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Interaction of Isoflavones with the BCRP/*ABCG2* Drug Transporter

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Abstract

This review will provide a comprehensive overview of the interactions between dietary isoflavones and the ATP-binding cassette (ABC) G2 efflux transporter, which is also named the breast cancer resistance protein (BCRP). Expressed in a variety of organs including the liver, kidneys, intestine, and placenta, BCRP mediates the disposition and excretion of numerous endogenous chemicals and xenobiotics. Isoflavones are a class of naturally-occurring compounds that are found at high concentrations in commonly consumed foods and dietary supplements. A number of isoflavones, including genistein and daidzein and their metabolites, interact with BCRP as substrates, inhibitors, and/or modulators of gene expression. To date, a variety of model systems have been employed to study the ability of isoflavones to serve as substrates and inhibitors of BCRP; these include whole cells, inverted plasma membrane vesicles, *in situ* organ perfusion, as well as *in vivo* rodent and sheep models. Evidence suggests that BCRP plays a role in mediating the disposition of isoflavones and in particular, their conjugated forms. Furthermore, as inhibitors, these compounds may aid in reversing multidrug resistance and sensitizing cancer cells to chemotherapeutic drugs. This review will also highlight the consequences of altered BCRP expression and/or function on the pharmacokinetics and toxicity of chemicals following isoflavone exposure.

Keywords

BCRP; isoflavones; genistein; daidzein; biochanin A; phase II metabolites

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Conflicts of Interest

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Introduction

Isoflavones

Isoflavones are secondary metabolites that occur naturally in plants of the *Leguminosae* family and include soybeans, red clover, peanuts, chickpeas, and alfalfa [1]. Isoflavones attract bacteria to the roots to aid in nodulation and nitrogen fixation [2]. Collectively, isoflavones represent a subset of the larger class of chemicals, flavonoids, which are defined by a polyphenolic three ring structure (A, B, and C) (Figure 1A). The location of the B ring at position C3 rather than C2 separates the isoflavones from the remaining flavonoids (Figure 1B). This review article will focus on the most commonly studied isoflavones: genistein, daidzein, biochanin A, glycitein, and formononetin (Figure 2).

The most abundant source of isoflavones is soybeans, which contain primarily genistein (approximately 2.3 mg/g), daidzein, and glycitein [1], in addition to trace amounts of formononetin and biochanin A [3]. It is important to note that soybeans are used in the production of numerous manufactured dietary products including tofu, tempeh, soy infant formula, miso, soybean oil, cereal, and bacon bits [1]. Red clover is another naturally abundant source of isoflavones containing primarily formononetin, in addition to biochanin A, daidzein, and genistein [3]. Within plants, isoflavones are present as two different forms: 1) glycoside (Figure 3) (i.e., genistin or genistein-7-O-β-D-glucoside) and 2) aglycone (i.e., genistein) (reviewed in [4]). Glycosides occur at greater concentrations than aglycones in soybeans and other plants [5]; however fermented foods (i.e., miso, tempeh) contain a greater proportion of aglycones compared to unfermented soy due to the hydrolysis of the glycosides to aglycones by microbial β-glucosidases during the fermentation process (reviewed in [4]).

Following ingestion, isoflavone glycosides are converted to their aglycone form by epithelial and microbial β-glucosidases in the oral cavity [6] and small intestines [7]. This is important because it is primarily the aglycone form of isoflavones that pass across the intestinal epithelium [8–10] and possess biological activity [11, 12]. Approximately 70–90% of isoflavones are metabolized to glucuronide and sulfate conjugates by UDP-glucuronosyltransferases (UGTs; UGT1A1, 1A8, 1A9, 1A10) and sulfotransferases (SULTs; SULT1A1*2, 1E, 2E1), respectively, in the intestines and liver [13, 14]. Conjugation occurs primarily at the C7 and C4′ positions on the parent ring system (Figure 1B), to form monoconjugates (i.e., genistein-7-glucuronide, G-7-G; genistein-4′-glucuronide, G-4′-G; genistein-7-sulfate, G-7-S; genistein-4′-sulfate, G-4′-S) or diconjugates (i.e., genistein-7-glucuronide-4′-glucuronide; genistein-7-sulfate-4′-sulfate; genistein-7-glucuronide-4′-sulfate; genistein-7-sulfate-4′-glucuronide) [15]. The phase II metabolites are excreted into the intestinal lumen and bile duct and subsequently eliminated in the feces or deconjugated by gut microflora which results in reabsorption and enterohepatic recirculation of the isoflavones [16]. Though it is not the primary pathway of isoflavone metabolism, phase I oxidative metabolites of genistein, daidzein [17], and formononetin [18] have been identified in the urine of healthy volunteers. Confirmed using human liver microsomes, the cytochrome P450 enzymes are responsible for the oxidation of isoflavones in the liver [17, 18]. Additional phase I metabolism of these chemicals include oxidative demethylation,

particularly for biochanin A and formononetin. Incubation of biochanin A and formononetin with human liver microsomes resulted in the formation of genistein and daidzein, respectively [18]. Another example of phase I metabolism of isoflavones is the formation of equol from daidzein by intestinal bacteria [19]. Equol is more effective in reducing prostate cancer growth *in vitro* than daidzein [20] and its presence in urine has been associated with a decreased risk of breast cancer [21]. Evidence suggests that approximately 30–50% of the population can produce equol from daidzein [22, 23]. This interindividual heterogeneity in the conversion of daidzein to equol may be a potential contributor to the inconclusive findings in clinical trials that examine the effects of a soy diet on various disease states.

Genistein is the most frequently studied isoflavone due to its presence in diverse dietary sources and an increasing number of reports that suggest genistein and other isoflavones have biological activity that may modulate disease progression [24–27] (reviewed in [28]). Specifically, there is evidence that genistein and other isoflavones possess cancer preventative and/or therapeutic activity, and can improve cardiovascular, bone and post-menopausal health [25, 29–34]. Much of this research has been supported by epidemiological findings that demonstrate health benefits conferred to Asian populations that consume a soy rich diet as compared to populations that consume a western diet [26, 27]. Nevertheless, previous studies also suggest that genistein and other isoflavones may be adverse to human health by promoting cancer progression and/or interfering with reproductive development early in life [35–38]. Additional work is needed to address the controversy as to whether these natural compounds have a negative impact on the health of humans clinically.

Many of the biological responses produced by isoflavones are believed to be mediated by cellular proteins including the estrogen receptor and tyrosine kinase receptors. In fact, due to the early discovery of isoflavones as estrogen receptor agonists [39] and modulators of reproductive function [40], these compounds have been termed phytoestrogens. The phenolic ring structure of isoflavones, allow the compounds to bind to estrogen receptors [39] and initiate transcription of estrogen receptor-responsive genes. Typically, isoflavones bind with greater affinity to the estrogen receptor β than estrogen receptor α [41]. Moreover, in 1987, Akiyama et al. reported that isoflavones can inhibit tyrosine kinase enzymes in A431 epidermoid cancer cells, including the epidermal growth factor receptor (genistein > prunetin >biochanin A >daidzein=genistin) [12]. Since then, a number of reports have confirmed these findings *in vitro* as well as *in vivo* [24, 42–45] and suggest that by targeting these molecular pathways often involved in cancer, isoflavones may possibly be combined with chemotherapeutic regimens to improve the efficacy of cancer treatment.

In addition to estrogen and tyrosine kinase receptors, isoflavones also interact with the breast cancer resistance protein (BCRP) efflux transporter, which is involved in the disposition of xenobiotics and endogenous compounds. These compounds are substrates of BCRP as well as inhibitors of its function. Further, many of the isoflavones modulate chemoresistance and *in vivo* pharmacokinetics of other BCRP substrates that are administered concomitantly.

BCRP

BCRP is a member of the ATP-binding cassette (ABC) superfamily of transmembrane transporters. Localized to the apical plasma membrane of cells, BCRP actively transports xenobiotic and endogenous substrates out of the cell with energy derived from the hydrolysis of ATP. In the 1990's, BCRP was discovered in multiple laboratories using cell lines that conferred resistance to mitoxantrone, and other xenobiotics, and that lacked the previously characterized multidrug resistance proteins, multidrug resistance protein 1 (MDR1) and the multidrug resistance-associated protein 1 (MRP1) [46–48]. The discovery of the new transporter in a number of models including a breast cancer cell line (MCF-7/AdrVp) [46], human placenta [47], and mitoxantrone-selected human colon carcinoma cells (S1-M1-80) [48], resulted in the concurrent publication of multiple names for the same transporter: BCRP, "ABC transporter highly expressed in the placenta" (ABCP), and the "mitoxantrone resistance (MXR) transporter". Subsequently, the human gene nomenclature committee officially designated the transcript as the second member of the G subfamily of the ABC superfamily of transporters, *ABCG2* [49].

Since its discovery in cancer cells, BCRP has been found to play an important role in mediating the disposition of substrates in normal human tissues thereby preventing chemical accumulation and subsequent toxicity [50]. BCRP is expressed in the epithelium of tissues such as the intestines, liver, and kidney, which are involved in the pharmacokinetics of chemicals. Localization of BCRP to the apical or luminal surface favors active transport of substrates into the intestinal lumen, bile duct, and proximal tubule lumen, respectively [50]. In addition to playing a role in excretion, BCRP aids in the formation of blood-organ barriers as it is expressed in syncytiotrophoblasts of the placenta, capillary endothelial cells of the blood-brain barrier, and luminal capillary endothelial cells of the blood-testis barrier [50–52]. BCRP mRNA and protein are expressed most abundantly in the human placenta, earning its name as "the placental transporter" [47].

In vitro, *in vivo*, and *in situ* model systems have been employed to detect BCRP substrates and inhibitors including several drugs, dietary components, and endogenous compounds (Table 1). While previous review articles have summarized the interactions of flavonoids with ABC transporters [53, 54], this review article provides an up-to-date summary of the evidence for the interaction of isoflavones specifically with BCRP. This includes a discussion of isoflavones as BCRP substrates (Table 2, 3, 4), inhibitors of BCRP transport activity (Table 5), and regulators of BCRP expression (Table 6) with commentary on the implications of these interactions.

Evidence for Isoflavones as Substrates of BCRP

In Vitro

Transport of Genistein—Genistein was first described as a substrate for BCRP-mediated transport in 2004 (Table 2). Imai et al. demonstrated a significant decrease in the accumulation of ^{3}H-genistein in human K562 myelogenous leukemia cells that overexpressed the human BCRP/*ABCG2* gene (K562/BCRP cells) compared to the parent cells that lacked BCRP (K562 cells) [71]. In addition, the transcellular transport of genistein

was assessed using pig kidney epithelial cells (LLC-PK1 cells) that overexpressed the human BCRP/*ABCG2* gene (LLC/BCRP cells). When grown in monolayers, BCRP protein localizes to the apical membrane of LLC-PK1 cells [62, 105]. For these experiments, cells are grown on a thin filter in between two chambers that recapitulate the polarization of cells (apical and basolateral) typically seen *in vivo*. These studies revealed that genistein is selectively transported by BCRP in the basolateral-to-apical (BL-to-AP) direction as compared to the apical-to-basolateral (AP-to-BL) direction [71]. The preference for BL-to-AP transport was abolished by the BCRP specific inhibitor, fumitremorgin C (FTC, 3 μM), further confirming a critical role for BCRP in the transcellular transfer of genistein [71]. Since this initial report, a number of studies have characterized the bidirectional transport of genistein in other BCRP-overexpressing (i.e., Madin-Darby canine kidney II; MDCK/BCRP) and endogenously expressing (i.e., Caco-2) cell lines [69, 95, 96]. Similar to the LLC/BCRP cells, the BL-to-AP transport of genistein (as quantified by LC/MS) was favored in the MDCK/BCRP cells with a efflux ratio that was 2-fold higher in the overexpressing cells as compared to the empty vector cells that lacked BCRP protein [69]. In such bidirectional permeability assays, an efflux ratio ≥ 2 indicates active efflux in the BL-to-AP direction as the value is calculated by dividing the apparent permeability (P_{app}) of a compound in the BL-to-AP direction by the P_{app} of the compound in the AP-to-BL direction ($P_{app\ BL\text{-}AP}/P_{app\ AP\text{-}BL}$) [95, 106]. The human colorectal adenocarcinoma cell line, Caco-2 is an *in vitro* model of intestinal transport. In culture, Caco-2 cells form monolayers and the BCRP protein localizes to the apical, or microvillous membrane, similar to its known trafficking in tissues [107–109]. In Caco-2 monolayers, the BCRP specific inhibitor, FTC (1 μM) decreased the efflux ratio of genistein by 3-fold, confirming that genistein is actively transported by BCRP in the BL-to-AP direction [95]. BCRP-mediated transport of genistein in Caco-2 cells was confirmed in another study which demonstrated that the BCRP inhibitor, estrone-3-sulfate increased the permeability of genistein by 40% in these cells [96]..

Additional studies indicate that genistein is also transported by the mouse isoform of the Bcrp protein overexpressed in MDCK/Bcrp cells as the efflux ratio was approximately 2-fold higher than the cells harboring the control expression vector in two studies [69, 97]. The similar ability of the mouse and human Bcrp/BCRP proteins to transport genistein *in vitro*, suggests that the mouse is an appropriate model for studying the *in vivo* transport of genistein by Bcrp/BCRP. This is in line with other compounds, such as cimetidine and aflatoxin B_1, which are transported by both the human and mouse BCRP/Bcrp orthologs [55, 56].

Transport of Daidzein—Similar to genistein, daidzein was first suggested to be a substrate of BCRP in 2004 using the ATPase assay [68]. Briefly, the ATPase assay uses plasma membranes isolated from cells or tissues that express BCRP and detects the ability of a compound to stimulate ATP hydrolysis as an indirect measure of substrate transport. In membranes isolated from the bacteria *L. Lactis* that overexpressed the human BCRP gene, daidzein (3–50 μM) stimulated the baseline ATP-hydrolysis relative to control membranes. Direct transport of daidzein by BCRP was verified in monolayers of MDCK/BCRP and Caco-2 cells [69, 96]. In the MDCK/BCRP cells, the efflux ratio was 3-fold greater than the empty vector cells [69] while the BCRP inhibitor, estrone-3-sulfate increased the relative

permeation (apical-to-basolateral transport) of daidzein by 80% compared to vehicle-treated control Caco-2 cells [96]. In addition to interacting with human BCRP, daidzein was also transported in MDCK cells by mouse Bcrp (MDCK/Bcrp), which was evidenced by an approximately 2-fold greater efflux ratio than the empty vector cells in two separate studies [69, 97].

Transport of Isoflavone Conjugates—Approximately 70–90% of an orally administered dose of isoflavones undergo phase II conjugation by glucuronidation and sulfation in the intestines and liver [13, 14, 110]. Phase-II conjugates of genistein are also transported by BCRP in Caco-2 cells as demonstrated by the reduced BL-to-AP transport of G-7-G, G-4′-G, G-7-S, and G-4′-S in the presence of the BCRP inhibitor Ko143 (5 μM) [73]. To better understand the transport of genistein-phase II metabolites by BCRP, human cervical carcinoma HeLa cells that endogenously expressed BCRP were genetically-engineered to overexpress the glucuronidation enzyme, UDP-glucuronosyltransferase (UGT) 1A9 [99]. Following a 2 h incubation with genistein (10 μM) and Ko143 (5 and 10 μM), these cells demonstrated reduced elimination of genistein-glucuronide and increased intracellular accumulation of the phase-II metabolite suggesting that genistein-glucuronide is transported by BCRP in the genetically-engineered HeLa cells [99]. Despite having a moderate and statistically significant effect on the overall glucuronidation rate in these cells, the authors still suggested that Ko143 did not reduce BCRP transport of genistein-glucuronide by decreasing the *in situ* formation of genistein-glucuronide [99]. To more thoroughly interpret these results, future experiments should address the ability of Ko143 to inhibit UGT function.

In addition to cells that overexpress the BCRP gene, there are alternative *in vitro* assays for identifying BCRP substrates that focus on plasma membrane transport. One common model are membrane vesicles isolated from *Spodoptera frugiperda* (*Sf9*) insect cells that overexpress human BCRP and are configured in an inverted (or inside-out) orientation. Because of this orientation, one can measure intravesicular concentrations of BCRP substrates directly in the presence of ATP. Using *Sf9*-BCRP vesicles, van de Wetering and Sapthu (2012) demonstrated a high rate of ATP-dependent transport of genistein-sulfate and daidzein-sulfate collected from the urine of Bcrp −/− mice (FVB) [98]. The mice were not dosed with the isoflavones *per se*, but rather consumed a normal rodent diet (AM-II) which contained isoflavones at low levels. This experimental approach allowed for the *in vivo* generation of isoflavone metabolites at physiologically relevant concentrations. BCRP-dependent transport of six isoflavone secondary metabolites (genistein-sulfate I, genistein-sulfate II, daidzein-4′-sulfate, daidzein-7-sulfate, glycitein-sulfate, and formononetin-sulfate) was confirmed using the inverted membrane vesicle system and ^{35}S-labeled isoflavone metabolites [98]. These studies suggested that BCRP is particularly important in the transport of sulfated isoflavone conjugates.

An and Morris (2011) demonstrated that biochanin A-sulfate, not biochanin A, was selectively transported in the BL-to-AP direction in MDCK/Bcrp cells as compared to the AP-to-BL direction [67]. The BCRP specific inhibitor, FTC did not increase the accumulation of biochanin A in MCF-7/MX100 cells overexpressing BCRP, further confirming that the aglycone, biochanin A was not a substrate for BCRP-mediated transport

[67]. In this same experiment, the contribution of human BCRP to the transport of biochanin A phase II metabolites could not be identified as low concentrations of phase II metabolites were detected intracellularly [67]. The authors suspect this was due to the low expression of phase II metabolizing enzymes in the MCF-7 cells [67]. Moreover, following the addition of biochanin A to the apical and basolateral chambers, genistein was detected in the medium of MDCK/BCRP as a phase I metabolite (oxidative demethylation) of biochanin A [67]. Similar to studies that added genistein to the medium, An and Morris (2011) confirmed that genistein is transported to a greater extent in the BL-to-AP direction compared to the empty vector cells. Another study found that after adding biochanin A to the apical chamber of a Caco-2 polarized monolayer, the AP-to-BL and BL-to-AP transport of biochanin A-glucuronide was reduced by the BCRP inhibitor dipyridamole (10 uM) [66]. The authors suggested that the abolished BL-to-AP transport is a result of reduced BCRP activity by dipyridamole [66], however the authors acknowledged that dipyridamole is also an inhibitor of MDR1 function [111]. Because MDR1 also localizes to the apical membrane of Caco-2 cells [112], this transporter cannot be ruled out as playing a role in biochanin A-glucuronide transport. Furthermore, dipyridamole is an inhibitor of MRP1 that is localized to the basolateral membrane of Caco-2 cells [113]. This indicates that inhibition of MRP1 transport may be responsible for the reduced AP-to-BL transport of biochanin A-glucuronide in Caco-2 cells. Interestingly, formononetin-glucuronide transport was not altered with the addition of dipyridamole in a similar experiment [66].

In Vivo

Bioavailability of Individual Isoflavones—Pharmacokinetic studies using Bcrp knockout (Bcrp −/−) mice have been conducted to assess the *in vivo* contribution of Bcrp to the disposition of various compounds including isoflavones and isoflavone metabolites. A number of studies have emphasized the ability of intestinal and hepatic Bcrp to transport isoflavones because the relevant route of exposure in the human population is oral ingestion. In two studies, oral (p.o.) administration of genistein [69, 73] or daidzein [69] resulted in significantly greater plasma concentrations of the respective isoflavone over time (or area-under-the-curve AUC) in Bcrp −/− mice on a FVB background strain as compared to wild-type mice. These data suggested that Bcrp was responsible for limiting the oral bioavailability of genistein and daidzein [69, 73]. In a similar study performed by Alvarez et al. (50 mg/kg, 3 h) [70], the plasma AUC values for the aglycones, genistein and daidzein, alone in the Bcrp−/− mice were elevated by 1.5- and 2-fold, respectively, which was not significantly greater than the wild-type values, unlike the other two studies which noted statistical significance [69, 73]. Alvarez et al. reported that the plasma AUC values for total genistein or daidzein (aglycone and metabolites) were significantly greater (up to 9-times) in Bcrp −/− mice compared to wild-type mice and proposed that the difference was due primarily to the increased concentration of phase II metabolites in the plasma of Bcrp −/− mice [70]. For example, the plasma daidzein-sulfate concentration was significantly increased 6-fold in the Bcrp −/− mice compared to wild-type mice [70]. Also, daidzein-glucuronide was detected in Bcrp −/− mice while the metabolite was undetectable in wild-types [70]. In two different studies, systemic genistein-sulfate concentrations were elevated up to 26- and 7-fold in Bcrp −/− mice administered 20 mg/kg genistein [73] and 50 mg/kg genistein [70], respectively, as compared to wild-type mice. In the same studies, genistein-

glucuronide concentrations were elevated up to 16- and 8-fold in Bcrp −/− mice following administration of 20 mg/kg [73] and 50 mg/kg genistein [70], respectively. This evidence is in line with an intestinal *in situ* perfusion study performed by Zhu et al. which demonstrated that the excretion of genistein-sulfate and genistein-glucuronide was significantly decreased in the small intestines of Bcrp −/− mice compared to wild-type mice, suggesting that genistein conjugates are substrates for Bcrp-mediated transport [72].

The exact mechanism by which isoflavones and their phase II metabolites occur at high concentrations in the plasma of Bcrp −/− mice is a topic of great controversy [114]. Alvarez et al. offered an explanation for the increased plasma concentration of only the phase II metabolites by suggesting that decreased excretion of the aglycones due to the lack of intestinal Bcrp in Bcrp −/− mice increased exposure of the isoflavones to phase II metabolizing enzymes in the intestine and subsequently enhanced plasma concentrations of isoflavone metabolites [70]. Enokizono et al. suggested that because glucuronidation is the primary metabolic pathway contributing to the elimination of gensitein [13, 110], and there is no difference in the intestinal rate of glucuronidation between wild-type and Bcrp−/− mice [115], that increased intestinal absorption of genistein is the primary cause of elevated plasma genistein concentrations (rather than altered glucuronide formation) in Bcrp−/− mice [69]. Yang et al. countered this assertation and demonstrated that there was no difference in the intestinal absorption of genistein using *in situ* perfusion of wild-type and Bcrp−/− mouse intestines and human Caco-2 cells in the presence and absence of Bcrp specific inhibitor, Ko143 [73]. Instead, Yang et al. suggested that in Bcrp −/− mice, genistein conjugates were increasingly excreted into the blood rather than into the intestinal lumen and bile duct (due to loss of Bcrp function) and that any elevated genistein concentration in the plasma was likely due to the hydrolysis of genistein-glucuronides conjugates back to the aglycone form, in the blood [73]. The multidrug resistance-associated protein 3 (MRP3) is expressed on the basolateral membrane of the rat intestine [116] and Yang et al. suggested that this transporter may be a candidate for the active transport of genistein conjugates into the portal blood [117], providing a possible mechanistic explanation for increased plasma concentrations of isoflavone phase II metabolites in Bcrp −/− mice [73]. It is important to note that a number of experimental methods varied between studies and may have contributed to the differences in the isoflavone (aglyone and metabolite) plasma profiles; these included the time period (i.e., 3 h vs 4 h vs 24 h) and/or isoflavone dose (i.e., 50 mg/kg vs 8.1 mg/kg vs 20 mg/kg).

In another study, Bcrp −/− knockout mice consumed a normal chow diet containing trace amounts of isoflavones, and exhibited significantly greater concentrations of daidzein-sulfate and genistein-sulfate in the plasma of male and female mice, respectively, compared to the wild-types [98]. A study of this nature suggests that BCRP/Bcrp may play a role in the disposition of isoflavones at a wide range of concentrations, however these findings must be interpreted with caution as the exact dose of isoflavone that each mouse received was unknown.

Bioavailability of Isoflavone Mixtures—To recapitulate a more relevant dietary exposure to isoflavones mixtures, Alvarez et al. also examined the plasma profiles of the individual compounds following the intragastric co-administration of genistein (25 mg/kg)

and daidzein (25 mg/kg) [70]. Interestingly, the findings of this experiment were similar to the results of studies in which mice were treated with individual isoflavones [70]. Notably, the AUC values for both aglycones, genistein and daidzein, were increased 1.6- and 2-fold in the Bcrp−/− mice but still not significantly different from wild-type mice [70]. Further, the total isoflavones and phase II metabolites were significantly greater in Bcrp−/− than wild-type mice [70]. In the animals dosed with a mixture of isoflavones, the phase II metabolite concentrations were approximately equal to the concentrations of the same metabolites in animals treated with individual isoflavones [70].

Intestinal Metabolism and Transport of Isoflavones—To better understand the role of intestinal Bcrp in the disposition of isoflavones, Enokizono et al. [69] and Yang et al. [73] examined plasma profiles of isoflavones following alternate routes of exposure. Following a continuous intravenous (i.v.) infusion of genistein (i.v., 1.4 mg/kg/h, 2h) in the right jugular vein, or an intraperitoneal (i.p.) administration (20 mg/kg, 24 h), there was no difference in the plasma concentration of genistein between the Bcrp −/− and wild-type mice suggesting that Bcrp in the intestine plays a primary role in altering plasma genistein concentrations [69, 73]. The plasma profiles of the genistein secondary metabolites in the i.p. dosing study were similar to the oral dosing studies in that the AUC values of G-7-G, G-4′-S, and G-7-S were significantly greater in Bcrp −/− mice compared to wild-type mice [73]. Interestingly, in the study performed by Enokizono et al., i.v. administration of daidzein (1.4 mg/kg/h, 2 h) resulted in a significantly increased plasma concentration of the aglycone in the Bcrp−/− mice as compared to the wild-type mice, but to a lesser extent than the p.o. dose [69]. The authors note that the increased plasma concentration of daidzein following i.v. infusion was unexpected, but suggest that “impaired urinary excretion” of daidzein may be a contributing factor since about 10% of daidzein is eliminated via the urine in rats administered an oral dose of daidzein (100 mg/kg) [69, 118].

Biliary Transport of Isoflavones and Conjugates—In the study performed by Yang et al., genistein-sulfate (G-7-S and G-4′-S) bile levels were significantly reduced by 93% compared to wild-type mice following a 2.5 h *in situ* intestinal perfusion experiment [73]. Interestingly, there were no changes in the parent or genistein-glucuronide concentrations between genotypes suggesting that hepatic Bcrp selectively transports genistein-sulfate metabolites. Another study examined the role of Bcrp in the hepatic excretion of daidzein-sulfate in Bcrp −/− mice fed a normal rodent diet with trace amounts of isoflavones [98]. Daidzein-sulfate was detected in the bile of male and female Bcrp −/− mice at significantly lower concentrations (~60%) than the wild-types.

Distribution of Isoflavones—The transport of isoflavones by Bcrp in other organs has also been investigated in mice. Tissues with low basal expression of Bcrp, such as the stomach, have preferential isoflavone accumulation further supporting the contention that Bcrp limits isoflavone accumulation in an organ-specific manner [69, 97, 119]. In wild-type mice, the Bcrp protein is localized to the capillary endothelial cells of the blood brain barrier [120], endothelial cells within the testis [69, 121], and the epithelial (head) and endothelial cells (body) of the epididymis. Two studies examined the brain concentrations of genistein and daidzein following a 120 minute i.v. infusion of genistein or daidzein into the right

jugular vein of adult FVB wild-type and Bcrp −/− mice [69, 97]. Both studies found that the brain concentrations of the two isoflavones were increased significantly in Bcrp −/− mice compared to wild-type mice, demonstrating that Bcrp limits the distribution of genistein and daidzein to the brain. In the same study by Enokizono et al. [69], testis concentrations of genistein and daidzein were significantly increased by 200% and 400%, respectively, in Bcrp −/− mice compared to wild-type mice suggesting that Bcrp limits accumulation of isoflavones in this reproductive organ. Further, epididymal concentrations of genistein were increased 150% in Bcrp −/− mice compared to wild-type mice. No difference was observed in ovarian concentrations of genistein between Bcrp −/− and wild-type mice [69] even though Bcrp mRNA is detectable in mouse ovaries [121, 122]. Bcrp protein, however is not consistently expressed throughout the ovary but when present, it is localized to the small capillaries of the ovary endothelium [121]. Dankers et al. suggested that the varying protein expression throughout the organ may be a result of “the dynamic environment of the estrous cycle, where there is a constant regeneration and degradation of capillaries” [121]. Constantly changing protein expression may result in unclear findings in the tissue distribution of Bcrp substrates. Future studies should control for rodent estrous cycle stage to examine the accumulation of isoflavones in the ovary.

In the placenta, the BCRP protein localizes to the apical membrane of syncytiotrophoblasts which are in direct contact with the maternal circulation [50]. Transporter function and localization in this important organ of pregnancy suggest that BCRP is responsible for protecting the fetus from exposure to harmful chemicals that may be present in the maternal circulation. The mouse orthologue, Bcrp, in the placenta limited fetal exposure to Bcrp substrates including topotecan [60] and is therefore a useful model for studying the *in vivo* function of placental Bcrp/BCRP. To evaluate the role of placental Bcrp in the *in utero* distribution of genistein, pregnant FVB wild-type and Bcrp −/− mice were infused via the right jugular vein with genistein (1.25 mM) for 120 min and there was no difference in the plasma concentration between the wild-type and Bcrp −/− dams [69]. Interestingly, Bcrp −/− fetuses accumulated significantly more (1.6-fold) genistein than the wild-type fetuses [69]. Further, significantly greater concentrations of genistein were detected in the brains of the Bcrp −/− fetuses [69] than the wild-type fetal brains, demonstrating the importance of Bcrp in protecting against genistein penetration of the developing blood-brain barrier. Of note, there is evidence that isoflavones such as genistein alter the epigenome of developing mice [123, 124]. Taken together, future research should focus on risk factors that may reduce BCRP activity in the placenta and impair the development of offspring following *in utero* exposure to isoflavones.

Evidence for Isoflavones as Inhibitors of BCRP Function

Recent research has begun to explore the ability of isoflavones to alter the pharmacokinetics or pharmacodynamics of chemicals that are transported by BCRP/Bcrp. This may be beneficial in situations where isoflavones enhance the efficacy of a particular treatment, such as a chemotherapeutic drug. However, negative outcomes such as an increase in the toxicity or a reduction in the efficacy of the drug may also occur as a result of BCRP inhibition by isoflavones. To date, a variety of *in vitro* and *in vivo* experimental models have been used to detect isoflavone-inhibitors of BCRP/Bcrp function and to better understand

the potential implications of concomitantly consuming isoflavones (i.e., a soy rich diet) with drugs that are transported by BCRP.

Altered Chemoresistance

Many chemotherapy drugs, including mitoxantrone, SN-38 (the active metabolite of irinotecan), topotecan, and methotrexate are substrates of BCRP (Table 1). These drugs are routinely used to screen for potential inhibitors of BCRP function indirectly by assessing the cytotoxic effect of the chemotherapy drug on cell growth in the presence of the potential inhibitor. In the presence of BCRP inhibitors, cells that express the BCRP protein cannot effectively remove the cytotoxic chemical which leads to a potentiation of the chemotherapeutic response. Human breast cancer and lung cancer cells expressing BCRP, MCF-7/MX100 and NCI-H460/MX20, respectively, are commonly used as models for measuring BCRP activity. MCF-7 and NCI-H460 cells that express BCRP at high levels are selected for using 100 nM or 20 nM mitoxantrone, respectively, prior to experiments [87]. Forty-eight hour exposure with genistein (10–50 μM) or biochanin A (5–50 μM) reversed mitoxantrone resistance in MCF-7/MX100 cells in a concentration-dependent manner using the sulforhodamine B assay to assess cell density [87, 100]. Furthermore, genistein inhibited the cell growth of human leukemia cells that overexpressed the BCRP transporter (K562/BCRP) in the presence of SN-38 compared to cells cultured without isoflavones [71, 101]. Daidzein inhibited the cell growth of K562/BCRP cells in the presence of SN-38 but to a lesser extent than genistein [101]. Genistein (10 μM) alone did not reduce the cell viability of MCF-7 breast cancer cells following 72 h incubation [125], whereas 48 h incubation of K562 leukemia cells with genistein (10–100 μM) modestly reduced cell viability by about 15% [126]. The inconsistency of these findings may arise from innate differences in the protective mechanisms of each cell type. For example, moderate levels of ABC transporters (MDR1 and BCRP) [125] are expressed in the MCF-7 cells and may participate in the removal of genistein or other toxic metabolites thereby protecting the cells from potential toxic effects of the compounds. Additionally, two different methods were used to detect cell viability. Zhang et al. utilized a more sensitive and direct measure of cell viability, trypan blue, which quantified the number of viable cells [126], while Pick et al. drew conclusions based on the ATP assay which indirectly indicates the amount of ATP generated which was positively correlated with cell viability [125].

Probe Substrates

Well-characterized and easily detected BCRP substrates (i.e., fluorescent and radiolabeled compounds) are commonly used as probe substrates for detecting inhibitors of BCRP-mediated transport. Using whole cell model systems, BCRP function is assessed by determining the intracellular accumulation of a probe substrate in the absence and presence of an inhibitor. Membrane-based assays including inverted plasma membrane vesicles and ATPase assays are isolated subcellular systems that are used to better characterize transporter kinetics and stimulation of transporter ATPase activity by a substrate in the presence of an inhibitor.

Cell-Based Assays—In addition to being a chemotherapy drug, mitoxantrone also has fluorescent properties and intracellular accumulation of the drug can be quantified by flow

cytometry. A 30-minute co-incubation of mitoxantrone with genistein, daidzein, or biochanin A in MCF-7/MX100 and NCI-H460/MX20 cells, significantly increased the accumulation of mitoxantrone up to 4-fold compared to the control cells treated with mitoxantrone alone [87, 100]. In addition, intracellular topotecan fluorescence increased in K562/BCRP cells in the presence of genistein (30 and 100 μM) relative to the parent K562 cells [71]. Pick et al. described genistein as an enhancer of fluorescent Hoechst 33342 accumulation in MDCK/BCRP and MCF-7/MX cells with IC_{50} values for BCRP efflux of 6.9 μM and 8.8 μM, respectively, in each cell type [125]. Daidzein significantly increased the accumulation of mitoxantrone in MCF-7/MX cells and K562/BCRP cells up to 3-fold which was similar to the fluorescence intensity detected in the respective parental cells [68]. In the same cell types, daidzein increased the accumulation of the fluorescent derivative of the BCRP substrate and blood pressure medication, prazosin (BODIPY-prazosin) to levels comparable to that in the parental cell line [68].

Similar to their interaction with human BCRP, the mouse Bcrp protein is also sensitive to isoflavone-mediated inhibition. Biochanin A (2.5–25 μM) significantly increased the intracellular fluorescence of mitoxantrone up to 4 and 2.5-fold in MDCK cells that expressed mouse or human Bcrp/BCRP, respectively [88]. In addition, the BL-to-AP transport of mitoxantrone was attenuated by biochanin A (5 μM) in MDCK/Bcrp cells [88]. Together, genistein and daidzein (100 μM each) reduced the BL-to-AP transport of BCRP substrates, nitrofurantoin and danofloxacin, in MDCK/BCRP and MDCK/Bcrp cells [89, 127], respectively. Even further, a combination of multiple flavonoids including biochanin A and genistein (apigenin, biochanin A, chrysin, genistein kaempferol, 1 μM total flavonoid) increased the accumulation of mitoxantrone in MCF-7/MX100 cells to a greater extent (EC_{50}: 0.23 ± 0.08 μM) than each individual flavonoid (i.e., genistein EC_{50}: 14.9 ± 2.69 μM). These data suggest that low concentrations of various isoflavones or flavonoids may have an additive or synergistic effect on the inhibition of BCRP transport [100].

Membrane-Based Assays—Inhibitors of BCRP function can be detected using BCRP inverted plasma membrane vesicles. In this assay, inhibitors are recognized by a significant decrease in the vesicular accumulation of a BCRP probe substrate compared to vesicles without the inhibitor. In BCRP vesicles, there was a trend for aglycones (genistein> biochanin A> glycitein> formononetin> daidzein) to reduce the ATP-dependent transport of the BCRP substrate, ^{3}H-methotrexate (100 μM) to a greater extent than their respective glucosides (biochanin A-glucoside, sissotrin> genistin> formononetin-glucoside, ononin> glycitin>daidzin) as detected by liquid scintillation counting [90]. In general, because the glucosides are quickly converted to the aglycone forms *in vivo*, the former finding holds more bearing on the potential for BCRP inhibition *in vivo*. Importantly, the concentration of genistein that inhibits BCRP function in inverted vesicles, is within the range of plasma concentrations of genistein (1–3 μM, n=12 healthy, human volunteers) that may be found circulating following dietary consumption of soy products [128]. Interestingly, soybean extract (1 mg/ml) reduced the uptake of ^{3}H-methotrexate in BCRP-overexpressing vesicles to the same extent as BCRP inhibitor, FTC (5 μM) suggesting that the multiple compounds present in a soy diet, including genistein, daidzein, glycitein and their glucosides, collectively reduce BCRP function [90].

Another plasma membrane-based method used to detect functional inhibitors of BCRP-mediated transport is the ATPase assay. ATP hydrolysis is measured as an indirect indicator of substrate transport and is reduced in the presence of a BCRP-specific functional inhibitor [129]. In the mammary gland, BCRP is expressed on the apical membrane of alveolar epithelial cells and actively concentrates substrates in the milk of lactating animals (mouse and cow) and humans [130]. In plasma membranes isolated from the mammary gland of a lactating cow, genistein (10 μM) reduced the Bcrp ATPase activity stimulated by mitoxantrone [80], suggesting that genistein may alter the transport of drugs into the milk of lactating animals and potentially humans.

Altered Pharmacokinetics of Other Drugs

The ability of dietary constituents, including isoflavones, to alter the *in vivo* pharmacokinetics and pharmacodynamics of a drug is important to consider since their sale and consumption are unregulated. This is especially of concern when a drug that is a BCRP substrate is administered during lactation. Inhibition of chemical transfer into the milk may provide benefits or deficits to the offspring resulting in protection of the infant from the toxic effects of the transferred drug, or reduced transfer of antiviral medications intended to limit the infant's exposure to HIV, respectively. Enrofloxacin is an antibacterial drug that has been used in veterinary medicine to treat gram-negative and gram-positive bacterial infections in ruminants such as cows and sheep. Its metabolite, ciprofloxacin, is also efficacious *in vivo* and is used in humans for the treatment of bacterial infections. Importantly, ciprofloxacin is recognized by the American Academy of Pediatrics as a maternal medication usually compatible with breastfeeding [131]. Both the parent enrofloxacin compound and its metabolite ciprofloxacin are recognized substrates of BCRP [78, 80]. To understand the effect of genistein on the milk concentrations of concomitantly administered enrofloxacin in sheep, 12 lactating sheep were infused with enrofloxacin either with or without genistein via the left jugular vein [80]. There was no change in the plasma concentration of enrofloxacin (area under the curve, AUC or maximal concentration, C_{max}) between the sheep that received genistein and those that did not. However, genistein decreased the enrofloxacin milk content which was demonstrated by a 1.5-fold reduction in the enrofloxacin milk AUC value [80]. These data suggest that in dairy animals, genistein may reduce milk concentrations of enrofloxacin and shorten the time in which the animals are withdrawn from producing usable milk. Further, in humans genistein may protect the developing infant from unnecessary exposure to ciprofloxacin.

Because genistein and daidzein are often found together in nature, Perez et al. [127, 132] examined the effect of a combined isoflavone exposure on the distribution of BCRP substrates danofloxacin and nitrofurantoin into the milk of lactating sheep. Danofloxacin is an antibiotic used in veterinary medicine to treat a broad range of bacterial infections. Nitrofurantoin is an antibiotic that is often prescribed for the treatment of urinary tract infections in humans and animals. In particular, nitrofurantoin is prescribed to pregnant [133] and lactating women as it is recognized by the American Academy of Pediatrics as a maternal medication typically compatible with breast feeding [131]. The compounds concentrate in the milk of lactating animals (danofloxacin and nitrofurantoin) [82, 134] and/or women (nitrofurantoin) [135]. In the nitrofurantoin experiment, sheep exposed to

genistein and daidzein via the standard diet or exogenous dosing with the isoflavones (10 mg/kg genistein + 10 mg/kg daidzein) by oral gavage, experienced a significant decrease in the milk AUC of nitrofurantoin [132]. Additionally, the nitrofurantoin-treated control animals experienced higher plasma AUC and C_{max} nitrofurantoin as compared to those that were exposed to isoflavones [132]. Because this is not in line with the Bcrp-inhibition paradigm, the authors suggest that the isoflavone treated sheep may have experienced a reduced plasma concentration of nitrofurantoin due to decreased absorption or enhanced elimination of nitrofurantoin. Unfortunately, they do not offer mechanistic evidence to explain the reduced systemic exposure to the drug. In the danofloxacin experiment, lactating sheep received a 15-day soy supplemented diet that significantly reduced the milk AUC and C_{max} of danofloxacin compared to the sheep that received regular forage without isoflavones [127]. Interestingly, the sheep that received an oral dose of genistein and daidzein (10 mg/kg genistein + 10 mg/kg daidzein) did not demonstrate a similar decrease in milk concentrations of danofloxacin suggesting that the form in which the compounds are administered (i.e., liquid vs. solid, aglycone vs. glycone) or presence of other compounds in the diet may determine its effect on the pharmacokinetics of other chemicals [127]. The authors list the various constituents of the soy diet but did not mention the amount of genistein, daidzein, or other isoflavones present in each diet [127].

Bcrp −/− mice can be used to elucidate the functional role of BCRP/Bcrp in mediating potential nutrient-drug interactions in an *in vivo* situation. In the absence of isoflavones, nitrofurantoin was confirmed as a BCRP substrate due to increased plasma AUC of nitrofurantoin in Bcrp −/− mice compared to wild-type mice [82],[89]. While treatment of wild-type mice with genistein and daidzein had no effect on the plasma AUC of nitrofurantoin over a 2 hr period compared to vehicle-treated controls, higher plasma nitrofurantoin levels were in fact observed at 30 min [89]. At that same time point, genistein and daidzein significantly reduced the nitrofurantoin concentrations in the milk and bile of wild-type mice as compared to the vehicle-treated mice [89]. This study suggests that examination of early time points may be necessary to observe changes in drug pharmacokinetics brought on by isoflavones. These data combined with those in the sheep indicate that a soy diet may reduce the potential for infant exposure to nitrofurantoin in milk.

Only one study has investigated whether biochanin A can alter the pharmacokinetics of other BCRP/Bcrp substrates [88]. Male wild-type ND4 Swiss Webster mice were administered an intravenous dose of mitoxantrone (5 mg/kg) 5 minutes following an intravenous dose of biochanin A (10 mg/kg) [88]. Biochanin A actually enhanced the mitoxantrone elimination from the kidney and spleen as there was a trend for those organs to accumulate less mitoxantrone than the vehicle-treated mice, though this was not statistically significant [88]. These findings were inconsistent with the *in vitro* results of this same study that found biochanin A to be an inhibitor of the Bcrp-mediated transport of mitoxantrone, indicating that there may be some disconnect between *in vitro* and *in vivo* models for measuring inhibition of BCRP function. Future research should examine the effects of isoflavones on the pharmacodynamics of a drug, such as measuring the contribution of the isoflavones to the exacerbation of a toxic side effect. Such evidence may provide the impetus for improving the current guidelines for prescribing drugs that are BCRP substrates.

The Regulation of BCRP by Isoflavones

Genistein interacts with a variety of cellular proteins that modulate gene expression and/or protein localization such as the estrogen receptor, aryl hydrocarbon receptor, and the epidermal growth factor receptor [12, 39, 41, 42, 136]. Because BCRP expression is regulated by the aryl hydrocarbon receptor [136], Ebert et al. examined the ability of genistein (1–50 μM) to alter BCRP mRNA expression in Caco-2 cells and described no effect compared to the vehicle-treated cells following a 48 h incubation [102]. This finding was confirmed when Arias et al. reported no change in BCRP protein expression in Caco-2 cells following 48 h exposure to genistein (0.1–10 μM) [103]. Further, a 5-day exposure to genistein (3 and 10 μM) did not alter the BCRP protein expression in K562 cells that expressed BCRP [71]. Conversely, genistein (15 μM, 24 h) reduced the mRNA expression of BCRP by approximately 70% in gastric cancer (MGC-803) cells [104]. Taken together, the effect of genistein and possibly other isoflavones on BCRP regulation is likely tissue-specific. Further investigation of the effect of isoflavones on BCRP expression in other cell lines and tissue types that rely on BCRP function for protection against xenobiotic accumulation, such as the placenta and kidneys, is required to fully understand the role of isoflavones in the regulation of BCRP expression and ultimately function.

Conclusions

Isoflavones interact with the BCRP efflux transporter through a variety of ways including direct transport, chemical antagonism, and altered transporter expression. Evidence for the selective removal of isoflavones from target organs which express BCRP/Bcrp [69, 73, 97], suggests that the transporter plays an important role in the *in vivo* disposition of isoflavones. Therefore the potential effects of isoflavones on human health may be influenced by the efficiency of BCRP function. In addition to transporting parent isoflavones, BCRP also transports sulfated and glucuronidated isoflavone metabolites [70, 73, 98, 99] which, too, possess some biological activity [137]. Furthermore, sulfatase and glucuronidase deconjugating enzymes are present in the liver, intestines, blood, and fetus [16, 73, 138, 139] and may convert the metabolites back to the parent compound. Because of these hydrolytic reactions, the ability of BCRP to transport both parent isoflavones and their metabolites is important. Due to the potential for isoflavones to disrupt the endocrine system, future research should focus on understanding the role of BCRP/Bcrp in reproductive organs and how disruption of transport may lead to inappropriate exposure of isoflavones to target organs and developing tissues.

As a result of being directly transported by BCRP, isoflavones may competitively inhibit the transport of other chemicals including pharmaceuticals which depend on BCRP for appropriate distribution, metabolism, and elimination. In addition to competitive inhibition, evidence suggests that isoflavones may also non-competitively inhibit BCRP-mediated transport as there are more isoflavone and flavonoid compounds that inhibit BCRP function than those that are directly transported [71, 90, 101]. Further examination of the molecular interactions of isoflavones with ABC transporters and other proteins that contain a nucleotide binding domain, reveal that the compounds may disrupt BCRP function through interference with ATP hydrolysis via association with the BCRP nucleotide binding domain

[140] (reviewed in [141]). It is important to note that affinity of a compound for a particular site within the BCRP protein will determine the nature and extent of the chemical-transporter interaction. A high-resolution 3D structure of the human BCRP protein is not yet available, though working towards completing this task will help to elucidate the complexities of BCRP-isoflavone interactions.

According to self-reported surveys, the average consumption of total isoflavones ranges between 2 and 50 mg/day and is vastly different depending upon ethnicity and geographic location [142–144]. Consumption of a soy food diet in this range resulted in total plasma isoflavone concentrations of approximately 19–500 nM. However, in a pharmacokinetic study which enlisted 12 healthy volunteers to consume soy food (96 mg/day) with 3 meals every day for 7 days, led to a total plasma isoflavone concentration of about 5 μM on day 7 [128]. These data suggest that the isoflavone concentrations which inhibited BCRP function *in vitro* (low μM range [87, 88, 90, 125]) may be applicable to a population that consumes soy regularly. To recapitulate a soy diet, a few investigations examined the effect of multiple isoflavones or flavonoids on BCRP function [70, 90, 100]. These preliminary studies revealed the compounds may have additive or synergistic effects, supporting the need for future studies to address the effect of isoflavone mixtures on BCRP function. This is of particular importance because the population which consumes soy is rapidly increasing, particularly in the United States. Since 1996, soy food sales in the United States dramatically increased from 1 billion to about 5.2 billion dollars spent per year by 2011 according to the Soy Foods Association of North America [145], which is likely a result of multiple reports suggesting the health benefits of soy consumption. Furthermore, there has been a steady increase in the overall prescription of drugs in the United States over the past 10 years [146]. Taken together with the findings that suggest that isoflavones alter the pharmacokinetics of drugs which are substrates for BCRP, the risk for a nutrient-drug interaction mediated by BCRP is conceivable and warrants further investigation to help improve the guidance for the prescription of drugs that are BCRP substrates.

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Abbreviations

−/−	knockout
ABC	ATP-binding cassette
ABCP	ABC transporter highly expressed in the placenta
AP-to-BL	apical-to-basolateral
AUC	area under the curve
BCRP/Bcrp	human/mouse breast cancer resistance protein
BL-to-AP	basolateral-to-apical
C_{max}	maximal concentration

FTC	fumitremorgin C
G-7-G	genistein-7-glucuronide
G-4′-G	genistein-4′-glucuronide
G-7-S	genistein-7-sulfate
G-4′-S	genistein-4′-sulfate
HPLC-DAD-MS/MS	high performance liquid chromatography diode array detection tandem mass spectrometry
HPLC-ECD	high performance liquid chromatography electrochemical detection
i.p	intraperitoneal
i.v	intravenous
LC/MS	liquid chromatography/mass spectrometry
LC-MS/MS	liquid chromatography/tandem mass spectrometry
LSC	liquid scintillation counting
MDCK	Madin-Darby canine kidney II
MDR1	multidrug resistance protein 1
MRP	multidrug resistance-associated protein
MXR	mitoxantrone resistance transporter
p.o	oral
Sf9	*Spodoptera frugiperda*
SULT	sulfotransferase
UGT	UDP-glucuronosyltransferase
UPLC-DAD-MS/MS	ultra-performance liquid chromatography diode array detection tandem mass spectrometry
UPLC-MS/MS	ultra-performance liquid chromatography tandem mass spectrometry

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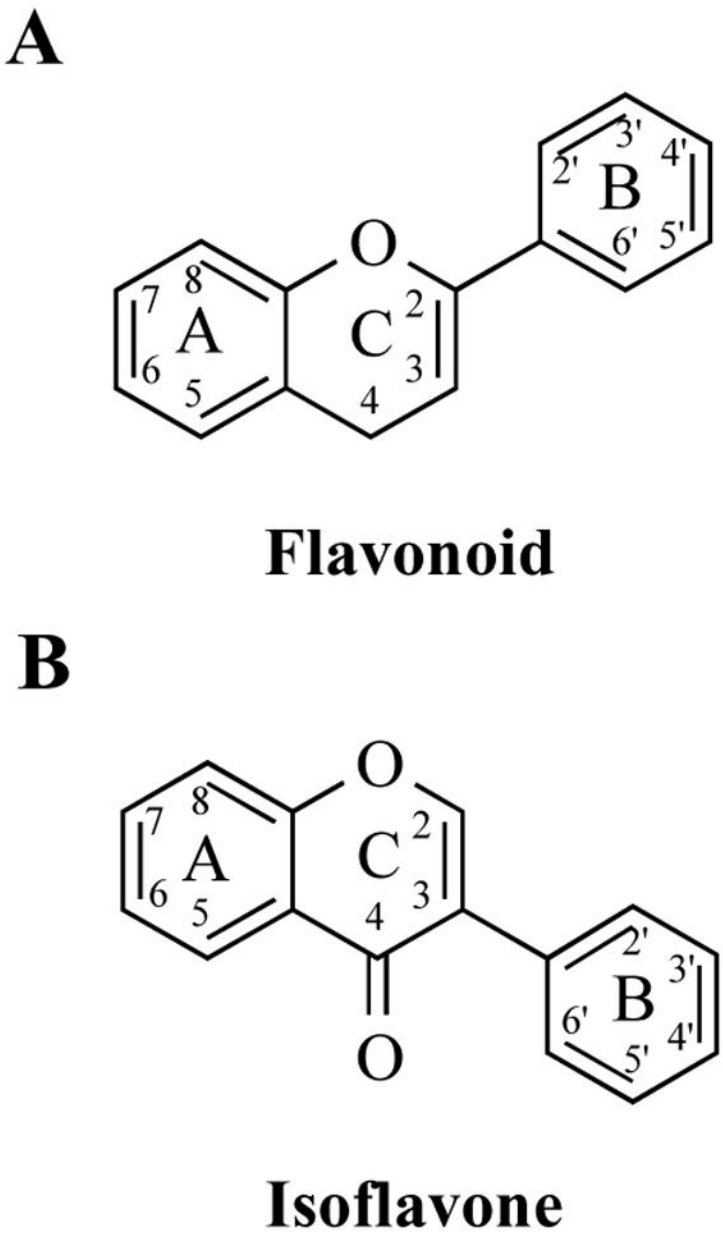

Figure 1.
The parent ring structures of A) flavonoids and B) isoflavones.

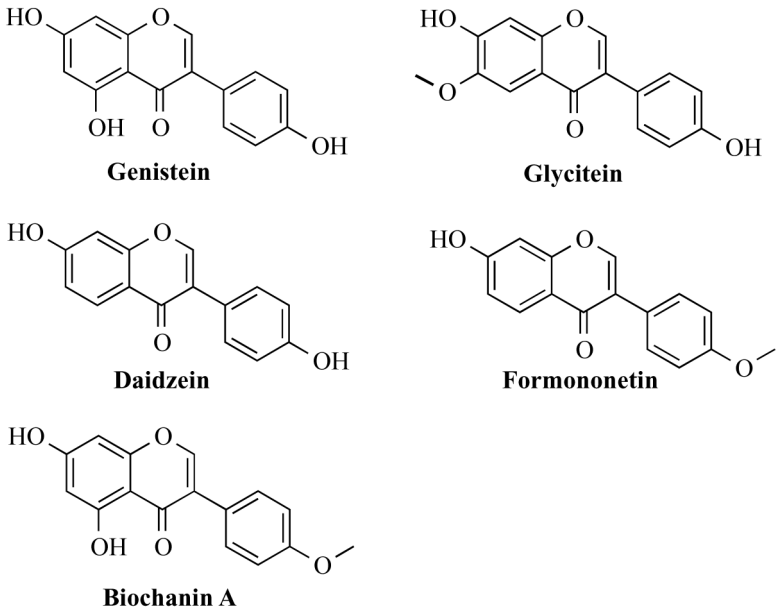

Figure 2.

Chemical structures of the most commonly studied isoflavones: genistein, daidzein, biochanin A, glycitein, and formononetin.

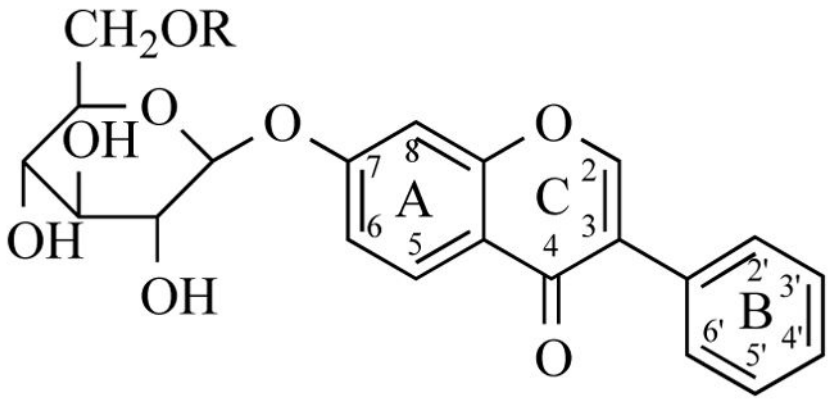

Figure 3.
Basic chemical structure of an isoflavone-glycoside.

Table 1

Substrates and Inhibitors of BCRP/Bcrp.

Substrates	**References**
Carcinogens	
Aflatoxin B_1	[55]
2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP)	[56]
Chemotherapy drugs	
Etoposide	[57]
Imatinib	[58]
Mitoxantrone	[46–48]
Methotrexate	[59]
Topotecan	[60]
SN-38	[61]
Endogenous compounds	
Estrone-3-sulfate	[62]
Folic acid	[63]
Protoporphyrin IX	[64]
Uric acid	[65]
Flavonoids	
Biochanin A-glucuronide	[66]
Biochanin A-sulfate	[67]
Daidzein	[68, 69]
Daidzein-sulfate	[70]
Genistein	[69, 71]
Genistein-glucuronide	[70, 72, 73]
Genistein-sulfate	[70, 72, 73]
Quercetin	[74]
Fluorescent dyes	
BODIPY-Prazosin	[75]
Hoechst 33342	[76]
Pheophorbide a	[60, 77]
Rhodamine 123	[75]
Other pharmaceutical drugs	
Cimetidine	[56]
Ciprofloxacin	[78]
Danofloxacin	[79]
Enrofloxacin	[80]
Glyburide	[81]
Nitrofurantoin	[82]
Prazosin	[83]
Inhibitors	
Chemotherapy drugs	

Substrates	**References**
Gefitinib	[84]
Imatinib	[85]
Fungal toxins	
FTC	[48]
Ko143	[86]
Flavonoids	
Biochanin A	[87, 88]
Daidzein	[87, 89]
Formonetin	[90]
Genistein	[87, 89, 90]
Glycitein	[90]
Endogenous compounds	
17β-estradiol	[91]
Estrone	[91]
Pharmaceutical drugs	
Cyclosporin A	[92]
Omeprazole	[93]
Other	
GF120918	[94]

Table 2

Summary of *in vitro* and *in vivo* experiments describing genistein as a substrate of BCRP/Bcrp.

Experiment Type	Model	Genistein Concentration	Detection Method	Time	BCRP Inhibitor	References
In Vitro						
Cellular uptake	K562/BCRP cells	30 nM (^{3}H)	LSC	4 h	-	[71]
Trancellular transport	LLC-PK1/BCRP cells	30 nM (^{3}H)	LSC	4 h	3 μM FTC	[71]
	MDCK/BCRP cells	3 μM	LC/MS	4 h	-	[69]
	Caco-2 cells	100 nM (^{14}C)	LSC	1 h	1 μM FTC	[95]
	Caco-2 cells	50 μM	HPLC-ECD	50 min	Estrone-3-sulfate	[96]
	MDCK/Bcrp	3 μM	LC/MS	4 h	-	[69]
	MDCK/Bcrp	1–3 μM	LC/MS	3 h	-	[97]
In Vivo Pharmacokinetics						
Oral (p.o.)						
Plasma	FVB Bcrp −/− mice	8.1 mg/kg	LC/MS	4 h	-	[69]
	FVB Bcrp −/− mice	2–20 mg/kg	UPLC/MS/MS	24 h	-	[73]
Intravenous (i.v.) infusion						
Brain	FVB Bcrp −/− mice	1.4 mg/kg/h	LC/MS	2 h	-	[69]
	FVB Bcrp −/− mice	0.8 mg/kg initial, 4.3 mg/kg/h	LC-MS/MS	2 h	-	[97]
Testis	FVB Bcrp −/− mice	1.4 mg/kg/h	LC/MS	2 h	-	[69]
Epididymis	FVB Bcrp −/− mice	1.4 mg/kg/h	LC/MS	2 h	-	[69]
Placenta	Pregnant FVB Bcrp −/− mice	1.4 mg/kg/h	LC/MS	2 h	-	[69]
Fetal Brain	Pregnant FVB Bcrp −/− mice	1.4 mg/kg/h	LC/MS	2 h	-	[69]

Table 3

Summary of *in vitro*, *in situ*, and *in vivo* experiments describing genistein-sulfate as a substrate of BCRP/Bcrp.

Experiment type	Model	Genistein concentration	Detection Method	Time	BCRP Inhibitor	References
In Vitro						
Transcellular transport	Caco-2	2–10 μM	UPLC-MS/MS	4 h	5 μM Ko143	[73]
Vesicle uptake	*Sf*9-BCRP vesicles	Chow (Amount unknown; mouse urine)	HPLC/MS	40 s	-	[98]
	*Sf*9-BCRP vesicles	^{35}S-genistein-sulfate	LSC	40 s	-	[98]
In Situ Intestinal Perfusion						
SI and colon excretion	FVB Bcrp −/− mice	10 μM	UPLC-DAD-MS/MS	120 min	-	[72]
Biliary excretion	FVB Bcrp −/− mice	10 μM	UPLC-MS/MS	2.5 h	-	[73]
In Vivo Pharmacokinetics						
Oral (p.o.)						
Plasma	FVB Bcrp −/− mice	50 mg/kg	HPLC-DAD-MS/MS	3 h	-	[70]
	FVB Bcrp −/− mice	2–20 mg/kg	UPLC-MS/MS	24 h	-	[73]
	FVB Bcrp −/− mice	Chow (Amount unknown)	HPLC/MS	-	-	[98]
Intraperitoneal (i.p.)						
Plasma	FVB Bcrp −/− mice	20 mg/kg	UPLC/MS/MS	24 h	-	[73]

Table 4

Summary of *in vitro*, *in situ*, and *in vivo* experiments describing genistein-glucuronide as a substrate of BCRP/Bcrp.

Experiment type	Model	Genistein concentration	Detection Method	Time	BCRP Inhibitor	References
In Vitro						
Cellular uptake	HeLa/UGT1A9 cells	10 μM	UPLC-MS/MS	120 min	5–10 μM Ko143	[99]
Transcellular transport	Caco-2	2–10 μM	UPLC-MS/MS	4 h	5 μM Ko143	[73]
In situ Intestinal perfusion						
SI Excretion	FVB Bcrp −/− mice	10 μM	UPLC-DAD-MS/MS	120 min	-	[72]
In Vivo Pharmacokinetics						
Oral (p.o.)						
Plasma	FVB Bcrp −/− mice	50 mg/kg	HPLC-DAD-MS/MS	3 h	-	[70]
	FVB Bcrp −/− mice	2–20 mg/kg	UPLC/MS/MS	24 h	-	[73]
Intraperitoneal (i.p.)						
Plasma	FVB Bcrp −/− mice	20 mg/kg	UPLC/MS/MS	24 h	-	[73]

Table 5

Summary of *in vitro*, *in situ*, and *in vivo* experiments describing genistein as an inhibitor of BCRP/Bcrp function.

Experiment type	Model	Genistein concentration	Detection method	Time	BCRP Substrate	References
In Vitro						
Chemo-resistance	MCF-7/MX100 cells	10–50 μM	Sulforhodamine B assay	48 h	0–1000 μM Mitoxantrone	[87, 100]
	K562/BCRP cells	3 μM	Cell counter	5 days	SN-38	[71]
	K562/BCRP cells	NR	Cell counter	5 days	SN-38	[101]
Cellular uptake	MCF-7/MX100 cells	50 μM	Flow cytometry	30 min	3 μM Mitoxantrone	[87, 100]
	NCI-H460/MX20 cells	50 μM	Flow cytometry	30 min	3 μM Mitoxantrone	[87]
Vesicle uptake	*Sf*9-BCRP vesicles	10–100 μM	LSC	2 min	100 μM ^{3}H-Mitoxantrone	[90]
ATPase activity	Lactating cow mammary gland plasma membranes	10 μM	Colorimetric detection of P_i	40 min	50 μM Mitoxantrone	[80]
In Vivo						
Oral (p.o.)						
Plasma	Lactating sheep	0.8 mg/kg	HPLC	24 h	Enrofloxacin	[80]

Table 6

Summary of experiments describing the regulation of BCRP by genistein.

Model	Genistein Concentration	Treatment Duration	Effect on BCRP mRNA	Effect on BCRP protein	References
Caco-2 cells	1–50 μM	48 h	↔	N.D.	[102]
	0.1–10 μM	48 h	N.D.	↔	[103]
K562/BCRP cells	3–10 μM	5 days	N.D.	↔	[71]
MGC-803 cells	15 μM	24 h	↓	↔	[104]