13]. It has become clear that flavonoids are important modulators or substrates of transport proteins including Pgp, MRPs and BCRP [14–24]. However, these studies were merely all performed with the flavonoid aglycones under experimental conditions which do not allow evaluation of the effects of flavonoid metabolism, expected to occur to a significant extent *in vivo* [25], on their MRP1 and MRP2 inhibitory capacity. Phase II metabolism of flavonoids is a generally recognized determinant of their biological activities and may also influence their interaction with MRPs [25–28]. Naturally occurring flavonoids in plants are glycosylated [29], but may become deconjugated during passage across the small intestine [30] or by bacterial activity in the colon [31]. Studies on the bioavailability of quercetin revealed

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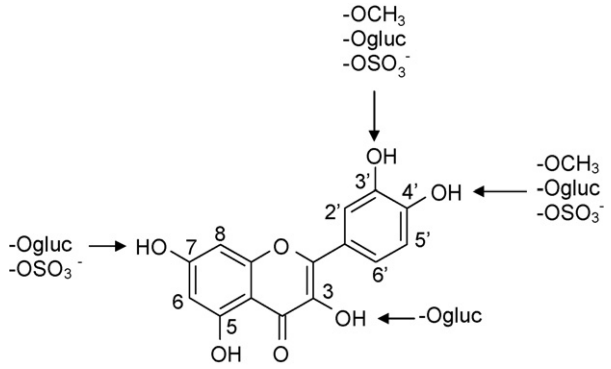

Fig. 1 – Structural formula of quercetin and the identified sites of conjugation [24].

the metabolism of quercetin to methylated, glucuronidated and/or sulphated conjugates [32–35]. Fig. 1 presents an overview of the type of phase II reactions reported to be relevant for the flavonoid quercetin and their regioselectivity. The plasma phase II metabolite pattern is likely to be the result of the interplay of different organs with metabolizing capacity, especially the liver and the small intestine [35].

The objective of the present study was to investigate the effect of phase II metabolism of the model flavonoid quercetin on its capacity to inhibit MRP1 and MRP2. To this end, the MRP1 and MRP2 inhibitory potency of several phase II quercetin metabolites and metabolite mixtures was studied using *Sf*9 inside-out vesicles. Quercetin was used as a model flavonoid since the phase II metabolism of this flavonoid is well described [32–35] and it is a known inhibitor of MRP1 and MRP2 [23]. For this study, both commercially available quercetin metabolites as well as characterised quercetin metabolite mixtures produced by incubation of quercetin with specific metabolising cell lines [35] were used. Together, the data reveal that phase II metabolism of quercetin results in powerful MRP1 and MRP2 inhibiting metabolites of quercetin.

2. Materials and methods

2.1. Materials

Quercetin was obtained from Acros organics (New Jersey, USA). 3′-*O*-Methylquercetin (isorhamnetin) and 4′-*O*-methylquercetin (tamarixetin) were purchased from extrasynthese (Genay Cedex, France). Rutin (quercetin-3-*O*-rhamnosylglucoside) and isoquercitrin (quercetin-3-*O*-β-glucoside) were obtained from Indofine (Somerville, USA). Dimethylsulfoxide (DMSO) was obtained from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Lab-Scan Ltd. (Dublin, Ireland). Fetal calf serum, Dulbecco's MEM, Dulbecco's MEM/F12 NutMix (HAM), fungizone, gentamycin and Hank's balanced salt solution (HBSS) were purchased from Gibco Ltd. Life Technologies (Paisley, UK). Calcein, adenosine-5′-triphosphate-disodium salt (ATP), adenosine 5′-monophosphate-sodium salt (AMP), creatine phosphate and DL-dithiothreitol (DTT) were obtained from Sigma (St. Louis, MO, USA). Creatine kinase was purchased from Roche (Almere, the Netherlands) and $MgCl_2$·hexahydrate from Merck (Darmstadt, Germany). Monoclonal antibodies to human MRP1 (MRPr1) and human MRP2 (M_2III-6) were obtained from Alexis Biochemicals (Kordia, Leiden, the Netherlands). *Sf*9 cells were obtained from Invitrogen (Groningen, the Netherlands). Recombinant baculoviruses containing either the human MRP1 cDNA or the human MRP2 cDNA were a kind gift from Prof. Dr. B. Sarkadi, National Institute of Haematology and Immunology, Research Group of the Hungarian Academy of Sciences, Budapest, Hungary.

2.2. Cell lines

The human cell line HT29 (colon carcinoma) and the rat cell line H4IIE (hepatocellular carcinoma) were purchased from the European collection of cell cultures (ECACC). Both cell lines were grown in 75 cm^2 plastic cell culture flasks in MEM-alpha medium supplemented with 10% fetal calf serum, 1% fungizone and 0.1% gentamicin.

2.3. Preparation of phase II metabolites of quercetin

For metabolism studies, HT29 and H4IIE cells were grown to confluency in cell culture flasks (75 cm^2). Before exposure, medium was removed and cells were washed with 10 mL HBSS. Then, 10 mL exposure medium was added to the cells consisting of Dulbecco's MEM/F12 NutMix (HAM) without phenol red, containing 15 mM HEPES, L-glutamine and pyridoxine which was supplemented with 100 μM quercetin from a 200 times concentrated stock solution in DMSO and with 1 mM ascorbic acid to prevent autoxidation of quercetin [35]. Control incubations were exposed to 0.5% DMSO in medium containing 1 mM ascorbic acid. Cells were exposed in duplicate and samples of the exposure medium were taken 24 h after starting the incubation. These samples were subsequently freeze dried and stored at −80 °C until analysis. After storage at −80 °C, the freeze-dried samples were resolved in Tris–sucrose (TS buffer: 10 mM Tris, 250 mM sucrose, pH 7.4) for the vesicle experiments. Prior to use in the vesicle transport experiments, HPLC analysis of the Tris–sucrose mixtures was performed essentially as described by Van der Woude et al. [35], to assure the quality and quantity of the metabolites in the samples.

2.4. HPLC-based identification and quantification of quercetin metabolites

To compare the quercetin phase II metabolite patterns made by the different cell lines, identification and quantification of the different metabolites was performed as previously described [35]. HPLC was performed on a waters M600 liquid chromatography system, using an Alltima C18 5U column (4.6 mm × 150 mm; Alltech, Breda, the Netherlands). Before injection, the incubation mixtures were centrifuged for 4 min at maximum speed. In a typical run, aliquots of 10 μL of the supernatant were injected. Samples were eluted at a flow of 1 mL/min with the following gradient: from 20% acetonitrile in nanopure water containing 0.1% trifluoroacetic acid, to 25% acetonitrile in 15 min, to 35% acetonitrile in 5 min, isocratic elution for 15 min at 35% acetonitrile, followed by an increase

to 80% acetonitrile in 2 min, keeping this percentage for 1 min, after which it was decreased to 0% acetonitrile in 1 min. This was kept for 1 min, after which the column was equilibrated at the initial conditions. Detection was performed between 220 and 445 nm using a waters 996 photodiode array detector. Chromatograms used are based on detection at 370 nm.

For commercially available standards calibration curves were made by plotting the peak area against the concentration. To quantify the amount of quercetin phase II metabolites produced by the cell lines, the peak area for the identified metabolites, was compared with the peak area–concentration curve obtained for the commercially available quercetin metabolites. The quercetin glucuronides were quantified using the rutin (quercetin-3-*O*-rhamnosylglucoside) calibration curve. The limit of detection of this HPLC method for quantification of quercetin and its metabolites was 0.1 μM (injection volume 10 μL).

2.5. *Expression of MRP1 and MRP2 in insect cells*

*Sf*9 insect cells were infected with recombinant baculoviruses containing either MRP1 cDNA or MRP2 cDNA as described previously [36]. Briefly, cells were cultured in spinner flasks in Grace's insect medium with 10% fetal calf serum and 10 μg/mL gentamycin at 27 °C. For infection, cells were cultured on 145 cm^2 culture disks and infected with a baculovirus (multiplicity of infection of 5) for 3 days essentially as described by Zaman et al. [37]. Virus-infected *Sf*9 cells were harvested and frozen at −80 °C until membrane preparation.

2.6. *Sf9 Vesicle preparation and immunoblotting*

Membranes from infected *Sf*9 cells were isolated as described previously [36]. Membrane protein concentrations were determined according to Bradford [38] adapted for 96-well measurements on a BioRad 3550 microplate reader. Vesicles were prepared by passing the suspension 30 times through a 26-gauge needle with a syringe. Aliquots of 25 μL membrane vesicles containing 1 mg protein/mL were quickly frozen in liquid nitrogen and stored at −80 °C until use. The expression of MRP1 and MRP2 in *Sf*9 membranes was assessed using immunoblotting with monoclonal antibodies MRPr1 and M_2III-6, raised against human MRP1 and human MRP2, respectively. The results obtained indicate an apparent M_r of 150 kDa in the *Sf*9 transfected cells [36]. This is in line with literature data describing that human MRPs are produced in an underglycosylated form in *Sf*9 cells which has been demonstrated not to affect their transport functions [39,40].

2.7. *Calcein uptake in Sf9 membrane vesicles*

Uptake of the fluorescent MRP1 and MRP2 substrate calcein in isolated *Sf*9 cell membrane vesicles was measured as follows [36]. Vesicles were rapidly thawed and pre-incubated for 1 min at 37 °C in Tris–sucrose buffer (TS buffer: 10 mM Tris, 250 mM sucrose, pH 7.4) containing 4 mM ATP 10 mM DTT, 10 mM $MgCl_2$, 10 mM creatine phosphate, 100 μg/mL creatine kinase and a concentration range of quercetin (0–50 μM), or 20 μM of quercetin or its metabolites: 3′-*O*-methylquercetin (isorhamnetin), 4′-*O*-methylquercetin (tamarixetin), rutin (quercetin-3-rutinose), isoquercitrin (quercetin-3-*O*-β-glucoside) or the metabolite mixtures produced by the HT29 or H4IIE cells. The reaction was started by addition of calcein to a final concentration of 40 μM. After 10 min incubation the reactions were stopped by adding 1 mL of ice-cold TS buffer. Then, the samples were rapidly filtered through pre-soaked nitrocellulose filters (0.45 μm pore size) (Schleicher and Schuell, Dassel, Germany) in a 1225 sampling manifold filtration unit (Millipore, Ettenleur, the Netherlands). Filters were rinsed with 10 mL TS buffer and the filters containing the vesicles were put in a 6 wells plate (corning) and 50 μL TS buffer was added on top of the filters. Fluorescence of the filters (excitation wavelength 485 nm, emission wavelength 530 nm) was measured using a cytofluor 2300 (Millipore, Ettenleur, the Netherlands). In control experiments, ATP was replaced by 4 mM AMP-PCP (α,β-methylene adenosine 5′-triphosphate) to measure background, ATP-independent, uptake of calcein. ATP-dependent calcein uptake was calculated by subtracting the values obtained in the presence of AMP-PCP from those in the presence of ATP.

2.8. *Data analysis*

Two-way analysis of variance test was used for all data analysis ($P < 0.05$) using SPSS 10.1.0 software from SPSS Inc.

3. Results

3.1. *MRP1 and MRP2 inhibition by quercetin*

Fig. 2 shows the effects of increasing concentrations of quercetin on MRP1 and MRP2 mediated calcein uptake by *Sf*9 vesicles. From the data presented it follows that 20 μM quercetin inhibits MRP1 mediated calcein uptake by 54 ± 6%. Thus, in comparison to 20 μM quercetin, both stronger and weaker inhibitory potencies of the quercetin metabolites can be detected. Therefore, 20 μM quercetin and/or its metabolites appeared the optimal concentration for studies on the consequences of quercetin phase II metabolism on its MRP1 inhibiting potential. Fig. 2 also shows that MRP2 is only mildly

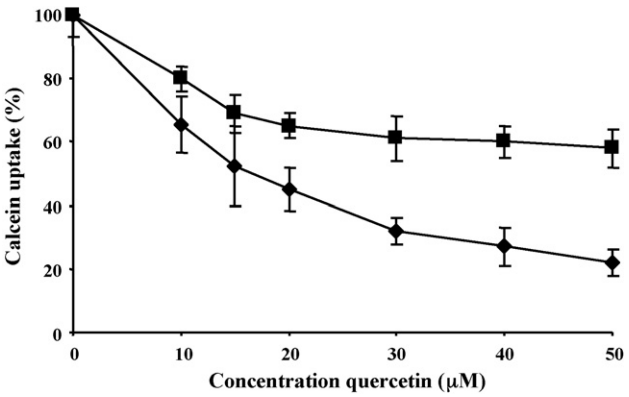

Fig. 2 – Concentration-dependent inhibition of MRP1 (◆) and MRP2 (■) mediated uptake of calcein by quercetin in inside out *Sf*9 vesicles. All values differ significantly from control (vehicle only) ($P < 0.05$).

Table 1 – Phase II metabolites synthesized by HT29 cells and H4IIE cells

Compound	Retention time (min)	Concentration (HM)
HT29 cells		
3-O-Glucuronosyl quercetin	7.4	26 ± 0.6
3-O-Glucuronosyl 3′-O-methylquercetin	12.3	3 ± 0.1
4′-O-Glucuronosyl quercetin	13.6	27 ± 0.2
3′-O-Glucuronosyl quercetin	15.3	5 ± 0.3
4′-O-Glucuronosyl 3′-O-methylquercetin	16.4	15 ± 0.4
3′-O-Glucuronosyl 4′-O-methylquercetin	17.8	3 ± 0.3
Quercetin	24.0	19 ± 0.3
3′-O-methylquercetin	30.7	2 ± 0.1
4′-O-Methylquercetin	31.2	1 ± 0.1
H4IIE cells		
7-O-Glucuronosyl quercetin	7.3	84 ± 6.5
3-O-Glucuronosyl 4′-O-methyl quercetin	13.9	10 ± 0.4
7-O-Glucuronosyl 4′-O-methylquercetin	15.1	2 ± 0.3
3′-O-Glucuronosyl quercetin	15.6	6 ± 0.9
Quercetin	24.0	1 ± 0.2

The cell lines were exposed to 100 μM quercetin in presence of 1 mM ascorbic acid for 24 h. The samples were analysed by HPLC. The percentage of the metabolites is based on the average concentration ± S.D. of two samples.

inhibited by quercetin. Even at 50 μM, quercetin does not inhibit MRP2 mediated calcein uptake by 50%. Nevertheless, to allow comparison to the MRP1 inhibition experiments MRP2 inhibition experiments were also performed at 20 μM quercetin or its metabolites. At this concentration of quercetin MRP2 was inhibited by 35 ± 8%.

3.2. *Formation of quercetin phase II metabolites by HT29 and H4IIE cells*

Table 1 presents the complete composition of the quercetin phase II metabolites synthesized by HT29 cells and H4IIE cells upon 24 h incubation. This table also summarizes the retention times of the peaks on HPLC as identified before [35] and used in the present study for their identification. The HT29 cells produced three major metabolites: 4′-O-glucuronosyl 3′-O-methylquercetin (15%), 3-O-glucuronosyl quercetin (26%) and 4′-O-glucuronosyl quercetin (27%). After 24 h incubation, approximately 19% of the native quercetin is still present in the exposure medium. The H4IIE cells produced one major metabolite: 7-O-glucuronosyl quercetin (84%) and a few other metabolites of which 3-O-glucuronosyl 4′-O-methyl quercetin (10%) is the most abundant one. After 24 h incubation only 1% of the native quercetin is still present in the exposure medium.

3.3. *Inhibition of MRP1 and MRP2 activity by quercetin and its metabolites*

Fig. 3A shows the effects of quercetin and its metabolites on MRP1-mediated uptake of the fluorescent substrate calcein by Sf9 vesicles. From the results presented it can be seen that 20 μM quercetin inhibits MRP1 activity by approximately 54 ± 13%. Of the metabolites tested 20 μM of the H4IIE synthetized quercetin metabolite mixture showed a significantly stronger inhibition (68 ± 4%, $P < 0.05$) compared to 20 μM quercetin. One metabolite tested, 4′-O-methylquercetin inhibited calcein uptake by MRP1 by 36 ± 6% which is significantly less than the MRP1 inhibition by quercetin ($P < 0.05$). Moreover, the quercetin-glycoside isoquercitrin did not inhibit MRP1-mediated calcein uptake at all whereas the other quercetin-glycoside tested, rutin, was a significant stronger inhibitor of MRP1 than quercetin ($P < 0.05$). The other

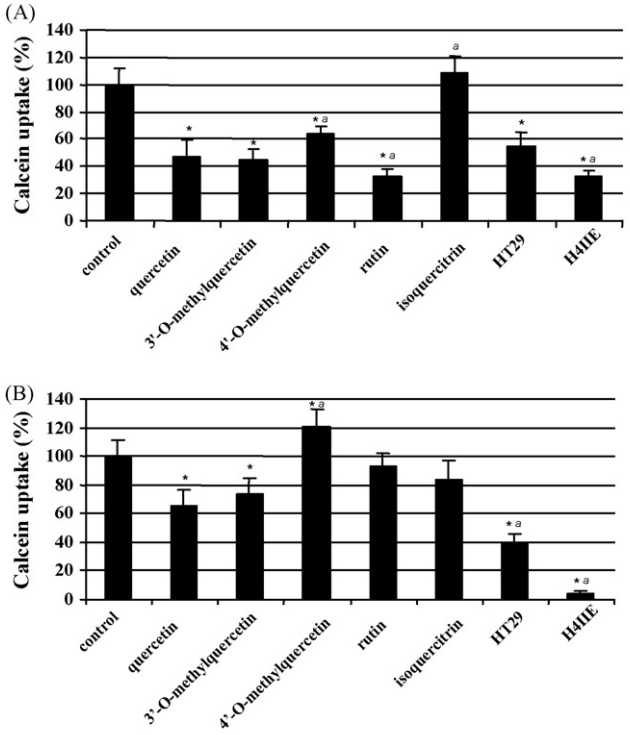

Fig. 3 – Calcein uptake by MRP1 (A) and MRP2 (B) upon exposure to vehicle only (control), quercetin and the metabolites or metabolite mixtures (all at 20 μM total final concentration). The metabolite mixtures produced by the HT29 and H4IIE cells are described in Table 1. The bars represent the mean ± S.D. ($n = 6$). Asterisk (*) represents values that differ significantly from control (vehicle only) ($P < 0.05$). Letter (a) represents values that differ significantly from 20 μM quercetin ($P < 0.05$).

metabolites tested showed MRP1 inhibitory potencies similar to 20 μM quercetin.

Fig. 3B shows the effects of quercetin and its metabolites on MRP2-mediated uptake of the fluorescent substrate calcein. The data presented reveal that 20 μM quercetin inhibits MRP2-mediated calcein uptake by approximately 35 ± 11%. Of the metabolites tested both the HT29 and H4IIE generated mixtures, containing 20 μM quercetin metabolites, inhibited MRP2 mediated calcein uptake by 61 ± 6% and 95 ± 2%, respectively, which is significantly stronger than the MRP2 inhibition by 20 μM quercetin ($P < 0.05$). Interestingly, the quercetin metabolite mixture formed by the H4IIE cells, containing especially (84%) 7-*O*-glucuronosyl quercetin showed almost complete inhibition of MRP2-mediated uptake of calcein. Two other compounds tested, the quercetin-glycosides rutin and isoquercitrin inhibited MRP2 mediated calcein uptake only by 7 ± 7% and 17 ± 12%, respectively, which is significantly less than the MRP2 inhibition by quercetin ($P < 0.05$). Moreover, one metabolite: 4′-*O*-methyl-quercetin, did even stimulate calcein uptake compared to vehicle only incubations (control) by 20 ± 12% ($P < 0.05$). The other metabolites tested showed MRP2 inhibitory potencies similar to quercetin.

4. Discussion

Recently we presented a review on flavonoid-mediated inhibition of ABC transporters in different model systems and how this may affect the oral bioavailability of drugs, food-borne toxic compounds and bioactive ingredients [24]. Several examples of an effect of flavonoids on different ABC transporters are reported in the literature [14–23] and it can be concluded that flavonoid-mediated inhibition of ABC transporters may affect the bioavailability of drugs, bioactive food ingredients and/or food-borne toxic compounds upon oral uptake. Flavonoid-mediated interactions at the level of the intestinal ABC transport proteins may be an important mechanism for unexpected food–drug, food–toxin or food–food interactions. The overview also indicated that future studies should focus on (i) *in vivo* validation of the flavonoid-mediated effects on bioavailability of drugs, toxins and beneficial bioactive food ingredients detected in *in vitro* models, and on (ii) the role of flavonoid phase II metabolism in modulating the activity of the flavonoids to act as ABC transporter inhibitors and/or substrates. The present study provides information on this important second aspect of flavonoid mediated interactions with ABC transporters.

Phase II metabolism determines the fate and biological activity of quercetin in the human body [25]. Not only are phase II metabolites of quercetin the most important forms present in plasma, phase II metabolism is also known to influence the biological activity of the flavonoid [26].

The phase II metabolism of quercetin by the H4IIE and HT29 cell lines was described before [35]. Comparison of the data set in Table 1 with the data obtained from this previous study reveals that the quercetin metabolite pattern formed by the two cell lines was similar to that observed before. Similar to Van der Woude et al. [35], after 24 h incubation the 100 μM exposure concentration was fully recovered indicating that little or no quercetin was accumulated in the cells. The present study shows that phase II metabolism of quercetin mostly results in metabolites that are at least equally good inhibitors of MRP1 and MRP2 activity as quercetin itself. 3′-*O*-Methylation of the catechol moiety does not affect the MRP inhibitory potential of quercetin, whereas 4′-*O*-methylation appeared to decrease the MRP1 inhibitory potency and appeared to stimulate MRP2 mediated uptake of calcein. The mechanism responsible for this phenomenon is unknown. However, it should be noted that 4′-*O*-methylquercetin is hardly present in human plasma because it is readily excreted via the bile [32,35]. In a previous study using MRP1 transfected MDCKII cells, it was found that methylation of quercetin resulted in stronger MRP1 inhibitors compared to quercetin itself [12]. The results of the present study reveal that the intrinsic capacity of 3′-*O*- and 4′-*O*-methylquercetin to inhibit MRP1 and MRP2 mediated uptake is not higher than that of quercetin itself. Therefore, it can now be concluded that the higher inhibitory capacity as a result of methylation observed in MRP1 and MRP2 transfected cell models [12] is most likely the result of higher uptake of the methylated form by the cells, rather than higher intrinsic inhibitory capacity as a result of this methylation.

Regarding glucuronidation, the MRP1 inhibitory potential of the different glucuronosyl mixtures produced by the two cell lines indicates that not only the type of conjugation is of importance for quercetin mediated MRP1 inhibition but also the regioselectivity of the phase II metabolism. Apparently, 7-*O*-glucuronosyl quercetin is a stronger inhibitor of MRP1 than the glucuronosyl metabolites present in the HT29 mixture containing 3-*O*-glucuronosyl quercetin, 4′-*O*-glucuronosyl-3′-*O*-methylquercetin and 4′-*O*-glucuronosyl quercetin. Glucuronidation of quercetin also improves the MRP2 inhibitory potential of quercetin as demonstrated by the HT29 and H4IIE quercetin metabolite mixtures. Again, regioselectivity of glucuronidation plays a role since the H4IIE quercetin metabolite mixture, containing 84% 7-*O*-glucuronosyl quercetin, is a stronger inhibitor of MRP2 activity than the HT29 quercetin metabolite mixture.

For MRP2, both rutin and isoquercitrin showed significant weaker inhibition than quercetin. Moreover, isoquercitrin also showed reduced inhibition of MRP1 than 20 μM quercetin. Isoquercitrin and rutin are examples of important types of glycosylated quercetin available in food. These glycosides may become deconjugated during passage across the small intestine [30] or by bacterial activity in the colon [31]. It is not likely that glycosylated forms of quercetin are present in human plasma [41]. The methyl- and glucuronide-type metabolites tested in this study represent the two major groups of metabolites present in human plasma upon consumption of quercetin [32–35]. The effect of quercetin sulphation remains a topic for future study although it is likely that also these conjugates will be able to inhibit MRP1 and MRP2 activity since sulphate conjugates are known to be common substrates of these MRPs [4].

Altogether the results of the present study demonstrate for both transporters that quercetin phase II metabolism is an important factor influencing its MRP1 and MRP2 inhibiting potential. Glucuronidated quercetin metabolites seem to be better inhibitors of both MRP1 and MRP2 than the parent compound or its methylated metabolites. A possible mechanism by which flavonoids interact with MRPs has

been described for human colonic carcinoma Caco-2 cells [42,43]. These reports show that flavonoids as well as their glucuronide- and sulphate-conjugates can act as MRP2 substrates and are efficiently transported by this transporter. This observation indicates an interaction of the flavonoid and its metabolites with the substrate binding site of MRP2. A similar mechanism has been described for ABCG2 (BCRP1) [44] where it was shown that BCRP1 limits net intestinal absorption of quercetin by pumping quercetin glucuronides back into the lumen. In a previous study from our group, the flavonoid robinetin was shown to be a competitive inhibitor of MRP1- and MRP2-mediated efflux of calcein from MRP1 and MRP2 transfected MDCKII cells, thereby corroborating the possible interaction of the flavonoid with the substrate binding site [12]. Elevation of plasma levels to levels approaching the 100 μM quercetin, the inhibitory concentrations shown to be effective in the present study, requires intravenous injection, since low bioavailability of quercetin and the high first pass effect makes elevation of quercetin levels to these high concentrations via oral supplementation unlikely. Human pharmacokinetic studies have demonstrated serum concentrations of the quercetin to range from 1 to 400 μM after a non-toxic i.v. dose quercetin with a half-life of 1–2 h [45]. Therefore, repeated i.v. doses or infusion seems to be the preferred way to administer quercetin as MRP inhibitor in *in vivo* studies. On the other hand the results obtained in the present study have also demonstrated that upon metabolism quercetin becomes a more effective inhibitor likely to be already active as an inhibitor at concentrations lower than 100 μM.

Overall, the results of this study reveal that the major phase II metabolites of quercetin are equally potent or even better inhibitors of MRP1 and MRP2. Also for other flavonoids, it is likely that phase II metabolism will not reduce their inhibitory capacity for MRP1 and MRP2 since their pattern of phase II metabolism is comparable to quercetin.

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