Toxicology and Applied Pharmacology 303 (2016) 45–57

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Invited Review Article

Modulation of expression and activity of intestinal multidrug resistance-associated protein 2 by xenobiotics

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ARTICLE INFO

Article history:
Received 12 February 2016
Revised 29 April 2016
Accepted 1 May 2016
Available online 04 May 2016

Keywords:
Drug transport
Drug-drug interactions
Intestinal chemical barrier
Multidrug resistance-associated protein 2
Nutrient-drug interaction
Pregnane-X-receptor

ABSTRACT

The multidrug resistance-associated protein 2 (MRP2/ABCC2) is a transporter that belongs to the ATP-binding cassette (ABC) superfamily. In the intestine, it is localized to the apical membrane of the enterocyte and plays a key role in limiting the absorption of xenobiotics incorporated orally. MRP2 may also play a role in systemic clearance of xenobiotics available from the serosal side of the intestine. MRP2 transports a wide range of substrates, mainly organic anions conjugated with glucuronic acid, glutathione and sulfate and its expression can be modulated by xenobiotics at transcriptional- and post-transcriptional levels. Transcriptional regulation is usually mediated by a group of nuclear receptors. The pregnane X receptor (PXR) is a major member of this group. Relevant drugs described to up-regulate intestinal MRP2 via PXR are rifampicin, spironolactone and carbamazepine, among others. The constitutive androstane receptor (CAR, NR1I3) was also reported to modulate MRP2 expression, phenobarbital being a typical activator. Dietary compounds, including micronutrients and other natural products, are also capable of regulating intestinal MRP2 expression transcriptionally. We have given them particular attention since the composition of the food ingested daily is not necessarily supervised and may result in interactions with therapeutic drugs. Post-transcriptional regulation of MRP2 activity by xenobiotics, e.g. as a consequence of inhibitory actions, is also described in this review. Unfortunately, only few studies report on drug-drug or nutrient-drug interactions as a consequence of modulation of intestinal MRP2 activity by xenobiotics. Future clinical studies are expected to identify additional interactions resulting in changes in efficacy or safety of therapeutic drugs.

Contents

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http://dx.doi.org/10.1016/j.taap.2016.05.002
0041-008X/

1. MRP2. General features

The multidrug resistance-associated protein 2 (MRP2, ABCC2) was introduced in the literature for the very first time by Jansen et al. (1985), although with a different nomenclature. The authors described a hyperbilirubinemic strain of rats defective for hepatic anion transport. The mutant phenotype was hereditary and resembled the Dubin-Johnson syndrome characterized decades before (Dubin and Johnson, 1954). Years later, Ishikawa et al. (1990) linked this transport system with an ATP-dependent mechanism and showed its ability to pump glutathione conjugates. Finally, the encoding gene was cloned and called cMrp, since it represented the liver canalicular isoform of the previously described multidrug resistance associated protein (already known as MRP1) (Büchler et al., 1996). The same group reported cMRP to be the transport system absent in Dubin-Johnson syndrome (Kartenbeck et al., 1996). A short time later, the protein was renamed MRP2 to denote a difference with other transporters belonging to this family (Keppler and König, 1997).

Intestinal expression and specific localization of MRP2 in the enterocyte apical membrane was first described by Mottino et al. (2000). The expression pattern displayed a proximal to distal gradient with the highest protein expression in duodenum and jejunum, decreasing towards the ileum. An expression gradient was also shown along the villus-crypt axis, with a higher expression in the upper villus that decreases in direction of the crypt. A similar pattern of distribution observed for conjugating enzymes suggests coordinated activity with MRP2, consistent with the finding that several conjugated anions are prototypical substrates of MRP2. MRP2 mRNA levels differ only slightly between proximal and distal intestine, suggesting the presence of post-transcriptional regulatory mechanisms.

2. MRP2 and the intestine

Exposure to a diverse range of xenobiotics occurs daily, with interactions between a select number of tissues in the body and the environment. The intestinal tract represents the largest mucosal surface where this exchange takes place. Moreover, due to the continuous inflow of food and liquids, the intestine plays a key role in mediating the absorption of macro- and micronutrients as well as in limiting the absorption of potential harmful compounds. This latter function, better known as chemical or biochemical barrier, relies on both anatomical and physiological features. First, the plasma membrane of the epithelial cell (enterocyte) represents an impermeable barrier to most hydrophilic solutes. In addition, an apical junctional complex consisting of tight junctions, *adherens* junctions and desmosomes maintains cellular proximity and seals the paracellular pathway between cells (Turner, 2009). However, a wide range of chemicals routinely reaching the small intestine may be small or hydrophobic enough to diffuse across the cell membrane or the epithelial junctions to finally reach the systemic circulation. At the plasma membrane of the enterocyte, mammals exhibit a complex set of transporters belonging to the superfamily of ATP binding cassette (ABC) proteins. They modulate the permeability of their own substrates, thus contributing not only to protect the enterocyte against chemical insults, but also to prevent intestinal absorption; this latter function could be considered as a chemical barrier. Among the ABC family members, MRP2 (ABCC2) plays a key role in limiting the absorption of xenobiotics like prescription drugs and food contaminants. Alternatively, MRP2 may play a role in systemic clearance of xenobiotics available from the serosal side of the intestine. Fig. 1 illustrates the concept of barrier function that ABC transporters localized apically confer to the intestinal epithelium, with MRP2 used as a particular example. The same figure also illustrates the possibility that MRP2 participates in the disposition of xenobiotics incorporated systemically.

3. MRP2. Structure and substrates

MRP2, as well as other members of the ABC family, is characterized structurally by two membrane spanning domains comprising six transmembrane segments each and a nucleotide binding domain at the C-terminal end of each transmembrane domain. In addition, MRP2 exhibits an extra N-terminal membrane spanning domain, which contains five transmembrane segments (Borst et al., 2006). This N-terminal segment is localized to the extracellular side of the plasma membrane (Fig. 2).

MRP2 transports a wide range of substrates including endo- and xenobiotics. Organic anions conjugated with glucuronic acid, glutathione and sulfate are well recognized MRP2 substrates, many of them being drugs of therapeutic use. Furthermore, non-conjugated drugs such as methotrexate, ritonavir, saquinavir and vinblastine, among others, are also MRP2 substrates (Klaassen and Aleksunes, 2010; Shibayama et al., 2011). As a consequence, an influence of intestinal MRP2 activity on the pharmacokinetics of many therapeutic agents is strongly expected. Moreover, MRP2 is itself subject to regulation by a wide range of drugs. Increased activity would result in a limited oral bioavailability and therapeutic efficacy of MRP2 substrates of clinical use. Conversely, a reduction of intestinal MRP2 activity would result in an increased oral bioavailability of substrates of this transporter, with a concomitant risk of toxicity. It is noteworthy that food contaminants of toxicological relevance like ochratoxin A and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) are also transported by MRP2 (Dietrich et al., 2001; Schrickx et al., 2006). Thus, MRP2 plays a major role not only in limiting the exposition of the enterocytes to a significant number of food contaminants but also in decreasing their intestinal absorption. Micronutrients such as trans-resveratrol are also transported by intestinal MRP2 after phase II transformation in the enterocyte. The intestinal barrier function of MRP2 results in limitation of the absorption of this natural compound (Planas et al., 2012), that could be eventually extended to other micronutrients of similar structure.

A role for MRP2 in the elimination of xenobiotics incorporated systemically was also demonstrated. Gotoh et al. (2000) were the first to prove such function using normal and Eisai hyperbilirubinemic rats (EHBR), in which MRP2 is hereditarily defective. After intravenous administration of 1-chloro-2,4-dinitrobenzene (CDNB), intestinal excretion of its glutathione conjugate 2, 4-dinitrophenyl-S-glutathione

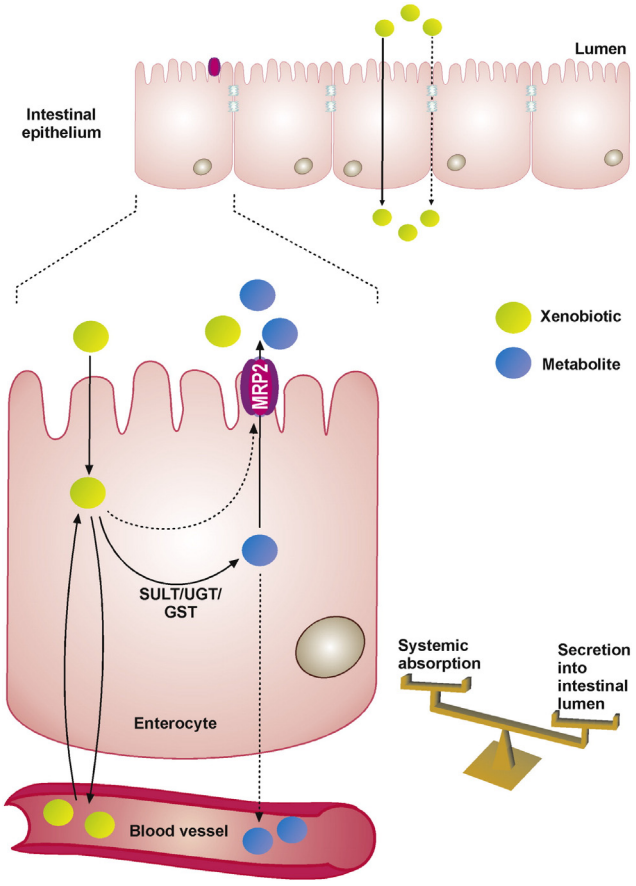

Fig. 1. MRP2 and intestinal barrier. The xenobiotics (yellow spheres) incorporated luminally enter the absorptive cells by simple diffusion or by a transporter-mediated process, followed by further incorporation to the systemic circulation at the basolateral pole. Depending on their size and hydrophobicity, xenobiotics may get to the systemic circulation by the paracellular pathway. After being metabolized in the enterocytes, *e.g.* by sulfotransferases (SULT), UDP-glucuronosyltransferases (UGT) or glutathione-S-transferases (GST), the conjugated derivatives (blue spheres), are secreted to the intestinal lumen by MRP2. A wide range of amphiphilic compounds are substrates of MRP2, so that some of them can eventually be secreted back to the intestinal lumen without transformation. The depicted balance illustrates the concept of the barrier function: the higher the rate of secretion by MRP2 into the lumen, the lower the chances of the intact xenobiotics or their metabolites to be absorbed to the systemic circulation. MRP2 may also play a role in systemic clearance of xenobiotics available from the serosal side of the intestine. In that case, the uptake at the basolateral membrane is followed by the action of conjugating enzymes, ultimately resulting in elimination of the conjugates through the brush border membrane by MRP2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(DNP-SG), a model substrate for MRP2, was significantly reduced in EHBR rats. This function of MRP2 was confirmed by the finding that Wistar rats exhibiting increased expression of MRP2 in response to administration of glucagon-like peptide 2, an intestinal trophic factor, also exhibited increased intestinal excretion of DNP-SG after intravenous administration of CDNB (Villanueva et al., 2010).

4. Regulation of MRP2 expression

MRP2 expression can be modulated at transcriptional and post-transcriptional levels. Transcriptional regulation implies changes in mRNA synthesis rate due to a modified activity of the MRP2 promoter. Post-transcriptional mechanisms comprise regulatory processes that do not imply changes in mRNA synthesis.

4.1. Transcriptional regulation

MRP2 transcriptional regulation is frequently mediated by nuclear receptors activated by xenobiotics, followed by further binding to response elements in the MRP2 promoter. This in turn results in increased MRP2 mRNA synthesis which usually translates into higher MRP2 protein levels at the plasma membrane, ultimately resulting in higher MRP2 activity. The pregnane X receptor (PXR, NR1I2) is one of the main and better studied nuclear receptors. PXR contains a flexible hydrophobic ligand binding pocket involved in the interaction with drugs, environmental pollutants and natural compounds of variable chemical structures (reviewed in di Masi et al., 2009). PXR forms a heterodimer with the retinoid X receptor alpha (RXRα, NR2B1) in order to bind to direct or everted repetition sequences (Geick et al., 2001; Kast et al., 2002). In the inactivated state (*i.e.* in the absence of an agonist) PXR is mainly bound to co-repressor proteins such as the silencing mediator of retinoid and thyroid hormone receptor (SMRT) (Johnson et al., 2006) and the nuclear receptor co-repressor protein (NCoR) (Ding and Staudinger, 2005). After binding to the agonist, co-repressors dissociate from PXR and the nuclear receptor recruits co-activators like the steroid receptor co-activator 1 (SRC-1) (di Masi et al., 2009; Sharma et al., 2013). Once bound to the co-activators, chromatin decompaction takes place and the activated complex couples to the gene promoter ultimately resulting in gene transcription. It is noteworthy that PXR is capable of being activated by many therapeutic agents used in the clinical practice (reviewed in di Masi et al., 2009). This is of particular importance in polymedicated patients, since changes in MRP2 activity can be associated to an altered clearance of co-administered drugs, thus leading to undesirable drug-drug interactions.

The constitutive androstane receptor (CAR, NR1I3) was also reported to modulate MRP2 expression. In non-activated state, CAR resides in the cytosol in a complex with the heat shock protein 90 (Hsp90) and the CAR cytoplasmatic retention protein (CCRP). In the presence of an agonist, the nuclear receptor becomes dephosphorylated and translocates to the nucleus. Unlike PXR, CAR activation by some agonists can also take place without binding to the nuclear receptor. That is the case of phenobarbital (PB), described to activate protein phosphatase 2A leading to CAR dephosphorylation, which in turn results in its nuclear translocation. Once in the nucleus, CAR heterodimerizes with RXRα and binds to response elements in the promoter of target genes, thus activating their transcription (di Masi et al., 2009; Cerveny et al., 2007; Kawamoto et al., 1999). Although most of the studies on this nuclear receptor were performed in liver, Petrick and Klaassen (2007) have described CAR expression also in duodenum, jejunum and ileum. This suggests that CAR may play a similar regulatory role in intestine.

Besides PXR and CAR, the farnesoid X receptor (FXR, NR1H4) and the liver X receptor α (LXRα, NR1H3) have been shown to modulate MRP2 transcriptionally through binding to response elements similar to those of PXR (Chisaki et al., 2009; Kast et al., 2002). FXR is activated by bile salts (Kast et al., 2002), whereas LXRα and LXRβ (NR1H2) are activated by oxysterols (Traversari et al., 2014).

In the case of non-hydrophobic compounds, the initial mechanism is the binding to membrane receptors or interaction with specific membrane proteins. One major signal pathway is associated to an increase in adenylyl cyclase activity resulting in increased synthesis of adenosine 3′,5′-cyclic monophosphate (cAMP). The effect of persistent increases in cAMP on intestinal MRP2 expression was recently evaluated by Arana et al. (2015). In their study, differentiated Caco-2 cells were used as an experimental model of human enterocytes. Increased intracellular cAMP levels were first mimicked by incubation with the membrane permeable analog of cAMP, dibutyryl cAMP. This resulted in significant induction in MRP2 protein and mRNA expression. Incubation with the adenylyl cyclase activator forskolin allowed the authors to confirm cAMP action. Further experiments through reporter gene and chromatin immunoprecipitation techniques demonstrated an increased binding of the transcription factors c-jun and activating transcription

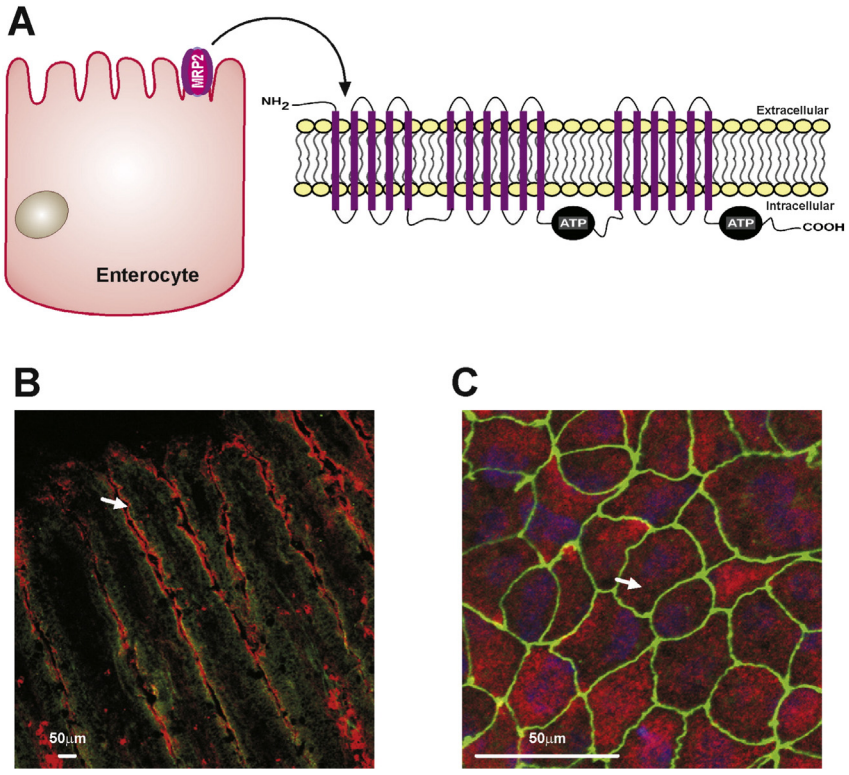

Fig. 2. MRP2 structure and apical membrane localization in intestinal cells. A: Depicted are the two membrane spanning and nucleotide binding domains of MRP2, typical of ABC transporters, and the additional, more specific, N-terminal domain. B and C: Confocal microscopy images of rat intestinal villi (B) and human intestinal Caco-2 cells (C), showing MRP2 detection in red and the tight junction protein Zonula Occludens 1 (ZO-1) in green. MRP2 is detected on the surface of the villus in the rat intestine, consistent with its localization to the apical pole of the enterocyte. It is noticeable a preferential expression at the tip of the villus, with decreasing expression towards the crypt, whereas ZO-1 is homogeneously distributed along the villus. Red signal at the central lacteal corresponds to secondary antibody unspecific staining. Polarized, well-differentiated Caco-2 cells show MRP2 detection at the frontal surface (C), consisting with apical localization, whereas ZO-1 is detected along cell contact regions. Barely detected are cell nuclei in blue (Hoechst staining). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

factor-2 (ATF-2) to MRP2 at a regulatory cluster region containing activator protein 1 (AP-1) and cAMP response element (CRE) sites.

The study of MRP2 promoter revealed additional binding sites that recognize transcriptional factors like the nuclear factor-erythroid 2-related factor-2 (Nrf2), the aryl hydrocarbon receptor (AhR), the peroxisome proliferator activated receptor alpha (PPARα), the CCAAT-enhancer-binding protein β (C/EBPβ) and a group of hepatic nuclear factors (HNFs) (Maher et al., 2005; Stöckel et al., 2000; Tanaka et al., 1999; Vollrath et al., 2006). Since they are expressed in intestinal tissue, a role of these factors in the mediation of intestinal MRP2 regulation by exogenous compounds should be considered as possible.

4.2. Post-transcriptional regulation

MRP2 protein expression can also be regulated in absence of changes in the transcription rate. There are different levels at which post-transcriptional regulation may occur. Post-transcriptional regulation itself consists of altered mRNA levels in response to changes in mRNA stability, processing or export, which may result in dissociation between mRNA and protein levels. As an example, an alteration in mRNA splicing was demonstrated to underlie Dubin-Johnson syndrome, characterized by a truncated or non-functional hepatic MRP2 (Tate et al., 2002; Mor-Cohen et al., 2005). Translational modulation constitutes an alternative mechanism to regulate MRP2 cellular levels. Jones et al. (2005) found increased detection of MRP2 protein in response to treatment with the synthetic steroid pregnenolone-16α-carbonitrile (PCN) as well as its down-regulation in pregnant rats, both in the absence of changes in mRNA levels. These observations could not be attributed to a modified protein half-life. More recently, Zhang et al. (2007, 2010) demonstrated the presence of several MRP2 transcription initiation sites. The alternative use of these sites would lead to synthesis of different MRP2 mRNAs exhibiting different translational efficiencies. A potential explanation consist in a differential response to miRNAs of such different mRNAs, and consequently, different susceptibilities to factors affecting miRNA expression. For instance, Werk et al. (2014) reported the presence of single nucleotide polymorphisms in MRP2 5'UTR which result in differential suppression of MRP2 synthesis by the miRNA-379. More common is the binding of miRNAs to the 3'UTR of MRP2 mRNA, a mechanism shared by most miRNAs (He and Hannon, 2004). Indeed, an *in silico* scanning (Betel et al., 2008) revealed the presence of multiple putative miRNA binding sites in MRP2 3'UTR. In this regard, some miRNAs have been already validated as regulators of MRP2 expression (Haenisch et al., 2011; Loeser et al., 2015).

5. Regulation of MRP2 activity

Changes in MRP2 activity not associated with changes in protein expression may also occur. One example is the short-term regulation involving modifications in MRP2 localization as a consequence of the dynamic interchange between the apical membrane and a subapical compartment. This process has been extensively described in liver and demonstrated to be regulated under several physiological and pathophysiological conditions (Crocenzi et al., 2012). In intestine, however, there is much less known. Nakano et al. (2009) demonstrated that intestinal MRP2 can be internalized in a process mediated by conventional protein kinase C, leading to a decrease in its transport activity. The authors associated this effect with an alteration in the phosphorylation status of ezrin-radixin-moesin proteins, which normally anchor MRP2 to the actin cytoskeleton.

A direct interaction of the MRP2 molecule with xenobiotics represents an alternative process that conditions transport activity. This may be originated in the differential interaction of the xenobiotic and the MRP2 substrate with MRP2 active sites (reviewed in detail in Zelcer et al., 2003), probenecid (PRO) being an example. Interestingly, depending on the substrate, PRO could act as activator (estradiol 17ß-D-glucuronide) or inhibitor (methotrexate, DNP-SG). Interaction may result alternatively in changes in MRP2 ATPase activity; that is the case of a wide range of flavonoids (reviewed in detail in Di Pietro et al., 2002). Competitive inhibition is also possible and results in decreased transport of substrates of MRP2 usually exhibiting lower affinity (Keppler and König, 1997). That way, a high affinity substrate is expected to be inhibitor of a low affinity substrate. Other xenobiotics affecting MRP2 activity post-transcriptionally present mechanisms of action that still remain unknown.

6. Modulation of intestinal MRP2 expression by drugs

Agents affecting MRP2 expression have been studied using *in vivo* models, *in situ* intestinal perfusions, *in vitro* tissue preparations (Ussing chambers, intestinal rings/vesicles, intestinal everted sacs) or colon carcinoma-derived cell lines (Caco-2, LS180, T84, LS174T). Despite their origin, Caco-2 cells display morphological and biochemical characteristics of mature, differentiated enterocytes and often constitute researcher's first choice given the inherent difficulties in isolating and culturing intestine epithelial cells or in establishing enterocyte-derived cell lines. MRP2 expression in these cells resembles the normal expression in proximal regions of the small intestine in rodents and humans (see panels B and C in Fig. 2).

What follows is a detailed description of therapeutic drugs along with other synthetic compounds shown to modulate intestinal MRP2 expression. The information is summarized in Table 1. If available, reference to the underlying molecular mechanism is also made.

6.1. Antibiotics and antiparasitics

Rifampicin (RIF), a well-known PXR agonist, has been shown to induce MRP2 expression and activity in different intestinal models. Fromm et al. (2000) reported the induction of MRP2 mRNA and protein levels in duodenal biopsies of healthy volunteers treated with RIF. A similar result was seen in another study with healthy volunteers. The authors demonstrated that up-regulation of intestinal MRP2 by RIF is associated with decreased AUC and reduced efficacy of the cholesterol-lowering drug ezetimibe following its oral administration (Oswald et al., 2006). The effect was later confirmed *in vitro* using the human intestinal cell line LS180 (König et al., 2010).

A similar effect on intestinal MRP2 expression and activity was shown for the tripanocide benznidazole (BZL), also a PXR agonist (Rigalli et al., 2012). In this regard, Perdomo et al. (2013) described higher MRP2 expression at the protein level in jejunum of BZL treated rats (100 mg/kg b.w., for 3 consecutive days, i.p.). This well correlated with an increased transport activity, demonstrated by the higher excretion of DNP-SG to the intestinal lumen.

6.2. Contraceptives and other steroids

Ethynylestradiol (EE), a synthetic estrogen incorporated to contraceptive formulations, has also been demonstrated to modulate intestinal MRP2 expression showing a clear dose- and model-dependence. For instance, Arias et al. (2009) administered a supra-pharmacological dose of EE previously associated with induction of intrahepatic cholestasis (5 mg/kg b.w. day, for 5 consecutive days, s.c.) and observed a decrease in MRP2 protein expression and activity in rat proximal

Table 1
Regulation of intestinal MRP2 expression by drugs.

Compound	Model	Effect	Possible mechanism	Reference
5-Fluorouracil	LS180 cells	mRNA up-regulation	Unknown	Theile et al. (2009)
5-Fluorouracil	Rat jejunum	Protein down-regulation	Unknown	Shibayama et al. (2011)
Aluminium	Rat duodenum	Protein up-regulation	Unknown	Orihuela et al. (2005)
Atorvastatin	T84 cells	mRNA down-regulation	PXR down-regulation	Haslam et al. (2008)
Benznidazole	Rat jejunum	Protein up-regulation	PXR activation	Perdomo et al. (2013)
Bortezomib	SW-480 cells	mRNA up-regulation	Nrf2 up-regulation	Ebert et al. (2011)
Bosentan	LS180 cells	mRNA up-regulation	PXR up-regulation	Weiss et al. (2011)
Carbamazepine	Healthy volunteers	mRNA and protein up-regulation	PXR activation	Giessmann et al. (2004)
Cimetidine	T84 cells	mRNA up-regulation	PXR up-regulation	Haslam et al. (2008)
Cisplatin	LS174T cells	mRNA up-regulation	Unknown	Herraez et al. (2012)
Efavirenz	LS180 cells	mRNA up-regulation	PXR activation	Weiss et al. (2009)
Epirubicin	Caco-2 cells	mRNA down-regulation	ROS increase	Lo (2013)
Ethanol	LS180 cells	mRNA down-regulation (short-term regulation); mRNA induction (long-term regulation)	Unknown	Theile et al. (2013)
Ethynylestradiol	Rat jejunum	Protein down-regulation	Translational mechanism	Arias et al. (2009)
Ethynylestradiol	Caco-2 cells	Protein up-regulation	Translational mechanism	Arias et al. (2014)
Irinotecan	T84 cells	mRNA down-regulation	PXR and CAR down-regulation	Haslam et al. (2008)
Isopentanol	LS180 cells	mRNA down-regulation (short-term regulation); mRNA induction (long-term regulation)	Unknown	Theile et al. (2013)
Mitotane	LS180 cells	mRNA up-regulation	PXR activation	Theile et al. (2015a, 2015b)
Obatoclax	LS180	mRNA up-regulation	PXR independent. AhR[a]	Theile et al. (2015a, 2015b)
Pregnenolone-16α-carbonitrile	Mice jejunum	mRNA up-regulation	PXR activation and up-regulation	Cheng and Klaassen (2006)
Quinidine	T84 cells	mRNA up-regulation	PXR and CAR up-regulation	Haslam et al. (2008)
Rifampicin	Healthy volunteers	mRNA and protein up-regulation	PXR activation	Fromm et al. (2000), Oswald et al. (2006)
Rifampicin	LS180 cells	mRNA up-regulation	PXR activation	König et al. (2010)
Saquinavir	LS180 cells	mRNA up-regulation	PXR activation	König et al. (2010)
Spironolactone	Rat jejunum	mRNA and protein up-regulation	PXR activation	Ruiz et al. (2009)
tert-Butyl hydroquinone	Caco-2 cells	Protein up-regulation	Unknown	Bock et al. (2000)
Topotecan	T84 cells	mRNA down-regulation	PXR down-regulation	Haslam et al. (2008)
Vincristine	LS174T cells	mRNA up-regulation	Unknown	Huang et al. (2006)

[a] Mechanism remaining to be proven.

jejunum. Messenger RNA levels were not modified, suggesting a post-transcriptional mechanism. Conversely, a concentration-response study within the therapeutic range found in women taking contraceptive pills (0.5 to 5.0 pm) showed MRP2 induction in the Caco-2 cell model. This was associated with an increased efflux of the model substrate DNP-SG and with an enhanced protection against the cytotoxicity exerted by the parent compound CDNB. Expression at the mRNA level remained unchanged, also suggesting a post-transcriptional mechanism. In spite of the post-transcriptional regulation, the effect was dependent on estrogen receptors, since it was prevented by the antagonist ICI 182,780 (Arias et al., 2014). The authors suggested a miRNA dependent mechanism since EE simultaneously induced estrogen receptor β (ERβ) expression, which has been already shown to down-regulate the expression of miRNAs, in association with colon cancer prevention (Edvardsson et al., 2013).

The synthetic steroid PCN was described as a potent PXR agonist in rodents (Jones et al., 2000). In line with this observation, treatment of C57BL/6 mice with PCN (200 mg/kg, i.p.) for 4 days resulted in an induction of MRP2 mRNA expression in jejunum. The effect was not observed in PXR knock-out mice, confirming the participation of this nuclear receptor. Concomitantly, an induction of PXR mRNA expression was observed (Cheng and Klaassen, 2006). Unexpectedly, the authors did not observe changes in hepatic MRP2 mRNA levels, suggesting that a translational mechanism is involved. Studies by Jones et al. (2005) also provide evidence on a translational regulation of hepatic MRP2 by PCN.

The diuretic drug spironolactone was reported to induce intestinal MRP2 in rat jejunum, either at mRNA and protein levels, resulting in increased transport activity (Ruiz et al., 2009). The prevention of MRP2 induction by the PXR antagonist ketoconazole suggests that PXR mediates spironolactone effects.

6.3. Anticancer agents

MRP2 modulation was formerly and largely reported during exposure to anticancer agents, as was also reported for other ABC transporters. Since many chemotherapeutic drugs are themselves ABC substrates, increased transport activity was tightly associated with generation of drug resistance; this has been named multidrug resistance. Conversely, a decrease in transporter expression is expected to enhance cell chemosensitivity. Controversial effects are often observed for a single drug. Such is the case for 5-fluorouracil, an agent administered to patients with colorectal cancer. An induction in MRP2 expression was first reported in intestinal LS180 cells at protein and mRNA levels (Theile et al., 2009). However, Shibayama et al. (2011) showed a decrease of intestinal MRP2 expression after administration of the drug to rats, also at protein and mRNA levels. This effect was time-dependent, since samples were analyzed every 2 days for 2 weeks and MRP2 protein down-regulation was observed only at days 2 and 4 followed by normalization. Discrepancies between these two reports can rely on the different natures of each model, as well as on the different ranges of dosage and scheme of treatments.

For other drugs, the effect was more consistent and associated with either induction or down-regulation of MRP2. For instance, vincristine and cisplatin induced MRP2 expression in LS174T cells (Herraez et al., 2012; Huang et al., 2006). The effect of mitotane, a drug frequently applied as adjuvant chemotherapy of adrenal cancer, was assessed in the LS180 cell line. A significant MRP2 induction was observed when used at a similar range of concentrations found in patients under treatment with the drug. Simultaneous activation of PXR suggests mediation by this nuclear receptor (Theile et al., 2015a). Similarly, the experimental bcl-2 inhibitor obatoclax induced MRP2 in the same cell line. However, in contrast to mitotane effect, induction by obatoclax was independent of PXR activation but associated to an increase in the activity of the aryl hydrocarbon receptor (AhR), previously shown to modulate hepatic MRP2 expression. Whether AhR is indeed the mediator in intestinal cells has not been determined yet (Theile et al., 2015b). On the contrary, topotecan and irinotecan reduced MRP2 expression in T84 colon adenocarcinoma cells. The effect was associated with a decrease in PXR expression after treatment with both drugs. Under these conditions, a lower amount of PXR interacting with co-activator proteins can be expected, thus explaining lower MRP2 expression (Haslam et al., 2008). In another study, epirubicin reduced MRP2 mRNA expression in Caco-2 cells, the effect being potentiated by co-incubation with 8-hydroxydaidzein, a metabolite of the isoflavone daidzein. The effect was associated with an increase in reactive oxygen species (ROS) and was prevented by *N*-acetyl cysteine, suggesting a role for ROS in the modulation of intestinal MRP2 expression (Lo, 2013).

The proteasome inhibitor bortezomib was shown to up-regulate MRP2 mRNA in SW-480 human colon cancer cells (Ebert et al., 2011). Since bortezomib produces oxidative stress and a parallel up-regulation of Nrf2, this transcriptional factor could be involved as mediator. This finding is compatible with previous reports that demonstrate Nrf2-mediated MRP2 induction in other tissues like brain (Ghanem et al., 2015), kidney (Aleksunes et al., 2010) and liver (Maher et al., 2007) in response to oxidative stress.

6.4. Antiviral agents

The protease inhibitor saquinavir (König et al., 2010) and the non-nucleoside reverse transcriptase inhibitor efavirenz (Weiss et al., 2009) induced MRP2 expression in LS180 cells. PXR could be the potential mediator since both antivirals are recognized PXR agonists (Sharma et al., 2013). Considering that these antiviral drugs are administered chronically, eventual, undesirable drug-drug interactions may occur if patients are treated with additional drugs substrates of MRP2. In additional studies, elvitegravir, etravirine, maraviroc, raltegravir, rilpivirine, telepravir and vicriviroc were tested for their effect on MRP2 expression in LS180 cells. Transcriptional activation of other PXR target genes by some of these compounds was demonstrated. Thus, an effect on the transcription of MRP2 is expected. However, neither of these agents were able to modulate MRP2 expression (Weiss and Haefeli, 2013; Weiss et al., 2014; Zembruski et al., 2011a, 2011b). These results could be rationalized considering that the effects of PXR agonists are promoter-dependent (Masuyama et al., 2005). Thus, activation of the nuclear receptor and the consequently induction of a target gene by a particular agonist, cannot be extrapolated to all reported target genes and points out the necessity of investigating each particular case.

6.5. Endothelin receptor antagonists

Endothelin receptor antagonists are used for the treatment of pulmonary arterial hypertension. Many of them have been tested for MRP2 modulation in LS180 cells. As an example, bosentan induced MRP2 mRNA. Since the same treatment also induced PXR expression and activity, a participation of this nuclear receptor seams feasible. Incubation of cells with the bosentan metabolites hydroxyl bosentan and hydroxyl desmethyl bosentan failed to induce MRP2 expression, suggesting a minor contribution of these derivatives to the induction previously reported (Weiss et al., 2015). Ambrisentan, atrasentan and macitentan were also tested for their potential to modulate MRP2 expression in LS180 cells. No significant effect on transporter expression was observed (Weiss and Haefeli, 2011; Weiss et al., 2011, 2013). Mechanisms underlying the differential effect of the different endothelin receptor antagonists on MRP2 are unclear. PXR is activated by both bosentan and macitentan, the latter resulting in no MRP2 induction. Thus, a differential activation of this nuclear receptor can be ruled out (Weiss et al., 2013). Interestingly, MDCK cells overexpressing MRP2 exhibited a higher bosentan efflux than wild-type cells, clearly indicating a role for MRP2 in the efflux of this drug (Fahrmayr et al., 2013). Transport of other endothelin receptor antagonists by MRP2 has not been described yet.

6.6. Alcohols and food additives/contaminants

The effect of exposure to ethanol and isopentanol, a secondary product of fermentation, was evaluated in LS180 cells at different time points. MRP2 mRNA was down-regulated both by ethanol and by isopentanol after 3 days of exposure, whereas a slight induction was observed after 1 week of exposure (Theile et al., 2013). This would result in turn in altered drug elimination in alcoholic patients.

Aluminium is a xenobiotic ubiquitously distributed to which intestine is frequently exposed. It comes from a wide variety of sources including drinking water, pharmaceutical products and food, the latter being generally the major source. Orihuela et al. (2005) reported induction of expression of MRP2 in rat duodenum after oral treatment with $AlCl_3$ (100 and 200 mg/kg b.w. day; 7 days). The authors propose such up-regulation to be a compensatory detoxifying response to deal with the excess of oxidized glutathione, consequence of the unbalanced redox status generated by aluminium. The mechanism of induction of MRP2 remains unknown.

Additionally, the food preservative *tert*-butylhydroquinone (TBHQ, 80 μM, 72 h), used as antioxidant, was reported to induce MRP2 protein expression in Caco-2 cells. Since the effect takes place with a concomitant induction of uridine diphosphate glucuronosyltransferase 1A6 (UGT1A6) expression, the authors suggest an enhanced chemoprotection against phenolic compounds after exposure to TBHQ (Bock et al., 2000).

6.7. Anticonvulsants

Phenobarbital (PB) is a known CAR activator previously shown to induce MRP2 expression in rat liver (Maher et al., 2005; Petrick and Klaassen, 2007). The effect of PB on intestinal MRP2 expression was investigated by Martin et al. (2008), who showed an induction both at mRNA- and protein levels in Caco-2 cells. If a similar effect took place *in vivo*, it would result in a reduced absorption and higher efflux of MRP2 substrates, including phase II metabolites of PB (Patel et al., 2003).

An increase in intestinal MRP2 expression both at mRNA and protein levels was also described after administration of carbamazepine to healthy volunteers. In this case, the higher transporter expression led to a reduced oral absorption of the MRP2 substrate talinolol (Giessmann et al., 2004). The effect is likely mediated by PXR, as carbamazepine was shown to be a PXR activator (Luo et al., 2002).

6.8. Miscellaneous

The cholesterol-lowering drug atorvastatin reduced MRP2 expression in association with reduction of PXR expression in T84 cells (Haslam et al., 2008). In contrast, the drugs quinidine and cimetidine increased both PXR and MRP2 expression in the same model. Although their role as PXR agonists was not demonstrated, a basal interaction of PXR with co-activator proteins was found to occur even in the absence of an agonist (Rigalli et al., 2013). Thus, it is possible that the solely induction of PXR expression and concomitant association with its co-activators increase the transcription of target genes. This postulate needs further and more direct demonstration.

7. Modulation of intestinal MRP2 expression by natural compounds

As well as therapeutic agents, dietary compounds, including micronutrients and other natural products, are capable of regulating intestinal MRP2 expression. This subject was given particular attention as the composition of the food ingested daily is not necessarily supervised and may result in potential interactions with therapeutic drugs. In the following section, we present the information available on such compounds, with special emphasis on massive dietary products. As with the precedent section, data are summarized in a table (Table 2).

7.1. Soy-derived compounds

MRP2 expression and activity have been demonstrated to be regulated by soy-derived compounds. This gains particular relevance considering that soybeans and soy-derived food products are major components of oriental diets. Moreover, due to their beneficial properties, soy products are being increasingly consumed in occidental populations. Bogacz et al. (2014) reported induction of MRP2 activity by a standardized soybean extract after 21 days of administration. A dose of 100 mg/kg b.w. of this extract increased MRP2 mRNA expression in rat intestinal epithelium, as analyzed by real-time PCR. Soy isoflavones are believed to exert the most potent effect on MRP2 since they activate nuclear receptors involved in its transcriptional regulation. In this regard, genistein (GNT) has been shown to up-regulate MRP2 protein expression and activity in Caco-2 cells (Arias et al., 2014). Similar to what was observed after exposition to EE (see Section 6.2), MRP2 induction was mediated by ERβ and was not associated with changes in mRNA levels, suggesting a post-transcriptional regulation. The modulation was clearly dose-dependent since it was observed after 48 h of incubation with 1 μM GNT, but not at 0.1 or 10 μM concentrations. Interestingly the 1 μM concentration is within the range of plasma concentrations associated with moderate soy ingestion; by extrapolation, changes in availability of MRP2 substrates could occur in a significant proportion of the population, particularly in Asian countries. The up-regulation observed in Caco-2 cells was associated with increased cell survival upon exposure to the cytotoxic CDNB. The authors highlighted the significance of this cytoprotection as a specific and novel effect since at the 1 μM dose GNT did not affect glutathione levels, thus ruling out a connection with an eventual antioxidant effect of the isoflavone. Interestingly, other phytochemicals present in soybeans appear to act in the opposite direction. Examples are the

Table 2
Regulation of intestinal MRP2 expression by natural compounds.

Compound	Model	Effect	Possible mechanism	Reference
8-Hydroxydaidzein	Caco-2 cells	mRNA down-regulation	ROS increase	Lo (2013)
Erucin	Caco-2 cells	mRNA up-regulation	Activation of PI3K/AKT pathway	Jakubíková et al. (2005)
Erucin	Caco-2 cells	Protein up-regulation	Unknown	Harris and Jeffery (2008)
Genistein	Caco-2 cells	Protein up-regulation	Post-transcriptional regulation (ER-dependent)	Arias et al. (2014)
Glyceollins	Caco-2 cells	mRNA and protein down-regulation	Unknown	Chimezie et al. (2015)
Quercetin	Caco-2 cells	Protein up-regulation	Unknown	Bock et al. (2000)
Resveratrol	Caco-2 cells	mRNA and protein down-regulation	Inhibition of IGF-1R/AKT/ERK pathway	Jia et al. (2015)
Resveratrol	Rat intestine	mRNA and protein down-regulation	Unknown	Jia et al. (2015)
Soybean extract	Rat intestine	mRNA up-regulation	Unknown	Bogacz et al. (2014)
Sulforaphane	Caco-2 cells	mRNA up-regulation	Unknown	Traka et al. (2005)
Sulforaphane	Caco-2 cells	mRNA up-regulation	Activation of PI3K/AKT pathway Nrf2[a]	Jakubíková et al. (2005)
Sulforaphane	Caco-2 cells	Protein up-regulation	Nrf2[a]	Harris and Jeffery (2008)

[a] Mechanism remaining to be proven.

above-mentioned 8-hydroxydaidzein (Lo, 2013) as well as glyceollins, a group of polyphenolic estrogen-like compounds produced by soybeans under stress conditions. Exposure to 100 μM of a mixture of three species of glyceollins reduced MRP2 mRNA and protein expression in Caco-2 cells (Chimezie et al., 2015).

A wide variety of soy derived nutritional and hormonal supplements are nowadays available as antioxidants or to alleviate menopause symptoms. In view of the specific and significant effects they produce on intestinal MRP2 and considering that they constitute over the counter supplements, undesirable nutrient-drug interactions may occur with frequency. Noteworthy, consumption of these natural products is not routinely considered in medical anamnesis or in the design of treatment schemes.

7.2. Isothiocyanates from cruciferous vegetables

Cruciferous vegetables contain high levels of different glucosinolates. Either before their consumption or soon after being ingested, they are converted into isothiocyanates by enzymatic hydrolysis. The isothiocyanate sulforaphane (SF) constitutes one of these metabolites. Its chemopreventive, anti-inflammatory and anti-cancer properties have been well documented (Bai et al., 2015; Sestili and Fimognari, 2015). SF was found to up-regulate intestinal MRP2 expression in Caco-2 cells. There are, however, slight discrepancies regarding the concentration at which SF is effective. For instance, Traka et al. (2005) performed a microarray analysis which revealed an increase in MRP2 expression after treatment with 50 μM SF for 24 h. This induction was further confirmed by RT-PCR studies. Likewise, Jakubíková et al. (2005) reported a dose-dependent MRP2 mRNA induction after 24 h of treatment with SF and erucin, a reduced analog of SF derived also from *Eruca sativa*. Maximum expression levels were observed at 20 μM of both compounds, whereas at 40 μM they exhibited no effect. MRP2 induction was mediated in part by PI3K/Akt, as the use of the PI3K/Akt inhibitor LY294002 partially abolished such induction. Since Nrf2 is activated by SF (Wang et al., 2014), this transcription factor may be also involved. In a different study, Harris and Jeffery (2008) showed a similar dose-dependent induction of MRP2 protein in response to SF and erucin in a concentration range of 1 to 20 μM.

7.3. Flavonoids

Resveratrol (RES), found in grape skin, has gained scientific importance particularly after the evidence of the cardioprotective effects of the red wine. Since then, a wide variety of health benefits have been reported for this phytochemical. Regarding the potential effects on MRP2, recently, Jia et al. (2015) demonstrated MRP2 mRNA and protein down-regulation by RES in rat intestine (30 mg/kg b.w., 14 days). These changes were accompanied by increased intestinal absorption of the chemotherapeutic agent bestatin, which was evidenced by *in vivo* and *in situ* studies. Similarly, expression of MRP2 mRNA and protein was decreased in Caco-2 cells treated with 1 μM RES for 72 h, with concomitant decrease in MRP2 transport activity. This effect was associated with a direct inhibition of the IGF-1R/AKT/ERK pathway.

In contrast to RES, the more widely distributed flavonoid quercetin was shown to induce MRP2 expression in Caco-2 cells (50 and 100 μM; 72 h). This induction occurred in parallel with that of UGT1A6; this would result in limited absorption of compounds such as acetaminophen, a typical substrate of UGT1A6, whose resultant glucuronide is in turn a typical substrate of MRP2 (Bock et al., 2000).

8. Modulation of MRP2 activity by drugs and natural compounds

Acute regulation of MRP2 activity may occur with no need for modification of the expression. When occurring, they may lead to pharmacological interactions as well. Some of the drugs interacting with MRP2 and further affecting its activity may also be transported by MRP2. Table 3 provides a compilation of xenobiotics affecting MRP2 activity that do not necessarily affect its expression. The uricosuric agent probenecid (PRO) was one of the first compounds shown to inhibit MRP2 activity (Bakos et al., 2000). This was corroborated in Caco-2 cells, where PRO inhibited the efflux of the MRP2 model substrate DNP-SG (Arias et al., 2014). An inhibition of intestinal secretion of grepafloxacin by PRO was observed in Sprague-Dawley rats *in vivo* but not in EHBR rats, thus unambiguously demonstrating its ability to modulate MRP2 activity (Naruhashi et al., 2002). In view of such property, PRO was used routinely as MRP2 inhibitor in experiments devoted to demonstrate the involvement of MRP2 in transport studies. However, due to its only partial selectivity, newly developed, more selective inhibitors increasingly substitute its use. That is the case of MK571.

Several non-steroidal anti-inflammatory drugs (NSAIDs) modulate MRP2 activity (El-Sheikh et al., 2007). This could partially explain the adverse drug-drug interactions observed in patients receiving simultaneously the MRP2 substrate methotrexate and NSAIDs. Indomethacin was found to inhibit MRP2 and to increase permeability of the substrate sulfasalazine both in Caco-2 cells and in the *in situ* model of rat jejunum single-pass perfusion. Such interaction may lead to a

Table 3
Regulation of MRP2 activity by drugs and natural compounds.

Compound	Model	Effect	Possible mechanism	Reference
D-Glucose	Rat jejunum	Activity increase (increased fluorescein transport)	Unknown	Legen and Kristl (2004)
Genistein	Rat small intestine	Activity inhibition	Competitive inhibition[a]	Yokooji et al. (2013)
Glyceollins	Caco-2 cells	Activity inhibition (reduced 5-(6)-carboxy-2′,7′-dichloroflourescein transport)	Concentration-dependent	Chimezie et al. (2015), Schexnayder and Stratford (2015)
Grapefruit/orange juice extracts	Caco-2 cells	Activity inhibition (reduced [$_3$H]vinblastine and [$_{14}$C]saquinavir transport)	Unknown	Honda et al. (2004)
Indomethacin	Rat small intestine and Caco-2 cells	Activity inhibition (reduced colchicine, fluvastatin and sulfosalazine transport)	Competitive inhibition	Lindahl et al. (2004), El-Sheikh et al. (2007), Dahan et al. (2009), Dahan and Amidon (2010)
Poly(acrylic acid) cysteine conjugates	Rat small intestine in Ussing chambers	Activity inhibition	Formation of disulfides in the MRP2 active site	Grabovac et al. (2015)
Probenecid	Rat small intestine and Caco-2 cells	Activity inhibition (reduced grepafloxacin efflux)	Unknown	Naruhashi et al. (2002); Arias et al. (2014)
Tryptanthrin	Caco-2 cells	Activity inhibition (reduced pravastatin transport)	Unknown	Zhu et al. (2011)

[a] Mechanism remaining to be proven.

reduced intracolonic concentration of sulfasalazine and thus, to a diminished therapeutic effect (Dahan and Amidon, 2010). In line with these results, it was described an increased apical to basolateral permeability and a reduced basolateral to apical permeability to colchicine (Dahan et al., 2009) and to fluvastatin (Lindahl et al., 2004) in Caco-2 cells coincubated with indomethacin.

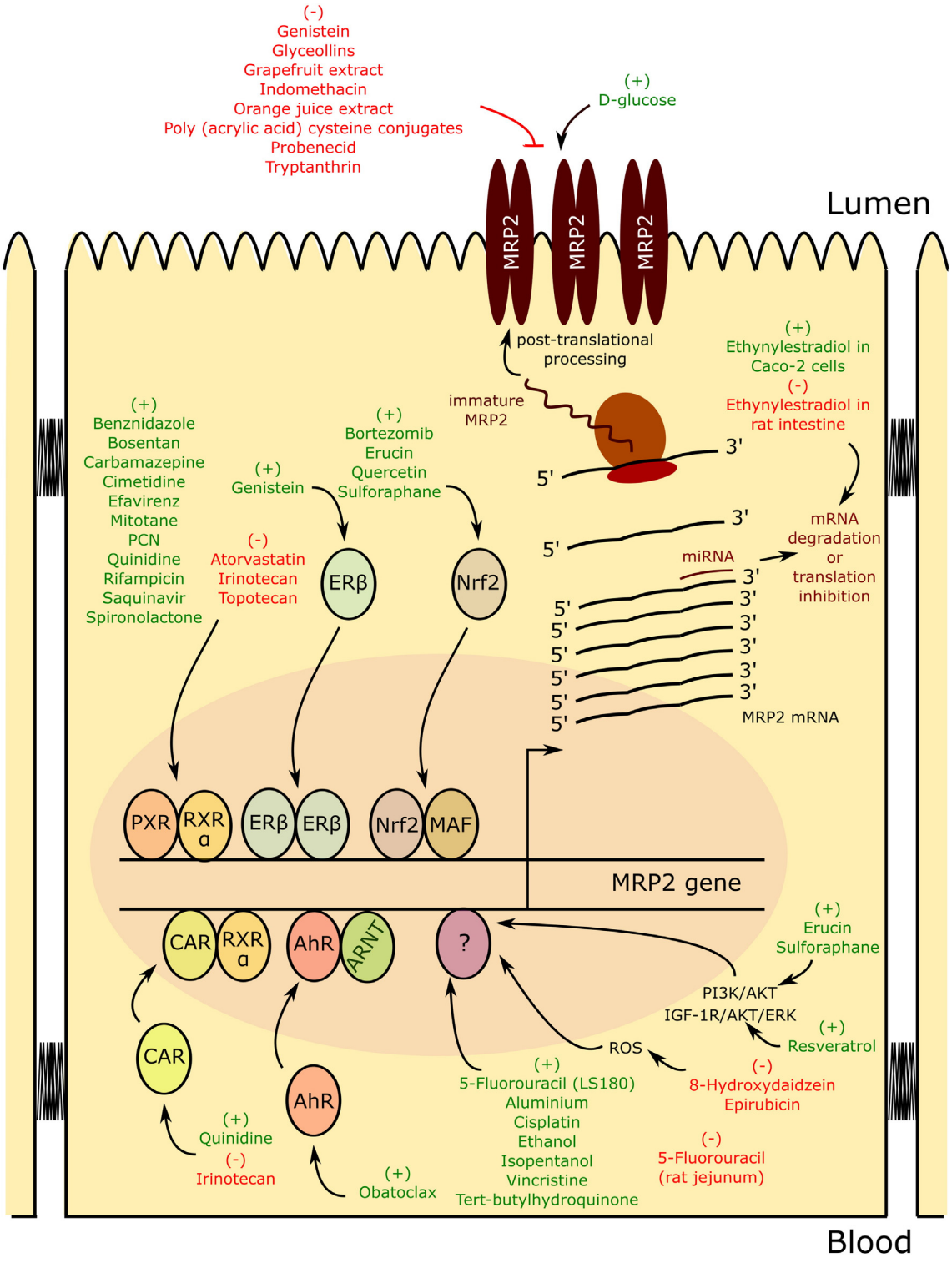

Fig. 3. Modulation of intestinal MRP2 expression and activity by xenobiotics. Drugs and natural compounds that positively (+) or negatively (−) regulate MRP2 expression and activity are summarized in a single enterocyte model. If known, the corresponding mechanism of action is also depicted. Once into the enterocyte, xenobiotics can interact with different nuclear receptors, which in turn modify the synthesis rate of MRP2 mRNA. Nuclear receptors like PXR, CAR and Nrf2 are important transcriptional regulators of MRP2, being activated by rifampicin, phenobarbital and sulforaphane respectively, among others. Modulation of MRP2 expression may also occur *via* a post-transcriptional mechanism, *e.g.* involving modulation of mRNA stability or translational efficiency. Ethynylestradiol effect is an example of this kind of regulation. Xenobiotics can also modulate MRP2 transport activity in an acute fashion. A direct interaction with the MRP2 molecule is a possible mechanism implicated. Examples of xenobiotics acting this way are indomethacin (competitive inhibition) and poly(acrylic acid) cysteine conjugates (activity modulation). Abbreviations not given in text: ARNT, aryl hydrocarbon receptor nuclear translocator; MAF, musculoaponeurotic fibrosarcoma.

Table 4
Functional impact of modulation of MRP2 expression by xenobiotics.

Compound	Effect on MRP2 expression	Effect on MRP2 activity	Model	Substrate studied	Reference
Benznidazole	Up-regulation	Up-regulation	Rat intestine – *in situ* perfusion	DNP-SG efflux	Perdomo et al. (2013)
Ethynylestradiol	Down-regulation	Down-regulation	Rat jejunum — everted sacs	DNP-SG efflux	Arias et al. (2009)
Ethynylestradiol	Up-regulation	Up-regulation	Caco-2 cells	DNP-SG efflux	Arias et al. (2014)
Spironolactone	Up-regulation	Up-regulation	Rat jejunum — everted sacs	DNP-SG efflux	Ruiz et al. (2009)
Genistein	Up-regulation	Up-regulation	Caco-2 cells	DNP-SG efflux	Arias et al. (2014)
Glyceollins	Down-regulation	Down-regulation	Caco-2 cells	CDF transport	Chimezie et al. (2015); Schexnayder and Stratford (2015)

DNP-SG: 2,4-dinitrophenyl-S-glutathione; CDF: 5-(6)-carboxy-2′,7′-dichlorofluorescein.

Inhibiting MRP2 activity remains a valid approach to increase the oral absorption of substrates of therapeutic use. It was described that poly(acrylic acid)-cysteine conjugates increase the absorption of sulforhodamine 101 and penicillin G, MRP2 model substrates, in rat small intestine mounted in Ussing chambers. The inhibitory effect was due to the presence of thiol groups in the polymers, which react with thiol groups in the MRP2 active site decreasing its transport activity. The extent of inhibition was dependent on both the molecular weight and content in thiol groups of the polymers. Since poly(acrylic acid)-cysteine conjugates are not absorbed, they are expected to not generate systemic interactions and thus they could be used to increase intentionally the oral bioavailability of drugs substrates of MRP2 (Grabovac et al., 2015).

Naturally occurring compounds can also modulate intestinal MRP2 activity in an acute fashion. Chimezie et al. (2015) demonstrated that glyceollins not only reduce MRP2 expression in Caco-2 cells after sustained treatments, but they inhibit the MRP2-mediated transport of 5-(6)-carboxy-2′,7′-dichlorofluorescein (CDF) after 2 h exposure, with a potency similar to the MRP2 inhibitor MK571 (Schexnayder and Stratford, 2015). MRP2 activity in Caco-2 cells is also impaired by tryptanthrin, a biologically active alkaloid from the medicinal plants *Polygonum tinctorium* and *Strobilanthes cusia*. Zhu et al. (2011) showed that the basolateral to apical permeability of pravastatin, a specific MRP2 substrate, was diminished in the presence of tryptanthrin (4 μM; 2 h). Whole extracts from orange and grapefruit juice, as well as their components tangeretin, hepatomethoxyflavone, nobiletin, bergamottin and 6′,7′-dihydroxybergamottin inhibited MRP2-mediated drug transport in Caco-2 cells (Honda et al., 2004). The flavonoid GNT also inhibits intestinal MRP2 activity. Using an intestinal perfusion model Yokooji et al. (2013) demonstrated that after 1 h of intravenous administration of GNT, MRP2-mediated secretion of irinotecan hydrochloride (CPT-11) and its metabolites was reduced. The authors also reported that GNT decreased the biliary excretion of CPT-11 and derivatives as an additional factor decreasing luminal exposition of the enterocytes to these agents. Since CPT-11 induces a delayed-type, severe diarrhea, they propose the co-administration of GNT to be a possible strategy to ameliorate the intestinal side effect of irinotecan treatment. It is worth noting that the plasma concentration of GNT in these experiments was in the 100 μM range, a level that cannot be reached after normal dietary exposition or ingestion of GNT-enriched supplements (Mathey et al., 2006). A previous study demonstrated that GNT inhibits competitively MRP2 transport in the isolated and perfused rat liver model (Jäger et al., 1997). A similar mechanism could be responsible for the inhibitory effect reported in intestine by Yokooji et al. (2013).

Other natural compounds exhibited a positive modulatory effect. Legen and Kristl (2004) demonstrated that D-glucose increases MRP2 activity, as 10 mM of this sugar incorporated to the mucosal side of rat jejunum increased serosal-to-mucosal transport of fluorescein. In their paper, the authors exclude the possibility of enrichment of energy supply or acidification of the mucosal surface to be involved. Although the mechanism still remains uncertain, a short-term modulation due to dynamic changes in MRP2 localization seems possible. This physiological mechanism has been described already for hepatic MRP2 (Roma et al., 2008) and might also account for acute changes in intestinal MRP2 activity.

9. Conclusion

The intestinal epithelium is highly exposed to a broad range of xenobiotics such as natural compounds, dietary contaminants and therapeutic drugs. As a consequence, alterations in MRP2 expression or activity are expected to occur with frequency. In this review we have compiled the evidence found in the literature regarding regulation of expression and/or activity by xenobiotics, together with the mechanism involved and functional consequences, when available. Fig. 3 summarizes these potential regulatory influences on MRP2 function.

Studies performed in experimental animals or human cell lines demonstrated important effects on MRP2 expression either at the transcriptional or post-transcriptional level. Nuclear receptors like PXR and CAR, among others, were frequently involved. Few studies documented both changes in expression and activity in the same experiment, and these findings are summarized in Table 4. Examples are the therapeutic agents benznidazole and spironolactone, which increased the expression and activity of intestinal MRP2 *in vivo* in rats. The effect of the synthetic estrogen ethynylestradiol was variable and dependent on the dose and model studied. Different soy-derived compounds exerted different effects. That is the case of genistein and a group of glyceollins that up-regulated or down-regulated MRP2 expression and activity in Caco-2 cells, respectively.

Alternatively, regulation of MRP2 activity by xenobiotics may occur with no need for modification of the expression. Examples are grapefruit/orange juice extracts, the natural compound tryptanthrin and the therapeutic drug indomethacin.

It should be noted that several of the xenobiotics currently reviewed altered the expression of drug-metabolizing enzymes as well as other intestinal drug transporters, apart from MRP2, indicating multiple and complex effects not contemplated in this review.

The impact of xenobiotic regulations on availability and pharmacokinetics of co-administered drugs, a concept associated with the membrane barrier function of MRP2, is poorly understood. Carbamazepine and rifampicin, both in human, and poly(acrylic acid)-cysteine conjugates in rats, are examples of xenobiotics affecting intestinal absorption of the MRP2 substrates talinolol, ezetimibe and sulforhodamine 101/penicillin G, respectively. Unfortunately, only few reports are available in the literature on drug-drug interactions associated with intestinal MRP2. Future clinical studies should help to identify additional interactions, including those related to natural compounds, which could result in changes in efficacy or safety of therapeutic drugs.

Conflict of interest

The authors declare that they have no conflict of interest.

Transparency Document

The Transparency Document associated with this article can be found, in online version.

Acknowledgements

This review study was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) [grant number PIP 2012-0240 (to A.D.M.)], and from Fondo para la Investigación Científica y Tecnológica (FonCyT) [grant number PICT 2014-0476 (to A.D.M.) and grant number PICT 2014-1121 (to S.S.M.V.)].

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