ATP binding cassette transporter gene expression in rat liver progenitor cells

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Background and aim: Liver regeneration after severe liver damage depends in part on proliferation and differentiation of hepatic progenitor cells (HPCs). Under these conditions they must be able to withstand the toxic milieu of the damaged liver. ATP binding cassette (ABC) transporters are cytoprotective efflux pumps that may contribute to the preservation of these cells. The aim of this study was to determine the ABC transporter phenotype of HPCs.

Methods: HPC activation was studied in rats treated with 2- acetylaminofluorene (2-AAF) followed by partial hepatectomy (PHx). ABC transporter gene expression was determined by real time detection reverse transcription-polymerase chain reaction in isolated HPCs, hepatocytes, cholangiocytes, and cultured progenitor cell-like RLF cells and by immunohistochemistry of total liver samples. ABC transporter efflux activity was studied in RLF cells by flow cytometry.

Results: 2-AAF/PHx treated animals showed increased hepatic mRNA levels of the genes encoding multidrug resistance proteins Mdr1b, Mrp1, and Mrp3. Immunohistochemistry demonstrated expression of Mrp1 and Mrp3 proteins in periportal progenitor cells and of the Mdr1b protein in periportal hepatocytes. Freshly isolated Thy-1 positive cells and cultured RLF cells containing Thyl-1 positive cells and cultured RLF cells were only minimally expressed. Blocking Mrp activity by MK-571 resulted in accumulation of the Mrp specific substrate carboxyfluorescein in RLF cells.

Conclusion: HPCs express high levels of active Mrp1 and Mrp3. These may have a cytoprotective role in conditions of severe hepatotoxicity.

Liver regeneration occurs after loss of liver tissue due to toxic injury or partial heptectomy (PHx) and is predominantly accomplished by replication of hepatocytes. Under conditions in which hepatocytes cannot proliferate, liver damage results in activation of the hepatic progenitor cell (HPC) compartment. In humans, this occurs during severe liver injury when a significant number of hepatocytes are lost. HPC proliferation can be achieved by treatment with 2-acetylaminofluorene (2-AAF) in combination with PHx or carbon tetrachloride (CCl4) administration. Treatment with 2-AAF followed by PHx causes a block in hepatic progenitor cell proliferation at the G1/S restriction point due to decreased expression of cyclin E and increased hepatic expression of p53 and p21. HPCs, located in the canals of Hering, escape from the 2-AAF induced blockade and proliferate in the 2-AAF/PHx model. Their proliferation reaches a maximal level 9–11 days after hepatic injury. HPCs resemble fetal hepatic cells as they express fetal markers such as α-fetoprotein and Thy-1 at high levels and are considered to be the bipotential precursors of hepatocytes and cholangiocytes.

HPCs are only activated after severe liver damage and are thought to represent a back up mechanism for hepatic regeneration. We hypothesise that, in view of their critical role in hepatic repair after excessive damage, HPCs should be able to protect themselves against toxic metabolites and xenobiotics, concentrations of which increase during reduced liver function. One mechanism of cellular protection is expression of efflux pumps that belong to the ATP binding cassette (ABC) superfamily of membrane transporters.

The ABC transporter family consists of approximately 48 members that have been divided into seven subclasses (A–G) based on their amino acid sequence homologies. Members of the Abcb (multidrug resistance proteins) subfamily that are expressed in the liver include efflux pumps for hydrophobic compounds and chemotherapeutic agents (Mdr1a/b in rodents, gene symbol Abcb1a/b, MDR1 in humans), a flipase that translocates phosphatidylcholine across the membrane (Mdr2, Abcb4, MRD3 in humans), and the export pump for biliary salts Bsep (Abcb11). These transporters are all located in the canalicular membrane of hepatocytes, transporting their substrates into bile (reviewed in Houweling and colleagues).

The Abcc (multidrug resistance associated protein) subfamily consists of 12 members, of which at least four (Mrp1, Mrp2, Mrp3, and Mrp6) are expressed in normal liver (reviewed by Borst and Oude Elferink). Mrp2 (Abcc2) is present in the canalicular membrane of hepatocytes. Mrp1 (Abcc1) is located in the basolateral membrane but is expressed only at low levels in normal liver. Mrp1 expression is induced in proliferating hepatocytes. Mrp1 and Mrp2 have a similar substrate specificity and transport glutathione- S-conjugates, cysteinyl-leukotrienes, bilirubin glucuronides, oestrogen glucuronides, and glutathione disulphide. Hepatic expression of Mrp3, Mrp4, and Mrp6 has been reported recently. In normal liver, Mrp3 (Abcc3) is expressed in the basolateral membrane of pericentral localised hepatocytes and in cholangiocytes. Mrp3 can transport mono- and bivalent bile salts as well as glucuronide conjugates. Its expression is increased during cholestasis and in conditions of conjugated or unconjugated hyperbilirubinaemia (Gunn rats and Mrp2 deficient mutant rats). Mrp4 (Abcc4) is an export

Abbreviations: ABC, ATP binding cassette; 2-AAF, 2-acetylaminofluorene; CCL4, carbon tetrachloride; CFDA, carboxyfluorescein diacetate; CF, carboxyfluorescein; HPC, hepatic progenitor cell; PBS, phosphate buffered saline; PHx, partial heptectomy; RT-PCR, reverse transcription-polymerase chain reaction.

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ABC transporter expression in rat liver progenitor cells

pump for organic anions but is also capable of transporting cyclic nucleotides and nucleotide analogues.\textsuperscript{13, 21} In normal liver, Mrp4 is expressed at low levels.\textsuperscript{13} However, enhanced hepatic concentrations of bile salts increase Mrp4 expression in liver.\textsuperscript{22} Mrp6 (Abcc6) is expressed at high levels in normal liver, predominantly in the basolateral membrane of hepatocytes.\textsuperscript{14} Its substrate specificity is presently less well defined.

A number of these transporters have been linked to cellular protection. Mdr1b functions as an efflux pump for toxins and may also have an antiapoptotic role.\textsuperscript{15, 16} Mrp1 is a transporter of leukotriene C\textsubscript{4}, and the glutathione conjugate of prostaglandin A\textsubscript{2}, factors involved in inflammation and cell cycle arrest. Moreover, Mrp1 mediated transport of glutathione disulphide and glutathione conjugated 4-hydroxynonenal suggests that Mrp1 functions as part of the cellular defence system against oxidative stress (reviewed by Renes and colleagues\textsuperscript{17}). Mrp3 may have an important role in protecting cells from excessive amounts of endogenous bile salts by extruding these into blood.\textsuperscript{18, 19}

Two additional members of the ABC family that may be relevant for progenitor cell survival are Abca1 and Bcrp (Abgb2). Abca1 is highly expressed in fetal liver where its expression correlates with apoptotic areas.\textsuperscript{20} In adult life, Abca1 is ubiquitously expressed in various tissues and organs.\textsuperscript{21} It is essential for HDL formation\textsuperscript{22} but its specific function in adult liver is currently unknown. Bcrp is expressed at relatively low levels in the apical membrane of hepatocytes.\textsuperscript{23} Expression of Bcrp can, in common with Mdr1a/b and Mrp1, cause multidrug resistance.\textsuperscript{24} Bcrp has been demonstrated to have a cytoprotective role in a subgroup of haematopoietic stem cells.\textsuperscript{25}

The expression profile of ABC transporters in the HPC compartment is not known. As these cells represent a potential proliferative reservoir of a severely damaged liver, we speculate that these cells must be well protected. We therefore studied ABC transporter expression in HPCs.

**MATERIALS AND METHODS**

**Animals**

Specified pathogen free male Wistar and Fisher 344 rats (130–170 g) were purchased from Harlan-CPB, Zeist, the Netherlands, and were kept under routine laboratory conditions with a 12 hour light-dark cycle at the Central Animal Laboratory of the University of Groningen. Rats received standard laboratory chow and had free access to food and water. This study was approved by the Local Committee for Care and Use of Laboratory Animals.

**Animal experiments**

2-AAF/PHx induced oval cell activation in rats

Seven days before PHx, 2-AAF pellets (70 mg/pellet over a 28 day release, 2.5 mg/day; Innovative Research Inc., Sarasota, Florida, USA) or placebo pellets were placed subcutaneously in Wistar rats. PHx was performed according to the technique of Higgins and Anderson, removing two thirds of the liver.\textsuperscript{26} Sham operated animals underwent the same treatment protocol, including manipulation of the intestine and liver, but without hepatectomy. All surgery was performed under halothane anaesthesia. Nine days after surgery, livers were perfused with phosphate buffered saline (PBS) via the portal vein, excised, cut into small pieces, and snap frozen in liquid nitrogen for RNA isolation or frozen in cold 2-methyl-butan for immunohistochemistry. Tissue was stored at −80°C prior to use.

PHx induced hepatocyte proliferation in rats

To determine transporter expression in proliferating hepatocytes, Wistar rats were either sham operated or underwent PHx.\textsuperscript{27} At 24 hours post PHx, hepatocytes were isolated using a two step collagenase perfusion technique, as described previously.\textsuperscript{28} Cell fractions were frozen in liquid nitrogen prior to RNA isolation.

**Isolation of hepatic cell fractions**

Specific hepatic cell fractions were isolated from male Fischer 344 rats. Cholangiocytes were isolated as described by Ishii and colleagues\textsuperscript{29} from five rats to obtain sufficient amounts of RNA. Oval cells were isolated from rats 12 days after 2-AAF/CCl\textsubscript{4} treatment, as described by Petersen and colleagues.\textsuperscript{30} Hepatocytes were isolated according to Moshage and colleagues\textsuperscript{31}.

**Cell culture**

The progenitor cell-like cell line RLF \# 13 was a kind gift from Dr SS Thorgeirsson (Laboratory of Experimental Carcinogenesis, Division of Basic Sciences, National Cancer Institute, Bethesda, USA) and has been described previously.\textsuperscript{32} Cells were maintained in Ham’s F12 (Invitrogen Life Technologies, Paisley, UK) supplemented with 10% fetal calf serum and 50 µg/ml gentamycin (Invitrogen Life Technologies). Cells were grown to 90–95% confluence prior to experiments in a humidified incubator at 37°C/5% CO\textsubscript{2}.

**Antibodies**

The mouse monoclonal antibody C219 (Dako, Glostrup, Denmark) was used for detection of all P-glycoproteins. The goat polyclonal anti-MRP1 antibody SC-7774 (Santa Cruz Biotechnology, Santa Cruz, California, USA) was used for detection of Mrp1. The rabbit polyclonal anti-rat Mrp3 antibody was a kind gift from Dr H Suzuki (University of Tokyo, Tokyo, Japan).\textsuperscript{33} The OV-6 antibody was a kind gift from Dr S Sell (Albany Medical College, Albany, New York, USA).\textsuperscript{34}

**Immunohistochemistry**

For immunohistological studies, 4 µm sections were cut from frozen liver tissue. After drying, sections were fixed in acetone for 10 minutes at room temperature and washed in PBS, immediately before use. Primary antibodies were incubated for 30 minutes at room temperature. For monoclonal antibodies, a peroxidase staining method was used with secondary antibodies, preabsorbed with rat serum (Sigma, St Louis, Missouri, USA). For the rabbit polyclonal antibodies, the rabbit-Envision staining method (Dako) was used. For the goat polyclonal antibody, the secondary step consisted of swine anti-goat IgG (Dako), followed by goat peroxidase-anti-peroxidase (Dako). Secondary and tertiary antibodies were diluted in PBS containing 10% normal rat serum. All incubation steps were performed for 30 minutes at room temperature and were followed by three washes in three changes of PBS for five minutes.

For semi quantitative estimations of protein expression, a series of dilutions of the primary antibodies were used (1/10; 1/30; 1/50; 1/100; 1/200; 1/500; 1/1000) and staining intensities were determined by microscopy. When staining was at the limit of detectability and disappeared at a higher dilution, it was evaluated as “critical staining”. For each liver specimen, this critical staining was evaluated in each cell compartment separately. The dilution at which critical staining was observed is indicative for the relative up- or downregulation of protein levels in each liver cell compartment.

**RNA isolation and quantitative PCR**

Total RNA was isolated from tissue or cells using TRIzol (Invitrogen Life Technologies) followed by the SV Total RNA isolation system (Promega, Madison, Wisconsin, USA) according to the manufacturer’s instructions. Reverse transcription (RT) was performed on 5 µg of total RNA using random primers in a final volume of 75 µl (Reverse Transcription System; Promega).
cDNA levels of the various genes were measured by real time polymerase chain reaction (PCR) using the ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, California, USA). Cycle numbers at which the sample fluorescence signal increases above a fixed threshold level (CT value) correlate inversely with mRNA levels. We used 4 μl of a 20-fold diluted cDNA in each PCR reaction in a final volume of 20 μl, containing 900 nM of sense and antisense primers, 200 nM of fluorogenic probe, 5 mM MgCl₂, KCl, Tris HCl, 0.2 mM dATP, dGTP, dTTP and dUTP and 0.5 U of AmpliTaq DNA polymerase (qPCR Core Kit; Eurogentech, Seraing, Belgium). Sequences of the primers and probes used are listed in table 1. Probes were labelled by a 5′ FAM (6-carboxy-fluorescein) reporter and a 3′ TAMRA (6-carboxy-tetramethyl-rhodamine) quencher. The PCR program was 95°C for 10 minutes, followed by 40 cycles of 15 seconds at 95°C and one minute at 60°C. Each sample was analysed in duplicate. For quantification of mRNA expression, calibration curves were constructed expressing the log of the input amount as x and Cₓ as y. 18S expression levels were used as endogenous controls.

Data resulting from the experimental groups are expressed as mean (SD). Differences between the four experimental groups in the 2-AAF/PHx experiment were determined by one way ANOVA analysis, with post hoc comparison by the Student-Newman-Keuls test (SPSS software). An unpaired Student’s t test was used to compare the means of the two groups. A p value <0.05 was considered significant.

### Flow cytometric detection of functional efflux

Functional activity of the MRP transporter proteins was demonstrated essentially according to Van der Kolk and colleagues. Cells were exposed to 0.1 μM carboxyfluorescein diacetate (CFDA) (Sigma) in combination with the Mrp inhibitor MK571 (kindly provided by Dr Ford-Hutchinson, www.gutjnl.com

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Sequences of polymerase chain reaction (PCR) primers and probes used for real time detection PCR analysis</th>
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<tbody>
<tr>
<td><strong>cDNA</strong></td>
<td><strong>Primer</strong></td>
</tr>
<tr>
<td>Mdr1a</td>
<td>Sense 5′-GCA GGT TGG CTG GAC AGA TT-3′</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′-GGA GGC CAA TCC TTC CAT GGA TA-3′</td>
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<tr>
<td></td>
<td>Probe 5′-FAM-CCG CCA GAG TTC CCA GCA GCA TG-TAMRA 3′</td>
</tr>
<tr>
<td>Mdr1b</td>
<td>Sense 5′-AAA CAT GGC ACG TAA CCA AGG TT-3′</td>
</tr>
<tr>
<td></td>
<td>Antisense 5′-AAA ATG TGG CCG TGT TTA ATG ATT-3′</td>
</tr>
<tr>
<td></td>
<td>Probe 5′-FAM-CTA TAA AGG TTA TTT CAT CAA GAC GAG AAG CCT TC-TAMRA 3′</td>
</tr>
<tr>
<td>Bsep</td>
<td>Sense 5′-CCA CAC TGC CAA GGA GGA AAG ATC CA-3′</td>
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<td></td>
<td>Antisense 5′-CCA CAC ATC TAT AGA GCC AAG CAA GCA CCT GAG CC-TAMRA 3′</td>
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<td>Probe 5′-FAM-AGC CAC ATT TAT AGA GCC AAG CAA GCA GCA CTA-TAMRA 3′</td>
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<td>Mrp1</td>
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<td>Antisense 5′-GCC AGA CCA GAA GGC AAG ATA CA-3′</td>
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<td>Probe 5′-FAM-AGC CAC TTG CTA TTT GAA CCA GCA GCA CTG-TAMRA 3′</td>
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<tr>
<td></td>
<td>Antisense 5′-CCA ATC ACA CCG CTT CCA AA-3′</td>
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<tr>
<td></td>
<td>Probe 5′-FAM-CCG GCA AAT TAC CCA CTC CCG A-TAMRA 3′</td>
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Merck Sharp, Canada). Carboxyfluorescein (CF) fluorescence of 5000 cells was measured at a laser excitation wavelength of 488 nm through a 530 nm band pass filter using a FACS calibur flow cytometer (Becton Dickinson Medical Systems, Sharon, Massachusetts, USA). The efflux blocking factor of the inhibitor was expressed as median fluorescence intensity in inhibitor treated cells divided by median fluorescence intensity in untreated cells.

**RESULTS**

**Characteristics of partial hepatectomised rats**

Rats were treated with 2-AAF or placebo for seven days before undergoing PHx or sham operation. Mean body weight of the rats was 196 (13.1) g when 2-AAF or placebo pellets were placed and 242 (17.1) g at the time of surgery. There was no significant difference in body weight between rats receiving placebo pellets and those receiving 2-AAF containing pellets. On average, 7.6 (0.7) g of liver were excised during PHx. Animals undergoing 2-AFF/PHx treatment gained approximately 20 g in weight over the nine days after PHx compared with approximately 40 g for rats in the placebo/sham, placebo/PHx, and 2-AAF/sham groups. Liver weights from sham operated animals were used to calculate the per cent liver weight/body weight (4.5%). From this, the percentage of liver removed from the partial hepatectomised rats was estimated to be 71%.

**ABC transporter gene expression in total liver samples during progenitor cell activation**

Figure 1 shows hepatic expression of the ABC genes in the four experimental groups, nine days after PHx or sham operation. Expression of Mrp1 mRNA was strongly increased in both groups treated with 2-AAF: 79 (20)-fold in 2-AAF-treated rats and 120 (53)-fold in 2-AFF/PHx treated rats (relative to 1.0 (0.6) and 0.3 (0.07) in the placebo/sham and placebo/PHx groups). In contrast, hepatic mRNA levels of Mdr2 (2.0-fold), Mrp1 (2.5-fold), Mrp3 (10-fold), and Mrp4 (2.0-fold) were significantly increased only in 2-AFF/HPx treated rats. mRNA expression levels of Mdr1a, Bsep, Mrp2, Mrp6, Abca1, and Bcrp1 were comparable for all four groups.

**Hepatic progenitor cells contain high levels of Mrp1 and Mrp3**

To determine the predominance of HPCs in rats treated with 2-AAF or 2-AFF/PHx, we performed immunohistochemical stainings using the OV6 antibody. Rats treated with 2-AAF alone showed mild proliferation of HPCs in their livers (fig 2A). Massive HPC proliferation was observed in livers of 2-AFF/PHx treated rats (fig 2B).

Next, localisation of selected ABC transporters was determined by immunohistochemistry. Figure 2C shows the clear canalicular staining pattern of C219, a monoclonal antibody recognising all P-glycoproteins, in normal rat liver. C219 staining intensity was much more pronounced in livers of 2-AFF/PHx treated rats and was observed predominantly in periportal hepatocytes in both groups (shown for 2-AFF/PHx in fig 2D). Comparison of the staining patterns of OV6 (fig 2B) and C219 (fig 2D) in serial liver sections does not reveal any significant colocalisation of these two markers in HPCs. In an attempt to quantify the relative increase in Mdr1b protein levels, we performed immunohistochemical experiments with a series of dilutions of the primary antibody (C219) and determined the upper limit of dilution.
for signal detection ("critical staining" level). Using this method, the increase in Mdr1b protein level expression for 2-AAF/PHx treated rats was estimated to be over 50-fold compared with controls.

In normal rat liver, expression of Mrp1 is low. We were not able to detect a specific signal in immunohistochemical experiments using an antibody against Mrp1 in these livers.
Comparison of transporter expression in both unique ABC phenotype in that they contained high levels of Mdr2, were observed in hepatocytes. In contrast, cholangiocytes contained high levels of Mrp6, and Thy-1 positive cells were isolated from 2-AAF/CCl4 treated rats, whole liver and subjected these fractions to RT-PCR analysis. We purified hepatocytes, cholangiocytes, and HPCs from types ABC transporter gene expression in purified hepatic cell types. Relative expression levels of various ABC transporter genes in the different cell fractions were determined by real time detection reverse transcription-polymerase chain reaction, as described in materials and methods.

Mdr1b mRNA was overexpressed in the RLF φ 13 cell line.

**Mrp proteins function as active efflux pumps in RLF φ 13 cells**

To confirm that expression of Mrp1 and Mrp3 results in efficient efflux of Mrp substrates from HPCs, we performed flow cytometric assays using CFDA, which is intracellularly converted to the Mrp substrate CF. Incubation of RLF φ 13 cells with CFDA resulted in a relative fluorescence of 25.0 (2.4) units. Performing the same experiment in the presence of the Mrp inhibitor MK571 (10 and 20 µM) caused retention of CF of 139.7 (2.2) and 224.9 (20.5) relative fluorescence units, respectively. Thus MK571 inhibited efflux 5.6- and 9.1-fold. A representative fluorescence histogram is shown in fig 4.

**ABC transporter gene expression in proliferating hepatocytes**

In the experiments described above, the proliferating HPCs from the 2-AAF/PHx treated rats were compared with hepatocytes and cholangiocytes from normal liver. To determine whether the specific Mrp1, Mrp3, and Abca1 phenotype is related to the proliferative state of these cells, we also determined the ABC expression profile of proliferating hepatocytes. Therefore, rats underwent PHx. Hepatocytes were isolated 24 hours later. At this time, most hepatocytes are going through cell division, as determined by BrdU incorporation (not shown). The ABC transporter profile of these cells is shown in table 2. Hepatocytes isolated from sham operated rats were used as controls. As described previously, proliferating hepatocytes contain high levels of Mdr1b. Apart from this characteristic, no strong differences in ABC transporter profiles were observed.

**ABC transporter gene expression in purified hepatic cell types**

To quantify cell type specific ABC transporter gene expression, we purified hepatocytes, cholangiocytes, and HPCs from whole liver and subjected these fractions to RT-PCR analysis. Thy-1 positive cells were isolated from 2-AAF/PHx, treated rats, 12 days after PHx exposure. These cells were compared with cholangiocytes and hepatocytes isolated from normal rats and with the progenitor cell-like cell line RLF φ 13. Figure 3 shows that, as expected, high level expression of Mrp1, Mrp2, Mrp6, and Bcrp, and low level expression of Mdr1b, Mrp1, and Mrp3 was observed in hepatocytes. In contrast, cholangiocytes contained high levels of Mrp3 and low levels of Mdr1b, Mrd2, Bsep, Mrp2, Mrp6, and Abca1. Thy-1 positive cells had a unique ABC phenotype in that they contained high levels of both Mrp1, Mrp3, and Abca1, a combination not observed in hepatocytes or cholangiocytes. For the other ABC transporters, the expression profile was mostly comparable with that of cholangiocytes. Comparison of transporter expression in RLF φ 13 cells with that of Thy-1 positive cells showed that RLF φ 13 cells had similar expression characteristics as Thy1 positive cells. The exception in this respect was Mdr1b mRNA which was overexpressed in the RLF φ 13 cell line.

**Mrp proteins function as active efflux pumps in RLF φ 13 cells**

To confirm that expression of Mrp1 and Mrp3 results in efficient efflux of Mrp substrates from HPCs, we performed flow cytometric assays using CFDA, which is intracellularly converted to the Mrp substrate CF. Incubation of RLF φ 13 cells with CFDA resulted in a relative fluorescence of 25.0 (2.4) units. Performing the same experiment in the presence of the Mrp inhibitor MK571 (10 and 20 µM) caused retention of CF of 139.7 (2.2) and 224.9 (20.5) relative fluorescence units, respectively. Thus MK571 inhibited efflux 5.6- and 9.1-fold. A representative fluorescence histogram is shown in Fig 4.
mRNA levels by 2-AAF in an in vitro model. They demonstrated that transcription of Mdr1b is induced by nuclear factor kB. Activation of nuclear factor kB is the ultimate result of oxidative stress generated by 2-AAF. Moreover, 2-AAF has been demonstrated to increase p53 expression, p53, in turn, can induce Mdr1b expression. The strong C219 staining pattern in rats treated with 2-AAF alone can thus, at least in part, be explained by direct effects of 2-AAF on hepatocytes. After 2-AAF/PHx treatment, when there is extensive oval cell activation, Mdr1b protein expression seemed to be confined to portal hepatocytes and did not occur in the OV-6 positive cell compartment or in bile duct epithelial cells. Notably, this observation contrasts with the human situation. In liver biopsies of patients with submassive necrosis, chronic hepatitis C, or primary biliary cirrhosis, we observed greatly increased expression of MRD1 not only in the remaining hepatocytes but also in regenerating ductules that represent putative progenitor cells (Ros et al, in press). The reason for this difference is not known at present.

Strongly increased expression of Mrp1 (2.5-fold) and Mrp3 (10-fold) was seen in livers of 2-AAF/PHx treated rats. In contrast with Mdr1b, this increase was not observed in 2-AAF/sham treated rats. In normal liver, both Mrp1 and Mrp3 are expressed at low levels with Mrp3 localised only in pericentral hepatocytes and cholangiocytes. In 2-AAF/PHx treated rats, both Mrp1 and Mrp3 were predominantly detected in HPCs. To further substantiate that Mrp1 and Mrp3 are indeed markers for HPCs, we determined the expression profiles of ABC transporters in purified cell fractions enriched in hepatocytes, cholangiocytes, or HPCs (Thy-1 positive cells). In addition, we included the hepatic progenitor cell-like RLF φ 13 cell line in these studies. Compared with hepatocytes, freshly isolated Thy-1 positive cells showed high level expression of Mrp1 and Mrp3 and low expression of Mdr2, Bsep, Mrp2, and Mrp6. Thus the ABC transporter phenotype of HPCs resembles that of cholangiocytes, except for Mrp1 and Abca1, which are highly expressed in progenitor cells but not in cholangiocytes. The ABC transporter phenotype of RLF φ 13 cells resembles that of freshly isolated Thy-1 positive cells, except for Mdr1b which is highly expressed in RLF φ 13 cells only. However, high expression levels of Mdr1b are frequently observed in cultured cells and cannot be regarded as a cell type specific feature. Our functional assays using specific Mrp inhibitors confirmed that RLF φ 13 cells are indeed able to efficiently extrude Mrp specific substrates.

High expression levels of Mrp1 and Mrp3 observed in rat HPCs is in agreement with the expression pattern seen in severely diseased human liver in which we also observed high level expression of Mrp1 and Mrp3 in regenerating ductules, the site of progenitor cells (Ros et al, in press).

It has recently been shown that primitive haematopoietic stem cells highly express Bcrp, the breast cancer resistance protein. As HPCs display primitive features, we speculated that these cells may also have high Bcrp expression. However, in the 2-AAF/PHx model, Bcrp mRNA expression was not induced. Neither RLF φ 13 nor Thy-1 positive cells were particularly rich in Bcrp. In contrast with haematopoietic (side population) cells, Bcrp is not highly expressed in HPCs.

Taken together, these data demonstrate that HPCs have high expression levels of functional Mrp1 and Mrp3. The current view is that Mrp1 functions mainly as a cellular efflux pump of cysteinyl-leukotrienes and glutathione-S-conjugates and Mrp3 as a pump for glucuronides and mono- and divalent bile salts. Thus Mrp1 and Mrp3 may have a role in removing both exogenous and endogenous toxic drugs/metabolites from progenitor cells.

In conclusion, our findings show that strong induction of Mdr1b in the 2-AAF/PHx model is confined to hepatocytes while expression of Mrp1 and Mrp3 occurs mainly in HPCs. HPCs appear to be well protected by these ABC transporters which may enable them to survive in conditions associated with oxidative stress generated by 2-AAF.
with excessive metabolic stress and serve as a proliferative reservoir when the liver is severely damaged.

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