Cisplatin induces renal expression of P-glycoprotein and canalicular multispecific organic anion transporter

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Demeule, Michel, Mathieu Brossard, and Richard Béliveau. Cisplatin induces renal expression of P-glycoprotein and canalicular multispecific organic anion transporter. Am. J. Physiol. 277 (Renal Physiol. 46): F832–F840, 1999.—The expression of two members of the ATP-binding cassette family of transport proteins, P-glycoprotein (P-gp) and the canalicular multispecific organic anion transporter (cMOAT or Mrp2), was evaluated in renal brush-border membranes (BBM) and various rat tissues after cisplatin treatment. One administration of cisplatin (5 mg/kg) increased P-gp expression by >200–300% in renal BBM and in crude membranes from liver and intestine. The increase in P-gp expression in the kidney was also detected in photolabeling experiments, suggesting the induction of functional P-gp. cMOAT expression was increased by >10-fold in renal BBM after cisplatin administration, although it had no effect on liver cMOAT expression. The increase in the levels of both proteins was maximal at 2 days after cisplatin treatment and lasted for at least 8 days. These results indicate that a single administration of cisplatin induces overexpression of P-gp and cMOAT in specific tissues. This may be of significant relevance to the design of clinical trials using cisplatin as a single chemotherapeutic agent or in combination with other drugs.

CANCER CELLS MAY BECOME resistant to a range of drugs that have different structures and cellular targets during chemotherapeutic treatment with a single drug (14, 23). In humans, multidrug resistance (MDR) is associated with membrane proteins that are members of the ATP-binding cassette (ABC) family. Two members of this family, P-glycoprotein (P-gp) and canalicular multispecific organic anion transporter (cMOAT), seem very important for the establishment of the MDR phenotype in cancer cells (14, 35).

P-gp was identified as an ATP-dependent transporter that excludes from cells a wide variety of unmodified hydrophobic substrates, including Vinca alkaloids, colchicine, antibiotics, and anthracyclines (12, 14, 23). P-gp is expressed in a variety of normal secretory tissues such as kidney, intestines, liver and at high levels in the endothelial cells of brain capillaries (5, 14, 19).

A high ATP-dependent transport activity for organic anions was reported in the canalicular membrane of hepatocytes and was identified as the cMOAT (Mrp2; see Refs. 16–18, 24, and 29). This transporter may contribute to drug resistance by transporting a wide range of glutathione, glucoronate, and sulfate conjugates out of cells by an ATP-dependent mechanism. This GS-X pump, a 190-kDa membrane glycoprotein, is mainly expressed in the canalicular membrane of hepatocytes but was also shown to be present in renal brush-border membrane (BBM), intestines, and in several multidrug-resistant cell lines selected for cisplatin resistance (9, 35).

Cisplatin [cis-dichlorodiammineplatinum(II)] is an effective antineoplastic agent used in the treatment of various solid tumors. However, cancer cells may acquire resistance during cisplatin treatment by limiting its cellular concentration and by altering its interaction with DNA. Cancer cells may also have the ability to sequester or inactivate cisplatin and hence reduce its toxicity. The resistance to cisplatin, which is not a P-gp substrate, is not associated with P-gp or MRPI expression (15). However, it was also shown that cisplatin may increase the expression of P-gp in some cisplatin-resistant cell lines (27). A direct interaction between cisplatin and glutathione (1:2 molar ratio) has also been reported (35). This cisplatin-glutathione conjugate was transported in an ATP-dependent manner by cMOAT, and a striking correlation was obtained between cisplatin resistance and cMOAT expression in cisplatin-resistant cell lines.

In the present study, the expression of P-gp and cMOAT was evaluated in rat tissues after cisplatin treatment. P-gp and cMOAT levels were evaluated by Western blot analysis using the monoclonal antibody (MAb) C219, which recognizes P-gp, and with a polyclonal antibody (PAb) directed against the COOH-terminal portion of cMOAT. Our results suggest that cisplatin, a nonstandard P-gp substrate, can induce P-gp expression in specific tissues. Furthermore, we are also reporting that a single administration of cisplatin induces cMOAT expression in renal BBM. Because cisplatin is widely used, therapies using this agent must therefore be carefully designed to be efficient.

MATERIALS AND METHODS

Materials. Cisplatin was obtained from Bristol Myers Squibb (Montreal, PQ) and Sigma Chemical (Oakville, ON). A Mini-Protean II apparatus for electrophoresis and electrophoresis reagents were from Bio-Rad (Mississauga, ON). Polyvinylidene difluoride membranes and a Milliblot-Graphite electrobetter were from Millipore (Mississauga, ON). MAb C219 directed against P-gp and MAbR directed against MRPI (QCRL-1 and MRP1) were from ID Laboratories (London,
ON). MAb 6/1C, which is specifically directed against human MDR1, was purchased from Kamiya Biomedical (Seattle, WA). Anti-mouse and anti-rabbit IgG horseradish peroxidase-linked whole antibody and enhanced chemiluminescence (ECL) reagents were purchased from Amersham (Oakville, ON). [125I]iodoaryl azidoazoprazin (IAAP) was purchased from DuPont-New England Nuclear (Markham, ON). All other reagents were from Sigma Chemical (Oakville, ON).

Cisplatin treatments. Male Sprague-Dawley rats weighing 300–350 g were treated for 11 days with a single subcutaneous injection of cisplatin (5 mg/kg) diluted in sterile saline. The control and the treated groups received single injections of saline or cisplatin, respectively. Rats were killed 1, 2, 4, 6, 8, and 15 days after cisplatin administration, and tissue sampling was performed.

Isolation of tissue crude membrane fractions, renal BBM, and brain capillaries. Crude membrane fractions were prepared from intestine, liver, heart, lung, and stomach of control and treated rats. Tissues from individual animals were pooled and homogenized in a buffer containing 250 mM sucrose and 5 mM HEPES-Tris (pH 7.4), with a Polytron tissue homogenizer (Brinkmann Instruments, Rexdale, ON), and the homogenates were centrifuged at 3,000 g for 10 min. The supernatants were then centrifuged at 33,000 g for 30 min, and the pellets containing the crude membrane fractions were resuspended in 50 mM mannitol and 20 mM HEPES-Tris, pH 7.5, and were stored at –80°C. Renal BBM were prepared by the MgCl2 precipitation method of Booth and Kenny (2). Purified membranes were resuspended in 50 mM mannitol and 20 mM HEPES-Tris, pH 7.5, and were stored at –80°C. Brain capillaries of control and treated rats were purified from brain cortex by the method of Dalilaire et al. (6). Protein content was determined in all experiments with the method of Bradford (3).

Detection of P-gp and cMOAT. P-gp and cMOAT were detected by Western blot analysis. SDS-PAGE was performed according to the method of Laemmli (22). Membranes were resuspended in sample buffer to a final concentration of 1 mg/ml and were loaded on 6.25 or 7.5% acrylamide-bisacrylamide (29:1:0.9) gels without prior heating. P-gp was detected using MAB C219 as described previously (19), whereas cMOAT was evaluated using a PAB directed against its COOH-terminal portion. Horseradish peroxidase-conjugated antibodies directed against mouse and rabbit IgGs were used as secondary antibodies. Detection was made with ECL reagents according to the manufacturer’s instructions. The blots were exposed to preflashed Fuji films.

Photoaffinity labeling with [125I]IAAP. Renal BBM proteins (100 µg) from control and cisplatin-treated rats were incubated with 20 nM of [125I]IAAP in 20 mM Tris-HCl, pH 7.5, supplemented with proteinase inhibitors (2 µg/ml aprotinin, 10 µg/ml pepstatin, and 100 µg/ml bacitracin). The incubation was carried out for 1 h at 25°C in the dark and was followed by cross-linking under a Spectroline ultraviolet lamp (Fisher Scientific, Montreal, PQ) at 254 nm for 5 min at 4°C. The labeled P-gp was recovered by immunoprecipitation with MAB C219 and protein A-Sepharose beads, as described in Jé et al. (19).

Enzyme assays. Leucine aminopeptidase activity in membrane samples was measured spectrophotometrically at 820 nm after 10 min at 37°C using leucine p-nitroanilide (1 mM) as the substrate (11). γ-Glutamyl transpeptidase activity was assayed using L-γ-glutamyl-p-nitroanilide as the substrate. The reaction was performed at 37°C for 5–15 min as described previously (19).

Immunization and antibody purification. The peptide EAGLEVNHTEL, corresponding to a COOH-terminal portion of cMOAT (30), was synthesized using multiple antigenic peptide system (MAPS) chemistry and was obtained from Service de Séquence de Peptides de l’Est du Québec (Centre Hospitalier de l’Université Laval). On day 0, two rabbits were injected subcutaneously at two sites with a total of 400 µg of the cMOAT peptide mixed with Freund’s complete adjuvant (Pierce, Rockford, IL) in a 1:1 volume ratio, i.e., 0.5 ml adjuvant and 0.5 ml of solution containing 400 µg peptide. Booster injections with 100 µg of antigen were administered on day 28 using Freund’s incomplete adjuvant. The final injection (day 50) consisted of 150 µg of peptide adsorbed to aluminum hydroxide (Imject Alum; Pierce), administered subcutaneously at two sites. The rabbits were bled 18 days after the final injection. The blood was allowed to coagulate at room temperature for 1 h and was stored overnight at 4°C to allow the clot to retract. The serum was collected after removal of blood cells by centrifugation at 10,000 g for 10 min. The serum was passed through a 22-ml protein A-Sepharose 4 Fast Flow column (Pharmacia, Dorval, PQ) equilibrated with 50 mM Tris-HCl, pH 8.6, using a Fast Protein Liquid Chromatography apparatus (FPLC; Pharmacia). The IgGs were eluted with 0.2 M glycine, pH 2.2. The fractions (5 ml) were collected in 0.8 ml of 1 M Tris-HCl, pH 9. The antibodies were further purified by affinity chromatography on a peptide-Sepharose column prepared as previously described. The IgGs were passed through this column equilibrated with 100 mM NaCl and 20 mM Tris-HCl, pH 7.5 (buffer A). After extensive washing, the antibodies were eluted from the column with 0.2 M glycine, pH 2.2. The neutralized fractions that contained IgGs directed against cMOAT peptide were pooled and dialyzed overnight against buffer A and adjusted to the desired concentration by ultrafiltration.

Densitometric and statistical analyses. The intensities of the bands obtained from Western blot analysis and the photolabeling studies were estimated with a Personal densitometer SI (Molecular Dynamics, Sunnyvale, CA). Statistical analyses were made with the Student’s paired t-test using Microsoft Excel. P < 0.05 was considered significant.

RESULTS

Detection of P-gp in tissues from cisplatin-treated rats. Rats were treated with cisplatin to study its effects on P-gp expression in normal tissues. Tissues were isolated from rats 4 days after a single subcutaneous injection of cisplatin (5 mg/kg). P-gp expression was evaluated by Western blot analysis with MAB C219 (Fig. 1). P-gp was detected by MAB C219 as a 140- to 180-kDa protein in membranes isolated from control and treated rats. After cisplatin administration, an increase in the expression of P-gp was detected in specific tissues, including intestine, kidney, and liver. The increase in P-gp expression was quantified by laser densitometry, and levels of P-gp were expressed as a percentage of the corresponding control groups (Fig. 2). In liver, kidney, and intestine, the amount of P-gp detected was increased to 330, 270, and 250% compared with control groups, respectively. Western blot analysis using a MAb directed against human MDR1 (MAB 6/1C) was performed in an attempt to identify the P-gp isoform induced by cisplatin. Unfortunately, no reliable signal was obtained for membrane preparations from control and cisplatin-treated rats.
Photolabeling of P–gp by [125I]IAAP. Photoaffinity labeling experiments were performed on BBM isolated from control and treated groups (Fig. 3) to measure the binding activity of P–gp. The amount of photolabeled P–gp in BBM from treated rats was increased by 2.75-fold. The increase in P–gp measured by photolabeling is very similar to the value measured by Western blot analysis. Photoaffinity labeling experiments were also performed on intestine and liver isolated from control and treated groups. We were unable to detect photolabeled P–gp in these tissues, probably because the expression of P–gp is too low (data not shown).

Detection of cMOAT in tissues from cisplatin-treated rats. An antibody directed against the EAGIEN-VNHTEL peptide of cMOAT was produced in rabbit and purified on a protein A-Sepharose column followed by a peptide-affinity column. In Western blot analysis, this antibody recognized a 190-kDa protein in normal liver (Fig. 4A). In the kidney, a faint band was immuno-detected at the same molecular weight. Aside from cMOAT, this antibody also detected other proteins with molecular weights of 155, 110, and 68 kDa. After cisplatin treatment, there was a strong increase in the cMOAT level in renal BBM, whereas all other detected proteins were unaffected. The 190-kDa band corresponding to cMOAT was scanned by laser densitometry. Cisplatin induced a 7.6-fold increase of cMOAT expression in renal BBM, whereas its level was unchanged in liver. When the samples were boiled before SDS-PAGE, the band corresponding to cMOAT in renal BBM and liver on Western blots completely disappeared, whereas the other nonspecific bands were still detected (Fig. 4A). This experiment strongly suggests that the 190-kDa band induced by cisplatin in renal BBM is indeed cMOAT, since it was previously detected by Western blots in unheated samples (30), indicating its heat sensitivity.

In addition to cMOAT, the PAb recognized a number of lower molecular weight bands. However, these bands were also observed using preimmune serum (Fig. 4A), suggesting that they were not related to cMOAT. In addition, negative controls were performed using heart membranes, brain capillaries, and lung membranes from control and cisplatin-treated animals (Fig. 4B). In these tissues, cMOAT was undetected in control and cisplatin-treated samples. Lower nonspecific molecular weight bands were still detected in these tissues.

Because previous studies reported that lipopolysaccharides (LPS) downregulate cMOAT in liver (30, 38), rats were treated with LPS to confirm that the 190-kDa band was cMOAT (Fig. 4C). As expected, the detection of the 190-kDa protein decreased in liver crude membranes isolated from LPS-treated rats, whereas nonspe-
Specific bands of lower molecular weight were unaffected by the treatment. These results presented in Fig. 4 show that the 190-kDa band corresponds to cMOAT and that cisplatin induced its expression in the kidney. Attempts were also made to detect MRP1 in these membrane preparations by Western blot analysis using two MAbs (QCRL-1 and MRPr1) that are commercially available. No signal was detected with either MAb because of their low sensitivity or due to the low expression of MRP1 in these tissues (data not shown).

Effect of cisplatin treatment on membrane markers. At 4 days after cisplatin treatment, the activity of various membrane marker enzymes was measured on preparations from kidney, liver, brain capillaries, and intestine (Table 1). Leucineaminopeptidase and \(\gamma\)-glutamyltranspeptidase were unaffected by cisplatin, suggesting that the increased levels of P-gp in these membranes are specific to this protein. The enrichment of these membranes from control and treated rats was also similar for all cisplatin treatments (data not shown), suggesting that cisplatin does not affect those physicochemical properties of membranes that are used for their isolation. Alkaline phosphatase activity in intestine was reduced by 45% by cisplatin, whereas it remained unaffected by the drug in renal BBM and liver. In addition, cytochrome P-450 was measured in homogenates from liver, kidney, and intestine by Western blot using a PAb that recognizes the 3A1 and 3A2 isoforms (Fig. 5). Levels of cytochrome P-450 were unaffected by cisplatin in liver and kidney homogenates. This latter result indicates that there is no correlation between the increase in P-gp and cMOAT.

Table 1. Effect of cisplatin on membrane markers in kidney, liver and intestine

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Alkaline Phosphatase, nmol·min(^{-1})·mg(^{-1})</th>
<th>Leucineaminopeptidase, nmol·min(^{-1})·mg(^{-1})</th>
<th>(\gamma)-Glutamyltranspeptidase, nmol·min(^{-1})·mg(^{-1})</th>
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<tbody>
<tr>
<td>Renal BBM</td>
<td>6,750 ± 950</td>
<td>430 ± 60</td>
<td>9,370 ± 300</td>
</tr>
<tr>
<td>Liver</td>
<td>7.3 ± 1.3</td>
<td>24 ± 1</td>
<td>NA</td>
</tr>
<tr>
<td>Intestine</td>
<td>9,820 ± 370</td>
<td>159 ± 7</td>
<td>115 ± 4</td>
</tr>
</tbody>
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Values represent means ± SD obtained from 3 different treatments. BBM, brush-border membrane. Alkaline phosphatase, leucineaminopeptidase, and \(\gamma\)-glutamyltranspeptidase activities were measured in various samples isolated from control and cisplatin-treated rats. NA, not available. *\(P < 0.05\).

Fig. 4. Immunodetection of canalicular multispecific organic anion transporter (cMOAT) after cisplatin and lipopolysaccharide (LPS) treatments. Rats were treated with a single administration of saline or 5 mg/kg of cisplatin and were killed on day 4. A: Western blot analysis: protein samples (50 µg) of renal BBM and liver membranes from control and cisplatin-treated rats were loaded onto SDS-PAGE, with (+) or without (−) boiling, and were subjected to electrophoresis. Immunoblots were performed using PAb directed against cMOAT or preimmune serum and enhanced chemiluminescence (ECL) reagents, as described in MATERIALS AND METHODS. B: Western blots were also performed on protein samples (50 µg) of brain capillaries and heart and lung membranes from control and cisplatin-treated rats. cMOAT in renal BBM from cisplatin-treated rats was detected as a positive control. C: rats were treated with a single intraperitoneal injection of saline or LPS (4 mg/kg) and were killed 24 h later as previously described (38). cMOAT was immunodetected by Western blots in protein samples (50 µg) of crude membranes isolated from saline (−) or LPS-treated (+) rats. Kidney BBM from cisplatin-treated rats (+) was used again as a positive control.
expression and the cytochrome P-450 isoforms in the tissues studied.

Time course of the induction of P-gp expression. Western blot analysis was used to follow P-gp expression in membranes from liver (Fig. 6A), kidney (Fig. 6B), and intestine (Fig. 6C) isolated 1, 2, 4, 6, 8, and 15 days after a single administration of cisplatin. The density of the band corresponding to P-gp was estimated by laser densitometry. Levels of P-gp were described as a percentage of the values obtained from animals injected with saline (Fig. 7). The increase in P-gp levels in these three tissues increased rapidly and was detectable 1 day after cisplatin injection. In these tissues, the increased P-gp expression was maximal between days 2 and 6. Levels of P-gp remained elevated for a longer time in liver than in kidney and intestine. In intestine, many bands of lower molecular weight than P-gp were detected (Fig. 1). Because this tissue contains a high concentration of proteases, these bands could be P-gp degradation products. Western blot studies of crude membranes from intestine were difficult to perform due to these high protease levels. Nevertheless, the elevated P-gp expression returned to control values at 8 and 15 days after cisplatin treatment in

Fig. 5. Immunodetection of cytochrome P-450 3A,1/2 (Cyt P450) isoforms after cisplatin treatment. Rats were treated with a single administration of cisplatin (5 mg/kg) and were killed on day 4. Western blot analysis: protein samples (40 µg) of homogenates from liver and kidney isolated from control and cisplatin-treated rats were resolved by SDS-PAGE. Immunoblots were performed using polyclonal antibody (PAb) directed against cytochrome P-450 3A,1/2 isoforms and ECL reagents, as described in MATERIALS AND METHODS. One representative experiment is shown; 4 experiments were performed.

Fig. 6. Time course of P-gp expression after cisplatin treatment. Rats were treated with one administration of saline or 5 mg/kg of cisplatin and were killed 1, 2, 4, 8, and 15 days after the treatment. Protein samples (50 µg) of membranes from liver (A), kidney (B), and intestine (C) isolated from control and cisplatin-treated rats were resolved by SDS-PAGE with a 6.25% acrylamide gel. Immunoblots were performed with MAb C219, as described in MATERIALS AND METHODS.

Fig. 7. Quantification of P-gp expression after cisplatin treatment. Immunoreactive bands detected by Western blot analysis in Fig. 6 were evaluated by laser densitometry. P-gp levels are expressed as a percentage of the total amount of immunoreactive protein present in liver (A), kidney (B), and intestine (C) membranes isolated from control rats. Values represent means ± SE for 2 independent experiments for liver and kidney and means ± SD for 1 experiment for intestine. Immunodetection of P-gp for each group of rats was performed in duplicate at least.
intestine and kidney, respectively, whereas it was still twofold higher in liver after 15 days.

Time course of the induction of cMOAT expression. cMOAT expression in liver and kidney was also estimated at 1, 2, 4, 6, 8, and 15 days after cisplatin treatment (Fig. 8). In liver, cMOAT expression was unaffected by this cisplatin treatment (Fig. 8A). In contrast, cMOAT expression was strongly increased in renal BBM isolated from cisplatin-treated rats (Fig. 8B). The 190-kDa band corresponding to cMOAT in the samples was scanned, and the results were expressed as a percentage where 100% equalled the value from control rats (Fig. 9). Although cMOAT levels remained similar in liver from control and cisplatin-treated rats, its expression in renal BBM increased by at least 10-fold 2 days after cisplatin administration. This increase in cMOAT expression was even stronger than that measured in previous treatments (Fig. 4). The cMOAT level in renal BBM remained high for at least 8 days and then returned to control values by 15 days after cisplatin treatment.

DISCUSSION

We investigated the effect of a single injection of cisplatin (5 mg/kg) on the endogenous expression of P-gp and cMOAT in normal tissues. This dose of cisplatin corresponds to chemotherapeutic levels known to induce mild renal failure in rats (13, 34). In addition, because renal insufficiency and both morphological and functional alterations in renal cortex mitochondria have also been reported to be maximal on days 3–5 after cisplatin treatment (13, 33), we chose day 4 after cisplatin treatment for collecting tissues. We used an immunoblotting procedure to detect the expression of both proteins. After this injection, P-gp levels were increased in liver, kidney, and intestine, whereas cMOAT level was increased only in kidney. These results show that cisplatin can modulate the expression of both proteins in specific tissues in vivo.

Alkaline phosphatase, $\gamma$-glutamyltranspeptidase, and leucineaminopeptidase activities in the kidney were shown to be inhibited by cisplatin in vitro, whereas they were unaffected by cisplatin in vivo (7). As was reported by the latter study, our results indicate that these three enzyme activities were similar after in vivo treatment with cisplatin, not only in the kidney but also in liver. The only enzyme affected by the drug was intestinal alkaline phosphatase. Cisplatin, which causes profound gastrointestinal symptoms for many days after drug administration, was also shown to produce morphological changes in the small intestinal mucosa and to affect the activities of intestinal enzymes (1, 20). Nevertheless, our results suggest that the effects of cisplatin treatment on P-gp and cMOAT expression are rather specific, since the two other marker enzymes were unaffected in these three tissues.

Overlaps in substrate specificities between cytochrome P-4503A and P-gp have been reported, suggesting that these enzymes have complementary roles in the pharmacokinetics of drug absorption and elimination that are particularly relevant to cancer chemotherapy (37). Changes in cytochrome P-4503A and P-gp levels in rat liver were also observed after administration of hydroxyethyl cyclosporin A (39). Altered levels of these two enzymes could drastically affect the delivery of cancer chemotherapeutic agents. Cisplatin treatment was shown to increase the level of P-4502C3 in renal microsomes and of P-4502A1, 2C7, 2E1, 4A2, and 4A3 in hepatic microsomes and to decrease the levels of P-4502C11 and 3A2 in rat liver (28). In the present

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**Fig. 8.** Time course of cMOAT expression after cisplatin treatment. Rats were treated with one administration of saline or 5 mg/kg of cisplatin and were killed 1, 2, 4, 8, and 15 days after the treatment. Protein samples (50 $\mu$g) of membranes from liver (A) and kidney (B) isolated from control and cisplatin-treated rats were resolved by SDS-PAGE with a 6.25% acrylamide gel. Immunoblots were performed with PAb directed against cMOAT, as described in MATERIALS AND METHODS.

**Fig. 9.** Quantification of cMOAT expression after cisplatin treatment. Immunoreactive bands detected by Western blot analysis in Fig. 7 were evaluated by laser densitometry. cMOAT levels are expressed as a percentage of the total amount of immunoreactive protein present in liver crude membranes (○) and renal BBM (●) isolated from control rats. Values represent means ± SE for 2 independent experiments. Immunodetection of cMOAT after each treatment was performed at least in duplicate.
cMOAT (Mrp2), a novel ATP-dependent export pump for amphiphilic anionic conjugates, which has been cloned from liver, was also identified in rat kidney and was localized to the apical membrane domain of proximal tubule epithelia (30). In rat, mrp2 mRNA was highly expressed in liver and at lower levels in kidney, duodenum, and ileum (9, 29). Using the amino acid sequence EAGIENVNHTEL at the COOH-terminal region of the rat Mrp2 protein, as described previously (8, 30, 38), we raised a PAb directed against this protein. As previously reported, the level of cMOAT immunodetected was higher in liver than in the renal BBM of normal rats. Previous studies demonstrated that cisplatin-resistant cell lines contained increased levels of cMOAT (9, 35). However, its expression does not seem essential for all cases of cisplatin resistance, since some highly resistant cell lines do not contain detectable levels of cMOAT. Nevertheless, data reported by various groups suggested that cMOAT could contribute to cisplatin resistance by exporting the cisplatin-glutathione complex, which is formed by one molecule of cisplatin and two molecules of glutathione (9, 18, 35). cMOAT function is also impaired in various experimental models of intrahepatic and obstructive cholestasis (36) or in Groningen yellow/transport-deficient and Eisai hyperbilirubinemic rats (18). Steady-state levels of mRNA for cMOAT were also examined in human lung cancer specimens in relation to platinum drug resistance (25). It was found that cMOAT expression levels did not correlate with platinum drug exposure but that MRP and γ-glutamylcysteine synthetase genes correlated with the exposure of human lung cancers to platinum drugs. Unfortunately, we were unable to detect cMOAT in lung, even after cisplatin treatment, suggesting that its expression in this tissue in rats may be too low for Western blotting analysis.

In normal rats, ~47% of the initial cisplatin dose is excreted by the kidney, whereas 1–5% is excreted by the liver. Urinary excretion of cisplatin involves glomerular filtration and tubular secretion (32). In the present study, only one injection of cisplatin was necessary to induce the expression of cMOAT in renal BBM. In addition, the large increase in the expression of cMOAT in renal BBM suggests that this transporter may be involved in the handling or excretion of cisplatin by the kidney. In contrast, the lack of induction of cMOAT expression in liver by cisplatin could be a consequence of the already high endogenous levels of cMOAT (16, 24, 29) or may be because this tissue is not the principal one involved in the elimination of the drug.

In conclusion, there was a rapid modulation of P-gp and cMOAT expression in specific tissues. The increase in expression for both proteins is rapid (1 day of treatment). In comparison, 5 days of administration of cyclosporin A, a P-gp substrate that is also nephrotoxic, were needed to increase P-gp expression by 50–100% in various rat tissues (19), whereas one administration of cisplatin increased P-gp expression by >200–300% in the described experiments, indicating that P-gp levels in these tissues are too low to be labeled by IAAP.

In the present study, the increase in P-gp levels caused by cisplatin, which is not a classic substrate for P-gp, suggests that P-gp is induced in specific tissues in response to cisplatin treatment. Intracellular events leading to the overexpression of P-gp by cisplatin remain to be established. However, previous studies reported that the human Y-Box binding protein (YB-1), a member of a DNA-binding protein family, was directly involved in MDR1 gene activation in response to genotoxic stress (27). The YB-1 concentration was shown to be much higher in cisplatin-resistant cell lines than in equivalent cisplatin-sensitive cell lines (26, 27). Furthermore, transfectants with lower levels of YB-1 presented increased sensitivity to cisplatin, mitomycin, and ultraviolet light, suggesting that YB-1 may protect cells from the cytotoxic effects of agents that induce cross-linking of DNA (26, 27). Thus cisplatin may induce P-gp expression as do many of its substrates, by a mechanism that involves YB-1. These studies suggest that the induction of P-gp in specific tissues (liver, kidney, and intestine) may be related to tissue toxicity and also to YB-1 distribution. Because it was previously reported that cisplatin is not a P-gp substrate (15), we can speculate that the overexpression of P-gp by cisplatin will not modify the pharmacokinetics of this drug. However, the pharmacokinetics and the distribution of coadministered drugs that are P-gp substrates may be strongly changed after the induction of P-gp in these secretory tissues.

The increase of P-gp expression in the kidney after cisplatin treatment may be related to its renal toxicity, which is morphologically characterized by tubular necrosis, loss of microvilli, alterations in number and size of lysosomes, and mitochondrial vacuolization in proximal tubular epithelial cells (21). The wide tissue distribution of P-gp suggests that this protein may be involved in protective mechanisms against occasionally encountered toxins or commonly encountered toxic natural products (5, 10). In mdr1a knockout mice, Pgp was shown to play an important role in the defense of the organism, since its absence affected the pharmacokinetics and tissue distribution of vinblastine and ivermectin (31). Modulation of P-gp expression in vitro by cytotoxic drugs was reported (4). Chronic administration of the chemosensitizer cyclosporin A (CsA) to rats also induced overexpression of P-gp in various tissues such as kidney, intestine, liver, stomach, heart, lungs, testis, and spleen (19). The increased P-gp expression in kidney after cisplatin treatment may be involved in the defense of the kidney against the stress caused by this drug. In addition, the increased P-gp expression in kidney caused by cisplatin was also detected by photoaffinity labeling, suggesting the induced P-gp is functional. In intestine and liver, the induction of P-gp expression was undetected with photoaffinity labeling experiments, indicating that P-gp levels in these tissues are too low to be labeled by IAAP.

The induction of P-gp or cMOAT caused by cisplatin and the results indicate that there is no parallel between the unaffected after cisplatin administration. Thus our homogenates from liver, kidney, and intestine were functional. In intestine and liver, the induction of P-gp in kidney caused by cisplatin was also detected by photoaffinity labeling, suggesting the induced P-gp is functional. In intestine and liver, the induction of P-gp expression was undetected with photoaffinity labeling.
liver, kidney, and intestine. Furthermore, in the present study, cMOAT expression increased >10-fold in renal BBM after cisplatin treatment. cMOAT and P-gp levels remained high for many days after the treatment. These results suggest that the increase of both protein levels depends on early events induced by cisplatin rather than on later responses caused by irreversible binding of cisplatin to macromolecules (32). The return of both proteins to control values may depend on cisplatin elimination and also on the turnover of these proteins. Because cisplatin is widely administered in different situations, the determination of the molecular mechanisms involved in the modulation of these two important ABC transporters is crucial for future clinical trials using this drug.

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