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Hepatic and Intestinal Multidrug Resistance-Associated Protein 2: Transcriptional and Post-transcriptional Regulation by Xenobiotics

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Abstract

We are daily exposed to a large number of pharmacological drugs, environmental pollutants, and natural toxins, which represent a potential toxic insult. The organism possesses a sophisticated system of detoxification particularly expressed in the liver, intestine, and kidney. This system consists of intracellular biotransformation enzymes that convert the toxins into more hydrophilic derivatives followed by their elimination through membrane transporters. Multidrug resistance-associated protein 2 (MRP2, ABCC2) is an important member of the ATP-binding cassette (ABC) superfamily of transporters localized at the apical membrane of polarized cells, such as hepatocytes, enterocytes, and renal tubular cells. MRP2 is proposed as a major actor in the elimination of endo- and xenobiotics, mainly conjugated with glucuronic acid, glutathione, and sulfate. The small intestine and the liver constitute relevant detoxification organs expressing MRP2 and therefore preventing absorption and promoting the hepatobiliary clearance of xenobiotics. MRP2 expression and/or function can be modulated by therapeutic drugs, herbal products, dietary compounds, and environmental pollutants. Consequently, MRP2 modulation could cause changes in tissue exposure, with potential toxicological and pharmacological consequences. This chapter reviews the information available on the role of hepatic and intestinal MRP2 in detoxification processes, and their regulation by xenobiotics, considering in particular its toxicological relevance.

Keywords: MRP2, intestine, liver, detoxification, clearance, toxicity

1. Introduction

1.1. Detoxification of xenobiotics

Most organisms are daily exposed to a large number of different xenobiotics, such as thera-peutic drugs, environmental pollutants, and natural toxins. The major routes of exposure to these chemicals comprise incorporation with the diet, inhalation or absorption through the skin. Preservation of health depends largely on the body's ability to eliminate these harmful substances. In this regard, the organism possesses a sophisticated system of detoxification mainly expressed in organs such as the liver, intestine, and kidney. The detoxification process consists of intracellular biotransformation enzymes that neutralize the toxins and membrane transporters for their subsequent elimination from the cell. The biotransformation process is carried out by the same biochemical machinery that metabolizes endogenous compounds, often of similar chemical structure. Even though a compound can be excreted without undergoing any change, it is usually converted by biotransformation enzymes into a more hydrophilic derivative prior to elimination. The biotransformation reactions are carried out by phase I enzymes such as cytochrome P450 (CYP) members and by phase II conjugating enzymes such as glutathione S-transferase (GST; EC 2.5.1.18), UDP-glucuronosyltransferase (UGT; EC 2.4.1.17), and sulfotransferase (SULT; EC 2.8.2.) [1, 2]. Phase I enzymes usually act in tandem with phase II enzymes, which ultimately results in incorporation of anionic groups into the xenobiotic molecule. The hydrophilic derivatives can be excreted from the cells by phase III or membrane transport systems, with the anionic groups acting as *affinity markers* for a series of transporters of the multidrug resistance-associated protein (MRP) family. These proteins are members of the ATP-binding cassette (ABC) drug efflux transporters and mediate the active extrusion of biotransformation products for their subsequent elimination from the body [1, 2]. Direct elimination of xenobiotics without suffering any biotransformation is also possible and is denominated *"phase 0 metabolism"* [3].

What follows is a description of the role of the multidrug resistance-associated protein 2 (MRP2, ABCC2) as an important component of the ABC family involved in xenobiotic disposition by liver and intestine.

1.2. Multidrug resistance-associated protein 2 and its role in the detoxification of xenobiotics

MRP2 structure consists of a large core segment, containing two cytosolic nucleotide-binding domains (NBDs), two membrane-spanning domains (MSDs), and a linker segment L1, shared by other ABC members. Additionally, MRP2 contains a third NH_2-terminal membrane-spanning domain MSD0, also called terminal transmembrane domain, with five transmem-brane helices resulting in an extracellular NH_2-terminus and an intracellular linker segment 0 (L0) (**Figure 1**) [4]. MRP2 is characteristically expressed at the apical membrane of polarized cells such as hepatocytes, enterocytes, and renal tubular cells [4–6], where it plays a primary role in the elimination of specific compounds. Particularly, the highest concentration of this transporter is found in the liver and intestine [7], two organs playing a prominent role in xenobiotic detoxification commonly known as "first-pass metabolism and clearance". Protein expression of MRP2 is highest in enterocytes of the proximal small intestine, decreasing in

direction to the terminal ileum [8], gradient shared with the expression of phase II metabolizing enzymes such as GST and UGT [9, 10], thus suggesting that metabolism and transport processes may act coordinately. In the liver, the major biotransformation organ, MRP2 is abundantly expressed in the bile canalicular membranes of the hepatocyte [11], where it plays a role in bile formation through secretion of endogenous substrates such as glutathione (GSH) and glutathione conjugates. Canalicular MRP2 also constitutes the main route of elimination of xenobiotics conjugated with GSH, sulfate or glucuronic acid [12]. Similarly, immunohistochemical analysis in normal rat liver demonstrated that UGTs and rMrp2 are localized in the same regions [13, 14], also indicating that they may work in cooperation. MRP2 in either localization contributes significantly to disposition of potentially harmful compounds thus decreasing their toxicity.
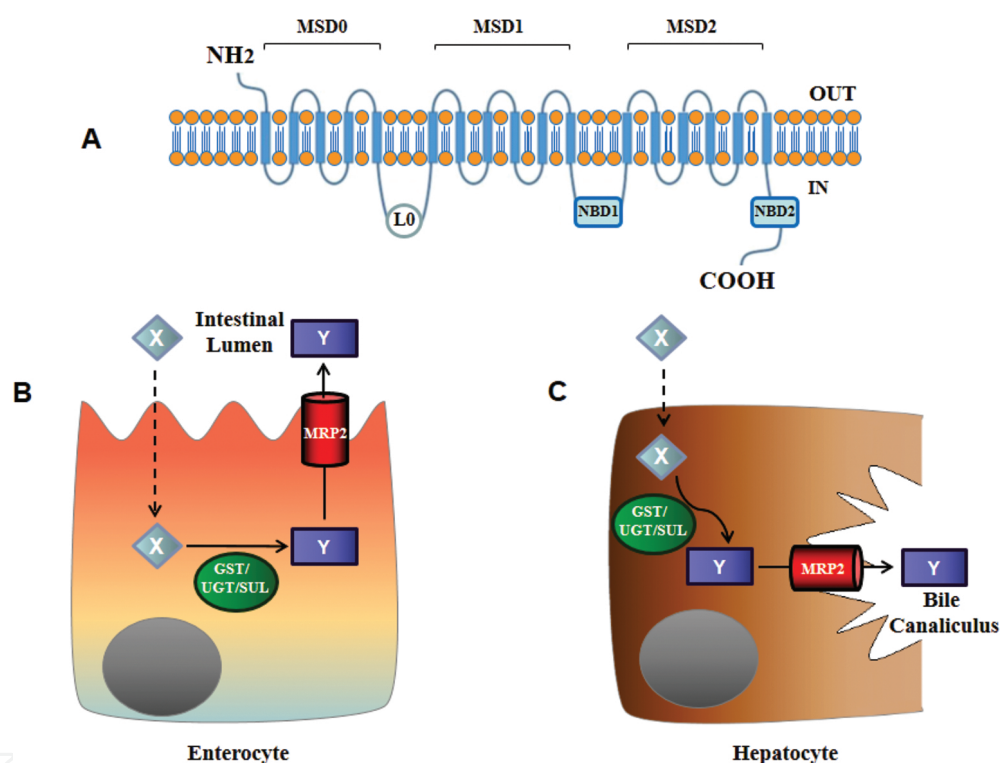

Figure 1. (A) Molecular structure of MRP2/ABCC2. MSDs: membrane-spanning domains; NBDs: nucleotide-binding domains; L0: linker segment 0. (B) Coordinated action between phase II metabolizing enzymes and MRP2 in the enterocyte. Hydrophobic xenobiotics (X) may enter the cell by diffusion through the apical membrane of the enterocyte. After that, they may suffer metabolic phase I reaction by cytochrome P450 and/or subsequent conjugation by phase II enzymes, such as UDP-glucuronosyltransferase (UGT) localized in the endoplasmic reticulum or glutathione S-transferase (GST) or sulfotransferase (SUL) localized in the cytosol. In the phase III, the more hydrophilic metabolites (Y) may be actively secreted into the intestinal lumen by MRP2. (C) Coordinated action between phase II metabolizing enzymes and MRP2 in the hepatocyte. Here, hydrophobic xenobiotics (X) enter the cell through the basolateral pole of the membrane in the hepatocyte. After conjugation by phase II metabolizing enzymes, the final product (Y) may be secreted into bile by MRP2.

Selected substrates of MRP2 are included in **Table 1**. Studies using Groningen Yellow (GY)/ TR- Wistar [15, 16] or Eisai hyperbilirubinemic rats (EHBR) [17] that are rMrp2 deficient as a result of mutations leading to premature stop codons have helped to identify transporter

substrates initially. Interestingly, ingredients of our daily diet are substrates for MRP2. That is the case of the tea component epicatechin, the dietary supplement chrysin [18, 19], and the meat-derived dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [20]. Finally, it is important to emphasize that there are MRP2 substrates which influence transporter expression thus influencing its own bioavailability. That is the case, for example, of tamoxifen [21].

Endogenous compounds	Exogenous compounds
Glutathione	*Anticancer drugs*: doxorubicin, epirubicin, etoposide, irinotecan, methotrexate,
Bilirubin glucuronides	mitoxantrone, cisplatin, tamoxifen, vincristine, vinblastine, camptothecin
Conjugated bile salts	*Antibiotics*: ampicillin, azithromycin, cefodizime, ceftriaxone, grepafloxacine,
Leukotrienes C4, D4, E4	irinotecan
Steroids	*HIV drugs*: adenovir, cidofovir, indinavir, lopinavir, nelfinavir, ritonavir,
(17β-glucuronosyl estradiol)	saquinavir
Triiodo-L-thyronine	*Other drugs*:genistein-7-glucoside, p-aminohippurate, olmesartan, phloridzin,
ethinylestradiol-3-O-glucuronide	pravastatine, quercetin 4'-β-glucoside, temocaprilate, conjugates of
	acetaminophen, indomethacin, phenobarbital, sulfinpyrazone.
	Toxicants: S-glutathionyl-2,4-dinitrobenzene, S-glutathionyl ethacrynic acid,
	ochratoxin A, 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridin, 4-
	(methylnitrosamino)-1-(3-pyridyl)-1-buta-nol, α-naphtylisothio-cyanate,
	heavy metal complexes (arsenic glutathione, Sb, Zn, Cu, Mn, Cd)
	Dyes: fluo-3, carboxydichloro fluorescein, sulfobromophthalein
	Flavonoids: epicatechin, chrysin

Table 1. Selected MRP2 substrates.

2. Regulation of expression and activity of MRP2 by xenobiotics

Expression of MRP2 can be regulated at different levels [22], which can be grouped in two main categories: (i) transcriptional level, implying changes in mRNA synthesis rate, or (ii) post-transcriptional level, comprising complex processes involving or not changes in MRP2 mRNA levels. Regarding the transcriptional regulation, the MRP2 promoter contains a number of binding sites recognizing a variety of transcription factors, which in turn can be activated by either endo- or xenobiotics [23, 24]. Thus, a wide variety of drugs, environmental pollutants, and natural toxins behave as ligands of transcription factors/nuclear receptors such as farnesoid X-activated receptor (FXR), pregnane X receptor (PXR), liver X receptor α (LXRα), and constitutive androstane receptor (CAR), to ultimately induce the synthesis of MRP2 mRNA [25]. They modulate MRP2 transcriptionally through binding to the response element

ER-8, which extends from −401 to −376 bp of the MRP2 promoter [25]. Additionally, the study of MRP2 promoter identified binding sites for other transcription factors such as the nuclear factor-erythroid 2-related factor-2 (Nrf2), the peroxisome proliferator-activated receptor alpha (PPARα), CCAAT/enhancer-binding protein-β (C/EBPβ), and hepatic nuclear factors (HNFs), which can also influence the MRP2 expression at the transcriptional level. Finally, a particular transcriptional regulation involves the intracellular nucleotide cyclic adenosine monophosphate (cAMP) pathway, likely triggered by xenobiotics acting predominantly through binding to plasma membrane receptors. This was so far demonstrated in the intestine [26], where the treatment of Caco-2 human intestinal cells with the membrane-permeable analogue dibutyryl cAMP or the adenylyl cyclase activator forskolin led to a significant induction in hMRP2 protein and mRNA expression. Reporter gene and chromatin immunoprecipitation assays performed in this same study showed an increased binding of the transcription factors c-JUN and activating transcription factor-2 (ATF2) to a regulatory region containing activator protein-1 (AP-1) and cAMP response element (CRE) binding sites within the MRP2 promoter.

On the other hand, post-transcriptional MRP2 regulation can involve the dynamic endocytic retrieval and exocytic insertion of this transporter between the canalicular membrane and an intracellular pool of vesicles [27]. A variety of signal transduction pathways involving the activation of the mitogen-activated protein kinases (MAPK) A and C take part in these events [27–29]. Also, mRNA splicing may account for post-transcriptional regulation. As an example, alternative splicing of hMRP2 mRNA has been shown to be a cause underlying Dubin–Johnson syndrome as a consequence of synthesis of nonfunctional protein [30, 31]. Additionally, MRP2 can be translationally modulated. In this regard, Jones et al. [32] observed that under certain situations rMrp2 protein in rats is modified without changes in mRNA levels. This is not just attributed to a modified rate of protein degradation but to the presence of several rMrp2 transcription initiation sites in the 5' untranslated region [7, 33]. The alternative use of these sites leads to the production of different rMrp2 mRNAs with differential translational efficiency.

What follows is a description of the regulatory properties of MRP2 as an important component of the ABC family in liver and intestine. The effects of xenobiotics on MRP2 expression and activity were particularly considered. Transcriptional and post-transcriptional regulations were described separately.

2.1. Transcriptional regulation

Several xenobiotics including therapeutic drugs, environmental pollutants, and natural toxins have shown to activate different transcription factors and nuclear receptors thus exhibiting the potential of increasing MRP2 expression [34]. However, not all xenobiotics induce the expression of MRP2. Indomethacin, a nonsteroidal anti-inflammatory drug, reduced the expression of rat rMrp2 at the mRNA and protein levels in the liver [35]. This reduction was associated with a diminished mRNA expression of the hepatic nuclear receptors CAR, FXR, PXR, retinoic acid receptor α (RARα), and retinoid X receptor α (RXRα). This down-regulation of nuclear receptors is consistent with observations in endotoxin-treated rats that also proved to cause rMrp2 down-regulation at the transcriptional level [36, 37]. Intestinal injury caused

by indomethacin can increase endotoxin levels in portal blood [38], which in turn induces several immune responses and oxidative stress, as shown by the reduced levels of hepatic GSH and increased levels of nitric oxide (NO) and nitrosothiols in portal blood [35]. In this regard, El Kasmi et al. demonstrated that dextran sulfate sodium-induced intestinal injury also down-regulates hepatic mMrp2 expression in mice liver and that the intestinal microbiota and TLR4 (Toll-like receptor 4) are involved in this effect [39]. In addition, it was shown that mice with intestinal injury that received soy oil-based parenteral nutrition containing phytosterols exhibited an exacerbated decrease in mMrp2 mRNA levels. The phytosterol stigmasterol was at least partially involved and associated with increased levels of interleukin 6 (IL-6) mRNA and reduced levels of FXR mRNA in liver [39].

In contrast to indomethacin, several drugs are able to induce the expression of MRP2. For example, spironolactone (SL), a drug used to treat patients with edema and ascites, has been shown to increase bile flow in rats due to the up-regulation of rMrp2 at the transcriptional level, probably in response to increased PXR levels [40]. This up-regulation in rMrp2 resulted in increased efflux activity of the model substrate of rMrp2 dinitrophenyl-S-glutathione (DNP-SG) *in vitro*. These findings were in accordance with observations in patients exhibiting increased clearance of drugs co-administered with SL [41, 42]. Benznidazole (BZL), a nitroimidazole administered for treatment of Chagas disease, was also shown to induce rMrp2 protein expression [43] in rats. This induction is presumably mediated by PXR, since knockdown of the nuclear receptor in the hepatic cell line HepG2 prevented the induction of hMRP2 by this drug [44]. The antituberculosis agents rifampicin and isoniazid that cause liver injury also up-regulate hepatic rMrp2 protein expression in rats [45]. Interestingly, monoammonium glycyrrhizin, commonly present in Chinese herbal formulas used for hepatic protection, prevented such increases in rMrp2 expression. In addition rifampicin and isoniazid increased lipid peroxidation and GSH levels in hepatic tissue, indicating the presence of oxidative stress, which was also prevented by monoammonium glycyrrhizin.

Acetaminophen (APAP) represents one of the most common over-the-counter drugs, used as an effective and safe analgesic and antipyretic. Nevertheless, APAP overdose is very frequent and associated with severe liver injury. Although the mechanism underlying APAP toxicity is not completely understood, the CYP-biotransformation product N-acetyl-p-benzoquinone imine (NAPQI) was described as a mediator of APAP toxic effects since it promotes GSH depletion and binds itself to cellular proteins [46]. APAP phase II metabolites resulting from conjugation with glucuronic acid and sulfate are known MRP2 substrates. Similarly, MRP2 transports the GSH conjugate of NAPQI, thus contributing to reduce the toxic burden exerted by APAP. In this regard, the administration of a single hepatotoxic dose of APAP to Wistar rats resulted in an increase in rMrp2 expression in liver plasma membranes [47]. In a similar study, an induction hepatic mMrp2 following Nrf2 activation was demonstrated in mice, clearly suggesting the presence of an adaptive mechanism to the APAP-triggered injury [48, 49]. In line with these observations, therapeutic activation of Nrf2 has been proposed as a possible strategy to ameliorate APAP-associated hepatotoxicity [50–52]. Nrf2 bears a special toxicological relevance due to its activation by pro-oxidant compounds and reactive metabolites usually associated with situations of drug overdose or exposure to environmental toxicants. Under

homeostatic conditions, Nrf2 is sequestered in the cytosol by the Kelch-like ECH-associated protein 1 (Keap1) which promotes Nrf2 ubiquitination and proteosomal degradation. Upon an oxidative stimulus, it takes place a modification in the oxidation status of particular cysteine residues in the Keap1 molecule leading to Nrf2 dissociation and migration to the nucleus where it binds to antioxidant response elements (ARE) within the promoters and activates the transcription of target genes [53] including antioxidant and GSH synthesis enzymes and also drug transporters like MRP2 [54]. Management of Nrf2 activation could be applied to other cases of oxidative stress-associated hepatic injury. For instance, the Nrf2 activator N-acetyl-cysteine was described to ameliorate the rMrp2 down-regulation exerted by the pro-oxidant phytochemical timosaponin A3 [55]. Similarly, other Nrf2 activators are being tested in clinical trials for the treatment of hepatic and extrahepatic diseases [53].

Environmental pollutants such as arsenite significantly increased rMrp2 protein expression in rat liver after 2 weeks of exposure. Longer exposure treatment (4 or 6 weeks) also increased rMrp2 expression but to a lesser extent [56]. Arsenite not only regulates Mrp2 but is also an MRP2 substrate, so transporter induction may help to counteract the toxic effects of arsenite in the liver. In agreement with this, arsenite content in bile decreased with the exposure time in the same manner as rMrp2 protein induction. Lipid peroxidation was increased and GSH peroxidase activity was reduced in the liver at 4 and 6 weeks of arsenite exposure, indicating a probable effect of oxidative stress in attenuating hepatic rMrp2 induction. This regulation of Mrp2 may explain the dual effects reported for arsenite exposure [57].

The T-2 mycotoxin is commonly found in different crops. Prolonged exposure (3 weeks) of poultry to T-2 reduced cMrp2 mRNA expression in the liver of broiler chickens [58]. Although the authors suggest that PXR may be involved in the down-regulation observed, no studies were conducted to prove that. The organochlorine pesticides 2,4'-dichlorodiphenyltrichloro-ethane (DDT), 4,4'-DDT, chlordane, heptachlor, dieldrin, and lindane, highly resistant to degradation, were shown to increase hMRP2 mRNA in HepaRG cells after a 48-h exposure [59]. This regulation could be mediated by PXR, since this nuclear receptor expression was increased in human hepatocytes after treatment with chlordane, dieldrin, and endosulfan [60].

Bisphenol A (BPA) is a typical contaminant of food, water, and air. A study evaluating the association between the expression of drug transporters in fetal liver and BPA exposure showed a positive correlation between BPA levels and hMRP2 expression. A similar correlation was described between BPA levels and Nrf2 expression, suggesting also a mediation of this transcription factor in the hMRP2 regulation by BPA and agreeing well with a previous report showing Nrf2 activation by BPA *in vitro* [61, 62].

Transcriptional regulation of MRP2 by xenobiotics was also demonstrated in extrahepatic tissues like the small intestine. An example of therapeutic drugs affecting MRP2 gene expression is rifampicin, a well-known PXR agonist which has been demonstrated to increase hMRP2 expression and activity in healthy volunteers. Fromm et al. reported an up-regulation of hMRP2 mRNA and protein in duodenal biopsies [63]. Later, Oswald et al. showed the impact of this regulation on oral availability and efficacy of the coadministered drug ezetimibe [64]. The same inducing effect was reported using the human-derived cell line LS180 [65]. Similarly, carbamazepine is another PXR agonist [66] shown to increase hMRP2 mRNA and protein levels

in human healthy volunteers and also to reduce intestinal absorption of the co-administered MRP2 substrate talinolol [67].

Even though there are only few reports on drug–drug interactions associated with PXR-dependent modulation of intestinal MRP2, many other agents have been shown to regulate its expression via PXR. Pregnenolone-16α-carbonitrile, a synthetic steroid, induced mMrp2 mRNA expression in jejunum of C57BL/6 mice (200 mg/kg, i.p., 4 days). A concomitant up-regulation of PXR mRNA was observed and the involvement of this nuclear receptor was confirmed using PXR knockout mice [68]. The antiviral agents and PXR agonists efavirenz and saquinavir [69] also up-regulated hMRP2 expression in LS180 cells [65, 70]. Since these drugs are usually involved in long-term treatments, coadministration with MRP2 substrates could result in unwanted drug–drug interactions. In the same human intestinal cell line, the effect of the endothelin receptor antagonist bosentan [71] and the antineoplastic mitotane [72] was demonstrated. They both exerted a significant hMRP2 induction concomitantly with PXR activation at pharmacologically relevant concentrations.

In some studies, the increases in MRP2 expression were correlated experimentally with increased transport activity. Examples of these drugs are BZL and SL, both PXR agonists. Perdomo et al. reported an induction of rMrp2 protein in jejunum of BZL-treated rats (100 mg/kg, i.p., 3 days) [43]. This change was accompanied with an increased efflux of DNP-SG to the intestinal lumen. These results not only demonstrate higher secretion of rMrp2 substrates but also strongly suggest a restriction in the absorption of xenobiotics incorporated luminally in BZL-treated rats. Similarly, SL was demonstrated to increase serosal to mucosal transport of DNP-SG well correlating with increased rMrp2 mRNA and protein expression in rat proximal jejunum. The PXR antagonist ketoconazole was able to prevent this induction, suggesting mediation by this nuclear receptor [73].

Cimetidine and quinidine have been shown to increase both hMRP2 and PXR expressions in T84 cells [74]. Similarly, the anticonvulsant phenobarbital induced PXR mRNA and hMRP2 mRNA and protein at the same time in Caco-2 cells [75]. On the contrary, MRP2 expression is down-regulated by drugs that decrease PXR expression. Haslam et al. reported such an effect in T84 cells for the cholesterol-lowering drug atorvastatin and the anticancer agents topotecan and irinotecan [74].

Xenobiotics can regulate expression of intestinal MRP2 interacting with nuclear receptors other than PXR. That is the case of the bcl-2 inhibitor obatoclax, which at nanomolar concentrations induced hMRP2 mRNA in LS180 cells through activation of the aryl hydrocarbon receptor [76]. Another example is the proteasome inhibitor bortezomib, which also increases hMRP2 mRNA expression in SW-480 human cells [77]. Even though the mediator has still to be identified, the transcription factor Nrf2 appears to be the main candidate considering its simultaneous up-regulation and also the well-demonstrated relationship between MRP2 induction and Nrf2 activation in tissues like liver [49], kidney [78], and brain [79].

Among the natural compounds modulating intestinal MRP2, the isothiocyanates sulforaphane (SF) and erucin (ER) were well studied. Derived from cruciferous vegetables, they have drawn the attention of the researchers due to their anticancer properties [80]. After microarray studies,

Traka et al. reported hMRP2 up-regulation in Caco-2 cells treated with SF (50 μM, 24 h) [81]. They confirmed this finding by RT-PCR analysis. Using the same model, Jakubíková et al. showed a similar hMRP2 mRNA increase after treatment with SF and with ER (20 μM, 24 h) [82]. This effect appears to be partly mediated by phosphoinositide 3-kinase (PI3K)/AKT, considering the inhibition exhibited by the PI3K/AKT inhibitor LY294002. Later, in analogous experimental conditions, Harris and Jeffery demonstrated an induction also at protein level [83]. Considering that Nrf2 is activated by SF [84], this transcription factor may play a role in MRP2 up-regulation.

Other naturally occurring xenobiotics found to modulate intestinal MRP2 are the polyphenols quercetin and resveratrol (RES). The first one was demonstrated to up-regulate hMRP2 at protein level in Caco-2 cells (50 and 100 μM, 72 h). A coordinated induction of the phase II enzyme UGT1A6 was also observed, suggesting reduced absorption and enhanced secretion of glucuronides at intestinal level [85]. Studies using RES have become of strong interest after the increasing evidence regarding its beneficial health effects. A short time ago, RES ability to down-regulate MRP2 at mRNA and protein levels has been proven in rat intestine and Caco-2 cells [86]. The functional impact was evaluated in the latter model by determination of intracellular retention of the MRP2 substrate methotrexate (MTX). Treatment with RES increased the accumulation of MTX, suggesting a suppressed efflux activity. Moreover, in an attempt to clarify the mechanism, the authors found a concomitant inhibition of the insulin-like growth factor receptor 1 (IGF-1R)/AKT/ERK signaling pathway.

2.2. Post-transcriptional regulation

As anticipated, post-transcriptional regulation of MRP2 activity may also occur in response to exposition to xenobiotics. Faldaprevir is a drug used to treat patients with hepatitis C in spite that it causes hyperbilirubinemia. Acute treatment of human and rat hepatocytes with faldaprevir inhibited hMRP2-/rMrp2-mediated efflux of bilirubin glucuronides into bile [87]. This may partially explain the impaired bilirubin disposition by faldaprevir. However, toxicology studies in monkeys and patients showed that the bilirubin accumulated during faldaprevir treatment was mainly unconjugated, suggesting that the main cause is probably inhibition of glucuronidation ather than excretion of the conjugated metabolites. Simeprevir, another drug used to treat hepatitis, causes hyperbilirubinemia composed by conjugated and unconjugated bilirubin, which supports an impaired hMRP2 efflux activity [88].

Ethynylestradiol (EE) and genistein (GNT) are estrogenic compounds of particular relevance considering their oral incorporation as components of contraceptives formulations and soy-derived food, respectively. Interestingly, they were demonstrated to regulate intestinal MRP2 at post-transcriptional level, although showing a noticeable dose and model dependence. Thus, it was initially found that EE at a cholestatic dose (5 mg/kg b.w. day, for 5 consecutive days, s.c.) down-regulates rMrp2 expression and function in rat proximal jejunum, without changes in mRNA levels [89]. However, at pharmacological concentrations (0.5–5 pM) EE up-regulates hMRP2 expression and activity in Caco-2 cells. This hMRP2 induction was estrogen receptor β (ERβ)-mediated but not associated with changes at the mRNA level, thus suggesting a post-transcriptional regulation [90]. The implication of ERβ in such kind of regulation is

possible since it was reported to regulate miRNAs expression [91] which, in turn, can alter hMRP2 expression [92]. Similarly, Caco-2 cells exposed to GNT (1 μM, 48 h) exhibited an ERβ-mediated hMRP2 induction at protein level without changes in expression at the mRNA level. This was associated with increased transporter activity and enhanced protection against 1-chloro-2,4-dinitrobenzene, an MRP2 substrate precursor [90]. GNT can also modulate MRP2 activity in an acute fashion, i.e., without changes in transporter expression. Some years ago, it was reported that this isoflavone competitively inhibits hepatic rMrp2 in isolated and perfused rat liver model [93]. In line with this result, Yokooji et al. demonstrated that GNT administered intravenously reduced rMrp2-mediated secretion of irinotecan hydrochloride and its metabolites in rat liver and intestine [94].

The uricosuric drug probenecid represents another example of xenobiotics affecting intestinal MRP2 activity without modifying MRP2 expression. Probenecid is a classical competitive inhibitor of organic anions transport also used in the clinical practice to enhance plasma levels of antibiotics. Although nonspecific, its inhibitory effect on intestinal MRP2 was clearly demonstrated in rats [95] and in Caco-2 cells [90]. Nowadays, more potent and specific MRP inhibitors like MK571 are preferred for characterization of transport specificity. Finally, various members of the large family of nonsteroidal anti-inflammatory drugs are recognized MRP2 modulators [96]. In intestine, indomethacin was shown to inhibit MRP2 and to increase sulfasalazine transepithelial permeability, both in rat small intestine and in Caco-2 cell monolayers [97]. In agreement with these findings, Caco-2 cells coincubated with indomethacin exhibited an increased permeability to the hMRP2 substrates fluvastatin [98] and colchicine [99].

3. Conclusion

Although the organisms are permanently exposed to a wide range of xenobiotics such as therapeutic drugs, environmental pollutants, and natural toxins, they possess a sophisticate system of detoxification in which metabolizing enzymes and transport proteins play an essential role. Evidence from *in vitro* and *in vivo* studies unambiguously demonstrates that MRP2 is a crucial actor in protecting specific tissues from xenobiotic toxicity. It is noteworthy that MRP2 also plays a crucial role in elimination of endogenous metabolites. A list of relevant and well-recognized substrates of MRP2 is presented in **Table 1**. Two relevant tissues expressing MRP2 are the liver and the small intestine. It should be noted that other participants not contemplated in this review, such as a bunch of metabolizing enzymes as well as members of the family of ABC transporters different from MRP2, are additionally involved in xenobiotic detoxification.

Expression and activity of MRP2 can be modulated at both transcriptional and post-transcriptional levels. The nuclear receptor PXR plays a major role in transcriptional regulations. PXR functions as sensor for many agents and its activation leads to a coordinated response on biotransformation enzymes and transport systems. Examples of these agents are rifampicin, carbamazepine, pregnenolone-16α-carbonitrile, efavirenz, saquinavir, BZL, SL, etc. Other

xenobiotics such as bortezomib, BPA, and sulforaphane regulate MRP2 as a result of the interaction with Nrf2. Alternatively, regulation of MRP2 activity by xenobiotics can occur without changes in transporter expression, as it was demonstrated to GNT. Finally, it is important to note that regulation of MRP2 activity by therapeutic agents can result in changes in their therapeutic efficacy or safety, or alternative in drug–drug interactions if other drugs, substrates of MRP2, are simultaneously administered.

Abbreviations: ABC, ATP-binding cassette; AP-1, activator protein-1; APAP, acetaminophen; ARE, antioxidant response elements; ATF2, activating transcription factor-2; BPA, bisphenol A; BZL, benznidazole; C/EBPβ, CCAAT/enhancer-binding protein-β; cAMP, cyclic adenosine monophosphate; CAR, constitutive androstane receptor; CRE, cAMP response element; CYP, cytochrome P450; DDT, dichlorodiphenyltrichloroethane; DNP-SG, dinitrophenyl-S-glutathione; EE, ethynylestradiol; EHBR, Eisai hyperbilirubinemic rats; ER, erucin; ERβ, estrogen receptor β; FXR, farnesoid X-activated receptor; GNT, genistein; GY, Groningen Yellow; GSH, glutathione; GST, glutathione S-transferase; HNFs, hepatic nuclear factors; IGF-1R, insulin-like growth factor receptor 1; IL-6, interleukin 6; Keap1, Kelch-like ECH-associated protein 1; L0, linker segment 0; LXRα, liver X receptor α; MAPK, mitogen-activated protein kinases; MRP, multidrug resistance-associated protein; MRP2, multidrug resistance-associated protein 2; hMRP2, human MRP2; rMrp2, rat Mrp2; mMrp2, mouse Mrp2; cMrp2, chickenMrp2; MSDs, membrane-spanning domains; MTX, methotrexate; NAPQI, N-acetyl-p-benzoquinone imine; NBDs, nucleotide-binding domains; NO, nitric oxide; Nrf2, nuclear factor-erythroid 2- related factor-2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; PI3K, phosphoinositide 3-kinase; PPARα, peroxisome proliferator activated receptor alpha; PXR, pregnane X receptor; RARα, retinoic acid receptor α; RES, resveratrol; RXRα, retinoid X receptor α; SF, sulforaphane; SL, spironolactone; SULT, sulphotransferase; TLR4, tool-like receptor 4; UGT, UDP-glucuronosyltransferase.

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