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

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ARTICLE INFO

Article history:
Received 5 December 2006
Accepted 3 April 2007

Keywords:
ABC transporters
MRP1
MRP2
Quercetin
Flavonoids
Phase II metabolism

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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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.04.002

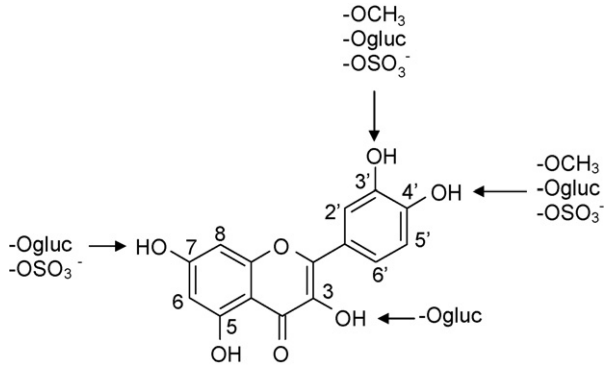

Fig. 1 – Structural formula of quercetin and the identified sites of conjugation [24].

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