ELSEVIER

Biochemical Pharmacology 65 (2003) 479–491

Biochemical Pharmacology

www.elsevier.com/locate/biochempharm

Metabolism of quercetin-7- and quercetin-3-glucuronides by an *in vitro* hepatic model: the role of human β-glucuronidase, sulfotransferase, catechol-*O*-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism

Karen A. O'Leary[a,b], Andrea J. Day[c], Paul W. Needs[a], Fred A. Mellon[a], Nora M. O'Brien[b], Gary Williamson[a,*]

[a]*Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK*
[b]*Nutritional Sciences, Department of Food Science, Food Technology and Nutrition, University College Cork, Cork, Ireland*
[c]*Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, UK*

Received 21 March 2002; accepted 3 September 2002

Abstract

Quercetin-3- and quercetin-7-glucuronides are major products of small intestine epithelial cell metabolism (J. Nutr. 130 (2000) 2765) but it is not known if quercetin glucuronides can be further processed in the liver or if they are excreted directly. Using the HepG2 hepatic cell model, we show that highly purified quercetin-7- and quercetin-3-glucuronides can follow two pathways of metabolism: (i) methylation of the catechol functional group of both quercetin glucuronides (44% of quercetin-7-glucuronide at a rate of 2.6 nmol/hr/10^6 cells, and 32% of quercetin-3-glucuronide at a rate of 1.9 nmol/hr/10^6 cells, over 48 hr) or (ii) hydrolysis of the glucuronide by endogenous β-glucuronidase followed by sulfation to quercetin-3′-sulfate (7% of quercetin-7-glucuronide at a rate of 0.42 nmol/hr/10^6 cells and 10% of quercetin-3-glucuronide at a rate of 0.61 nmol/hr/10^6 cells, over 48 hr). In contrast, quercetin-4′-glucuronide was not metabolised, and interestingly this is not a major product of the small intestine absorption process. The conversion of the quercetin-7- and quercetin-3-glucuronide to the mono-sulfate conjugate shows intracellular deglucuronidation by β-glucuronidase activity, allowing transient contact of the free aglycone with the cellular environment. Inhibition of methylation using a catechol-*O*-methyltransferase inhibitor shifted metabolism towards sulfation, as indicated by an increase in quercetin-3′-sulfate formation (increase in rate to 1.13 and 1.43 nmol/hr/10^6 cells for quercetin-7-glucuronide and quercetin-3-glucuronide, respectively). Efflux of quercetin metabolites from HepG2 cells (methylated glucuronide and sulfate conjugates) was not altered by verapamil, a *p*-glycoprotein inhibitor, but efflux was competitively inhibited by MK-571, a multidrug resistant protein inhibitor, indicating a role for multidrug resistant protein in the efflux of quercetin conjugates from HepG2 cells. These results show that HepG2 cells can absorb and turnover quercetin glucuronides and that human endogenous β-glucuronidase activity could modulate the intracellular biological activities of dietary antioxidant flavonoids.

Keywords: Human; Metabolism; Glucuronides; Flavonoids; β-Glucuronidase; HepG2 cells

1. Introduction

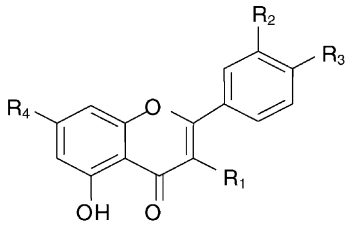
Flavonoids are polyphenolic compounds with antioxidant properties that occur ubiquitously in foods of plant origin [1]. A high dietary intake of flavonols, a subclass of flavonoids, has been inversely associated with the incidence of cardiovascular disease [2,3]. A number of different mechanisms may be involved in the biological activities exerted by the flavonols including free radical scavenging, chelation of metal ions and/or through association with different endogenous proteins [4–7].

* Corresponding author. Present address: Nestlé Research Centre, P.O. Box 44, CH-1000, Lausanne 26, Switzerland. Tel.: +41-21-785-8546; fax: +41-21-785-8544.

E-mail address: gary.williamson@rdls.nestle.com (G. Williamson).

Abbreviations: COMT, catechol-*O*-methyltransferase; UGT, UDP-glucuronosyltransferase; ST, sulfotransferase; MRP, multi-drug resistant protein; LC–MS, liquid chromatography–mass spectrometry; LDH, lactate dehydrogenase; OATP, organic anionic transport polypeptide; LPH, lactase phlorizin hydrolase; CBG cytosolic β-glucosidase; PR, phenol red; IS, internal standard.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01510-1



Flavonoid	R_1	R_2	R_3	R_4
Quercetin	OH	OH	OH	OH
Quercetin-7-glucuronide	OH	OH	OH	O-glucuronic acid
Quercetin-3-glucuronide	O-glucuronic acid	OH	OH	OH
Quercetin-4'-glucuronide	OH	OH	O-glucuronic acid	OH

Fig. 1. Structure of quercetin and quercetin glucuronides.

Flavonols, a widely distributed group of plant polyphenolic compounds, are absorbed from food [8]. The first step in the metabolism of dietary flavonoid glycosides is deglycosylation by the small intestine. CBG and LPH are both involved in small intestine metabolism [9,10], followed by conjugation primarily with glucuronic acid (Fig. 1) in the small intestine epithelial cells [11–13]. Using the rat everted-gut model with quercetin or quercetin glucoside in the mucosal compartment, the major products to pass into the serosal side were the 3- and the 7-glucuronide of quercetin [11], implying that, *in vivo*, *via* the hepatic portal vein, glucuronide conjugates reach the liver. Also, recently it was demonstrated that human small intestinal microsomes can glucuronidate quercetin in the -3-, -7-, -4′- and -3′-position [14]. These results indicate that typical dietary intakes of quercetin facilitate full conjugation in the small intestinal cells. In contrast, pharmaceutical doses of drugs saturate the small intestine conjugating enzymes and so they pass into the hepatic portal vein in a free (unconjugated) form.

Generally, glucuronidation of drugs is regarded as an inactivation step for excretion. It is important to assess if the products of small intestine metabolism, the quercetin glucuronides, can be further metabolised in the liver or if they are excreted directly by the kidney. Metabolism of xenobiotics in the liver is highly dependent on the presence of conjugating enzymes in the liver cell such as COMT [15], UGT [16] and ST [17]. The nature of the conjugates transported into and out of the liver and thus found circulating *in vivo* will have profound effects on their biological activities [18]. Biliary excretion of glucuronides often occurs [19]; however, bacteria present in the colon are capable of hydrolysing these conjugates and enable re-absorption of released quercetin aglycone, resulting in enterohepatic cycling.

To date, little attention has focused on the role of enzymes such as β-glucuronidase, present in the liver, on intracellular turnover of flavonoid glucuronides. β-Glucuronidase is a glycoprotein present in many human tissues and is capable of hydrolysing various xenobiotic glucuronides [20]. β-Glucuronidase is intracellularly distributed in the lysosomes (67%) and in the microsomal fraction (33%) of the cell [21], with large inter-individual variation [22]. The lysosomal β-glucuronidase is involved in steroid metabolism [23]. However, whether microsomal β-glucuronidase serves solely as a precursor of the lysosomal enzyme or whether it has a distinct physiological function is not yet clear. Previously, we have demonstrated that flavonoid glucuronides are substrates for recombinant human β-glucuronidase. Similarly, we have shown that quercetin glucuronides can be deconjugated by cell-free extracts of human liver, small intestine and blood neutrophils [24].

Any active transport mechanism of flavonoid glucuronide uptake into the liver remains to be elucidated. OATP2 is an organic anionic transport polypeptide, found almost exclusively in liver cells [25,26]. It is present on the basolateral membrane of the hepatocytes and it is involved in the uptake of mono and diglucuronides of bilirubin, 17-β-glucuronosylestradiol and sulfobromophthalein into liver cells [26]. Although little has been published on the OATP2 transporter, it is possible that it may be involved in the uptake of quercetin glucuronides into liver cells.

The transporter suggested to be involved in flavonoid efflux from the hepatocyte into the bile is MRP2. MRP2 is also known as the canalicular multi-specific organic anionic transporter (cMOAT) and it is an ATP-dependent conjugate pump found in many tissues of the body including the liver [27,28]. In liver cells, it is strictly and specifically located on the apical domain of the cell [27]. Recently, it has been suggested that it is the membrane export pump involved in the efflux of the isoflavone, genistin, and the flavonol, chrysin, from Caco2 cells [29,30]. It has also been demonstrated that the MRP2 transporter is highly expressed, with low expression of MRP1, in HepG2 cells [27,29–31]. However, the overall contribution of this export pump to quercetin conjugate efflux from hepatocytes remains unknown.

The aim of this work was to investigate whether flavonol glucuronides can undergo further hepatic metabolism using the well-established hepatic model system, HepG2 cells. We report on the contribution of both β-glucuronidase and

COMT to flavonoid metabolism by these cells and we also examine the role played by MRP2 and OATP2 in the transport of quercetin metabolites.

2. Materials and methods

2.1. Materials

All reagents were obtained from Sigma unless otherwise stated and were of analytical or HPLC grade where applicable. Tissue culture plastics were supplied by Life Technology. Water was purified *via* a Millex Q plus system (Millipore). MK-571 was obtained from Affiniti Chemicals. Quercetin-3-glucuronide was purified from green bean tissue [32]. Chemical synthesis of quercetin-4′-glucuronide, quercetin-7-glucuronide and enzymatic synthesis of quercetin glucuronides mixtures were previously described [24]. A mixture of 3′-methylquercetin glucuronide standards were enzymatically synthesised using a previously published method for quercetin [24]. Quercetin sulfates were kindly provided by Barron [33]. These compounds have previously been confirmed by MS and ^{1}H and ^{13}C NMR. All flavonoids and conjugates were checked for purity, prior to use, by HPLC and were found to be >98% pure. The purity of the quercetin conjugates, particularly any contamination with quercetin (aglycone), was important to determine before the metabolism and transport studies, as metabolism of any free quercetin would interfere with the results. The level of free quercetin in each of the standards was below the limit of detection (<0.1 nmoles/injection, <0.02%). All substrates were stored in aqueous methanol and were stable in these forms at −20° for over 6 months. Prior to an experiment, an aliquot of the flavonoid glucuronide stock was diluted in 20 mM sodium phosphate buffer pH 7.2. The final concentration of the methanol was less than 0.3% (v/v) in the reaction mixture, a level which did not interfere with the assay.

2.2. Cell culture

Human hepatoma HepG2 cells were obtained from the European Collection of Cell Culture and were maintained in Eagle's Minimal Essential Medium (EMEM) supplemented with 10% foetal calf serum, 1% (v/v) nonessential amino acids and 2 mM L-glutamine, penicillin (100 U/mL) and streptomycin (100 μg/mL). Cells were grown in a humidified incubator of 5% CO_2, 95% air at 37° and passaged every 8–10 days.

2.2.1. Deconjugation assay

Cell-free extracts of HepG2 cells were prepared by homogenisation of the cells in phosphate buffer (400 μL, 10 mM, pH 7.0). The cells were then frozen, thaw lysed three times to ensure release of the cell content, followed by centrifugation at 13,500 *g*, 4° for 10 min. The supernatant was retained and stored at −20° until use. Storage of the supernatant at this temperature did not affect the stability of the β-glucuronidase enzyme. β-Glucuronidase activity of HepG2 cells was determined as previously described by O'Leary *et al*. [24]. A quercetin glucuronide mixture (100 μM) was incubated with cell-free extracts of HepG2 cells at 37° over a time period of 180 min. Samples were taken between 0 and 180 min and were analysed by HPLC after extraction into methanol. Experiments were carried out in triplicate, with nine observations in total.

2.3. Cell treatments

2.3.1. Stability of quercetin glucuronides in cell culture

Freshly prepared media, and media that had been used to culture cells under normal cell culture conditions for 4 days, were incubated with quercetin-4′-, quercetin-3- and quercetin-7-glucuronide for 48 hr. The reaction was stopped by placing the media on dry ice and analysed as described below. To assess the cell membrane integrity of HepG2 cells following treatment of the cells with quercetin and quercetin glucuronides, LDH release into the cultured media was assayed based on the reduction of pyruvate to lactate and the oxidation of NADH to NAD^{+}, resulting in a change in absorbance at 340 nm.

2.3.2. Cytotoxicity assay

Cytotoxicity tests on the transporter inhibitors were assessed using the neutral red uptake assay as an index of cytotoxicity. Indocyanine green (an inhibitor of OATP2 [25]), verapamil (an inhibitor of *p*-glycoprotein [29]) and MK-571 (an inhibitor of MRP2 [28]) were dissolved in DMSO, where the final concentration of the solvent in the culture medium was <0.5% (v/v). A set of control cultures was exposed to the equivalent concentration of DMSO only and was found not to interfere with the cell growth or alter the results significantly. HepG2 cells were exposed to various concentrations of MK-571 (5–200 μM), verapamil (5–200 μM) and indocyanine green (5–500 μM) for 24 hr. Cytotoxicity was expressed as a percentage of cell viability relative to that of nontreated cells. Concentrations of the transporter inhibitors chosen were such that the cell viability did not fall below 95% for any of the treatments. For subsequent experiments, the concentrations selected were verapamil (50 μM), MK-571 (50 μM) and indocyanine green (100 μM).

2.3.3. Metabolism of quercetin glucuronides in HepG2 cells

Cells were seeded at a density of approximately 2×10^4 cells/cm^2 in 56 cm^2 dishes and allowed to adhere overnight. The cells were allowed to grow to confluency over a period of 6 days and the media were changed every second day. On the day of the experiment, the cells were washed twice in PBS and fresh media added (10 mL).

The cells were incubated with either quercetin, quercetin-7-glucuronide, quercetin-3-glucuronide or quercetin-4′-glucuronide (30 μM; <0.3% methanol) for 0, 3, 6, 9, 24 or 48 hr. Quercetin, quercetin-3-glucuronide or quercetin-7-glucuronide (30 μM) were also incubated with a COMT inhibitor (3,5-dinitrocatechol; 10 μM) for 0, 3, 6, 9, 24 or 48 hr. To further show that intracellular deglucuronidation occurred and that the observed results were not due to secreted β-glucuronidase, quercetin-7-glucuronide and quercetin-3-glucuronide (30 μM) were incubated with a β-glucuronidase inhibitor (saccharic acid 1,4-lactone; 1 mM) for 0, 3, 6, 9, 24 and 48 hr. All metabolic experiments were carried out in triplicate, with nine observations in total.

To quench all incubation reactions, the media were removed (≈10 mL) and placed immediately on dry ice. The media were processed for HPLC analysis as follows: 15 mL of methanol containing 1 mM ascorbic acid was added to the samples. The samples were acidified by addition of acetic acid. Samples were centrifuged at 13,500 *g* for 10 min at 4°, concentrated to dryness by rotary evaporation and resuspended in 1 mL methanol:-water (80:20, v/v). Samples were filtered through 0.22 μm filter units prior to analysis by HPLC.

2.3.4. Flavonoid glucuronide transport studies

Cells were seeded at a density of approximately 2×10^4 cells per cm^2 in 56 cm^2 dishes and allowed to adhere overnight. The cells were allowed to grow to confluency over a period of 6 days and the media were changed every second day. On the day of the experiment, the cells were washed twice in PBS and media were replaced (10 mL). The cells were incubated with either quercetin-7-glucuronide or quercetin-3-glucuronide (30 μM; <0.3% methanol) and an OATP2 inhibitor (indocyanine green; 100 μM) for 0, 3, 6, 9 and 24 hr. Quercetin-3- and quercetin-7-glucuronide (30 μM) were also incubated with an MRP inhibitor (MK-571; 50 μM) and a *p*-glycoprotein inhibitor (verapamil; 50 μM) for 0, 3, 6, 9, 24 and 48 hr. To quench reactions, the media was removed (≈10 mL) and placed immediately on dry ice. The media were processed for HPLC analysis as previously described.

2.3.5. Indirect determination of flavonoid metabolites

Portions of flavonoid conjugates/metabolites, generated during the various experiments described above, were treated with microbial β-glucuronidase, aryl-sulfatase or a combination of both enzymes, as previously described by Day *et al*. [33].

2.4. HPLC analysis

A modified version of a previously published analytical HPLC method was used [34]. Solvents A (water:tetrahydrofuran:trifluoroacetic acid, 98:2:0.1, v/v/v) and B (acetonitrile) were run at a rate of 1 mL/min, using a gradient of 17% B (2 min), increasing to 25% B (5 min), 35% B (8 min), 50% B (5 min) and then to 100% B (5 min). A column clean up stage was performed at 100% B (5 min) followed by a re-equilibration at 17% B (15 min). The column was packed with Prodigy 5 μm ODS3 reversed-phase silica, 250 by 4.6 mm i.d. (Phenomenex). The eluent was monitored at 270 and 370 nm with a diode array detector. IS (apigenin; 30 μM) was added to each sample to ensure reproducibility of experiments. An external standard of rutin (quercetin-3-rhamnoglucoside) was run approximately every six runs.

2.5. Liquid chromatography–mass spectrometry (LC–MS)

Positive ion electrospray LC–MS measurements were performed using a Micromass Quattro II (Micromass), equipped with a Z-spray™ source. Samples were introduced using a Hewlett Packard 1050 HPLC (Agilent) equipped with a diode array detector. Eluent flow (1 mL/min) was split between the diode array detector and the mass spectrometer ion source in the approximate ratio 8:1. The electrospray capillary voltage was set to 3.5 kV and the cone voltage to 28 V. Source block temperature was 140° and desolvation temperature 350°. Nitrogen was used as the drying and nebulising gas at flows of 400 and 20 L/hr, respectively. Full scan spectra were obtained in positive ion mode from *m/z* 50 to 1000 at a scan rate of 1.5 s. Selected ion monitoring was conducted on mass channels 303, 317, 383, 397, 479 and 483 with a scan window of 0.2, dwell times of 0.1 s/channel and an interchannel delay of cycle time of 0.03 s. Diode array spectra were scanned from 190 and 450 nm, with an interval of 2 nm. Instrument control, data acquisition and processing were performed using Micromass MassLynx™ version 3.4 data system and software.

3. Results

3.1. Deconjugation of quercetin glucuronides by cell-free extracts of HepG2 cells

The level of β-glucuronidase activity in HepG2 cells was determined as 1.57 ± 0.79 nmol/mg protein/min. This is 1.8-fold lower than the level we previously determined in human liver [24]. Incubation of a mixture of quercetin glucuronides (quercetin-3-, -7-, -4′- and -3′-glucuronide) with cell-free extracts of HepG2 cells demonstrated the potential of these cells to deconjugate quercetin glucuronides, forming quercetin as the only product. The rate of deconjugation (approximately 55% for each quercetin glucuronide at 100 μM total glucuronide) after 180 min was not affected by the position of glucuronide conjugation.

3.2. Quercetin glucuronide stability in cell culture conditions

To demonstrate that the HepG2 cells did not secrete any β-glucuronidase into the medium, cells were grown for 96 hr and the medium was then removed. Quercetin-7-glucuronide, quercetin-3-glucuronide or quercetin-4′-glucuronide were recovered (>98%) from this "used" media after incubation in the absence of cells for 48 hr. Neither free quercetin nor any metabolites were formed, demonstrating that β-glucuronidase was not secreted into the media by HepG2 cells in culture.

We demonstrate that the cell membrane integrity was not comprised during the experiment by measuring the LDH release. There was no significant difference in extracellular LDH activity between 0 and 48 hr after incubation with any of the test compounds, nor between the test compounds. If the membrane integrity had been breached, it would have been possible for β-glucuronidase to be released into the medium during 48 hr. A further control using a β-glucuronidase inhibitor is shown below.

3.3. Quercetin-7-glucuronide metabolism

Quercetin-7-glucuronide was incubated with HepG2 cells and the products were analysed. Methylation of quercetin-7-glucuronide occurred in HepG2 cells. Three major metabolites of quercetin-7-glucuronide metabolism were detected by HPLC UV diode array analysis (Fig. 2A and B), two of which were positively identified as 3′-methylquercetin-7-glucuronide and 4′-methylquercetin-7-glucuronide (confirmed by co-elution with standards and by mass spectrometry: $[M + H]^+$ ions at *m/z* 493 and a corresponding fragment ion at *m/z* 317; Fig. 2C).

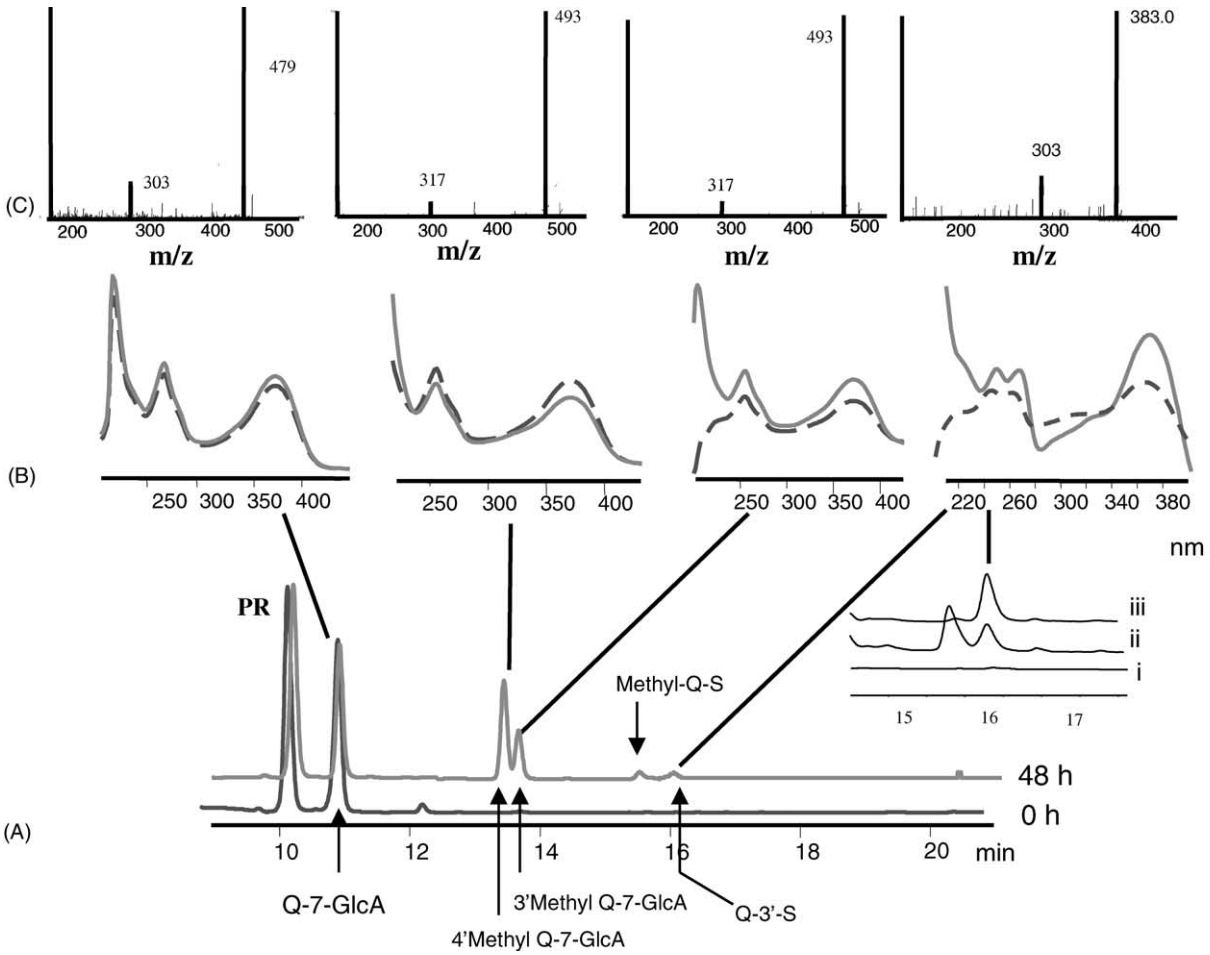


Fig. 2. HPLC chromatograms of cell culture medium after incubation of HepG2 cells with quercetin-7-glucuronide for 0 and 48 hr. Confluent cells were incubated with quercetin-7-glucuronide (30 μM) over a 48-hr period. (A) UV absorption at 370 nm; arrows on the chromatogram indicate the elution time of synthesised quercetin conjugate standards (3′-methyl-Q-7-GlcA, 3′-methylquercetin-7-glucuronide; 4′-methyl-Q-7-GlcA, 4′-methylquercetin-7-glucuronide; Q-3′-S, quercetin-3′-sulfate; Q-7-GlcA, quercetin-7-glucuronide). Also indicated are methyl-Q-S (methylquercetin-sulfate), confirmed by sulfatase treatment and MS, but the positions of substitution are unknown, and phenol red derived (PR) from the cell culture media. (B) UV spectra of detected metabolites (- - -), with peaks indicated by connecting lines, overlaid with co-eluted quercetin conjugate standards (—). (C) LC–MS analysis of indicated peaks using full scan mode, electrospray ionisation and positive ion detection (only principal ions shown normalised to 100%). Inset: expanded section of chromatograms showing the production of sulfates from quercetin-7-glucuronide (i) before incubation and in the (ii) absence and (iii) presence and of a COMT inhibitor (3,5-dinitrocatechol; 10 μM) at 48 hr.

Treatment of the sample with commercial microbial β-glucuronidase resulted in the disappearance of the two peaks and formation of 3′-methylquercetin and 4′-methylquercetin. Methylated quercetin-7-glucuronide accounted for 44% of the total quercetin in the HepG2 culture media after 48 hr (average rate of methylation: 2.6 nmol/hr/10^6 cells) in HepG2 cells over a period of 48 hr. The third peak was identified as quercetin-3′-sulfate (quercetin-3′-sulfate accounted for 7% of the total quercetin in the HepG2 culture media after 48 hr with a rate of 0.42 nmol/hr/10^6 cells), and was confirmed by co-elution with a synthetic standard, response to aryl-sulfatase treatment and LC–MS analysis (*m/z* at 383 and a fragment ion at *m/z* 303; Fig. 2C). No free quercetin was detected in any sample. These results show that quercetin-7-glucuronide follows one of two fates of metabolism: methylation in the 4′- and 3′-position (catechol moiety) or deglucuronidation followed by sulfation in HepG2 cells.

3.4. Quercetin-3-glucuronide metabolism

A similar profile of metabolism was observed for quercetin-3-glucuronide in HepG2 cells; methylated and sulfated derivatives were formed and were identified as 3′-methylquercetin-3-glucuronide, 4′-methylquercetin-3-glucuronide and quercetin-3′-sulfate (Fig. 3). Methylated quercetin-3-glucuronide accounted for 32% of the total quercetin in the HepG2 culture media after 48 hr (average rate of methylation: 1.9 nmol/hr/10^6 cells). Quercetin-3′-sulfate accounted for 10.2% of the total quercetin in the HepG2 culture media after 48 hr with a rate of 0.61 nmol/hr/10^6 cells. The identity of the metabolites was confirmed

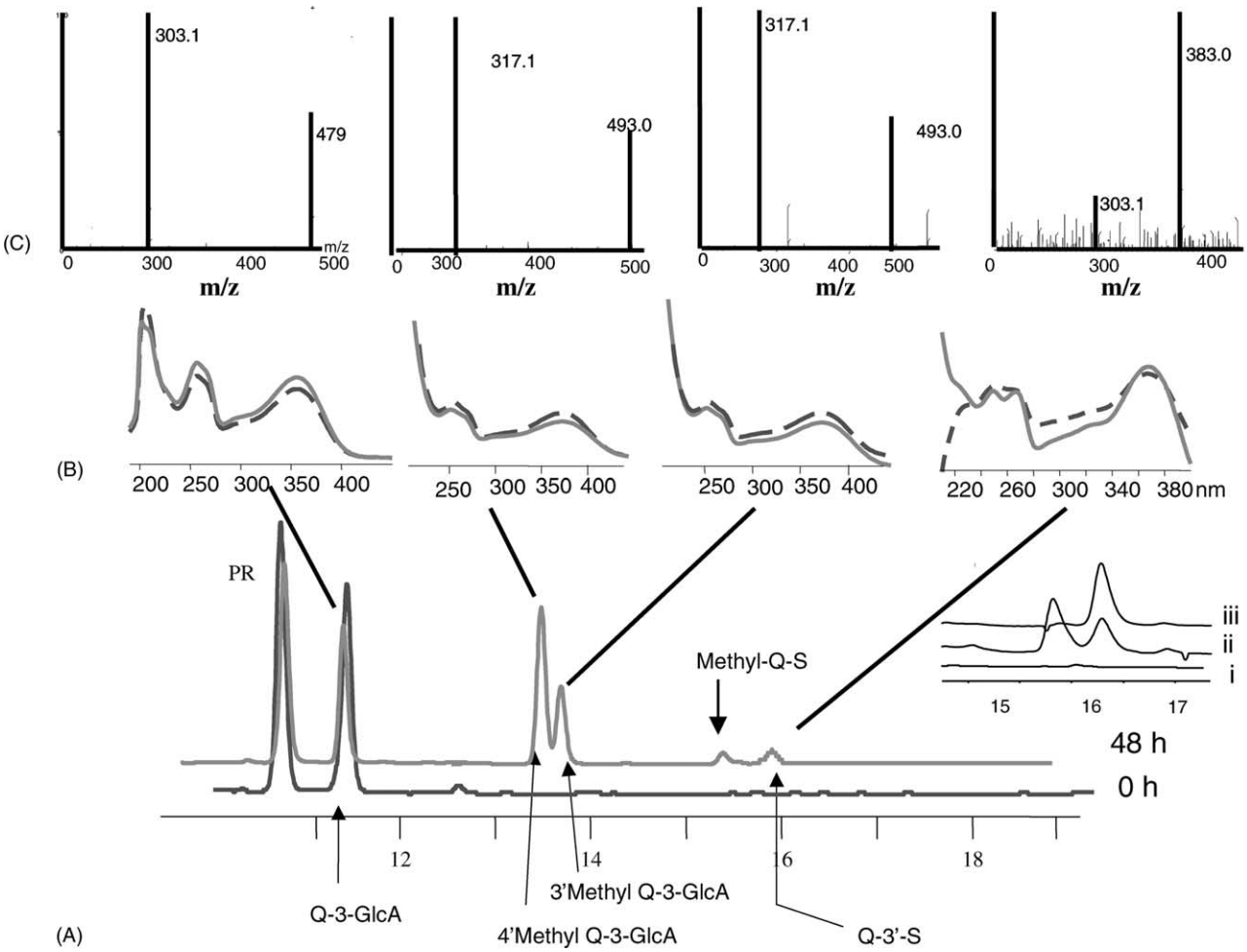


Fig. 3. HPLC chromatograms of cell culture medium after incubation of HepG2 cells with quercetin-3-glucuronide for 0 and 48 hr. Confluent cells were incubated with quercetin-3-glucuronide (30 μM) over a 48-hr period. (A) UV absorption at 370 nm; arrows on the chromatogram indicate the elution time of synthesised quercetin conjugate standards (3′-methyl-Q-3-GlcA, 3′-methylquercetin-3-glucuronide; 4′-methyl-Q-3-GlcA, 4′-methylquercetin-3-glucuronide; Q-3′-S, quercetin-3′-sulfate; Q-3-GlcA, quercetin-3-glucuronide). Also indicated are methyl-Q-S (methylquercetin-sulfate), confirmed by sulfatase treatment and MS, although the positions of substitution are unknown, and phenol red (PR) derived from the cell culture media. (B) UV spectra of detected metabolites (- - -), with peaks indicated by connecting lines, overlaid with co-eluted quercetin conjugate standards (—). (C) LC–MS analysis of indicated peaks using full scan mode, electrospray ionisation and positive ion detection (only principal ions shown normalised to 100%). Inset: expanded section of chromatograms showing the production of sulfates from quercetin-3-glucuronide (i) before incubation and in the (ii) absence and (iii) presence and of a COMT inhibitor (3,5-dinitrocatechol; 10 μM) at 48 hr.

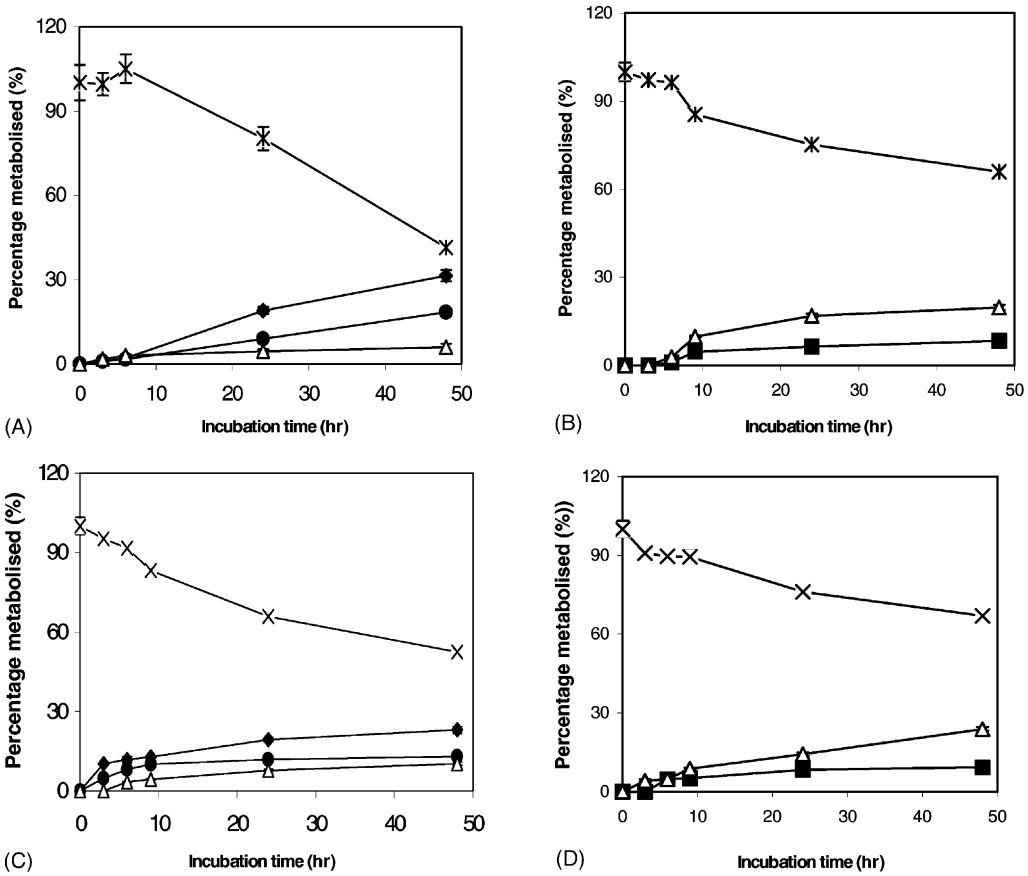


Fig. 4. Time course of metabolism by HepG2 cells of (A) quercetin-7-glucuronide, (B) quercetin-7-glucuronide in the presence of a COMT inhibitor, (C) quercetin-3-glucuronide and (D) quercetin-3-glucuronide in the presence of a COMT inhibitor. Confluent cells were incubated with quercetin-7-glucuronide (30 μM) or quercetin-3-glucuronide (30 μM) in the presence or absence of a COMT inhibitor (3,5-dinitrocatechol; 10 μM) over a 48-hr time period. Error bars represent mean ± SEM of three independent experiments. Error bars, where not visible, were smaller than the size of the symbol. (6-point star), quercetin-7-glucuronide; (cross), quercetin-3-glucuronide; (filled square), total quercetin glucuronide formed; (filled diamond), 3′-methylquercetin glucuronide; (filled circle), 4′-methylquercetin-glucuronide; (open triangle), quercetin-3′-sulfate.

by HPLC with UV diode array analysis, co-elution of the metabolites with known conjugate standards, response to added microbial β-glucuronidase/sulfatase treatment and LC–MS analysis. The time courses of formation of the metabolites of quercetin-7- and quercetin-3-glucuronide are shown in Fig. 4A and C.

3.5. Quercetin-4′-glucuronide metabolism

Unlike quercetin-3- and quercetin-7-glucuronide, when quercetin-4′-glucuronide was incubated with HepG2 cells for 48 hr, no significant metabolism was observed. Human recombinant β-glucuronidase also has a high activity on quercetin-4′-glucuronide [24], which further indicates that the results observed are not due to secreted β-glucuronidase activity (see below).

3.6. Incubation of quercetin glucuronides with a β-glucuronidase inhibitor (saccharic acid 1,4-lactone)

To confirm that it was not secreted β-glucuronidase in cell culture media that hydrolysed quercetin glucuronides to quercetin, we incubated HepG2 cells with quercetin-3-glucuronide or quercetin-7-glucuronide and a β-glucuronidase inhibitor over 48 hr. When saccharic acid 1,4-lactone was present in the media, there was no change in the metabolic profile in HepG2 cells indicating that there is no secreted β-glucuronidase present in the cell culture media capable of hydrolysing quercetin glucuronides to quercetin. Owing to its hydrophilicity, this inhibitor is unlikely to penetrate the cell membrane and so would not be taken up into the cells. We have previously shown that incubation of cell-free extracts of human liver with quercetin glucuronide mixtures resulted in complete hydrolysis in 30 min. However, in the presence of the β-glucuronidase inhibitor, no hydrolysis using cell-free extracts was observed [24].

3.7. Quercetin metabolism

To determine if the deglucuronidation step changes the metabolic profile, we examined whether the metabolites produced from free quercetin were the same as those from quercetin glucuronides. HepG2 cells were incubated with

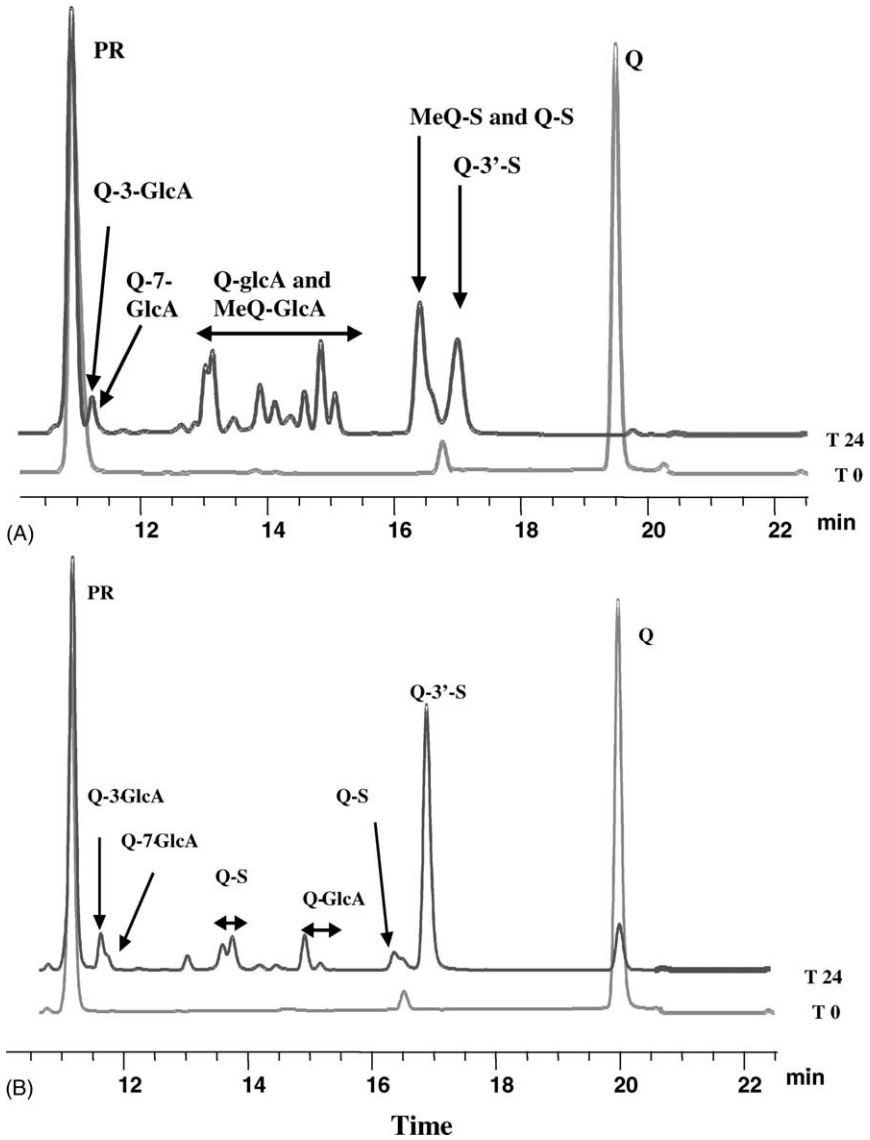


Fig. 5. HPLC chromatograms of cell culture medium from HepG2 cells at 0 and 24 hr: (A) quercetin metabolism; (B) quercetin metabolism in the presence of a COMT inhibitor. Confluent cells, grown for 7 days, were incubated with 30 μM quercetin in the presence or absence of a COMT inhibitor (3,5-dinitrocatechol) for 24 hr (UV absorption at 370 nm).

quercetin over a 24-hr period, and Fig. 5 illustrates the metabolic profile. Analysis of the cell culture medium indicated extensive metabolism yielding four classes of metabolites (Fig. 5). The first metabolite to appear was 3′-methylquercetin, which increased initially up to approximately 4 hr and disappeared by 24 hr. Several other peaks were produced more slowly over a 24-hr period; these were glucuronide conjugates of 3′-methylquercetin (3′-methylquercetin-3-, -7- and -4′-glucuronide) and quercetin (quercetin-3-, -7-, -4′- and -3′-glucuronide). The major glucuronides formed were that of the major 3′-methylquercetin with the quercetin glucuronides being formed to a lesser extent. The identity was confirmed by co-elution with glucuronide standards and β-glucuronidase treatment and by LC–MS (full scan). Quercetin-3′-sulfate was also identified as a major product. There was no detectable di-glucuronides or mixed glucurono-sulfate conjugates of quercetin using HPLC or LC–MS. These mixed conjugates have been previously reported as metabolites of quercetin in the plasma of rats [35], rat hepatocytes [36] and human plasma [33]. These data show that the metabolites of quercetin are qualitatively similar to those from quercetin-3- and quercetin-7-glucuronides, but that the overall rate of metabolism of quercetin is faster.

3.8. Role of COMT in flavonoid glucuronide metabolism

To further elucidate the mechanism, we inhibited methylation in HepG2 cells using a COMT inhibitor (3,5-dinitrocatechol). Quercetin-7-glucuronide and quercetin-3-glucuronide in the presence of the COMT inhibitor (Fig. 4B and D) were deglucuronidated and sulfated, with very little methylation. The rate of sulfation increased from 0.42 to 1.13 nmol/hr/10^6 cells with a maximum yield of

19% for quercetin-7-glucuronide and from 0.61 to 1.43 nmol/hr/10^6 cells with a maximum yield of 24% for quercetin-3-glucuronide. Re-glucuronidation was also observed, with the reglucuronidation at some different positions to the parent compounds: quercetin-7-glucuronide and quercetin-3-glucuronide produced some quercetin-4′-glucuronide, showing that deglucuronidation followed by reglucuronidation, but in a different position, had occurred. Similarly, Fig. 5 illustrates the metabolic profile of quercetin in the presence of a COMT inhibitor at 24 hr and Fig. 6 shows the time course of the formation of the metabolites. Methylation was inhibited, sulfation was increased and sulfated derivatives in three different positions were identified after 9 hr. These results show that inhibition of methylation shifts metabolism to favour deglucuronidation and sulfation, leading to a 2.7- and 2.4-fold increase in quercetin sulfate formation for quercetin-3-glucuronide and quercetin-7-glucuronide, respectively.

3.9. Role of MRP and p-glycoprotein in the efflux of quercetin conjugates from HepG2 cells

Incubation of quercetin with HepG2 cells in the presence of the *p*-glycoprotein inhibitor (50 μM verapamil) did not significantly alter the efflux of quercetin metabolites, indicating that the contribution of *p*-glycoprotein to quercetin conjugate efflux is minimal. The anionic multidrug resistance protein, MRP2, has previously been implicated in the efflux of flavonoids from Caco2 cells and it has been shown to be present in HepG2 cells. To investigate the contribution of MRP2 in the efflux of the quercetin metabolites from HepG2 cells, a selective inhibitor of MRP2, MK-571, was incubated simultaneously with quercetin. In the presence of the MRP2 inhibitor, efflux of glucuronides was more inhibited (by 73%) than production of sulfate metabolites (by 26%). The inhibition of efflux was found to be maximal at 5–6 hr, when the rate of metabolism appeared to be at its highest. After 24 hr, competitive inhibition of quercetin glucuronide and sulfate conjugates efflux was saturated and the effect of the MRP2 inhibitor was no longer apparent.

3.10. Role of organic anionic transport polypeptide (OATP2) of quercetin glucuronides into HepG2 cells

The mechanism of uptake of quercetin glucuronides into the liver is not known. Recently, OATP2, involved in the uptake of bilirubin glucuronides into the liver, has been identified [25,26]. We investigated whether quercetin-7-glucuronide and quercetin-3-glucuronide were taken up into HepG2 cells *via* this OATP2 transporter. Incubation of quercetin-7-glucuronide and an OATP2 inhibitor (indocyanine green; 100 μM) resulted in inhibition of quercetin-7-glucuronide uptake into HepG2 cells. Fig. 7 illustrates quercetin-7-glucuronide metabolism in the presence and absence of indocyanine green. No metabolites of quercetin-7-glucuronide could be detected. Conversely, incubation of quercetin-3-glucuronide with the OATP2 inhibitor

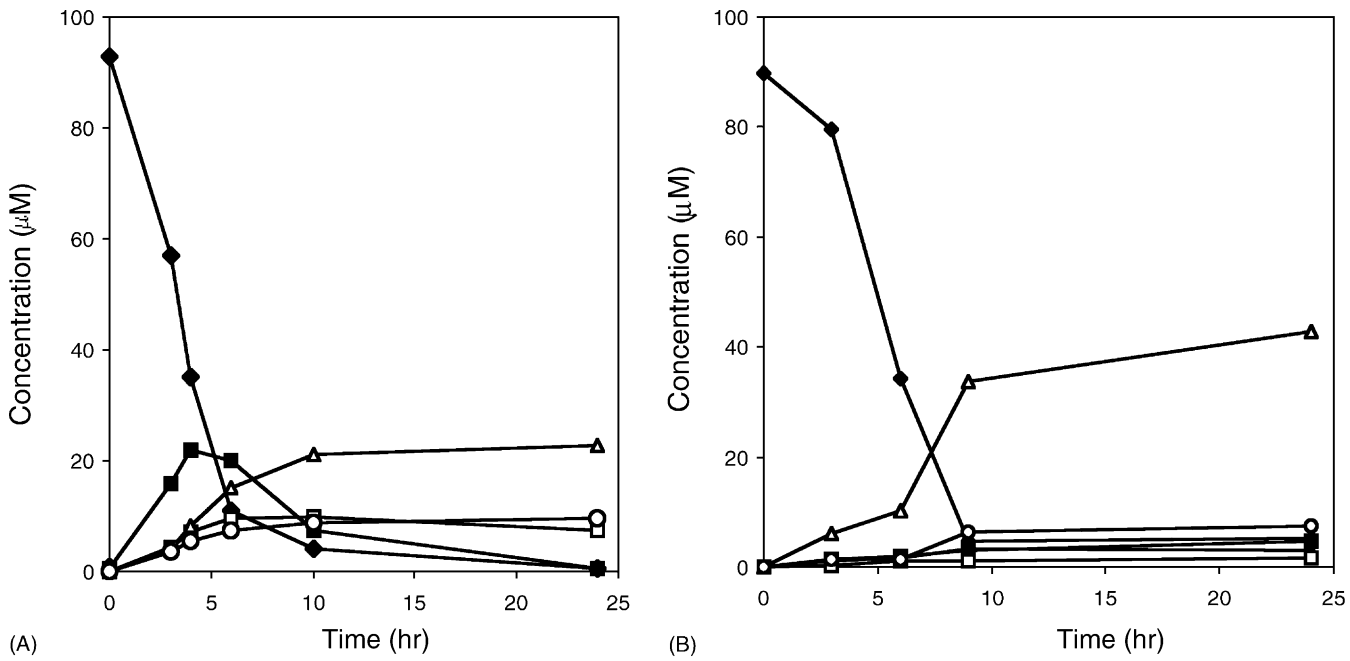


Fig. 6. Time course of metabolism by HepG2 cells: (A) quercetin; (B) quercetin in the presence of a COMT inhibitor. Confluent cells, grown for 7 days, were incubated with 30 μM quercetin in the presence or absence of a COMT inhibitor (3,5-dinitrocatechol; 10 μM). Error bars represent mean ± SEM of three independent experiments, and if not visible, are less than the size of the symbol. (A): (filled diamond), quercetin; (open circle), total quercetin glucuronide; (filled square), 3′-methylquercetin; (open square), total methylquercetin glucuronide; (open triangle), quercetin-3′-sulfate. (B): (filled diamond), quercetin; (open circle), quercetin-4′-glucuronide; (filled diamond), quercetin-7-glucuronide; (filled circle), quercetin-3-glucuronide; (open square), quercetin-3/7-sulfate; (open triangle), quercetin-3′-sulfate.

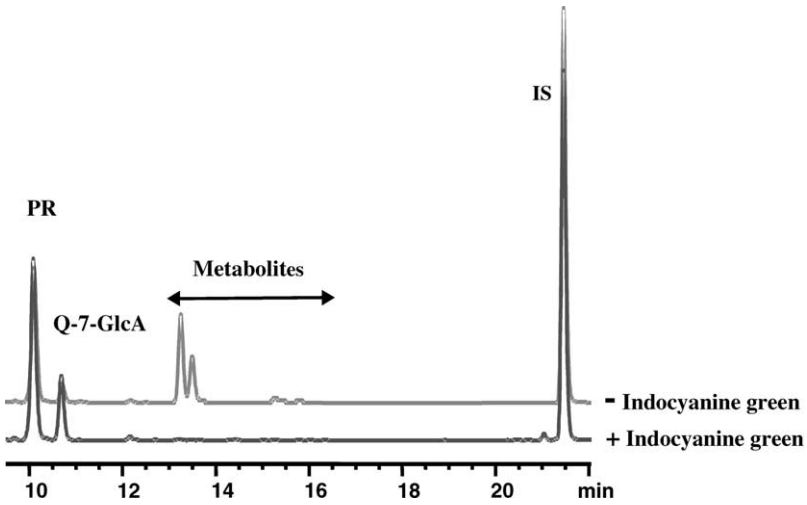


Fig. 7. Effect of indocyanine green on quercetin-7-glucuronide metabolism in HepG2 cells. Confluent cells, grown for 7 days, were incubated with 30 μM quercetin-7-glucuronide in the presence or absence of indocyanine green (100 μM), an OATP2 inhibitor, for 48 hr (UV absorption at 370 nm).

resulted in no significant change in the metabolic profile. However, we were unable to detect the presence of OATP2 in HepG2 cells using Western blotting, which indicates that quercetin-7-glucuronide, but not quercetin-3-glucuronide, is taken up into cells by an indocyanine green-inhibited transporter that does not appear to be OATP2.

4. Discussion

HepG2 cells are a well-characterised immortalised liver cell line of human origin and provide a useful model system for investigating human hepatic drug metabolism [37]. HepG2 cells retain many of their specialised functions even upon culturing and can carry out cytochrome P450-dependent mixed function oxidase and conjugation reactions [38]. Previously, flavonoid aglycones, such as chrysin, have been shown to be metabolised by HepG2 cells by glucuronidation and sulfation [39], although the only metabolite detected when quercetin was incubated with HepG2 cells for 24 hr was methylquercetin [40]. Our data provides evidence for extensive methylation, sulfation and glucuronidation of quercetin in HepG2 cells and the differences between our results may be due to cell density and metabolic capacity, since our first metabolite of quercetin was methylquercetin. Quercetin-3- and quercetin-7-glucuronide followed two metabolic fates: methylation or deglucuronidation/sulfation. This suggests a role for β-glucuronidase in the turnover of glucuronides in liver cells. Methylation by COMT was the dominant pathway for all compounds investigated, with the exception of quercetin-4′-glucuronide, which does not have a free functional catechol moiety. Following deglucuronidation, sulfation of quercetin appeared to be the favoured pathway, rather than re-glucuronidation.

Results from our study demonstrate that the metabolism of quercetin glucuronides in HepG2 cells depends on the position of conjugation of the glucuronic acid. Similar profiles of metabolism were observed for quercetin-7-glucuronide and quercetin-3-glucuronide, undergoing methylation, or deglucuronidation and sulfation reactions within HepG2 cells, whereas Q-4′-GlcA was not further metabolised. As quercetin-4′-glucuronide is a substrate for β-glucuronidase from HepG2 cell-free extracts, then the lack of further metabolism of this compound may be derived from a lower rate of transport into the cell. Specificity of influx transporters on the cellular membrane may play a role in the uptake of quercetin glucuronides into the hepatocyte. Uptake of quercetin glucuronides across the hepatic membrane may be controlled by a balance between uptake by an influx transporter and efflux by MRP2. While this balance may favour influx of quercetin-3-glucuronide and quercetin-7-glucuronide, the balance may shift to favour efflux by MRP for quercetin-4′-glucuronide. A similar hypothesis was suggested for quercetin glucoside uptake into Caco2 cells [41]. Further evidence to suggest that quercetin-4′-glucuronide may not be metabolised as rapidly as the other quercetin glucuronides stems from our previous specificity data. Results determining the affinity of recombinant human β-glucuronidase for various flavonoid glucuronide substrates also showed that quercetin-3-glucuronide and quercetin-7-glucuronide are marginally better substrates for β-glucuronidase (11.4×10^3 M^{-1} s^{-1} for quercetin-3-glucuronide; 6.9×10^3 M^{-1} s^{-1} for quercetin-7-glucuronide) than the quercetin-4′-glucuronide (5.4×10^3 M^{-1} s^{-1}) [24]. Therefore, if quercetin-4′-glucuronide is taken up into the cell, metabolism may occur at a slower rate than that of the other glucuronides investigated and thus the appearance of new metabolites may only be determined after longer incubation times. However, we continued the incubation for up to 72 hr and still did not detect any metabolites. It is interesting to note that metabolism by the rat small intestine gave quercetin-7- and

quercetin-3-glucuronides as the major metabolites [11], and very little quercetin-4′-glucuronide nor quercetin-3′-glucuronide, so that we can predict, *in vivo*, that the liver receives quercetin metabolites from the small intestine that it can further metabolise.

It is still unclear whether OATP2 plays a role in the uptake of quercetin glucuronides into HepG2 cells, although the position of conjugation of the flavonoid glucuronide appears to determine the influx mechanism or transporter involved. Although we could not detect OATP2 in HepG2 cells by Western blotting, quercetin-7-glucuronide was not metabolised in the presence of an OATP2 inhibitor, indicating it was not taken up into the cells. It is possible that a yet unidentified influx transporter similar to OATP2 or from the OATP family may be involved.

Quercetin can be methylated in the catechol moiety at the 3′- or 4′-position, to form either 3′-methylquercetin and 4′-methylquercetin, respectively, by COMT. Several studies of quercetin metabolism in rats have found both 3′- and 4′-methylquercetin in the plasma, bile and urine after deconjugation [33,35,42,43,44]. There are reports of 3′-methylquercetin being found in humans [45,46]. COMT is an intracellular enzyme, widely distributed throughout the organs of the body with the highest COMT activity is in the liver, followed by the kidneys and gastrointestinal tract [47]. From comparison experiments, COMT appears to be expressed to a greater extent in HepG2 cells than in human hepatocytes [36]. COMT clearly methylates glucuronides inside HepG2 cells. Inhibition of methylation shunts metabolism towards sulfation after deglucuronidation. Galijatovic *et al*. [39] showed that sulfation of chrysin was more efficient than glucuronidation in both HepG2 and Caco2 cells lines. These results are in agreement with our results for quercetin in the presence of COMT.

MRP2 has previously been implicated in the efflux of quercetin metabolites from the basolateral side of Caco2 cells. Our study showed that *p*-glycoprotein did not play a significant role in the efflux of quercetin metabolites from HepG2 cells. In contrast, we showed that MRP2 was involved in the efflux of glucuronide and sulfate conjugates of quercetin from HepG2 cells. A more potent inhibition of

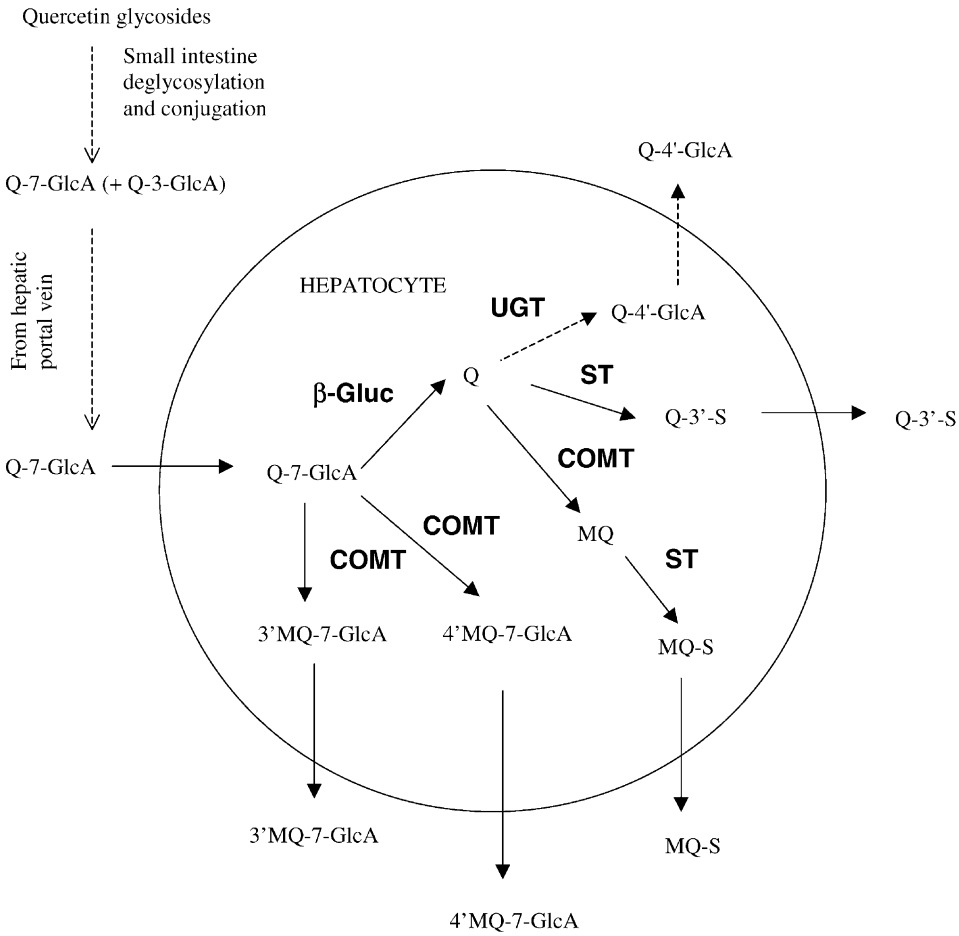


Fig. 8. Proposed mechanism of quercetin-7-glucuronide uptake and metabolism in mammalian liver. Quercetin-7-glucuronide enters into the hepatocyte by an unidentified transporter. Metabolism of the glucuronide involves deglucuronidation by β-glucuronidase, methylation by the enzyme COMT, possible re-glucuronidation by the enzyme UGT and sulfation by ST. Quercetin (derived from deglucuronidation of quercetin-7-glucuronide) can be additionally glucuronidated at different positions but this reaction was only seen for quercetin glucuronides when they were incubated with a COMT inhibitor in HepG2 cells. A comparable metabolic pathway for quercetin-3-glucuronide is also proposed.

glucuronide efflux was observed compared to the sulfate conjugates, indicating that another transporter for sulfate efflux may be present in liver cells. A similar difference between the efflux of glucuronide and sulfate conjugates of chrysin from Caco2 cells was observed [29].

Phase II conjugation of quercetin to methylquercetin, glucuronide and sulfate conjugates are efficient reactions *in vitro* and would be expected to occur even at low concentrations *in vivo*. Fig. 8 illustrates the possible reactions of quercetin-7-glucuronide that could occur *in vivo*, illustrating a route for deconjugation by β-glucuronidase, methylation, glucuronidation and sulfation. We propose that β-glucuronidase present in liver cells will hydrolyse the quercetin glucuronide to the free aglycone and that this deglucuronidation reaction does not occur extracellularly. Under some conditions, quercetin-7-glucuronide can be deglucuronidated and may be re-glucuronidated at the 4′-position, and this reaction also occurs with quercetin. Methylation is likely to be in the 3′- or 4′-position in the liver, with the position of sulfation in the 3′-position for quercetin.

In conclusion, β-glucuronidase present in human liver cells is capable of hydrolysing quercetin glucuronides that enter the liver cell. This results in the release of the aglycone, quercetin, which can then be further metabolised. With HepG2 cells, the metabolic pathway is directed mainly through methylation or sulfation, although in primary hepatocytes, the preferential pathway of metabolism for quercetin glucuronides will be subject to inter-individual variation. Hepatocytes will, under normal dietary intake, encounter conjugated quercetin metabolites produced during passage across the small intestine. It is, therefore, of importance to consider the role of the intact liver in the turnover of this important class of compounds.

Acknowledgments

The work was funded by the European Union (POLYBIND, QLK1-1999-00505), the Biotechnology and Biological Research Council, UK and by a fellowship to K. O'L. (National University of Ireland).

References

[1] Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxcol 1995;33:1061–80.
[2] Hertog MG, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342:1007–11.
[3] Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. Br Med J 1996;312:478–81.
[4] Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardiovascular effects and dietary sources. J Nutr Biochem 1996;96:66–76.
[5] Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933–56.
[6] Kandaswami C, Middleton E. Free radical scavenging and antioxidant activity of plant flavonoids. Adv Exp Med Biol 1994;366:351–76.
[7] van Acker SA, van Balen GP, van den Berg DJ, Bast A, van der Vijgh WJ. Influence of iron chelation on the antioxidant activity of flavonoids. Biochem Pharmacol 1998;56:935–43.
[8] Hollman PCH, de Vries JHM, van Leeuwen SD, Mengelers MJB, Katan MB. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am J Clin Nutr 1995;62:1276–82.
[9] Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJC, Morgan MRA, Williamson G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. FEBS Lett 1998;436:71–5.
[10] Day AJ, Canada FJ, Diaz JC, Kroon PA, McLauchlan R, Faulds CB, Plumb G, Morgan MRA, Williamson G. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett 2000;468:166–70.
[11] Gee JM, DuPont MS, Day AJ, Plumb GW, Williamson G, Johnson IT. Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. J Nutr 2000;130:2765–71.
[12] Crespy V, Morand C, Manach C, Besson C, Demigne C, Remesy C. Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. Am J Physiol 1999;277:G120–6.
[13] Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans CA. The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett 1999;458:224–30.
[14] Boersma MG, van der Woude H, Bogaards J, Boeren S, Vervoort J, Cnubben NHP, van Iersel MLPS, van Bladeren PJ, Rietjens IMCM. Regioselectivity of phase II metabolism of luteolin and quercetin by UDP-glucuronsyl transferases. Chem Res Toxicol 2002;15:662–70.
[15] Aksoy S, Klener J, Weinshilboum RM. Catechol-*O*-methyltransferase pharmacogenetics—photoaffinity-labeling and Western-blot analysis of human liver samples. Pharmacogenetics 1993;3:116–22.
[16] King CD, Rios GR, Green MD, Tephly TR. UDP-glucuronosyltransferases. Curr Drug Met 2000;1:143–61.
[17] Coughtrie MWH, Sharp S, Maxwell K, Innes NP. Biology and function of the reversible sulfation pathway catalysed by human sulfotransferases and sulfatases. Chem Biol Interact 1998;109:3–27.
[18] Day AJ, Bao Y-P, Morgan MRA, Williamson G. Conjugation position of quercetin glucuronides and effect on biological activity. Free Radic Biol Med 2000;29:1234–43.
[19] Donovan JL, Crespy V, Manach C, Morand C, Besson C, Scalbert A, Remesy C. Catechin is metabolized by both the small intestine and liver of rats. J Nutr 2001;131:1753–7.
[20] Paigen K. Mammalian beta-glucuronidase: genetics, molecular biology, and cell biology. Prog Nucleic Acid Res Mol Biol 1989;37:155–205.
[21] Swank RT, Pfister K, Miller D, Chapman V. The easgin gene affects the processing of oligosaccarides of lysosomal β-glucuronidase in liver. Biochem J 1986;240:445–54.
[22] Sperker B, Murdter TE, Schick M, Eckhardt K, Bosslet K, Kroemer HK. Inter-individual variability in expression and activity of human beta-glucuronidase in liver and kidney: consequences for drug metabolism. J Pharmacol Exp Ther 1997;28:914–20.
[23] Whiting JF, Narciso JP, Chapman V, Ransil BJ, Swank RT, Gollan JL. Deconjugation of bilirubin-IX alpha glucuronides: a physiologic role of hepatic microsomal beta-glucuronidase. J Biol Chem 1993;268:23197–201.
[24] O'Leary KA, Day AJ, Needs PW, Sly WS, O'Brien NM, Williamson G. Flavonoid glucuronides are substrates for human liver beta-glucuronidase. FEBS Lett 2001;503:103–6.
[25] Cui Y, Konig J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. J Biol Chem 2001;276:9626–30.

[26] Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am J Physiol Gastrointest Liver Physiol 2000;278: G156–64.
[27] Jedlitschky G, Leier I, Buchholz U, Hummel-Eisenbeiss J, Burchell B, Keppler D. ATP-dependent transport of bilirubin glucuronides by the multidrug resistance protein MRP1 and its hepatocyte canalicular isoform MRP2. Biochem J 1997;327:305–10.
[28] Konig J, Nies AT, Cui Y, Leier I, Keppler D. Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity, and MRP2-mediated drug Resistance. Biochim Biophys Acta 1999;1461:377–94.
[29] Walle UK, French KL, Walgren RA, Walle T. Transport of genistein-7-glucoside by human intestinal CACO-2 cells: potential role for MRP2. Res Commun Mol Pathol Pharmacol 1999;103:45–56.
[30] Walle UK, Galijatovic A, Walle T. Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. Biochem Pharmacol 1999;58:431–8.
[31] Lee TK, Hammond CL, Ballatori N. Intracellular glutathione regulates taurocholate transport in HepG2 cells. Toxicol Appl Pharmacol 2001;174:207–15.
[32] Price KR, Colquhoun IJ, Barnes KA, Rhodes MJC. The composition and content of flavonol glycosides in green beans and their fate during processing. J Agric Food Chem 1998;48:4898–903.
[33] Day AJ, Mellon FA, Barron D, Sarrazin G, Williamson G. Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. Free Radic Res 2001;36:934–8.
[34] Price KR, Bacon JR, Rhodes MJC. Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). J Agric Food Chem 1997;45:938–42.
[35] Manach C, Morand C, Demigne C, Texier O, Regerat F, Remesy C. Bioavailability of rutin and quercetin in rats. FEBS Lett 1997;409: 12–6.
[36] Day AJ. Uptake and metabolism of dietary flavonoid glycosides. Ph.D. Thesis. University of East Anglia, Norwich, UK; 2000.
[37] Knasmuller S, Parzefall W, Sanyal R, Ecker S, Schwab C, Uhl M, Mersch-Sundermann V, Williamson G, Hietsch G, Langer T, Darroudi F, Natarajan AT. Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. Mut Res 1998;402:185–202.
[38] Rebbeor JF, Wang W, Clifton D, Ballatori N. Glutathione S-conjugate formation and metabolism in HepG2 cells: a cell model of mercapturic acid biosynthesis. J Toxicol Environ Health A 1998;53:651–63.
[39] Galijatovic A, Otake Y, Walle UK, Walle T. Extensive metabolism of the flavonoid chrysin by human Caco-2 and HepG2 cells. Xenobiotica 1999;29:1241–56.
[40] Boulton DW, Walle UK, Walle T. Fate of the flavonoid quercetin in human cell lines: chemical instability and metabolism. J Pharm Pharmacol 1999;51:353–9.
[41] Walgren RA, Walle UK, Walle T. Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. Biochem Pharmacol 1998;55:1721–7.
[42] Ueno I, Nakano N, Hirono I. Metabolic fate of [14C]quercetin in the ACI rat. Jpn J Exp Med 1983;53:41–50.
[43] Manach C, Morand C, Texier O, Favier ML, Agullo G, Demigne C, Regerat F, Remesy C. Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. J Nutr 1995;125:1911–22.
[44] Manach C, Texier O, Regerat F, Agullo G, Demigne C, Remesy C. Dietary quercetin is recovered in rat plasma as conjugated derivatives of isorhamnetin and quercetin. J Nutr Biochem 1996;7:375–80.
[45] Manach C, Morand C, Crespy V, Demigne C, Texier O, Regerat F, Remesy C. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett 1998;426:331–6.
[46] Hollman PCH, van Trijp JM, Buysman MN, van der Gaag MS, Mengelers MJB, de Vries JHM, Katan MB. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett 1997;418:152–6.
[47] Karhunen T, Tilgmann C, Ulmanen I, Julkunen I, Panula P. Distribution of catechol-*O*-methyltransferase enzyme in rat tissues. J Histochem Cytochem 1994;42:1079–90.