

Increased Bioavailability of the Food-Derived Carcinogen 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in MRP2-Deficient Rats

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ABSTRACT

MRP2 is an apical transporter expressed in hepatocytes and the epithelial cells of the small intestine and kidney proximal tubule. It extrudes organic anions, conjugated compounds, and some uncharged amphipaths. We studied the transport of an abundant food-derived carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in vitro, using an MRP2 transfected epithelial cell line (MDCK II) and intestinal explants from Wistar and MRP2-deficient TR⁻ rats in Ussing chambers. In the experiments with the transfected cell line, we could demonstrate more than 3-fold higher transport from basolateral to apical than vice versa, whereas the transport in the parent cell line was equal in both directions. These results were confirmed in studies using isolated pieces of small intestine from Wistar and TR⁻ rats in the Ussing chamber. Subsequent in vivo ex-

periments demonstrated that after oral administration, absorption of PhIP was 2-fold higher in the TR⁻ rat than in the Wistar rat. Consequently, PhIP tissue levels in several organs (liver, kidney, lung, and colon) were 1.7- to 4-fold higher 48 h after oral administration. MRP2 mediated transport of unchanged PhIP probably involves intracellular GSH, because GSH depletion by BSO-treatment in Wistar rats reduced intestinal secretion in the Ussing chamber to the same level as in TR⁻ rats. In accordance, BSO treatment increased oral bioavailability in intact Wistar rats. This study shows for the first time that MRP2-mediated extrusion reduces oral bioavailability of a xenobiotic and protects against an abundant food-derived carcinogen.

MDR1 P-glycoprotein and the multidrug resistance proteins (MRPs) have been characterized as plasma membrane proteins that extrude amphipathic, toxic compounds from cells and thereby confer resistance against these compounds. Of the multidrug resistance transporters, MRP2 and MDR1 are localized in the apical membrane of epithelial cells including the intestine (Thiebaut et al., 1987; Keppler and König, 1997; Mottino et al., 2000). Thus, these transporters may play a role in the defense against orally ingested drugs, toxins, and carcinogens. Although the role of the MDR1 Pgp in oral bioavailability of drugs is currently being established (Lown et al., 1997; Sparreboom et al., 1997; van Asperen et al., 1997), little attention has been paid to MRP2 in this respect and to the role of both transporters in the defense against naturally occurring carcinogens, as has been done with MRP1 (Loe et al., 1997).

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MRP2 was first identified as a hepatocellular canalicular organic anion transporter (Jansen et al., 1993). The range of molecules transported by this protein is broad (Oude Elferink et al., 1995; König et al., 1999), mainly including amphipathic anions and glucuronide-, glutathione-, and sulfate-conjugates. The gene was first cloned in the rat (Buchler et al., 1996; Paulusma et al., 1996); mutations in the human isoform were found to cause Dubin-Johnson syndrome (Paulusma et al., 1997; Toh et al., 1999).

MRP2 confers multidrug resistance against such compounds as methotrexate (Hooijberg et al., 1999), vincristine, or cisplatin (Kawabe et al., 1999) and is induced by cisplatin, 2-acetylaminofluorene (Kauffmann et al., 1997), rifampicin, and tamoxifen (Kauffmann et al., 1998). The transport of uncharged compounds such as vincristine by MRP1 can be stimulated by GSH; it was suggested that this involves cotransport (Loe et al., 1998). Because MRP2 is also expressed in the rat small intestine (Gotoh et al., 2000; Mottino et al., 2000), we hypothesized that it actively extrudes different amphipathic substances from the intestinal mucosa and

ABBREVIATIONS: MDR, multidrug resistance transporter; MRP, multidrug resistance protein; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; HPLC, high performance liquid chromatography; BSO, buthionine-sulfoximine; GSH, glutathione.

thereby protects against food-derived xenobiotics, including carcinogens and drugs.

In the present study we tested this hypothesis by studying the role of rat MRP2 in the bioavailability of a food-derived carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). PhIP is a heterocyclic amine belonging to the family of the aminoimidazoazaarenes. It was first described by Felton et al. (1986). PhIP is the most abundant heterocyclic amine formed during cooking, frying, and grilling (Skog et al., 1998) and was shown to induce colon and breast carcinomas in rats (Ito et al., 1991). The metabolism of the compound in different species is well defined (Alexander et al., 1995; Kadlubar et al., 1995; Lang et al., 1999).

Much less is known about the transport of PhIP. Transport of the parent compound is necessary for absorption, disposition, and for uptake in the cells of organs. In a very recent publication, it was shown that there might be active secretion of PhIP from Caco-2 cells (Walle and Walle, 1999); from the data presented in this work, it was suggested that MDR1 and/or MRP2 can play a role in the defense against this type of compounds.

Polymorphisms of phase I and phase II metabolizing enzymes play a role in type and extent of metabolism (Kadlubar et al., 1995; King et al., 1997; Lang et al., 1999). It already has been shown that polymorphisms in the *MDR1* gene influence the bioavailability of digoxin (Hoffmeyer et al., 2000). It is obvious that polymorphisms in other transporter genes might also influence the absorption and distribution of xenobiotics in the body. In this study, we directly demonstrate MRP2-mediated transport of PhIP in MRP2-transduced epithelial cells, in small intestinal explants and in vivo.

Materials and Methods

Chemicals. [^{14}C]PhIP (specific activity, 10 mCi/mM) was obtained from Toronto Research Chemicals, Inc (North York, Ontario, Canada). Unlabeled PhIP was bought from ICN (Costa Mesa, CA). All batches were found to be pure >98% by HPLC. L-Buthionine-[*S,R*]-sulfoximine (BSO) was bought from Sigma (St. Louis, MO). All solvents used for HPLC separation (HPLC- or analysis-grade) were from Merck (Darmstadt, Germany).

Animals. Female Wistar rats were obtained from Harlan-CPB (Zeist, The Netherlands). Age- and weight-matched female TR⁻ rats were from our own breeding stock, which has been characterized previously (Jansen et al., 1985). Rats were housed in cages with a 12-h light/dark cycle and given access to food and water ad libitum. Rats were used for experiments at age 12 to 16 weeks (200–250 g). All animal experiments in this work have been carried out in accordance with the Declaration of Helsinki.

Cell Line Experiments. Transduction of the polarized canine kidney cell line MDCK II with human MRP2 and characterization of the respective product has been described previously (Evers et al., 1998). MDCK II cells were also transduced with human MDR1, using a method described previously for LLC-PK1 cells (Schinkel et al., 1995). Expression of the protein was verified using Western analysis immediately before experiments. All cell lines were cultured in 75 cm² flasks with Dulbecco's modified Eagle's Medium (DMEM; Life Technologies, Breda, The Netherlands), supplemented with 9% fetal calf serum (BioWhittaker, Verviers, Belgium), 2 mM glutamine, and 100 U/ml penicillin/streptomycin (both from Life Technologies, Breda, The Netherlands) in humidified atmosphere (10% CO₂). After trypsinization, cells were seeded in a density of 40,000 cells per milliliter of medium in filter inserts (4.7 cm², 3.0 μm pore size; Transwell, Costar-Corning, NY) and cultured 7 to 10 days, with a change of medium every other day. Filters were used for experiments

only when macroscopically tight. Filters also were checked for tightness after the experiment using [^{14}C]inulin.

For the experiments, filters were preincubated for 20 min with 2 ml of DMEM without phenol red (4.5 g/l glucose, 2 mM glutamine, 25 mM HEPES) in both compartments, then PhIP (50 nM) was added to the donor compartment (basolateral or apical). Samples (50 μl) were taken at time points 60 and 120 min without replacing the medium. Use of DMEM without serum prevented protein binding of PhIP but did result in higher unspecific diffusion after 120 min.

Ussing Chamber Experiments. Rats were anesthetized (57 mg/kg ketamine, 5.7 mg/kg xylazine, intramuscular injection). The abdomen was opened via midline incision and the jejunum was identified and ligated. Incisions next to the ligation allowed rinsing of the lumen with ice-cold saline solution. After ligating the blood supply, the jejunal segment was removed and immediately placed in ice-cold DMEM without phenol red. The muscular layer was rapidly removed by stripping, cut pieces of jejunum were put on polycarbonate filters (Schleicher & Schuell, Dassel, Germany) and mounted in Ussing chambers (0.5-cm² surface, 10-ml volume of both compartments). After 5-min equilibration with carbogenated DMEM without phenol red at 37°C, PhIP (10 μM) was added to the donor compartment (serosal or mucosal). Samples (500 μl) were taken at 30, 60, and 90 min and 500 μl of medium was replaced. Transepithelial potential difference was continuously monitored with AgCl electrodes and voltage deflections induced by 10 μA bipolar current pulses through platinum wires. Increasing loss of integrity of the intestine beyond 90 min made longer experiments impossible.

Bioavailability Studies (4 Rats Each Group). 2 μCi of [^{14}C]PhIP was given via gastric gavage in olive oil (500 μl). Blood samples (500 μl) were obtained 0.5, 1, 2, 4, 24, and 48 h after dosing by heart puncture (under light anesthesia with isoflurane). Feces and urine were collected in 24-h fractions.

In another experimental setting, [^{14}C]PhIP (dissolved in 500 μl of olive oil) was administered by intraduodenal injection after anesthesia (57 mg/kg ketamine, 5.7 mg/kg xylazine, intramuscular injection) and opening of the abdomen. Blood samples were obtained from the portal vein at time points 5, 15, 30, 60, and 120 min after injection.

BSO Pretreatment. To inhibit glutathione (GSH) synthesis, we treated Wistar rats with 4 mM BSO per kilogram 3 h and 1.5 h before beginning of the experiments (i.p. injection). In the bioavailability studies over 48 h, an additional 4 mM BSO per kilogram was given each 12 h after the first dosing. Cells were incubated with 100 μM BSO in culture medium 24 h immediately before the experiments were carried out. Efficiency of BSO-pretreatment was controlled by determining GSH levels [according to the method of Tietze (1969)] from gut tissue pieces or denatured cells.

Organs. After sample collection in each in vivo experiment animals were sacrificed by bleeding from the aorta. Liver, whole intestine (with feces), kidney, pancreas, heart, and lungs were excised. Feces were separated from the intestine and all organs were washed before storage. All material was stored at -80°C .

Sample Treatment. Directly after sampling, aliquots of cell medium samples were injected on the HPLC for quantification of PhIP. Weighed portions of the organs from at least two different sites were homogenized with the same volume (w/v) of ethanol then centrifuged, and the pellet was washed once more with ethanol. After another centrifugation step, dry pellets (as well as samples of whole blood) were completely solubilized in Soluene 350 (Packard, Meriden, CT) (1 ml per 100 mg of tissue or 100 μl of blood) and decolorized with 0.1 ml of H₂O₂. Feces were homogenized with 50% methanol in water (1:5, w/v); the homogenate was counted after adding scintillation fluid.

HPLC. Analysis and PhIP quantification was done on a system with Gynkotek-Pumps (Germering, Germany), connected to a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA) and an Inertsil 5 ODS 3 column (Chrompack, Bergen op Zoom, The Netherlands). Injection volume was 20 μl . We used an UV-Detector Spectroflow 757 (Kratos, Ramsey, NJ) at 315 nm, a fluorescence detector (FP-920;

Jasco, Tokyo, Japan) with excitation wavelength of 315 nm and emission wavelength of 370 nm. The mobile phase (0.4 ml/min) consisted of 10% methanol in 0.1% diethylamine, pH 6.0 (A), and 90% methanol in 0.1% diethylamine, pH 6.0 (B). The gradient was adjusted from 10% B to 75% B in 20 min. In this system, PhIP eluted as a single peak with a retention time of approximately 17 min.

Statistical Analysis. Data were compared using one-sided *t* test. $P < 0.05$ was considered to express significance.

Results

Cell Line Experiments. Nontransduced MDCK II cells show equal rates of transport of PhIP in the apical as well as in the basolateral direction (11.56 ± 0.74 nM versus 12.74 ± 1.23 nM in 2 h, respectively). In the MDCK II cells transduced with human *MRP2*, transport from the apical to the basolateral compartment was lower than that in the parent cells (9.17 ± 2.1 nM in 2 h, $p = 0.02$). Higher transport was observed from basolateral to apical in these cells (26.05 ± 2.2 nM in 2 h, $p < 0.001$, Fig. 1). This effect of *MRP2* transduction was completely reversed by pretreatment of the cells with BSO before the experiment (11.55 ± 0.83 nM in 2 h in basolateral direction, $p = 0.52$ compared with untreated transduced cells; 10.8 ± 1.2 nM in 2 h in apical direction, $p < 0.001$ compared with untreated transduced cells; no significance compared with parent cells) (Figs. 1 and 2).

Transport of PhIP in *MDR1* transduced cells was equal in both directions ($p = 0.45$). Compared with the parent cells, transport in the apical direction was slightly but significantly higher (13.2 ± 1.2 nM in 2 h, $p = 0.034$), whereas transport in the basolateral direction was identical.

These experiments were performed at a PhIP concentration of 50 nM in the donor compartment. At higher concentrations in the donor compartment, the differences between transport rates in the *MRP2* transduced cells became smaller; at 10 μ M, we found equal transport rates (data not shown). This demonstrated that transport was saturable. HPLC analysis of the samples in both compartments demonstrated that there was no detectable metabolism of PhIP in the untransduced as well as in the transduced cells.

Ussing Chamber Experiments. Explants from Wistar rat small intestine were mounted in Ussing chambers. Ad-

ministration of PhIP (10 μ M) to the serosal or mucosal compartment demonstrated a higher basal to apical transport than vice versa. In 90 min, the concentration on the mucosal side reached 51.2 ± 12.8 nM in the Wistar rat, but only 29 ± 5.9 nM in the TR⁻ rat ($p = 0.003$, Fig. 3A). Serosal transport in the TR⁻ rat (19.5 ± 6.2 nM) was still lower than mucosal transport ($p = 0.01$), but serosal and mucosal transport in the TR⁻ rat were not significantly different from serosal transport in the Wistar rat.

With BSO pretreatment, the mucosal secretion was reduced in the Wistar rat to the extent of the TR⁻ rat (26.6 ± 4.6 nM, $p = 0.002$ compared with untreated Wistar rat, Fig. 3B). Surprisingly, the transport to serosal was significantly reduced, too (15.8 ± 3.5 nM, $p = 0.005$ compared with serosal transport in untreated Wistar rats, $p = 0.003$ compared with mucosal transport in BSO-pretreated Wistar rats). We could not detect any metabolites of PhIP by HPLC.

Oral Bioavailability and Disposition over 48 h. Because the previous experiments suggested that the parent compound could be transported by *MRP2* from the gut mucosa into the lumen, we investigated whether *MRP2* also influences the oral bioavailability of PhIP. We administered PhIP (dissolved in olive oil) by gastric gavage. Blood samples were collected on various time points over a subsequent period of 48 h (see under *Materials and Methods*). Thirty minutes after ingestion, Wistar rats had $0.79\% \pm 0.2$ of the administered dose in their blood (assuming a blood volume of 4% of the body weight), whereas TR⁻ rats had a blood level that was 2-fold higher, $1.66\% \pm 0.38$ (Fig. 4A, $p = 0.006$). This difference was sustained during the first 4 h, but 24 and 48 h after administration, the two strains had comparably low levels. In TR⁻ rats, there was a second peak at 2 h, which seemed to be absent in the Wistar rats. The area under the curve in the TR⁻ rats was more than doubled compared with the Wistar (4820 and 2240, respectively over 48 h, mean values).

To exclude the effect of altered hepatic handling on blood levels of PhIP, we obtained blood samples directly from the portal vein after intraduodenal administration of PhIP dissolved in olive oil (Fig. 4B). Blood samples obtained in this

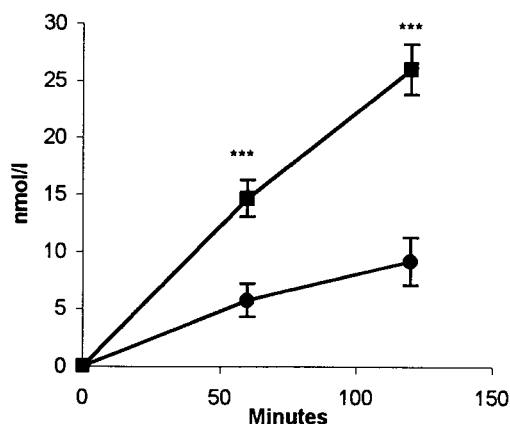


Fig. 1. Transport of PhIP in *MRP2* transduced MDCK II cells from basolateral to apical (■) and apical to basolateral (●). PhIP was added to either the basolateral or the apical compartment at a concentration of 50 nM. PhIP concentration in the acceptor compartment at indicated time points was determined by HPLC. Significance level compared with opposite direction: *** $p < 0.001$. Data represent mean \pm S.D. of four experiments.

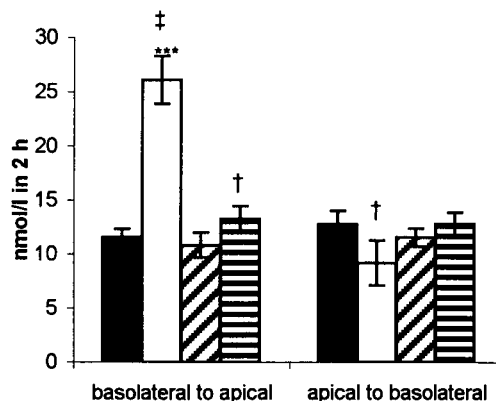


Fig. 2. Transport rate of PhIP over 2 h in untransduced MDCK II cells (■), *MRP2* transduced cells (▨), BSO-pretreated *MRP2* transduced cells (▤), and *MDR1* transduced cells (▩) in apical direction (left) and basolateral direction (right). Significance level, compared with the respective opposite direction of the same cell line, is *** $p < 0.001$. Significance levels, compared with the respective direction of the parent cell line (untransduced), are: † $p < 0.05$; ‡ $p < 0.001$. Data are given as means \pm S.D. ($n = 4$ for all groups).

experiment showed generally 3- to 4-fold higher levels of radioactivity than those obtained by heart puncture. Levels in the TR⁻ rat were 1.5- to 2-fold higher than that in the Wistar rat, resulting in a similar difference in area under the curve (1464 in the Wistar versus 2344 in the TR⁻ rat over 2 h, mean values).

We also determined tissue binding of radioactivity in the organs at 48 h after oral PhIP dosing (Fig. 5). We found significant differences in tissue binding of radioactivity in liver (637 ± 215 and 194 ± 70 dpm/100 mg of tissue in the TR⁻ and Wistar rats, respectively; $p = 0.01$), lung (185 ± 56 and 100 ± 9 dpm/100 mg of tissue in the TR⁻ and Wistar rats, respectively; $p = 0.03$), kidney (766 ± 408 in the TR⁻ and 212 ± 15 dpm/100 mg of tissue in the Wistar rats; $p = 0.03$), and colon (276 ± 152 in the TR⁻, 89 ± 36 dpm/100 mg of tissue in the Wistar rats, $p = 0.04$).

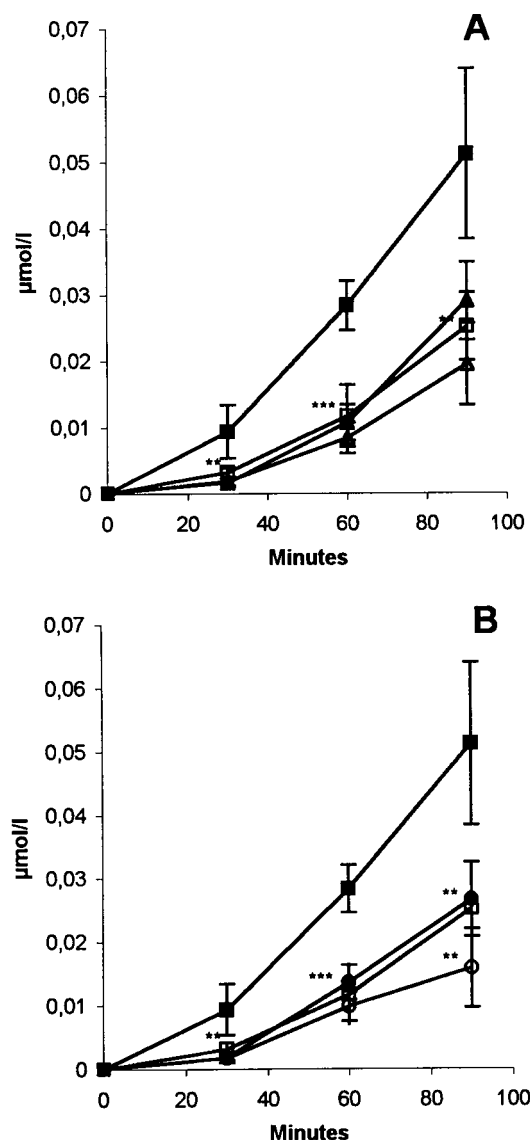


Fig. 3. Transport rate of PhIP over 90 min in isolated jejunum from (A) Wistar (■) and TR⁻ rats (▲) and (B) untreated Wistar (■) and BSO-pretreated Wistar rats (●) in both directions: serosal to mucosal (solid symbols) and mucosal to serosal (open symbols). PhIP was added at a concentration of 10 μM, samples were taken at indicated time points and analyzed by HPLC. Significance levels, compared with untreated Wistar rats, are ** $p < 0.01$; *** $p < 0.001$. Data are given as means \pm S.D. ($n = 6$ in all groups).

The total body excretion over 48 h was nearly complete in both the Wistar and the TR⁻ rat, but delayed in the latter. The TR⁻ rat excreted about 60% of the dose over 48 h in the urine, the Wistar only 25% (data not shown).

We also tested oral bioavailability in an experiment over 48 h with BSO-pretreated Wistar rats. The GSH levels from liver and small intestine from this experiment with BSO are given in Table 1. BSO-treated Wistar rats exhibited a higher blood level of PhIP 30 min after ingestion ($1.1\% \pm 0.1$ compared with $0.79\% \pm 0.2$ in the untreated Wistar, $p = 0.03$; Fig. 4C).

The organs of the BSO-treated Wistar rats were collected 48 h after oral administration of PhIP and tissue binding was determined. Almost all organs in these animals showed similar accumulation as in the TR⁻ rat, with significant elevations (compared with untreated Wistar) in liver, lungs, kidney, and colon (Fig. 5). The BSO-treated rats had significantly higher accumulation of radioactivity in the heart and the small intestine. This was not observed in TR⁻ rats.

Discussion

This study is the first to show that both human and rat MRP2 are involved in oral bioavailability of a xenobiotic. We present clear evidence that the heterocyclic amine PhIP, an abundant food-derived carcinogen, is transported by MRP2. This confirms and extends recent data, showing that inhibitors of MRP2 impair PhIP transport in Caco-2 cells (Walle and Walle, 1999). PhIP transport from the basolateral to the apical compartment in polarized epithelial cells transduced with the human MRP2 is almost 3-fold higher than vice versa. Corresponding to that result, we found that transport from serosal to mucosal approximately doubled in the isolated intestine from normal Wistar rats compared with MRP2-deficient TR⁻ rats. The difference in concentrations needed in the donor compartments in the two in vitro models (50 nM in MDCK II cells and 10 μM in intestinal explants) reflects the technical and anatomical differences of the used models: whereas the barrier in the cell line experiments is only a monolayer, in the Ussing chamber, the compound has to cross the mucus, epithelial, and submucosal layers. In addition, experiments with the Ussing chamber could not last longer than 90 min because of subsequent deterioration of the tissue. Thus, in the Ussing chamber, a higher PhIP concentration had to be added to the system to reach significant transport within the experimental period. It is possible that other transporters play a role as well in intestinal PhIP secretion, given the fact that the serosal to mucosal transport in the TR⁻ rat is still significantly higher than vice versa. Quantitatively, however, the contribution of MRP2 seems to

TABLE 1

Intracellular GSH content of liver and small intestine after the bioavailability study over 48 h.

Data are given in micromoles per gram of tissue (percentage of untreated Wistar rats in brackets) and represent means \pm SD of four independent experiments

Tissue	Control	BSO Treatment
Wistar liver	6.64 \pm 0.58 (100 \pm 8.7)	1.63 \pm 0.30 (24.5 \pm 5.0)
Wistar intestine	1.8 \pm 1.1 (100 \pm 15.35)	0.10 \pm 0.02 (5.6 \pm 1.1)
TR ⁻ liver	10.4 \pm 0.3 ^a (157 \pm 4.5)	N.D.
TR ⁻ intestine	3.1 \pm 0.3 (173.2 \pm 17.3)	N.D.

^a Paulusma et al., 1999.

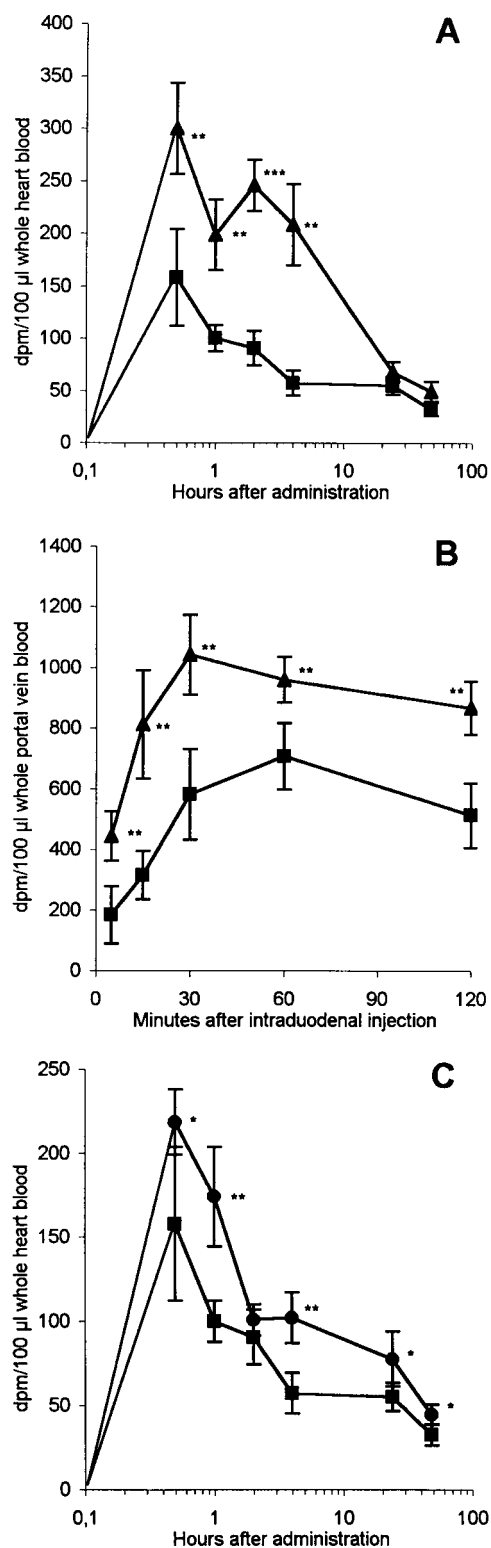


Fig. 4. Blood content of radioactivity after luminal administration of [^{14}C]PhIP in the gut. A, levels in peripheral blood (heart puncture) between 0.5 and 48 h after administration of [^{14}C]PhIP by gastric gavage in Wistar (■) and TR⁻ (▲) rats. B, levels in portal vein blood between 5 and 120 min after intraduodenal injection of [^{14}C]PhIP in Wistar (■) and TR⁻ (▲) rats. C, levels in peripheral blood (heart puncture) between 0.5 and 48 h after administration of [^{14}C]PhIP by gastric gavage in Wistar (■) and BSO-pretreated Wistar rats (●). Significance levels (* p < 0.05; ** p < 0.01; *** p < 0.001) of TR⁻ and BSO-pretreated rats are compared with untreated Wistar rat. Data are given as means \pm S.D. (n = 4 in all groups).

be most important. It is questionable whether MDR1 contributes significantly to transport of PhIP as suggested by Walle and Walle (1999). We found slightly but significantly higher transport of PhIP in the apical direction in MDR1 transduced cells compared with the untransduced cells, but compared with the basolateral direction of the transduced cells, the difference was not significant. This indicates that MDR1 plays a minor role, if any, in PhIP transport. It is an interesting finding that MRP2 contributes much more than MDR1, because PhIP is an uncharged amphipath.

To test the role of MRP 2 in genuine oral bioavailability of PhIP, we administered PhIP by gastric gavage to Wistar and TR⁻ rats and determined blood levels up to 48 h after administration. Blood levels of radioactivity were about twice as high in TR⁻ rats than in Wistar rats. There are two possible explanations for that effect: Firstly, higher absorption from the gut of the TR⁻ rat and secondly, reduced excretion by liver or kidney via bile or urine, respectively. To distinguish between these two possibilities we drew blood samples directly from the portal vein after intraduodenal administration of PhIP; the increased PhIP levels in portal blood supported the first hypothesis. The 3- to 4-fold higher portal blood levels in both animal groups (compared with the heart blood) demonstrated the importance of metabolism of PhIP in the liver; the difference in portal blood levels between Wistar and TR⁻ confirmed a higher initial PhIP absorption in the TR⁻ rats of ~2- to 3-fold and higher overall absorption of 1.5- to 2-fold, as can be calculated from the area under the curve.

Overall, the blood levels in the portal vein confirmed the in vitro results qualitatively and quantitatively. Thus, this physiological setting demonstrated an important role of Mrp2 in the direct elimination of PhIP from the gut mucosa, thereby reducing the oral bioavailability. Given the results from transduced cells and the fact that MRP2 is also expressed in human small intestine and is inducible there (Fromm et al., 2000), it is likely that this mechanism also plays a role in man, depending on the individual expression level.

HPLC analysis of samples from the cell line experiments and the Ussing chamber studies showed no metabolism in MDCK II cells or the rat intestine, indicating that the dem-

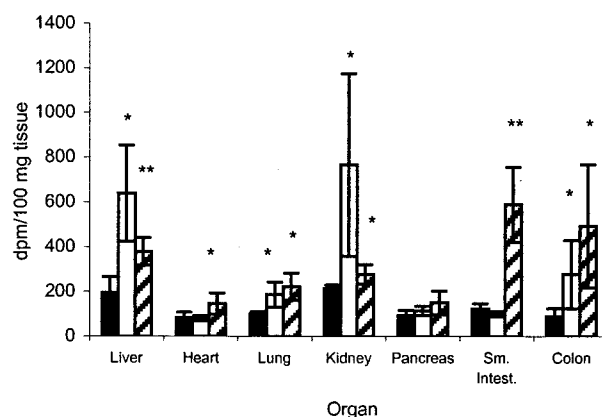


Fig. 5. Radioactivity content after ethanol extraction in several organs of Wistar (■), TR⁻ (□), and BSO-pretreated Wistar rats (▨) 48 h after luminal administration of [^{14}C]PhIP in the gut. Significance levels (* p < 0.05; ** p < 0.01) of TR⁻ and BSO-pretreated rats are those compared with untreated Wistar rat. Data are given as means \pm S.D. (n = 4 in all groups).

onstrated effect on bioavailability is caused by MRP2-mediated transport of the parent compound. However, PhIP that reaches the liver is metabolized extensively (Alexander et al., 1995).

We speculate that the transport of PhIP is taking place in cotransport with GSH, because PhIP itself is uncharged. We have shown previously that incubation of MRP2 transduced cells with 100 μ M BSO for 24 h before the experiment results in GSH depletion to 17% of controls and that this treatment did not affect ATP levels or cellular integrity (Paulusma et al., 1999). Transport in such pretreated cells, as well as in small intestine from BSO-pretreated animals in Ussing chambers, was reduced significantly. Blood levels of radioactivity were significantly higher in the BSO-pretreated animals than in normal Wistar rats. Although GSH depletion by BSO is an established method (Drew and Miners, 1984; Minchinton et al., 1984; Paulusma et al., 1999), we cannot totally exclude the possibility that other mechanisms are at play as well. In all of our experimental systems, however, BSO causes almost the same results as the absence of MRP2, which suggests that GSH plays a role in MRP2-mediated transport of PhIP.

If this is true, the role of GSH in defense against PhIP is complex. In the past it was shown that glutathione depletion caused a 5-fold increase of DNA adducts in the liver (Kaderlik et al., 1994a) or a 15-fold increase in hepatocytes (Kaderlik et al., 1994b), respectively, in the latter case without changing the metabolism profile. It was also shown that a concentration of 40 mM GSH in vitro inhibited DNA-binding by *N*-acetoxy-PhIP (Kaderlik et al., 1994b). Our data suggest that, in addition to reduction of DNA adduct formation, GSH may play a role in direct extrusion of the carcinogen. It is likely, therefore, that there is addition of several effects in GSH action, which may explain discordant results between BSO-pretreated Wistar and TR⁻ rats in the bioavailability studies.

We show here that the absence of MRP2 leads to altered GSH homeostasis in the small intestine of the TR⁻ rat (Table 1) as is the case in the liver (Paulusma et al., 1999). Thus, in this animal, one effect could counteract the other. On one hand, absence of MRP2 will lead to increased tissue levels of PhIP and reactive metabolites, as shown here. On the other hand, the increased tissue levels of GSH could reduce adduct formation. This may explain the relatively low tissue binding in the small intestine of the TR⁻ rats and the very high tissue levels of radioactivity in small intestine of the BSO-pretreated Wistar rats. However, BSO-pretreatment produced lower levels of radioactivity in the liver of Wistar rats than MRP2-deficiency in the liver of TR⁻ rats. Our data cannot resolve this difference, but it may be possible that this difference in susceptibility to tissue radioactivity binding between intestine and liver is a result of different degrees of GSH depletion (Table 1).

The importance of the described defense mechanism in MRP2 is highly emphasized by the differences in tissue levels 48 h after administration. In our study, MRP2 deficiency led to significantly higher, ethanol-insoluble radioactivity contents in several organs after 48 h. After 48 h, excretion and metabolism of the administered compound is almost completed (over 97% recovery of the dose in urine and feces). Therefore, ethanol-insoluble tissue contents of radioactivity at that time indicate covalent binding of reactive metabolites

to the tissue (Watkins et al., 1991). This may suggest, but does not yet prove, that there will be higher carcinogenicity. Because DNA-adduct formation cannot simply be used to estimate carcinogenicity (Ochiai et al., 1996), a long-term feeding study is, in our opinion, the only way to discover the relevance of this difference for carcinogenicity of PhIP.

Our results shed new light on the role of MRP2. Obviously, MRP2 is, in two respects, not only a canalicular multispecific organic anion transporter, as was initially assumed. First, in addition to its role in biliary transport, it plays an important role in the first-line defense in the intestine. Second, it transports a wide variety of neutral or amphipathic compounds, not only organic anions. As such, it probably plays a role in the oral bioavailability of many drugs, toxins, and carcinogens.

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