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In vitro and in vivo hepatic transport of the magnetic resonance imaging contrast agent B22956/1: role of MRP proteins

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Abstract

The molecular mechanisms of the hepatic transport of B22956/1, a new gadolinium complex from the class of intravascular contrast agents for MRI, which undergoes extensive biliary elimination, were studied. Biliary and urinary elimination of B22956/1 were measured in normal and in mutant MRP2 lacking rats (TR^-); cellular trafficking of the compound was assessed in wild and *MRP1* or *MRP2* transfected MDCKII cells. Eight hours after IV injection of B22956/1, $90 \pm 8\%$ of the dose was recovered in the bile of normal rats. By contrast, in TR^- rats, the biliary excretion was significantly lower ($14 \pm 3\%$) while $55 \pm 9\%$ of the compound was found in urine. In vitro, the cellular accumulation of B22956/1 was significantly lower in both *MRP1* and *MRP2* transfected cells as compared to wild type MDCKII cells, and the cellular efflux was prevented by the MRP inhibitor MK571, indicating the involvement of both MRP2 and MRP1 in the transport of B22956/1. Due to the distinct cellular localization of the proteins, MRP2 accounts for the biliary and urinary excretion of the compound, while MRP1 prevents cellular accumulation of the MRI agent. B22956/1 may be useful in clinical conditions where a defective biliary transport is present.

Keywords: Magnetic resonance imaging agents; MRI; MRP1; MRP2; Liver; ABC proteins hepatic transport; Organic anions; Drug targeting

B22956/1 is a new low molecular weight gadolinium chelate which belongs to the class of intravascular (blood pool) contrast agents for MRI. High vascular containment and persistence of enhancement are the most defining properties of a blood pool agent, since it restricts distribution to the vascular space thus leading to high plasma concentrations and specific enhancement of blood vessels. Vascular containment of B22956/1 depends on its ability to bind to plasma proteins. In vitro studies showed that 94% of B22956/1 is bound to human serum albumin [1,2]. The B22956 ion, which provides the contrasting effect, is known to enter hepatocytes, from where it is excreted into the bile to an extent depending on the animal species. Since preliminary pharmacokinetic studies in a healthy volunteer have shown that the biliary excretion is the primary route of elimination, the study of the mechanisms involved in the transport of the compound by the hepatocytes is crucial for the definition of the pharmacokinetic properties of B22956/1. While basolateral uptake in hepatocytes is still not completely defined for the different varieties of these organic anions, it was previously demonstrated that the biliary excretion of "lipophilic MRI contrast agents" having DTPA-derived structures is mediated by ABC proteins, belonging to the MRP subfamily, and in particular by MRP2 [3–5]. MRP1, another ABC protein located at the basolateral level of the cells, could also be involved in the efflux from hepatocytes.

A Wistar-derived mutant rat strain (TR^-), genetically lacking MRP2 expression [6], has been used to characterize the biliary excretion of several organic anions [7] including contrast agents [4,5]. The evaluation of the excretory mechanism(s) of B22956/1 in normal and in hyperbilirubinemic TR^- rats should supply information on the transport mechanisms of this compound

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across the bile canalicular membrane and provide hints on the biliary transport in an animal model mimicking the genetic defects in human Dubin–Johnson syndrome [8].

In this study, we report on the biliary and urinary excretion of the new contrast agent B22956/1 in normal and in TR^- rat models. To investigate the possible involvement on MRP transport proteins and to extrapolate data obtained in rats to human subjects, data were also collected in MDCKII cell lines transfected with human *MRP1* and *MRP2*.

Materials and methods

Materials. B22956/1 is the trisodium salt of a derivative of gadopentetate bearing on the methylene group of the centrally located acetate group in *S*-configuration, a propionic acid linker to the amino group of the 3β-amino-analogue of deoxycholic acid [9]. Its chemical name according to CAS is trisodium[(3β, 5β, 12α)-3-[[(4*S*)-4-[bis[2-[bis[(carboxy-κ*O*)methyl]amino-κ*N*]ethyl]amino-κ*N*]-4-(carboxy-κ*O*)-1-oxybutyl]amino]-12-hydroxycholan-24-oato (6-)]gadolinate (3-) and the proposed international nonproprietary name is gadocoletic acid trisodium salt. B22956 is the code name for the gadolinium complex with the ligand B22950. For studies on animals B22956/1 was pharmaceutically formulated as sterile and apyrogenic 0.25 M solution for injection, without additional excipients. In aqueous solution B22956/1 dissociates into sodium ions and the contrastographically active component B22956 ion. The anaesthetics Ketamine (Ketavet 100) and Xilazine (Rompun) were purchased from Farmaceutici Gellini, Aprilia, Italy and from Bayer AG, Leverkusen, Germany, respectively.

$^{147}PmCl_3$ in HC1 was purchased from ICN (Irvine, CA, USA), and was used to prepare stock solutions of ^{147}Pm–B22950 with a specific activity of 89.6 mCi $mmol^{-1}$. Labelled solutions of Gd–B22950 were obtained by adding the appropriate amount of the solution of ^{147}Pm complex to the solution of the corresponding Gd–B22950. ^{147}Pm and Gd complexes were prepared as sodium salts. The purity of each compound was greater than 99%, as assessed by HPLC. The labelled compound (^{147}Pm) used for in vitro studies will be referred to as Gd labelled B22950 throughout the text.

Analytical assays. Pharmacokinetic and biodistribution studies on B22956/1 were performed by using a spectroscopic method for total gadolinium, namely inductively coupled plasma-atomic emission spectrometry (ICP-AES) [10]. The ICP-AES assays were carried out on a Jobin–Yvon Mod 24 spectrometer operating with the following instrumental parameters: (1) sample flow: 1 mL min^{-1}; (2) plasma flame: 6000–10,000 °C; (3) wavelength: 342.247 nm; and (4) argon flow: nebulizer 0.3 L min^{-1}, transport gas 0.2 L min^{-1}, and cooling gas 12 L min^{-1}. Different preparations for each biological matrix were adopted. Liver was dried by means of a freeze drying process (indicatively with a shelf temperature ranging from −40 to 30 °C and a chamber pressure up to 0.1 mbar), and then homogenized by grinding in a mortar. Liver solutions were prepared by suspending 200 mg of tissue powder in 1.5 mL of nitric acid (65% v/v). The same procedure (1.5 mL of nitric acid, 65% v/v) was used for the preparation of kidney, plasma (1.0 mL), urine (0.3 mL), and bile (0.3 mL) samples.

The destruction of the organic matrix was achieved by subjecting the samples to a wet ashing process with a microwave oven system (MDS-2000 CEM). The dried residues were dissolved with 3.0 mL of HCL 5% (v/v) and then analysed by ICP-AES. Linearity was evaluated in standards containing increasing concentrations of Gd ranging from 0 to 20 mg (Gd) L^{-1} dissolved in HCl 5%, v/v. The total content of gadolinium calculated by the instrumental calibration line was expressed as μg (Gd) mL^{-1}.

"*In vivo*" *experimental procedures.* The urinary and biliary excretion of gadolinium were studied in 5 Crl:CD (SD)BR male rats (mean body weight: 280 g) and 3 Wistar TR^- male rats (mean body weight: 235 g). Animals were fasted for 16–18 h prior to the experiments but had free access to water. Animals were anaesthetized by intramuscular injection of Ketamine (Ketavet), 0.7 mL kg^{-1} plus Xilazine (Rompun); when necessary, further aliquots of anaesthetic (0.35 mL kg^{-1}) were injected to maintain anaesthesia throughout the experiment. The common bile duct was cannulated with an Intramedic PE 50 polyethylene catheter (Becton Dickinson, Parsipanny, NJ, USA), and a second catheter was inserted into the urinary bladder. Two ligatures were made, the first to fasten the catheter to the wall of the bladder and the second to reduce the volume between the mouth of the ureter and the catheter. The abdominal cavity was sutured, and the animal was placed on a surgical table warmed to 37 °C to maintain the body temperature within physiological limits. A femoral vein was exposed and B22956/1 was injected at the rate of 6 mL min^{-1}. Injection volumes were calculated on the basis of the dose and the animal weight.

Bile was collected for 30 min before the administration of the contrast agent and bile samples were collected 120, 240, and 480 min thereafter. Similar collection times were used for urine. At the end of the experiment, i.e., 480 min after administration, the animals received 1 mL kg^{-1} sodium heparin (Liquemin 5000 UI mL^{-1}, Hoffmann-La Roche AG, Grenzach, Wyhlen, Germany) by intracarotid injection and were exsanguinated through the carotid artery. Blood was collected into test tubes containing sodium heparin solution (5000 UI mL^{-1}) at the ratio of 1:50 (v/v). Plasma samples were obtained after centrifugation of blood samples (15 min at 1800*g*). After exsanguination, liver and kidneys were excised and weighed. All biological samples were stored at +4 °C until prepared for ICP-AES analyses (less than 1 month).

Cell culture. Wild type and *MRP1* or *MRP2* transfected MDCKII clones (108 and 227, respectively, a gift of Dr R. Oude Elferink, Academic Medical Center, Amsterdam, The Netherlands) [11,12] were cultured in DMEM (Life Technologies Italy, Milan, Italy) under standard conditions with 10% (v/v) FCS and 1% antibiotics (10,000 U mL^{-1} penicillin and 10 mg mL^{-1} streptomycin). The cells were routinely maintained in 75-cm^2 Falcon flasks for 3 days, then harvested by exposure to a solution of 0.25% trypsin and 0.02% EDTA, and transferred onto 35-mm petri dishes at a density of 2.5×10^6 cell/dish. After an additional day in culture, cells were used for the uptake experiments.

Uptake studies in transfected cells. All the transport experiments were performed at room temperature (22–24 °C). Cells were washed once with 1.5 mL of fresh culture medium (DMEM) and measurement of B22950 uptake was started by the addition of 1.5 mL uptake medium consisting of DMEM added of 100 μM Gd labelled B22950. After incubation for the desired length of time (from 0 to 30 min), uptake was stopped by removing the uptake medium and by washing the cells three times with 2 mL of ice-cold PBS. The cells were then solubilized into 1 mL of 2% SDS/0.2 N NaOH, 500 μL of the cell lysate was added to 10 mL of scintillation liquid (Filtercount, Packard, Groningen, The Netherlands), and radioactivity was counted. Overall transport was expressed as pmol (mg prot)$^{-1}$ while the percentage of retained compound was assessed after subtracting the value observed at time 0. Protein content was measured on an aliquot of the cell lysate, using the bicinchonic acid reagent [13]. To assess the inhibitory studies on MRP activity, 10 μM MK571 was added to the transport buffer and transport was measured as above. All the experiments were performed at least in triplicate.

Statistical analysis. Data are expressed as mean ± SD. Statistical analysis was performed by Student's *t* test for pair data and a *p* value less than 0.05 was considered statistically significant.

Results

"In vivo" studies

The overall biliary excretion of gadolinium, expressed as percentage of the administered dose, is reported in Table 1. In control rats, 480 min after intravenous administration of B22956/1, the biliary excretion of gadolinium accounted for $89.6 \pm 8.8\%$ of the injected dose. On the contrary, the biliary excretion was significantly lower in the TR^- rats, where the total gadolinium recovery in bile was $14.1 \pm 3.0\%$ ($p < 0.001$). In control rats the biliary excretion was almost maximal after 120 min when more than 80% of the injected doses was recovered. On the contrary, in TR^- rats the biliary excretion steadily increased over the time with the percentage excreted in the first 120 min being lower that that observed thereafter.

The difference in the biliary excretion between control and TR^- rats is further indicated in Table 2 where the biliary excretion rate of the compound and the bile flow are reported. In normal rats, the Gd excretion rate peaked between 0 and 120 min with a mean maximum value of 658 ± 63 μmol min^{-1} g^{-1}. The biliary flow values remained at approximately constant values during the entire experimental period. By contrast, in TR^- rats the biliary excretion rate of Gd peaked between 120 and 240 min after B22956/1 administration with a mean value of 39.8 ± 4.4 μmol min^{-1} g^{-1}, i.e., 6% over the maximal biliary excretion rate observed in control animals. Differently from what was observed in control rats, the biliary flow decreased immediately after B22956/1 administration, but remained almost constant thereafter. In line with the different biliary recoveries, the biliary B22956/1 concentration was maximal between 0 and 120 min after administration (7.5 ± 1.4 μmol mL^{-1}) in controls rats, while in TR^- rats, the biliary gadolinium concentration reached a peak (1.7 ± 0.5 μmol mL^{-1}) between 120 and 240 min after B22956/1 administration ($p < 0.01$). This finding indicates that the biliary excretion of the B22956/1 is reduced in TR^- rats, as also indicated in Table 1, and that the reduced bile flow of these animals [6] does not account for such a reduction.

The cumulative urinary excretion (up to 480 min) of gadolinium in normal and TR^- rats, expressed as percent of the administered dose, is reported in Table 3. The urinary excretion of B22956/1 in normal rats accounted for $15.6 \pm 1.9\%$ of the injected dose, while it was significantly higher ($p < 0.001$) in the TR^- rats which accounted for $54.8 \pm 8.9\%$. In addition, while in normal rats the urinary excretion was almost complete after 120 min, in TR^- rats persisted over the entire experimental period, although the percentage decreased particularly in the time frame between 240 and 480 min.

Eight hours after B22956/1 administration, the residual gadolinium in plasma, liver, and kidneys was very low of normal rats ($0.043 \pm 0.012\%$, $0.78 \pm 0.13\%$, and $0.121 \pm 0.020\%$ of the injected dose, respectively). On the contrary, the residual gadolinium in plasma, liver, and kidney was significantly higher ($p < 0.01$) in TR^- rats where the percentage of the injected dose of gadolinium was $6.17 \pm 0.70\%$ in plasma, $6.9 \pm 2.0\%$ in liver, and $1.17 \pm 0.36\%$ in kidneys, respectively. The gadolinium not recovered in urine, bile, liver, kidneys, and plasma is likely supposed to remain in the carcass.

Cellular studies

Fig. 1 shows the time course of the transport of Gd labelled B22950 obtained in either MDCKII wild type cells or in human *MRP1* or *MRP2* permanently transfected parental cells. As indicated above, transport was defined as the amount of compound retained at different

Table 1
Cumulative biliary excretion of gadolinium after intravenous administration of B22956/1 to anaesthetized normal and TR^- rats (dose: 0.1 mmol kg^{-1})

Time (min)	TR^- rat % of administered dose (mean ± SD, $n = 3$)	Normal rat % of administered dose (mean ± SD, $n = 5$)
0–120	2.6 ± 1.0	82.1 ± 8.6
120–240	4.8 ± 0.6	5.8 ± 1.3
240–480	6.8 ± 1.6	1.75 ± 0.55
0–480	14.1 ± 3.0	89.6 ± 8.8

Table 2
Biliary excretion rate of gadolinium and biliary flow after intravenous administration of B22956/1 to normal and TR^- rats (dose: 0.1 mmol kg^{-1})

Time (min)	Normal rats		TR^- rats	
	Biliary excretion rate (μmol min^{-1} g^{-1}) (mean ± SD, $n = 5$)	Biliary flow (μL min^{-1} kg^{-1}) (mean ± SD, $n = 5$)	Biliary excretion rate (μmol min^{-1} g^{-1}) (mean ± SD, $n = 3$)	Biliary flow (μL min^{-1} kg^{-1}) (mean ± SD, $n = 3$)
Basal	0	82 ± 0.12	0	33.0 ± 7.5
0–120	658 ± 63	90 ± 10	20.7 ± 8.8	21.6 ± 1.4
120–240	47 ± 10	80 ± 10	39.8 ± 4.4	24.8 ± 4.1
240–480	9.4 ± 3.0	67.8 ± 6.7	28.8 ± 6.5	27.8 ± 1.8

Table 3
Cumulative urinary excretion of gadolinium after intravenous administration of B22956/1 to normal and TR$^-$ rats (dose: 0.1 mmol kg^{-1})

Time (min)	TR$^-$ rat % of administered dose (mean ± SD, $n = 3$)	Normal rat % of administered dose (mean ± SD, $n = 5$)
0–120	28.52 ± 0.92	14.2 ± 2.6
120–240	12.9 ± 0.6	1.0 ± 1.3
240–480	13.4 ± 1.6	0.36 ± 0.45
0–480	54.8 ± 8.9	15.6 ± 1.9

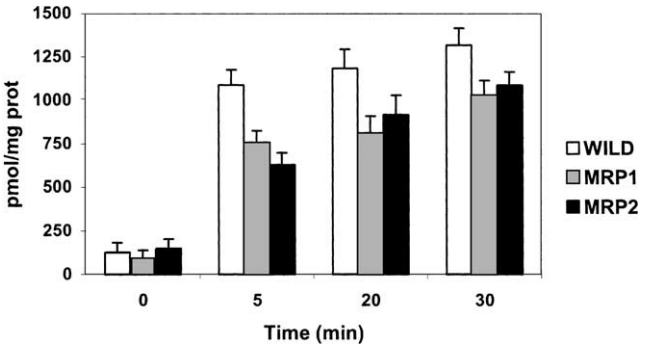

Fig. 1. Time-dependent uptake of Gd-labelled B22950 in MDCKII cells. Uptake of Gd-labelled B22950 in wild type (open bars) MDCKII cells or in cells stably transfected with human *MRP1* (gray bars) or human *MRP2* (black bars). Uptake was measured at a constant concentration of Gd-labelled B22950 (100 μM) at different times (0, 5, 20, and 30 min). Data are reported as mean ± SD (bars) of at least three different experiments.

time intervals after the exposure of the cell clones to a constant Gd labelled B22950 concentration of 100 μM. No difference was observed at time 0, indicating a comparable binding to the differently transfected and control cells. After 5 min, a clear reduction in the cellular accumulation of the compound was observed in both *MRP1* (758 ± 66 pmol (mg prot)$^{-1}$) and *MRP2* (628 ± 71 pmol (mg prot)$^{-1}$) transfected as compared to wild type cells (1087 ± 88 pmol (mg prot)$^{-1}$) ($p < 0.01$). The same pattern was observed after 20 and 30 min, when the intracellular content of Gd labelled B22950 was increased but was still lower in transfected than in control cells (1027 ± 112 vs. 1316 ± 89 pmol (mg prot)$^{-1}$, $p < 0.05$). No significant difference was found between *MRP1* and *MRP2* transfectant clones.

Fig. 2 shows the effect of the inhibition of the MRP activity obtained by the addition of MK571 [14,15]. As in Fig. 1, cells were exposed to a constant concentration of Gd labelled B22950 (100 μM) together with 10 μM of MK571, and the uptake was measured as described above. No difference was observed 5 min after addition of the inhibitor of MRP activity. On the contrary, after

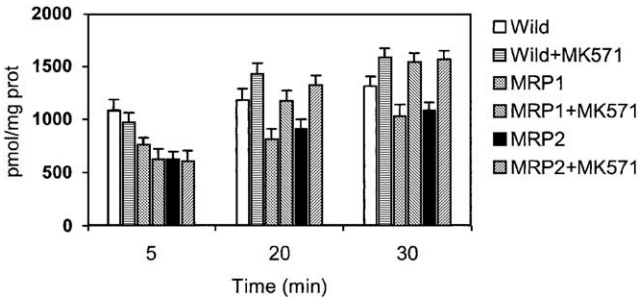

Fig. 2. Effect of the inhibition of MRP activity by MK571 on the cellular accumulation of Gd-labelled B22950 in MDCKII cells. The cellular content of Gd-labelled B22950 was assessed at different times (5, 20, and 30 min) after incubation of different cell types with 100 μM of Gd-labelled B22950 either in the absence or presence of 10 μM of MK571. Details are given on the legend in the figure. Data are reported as mean ± SD (bars) of at least three different experiments.

20 min, the inhibition of MRP activity resulted in an increased cellular retention either in the wild type or in transfected cells. This effect was even greater after 30 min when the inhibitor significantly ($p < 0.001$) increased the cellular contents, in particular in transfected cells. Of notice is the observation that, in the presence of MK571, the amount of Gd labelled B22950 found in wild and *MRP1* or *MRP2* transfected clones was almost identical (1583 ± 89, 1543 ± 112, and 1565 ± 85 pmol (mg prot)$^{-1}$, respectively).

Discussion

Many attempts have been performed to identify contrast agents which may show organ specificity and thus may be "targeted" to specific organs. As far as the study of hepatobiliary MRI is concerned, data have been provided that several Gd-complex MRI contrast agents way undergo an efficient hepatic uptake and subsequent biliary excretion. While the molecular mechanisms involved in the first step are still not fully unraveled, evidence has been provided indicating a role of MRP proteins in the biliary excretion [3].

The aim of the present study was to evaluate the hepatic transport of the new compound B22956/1, a candidate gadolinium based magnetic resonance (MR) imaging contrast agent for coronary angiography [1,2], which has been shown to undergo extensive biliary elimination. This issue has been addressed by the use of two different, but rather complementary experimental models: mutant Wistar rats (TR$^-$ rats) which lack *MRP2* expression [8] and cells transfected with either human *MRP1* or *MRP2* gene. The animal model allowed us to understand the role of MRP2 in the biliary excretion of B22956/1 while the in vitro model provided evidence on the possible additional role of MRP1 in the transport of the compound both in the liver and in those

organs where this ABC transports has been described [16]. In addition, the use of human genes in transfection may help in extrapolating the results obtained in vitro to human conditions and verify if the human homologue is active as the rat transporter.

The results obtained indicate that in the hyperbilirubinemic, MRP2 deficient TR^- rats, the biliary excretion 8 h after administration of B22956/1 is six times lower as compared to normal animals. Upon intravenous administration in normal rats, the MRI contrast agent is rapidly excreted into the bile where more than 80% of the dose is recovered within 120 min. This percentage is greater that what was found with other MRI contrast agents such as Gadobenate Dimeglumine (MultiHance) [17] or Gd-EOB-DTPA [5]. On the contrary, TR^- rats showed an almost nil biliary excretion up to 120 min, with a slight increase thereafter and in particular during the 120–240 min period (Table 2). This reduced biliary excretion is associated with a concomitant increment in urinary excretion where more than 50% of the administered dose is found in urine after 8 h, as compared to less than 16% in control rats (Table 3). Of notice is the observation that the urinary excretion does not increase over the time, as expected by the accumulation of the MRI agent, but rather progressively decreases, particularly in the 240–480 min interval. The data suggest that B22956/1 may be reabsorbed by the kidney and that increased concentrations of the contrast agents may upregulate this mechanism. In line with a much less efficient clearance of the MRI contrast agents is the percentage of the injected dose retained in plasma at the end of the experimental time (8 h) in the TR^- and control rats. While only less than 0.05% was found in normal rats, this percentage rose to 6% in TR^- animals. A similar difference was observed both in liver and kidney where the residual concentrations of B22956/1 were almost 10 times higher in MRP2 deficient rats.

The use of the transfected cells allowed a better understanding of the mechanisms involved in the transport of B22956/1. The expected involvement of MRP2 suggested by the results obtained in TR^- rats was confirmed by the observation that *MRP2* transfected cells retained less compound as compared to wild type MDCKII cells. The conclusion of the involvement of this ABC transporter is also supported by the inhibition exerted by the MRP inhibitor MK571 [15] on cellular export. Different immunofluorescence studies have demonstrated that human and rat MRP2 are localized under physiological conditions to the apical membrane domain of polarized cells, including hepatocytes and proximal tubule epithelia of the kidney [18,19]. Due to its localization, MRP2 in rats could contribute to the urinary excretion of B22956/1 through tubular secretion and also this activity should be impaired in TR^- rats.

The experiments performed with *MRP1* transfected cells revealed that this protein also accounts for the transport of B22956/1. Similarly to what was observed in *MRP2* transfected cells, the presence of MRP1 is associated with a reduced cellular accumulation of the MRI contrast agent and this effect may be prevented when MK571 was added to the cells. Contrary to MRP2, MRP1 is a transport protein which is expressed on the basolateral membrane of hepatocytes and tubular epithelial cells of the kidney [20]. This localization excludes its direct involvement in the biliary and urinary excretion of the contrast agent, but MRP1 may rather account for the residual presence of B22956/1 in the plasma. The involvement of MRP1 in the transport of B22956/1 is not surprising since an overlapping substrate specificity between MRP1 and MRP2 has been reported [21]. The additional role of other MRP proteins cannot be excluded, however, due to the frequent substrate homology between the members of this ABC subfamily. In line with this conclusion are the results from the inhibition studies with MK571 on wild type MDCKII cells. The obtained data showed that these canine kidney cells have an endogenous mechanism of efflux for B22956/1 (Fig. 2) that could be ascribed to multidrug resistance activity, as previously reported [22,23].

Collectively, these data point to the involvement of both MRP2 and MRP1 in the handling of the B22956/1 MRI contrast agent. Due to their different membrane localizations, the first allows biliary and urinary excretion while the second prevents cellular accumulation by export towards the plasma. The use of two different experimental models allowed us to define the relative role of these proteins (either) in vivo (or in vitro) and to anticipate the possible use of the new contrast agent in clinical conditions where a defective activity of canalicular transporter(s) has been described [24].

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References

[1] F.M. Cavagna, J. Zheng, V. Lorusso, F. Maggioni, D. Li, P.J. Finn, M.R. coronary angiographic: new insight and new contrast agents, Proc. Nort. Am. Soc. Cardiac. Imaging 4. Conference Proceeding, 1999.

[2] F.M. Cavagna, J. Zheng, V. Lorusso, F. Maggioni, D. Li, P.J. Finn, New protein binding gadolinium chelate with high vascular containement for magnetic resonance coronary angiography, Conference Proceeding, 2000.

[3] L. Pascolo, S. Petrovic, F. Cupelli, C.V. Bruschi, P.L. Anelli, V. Lorusso, M. Visigalli, F. Uggeri, C. Tiribelli, Abc protein transport of MRI contrast agents in canalicular rat liver plasma

vesicles and yeast vacuoles, Biochem. Biophys. Res. Commun. 282 (2001) 60–66.
[4] C. de Haen, V. Lorusso, P. Tirone, Hepatic transport of gadobenate dimeglumine in TR-rats, Acad. Radiol. 3 (Suppl. 2) (1996) S452–S454.
[5] M. Muller, R.P. Oude Elferink, H.J. Weinmann, Complete elimination of the hepatobiliary MR contrast agents in hepatic disfunction: an experimental study using transport-deficient, mutant rats, MAGMA 1 (1994) 134–139.
[6] P.L. Jansen, G.M. Groothuis, W.H. Peters, D.F. Meijer, Selective hepatobiliary transport defect for organic anions and neutral steroids in mutant rats with hereditary-conjugated hyperbilirubinemia, Hepatology 7 (1987) 71–76.
[7] P.L. Jansen, W.H. Peters, W.H. Lamers, Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport, Hepatology 5 (1985) 573–579.
[8] C.C. Paulusma, P.J. Bosma, G.J. Zaman, C.T. Bakker, M. Otter, G.L. Scheffer, R.J. Scheper, P. Borst, R.P. Oude Elferink, Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene, Science 271 (1996) 1126–1128.
[9] P.L. Anelli, L. Calabi, C. de Haen, L. Lattuada, V. Lorusso, A. Maiocchi, P. Morosini, F. Uggeri, Hepatocyte-directed MR contrast agents. Can we take advantage of bile acids? Acta Radiol. 412 (Suppl.) (1997) 125–133.
[10] E.M. Frame, E.E. Uzgiris, Gadolinium determination in tissue samples by inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectrometry in evaluation of the action of magnetic resonance imaging contrast agents, Analyst 123 (1998) 675–679.
[11] R. Evers, M. Kool, L. van Deemter, H. Janssen, J. Calafat, L.C. Oomen, C.C. Paulusma, R.P. Oude Elferink, F. Baas, A.H. Schinkel, P. Borst, Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA, J. Clin. Invest. 101 (1998) 1310–1319.
[12] C.C. Paulusma, M.A. van Geer, R. Evers, M. Heijn, R. Ottenhoff, P. Borst, R.P. Oude Elferink, Canalicular multispecific organic anion transporter/multidrug resistance protein 2 mediates low-affinity transport of reduced glutathione, Biochem. J. 338 (Part 2) (1999) 393–401.
[13] L. Pascolo, C. Fernetti, M.V. Garcia-Mediavilla, J.D. Ostrow, C. Tiribelli, Mechanisms for the transport of unconjugated bilirubin in human trophoblastic BeWo cells, FEBS Lett. 495 (2001) 94–99.
[14] J. Renes, E.G. de Vries, E.F. Nienhuis, P.L. Jansen, M. Muller, ATP- and glutathione-dependent transport of chemotherapeutic drugs by the multidrug resistance protein MRP1, Br. J. Pharmacol. 126 (1999) 681–688.
[15] I. Leier, J. Hummel-Eisenbeiss, Y. Cui, D. Keppler, ATP-dependent para-aminohippurate transport by apical multidrug resistance protein MRP2, Kidney Int. 57 (2000) 1636–1642.
[16] P. Borst, R. Evers, M. Kool, J. Wijnholds, A family of drug transporters: the multidrug resistance-associated proteins, J. Natl. Cancer Inst. 92 (2000) 1295–1302.
[17] V. Lorusso, T. Arbughi, P. Tirone, C. de Haen, Pharmacokinetics and tissue distribution in animals of gadobenate ion, the magnetic resonance imaging contrast enhancing component of gadobenate dimeglumine 0.5 M solution for injection (MultiHance), J. Comput. Assist. Tomogr. 23 (Suppl. l) (1999) S181–S194.
[18] M. Buchler, J. Konig, M. Brom, J. Kartenbeck, H. Spring, T. Horie, D. Keppler, cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats, J. Biol. Chem. 271 (1996) 15091–15098.
[19] J. Kartenbeck, U. Leuschner, R. Mayer, D. Keppler, Absence of the canalicular isoform of the MRP gene-encoded conjugate export pump from the hepatocytes in Dubin–Johnson syndrome, Hepatology 23 (1996) 1061–1066.
[20] P. Borst, R. Evers, M. Kool, J. Wijnholds, The multidrug resistance protein family, Biochim. Biophys. Acta 1461 (1999) 347–357.
[21] J. König, D. Rost, Y. Cui, D. Keppler, Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane, Hepatology 29 (1999) 1156–1163.
[22] Y. Cui, J. Konig, D. Keppler, Vectorial transport by double-transfected cells expressing the human uptake transporter SLC21A8 and the apical export pump ABCC2, Mol. Pharmacol. 60 (2001) 934–943.
[23] E. Bakos, R. Evers, G. Szakacs, G.E. Tusnady, E. Welker, K. Szabo, M. de Haas, L. van Deemter, P. Borst, A. Varadi, B. Sarkadi, Functional multidrug resistance protein (MRP1) lacking the N-terminal transmembrane domain, J. Biol. Chem. 273 (1998) 32167–32175.
[24] C.C. Paulusma, R.P. Oude Elferink, The canalicular multispecific organic anion transporter and conjugated hyperbilirubinemia in rat and man, J. Mol. Med. 75 (1997) 420–428.