Available online at www.sciencedirect.com
SCIENCE DIRECT®
Archives of Biochemistry and Biophysics 423 (2004) 148–161
ABB
www.elsevier.com/locate/yabbi

# Cellular uptake and metabolism of flavonoids and their metabolites: implications for their bioactivity

Jeremy P.E. Spencer,* Manal M. Abd El Mohsen, and Catherine Rice-Evans

*Antioxidant Research Group, Wolfson Centre for Age-Related Diseases, GKT School of Biomedical School, King's College London, Guy's Campus, London SE1 9RT, UK*

Received 16 September 2003, and in revised form 5 November 2003

**Abstract**

Flavonoids have been proposed to act as beneficial agents in a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders. The biological effect of these polyphenols and their in vivo circulating metabolites will ultimately depend on the extent to which they associate with cells, either by interactions at the membrane or more importantly their uptake. This review summarises the current knowledge on the cellular uptake of flavonoids and their metabolites with particular relevance to further intracellular metabolism and the generation of potential new bioactive forms. Uptake and metabolism of the circulating forms of flavanols, flavonols, and flavanones into cells of the skin, the brain, and cancer cells is reviewed and potential biological relevance to intracellular formed metabolites is discussed.


*Keywords:* Flavonoid; Cell uptake; Metabolism; Bioactivity

Flavonoids and flavonoid-rich extracts have been implicated as beneficial agents in a multitude of disease states (reviewed in [1,2]), most commonly cancer [3–7], cardiovascular disease [6,8–10], and neurodegenerative disorders [11–15]. However, it has become clear over the last few years that the bioactive forms of flavonoids in vivo are not necessarily the natural phytochemical forms, for example the aglycones or their various glycosides, but instead conjugates and metabolites arising from these on absorption (reviewed in [16–20]. In particular, there is now strong evidence for the extensive phase I deglycosylation and phase II metabolism of the resulting aglycones such as quercetin, hesperetin, naringenin, and epicatechin to glucuronides, sulphates, and O-methylated forms during transfer across the small intestine [21] and then again in the liver (Fig. 1). Further transformation has been reported in the colon where the enzymes of the gut microflora degrade flavonoids to simple phenolic acids, which may also be absorbed and subsequently further metabolised in the liver. Current interest in the elucidation of the potential mechanism of action of flavonoids in vivo has centred on the bioactivity of these phase I and II metabolites in various cell systems.

Circulating glucuronides, sulphates, and O-methylated forms are believed to be those most likely to exert bioactivity and express beneficial effects in humans and animals [22–26]. Although it is feasible that glucuronides, sulphates, and O-methylated forms (Fig. 1) may participate directly in plasma antioxidant reactions by scavenging reactive oxygen and nitrogen species in the circulation in a similar manner to their parent aglycones [27–31], their circulating levels per se may be too low to be relevant in this context. However, it remains unclear as to whether these forms express biological activities at the cellular level. The cellular effects of flavonoid metabolites will ultimately depend on the extent to which they associate with cells, either by interactions at the membrane or uptake into the cytosol. Information regarding uptake of flavonoids and their metabolites from the circulation into various cell types and whether they are modified further by cell interactions has become increasingly important as attention focuses on the new concept of flavonoids as potential modulators of intracellular signalling cascades vital to cellular function.

* Corresponding author. Fax: +44-207-848-6143.
*E-mail address:* jeremy.spencer@kcl.ac.uk (J.P.E. Spencer).

0003-9861/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2003.11.010

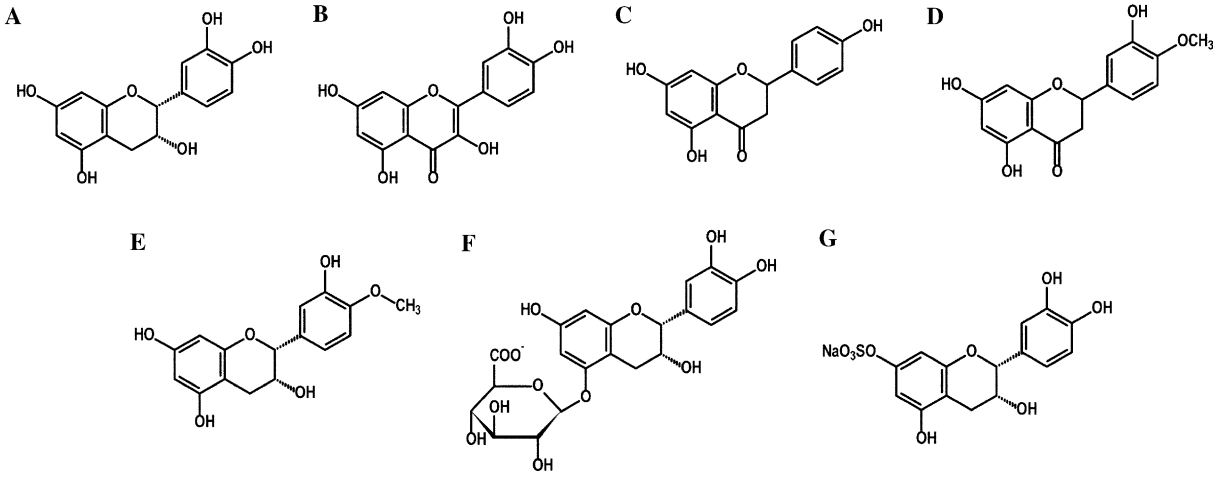


Fig. 1. Structures of flavonoids and their circulating metabolites: (A) epicatechin, (B) quercetin, (C) naringenin, (D) hesperetin, (E) 3′-*O*-methyl epicatechin, and (F) epicatechin-5-*O*-β-D-glucuronide, epicatechin-7-sulphate. Glucuronide and sulphate conjugates are formed with the majority of flavonoids in the small intestine and liver, whilst O-methylated forms are only formed where the flavonoid has a catechol B-ring.

This review summarises the current knowledge on the cellular uptake of flavonoids and their metabolites. The difference in the way various cell types respond to flavonoids and their metabolites is reviewed with particular reference to the intracellular metabolism and their modulation of biological function. We illustrate this by contrasting the uptake and metabolism of circulating forms of flavanols, flavonols, and flavanones into cells of the skin, the brain, and finally cancer cells. The potential biological relevance to intracellularly formed metabolites is discussed.

## Uptake and metabolism in different cell types

As mentioned, consideration of the potential bioactive mechanisms of flavonoids and their in vivo metabolites in cell systems takes account of their uptake and potential for subsequent cellular metabolism. The uptake of flavonoids and their in vivo metabolites is dependent on cell type. However, this is most probably due to a greater level of intracellular metabolism and faster rate of export from some cells rather than simply differing levels of passive diffusion. In this review, uptake data are expressed as ng of flavonoid and/or metabolite per mg protein. Calculation of intracellular concentration from these data is difficult due to the absence of precise data in the literature for the exact volume of the various cell types. It should be noted that the extraction protocols used in the majority of studies below do not distinguish between cytosolic flavonoid and that which is membrane-bound or otherwise cell-associated. Consequently, the use of the term "uptake" in this review is used to encompass both cytosolic accumulation of flavonoids and that which is membrane associated, unless otherwise stated. As the precise mechanism of flavonoid action is not clearly established, both forms of accumulation may be important as flavonoids may act via interactions with membrane receptors as well as with cytosolic proteins.

This review deals with the uptake of the main groups of flavonoids into different cell types of peripheral tissue. However, for the purpose of this review, we will exclude cells of the liver and the gastrointestinal tract as these are well reported to take up and metabolise flavonoids. It is now well established that the gastrointestinal tract plays a significant role in the metabolism and conjugation of polyphenols before entry into the systemic circulation and the liver [16,17,21,32–34]. Enterocytes in the jejunum and ileum of the small intestine transfer flavonoids from the luminal side of the gut to portal vein during which there is significant glucuronidation of nearly all flavonoids tested by the action of UDP-glucuronosyltransferase enzymes [17,18,35–39]. In addition, in the case of flavonoids containing a catechol containing B-ring there is also extensive O-methylation catalysed by the action of COMT [21]. A full assessment of uptake and metabolism of flavonoids by liver and GI tract cells is beyond the scope of this review but has been reviewed previously [16–20] and is summarised in Fig. 2.

The following sub-sections contain cell uptake data for both flavonoid aglycones and O-methylated forms. In vivo these forms would primarily exist as either glucuronide or sulphate conjugates [40]. However, there is the possibility that both flavonoid and O-methylated flavonoid glucuronides may be de-conjugated by the action of β-glucuronidases present in human tissues such as liver or small intestine [41] or during local conditions of inflammation. In this case, free aglycone or O-methylated forms will be released and may go on to express cellular effects. Indeed, β-glucuronidases are present in a number of tissues within the body [42] and

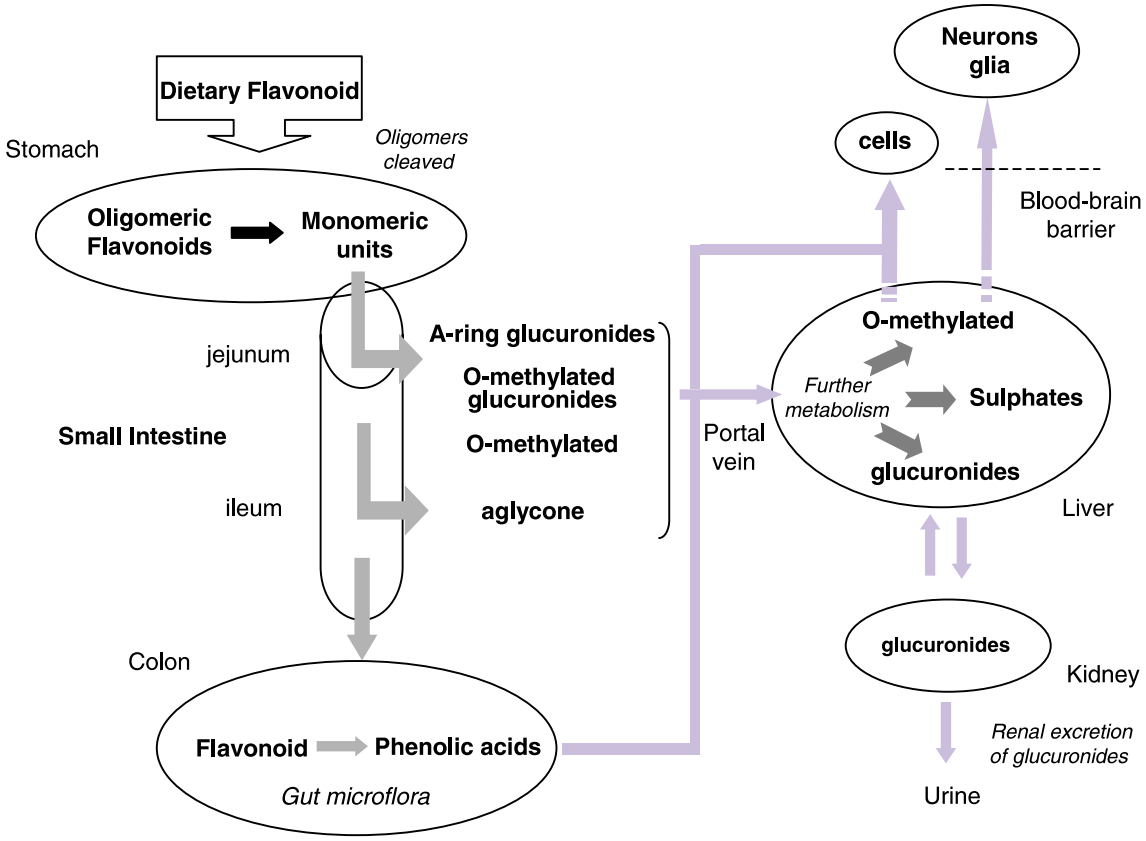


Fig. 2. Summary of the formation of gastrointestinal tract and hepatic metabolites and conjugates of flavonoids in humans. Cleavage of oligomeric flavonoids such as procyanidins may occur in the stomach in environments of low pH. All classes of flavonoids undergo extensive metabolism in the jejunum and ileum of the small intestine and resulting metabolites enter the portal vein and undergo further metabolism in the liver. Colonic microflora degrade flavonoids into smaller phenolic acids that may also be absorbed. The fate of most of these metabolites is renal excretion, although, some may enter cells and tissues.

may be released by certain cells. For example, histamine causes rapid exocytosis of lung macrophages [43] and luteolin monoglucuronide is cleaved to free luteolin by β-glucuronidase released from neutrophils stimulated with ionomycin [44,45].

*Cells of the skin*

To further the understanding of the potential protective actions of flavonoids against dermal injury, especially involving UV-induced photoaging [46], inflammation [47,48], and cancer [49–51], the uptake of flavonoids into cells derived from the skin has been investigated. There is strong evidence to suggest that flavonoids, such as those found in green tea, may act to inhibit UV-induced skin damage [52–55]. These investigations suggest that topical applications of flavanols such as EGCG to skin may afford some protection against UV-induced immunosuppression, photoaging, inflammatory dermatoses, and photocarcinogenesis [52,55]. Interestingly, there are an increasing number of commercial skincare products containing flavonoids or flavonoid-extracts even though at this time, their mechanism of action has not been elucidated nor has their beneficial effect been critically evaluated.

Studies utilising normal human dermal fibroblasts have highlighted the relative abilities for flavonoid uptake, and in some cases this uptake has been linked to protection against oxidative stress-induced cell injury [22,25]. Uptake experiments have revealed the association of flavanols into fibroblasts at both 2 and 18 h of exposure [25]. Epicatechin and its in vivo metabolites, 3′-*O*-methyl epicatechin, and 4′-*O*-methyl epicatechin, have been observed to be associated with fibroblasts, at relatively low levels (Table 1). However, no epicatechin-5-*O*-β-D-glucuronide was detected (Table 1) [25].

In contrast, the uptake of the more lipophilic flavonol quercetin into cultured fibroblasts was higher than that recorded for flavanols (Table 1). In addition, significant intracellular metabolism was observed. Uptake of quercetin led to a time-dependent appearance of three products, quercetin itself, 2′-glutathionyl quercetin, and a quercetin quinone/quinone methide [56]. The formation of the glutathione adduct of quercetin may occur in cells either via enzymatic transfer of the thiol to the flavonol by glutathione *S*-transferase or by nucleophilic

Table 1
Uptake and metabolism of flavonoids and their circulating metabolites into cells derived from the skin

| Flavonoid | Fibroblast | | |
|---|---|---|---|
| | 2 h | 18 h | Metabolites |
| Flavanol | | | |
| EC | 37.1 | 39.2 | — |
| 3OMeEC | 47.9 | 45.6 | — |
| 4OMeEC | 61.6 | 52.4 | — |
| EC glucuronide | 0.0 | 0.0 | — |
| Flavonol | | | |
| Quercetin | 53.8 | 12.7 | Oxidative Q-GSH |
| 3OMeQ | 192.4 | 87.4 | Oxidative demethylation |
| 4OMeQ | 129.6 | 181 | Demethylation |
| Q-glucuronide | 0.0 | 0.0 | — |
| Q-GSH | 1.3 | 0.6 | — |
| Q-3-glucoside | 0.0 | 0.0 | — |
| Kaempferol | 41.7 | 29.5 | Oxidative |
| Flavanone | | | |
| Naringenin | 546 | 235 | Glucuronide** |
| NG | 0.0 | 0.0 | — |
| Hesperetin | 1178 | 1297 | Glucuronides* |
| HG | 0.0 | 0.0 | — |

All data are mean values of uptake and are represented ng/mg protein.
* Indicates that metabolite was measured in the medium and not in the cell lysate.
** Indicates identification is based on spectral characteristics and the co-elution with authentic standard and not with LC-MS/MS. All other metabolites were characterised by LC-MS/MS and measured in both cell lysates and exposure medium.

addition of the thiol to quercetin quinone (Fig. 3). Furthermore, the appearance of both quercetin–glutathione conjugates in the medium with time indicates an active export of this intracellularly formed metabolite [56]. This suggests that in vivo, once in the extracellular environment they may be transported to tissues that are capable of accumulating these metabolites [57,58] and may conceivably undergo further processing before being ultimately excreted form the body (see Flavonoid–thiol conjugates).

In comparison with quercetin, one of its in vivo physiological metabolites, 4′-*O*-methyl quercetin, showed more extensive time-dependent uptake [56]. This uptake was accompanied by minor intracellular processing to quercetin. Previous studies have described a cytochrome P450-dependent demethylation of 4′-*O*-methyl quercetin in human liver microsomes, which is not specific for the 3′-O-methylated form [59]. Furthermore, the formation of oxidation products of 4′-O-methyl quercetin was not detected, presumably because this metabolite cannot take the same metabolic route as quercetin as it lacks conjugating hydroxyl groups allowing oxidation across the flavonol structure. In contrast, the 3′-*O*-methyl quercetin was observed to yield high levels of intracellular oxidation products similar to those formed from quercetin (Fig. 3). This metabolite has a free 4′-OH group on the B-ring and is able to autoxidise or is subjected to the action of peroxidases to form oxidation products such as 3′-*O*-methyl quercetin-5-quinone methide. It is possible that these intracellularly formed metabolites may participate in cellular reactions, for example, by direct modulation of signalling pathways such as the mitogen activated protein kinase (MAP kinase) or phosphoinositol-3-kinase (PI3 kinase)/Akt pathway [11,23,60] (Fig. 3). Finally, consistent with the observation with epicatechin glucuronide, no uptake or metabolism was observed with quercetin-7-*O*-β-D-glucuronide, presumably due to its inability to enter the cells. The uptake and metabolism of flavonols in fibroblasts is summarised in Fig. 3.

The flavanones hesperetin and naringenin demonstrated the greatest uptake into fibroblasts and were observed to undergo significant metabolism following uptake (Table 1). Whilst hesperetin was observed at high amounts in cell lysates, the glucuronide, as expected, did not appear to associate with the cells [61]. However, the appearance of hesperetin glucuronide in the medium of fibroblasts exposed to the aglycone hesperetin indicates metabolism in the form of intracellular glucuronidation, followed by rapid cellular export. Naringenin was also observed to be glucuronidated in fibroblasts with naringenin glucuronide apparent in the lysates as well as in the medium (Table 1; unpublished data). The levels of naringenin aglycone were low as compared to those of hesperetin aglycone. These data support the idea that human dermal fibroblasts contain UDP-glucuronosyl- transferases capable of glucuronidating flavanones. The fact that no glucuronidation was observed with the other flavonoids, such as epicatechin and quercetin, suggests either that these UDP-glucuronosyltransferases are only specific for flavanones or that export pumps are limited in the efficient export of flavanol and flavonol glucuronides. These observations suggest that glucuronidation may occur in vivo in tissues other than in small intestine and liver, and may denote the processing of flavanones by fibroblasts to aid export and removal of these compounds from cells. The UDP-glucuronosyltransferase isoform, UGT2B11, has been reported in skin cells (mainly located in the stratum corneum) [62–65] and a number of other cell types in the body. The rapid exclusion of flavanone glucuronides from the cells suggests that dermal fibroblasts may have conjugate export pumps similar to those found in the hepatocytes or at the blood–brain barrier (see Cells of the central nervous system) [66,67]. These pumps, which also presumably export quercetin glutathione conjugates from fibroblasts, are likely to be multidrug-resistance-associated transport proteins such as MRP-1 and MDR-1, which mediate ATP-dependent transport of a variety of lipophilic substances conjugated to glucuronic acid, glutathione or sulphate [68].

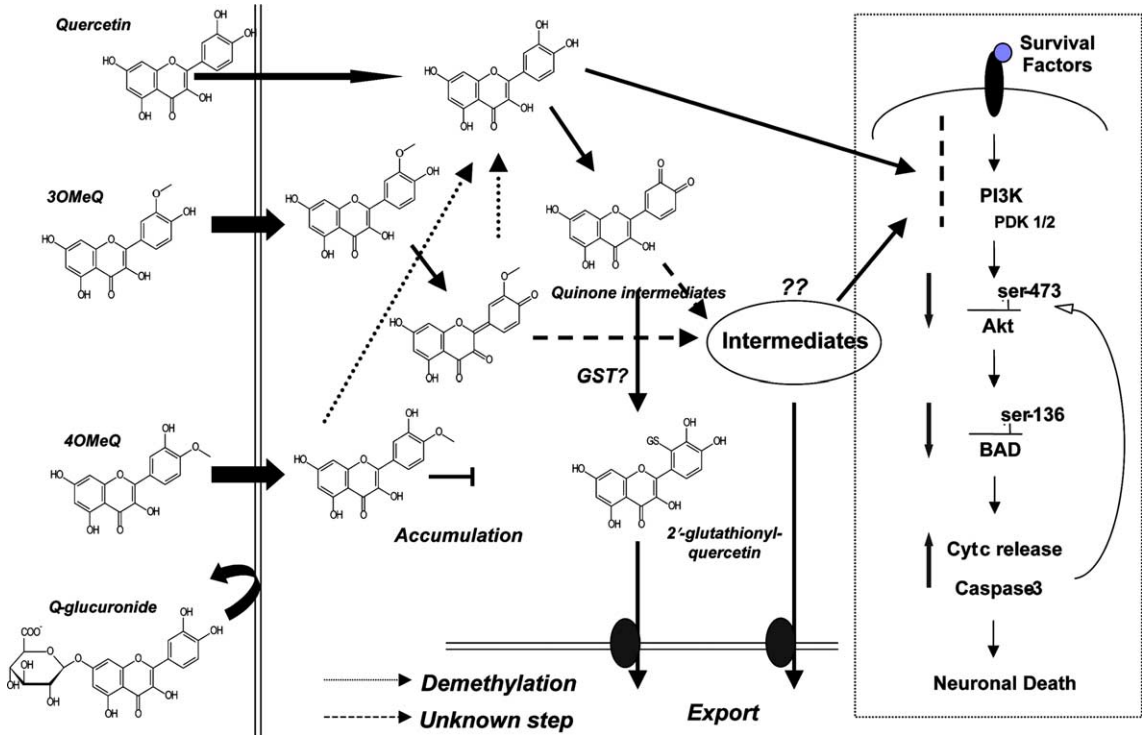


Fig. 3. Summary of the cellular metabolism of quercetin and its O-methylated metabolites in fibroblasts and their possible modulation of signalling pathways. O-methylated forms of quercetin associate with fibroblasts to a greater extent than quercetin, whereas the glucuronide does not enter. Quercetin and 3′-*O*-methyl quercetin undergo oxidative metabolism within the cell to yield quinones and quinone methide intermediates and a small amount of 4′-*O*-methyl quercetin is demethylated. Quercetin *O*-quinone may react directly with GSH or with glutathione *S*-transferase to form a 2′-glutathionyl conjugate. Both the GSH conjugate and oxidative products are exported from cells.

*Cells of the central nervous system*

Recent epidemiological and dietary intervention studies in both humans and animals have suggested that diet-derived phenolics, in particular the flavonoids, may play a beneficial role in the prevention of neurodegeneration, age-related cognitive and motor decline [64,65], and brain ischaemia/reperfusion injury [12]. For example, the neuroprotective action of one of the green tea flavonoids, epigallocatechin gallate (EGCG), has been shown in both oxidative stress- [69] and Aβ-induced [70] neuronal death models. Protective effects in these neuronal systems were linked to a modulation of signalling processes through protein kinase C and/or modulation of cell survival/cell cycle genes [69,70]. Furthermore, the actions of quercetin on neuronal viability are mediated by interactions within both Akt/PKB and ERK1/2 signalling pathways [23]. These mechanistic observations are significant, as actions exerted through signalling cascades are likely to proceed at intracellular concentrations lower than those required for antioxidant actions. This is important as the accumulation of flavonoids in the brain and their potential bioactivity may be restricted by processes at the blood–brain barrier (BBB) in that it may aid or prevent the entry of such compounds to the brain.

The citrus flavonoids, hesperetin, naringenin, and their relevant in vivo metabolites, as well as the dietary anthocyanins, cyanidin-3-rutinoside, and pelargonidin-3-glucoside, have been observed to be taken up by two brain endothelial cell lines from mouse (bEND5) and rat (RBE4) [71]. In both cell types, the uptake of hesperetin and naringenin was the greatest, relative to their more polar glucuronidated conjugates, to epicatechin and its metabolites, and to the dietary anthocyanins and specific phenolic acids derived from colonic biotransformation of flavonoids [71]. These results demonstrate that flavonoids and their metabolites are able to traverse the BBB but that this potential for permeation is dependent on compound lipophilicity. Consequently, the uptake of O-methylated metabolites of flavonoids, for example, 3′-*O*-methyl quercetin, may be greater than that of the parent aglycone. For the same reason, flavonoid glucuronides, such as quercetin-7-*O*-β-D-glucuronide, might not be expected to transverse the BBB due to their relatively low apparent permeability ($P_{app}$) values. However, studies have shown that certain drug glucuronides, such as morphine-6-glucuronide, are capable of crossing the blood–brain barrier [72] and mediating

pharmacological effects [73–75], thus suggesting that there may be a specific uptake mechanism for glucuronides in vivo. Furthermore, epicatechin glucuronides have been detected in brain following oral administration of epicatechin to rats [76] (Tissue accumulation following feeding studies), although it is not clear whether this reflects the BBB transfer to the glucuronide or formation within the brain.

Little is known about the distribution of flavonoids into different brain regions following BBB transfer. Although some studies have investigated flavonoid distribution to the brain following oral administration (see Tissue accumulation following feeding studies), these studies were unable to identify specific transport and/or metabolism mechanisms. The uptake of epicatechin and its O-methylated metabolites has been demonstrated in primary cultures of mouse cortical neurons [25] (Table 2). This uptake of epicatechin, and indeed its two O-methylated metabolites, was low in comparison to that observed in primary mouse astrocytes (Table 2). Interestingly, the uptake observed using astrocytes was comparable to that observed in dermal fibroblasts, although astrocytes were shown to metabolise the flavanols to some extent whilst no such metabolism was measured in fibroblasts. A monoglutathionyl adduct of epicatechin was present in both astrocyte lysates and medium following exposure to epicatechin. Similarly, glutathione conjugation was also observed following exposure of quercetin to astrocytes. Although no glutathione adducts of quercetin were observed in neurons, oxidative metabolites were measured in both brain cell types indicating a common pathway for metabolism of quercetin. Consistent with this, the structurally related flavonol, kaempferol, also underwent oxidative metabolism in both neurons and astrocytes.

As was observed with O-methylated metabolites of epicatechin, O-methylated forms of quercetin were also accumulated to a greater amount by both neurons and astrocytes. This accumulation was higher at 2 h than at 18, especially in the case of the 3′-*O*-methyl metabolite (Table 2). Whilst demethylation is a possible contributor to lower cellular levels of these compounds after prolonged exposure, oxidative metabolism of 3′-*O*-methyl form, as was observed in fibroblasts, is likely to be the primary basis for the clearance of these compounds from cells. Demethylation of the methylated forms of both epicatechin and quercetin was only identified in astrocytes and was not detected in neurons. It is clear from these studies that astrocytes accumulate higher levels of flavonoids and are capable of metabolising them via non-oxidative routes. These observations may have important implications in vivo where glial and neuronal populations coexist. Astrocytic accumulation and metabolism of flavonoids may occur before they are exposed to neurons (Fig. 4). Astrocytic metabolites, along with parent compounds, may then pass into neurons where they may influence neuronal function. Glial metabolites such as glutathionyl adducts of flavonoids may be important in mediating effects on neurons following their import. Alternatively, the uptake and metabolism of reactive flavonoids such as quercetin in astrocytes may act to limit their uptake into neurons, thus reducing the potential for neuronal

Table 2
Uptake and metabolism of flavonoids and their circulating metabolites into cells derived from the brain

| Flavonoid | Neuron | | | Astrocyte | | |
|---|---|---|---|---|---|---|
| | 2 h | 18 h | Metabolites | 2 h | 18 h | Metabolites |
| Flavanol | | | | | | |
| EC | 10.4 | 8.8 | — | 43.5 | 38.7 | EC-GSH |
| 3OMeEC | 30.4 | 29.1 | — | 87.8 | 77.1 | Demethylation |
| 4OMeEC | 42.6 | 45.6 | — | 92.3 | 85.4 | Demethylation |
| EC glucuronide | 0.0 | 0.0 | — | 43.5 | 38.7 | — |
| Flavonol | | | | | | |
| Quercetin | 32.1 | 20.2 | Oxidative | 74.3 | 11.2 | Oxidative Q-GSH |
| 3OMeQ | 112.1 | 47.8 | Oxidative | 187.9 | 56.4 | Oxidative demethylation |
| 4OMeQ | 123 | 97.2 | — | 204.3 | 187.3 | Demethylation |
| Q-glucuronide | 0.0 | 0.0 | — | 0.0 | 0.0 | — |
| Q-GSH | 2.3 | 0.7 | — | 0.5 | 0.8 | — |
| Q-3-glucoside | 0.0 | 0.0 | — | NT | NT | — |
| Kaempferol | 25.3 | 14.8 | Oxidative | NT | NT | Oxidative |
| Flavanone | | | | | | |
| Naringenin | 476.3 | 412.8 | — | 564.2 | 449.6 | — |
| NG | 0.0 | 0.0 | — | 0.4 | 0.0 | — |
| Hesperetin | NT | NT | — | NT | NT | — |
| HG | NT | NT | — | NT | NT | — |

All data are mean values of uptake and are represented ng/mg protein.

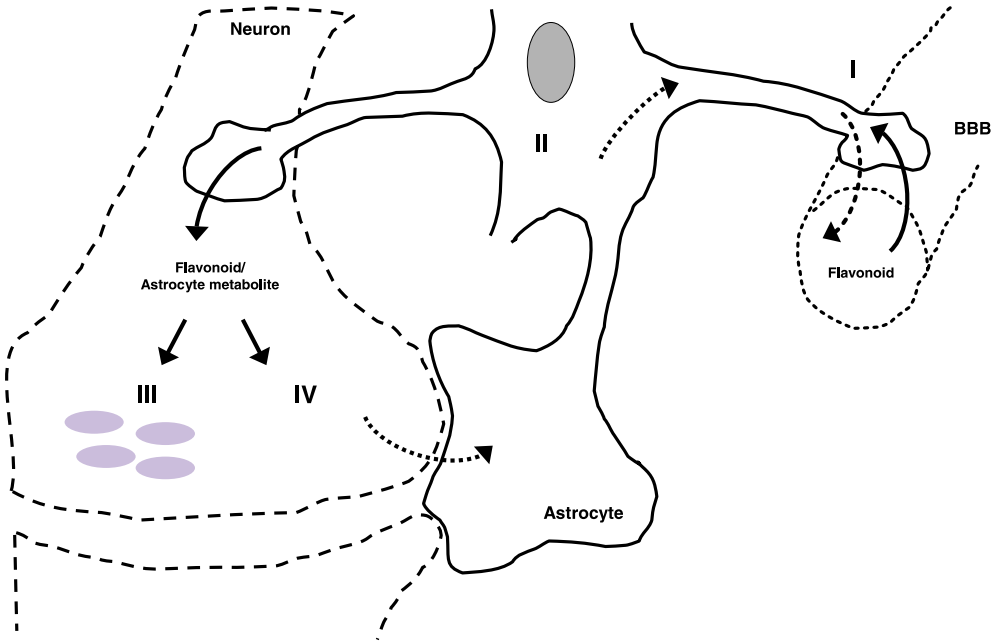


Fig. 4. Uptake and metabolism of flavonoids in the brain. Flavonoids cross blood–brain barrier (I) and enter astrocytes where they are metabolised to form various oxidative metabolites and thiol conjugates (II). Flavonoids and metabolites may then access neurons where they may exert biological function at mitochondria (III) or signalling cascades (IV). Astrocytic metabolism may act to protect neurons from exposure to excessive amounts of potentially reactive and/or toxic polyphenols such as quercetin. However, exposure of neurons to oxidative metabolites formed in astrocytes may have a potentially harmful influence on neuronal function.

damage [23]. Studies to investigate uptake and metabolism into neurons and glial cells in co-culture should shed further light on this issue.

*Cancer cells*

The ability of certain flavonoids to act as pro-oxidants in cell systems has been suggested to be a potential mechanism by which they may act as anti-carcinogenic compounds in vivo due to their abilities to promote cell death [7,77,78]. However, whilst flavonoids may readily undergo oxidation in culture medium in vivo such oxidation will be limited due to efficient transition metal ion sequestering and low oxygen tension. In addition, such pro-oxidant effects are usually only observed when relatively high concentrations of flavonoids (high μM to mM) have been applied to cells.

The ways in which flavonoids may act as chemopreventive agents may be divided into three distinct mechanisms: (1) prevention of carcinogen metabolic activation, (2) prevention of tumour cell proliferation by inactivation or down-regulation of pro-oxidant enzymes or signal transduction enzymes, and (3) by inducing tumour cell death (apoptosis) (reviewed in [7]). Clearly, the extent of uptake and the level of metabolism of flavonoids in cancer cells will be central to these mechanisms of actions. The uptake, metabolism, and elimination of quercetin have been investigated in a human hepatocarcinoma cell line Hep G2 using $^{14}$C-labelled compound [79]. These cells showed a 9.6-fold accumulation of quercetin and the formation of a 3′-O-methylated metabolite. However, a rapid elimination of quercetin, with no compound detectable beyond 8 h, was proposed to be the result of extensive oxidative metabolism/degradation [79]. The initial intermediate reaction appears to involve peroxidation, leading to a dioxetan, as evidenced by a LC/MS analysis. Subsequently, opening of the C-ring leads to the formation of carboxylic acids, the major one identified in this study as protocatechuic acid. A separate reaction results in a polymeric quercetin product that presumably forms following an initial oxidation step. It is postulated that these degradative and metabolic changes may contribute to the multiple cellular effects reported for quercetin in cell culture models and potentially in vivo. Indeed the potential of quercetin to act as a cytotoxic agent, a favourable property in the case of uptake into cancer cells, has been linked to its intracellular oxidative metabolism to *o*-quinones [23,56,80].

The cellular uptake of epicatechin, epigallocatechin gallate, gallic acid, and quercetin-3-glucoside and their effect on the cell cycle has been investigated in human colon adenocarcinoma cells (Caco-2) [81]. Epicatechin, epigallocatechin gallate, gallic acid, and quercetin-3-glucoside all showed significant uptake into Caco-2 cells at 24 h [81]. The authors report that, epicatechin achieved levels of $0.43 \pm 0.22$ ng/$10^6$ cells after 24 h of incubation and increased to $3.50 \pm 2.2$ ng/$10^6$ cells

following 72 h of exposure. In addition, the uptake of EC[1] and EGCG showed differences with EC increasing steadily between 12 and 72 h and EGCG reaching maximum levels at 48 h. However, whilst the uptake of both EC and EGCG was high at all exposure times, quercetin-3-glucoside was less readily taken up and was undetectable at the 72 h exposure time [81]. Possible reasons for the low accumulation of this glycoside include the inability of the polar flavonol glucoside to enter cells, as has been observed with flavonoid glucuronides and glucosides in fibroblasts and brain cells (Tables 1 and 2). Alternatively, this compound may be a better substrate for multi-drug resistance-associated protein MRP2 [82,83]. Indirect evidence of flavonoid uptake comes from the fact that the poly-O-methylated flavonoids, nobiletin and tangeretin, markedly inhibit human squamous cell carcinoma (HTB43) growth whereas quercetin and taxifolin have no significant effects [84]. The difference in activity may be due to the relatively greater membrane uptake of the poly-O-methylated flavonoids since methoxylation of the phenolic groups decreases hydrophilicity of the flavonoid. This phenomenon has been observed with other flavonoid metabolites and different cell types in that in all cases levels of accumulation of O-methylated flavonoids are higher than that of the native flavonoid aglycone (Tables 1 and 2).

*Tissue accumulation following feeding studies*

In addition to measurement of the cellular uptake of flavonoids, some studies have attempted to measure tissue levels of flavonoid following in vivo feeding experiments. Whilst these investigations are limited to providing accurate uptake and metabolism assessments, they provide essential information about the potential accumulation of flavonoids by cells in vivo. Most of these investigations have concentrated on the detection of levels of flavonoids in the brain. One such study has provided evidence for the ability of epicatechin and its physiological metabolites, epicatechin glucuronide and O-methylated glucuronide, to access the brain, although their presence in the brain may reflect formation of glucuronides within the brain rather than BBB transfer [76]. There is also evidence for the localization of the citrus flavonoids, naringenin, and hesperetin, within the brain [85,86]. However, in these studies the flavonoids were administered intravenously and thus are not representative of normal dietary consumption and the effects of gastrointestinal metabolism. Evidence also exists for the ability of the O-methylated flavonoid, tangeretin, to cross the blood–brain barrier and interact with brain cells [87]. Concentrations of tangeretin, or metabolites derived from it varied, with the brain stem and cerebellum having lowest concentrations (0.17 and 0.27 ng/mg tissue, respectively) whilst highest concentrations were seen in the hippocampus, striatum, and hypothalamus (2.0, 2.36, and 3.88 ng/mg tissue, respectively) [87].

Valuable information have also been gained via the use of radiolabelled flavonoids, which allows the accumulation of flavonoids and all metabolites derived from them, to be monitored in different organs and tissues following administration, although characterisation of the metabolites is not possible. In a recent study utilising $^{14}$C-labelled quercetin-4′-glucoside, only 6.4% of the recovered radioactivity was detected outside the gastrointestinal tract 60 min after consumption by rats [88], suggesting limited absorption and low levels of metabolites in plasma and peripheral tissues. Another feeding investigation using [$^{3}$H](−)-epigallocatechin gallate led to the detection of radioactivity in various organs, including the liver, lung, pancreas, mammary gland, skin, brain, kidney, uterus and ovary, and testes [89]. This study might suggest that flavonoids, or metabolites derived from them, may be present in, and interact with, many different organs in the body following oral intake. The use of labelled flavonoids in determination tissue accumulation in vivo is a powerful tool and should be exploited in future.

Tissue accumulation of flavonoids has also been assessed ex vivo following perfusion of isolated rat heart under normoxic conditions in a recirculating Langendorff model. Here, perfusion with the anthocyanin, cyanidin-3-*O*-β-glucopyranoside (10 or 30 μM), for 30 min provoked an intracellular accumulation of 0.021 and 0.056 μmol/g dry weight, respectively [90], indicating that association of flavonoids with cardiac cells may occur in vivo. The use of this type of investigation may be useful in future to link levels measured in cell studies with those recorded in vivo feeding experiments.

## Potential biological function of intracellular metabolites

Much research has now established that flavonoids are extensively metabolised during absorption in the small intestine and again in the liver (reviewed in [16–20]. Flavonoids not assimilated in the small intestine will enter the large intestine where they are subjected to degradation by enzymes of the colonic microflora. These events lead to the generation of a number of flavonoid metabolites of biological relevance [22,25,60]. Glucuronide and sulphate conjugates, O-methylated forms, and O-methylated glucuronidated adducts are now believed to be most biologically relevant in terms of their actions in vivo. However, cellular actions will also be dependent

[1] *Abbreviations used:* EC, epicatechin; 3OMeEC, 3′-*O*-methyl epicatechin; 4OMeEC, 4′-*O*-methyl epicatechin; EC-GSH, glutathionyl conjugate of epicatechin; Q, quercetin; 3OMeQ, 3′-*O*-methyl quercetin; 4OMeQ, 4′-*O*-methyl quercetin; Q-GSH, 2′-glutathionyl quercetin; NG, naringenin glucuronide; HG, hesperetin glucuronide.

on the uptake and metabolism of these circulating species, as discussed above, and on new cellular-generated metabolites, which themselves may exert biological actions. Evidence suggests that intracellular metabolism of flavonoids may be grouped into three categories: (1) conjugation with thiols, particularly GSH, (2) oxidative metabolism, and (3) P450-related metabolism. As mentioned, resulting cell metabolites may have varying biological activities that may be different to that of the parent molecule. Although very little is known about these metabolites, evidence from some studies hints that these novel cellular metabolites may mediate the effects of flavonoids in some cell studies [23,56,91].

*Flavonoid–thiol conjugates*

Thus far, little attention has been given to the identification of thiol metabolites of flavonoids in either the circulation or the urine. Similarly, few studies have investigated the potential biological effects of flavonoid–thiol adducts. The formation of GSH adducts of flavonoids has been shown in both fibroblasts [56] and B16F-10 melanoma cells [91]. Recently, it has been demonstrated that cysteine reacts faster with quercetin quinones than GSH and *N*-acetylcysteine [92] in vitro. However, this preferential scavenging by cysteine over GSH may not reflect in vivo situations where physiological concentrations of GSH are substantially higher than those of free cysteine. This is likely to shift the balance of thiol conjugate formation in favour of glutathionyl adducts in the body. It is also conceivable that in biological systems the covalent addition of quercetin quinone to tissue protein sulphydryl groups could occur. As mentioned, these protein–flavonoid interactions may be potentially important, especially as many enzymes contain important cysteine residues within catalytic or regulatory sites.

The biological action of polyphenolic–GSH conjugates may be limited by their export from the cells in which they are formed [56]. In addition, exposure of fibroblasts to 2′-glutathionyl quercetin indicated that its toxicity is significantly lower than that of quercetin, suggesting that this conjugate either has reduced cytotoxic potential or, more likely, a reduced potential for cell uptake. Indeed, uptake of 2′-glutathionyl quercetin into fibroblasts (Table 1) and brain cells (Table 2) is very low relative to quercetin. This is likely to be due to the increased polarity of the thiol conjugate, similar to those of flavonoid glucuronides, which also do not enter cells. However, the potential cytotoxicity of intracellularly formed thiol conjugates cannot be ruled out. In fact, glutathionyl conjugates originating from a variety of polyphenol quinones have been observed to display a wide array of cellular activities [57]. Indeed, the redox activity of polyphenols is frequently enhanced following conjugation with GSH [57,58] and thus does not necessarily result in detoxification. Only when GSH conjugation is coupled to the subsequent export of the adduct from cells, 'so-called' phase III metabolism [93], will detoxification be the predominant consequence. Indeed, once in the extracellular environment and/or circulation they may interact with cells capable of accumulating these metabolites, for example, those of the kidney [57]. Indeed, glutathionyl conjugates of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) express strong nephrotoxic effects by redox cycling reactions at the apical membrane of renal proximal tubular cells [94]. In addition, cytotoxicity may occur due to the intracellular formation of these conjugates, possibly due to a lowering of cellular thiol levels [95] or by the binding of quinone intermediates to cysteine residues at the active site of specific enzymes [96]. Similar chemistry is involved in the reaction of other catechols such as the catecholamines, dopamine, and L-DOPA. Their conjugation with cysteine or GSH [96–98] can lead to lower GSH levels and to the generation of mitochondrial toxins with relevance to Parkinson's disease [99]. Similarly, the formation of a quercetin glutathione conjugate in fibroblasts [56] and astrocytes (Table 2) may provide an insight into the toxic effects of quercetin [23].

The fate of flavonoid–glutathione conjugates is unclear. Glutathione adducts of flavonoids, formed in cells or in the liver, enter the circulation where they may be taken up into other cells of the body exerting biological actions. More likely, they are excreted into the bile as excretion from the liver depends partly upon molecular weight with compounds above 350 limited from release into the bloodstream. As all flavonoid glutathione conjugates would have molecular weights over 500, the biliary route of excretion would be favoured. Thus, flavonoid–glutathione adducts may enter the duodenum where glutamic acid and glycine are sequentially removed by enzymes in the bile duct or by micro-organisms in the GI tract leading to the formation of cysteinyl conjugates. Cysteine conjugates may be reabsorbed from the GI tract back into the circulation where they may undergo cellular uptake and/or excretion in the urine following the addition of an acetyl group in the kidney (Fig. 5). This pathway of glutathione conjugate processing has been demonstrated for xenobiotics [94]. Flavonoid–cysteine adducts may exert biological effects in vivo after possible re-uptake from the GI tract and may represent another route of elimination of these compounds from the body.

*Oxidative metabolites*

Certain flavonoids, in particular those with a di-hydroxylated or catechol B-ring, have the ability to oxidise under certain conditions [77,78]. In doing so, it is believed they may generate toxic quinone forms and even

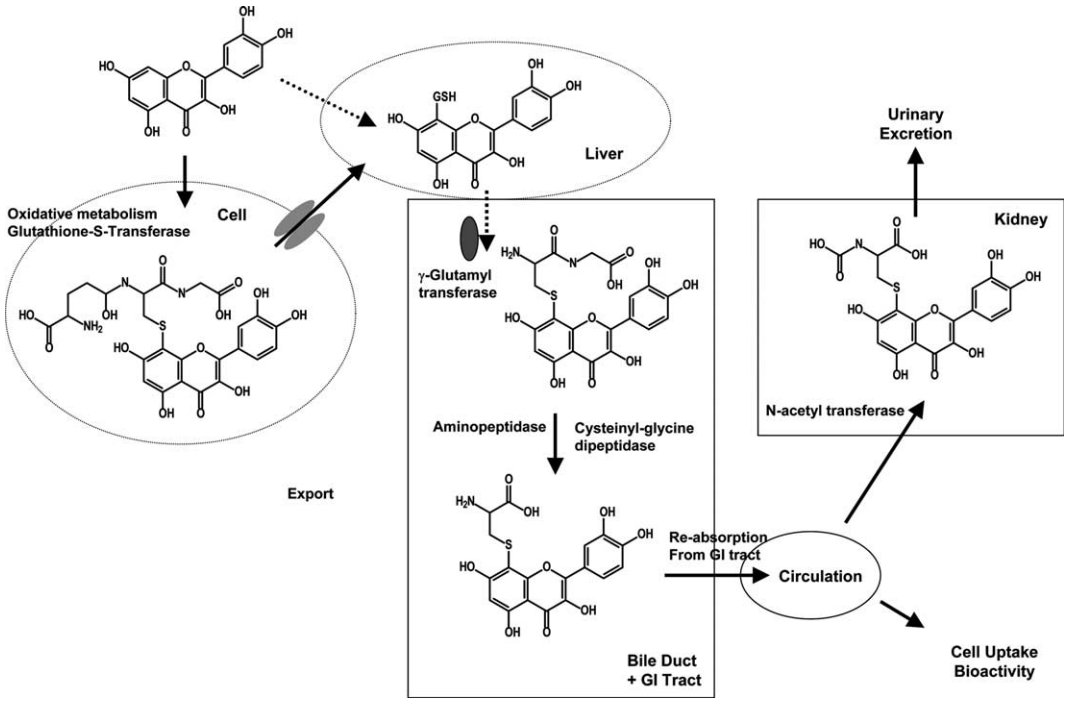


Fig. 5. Potential enterohepatic recirculation of flavonoid glutathione adducts. Glutathione conjugates formed in cells by enzymatic processing or oxidative metabolism enter the liver and are excreted into the bile. Following secretion into the GI tract conjugates undergo enzymatic cleavage to cysteinyl conjugates and may be re-absorbed into the circulation or excreted in the faeces. Re-absorption will result in ultimate elimination in the urine following acetylation in the kidney. However, circulating cysteinyl adducts may be capable of cell uptake and/or biological action.

produce cytotoxic amounts of hydrogen peroxide (Fig. 6). For example, the addition of high concentrations of strong reducing agents (high μM to mM), including flavonoids, to certain culture environments can lead to generation of substantial amounts of $H_2O_2$ (10–100 μM) [100,101]. This compound-dependent generation of reactive oxygen species may be viewed both negatively in that peroxide is a known inducer of both apoptosis and necrosis in cells, or positively in that, small amounts of generated peroxide may result in an adaptive response in cells that would act to protect them against a subsequent oxidative insult. There is also evidence to suggest that mono-hydroxylated flavonoids, such as naringenin, are also pro-oxidant, and have been found to partially oxidise hepatocyte GSH to GSSG and oxidise human erythrocyte oxy-haemoglobin more readily than polyphenolics with catechol rings [78].

The formation of quercetin quinone/quinone methide metabolites has been suggested both from indirect data, reflected in the formation of glutathionyl quercetin adducts, or directly by measurement of oxidised metabolites in cell lysates (Fig. 6). Quinone and quinone methide species are potentially long-lived species and have been shown to have varying biological half-lives. For example, *o*-quinone formed from the catechol oestrogen, 2-hydroxyoestrone, has a half-life of 47 s, whereas the 4-hydroxyoestrone *o*-quinone is considerably longer lived ($t_{1/2} = 12$ min) [102]. Such types of products may be linked to the toxicity of quercetin and 3′-*O*-methyl quercetin at high concentration and indeed many studies have shown that flavonoid catechol moieties can autoxidise in vitro as well as act as substrates for peroxidases, and other metalloenzymes, yielding quinone or quinone methide pro-oxidant and alkylating agents [80,103]. Quinones are Michael acceptors and can exert pro-oxidant activities after metabolic activation to semiquinone and quinoidal products with ensuing cellular damage from alkylation of cellular proteins or DNA [80] (Fig. 6). Quercetin is known to be mutagenic [104], presumably, in part through formation of quinone or quinone methide type metabolites [103,105,106] whereas the physiological form in humans, 4′-*O*-methyl quercetin, would not undergo such reactions and elicit these effects. It has been postulated that the cytotoxicity of quercetin may be mediated by its cellular metabolic activation to a semiquinone and/or quinone, which are known to facilitate the formation of superoxide and the depletion of GSH [80]. Because of the inherent nucleophilicity of the sulphydryl group, protein and non-protein sulphydryls represent a major target for quinones and the detoxification of quinones by GSH is generally considered cytoprotective [57,58,107,108]. In fact, the formation of glutathionyl quercetin adducts in a tyrosinase-containing melanoma cell line has been used as evidence for the formation of cellular oxidative metabolites [91].

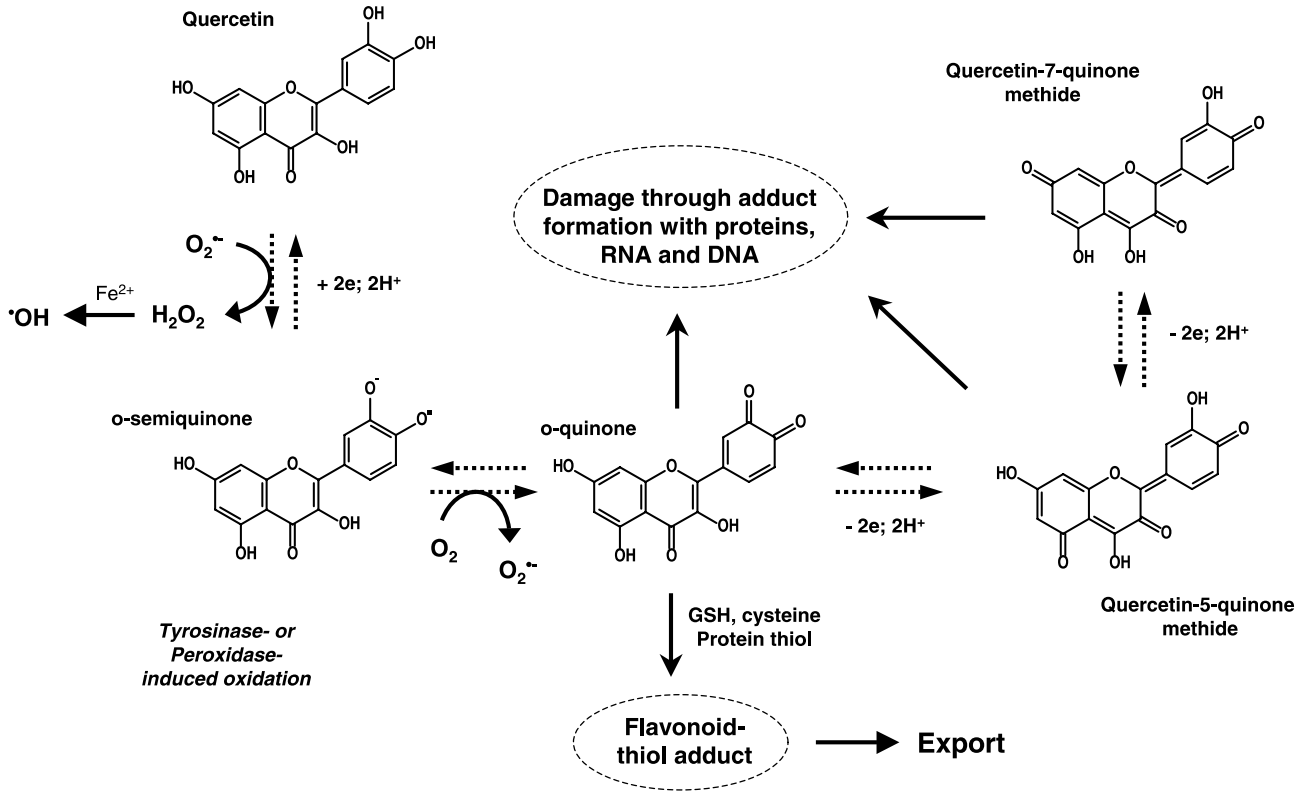


Fig. 6. Oxidation of flavonoids containing a catechol or di-hydroxylated B-ring (i.e., quercetin). Oxidation may proceed in physiological conditions in the in the presence of transition metals such as iron or copper. Alternatively, enzymes such as tyrosinase or peroxidases may catalyse quinone formation. Autoxidation may lead to production of reactive quinone and quinone methide species capable of reaction with cellular biomolecules and/or cellular thiols such as GSH and protein cysteine residues. Enzymatic oxidation of flavonoids may act to clear flavonoids from the cell via the reaction of the quinone with GHS and export of the conjugate.

*P450-related metabolites*

The glucuronidation or de-methylation/de-conjugation of circulating metabolites has important implications for resulting bioactivity. Previous studies have described a cytochrome P450-dependent (CYP2C9 and CYP1A2) demethylation of 4′-*O*-methyl quercetin in human liver microsomes, which is not specific for the 3′-O-methylated form [59]. In fibroblasts, there was evidence for the de-methylation of both 3′-*O*-methyl quercetin and 3′-*O*-methyl quercetin; although no evidence for isoforms of cytochrome P450 in fibroblasts has been described. The demethylation of O-methylated metabolites within cells is likely to have important implications for bioactivity. For example, demethylation of 3′-*O*-methyl quercetin has been observed in fibroblasts [56] and astrocytes, leading to the generation of the more reactive and cytotoxic quercetin intracellularly [23]. Alternatively, this processing of O-methylated metabolites may proceed to clear them from the cell more effectively via GSH conjugation and export. Hydroxylation of mono-hydroxylated flavonoids such as kaempferol is also possible, although not yet reported in cell studies. The hydroxylation of genistein, tangeretin, kaempferol, apigenin, and naringenin by P450 enzymes purified from liver microsomes and hepatocytes has been reported [59,109,110]. Furthermore, P450 enzymes isolated from liver microsomes have been shown to metabolise flavonoids to glucuronides [111] and glutathione conjugates [110,112], the latter suggesting that catechin could be metabolically activated by P450 peroxidase activity to form intermediate quinone species.

So far, there is limited information on the P450-mediated intracellular metabolism of flavonoids in intact cells. One such study has reported the glucuronidation of the flavanone, hesperetin, in human dermal fibroblasts. This is particularly interesting as hesperetin glucuronide but not the aglycone form of hesperetin was effective in protecting the cells against UVA-induced necrotic death [61]. Not only does this indicate that fibroblasts contain UDP-glucuronosyltransferases capable to glucuronidating flavanones but also suggests that cells may generate potentially beneficial metabolites intracellularly. Although similar observations have thus far not been demonstrated with other flavonoids, they do open up the possibility that flavonoid glucuronides may contribute to overall cell protection in vivo, even if

they have been shown to be ineffective against oxidative stress-induced damage [25].

## Summary

The uptake of flavonoids and their O-methylated, glucuronidated, and sulphated forms into cells will ultimately determine their biological actions. It is clear that uptake of flavonoids into cells is dependent on both the flavonoid and perhaps more importantly the cell type. It seems likely that these differences may reflect the variation in the way different cell populations handle flavonoids. For example, astrocytes metabolise flavonoids intracellularly whilst neurons do not. The generation of such intracellular metabolites, such as 2′-glutathionyl quercetin, is of great importance as they may also be capable of mediating potential beneficial or negative actions of flavonoids in vivo. Furthermore, intracellularly formed metabolites may represent novel in vivo metabolites of flavonoids and their presence in the circulation and urine may provide important information on the pharmacokinetics of flavonoids following ingestion.

It should be noted that the assessment of flavonoid cell uptake using in vitro cell culture models may not fully reflect uptake in vivo. As cells grow as a monolayer under normal culture conditions, the flavonoid will usually only be exposed to one side of the cell and consequently uptake may be underestimated. Furthermore, oxidation of flavonoids in culture medium during exposure will reduce the effective concentration interacting with the cells. In vivo, this oxidation is limited due to the tight regulation of oxygen in the cellular environment. Although there are limitations the above studies still provide important information on which flavonoids or their metabolites are most able to enter cells. The information can be used to predict localisations of flavonoids in different body tissues and furthermore which metabolite forms are likely to exert cellular actions. This is especially relevant as more studies begin to focus on potential non-antioxidant mechanisms of flavonoid action in vivo.

## Acknowledgments

The authors acknowledge the Biotechnology and Biological Sciences Research Council for financial support.

## References

[1] C. Rice-Evans, Curr. Med. Chem. 8 (2001) 797–807.
[2] B.H. Havsteen, Pharmacol. Ther. 96 (2002) 67–202.
[3] M. Lopez-Lazaro, Curr. Med. Chem. Anti-Cancer Agents 2 (2002) 691–714.
[4] L. Le Marchand, Biomed. Pharmacother. 56 (2002) 296–301.
[5] C.S. Yang, J.M. Landau, M.T. Huang, H.L. Newmark, Annu. Rev. Nutr. 21 (2001) 381–406.
[6] P.M. Kris-Etherton, K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel, T.D. Etherton, Am. J. Med. B 113 (Suppl. 9) (2002) 71S–88S.
[7] G. Galati, S. Teng, M.Y. Moridani, T.S. Chan, P.J. O'Brien, Drug Metabol. Drug Interact. 17 (2000) 311–349.
[8] J.A. Ross, C.M. Kasum, Annu. Rev. Nutr. 22 (2002) 19–34.
[9] F.M. Steinberg, M.M. Bearden, C.L. Keen, J. Am. Diet. Assoc. 103 (2003) 215–223.
[10] P.M. Kris-Etherton, C.L. Keen, Curr. Opin. Lipidol. 13 (2002) 41–49.
[11] H. Schroeter, C. Boyd, J.P.E. Spencer, R.J. Williams, E. Cadenas, C. Rice-Evans, Neurobiol. Aging 23 (2002) 861–880.
[12] O. Inanami, Y. Watanabe, B. Syuto, M. Nakano, M. Tsuji, M. Kuwabara, Free Radic. Res. 29 (1998) 359–365.
[13] J.A. Joseph, B. Shukitt-Hale, N.A. Denisova, R.L. Prior, G. Cao, A. Martin, G. Taglialatela, P.C. Bickford, J. Neurosci. 18 (1998) 8047–8055.
[14] J.A. Joseph, B. Shukitt-Hale, N.A. Denisova, D. Bielinski, A. Martin, J.J. McEwen, P.C. Bickford, J. Neurosci. 19 (1999) 8114–8121.
[15] K.A. Youdim, J.P. Spencer, H. Schroeter, C. Rice-Evans, Biol. Chem. 383 (2002) 503–519.
[16] J.P.E. Spencer, H. Schroeter, A.R. Rechner, C. Rice-Evans, Antioxid. Redox. Signal. 3 (2001) 1023–1039.
[17] J.P.E. Spencer, S.K. Srai, C. Rice-Evans, in: C. Rice-Evans, L. Packer (Eds.), Flavonoids in Health and Disease, Marcel Dekker, New York, 2003, pp. 363–390.
[18] T. Walle, R.A. Walgren, U.K. Walle, A. Galijatovic, J.B. Vaidyanathan, in: C. Rice-Evans, L. Packer (Eds.), Flavonoids in Health and Disease, Marcel Dekker, New York, 2003, pp. 349–362.
[19] A.J. Day, G. Williamson, in: C. Rice-Evans, L. Packer (Eds.), Flavonoids in Health and Disease, Marcel Dekker, New York, 2003, pp. 391–412.
[20] J.L. Donovan, A.L. Waterhouse, in: C. Rice-Evans, L. Packer (Eds.), Flavonoids in Health and Disease, Marcel Dekker, New York, 2003, pp. 413–440.
[21] J.P.E. Spencer, G. Chowrimootoo, R. Choudhury, E.S. Debnam, S.K. Srai, C. Rice-Evans, FEBS Lett. 458 (1999) 224–230.
[22] J.P.E. Spencer, H. Schroeter, G. Kuhnle, S.K. Srai, R.M. Tyrrell, U. Hahn, C. Rice-Evans, Biochem. J. 354 (2001) 493–500.
[23] J.P.E. Spencer, C. Rice-Evans, R.J. Williams, J. Biol. Chem. 278 (2003) 34783–34793.
[24] H. Schroeter, C.S. Boyd, R. Ahmed, J.P. Spencer, R.F. Duncan, C. Rice-Evans, E. Cadenas, Biochem. J. 372 (2003) 359–369.
[25] J.P.E. Spencer, H. Schroeter, A.J. Crossthwaithe, G. Kuhnle, R.J. Williams, C. Rice-Evans, Free Radic. Biol. Med. 31 (2001) 1139–1146.
[26] H.A. Guglielmone, A.M. Agnese, S.C. Nunez Montoya, J.L. Cabrera, Thromb. Res. 105 (2002) 183–188.
[27] Y. Miyake, K. Shimoi, S. Kumazawa, K. Yamamoto, N. Kinae, T. Osawa, J. Agric. Food Chem. 48 (2000) 3217–3224.
[28] J. Terao, S. Yamaguchi, M. Shirai, M. Miyoshi, J.H. Moon, S. Oshima, T. Inakuma, T. Tsushida, Y. Kato, Free Radic. Res. 35 (2001) 925–931.
[29] M. Shirai, J.H. Moon, T. Tsushida, J. Terao, J. Agric. Food Chem. 49 (2001) 5602–5608.
[30] N. Yamamoto, J.H. Moon, T. Tsushida, A. Nagao, J. Terao, Arch. Biochem. Biophys. 372 (1999) 347–354.
[31] E.L. da Silva, M.K. Piskula, N. Yamamoto, J.H. Moon, J. Terao, FEBS Lett. 430 (1998) 405–408.

[32] J.L. Donovan, V. Crespy, C. Manach, C. Morand, C. Besson, A. Scalbert, C. Remesy, J. Nutr. 131 (2001) 1753–1757.
[33] J.P.E. Spencer, H. Schroeter, B. Shenoy, S.K. Srai, E.S. Debnam, C. Rice-Evans, Biochem. Biophys. Res. Commun. 285 (2001) 588–593.
[34] G. Kuhnle, J.P.E. Spencer, H. Schroeter, B. Shenoy, E.S. Debnam, S.K. Srai, C. Rice-Evans, U. Hahn, Biochem. Biophys. Res. Commun. 277 (2000) 507–512.
[35] K. Shimoi, H. Okada, M. Furugori, T. Goda, S. Takase, M. Suzuki, Y. Hara, H. Yamamoto, N. Kinae, FEBS Lett. 438 (1998) 220–224.
[36] J.B. Vaidyanathan, T. Walle, Drug Metab. Dispos. 30 (2002) 897–903.
[37] U.K. Walle, T. Walle, Drug Metab. Dispos. 30 (2002) 564–569.
[38] A. Galijatovic, Y. Otake, U.K. Walle, T. Walle, Pharm. Res. 18 (2001) 374–379.
[39] M.B. Fisher, M.F. Paine, T.J. Strelevitz, S.A. Wrighton, Drug Metab. Rev. 33 (2001) 273–297.
[40] C. Manach, C. Morand, V. Crespy, C. Demigne, O. Texier, F. Regerat, C. Remesy, FEBS Lett. 426 (1998) 331–336.
[41] K.A. O'Leary, A.J. Day, P.W. Needs, W.S. Sly, N.M. O'Brien, G. Williamson, FEBS Lett. 503 (2001) 103–106.
[42] R.H. Tukey, C.P. Strassburg, Annu. Rev. Pharmacol. Toxicol. 40 (2000) 581–616.
[43] M. Triggiani, M. Gentile, A. Secondo, F. Granata, A. Oriente, M. Taglialatela, L. Annunziato, G. Marone, J. Immunol. 166 (2001) 4083–4091.
[44] K. Shimoi, N. Saka, R. Nozawa, M. Sato, I. Amano, T. Nakayama, N. Kinae, Drug Metab. Dispos. 29 (2001) 1521–1524.
[45] K. Shimoi, N. Saka, K. Kaji, R. Nozawa, N. Kinae, Biofactors 12 (2000) 181–186.
[46] D.E. Godar, J. Investig. Dermatol. Symp. Proc. 4 (1999) 17–23.
[47] A. Takashima, P.R. Bergstresser, Photochem. Photobiol. 63 (1996) 397–400.
[48] S. Kondo, D.N. Sauder, J. Dermatol. 22 (1995) 888–893.
[49] F.R. de Gruijl, H.J. van Kranen, L.H. Mullenders, J. Photochem. Photobiol. B 63 (2001) 19–27.
[50] P.H. Hart, M.A. Grimbaldeston, J.J. Finlay-Jones, Clin. Exp. Pharmacol. Physiol. 28 (2001) 1–8.
[51] S.B. Bratton, S.S. Lau, T.J. Monks, Chem. Res. Toxicol. 13 (2000) 550–556.
[52] S.K. Katiyar, H. Mukhtar, J. Leukoc. Biol. 69 (2001) 719–726.
[53] S.K. Katiyar, C.A. Elmets, Int. J. Oncol. 18 (2001) 1307–1313.
[54] S.K. Katiyar, M.S. Matsui, C.A. Elmets, H. Mukhtar, Photochem. Photobiol. 69 (1999) 148–153.
[55] S.K. Katiyar, F. Afaq, A. Perez, H. Mukhtar, Carcinogenesis 22 (2001) 287–294.
[56] J.P.E. Spencer, G.G. Kuhnle, R.J. Williams, C. Rice-Evans, Biochem. J. 372 (2003) 173–181.
[57] T.J. Monks, S.S. Lau, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 229–255.
[58] T.J. Monks, S.S. Lau, Chem. Res. Toxicol. 10 (1997) 1296–1313.
[59] V.M. Breinholt, E.A. Offord, C. Brouwer, S.E. Nielsen, K. Brosen, T. Friedberg, Food Chem. Toxicol. 40 (2002) 609–616.
[60] H. Schroeter, J.P. Spencer, C. Rice-Evans, R.J. Williams, Biochem. J. 358 (2001) 547–557.
[61] A.R. Proteggente, S. Basu-Modak, G. Kuhnle, M. Gordon, K.A. Youdim, R.M. Tyrrell, C.A. Rice-Evans, Photochem. Photobiol. 78 (2003) 256–261.
[62] M. Beaulieu, E. Levesque, D.W. Hum, A. Belanger, Biochem. Biophys. Res. Commun. 248 (1998) 44–50.
[63] R.A. Lobo, W.L. Paul, E. Gentzschein, P.C. Serafini, J.A. Catalino, R.J. Paulson, R. Horton, J. Clin. Endocrinol. Metab. 65 (1987) 711–714.
[64] D.S. Vienneau, U. DeBoni, P.G. Wells, Cancer Res. 55 (1995) 1045–1051.
[65] W.H. Peters, W.A. Allebes, P.L. Jansen, L.G. Poels, P.J. Capel, Gastroenterology 93 (1987) 162–169.
[66] J. Konig, A.T. Nies, Y. Cui, I. Leier, D. Keppler, Biochim. Biophys. Acta 1461 (1999) 377–394.
[67] J.H. Hooijberg, H.J. Broxterman, M. Heijn, D.L. Fles, J. Lankelma, H.M. Pinedo, FEBS Lett. 413 (1997) 344–348.
[68] J.M. Baron, D. Holler, R. Schiffer, S. Frankenberg, M. Neis, H.F. Merk, F.K. Jugert, J. Invest. Dermatol. 116 (2001) 541–548.
[69] Y. Levites, T. Amit, M.B. Youdim, S. Mandel, J. Biol. Chem. 277 (2002) 30574–30580.
[70] Y. Levites, T. Amit, S. Mandel, M.B. Youdim, FASEB J. 17 (2003) 952–954.
[71] K.A. Youdim, M.S. Dobbie, G. Kuhnle, A.R. Proteggente, N.J. Abbott, C. Rice-Evans, J. Neurochem. 85 (2003) 180–192.
[72] T.A. Aasmundstad, J. Morland, R.E. Paulsen, J. Pharmacol. Exp. Ther. 275 (1995) 435–441.
[73] H.K. Kroemer, U. Klotz, Clin. Pharmacokinet. 23 (1992) 292–310.
[74] B. Sperker, J.T. Backman, H.K. Kroemer, Clin. Pharmacokinet. 33 (1997) 18–31.
[75] R. Osborne, S. Joel, D. Trew, M. Slevin, Lancet 1 (1988) 828.
[76] M.M. Abd El Mohsen, G. Kuhnle, A.R. Rechner, H. Schroeter, S. Rose, P. Jenner, C.A. Rice-Evans, Free Radic. Biol. Med. 33 (2002) 1693–1702.
[77] T.S. Chan, G. Galati, A.S. Pannala, C. Rice-Evans, P.J. O'Brien, Free Radic. Res. 37 (2003) 787–794.
[78] G. Galati, O. Sabzevari, J.X. Wilson, P.J. O'Brien, Toxicology 177 (2002) 91–104.
[79] C.M. Watanabe, S. Wolffram, P. Ader, G. Rimbach, L. Packer, J.J. Maguire, P.G. Schultz, K. Gohil, Proc. Natl. Acad. Sci. USA 98 (2001) 6577–6580.
[80] D. Metodiewa, A.K. Jaiswal, N. Cenas, E. Dickancaite, J. Segura-Aguilar, Free Radic. Biol. Med. 26 (1999) 107–116.
[81] M. Salucci, L.A. Stivala, G. Maiani, R. Bugianesi, V. Vannini, Br. J. Cancer 86 (2002) 1645–1651.
[82] R.A. Walgren, J.T. Lin, R.K. Kinne, T. Walle, J. Pharmacol. Exp. Ther. 294 (2000) 837–843.
[83] R.A. Walgren, K.J. Karnaky Jr., G.E. Lindenmayer, T. Walle, J. Pharmacol. Exp. Ther. 294 (2000) 830–836.
[84] C. Kandaswami, E. Perkins, D.S. Soloniuk, G. Drzewiecki, E. Middleton Jr., Cancer Lett. 56 (1991) 147–152.
[85] T.H. Tsai, Y.F. Chen, J. Food Drug Anal. 8 (2000) 331–336.
[86] H.W. Peng, F.C. Cheng, Y.T. Huang, C.F. Chen, T.H. Tsai, J. Chromatogr. B 714 (1998) 369–374.
[87] K.P. Datla, M. Christidou, W.W. Widmer, H.K. Rooprai, D.T. Dexter, NeuroReport 12 (2001) 3871–3875.
[88] W. Mullen, B.A. Graf, S.T. Caldwell, R.C. Hartley, G.G. Duthie, C.A. Edwards, M.E. Lean, A. Crozier, J. Agric. Food Chem. 50 (2002) 6902–6909.
[89] M. Suganuma, S. Okabe, M. Oniyama, Y. Tada, H. Ito, H. Fujiki, Carcinogenesis 19 (1998) 1771–1776.
[90] A.M. Amorini, G. Lazzarino, F. Galvano, G. Fazzina, B. Tavazzi, G. Galvano, Free Radic. Res. 37 (2003) 453–460.
[91] H.M. Awad, M.G. Boersma, S. Boeren, H. van der Woude, J. van Zanden, P.J. van Bladeren, J. Vervoort, I.M. Rietjens, FEBS Lett. 520 (2002) 30–34.
[92] H.M. Awad, M.G. Boersma, S. Boeren, P.J. van Bladeren, J. Vervoort, I.M. Rietjens, Chem. Res. Toxicol. 16 (2003) 822–831.
[93] T. Ishikawa, Trends Biochem. Sci. 17 (1992) 463–468.
[94] M. Carvalho, G. Hawksworth, N. Milhazes, F. Borges, T.J. Monks, E. Fernandes, F. Carvalho, M.L. Bastos, Arch. Toxicol. 76 (2002) 581–588.

[95] R. Bugianesi, M. Serafini, F. Simone, D. Wu, S. Meydani, A. Ferro-Luzzi, E. Azzini, G. Maiani, Anal. Biochem. 284 (2000) 296–300.
[96] X.M. Shen, G. Dryhurst, Chem. Res. Toxicol. 9 (1996) 751–763.
[97] J.P.E. Spencer, P. Jenner, S.E. Daniel, A.J. Lees, D.C. Marsden, B. Halliwell, J. Neurochem. 71 (1998) 2112–2122.
[98] J.P.E. Spencer, M. Whiteman, P. Jenner, B. Halliwell, J. Neurochem. 81 (2002) 122–129.
[99] H. Li, X.M. Shen, G. Dryhurst, J. Neurochem. 71 (1998) 2049–2062.
[100] B. Halliwell, M.V. Clement, J. Ramalingam, L.H. Long, IUBMB Life 50 (2000) 251–257.
[101] P.C. Chai, L.H. Long, B. Halliwell, Biochem. Biophys. Res. Commun. 304 (2003) 650–654.
[102] S.L. Iverson, L. Shen, N. Anlar, J.L. Bolton, Chem. Res. Toxicol. 9 (1996) 492–499.
[103] H.M. Awad, M.G. Boersma, J. Vervoort, I.M. Rietjens, Arch. Biochem. Biophys. 378 (2000) 224–233.
[104] Z. Yao, K. Drieu, V. Papadopoulos, Brain Res. 889 (2001) 181–190.
[105] H.M. Awad, M.G. Boersma, S. Boeren, P.J. van Bladeren, J. Vervoort, I.M. Rietjens, Chem. Res. Toxicol. 14 (2001) 398–408.
[106] H.M. Awad, M.G. Boersma, S. Boeren, P.J. van Bladeren, J. Vervoort, I.M. Rietjens, Chem. Res. Toxicol. 15 (2002) 343–351.
[107] J.L. Bolton, Toxicology 177 (2002) 55–65.
[108] E. Cadenas, L. Ernster, Adv. Exp. Med. Biol. 264 (1990) 37–44.
[109] V.M. Breinholt, S.E. Rasmussen, K. Brosen, T.H. Friedberg, Pharmacol. Toxicol. 93 (2003) 14–22.
[110] Y. Otake, T. Walle, Drug Metab. Dispos. 30 (2002) 103–105.
[111] Y. Otake, F. Hsieh, T. Walle, Drug Metab. Dispos. 30 (2002) 576–581.
[112] M.Y. Moridani, H. Scobie, P. Salehi, P.J. O'Brien, Chem. Res. Toxicol. 14 (2001) 841–848.