Chapter I

General Introduction
MRP2 and the defence against drugs and toxins

General Introduction

1. Xenobiotic defence in the body
   1.1 Metabolism
   1.2 Transport
      1.2.1 MDR1/ABCB1
      1.2.2 MRP2/ABCC2
      1.2.3 BCRP/ABCG2
      1.2.4 ABCG5/G8
   1.3 Coordinated regulation of genes involved in xenobiotic defence

2. Molecular principles of bile formation and cholestasis
   2.1 The hepatocyte and bile formation
   2.2 Regulation of transporter expression in cholestasis

3. Cholestatic diseases
   3.1 Genetic hyperbilirubinemia (Dubin-Johnson, Gilbert and Crigler-Najjar syndromes)
   3.2 Byler syndrome (Progressive familial intrahepatic cholestasis, PFIC)
   3.3 Acquired cholestasis

4. Model compounds of toxicity and carcinogenicity
   4.1 The food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
   4.2 Arsenite
   4.3 ω-Naphthylisothiocyanate

5. Hypothesis and aim of this work

6. References
1. Xenobiotic defence in the body

1.1 Metabolism

Detoxification of xenobiotics including toxins, carcinogens and drugs is the central task of many metabolizing enzymes in the body. While this is a desirable and necessary mechanism of protection against harmful compounds taken up with the food, it represents a major obstacle to medical drug therapy. This is further complicated by the fact that exposition of cells to xenobiotics can lead to induction of such enzymes. On the one hand, a complex coordinated multi-step system of defence against the toxic effects of compounds is established by this induction. On the other hand, induction of some of these steps can actually lead to activation of xenobiotics and subsequent toxic effects in the body.

Two groups of enzymes are known to handle metabolism of harmful compounds. The group of cytochrome-P450-isoenzymes (CYP’s) comprises numerous isoforms (about 60 are expected in humans) leading to oxidation (mostly hydroxylation) of reactive moieties in molecules (for an overview see Ref. 2). The isoforms in the CYP groups 1, 2 and 3 mediate metabolism of many exogenous compounds. Several compounds, drugs and carcinogens require activation by CYP450-isoenzymes to obtain their reactive, i.e. alkylating, properties in the body. In that respect, CYP450-mediated metabolism is not always advantageous for the organism. Because CYP450-isoenzymes mostly catalyze the first step of biotransformation, this function is called phase I metabolism. Phase I metabolism precedes diverse conjugation steps, also called phase II metabolism. However, activation by phase-I-enzymes is not always a prerequisite for phase-II-metabolism which can also take place on non-metabolized compounds.

Phase-II-metabolism is mediated by several different enzymatic systems, the most important being the UDP-glucuronosyltransferases. Isoforms of this enzyme class conjugate compounds to glucuronic acid thereby making them more soluble and excretable in urine or bile. For most compounds, this metabolic step represents the final detoxification. Deconjugation can, however, take place since glucuronidase activity is present in several tissues and body fluids. Other important phase II systems are glutathione-S-transferases, sulfotransferases and acetyltransferases.

More recent research has demonstrated that we have to add two more steps to our models of xenobiotic defence. Both steps are mediated by transport proteins, translocating compounds in
an ATP-dependent fashion across the plasma membrane. Due to their expression in different tissues, these transporters reduce the local cellular burden of toxic compounds giving the individual cell a protection against toxic effects. More importantly, these transport proteins are primarily expressed in the apical membrane of epithelial cells, such as enterocytes, which are exposed to exogenous xenobiotics. In these cells the same transporters function on the one hand to reduce the entrance of harmful substances and on the other hand to eliminate their detoxification products. The latter step has been called “phase-III-metabolism”[^4], indicating the close connection to the oxidation and conjugation steps of detoxification. The first function, i.e. direct elimination of xenobiotics upon entrance in the cell, represents a first defence line against xenobiotics and likewise could be called “phase-0-metabolism”.

All transporters involved in these mechanisms are members of the family of ATP-binding-cassette transporters. They mediate cellular efflux in an active, ATP-dependent manner, against concentration gradients. To present knowledge, at least two transporters play a prominent role in phase 0 and phase III defence against xenobiotics. They are the multidrug resistance transporter 1 (MDR1) and multidrug resistance-associated protein 2 (MRP2). After the new nomenclature they were termed ABCB1 and ABCC2, respectively. However, a third transporter, more recently identified, called breast cancer related protein (BCRP) or ABCG2, is likely to be involved in this defence system as well.

1.2 Transport

1.2.1 MDR1/ABCB1

MDR1 has been first described in cancer cells where it extrudes chemotherapeutic agents out of the cell thereby conferring multidrug resistance[^5], a problem in treatment of cancer. Its physiological function, however, became only fully clear upon studies with several drugs in mdr1a -/-, later in combined mdr1a/b -/- mouse models[^6]^[7]. In contrast to humans who only have the *MDR1* gene, mice have two genes *mdr1a* and *mdr1b*, with overlapping substrate specificity and tissue distribution. Since the above mentioned studies it is widely accepted that MDR1 functions as a gatekeeper against xenobiotics in the gut[^8]. It cannot be excluded that there are endogenous substrates for MDR1 as well[^9]; in fact, MDR1-mediated transport was demonstrated for endomorphins[^10] as well as aldosterone[^11]. MDR1 also represents an important gatekeeper in the blood-brain-barrier[^6]. This function interestingly might be at least partly independent from that in the gut, because in mdr1a/b-knockouts, accumulation of the
opioid receptor agonist asimadoline in the brain was 9-fold increased while oral bioavailability remained unchanged. The gatekeeper function is desirable for toxins and carcinogens, but was found to limit the oral availability of drugs. Individual differences in activity and/or expression of the protein were shown to lead to changes in drug bioavailability. In kidney transplant recipients, MDR1 protein concentration predicted intestinal absorption of cyclosporine. Plasma levels of digoxin were elevated in healthy volunteers with the C3435T polymorphism of MDRI. In the latter study, reduced duodenal MDR1 expression was demonstrated in individuals homozygous for this polymorphism, indicating a direct influence of MDR1 function on oral bioavailability of digoxin. For nelfinavir, the same polymorphism was found to increase the immunological response (CD4 count) in HIV-positive patients, suggesting clinical relevance of MDR1 activity. On the other hand, no effect of the C3435T polymorphism on the oral bioavailability of fexofenadine, a well established MDR1 substrate, could be found in vivo in healthy subjects. The frequency of the first discovered polymorphism of MDRI, the C3435T polymorphism, was investigated recently in a large sample of Caucasian subjects; it was found that about a quarter of the subjects were homozygous for this polymorphism. Another study identified several other polymorphisms in the MDRI gene, partially linked to each other in the sense that the C3435T did occur together with two other polymorphisms (C1236T and G2677T) in most patients. In vitro studies, however, so far only defined change of activity or expression for single polymorphisms therefore being only partly valuable for identifying the influence of these frequently occurring alleles. It is difficult to assess the clinical importance of MDRI genetic polymorphisms from these in vitro data as additional parameters (e.g. compensatory up-regulation of other transporters) may contribute to overall bioavailability of drugs. It seems clear that the influence of MDR1 activity on the effect of drugs is substrate specific and therefore has to be investigated for each drug.

MDR1 transports a wide range of structurally diverse drugs, of which the most important are given in table 1. In the lower section of this table, drugs known as inhibitors are specified. It is possible that some of these drugs will turn out to be substrates of MDR1 as well, but up till now, only their potential of inhibiting MDR1-mediated transport of other substrates has been defined.
<table>
<thead>
<tr>
<th>MDR1 Substrates</th>
<th>MRP2 Substrates</th>
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<tr>
<td>Grepafloxacin (^{20,21})</td>
<td>Arsenite (^{40,41})</td>
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<tr>
<td>Octreotide (^{22})</td>
<td>Cisplatin (^{42})</td>
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<td>Saquinavir (^{23,24})</td>
<td>Methotrexate (^{43})</td>
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<td>Vinblastine (^{25-27})</td>
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<th>MDR1 Inhibitors</th>
<th>MRP2 Inhibitors</th>
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<td>Simvastatin (^{49})</td>
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<tr>
<td>Valspodar (PSC-833) (^{47})</td>
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<tr>
<td>Verapamil (^{48})</td>
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**Table 1:** (Incomplete) list of important drugs, which interact with MDR1 or MRP2 either as (predominantly) inhibitor and/or substrate (current state of knowledge). Several inhibitors might turn out to be substrates as well in the future. Vice versa substrates variably might also inhibit or stimulate MDR1 activity. Additional explanations as well as other xenobiotic substrates are given in the text.
The large number of drugs potentially influencing MDR1-activity makes interactions likely to occur in multi-drug therapy, especially given the fact that some drugs (e.g. doxorubicin) additionally influence the expression of MDR1 (see also below). It has to be kept in mind, though, that most of these substrates have been investigated in vitro, leaving the clinical effect of interactions undefined. One illustrative human study however, assessing the effect of co-treatment with loperamide (a MDR1 substrate) and quinidine (a MDR1 inhibitor), demonstrated a possible impact. Loperamide, an opioid derivative given for diarrhea, normally does not induce central effects. Under co-treatment with quinidine, healthy volunteers developed respiratory depression though plasma levels of loperamide remained unchanged. This study proved that the lack of central effects of loperamide is due to the gatekeeper role of MDR1 in the blood-brain-barrier and represents a “proof of principle” for drug interactions by MDR1 although oral bioavailability was not influenced. Interactions between multiple drugs influencing the latter MDR1 function were not established in humans yet, but probably exist and may be of clinical importance.

MDR1-blocking agents are used nowadays in clinical trials of chemotherapy in order to reduce multidrug resistance. PSC-833, or valspodar, predominantly inhibits MDR1 but is also transported by it, albeit very slow and at a insignificant rate. Whether the inhibitory effect of PSC-833 on MDR1, discovered and characterized in vitro, can be fully translated to patients, is still not clear and requires further studies. While studies in mice demonstrated significant effects, the results in humans are not that encouraging up till now. Whatever the efficacy is, it is clear that agents like PSC-833 do not only increase the uptake of chemotherapeutic drugs in tumor cells, but also their bioavailability, activity and toxicity as well as that of co-medications. This phenomenon has to be kept in mind when prescribing drugs in addition to PSC-833-containing regimens. Components of our daily nutrition can influence MDR1-activity as well. Grapefruit juice has been shown to influence MDR1 activity, although results of in vivo and in vitro studies are conflicting with regard to the extent to which this happens. Methoxyflavones in orange juice have been shown in vitro to inhibit MDR1 activity but whether this is relevant in vivo needs further investigations.

Both MDR1 and the most important drug-metabolizing CYP isof orm 3A4 are regulated by common nuclear receptors (see below), which makes drug interactions even more complex. In vitro systems have been developed to study the coordinated actions of this xenobiotic defence system. For this purpose, polarized epithelial cells where transfected to express both CYP 3A4 and MDR1. These cells were suitable for studying linked metabolism and transport of
several substrates, however they represent an extremely isolated system which probably is far from the *in vivo* situation.

In order to improve oral bioavailability of drugs, a combined therapy with specific inhibitors for each of the components of this system (MDR1 and CYP3A4) has been proposed \textsuperscript{64}, but would require additional medications. It is doubtful whether therapy with enzyme inhibitors represents a reasonable option in maximizing oral bioavailability of drugs.

1.2.2 MRP2/ABCC2

MRP2 was first functionally characterized as a canalicular multispecific organic anion transporter in the apical membrane domain of hepatocytes \textsuperscript{65}. As such, it mediates transport of numerous organic anions, especially conjugated compounds, into bile and therefore out of the body \textsuperscript{45}. As a consequence it influences the whole body load of endo- and xenobiotics. Recent data show, that MRP2 is not only a transporter for conjugated organic anions.

MRP2 is not only expressed in the liver and the kidney, but also in epithelial cells of the intestine \textsuperscript{66-69}, the placenta \textsuperscript{70} and at the blood brain barrier \textsuperscript{71}. Apart from conjugates MRP2 also transports amphipathic uncharged compounds \textsuperscript{26, 72, 73}, indicating a much broader substrate spectrum of this transporter. Various studies show that MRP2-mediated transport of uncharged or cationic substrates only works in the presence of reduced glutathione (GSH) \textsuperscript{26, 40, 41, 72}. Labile complexing of the substrates with reduced GSH or simple co-transport likewise seems to be a possible explanation for this phenomenon. The latter mechanism also has been described for transport of amphipathic compounds by another member of the MRP-family, MRP1 \textsuperscript{74}. Consequently, depletion of intracellular GSH inhibits MRP2-mediated export of uncharged compounds while that of anions is preserved.

The role of MRP2 in the disposal of conjugated xenobiotic metabolites has been documented in the liver. Here, lack of MRP2 leads to lower efficiency in glucuronidation of toxins *in vivo* \textsuperscript{72} while in an *in vitro* study, the presence of MRP2 augmented the detoxification through glutathione transferase \textsuperscript{75}. This effect probably is a result of the influence of MRP2 on the respective enzymatic reactions (i.e. removal of enzymatic products out of the cell in the latter case or, in the former case, a result of product inhibition through lack of removal). This phenomenon underlines the importance of coordinated actions of several consecutive detoxification enzymes.

The functional importance of MRP2 in the intestinal epithelium has not clearly been defined yet. In the rat, bioavailability of an abundant food-derived carcinogen (PhIP) is reduced by MRP2 \textsuperscript{73}. MRP2 expression in the human duodenum is inducible by rifampicin \textsuperscript{69}, indicating
possible interactions in multi-drug therapy. This induction has been shown to be the result of signalling by nuclear receptors which are induced by a variety of xenobiotics (see below) and represents one underlying mechanism for acquired differences in protein expression. Polymorphisms, as described for \textit{MDR1}, have now been found also in the \textit{MRP2} gene \(^7^6\), but neither frequency nor influence on transporter activity or expression are defined yet. Another possibility of acquired individual differences in protein expression is the prevalence of certain intestinal diseases (Crohn’s disease, celiac sprue). Given the fact that MRP2 (and especially its rat orthologue) is expressed in the tips of the intestinal villi \(^6^7^, \^6^9\), which are atrophic in celiac sprue, this might represent an important mechanism for reduction of intestinal MRP2. However, the influence of intestinal diseases on MRP2 expression or activity has not been investigated yet. The clinical relevance of this phenomenon therefore remains obscure. Substrates and inhibitors of MRP2 are included in table 1. Note that MDR1 and MRP2 share some substrates as well as inhibitors, which may lead to broader interactions in oral bioavailability of drugs. In addition, there are drugs (e.g. tamoxifen \(^7^7\)) which just influence expression of the protein thereby influencing bioavailability, activity and toxicity of the substrates. In contrast to MDR1, systematic clinical investigations regarding the influence of MRP2 inhibition on oral bioavailability of substrates are lacking up till now.

Components of our daily diet are also substrates for MRP2 such as the flavonoid epicatechin in tea \(^7^8\), chrysin and its metabolites \(^7^9\) and the meat-derived heterocyclic amine PhIP \(^7^2\). While the first two compounds are supposed to have antitumorous effects, PhIP is a carcinogen with genotoxic properties. Further studies are necessary to define the role of MRP2 in the defence against food-derived xenobiotics.

To elucidate the importance of different transporters in the gut, transcripts in the human jejunum have been analyzed, showing higher levels for \textit{MRP2} transcripts than for \textit{MDR1} \(^8^0\). This lets MRP2 in the gut appear at least as important as MDR1. It has to be kept in mind, however, that transcription analysis is not representative for protein expression, especially because MRP2 expression can be regulated translationally \(^8^1\). Additionally, protein levels will not be representative for the actual transport activity.

\subsection*{1.2.3 BCRP/ABCG2}

Breast cancer resistance protein (BCRP) was originally discovered, as implied by its name, in breast cancer cells \(^8^2\). It was also termed Mitoxantrone resistance protein (MXR) because of one of its substrates \(^8^3\). On the basis of homologies in sequence and domain arrangements, it has been added to the ABCG group. This group consists of so called “half-transporters”,
describing the molecular construction with only one transmembrane domain while all “full transporters” consist of two transmembrane domains, connected by the ATP-binding cassette. Only “full transporters” were found to be expressed in the plasma membrane of cells whereas “half transporters” exist in membranes of intracellular compartments. However, BCRP was the first “half transporter” identified in the plasma membrane, and is thought to function as homodimer.

BCRP has a relatively broad tissue distribution; the transporter was found in the small intestine, the colon and in the canalicular membrane of hepatocytes. This supports the hypothesis, that BCRP plays a similar role in the body as MDR1 and MRP2. Transcription of BCRP in the human jejunum is higher than that of MDR1 and comparable to that of MRP2. In mice BCRP reduces the oral bioavailability of topotecan, a topoisomerase inhibitor used in cancer chemotherapy.

BCRP is also expressed in the breast and the placental syncytiotrophoblast. In the study on topotecan disposition in mice cited above, it was shown that BCRP influences the fetal penetrance of topotecan. Other topoisomerase inhibitors such as irinotecan and its metabolite SN38 also belong to the substrates of this protein. By microarray analysis of cell lines highly resistant to NB-506 and J-107088, two novel topoisomerase inhibitors with a molecular structure distinct from topotecan, BCRP was identified as the most likely candidate for mediating this resistance. In this study, however, another cell line, transfected with a BCRP expression vector, was resistant against these newly developed topoisomerase inhibitors but not against methotrexate or topotecan, substrates for BCRP identified in earlier studies. The conclusion of the authors that BCRP might mediate multidrug resistance by differential physiological mechanisms, remained unproven. In contrast, another study found such differential phenotypes of multidrug resistance as the result of single amino acid mutations in the protein. While the wild type protein with an arginine at position 482 conferred resistance to mitoxantrone and irinotecan, R482T or R482G mutations (arginine replaced by threonine or glycine, respectively) resulted in additional transport of rhodamine and doxorubicin by BCRP. This suggests that single nucleotide polymorphisms can influence the substrate specificity of this transporter.

Currently, a novel topoisomerase inhibitor (ST 1481), designed to overcome BCRP-mediated multidrug-resistance in tumor cells, is in clinical evaluation and has been shown in vitro not to be a substrate for BCRP.

Very recently, knockout mice for the Bcrp/Abcg2 gene have been produced. As might have been expected these animals are hypersensitive towards mitoxantrone. The importance
of Bcrp function in the gut was highlighted by the studies of Jonker et al.\textsuperscript{91} who showed that Bcrp-/- mice develop phototoxic lesions on light-exposed areas of the skin. This phenotype only developed when the animals were fed with lab chow and not on a synthetic diet. It could be demonstrated that the phototoxicity was caused by pheophorbide, a chlorophyll-breakdown product that occurs in various plant-derived foods and food supplements. Bcrp transports pheophorbide and is highly efficient in limiting its uptake from ingested food.

1.2.4 ABCG5/G8

ABCG5 and ABCG8 also belong to the ABCG family of half-transporters. They are expressed in the apical membrane domains of the liver and the intestine. Together they are thought to form a functional and obligatory heterodimer, encoded by two genes arranged head-to-head\textsuperscript{93}. Transcription from these genes seems to be coordinately regulated. It has been shown very recently that coexpression of both transporters is necessary for either transporter to travel to the cell surface\textsuperscript{94}. Mutations in both transporters have been linked to sitosterolemia\textsuperscript{93}, a rare autosomal recessive disorder first described in 1974\textsuperscript{95}. Normally, only less than 1\% of plant sterols such as sitosterol in the food is absorbed while about 50–70\% of unmodified cholesterol is absorbed in the intestine. However, in humans with sitosterolemia, plant sterol absorption is increased up to 40\% and biliary excretion is reduced, leading to excessive plasma levels of phytosterols. Patients develop xanthomas, premature atherosclerosis and coronary heart disease. Additionally, hemolytic episodes, hypersplenism, platelet abnormalities and arthritis have been described. The fact that sitosterolemia patients also have hypercholesterolemia, had already led to the assumption that ABCG5/G8 might be involved in cholesterol secretion\textsuperscript{96}. This was demonstrated recently in a transgenic mouse model\textsuperscript{97}.

These two transporters together provide an important gatekeeper function in the intestine which protects the body from plant sterols.

1.3 Coordinated regulation of genes involved in xenobiotic defence

The human promoter sequences of MDR1 (GenBank accession number M 29423,\textsuperscript{98}) and MRP2 (GenBank accession number AJ 005200,\textsuperscript{99}) have been cloned and allow studies concerning regulation of gene expression. For BCRP data are lacking since the promoter has not been characterized yet. The regulation of expression of MDR1 and MRP2 in several experimental models such as bile duct ligation (extrahepatic cholestasis), endotoxin treatment
(intrahepatic cholestasis) and partial hepatectomy (proliferation) has been defined extensively in the liver (reviewed in [100]). For MDR1, Sp1 and factors interacting with the Y box element (NF-Y, YB-1) seem to play a role in transcriptional activation. Interestingly, YB-1, a Y box binding protein, has the opposite suppressive effect on rat MRP2 transcription (A. Geier et al., submitted). Nuclear factors in MRP2 transcription activation, judged from putative binding sites, may include C/EBPβ and different hepatocyte nuclear factors (HNF1 and 3β), which are predominantly expressed in liver and therefore may not have any importance for the intestine. MRP2 is further activated through binding of a heterodimer formed by the retinoid X receptor (RXR, NR2B1) and the retinoic acids receptor (RAR, heterodimer abbreviated as RXR:RAR). Downregulation of MRP2 expression in inflammatory response has been shown to be due to IL-1 mediated suppression of RXR:RAR binding [101], a result also obtained in liver but both proteins involved are also expressed in the intestine. Tissue specific mechanisms of regulation obviously are important; the RXR:RAR mediated suppression of MRP2 has been shown to be absent in the kidney [102]. The fact that MRP2 is also induced by binding of the farnesoid X receptor FXR (NR1H4; as heterodimer FXR:RXR), a nuclear receptor in liver and gut involved in bile acid homeostasis, suggests a role for MRP2 in enterohepatic cycling of bile acids [103]. Induction of one of the two rodent Mdr1 genes, Mdr1b can be mediated by NF-kappaB [104,105], which may represent an ubiquitous mechanism.

It is straightforward to hypothesize that there should be one mechanism in the body which, as a response to xenobiotic exposition, activates the whole set of enzymes necessary for detoxification and elimination of this xenobiotic. Since the transporters for elimination of the unmetabolized parent compound and for elimination of the detoxification products are the same, this reaction can build up a coordinated defence system with at least 3 lines of defence. Rifampicin (a drug) and 2-acetylamino-fluorene (2-AAF, a carcinogen) both have been characterized as inducers of MDR1 and MRP2 [106,107]. Such results have been obtained in vitro, but MRP2 induction by rifampicin was also demonstrated immunohistochemically in intestinal biopsies [69], showing relevance for the human in vivo situation in general and for the human intestine in particular. The newly discovered orphan receptor, pregnane X receptor (PXR, in humans sometimes termed steroid X receptor SXR, new nomenclature NR1I2), has been identified as the cause for upregulation of both transporters [103,108]. Apart from rifampicin and 2-AAF, hyperforin (from St. John's wort), taxol, clomitrazole, phenobarbital, ritonavir and dexamethasone as examples for exogenous ligands, but also lithocholic acid, ursodeoxycholic acid and C21-steroids, called pregnanes, activate PXR as endogenous ligands [109-112]. All these molecules exhibit diverse structures and differ greatly in size and
shape, but most of them are potentially harmful either directly or when accumulating as a result of impaired metabolism or excretion. PXR not only activates *MDRI* and *MRP2*, but also the most important drug-metabolizing cytochrome isoform, 3A4, and isoforms of the 2B and 2C class, all involved in metabolism of steroids and xenobiotics in the intestine and the liver. Additionally the constitutive androgen receptor CAR (NR1I3), another CYP-inducing receptor, can replace PXR in the binding together with RXR to the respective response element and also induce MRP2 expression, which creates more ligands for regulation of this transporter gene. Taken together, this establishes a defence system with a very broad specificity, which also includes endogenous substances. However, this system can cause a new type of drug interactions. In healthy volunteers, co-treatment with rifampicin and talinol resulted in significant reduction of oral bioavailability of the latter compound, caused by PXR-mediated induction of MDR1 by the former drug. Here, excretion of the MDR1-substrate talinol was altered although this drug itself is not able to influence expression of MDR1.

It can be concluded from other studies that there are additional mechanisms accounting for independent regulation of transporters and CYP isoforms by some xenobiotics, certainly PXR is not the only nuclear receptor in directing intestinal expression of these enzymes. Moreover it remains unclear whether PXR-substrates can influence CYP or transporter genes by mechanisms other than PXR.

Figure 1 depicts the coordinated regulation of these enzymes in the enterocyte, which establishes a defence system with several defence lines.
2. Molecular principles of bile formation and cholestasis

The liver is the central organ of metabolism and detoxification in the body, though in the gut – as outlined above - metabolism of endogenous or exogenous compounds takes place as well. The location of the liver (downstream of the intestinal capillary bed and before the systemic circulation) and its unique ability to dispose waste products into bile make this organ indispensible for the body.

2.1 The hepatocyte and bile formation

The liver is built up mainly of hepatocytes which represent the functional cell type of this organ. In these parenchymal cells, metabolism and excretion, i.e. detoxification, take place and protect the organism from being overflown by toxic waste products. In that context, the liver represents a second filter after the intestinal epithelium and before the systemic circulation. The basolateral side of the hepatocyte lines the sinusoidal vessels, being the branches of the portal vein. Between the basolateral membrane of the hepatocyte and the sinusoids, small endothelial cells form the space of Disse. The canalicular (or apical) side of the hepatocytes forms the bile canaliculi which merge to form the bile duct (figure 2). Compounds to be detoxified are extracted from the blood in the sinusoids, metabolized in the hepatocytes and excreted into bile in order to get eliminated with the faeces. There can be reuptake in the intestine though, as discussed below (enterohepatic cycle).

Bile formation is a complex task and a unique ability of the liver. Bile forms by active, ATP-dependent secretion of bile salts and other constituents into the bile canaliculi and subsequent passive diffusion of water through the tight junctions. Consequently bile formation is an osmotic process, which mainly is established by canalicular transport of osmotically active substances.

At the canalicular side of the hepatocyte, at least 6 transporters, all belonging to the class of ATP-binding cassette (ABC-) transporters, contribute to secretion of bile constituents (Multidrug resistance transporter 1 and 3 (MDR1 and 3), Multidrug resistance associated protein 2 (MRP2), Bile salt export pump (BSEP), Breast cancer related protein (BCRP) and the transporter associated to familial intrahepatic cholestasis (FIC1); see figure 3 and table 2).
Fig. 2. Organization of sinusoids, hepatocytes and bile ducts in the liver (taken from "The Molecular Biology of the Cell" by Alberts et al., Garland Science 1997).

Fig. 3. Transporters involved in bile formation, with their respective substrates.
<table>
<thead>
<tr>
<th>Transporter</th>
<th>Substrates</th>
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<td>MDR1 (ABCB1)</td>
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</tr>
<tr>
<td>MDR3 (ABCB4)</td>
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<td>MRP2 (ABCC2)</td>
<td>Anionic/GSH cotransported xenobiotics</td>
<td>Dubin-Johnson syndrome</td>
</tr>
<tr>
<td>BSEP (ABCB11)</td>
<td>Bile salts</td>
<td>PFIC type 2</td>
</tr>
<tr>
<td>BCRP (ABCG2)</td>
<td>Uncharged xenobiotics</td>
<td>?</td>
</tr>
<tr>
<td>FIC1 (ATP8B1)</td>
<td>Aminophospholipids?</td>
<td>PFIC type 1</td>
</tr>
<tr>
<td>ABCG5/G8</td>
<td>Phytosterols and cholesterol</td>
<td>Sitosterolemia</td>
</tr>
</tbody>
</table>

Table 2: ABC-transporters involved in bile formation at the canalicular membrane of the hepatocyte, with their substrates and clinical syndromes in the respective genetic defect.

The main determinant of bile flow is secretion of bile salts, the most important being taurocholate. Secretion of most bile salts is mediated by the canalicular bile salt export pump (BSEP, former name Sister of P-glycoprotein SPGP, code ABCB11). The extent, however, to which bile salt secretion determines bile flow, is species-dependent. The so-called bile-salt-independent bile flow, maintained by secretion of other compounds, the most important being bicarbonate and glutathione, also contributes to bile secretion. Secretion of glutathione (GSH) is mediated by MRP2 (code ABCC2), qualifying MRP2 as the second most important transporter in the generation of bile flow. Bile flow itself, however, is not an exclusive function of hepatocytes as about 40% of the total bile flow, measured at the end of the common bile duct, is contributed by the cholangiocytes lining up the bile duct.

The quantitative second most important constituents of bile (after bile salts) are the lipids (mostly cholesterol and phosphatidylcholine (PC)). Cholesterol transport out of the hepatocyte into bile is mediated by ABCG5/G8 (see above). PC, inserted in the inner leaflet of the plasma membrane, is flipped by MDR3 to the outer leaflet of the membrane, where it is extracted by secreted bile salts. In a model of biliary lipid secretion, PC together with other lipids forms vesicular formations which are extracted from the plasma membrane by the detergent bile salts in the bile canaliculi. This hypothesis is supported by electron-microscopic detection of such vesicular formations in the canaliculi. By this mechanism, PC also serves as protection of the hepatocyte plasma membrane before the toxic detergent effects of bile salts. The importance of this function is underlined by the progressive familial intrahepatic cholestasis type 3, a genetic disease provoked by mutations in the MDR3 gene (see below).
The lipids and bile salts in bile form mixed micelles which facilitate absorption of other essential lipids including vitamin A, E, D and K in the gut. While the pancreas produces digestive enzymes to cleave nutritional contents, bile mediates absorption of important factors out of chyme. These complementary functions of bile are the basis for the enterohepatic cycling of bile salts as well as of other bile constituents. In this cycle bile salts are secreted into bile and taken up again in the terminal ileum in the gut (by micellar function). By this mechanism, a relatively small bile salt pool (2 – 3 g) is able to supply the continuous bile salt secretion (20 – 30 g per day) with a relatively low de novo production (< 1 %) of bile salts. This economic effect is undermined in therapeutic intention in certain forms of hyperlipidemia. Since bile salts are produced in the liver from cholesterol (by the key enzyme cholesterol 7α-hydroxylase), medicinal binding of bile salts in the gut and subsequent faecal elimination raises the de novo production and reduces cholesterol levels. Finally, the export of metabolized compounds out of the liver into bile (“Phase-III-metabolism”, see above) represents an important final detoxification step. As outlined above, MDR1, MRP2 and probably BCRP are involved in this aspect of bile formation and utilization. Together with their expression in the intestine, these canalicular transporters perfectly fulfill a gatekeeper function for the body. At different other “gates” in the body (MDR1 at the blood-brain-barrier, MRP2 in the kidney, all three transporters in the placenta), this function also helps to protect sensitive body parts against toxic compounds.

The multifunctional role of bile formation, however, can cause several different symptoms in cholestatic patients (see below).

2.2 Regulation of transporter expression in cholestasis

In cholestasis, bile flow is reduced or totally abolished and compounds destined to be excreted via bile accumulate in the liver. This leads to damage to the hepatocyte which in turn activates mechanisms of compensation to escape this toxic state. The best investigated cholestatic animal model is that of common bile duct ligation (CBDL). Other models are generated by endotoxin treatment and administration of ethinyl estradiol. Molecular responses of transporter expression in all three animal models have been reviewed two years ago. Basically uptake of compounds to be metabolized or excreted in the hepatocyte is reduced by downregulation of basolateral influx transporters, especially the sodium dependent taurocholate transporter (Ntcp) and organic anion uptake transporter 1 (Oatp1). Canalicular...
transporters are, depending on the model, either preserved after initial reduction (Bsep in CBDL and estrogen cholestasis) or permanently downregulated (Mrp2 in all states of cholestasis, Bsep in endotoxin-induced cholestasis). The basolateral efflux pump Mrp3 is upregulated in order to reduce the cellular load of potentially toxic metabolites or waste products.

Human data are sparse. In a study of 24 patients with cholestasis of different origins and degrees\(^1\), mRNA levels of NTCP, OATP2 and BSEP were reduced to 50 – 65 % compared to 13 healthy controls. Immunostaining of proteins demonstrated downregulation for NTCP and BSEP proteins. MRP2 mRNA was unchanged, but protein was reduced, suggesting posttranscriptional regulation (probably by endocytosis and consecutive breakdown\(^2\)). Combining these data with those from animals and humans with PSC (see below), MRP2 is the only canalicular transport protein which is steadily downregulated in all kinds of cholestasis. In the light of its important role in xenobiotic defence, the susceptibility to toxic or carcinogenic effects of xenobiotics in cholestasis should be further investigated.

3. Cholestatic diseases

The functions of the liver can be impaired to different extent in various diseases, the most important being liver cirrhosis, which represents rather a general end stage of several other diseases than being an independent entity. Diseases leading to liver cirrhosis include viral (hepatitis B and C), metabolic (hemochromatosis, \(a\)-1-antitrypsin-deficiency, Wilson disease) and autoimmune etiologies (primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis) as well as toxic (especially ethanol) or obstructive biliary (gall stones) damages to the liver. Although the cause and development of cirrhosis differ between the underlying diseases, the consequences remain similar and in the end stage with liver cirrhosis or liver cancer, the patient can only be saved by liver transplantation. Cholestasis is a hallmark of several biliary diseases including end stage liver cirrhosis. In general, extrahepatic cholestasis as represented by obstruction in the bile ducts is distinguished from intrahepatic cholestasis, where the origin lies in the hepatocytes. The first gives rise to a marked elevation of predominantly conjugated bilirubin, which cannot be excreted due to obstructive changes in the biliary system.

Impairment of liver function is the central problem in the clinical management of cirrhotic patients. Liver transplantation is required early and mostly completely abolishes the disease.
However, in several disorders of the liver, different degrees of cholestasis and hyperbilirubinemia develop before or without severe impairment of liver function and might reduce the performance of the liver without directly threatening the life of the patient. In such patients impaired metabolism and/or excretion of toxic compounds metabolized in the liver might have consequences in terms of toxicity or carcinogenicity of endo- and xenobiotics. Therefore, study of these phenomena is warranted in order to elucidate limitations of the liver function in different diseases.

3.1 Genetic hyperbilirubinemia (Dubin-Johnson, Gilbert and Crigler-Najjar syndromes)

Two distinct syndromes lead to mild hyperbilirubinemia which does not require therapy. Other genetic forms of hyperbilirubinemia, however, have serious consequences and are fatal within the first years of life unless treated by liver transplantation. The different diseases, their genetic background, which determines the severity, and clinical properties are reviewed in the following section.

3.1.1 Dubin-Johnson syndrome

The Dubin-Johnson syndrome is associated with a predominantly conjugated hyperbilirubinemia and is caused by mutations in the MRP2 gene. In patients with this syndrome, MRP2 is completely absent in canalicular membranes of hepatocytes and apical membranes of enterocytes. The syndrome is very rare, however it is frequently observed in a subgroup of Jewish patients. Apart from the hyperbilirubinemia, routine clinical chemistry is normal. The pattern of urinary excretion of coproporphyrins is changed in these patients and may serve as a diagnostic tool. Liver histology is often normal, except for lysosomal accumulation of a black pigment, which is not a clear proof but very characteristic for the syndrome. The course of the disease is benign and therefore no treatment is necessary. It is tempting, however, to speculate whether in the light of the gatekeeper function of MRP2, patients with Dubin-Johnson-syndrome might be at risk for toxic effects of drugs or, even worse, for the carcinogenic effects of food-derived xenobiotics. Although several reports on primary hepatocellular carcinoma exist, the rarity of this syndrome precludes epidemiological studies.
In any case, there is no progression to cirrhosis, but in how far isolated MRP2 deficiency affects liver function is unknown.

Several animal models facilitated research into the etiology of the Dubin-Johnson syndrome. The TR' rat (Wistar background) as well as the EHBR rat (Sprague Dawley background) exhibit the same symptoms as patients and were found to have mutations in the same gene, Mrp2. Additionally, physiological data of the Corriedale sheep resemble closely the human Dubin-Johnson syndrome. These animal models can be used to study subclinical effects of MRP2 deficiency on the organism.

### 3.1.2 Gilbert syndrome

The Gilbert syndrome is a frequent disorder (~ 6 % of the caucasian population). The syndrome results in a mild, unconjugated hyperbilirubinemia (usually around 2 to 5 mg/dl total bilirubin) which exacerbates in certain stress situations. No treatment is necessary since the underlying genetic defect leads only to a partial reduction of glucuronidation (about 30 % of normal).

Patients attract the physician's attention because of the isolated unconjugated hyperbilirubinemia. Serum levels of liver enzymes (including alkaline phosphatase and γ-GT) are normal so that further diagnostic evaluation is not necessary. Apart from reports about higher drug toxicity (e.g. the chemotherapeutic drug irinotecan), no severe clinical sequela are known so far in patients with Gilbert syndrome.

Mutations in the promoter region of the \( UGT1 \) gene are the causative factor. Patients with Gilbert syndrome show 7 TA repeats in their TATA box in the UGT1 promoter instead of 6 as in the healthy counterparts. Interestingly, there also exist mutations which lead to a (TA)\(_5\) or (TA)\(_8\) genotype; functional studies with repeats between 5 and 8 revealed that the number of TA repeats is inversely correlated with the glucuronidation activity. Obviously this change alone is not enough to explain the different phenotypes of Gilbert patients. A second, frequently occurring defect is required for development of the full blown Gilbert phenotype. The cause of this defect is not established yet, but may be related to defective uptake of bilirubin into the hepatocyte. Additionally it has been proposed that more severe appearances of unconjugated hyperbilirubinemia (total bilirubin up to 10 mg/dl) are due to heterozygous mutations in exons of the \( UGT1 \) gene. Homozygosity of these same mutations leads to the Crigler-Najjar syndrome.
3.1.3 Crigler-Najjar syndrome I and II (CN I and II)

The unique genetic organization of the UGT1 gene is responsible for the fact that mutations in the same gene produce several clinically distinct syndromes, at one end of the spectrum harmless (Gilbert syndrome), at the other a disease which inevitably leads to liver failure in the first decade of life. Crigler-Najjar syndrome type I and II represent the other end of this spectrum and differ from each other in the severity of UGT deficiency. The UGT1 gene gives rise to many UGT isoforms. The gene consists of 5 exons. Exons 2 to 5 are common for all isoforms, but each isoform has its own promoter and exon 1, which encodes the substrate specific sequence of the protein. Mutations in the CN syndrome can be located in exons 2 to 5, the common exons of the UGT1 gene\(^ {136}\). These exons lead to identical C-termini in every isoform of the UGT1 family. Consequently, mutations in this part of the gene result in impairment of all isoforms which cannot compensated for by other isoforms or members of the UGT2 family. If a mutation is present in exon 1 only, the bilirubin conjugating isoform UGT1A1 is affected.

Crigler-Najjar Type II (CN II) is associated with bilirubin levels between 10 and 20 mg/dl. Central nervous system damage is rare and patients survive into adulthood without complications\(^ {129}\). Glucuronidation activity is usually less than 10 % of the normal in these patients, however, in their bile, conjugated bilirubin with a relative excess of monoglucuronidated bilirubin can be detected\(^ {137}\). The syndrome responds promptly to phenobarbital treatment which leads to a significant reduction of bilirubin levels\(^ {137}\). Most likely enzyme induction mediated by nuclear receptors (e.g. CAR and PXR\(^ {138}\)) is responsible for that effect. The mode of inheritance is variable, indicating possible connections to the Gilbert syndrome (CN II as homozygosity or compound heterozygosity from GS\(^ {129}\)). This is further supported by the fact that CN II patients often show at least on one allele mutations in exon 1 of the UGT1A gene, while mutations in CN I are exclusively located in exons 2 to 5\(^ {136}\).

Type I is associated with development of a more severe clinical picture with jaundice and kernicterus causing neurologic symptoms. Bilirubin levels can vary between 15 and 50 mg/dl. The bilirubin overload of cerebral structures leads to death in the first years of life. Though the primary defect is situated in the liver, this organ is not severely affected by the time of death. Liver transplantation is the only definitive treatment for this disease though phototherapy may provide some effects on bilirubin levels. CN I is rare, with only a few
hundred cases described in the literature. Transmission is autosomal recessive which is consistent with the fact that homozygosity for mutations in the coding region of the UGT1A gene is necessary for development. In contrast to CN II, phenobarbital therapy is useless.

The genetic defect is analogous to that in the Gunn rat (Wistar background) which exhibits a similar phenotype as CN I patients, however these rats do not die in childhood. The reason for this different behaviour in both species is unclear, maybe differential degrees of excretion of the unconjugated bilirubin via the intestine or species-specific metabolic compensation by CYP isoforms might contribute to this difference. Indeed, serum bilirubin levels in Gunn rats are considerably lower than in CN patients.

3.2 Byler disease and syndrome (Progressive familial intrahepatic cholestasis)

The classic Byler disease was first described in Amish people and is nowadays referred to as progressive familial intrahepatic cholestasis (PFIC) type 1. The genetic basis of this and other types of inherited cholestasis has been elucidated and four types are differentiated. Table 3 gives the four types, their genetic basis, differences in their behaviour and prognosis.

<table>
<thead>
<tr>
<th>PFIC type</th>
<th>Gene</th>
<th>Clinic</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FIC1 (ATP8B1)</td>
<td>Normal γ-GT, early onset, diarrhea.</td>
<td>OLT required</td>
</tr>
<tr>
<td>2</td>
<td>BSEP (ABCB11)</td>
<td>Normal γ-GT, early onset, rapid course.</td>
<td>OLT required</td>
</tr>
<tr>
<td>3</td>
<td>MDR3 (ABCB4)</td>
<td>High γ-GT, later onset, slower course.</td>
<td>Mostly OLT required in adolescence</td>
</tr>
<tr>
<td>4</td>
<td>Bile salt synthesis genes</td>
<td>Normal γ-GT, late onset, slower course, Bile acid supplementation</td>
<td>Seldom OLT required</td>
</tr>
</tbody>
</table>

Table 3: Types of progressive familial intrahepatic cholestasis (PFIC). OLT = orthotopic liver transplantation.

The three types of PFIC associated with defects in transporter genes are similar in age of onset, clinical picture and prognosis. However, there are subtle differences owing to the different properties of the affected proteins.

Type I is due to homozygous mutations in the FIC1 gene. The function of the protein is still not clear, preliminary studies suggest a direct or indirect role in bile acid transport. This is supported by the fact that the protein’s expression is high in the small intestine where it might
contribute to bile salt enterohepatic circulation. Additionally PFIC 1 patients have diarrhea which cannot be cured by liver transplantation. This latter symptom distinguishes these patients from patients with other PFIC types 144, 145. The γ-GT is normal (as in type 2), while bilirubin, transaminases and bile salts are elevated. Liver histology reveals fibrosis, portal inflammation and canalicular stasis 146. The prognosis, unless treated with liver transplantation, is poor (fatal within first or early second decade of life).

Interestingly, another cholestatic syndrome, benign recurrent intrahepatic cholestasis (BRIC), was also shown to be due to mutations in the FIC1 gene 147. Patients with this syndrome develop a benign clinical picture with episodic icterus with spontaneous recovery and without any sequela. Different mutations in rather less conserved regions of the gene (as compared to PFIC1) might be responsible for the benign course of the disease.

PFIC 2, formerly called Byler syndrome, is caused by homozygous mutations in the BSEP gene. BSEP is the major canalicular bile salt export pump which is crucial for bile salt secretion into bile (see above). Disruption of this protein’s function leads to a even more severe clinical picture than in type 1 including early cholestasis and subsequent fibrosis and cirrhosis 148. Apart from the absence of diarrhea and this more severe course, type 2 resembles type 1 with regard to clinical picture and prognosis.

PFIC 3 differs from the two former types. Patients have a high serum γ-GT besides the other markers of cholestasis and they lack phospholipids in their bile, in contrast to the previous two types where the reduction of the bile acids in bile is the predominating finding. The underlying genetic cause lies in the MDR3 gene 149. Homozygous or compound heterozygous mutations give rise to a loss of function of the protein. Absence of the protein abrogates biliary phospholipid secretion and thereby leaves the canalicular membrane unprotected to the detergent action of bile salts (see above), which permits direct toxic damage to the hepatocyte. Portal inflammation and ductular proliferation, leading to fibrosis and cirrhosis can be seen in histologic sections 150. PFIC 3 patients have a higher age of onset, develop milder pruritus than type 1 and 2 patients and bile salt levels in serum are only slightly elevated, because bile salt secretion into bile is at least initially not impaired 151. Patients often require liver transplantation after a long course of the disease with slower development of cirrhosis. Heterozygous mutations in PFIC 3 position might be responsible for a subset of intrahepatic cholestasis in pregnancy (ICP) 152.

Defects in bile salt synthesis are responsible for PFIC type 4. Several mutations in two genes (Δ^4-3-oxosteroid-5β-reductase and 3β-hydroxy-C_{27}-steroid-dehydrogenase/-isomerase (3β-HSD), responsible for the conversion from cholesterol to bile salts, have been identified in
PFIC 4 patients. Normally, the course of this disease is relatively benign, supplementation of bile salts often avoids liver transplantation.

3.3 Acquired cholestasis

Posthepatic or obstructive cholestasis is mostly due to several acquired cholestatic syndromes, which develop in adulthood. Blocking of the common bile duct by gallstones represents the most common etiology for acquired jaundice, followed by neoplastic reasons (especially malignancies of the pancreas). In the former case, mainly female patients with obesity are at risk for development of gall stones, but there are also genetic risk factors.

Primary sclerosing cholangitis (PSC), an autoimmune disorder leading to strictures in bile ducts, accounts for another group of posthepatic cholestasis. This disease, occasionally associated with inflammatory bowel disease, can be diagnosed through the typical endoscopic appearance of the bile ducts in connection with detection of antineutrophil cytoplasmatic antibodies (ANCA). It predisposes patients to malignancies of the bile duct, cholangiocellular carcinomas. Experimental data in a rat model of PSC as well as data from patients with this disease indicate that downregulation of MRP2 represents a major hepatocellular reaction to the chronic cholestasis in this disease. In primary biliary cirrhosis, cholestasis develops on the level of small bile ducts, leaving the large bile ducts unaffected. In such patients, antimitochondrial antibodies type 2 (AMA 2) can be detected. In both diseases, ursodeoxycholic acid represents the most promising form of treatment (15 mg/kg body weight), however some patients progress to liver cirrhosis or malignancies of the bile duct and eventually require liver transplantation.

There are many other rare diseases which might cause cholestasis but these are beyond the scope of this introduction.
4. Model compounds of toxicity and carcinogenicity

4.1 The food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is a heterocyclic amine and is formed in several forms of meat during cooking, frying or barbecuing. The principal discovery that food-derived carcinogens can form during food-preparation was made in 1977. It appeared soon, that the mutagenic potential of these compounds was comparable or even higher than that of already established carcinogens. PhIP was discovered, structurally characterized and identified as the most abundant carcinogen in prepared meat in 1986. It was shown to induce colon and mammary carcinomas in rats; pooled data from several animal models as well as human epidemiological data led to the conclusion that PhIP may also be involved in human carcinogenesis. PhIP itself has to be activated to generate carcinogenic activity. Metabolism leading to activation as well as to detoxification has been investigated in several species. Activation takes place by hydroxylation through CYP1A1 and 2, leading to N-OH-PhIP. This metabolite is already mutagenic per se, but can be metabolized further to N-acetoxy- or N-sulfoxy-PhIP, both metabolites with a high carcinogenic activity in different mutagenesis assays. Detoxification on the other hand is mediated mainly by glucuronidation with UGT1 isoforms, either directly of PhIP, or of the oxidation products N-OH-PhIP and the already nontoxic 4’-OH-PhIP. It is reported in chapter 2, that the complete lack of UGT1 isoforms, as in the Gunn rat, does not elevate carcinogenic potential of PhIP, but results in faster excretion of the compound due to compensatory action of sulfotransferases.

![Fig. 4: PhIP and its phase-I-metabolites. In phase II, glucuronidation as detoxification occurs at the 4’-position and the N2- and N3-position. Activation to genotoxic metabolites is confined to N-OH-PhIP (acetylation or sulfation).](image-url)
In vitro data already pointed to the possibility that Mrp2 might be involved in transport of PhIP \(^{174}\). Before such studies, it was assumed that highly amphipathic compounds such as PhIP more or less passively diffuse through cell plasma membranes. The cited study as well as the studies described in chapters 3 and 4 established for the first time an important role of defence for a transport protein against a food-derived carcinogen.

4.2 Arsenite

Arsenic has high importance in human health issues. On the one hand, different forms of arsenic, present in drinking water in several parts of the world, are responsible for cancers of the skin, the lung, the bladder, the kidney, the colon and the liver \(^{175,176}\). Blackfoot disease, a unique peripheral vascular sickness, is caused by continous arsenic exposure and predisposes to development of the above neoplasms \(^{176}\). On the other hand, inorganic arsenic salts are used in treatment of acute promyelocytic leukemia \(^{177}\). Metabolism and disposition of arsenic has been characterized \(^{178,179}\) without elucidating the excretory mechanism. It had been realized already earlier, however, that biliary secretion of arsenic is connected to higher GSH concentrations in bile and it was hypothesized that this happens as an arsenic-glutathione complex \(^{180}\). Recently, it has been established that MRP2 secretes arsenic in form of an arsenic-glutathione complex into bile \(^{40}\). This fits nicely with results showing that arsenic induces expression of MRP2 \(^{181}\). MRP1 might as well be involved in glutathione-dependent transport of inorganic arsenic \(^{182}\). Different forms of arsenic complex with either 3 or 4 molecules GSH exist \(^{40}\), however it has been noted several years ago that the rise in biliary glutathione secretion is much higher and appears to reflect a different stoichiometry \(^{183}\). This unproportional loss of glutathione could be explained by results presented in chapter 5.

4.3 α-Naphtylisothiocyanate

α-naphtylisothiocyanate (ANIT), used 40 years ago in pesticides, is a model compound for induction of intrahepatic cholestasis \(^{184}\). As such, its mechanism of inducing cholestasis in the liver was subject of several studies, which were able to visualize toxic effects of ANIT to hepatocytes as well as to cholangiocytes \(^{185-187}\). The relative contributions of these effects to overall toxicity and the development of cholestasis remained undefined in these studies,
because the chronological order of toxic effects on hepatocytes and cholangiocytes was controversial. ANIT is complexed with GSH in the hepatocyte and secreted as GS-ANIT into bile\textsuperscript{188}, a finding which prompted the hypothesis that MRP2 might be involved in biliary secretion of this complex\textsuperscript{187}. As for arsenite, analysis of biliary secretion after ANIT administration found an unproportionally high GSH concentration in ANIT-treated rats\textsuperscript{189}. The fact that the GS-ANIT complex is labile at physiologic pH values and falls apart, led to the investigation of the phenomenon of excess GSH secretion in one study described in chapter 5.

5. Hypothesis and aim of this work

Multidrug resistance is a phenomenon, which is highly undesirable because it obstructs chemotherapeutic efforts in patients with malignancies. It is straightforward, however, to search for the physiological purpose of this effect. The body is, apart from drugs, exposed to innumerable toxic compounds in daily life, trying to enter the organism through the airways or the gut. It is therefore logical to hypothesize that transporters involved in multidrug resistance originally were supposed to be defence setups against such potentially toxic or carcinogenic compounds.

MRP2 is only one of these transporters, but its unique tissue distribution at several barriers in the body (intestine, liver, kidney, placenta, blood-brain-barrier) implies an important role in the xenobiotic defence for this protein. The characterization of the contribution of MRP2 to this defence system consequently was the first aim of the studies collected in this book. In contrast to MDR1 which is able to transport compounds with different polarity on its own, MRP2 requires for the transport of amphipathic compounds the help of glutathione. The further characterization of the GSH-dependence of the xenobiotic defence by MRP2 was another aim of the work presented herein.

6. References


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53


