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Dexamethasone modulation of multidrug transporters in normal tissues

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Abstract The expression of P-glycoprotein (P-gp) and canalicular multispecific organic anion transporter (cMOAT or Mrp2) was evaluated by Western blotting analysis of rat tissues isolated following daily administration (1 mg kg^{-1} day^{-1}) of dexamethasone over 4 days. Dexamethasone rapidly increased P-gp expression more than 4.5- and 2-fold in liver and lung, respectively, while it was decreased 40% in kidney. cMOAT expression was increased 2-fold in liver and kidney following dexamethasone treatment. The levels of both proteins returned to control values by 6 days after the conclusion of dexamethasone administration. These results indicate that dexamethasone can modulate P-gp and cMOAT expression in specific rat tissues and may have significant relevance for patients treated with dexamethasone as a single agent or in combination therapy with other drugs.

Key words: Dexamethasone; P-glycoprotein; cMOAT; Mrp2; Protein expression

1. Introduction

Dexamethasone is a potent adrenal corticosteroid which has anti-inflammatory, immunosuppressant, anti-neoplastic and anti-emetic properties. Dexamethasone has also been shown to be transported by the P-glycoprotein from human adrenal gland when expressed in porcine LLC-PK1 cells [1]. P-gp was identified as an ATP-dependent transporter which exports a wide variety of unmodified hydrophobic substrates out of the cell including vinca alkaloids, colchicine, antibiotics and anthracyclines [2–5]. This protein is a 1280 amino acid integral membrane glycoprotein (150–180 kDa) with two homologous halves connected by a linker region. Hydropathy plots deduced from the amino acid sequence indicate that each of these two regions contains six transmembrane domains and a consensus sequence for an ATP binding domain [4,5]. P-gp was found to be expressed in a variety of normal tissues such as kidney, intestines, liver and at high levels in the endothelial cells of brain capillaries [6,7]. In humans, there are two P-gp encoded by distinct *MDR* genes, *MDR1* which confers multidrug resistance, and *MDR2* (also referred to as *MDR3*) which is not involved in cytotoxic drug transport [4]. Rodents have two homologues of *MDR1*, *mdr1a* and *mdr1b*, and one homologue of MDR2, *mdr2* [4].

More recently, a high ATP-dependent transport activity for organic anions was reported in the canalicular membrane of hepatocytes [8,9]. This activity was identified as the canalicular multispecific organic anion transporter (cMOAT) and has been suggested to be involved in the detoxification of heavy metals. This 190 kDa transporter, also known as Mrp2, has a sequence homology of 49% with the human multidrug resistance-associated protein (Mrp1). Studies have shown that cMOAT is a pump which may contribute to drug resistance by transporting a wide range of glutathione, glucuronate and sulfate conjugates out of the cells by an ATP-dependent mechanism [10]. This protein is mainly expressed in the canalicular membrane of hepatocytes but is also found in renal brush border membranes (BBM), intestines and several multidrug-resistant cell lines selected for cisplatin resistance [9,11,12].

Many in vitro studies have evaluated the effects of dexamethasone on P-gp expression. However, contradictory results were obtained [13–16] suggesting that the effects of dexamethasone on P-gp could be related to the type of cell line used. More recently, in vivo administration of dexamethasone was shown to reduce P-gp expression in liver from female rats while its expression was increased in male rats [17]. In contrast to P-gp, nothing is known of the effects of dexamethasone on cMOAT expression. In the present study, expression of these two members of the ATP binding cassette (ABC) family of transport proteins (P-gp and cMOAT) was evaluated in male rat tissues after dexamethasone treatment. Both protein levels were evaluated by Western blot analysis using two monoclonal antibodies (mAbs) for P-gp, C219 and MDR Ab-2, and a polyclonal antibody (pAb) directed against a portion of the C-terminal sequence of cMOAT. Both protein levels were modulated in specific tissues. Since dexamethasone is widely used in chemotherapy and in multicombination therapy, the modulation of P-gp and cMOAT expression in normal tissues may be relevant to treatment efficacy and to side effects of the drug.

2. Materials and methods

2.1. Chemicals

Dexamethasone was purchased from Sigma-Aldrich (St. Louis, MO). A Mini-Protean II apparatus for electrophoresis and electrophoresis reagents were from Bio-Rad (Mississauga, Ont.). Polyvinylidene difluoride (PVDF) membranes and a Milliblot-Graphite electroblotter I were from Millipore (Mississauga, Ont.). mAb C219, directed against P-gp, was from ID Labs (London, Ont.). MDR Ab-2, which is a mAb specifically directed against human MDR1, was purchased from Neo Markers (Fremont, CA). Anti-mouse and anti-rabbit IgG horseradish peroxidase-linked whole antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA) while enhanced chemiluminescence (ECL) reagents were purchased from Amersham (Oakville, Ont.). [^{125}I]Iodoaryl azidoprazosin (IAAP) was purchased from DuPont-New England Nuclear (Markham, Ont.). All other reagents were from Sigma Chemical (Oakville, Ont.).

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Abbreviations: BBM, brush border membranes; ECL, enhanced chemiluminescence; IAAP, [^{125}I]iodoaryl azidoprazosin; mAb, monoclonal antibody; pAb, polyclonal antibody; P-gp, P-glycoprotein; PVDF, polyvinylidene difluoride; spgp, sister P-glycoprotein; TBS-T, Tris buffered saline with 0.3% Tween 20

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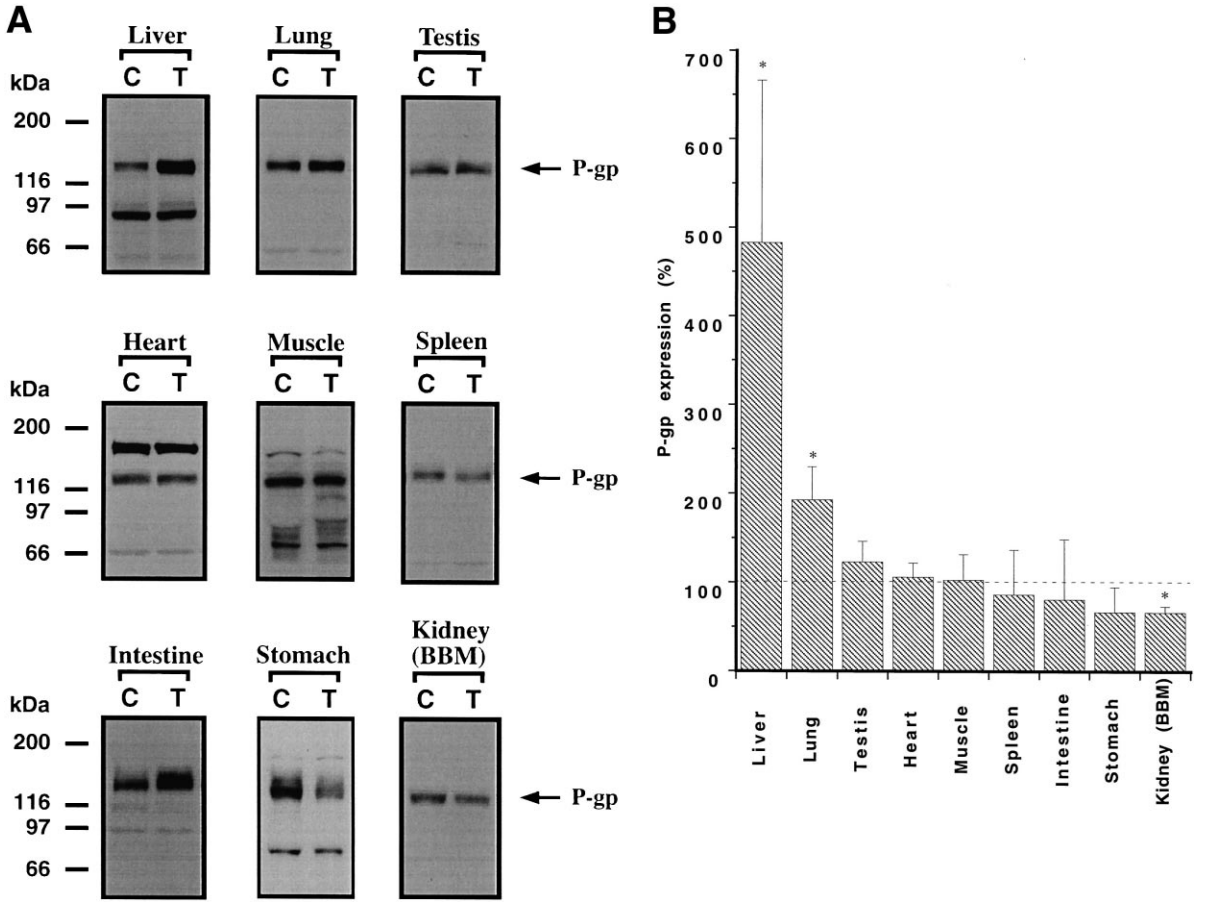

Fig. 1. Immunodetection of P-gp in tissues after dexamethasone treatment. Rats were treated with a daily dose (1 mg kg^{-1} day^{-1}) of dexamethasone for 4 days. A: Protein samples (40 μg) from kidney (BBM), and from crude membranes of liver, lung, muscle, heart, testis, stomach, spleen and intestine were isolated from control (C) and treated (T) rats and were resolved by SDS-PAGE. Immunoblots were performed with mAb C219, as described in Section 2. B: Quantification of P-gp expression in normal tissues after dexamethasone treatment. Immunoreactive protein bands corresponding to P-gp were evaluated by laser densitometry. P-gp levels are expressed as a percentage of the total amount of immunoreactive protein present in tissue fractions isolated from control rats. Values represent means ± S.E.M. for at least three independent experiments.

2.2. Dexamethasone treatments

Male Sprague-Dawley (Charles River) rats weighing 300–350 g were treated by gavage with dexamethasone (1 mg kg^{-1} day^{-1}) in corn oil. The control rats received only corn oil. Rats were treated with dexamethasone or corn oil for 1, 2, 3 or 4 days and killed 24 h after the last administration. The effect of interruption of dexamethasone administration was studied by treating rats daily with dexamethasone for 4 days and killing them 1, 3 or 6 days after cessation of treatment. For all treatments, each group of animals was made up of three rats.

2.3. Isolation of tissue crude membrane fractions and renal BBM

Crude membrane fractions were prepared from liver, lung, testis, heart, muscle, spleen, intestine and stomach from control and treated rats. The tissues obtained from individual animals of each group were pooled and homogenized in a buffer containing 250 mM sucrose and 10 mM HEPES-Tris (pH 7.4), with a Polytron tissue homogenizer (Brinkman Instruments, Rexdale, Ont.), and the homogenates were centrifuged at $3000 \times g$ for 10 min. The supernatants were then centrifuged at $33\,000 \times g$ for 30 min, and the pellets containing the crude membrane fractions were resuspended in 50 mM mannitol, 20 mM HEPES-Tris, pH 7.5, and stored at −80°C. Renal BBM from control and treated rats were prepared by $MgCl_2$ precipitation [18]. Purified BBM were resuspended in 300 mM mannitol, 20 mM HEPES-Tris, pH 7.5, and stored at −80°C. Protein concentration was determined with the Bradford assay [19].

2.4. Detection of P-gp and cMOAT

Proteins (40 μg) from various tissues were separated by SDS-PAGE on a 6.25% acrylamide gel according to the method of Laemmli [20]. P-gp was detected by Western blot analysis using mAbs C219 and MDR Ab-2 (1:1000) as described previously [7]. A horseradish peroxidase-conjugated anti-mouse IgG was used as secondary antibody and P-gp was revealed with ECL reagents according to the manufacturer's instructions. cMOAT was also detected by Western blot analysis using the pAb developed against the C-terminal portion of this transporter. A horseradish peroxidase-conjugated anti-rabbit IgG and ECL reagents were used to reveal cMOAT.

2.5. Enzyme assays

Alkaline phosphatase and leucine aminopeptidase were measured spectrophotometrically at 410 nm by standard methods as previously used [21]. γ-Glutamyltranspeptidase activity was assayed at 410 nm with γ-glutamyl-*p*-nitroanilide as a substrate as described previously [7].

2.6. Immunization and antibody purification

The peptide EAGIENVNHTEL, corresponding to a C-terminal portion of cMOAT, was synthesized using MAPS chemistry and was obtained from Service de Séquence de Peptides de l'Est du Québec (Centre Hospitalier de l'Université Laval, Québec, Que.). Rabbit immunization and antibody purification were performed as previously described [22].

2.7. Densitometric and statistical analysis

The intensity of the bands obtained from Western blot analysis and from the photolabeling experiments was estimated with a Personal

densitometer SI (Molecular Dynamics, Sunnyvale, CA). Molecular mass determination was performed with the following standards: myosin (200 kDa), β-galactoside (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (43 kDa). Statistical analyses were performed using Student's paired *t*-test in the Excel program (Microsoft Excel 4.0) on a Macintosh computer. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of dexamethasone treatment on P-gp expression in rat tissues

Western blot analysis using mAb C219, which recognized all P-gp isoforms [3], was used to evaluate P-gp expression in tissues from rats treated with dexamethasone (1 mg kg^{-1} day^{-1}) for 4 days (Fig. 1). In treated rats, an increase in the level of P-gp was observed in liver and lung. In contrast, P-gp expression was decreased in kidney while it was unchanged in testis, heart, muscle, spleen, intestine and stomach. The modulation of P-gp expression was evaluated by laser densitometry, and levels of P-gp were expressed as a percentage of the corresponding control group (Fig. 1B). In liver and lung, the level of P-gp detected was increased 4.8- and 1.9-fold compared to control rats whereas in renal BBM it was reduced 40%. Many gastrointestinal side effects are associated with dexamethasone administration, and this may explain the variations in P-gp levels in intestine and stomach observed during drug treatments. To determine whether the decrease in P-gp expression may be related to a generalized atrophy, the weight of tissues from control and treated rats was measured (data not shown). The weight of tissues from treated rats was very similar to that of control rats, except for spleen where it was reduced 50% by dexamethasone.

In order to determine which isoform was modulated by dexamethasone in Fig. 1, Western blot analysis using a mAb directed specifically against human MDR1 that does not recognize MDR3 (mAb MDR Ab-2) was also performed (Fig. 2). In treated rats, P-gp level was increased 140% in lung whereas it was decreased 40% in renal BBM (Fig. 2B). These results are very similar to the modulation of P-gp expression evaluated using mAb C219. In contrast with the results obtained with C219 in liver, P-gp level detected in this tissue using mAb MDR Ab-2 was weak and very similar in control and treated rats. These results suggest that dexamethasone modulation involved mdr1 isoforms in lung and renal BBM whereas it involved mdr2 in liver. In addition, dexamethasone reduced by 40% the photolabeling of P-gp by IAAP in renal BBM whereas in liver no labeled P-gp was detected (results not shown). Since IAAP labels mdr1 and not mdr2 [23], the photolabeling experiments confirmed the results obtained with mAb MDR Ab-2. However, we cannot rule out the possibility that dexamethasone could induce the sister of P-gp (spgp) since this novel putative canalicular ABC transporter of 160 kDa has two amino acid sequences (VQEALN and VQTALD) that are similar to the epitopes recognized by mAbC219 [24]. This spgp should be detected by mAb C219 but not by mAb MDR1 Ab-2 since the epitopes recognized by this antibody are not conserved. This protein contains an additional 40 amino acids (about 5 kDa) compared to mdr isoforms. Thus, the difference in the molecular weight between mdr isoforms and spgp should be detected with mAb C219 by Western blots. However, no band with a higher molecular weight than mdr isoforms and no doublet were detected suggesting that this spgp of 160 kDa was not induced by dexamethasone. In addition, Western blots using mAb C219 detected only one band in liver with an apparent molecular weight of 145 kDa, similar to the mdr1 isoforms detected with mAb Ab-2, suggesting once again that mAb C219 recognizes mainly mdr isoforms in these crude membranes.

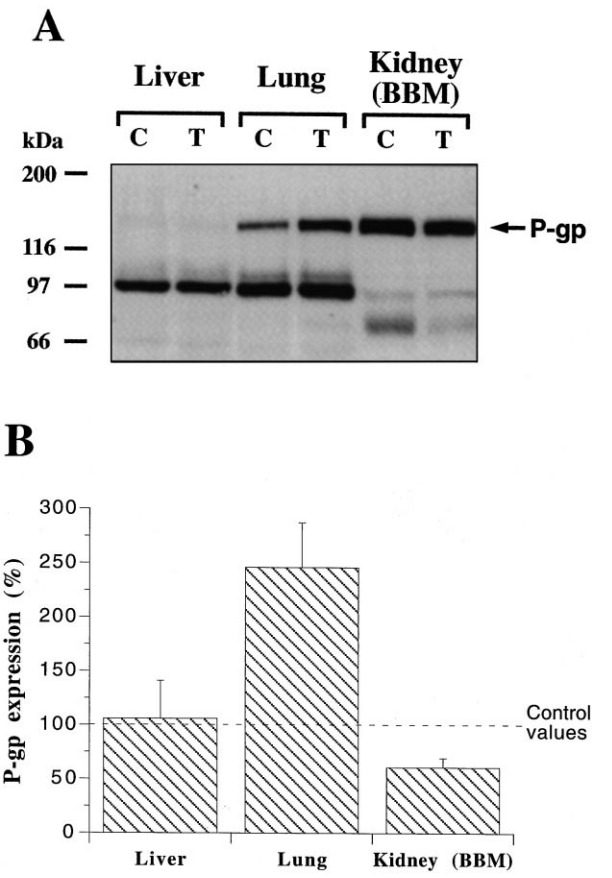

Fig. 2. P-gp detection by Western blots using mAb MDR Ab-2. Rats were treated daily with dexamethasone (1 mg kg^{-1}) for 4 days. They were killed 24 h after the last gavage. Liver, lung and renal BBM were isolated from control (C) and treated (T) rats. A: Protein samples (40 μg) were resolved by SDS-PAGE. Immunoblots were performed with mAb MDR Ab-2 (1:1000), as described Section 2. B: Quantification of P-gp detection using mAb MDR Ab-2 in normal tissues after dexamethasone treatment. Immunoreactive protein bands corresponding to P-gp were evaluated by laser densitometry. P-gp levels are expressed as a percentage of the total amount of immunoreactive protein present in tissue fractions isolated from control rats. Values represent means ± S.E.M. for at least three independent experiments.

Liver, lung and renal BBM were also isolated from rats treated daily with dexamethasone (1 mg kg^{-1} day^{-1}) for 1, 2, 3, or 4 days and at 1, 3, and 6 days after the end of 4 days treatment. Modulation of P-gp was followed by Western blot analysis using mAb C219 (Fig. 3A). The band corresponding to P-gp was analyzed by laser densitometry and the data were expressed as percentage of the P-gp expression in control rats (Fig. 3B). In liver and lung, P-gp expression was rapidly increased and reached a maximum after 2 days treatment with dexamethasone. In renal BBM, P-gp expression decreased after 2 days of dexamethasone administration. In these three tissues, the level of P-gp expression returned to control values after the interruption of dexamethasone treatment.

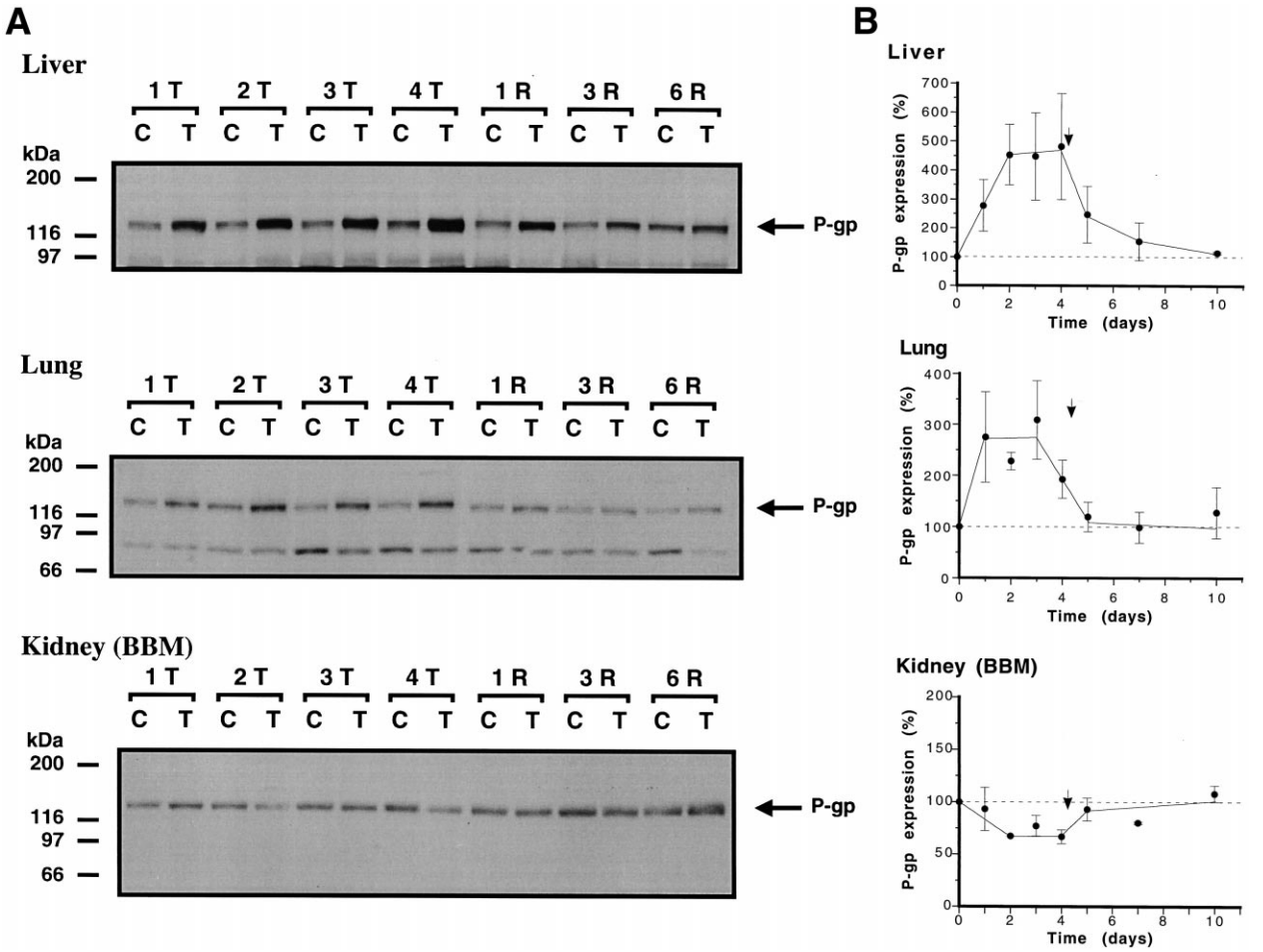

Fig. 3. Immunodetection of P-gp in normal tissues during treatment with dexamethasone. Rats were treated with a daily dose (1 mg kg^{-1} day^{-1}) of dexamethasone for 1, 2, 3, and 4 days and killed 24 h after each gavage. Membranes from liver, lung and kidney (BBM) were isolated from these rats as well as from rats which were treated daily with dexamethasone for 4 days and than killed at 1 (R1), 3 (R3) and 6 (R6) days after cessation of treatment. A: Protein samples (40 μg) from liver, lung and kidney isolated from control (C) and treated (T) rats were resolved by SDS-PAGE. Immunoblots were performed with mAb C219, as described in Section 2. B: Quantification of P-gp expression in liver, lung and kidney (BBM) after dexamethasone treatment. Immunoreactive protein bands corresponding to P-gp were evaluated by laser densitometry. P-gp levels are expressed as a percentage of the total amount of immunoreactive protein present in tissue fractions isolated from control rats. Arrows indicate the interruption of the treatment. Values represent means ± S.D. obtained from two independent experiments.

3.2. Effect of dexamethasone treatment on cMOAT expression

The expression of cMOAT was also evaluated in liver and kidney by Western blot using a polyclonal antibody that we developed against the C-terminal portion of cMOAT. This transporter was detected as a 170–190 kDa protein depending on the acrylamide concentration of the gel. The level of cMOAT was higher in liver than in renal BBM from control rats (Fig. 4). cMOAT expression was evaluated in these two tissues from rats treated daily with dexamethasone for 1, 2, 3, and 4 days and from rats at 1, 3 and 6 days after the end of the treatment. cMOAT levels were rapidly increased in both tissues by dexamethasone. A strong band of lower molecular weight was also detected in liver, whereas in kidney it was very faint. This protein does not seem to be related to a degradation product since its detection in both tissues does not correlate with variations in cMOAT levels caused by dexamethasone. Our results suggest that this pAb directed against cMOAT also detects unrelated proteins of lower mo-

Table 1
Effect of dexamethasone on membrane markers in renal BBM, liver and lung

Tissue	Alkaline phosphatase (nmol min^{-1} mg^{-1})		Leucine aminopeptidase (nmol min^{-1} mg^{-1})		γ-Glutamyltranspetidase (nmol min^{-1} mg^{-1})	
	Control	Treated	Control	Treated	Control	Treated
Renal BBM	3340 ± 630	2200 ± 340*	297 ± 31	247 ± 24	8660 ± 1130	8470 ± 1210
Liver	5.9 ± 0.5	5.0 ± 0.4	20 ± 2	15 ± 2	BD	BD
Lung	130 ± 21	96 ± 12	27 ± 1	29 ± 4	22 ± 1	29 ± 7

Alkaline phosphatase, leucine aminopeptidase and γ-glutamyltranspeptidase activities were measured in various samples isolated from control and treated rats. Values represent means ± S.E.M. obtained from at least three different treatments.
*$P < 0.05$; BD = below detectable levels.

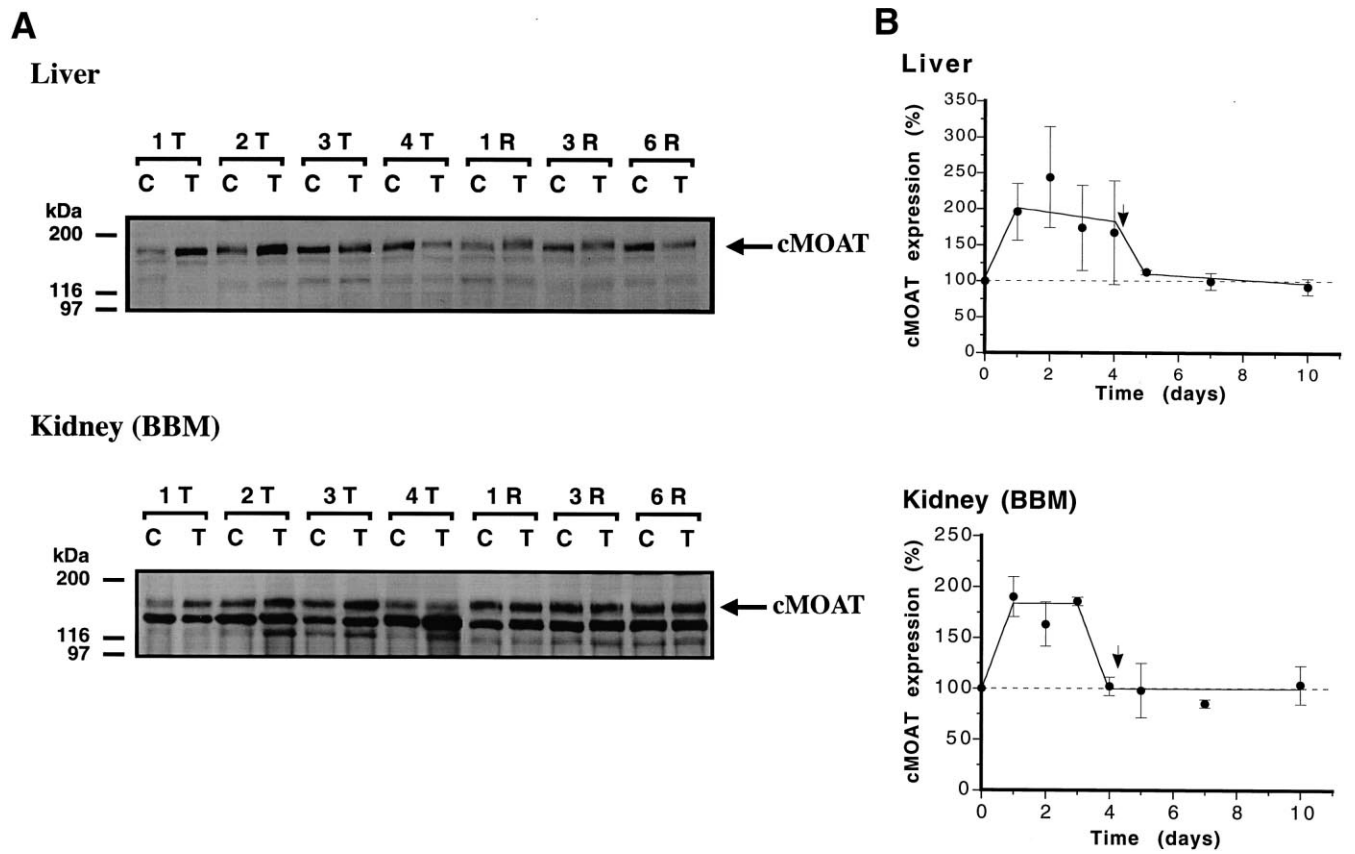

Fig. 4. Immunodetection of cMOAT in liver and in kidney during treatment with dexamethasone. Rats were treated with a daily dose (1 mg kg^{-1} day^{-1}) of dexamethasone for 1, 2, 3, and 4 days and killed 24 h after the last gavage. Membranes from liver and kidney (BBM) were isolated from these rats as well as from rats which were treated daily with dexamethasone for 4 days and then killed at 1 (R1), 3 (R3) and 6 (R6) days after cessation of treatment. A: Protein samples (40 μg) from crude membranes of liver and kidney (BBM) isolated from control (C) and treated (T) rats were resolved by SDS-PAGE. Immunoblots were performed with a pAb directed against cMOAT, as described in Section 2. B: Quantification of cMOAT expression in liver and in renal BBM after dexamethasone treatment. Immunoreactive protein bands corresponding to cMOAT were evaluated by laser densitometry. CMOAT levels are expressed as a percentage of the total amount of immunoreactive protein present in tissue fractions isolated from control rats. Arrows indicate the interruption of the treatment. Values represent means ± S.D. obtained from two independent experiments.

lecular weight. The results obtained for cMOAT were analyzed by laser densitometry and the data were expressed as the percentage of cMOAT expression in tissues from control animals (Fig. 4B). The cMOAT levels increased approximately 2-fold during the first 3 days of dexamethasone administration, decreased the following day and returned to control values after cessation of the treatment. We have also verified whether this antibody could cross-react with other MRP family members. The C-terminal portion of MRPs and cMOATb is not very well conserved suggesting that this antibody does not cross-react with other MRPs. For example, only one of these 12 amino acids is identical between Mrp1 and Mrp2.

3.3. Effect of dexamethasone treatment on membrane enzymes

The activity of membrane marker enzymes was also evaluated in various samples isolated from control and treated rats (Table 1). In renal BBM, the activity of leucine aminopeptidase and γ-glutamyltranspeptidase were similar in control and dexamethasone-treated rats. However, the specific activity of alkaline phosphatase decreased 35% in renal BBM after 4 days of drug treatment as well as in intestine (data not shown). The activity of the three enzymes was much lower in crude membranes from other tissues than in renal BBM. In liver membranes γ-glutamyltranspeptidase was undetectable while the two other enzyme activities were similar in control and treated rats. The enzyme activities measured in lung were also unaffected by the drug. These results suggest that the effects of dexamethasone on P-gp and cMOAT in liver, lung and kidney are specific and not related to general tissue degradation.

4. Discussion

The results reported here show that in vivo administration of dexamethasone modulates P-gp and cMOAT expression. An increase in P-gp levels following dexamethasone administration was detected in liver and lung. This increase was rapid since it was observed after only 1 day of treatment. For example, subcutaneous injections of cyclosporin A (10 mg kg^{-1} day^{-1}) are required for 4 or 5 days to induce P-gp expression by 50–100% in various tissues (liver, kidney, intestine, lung, heart and stomach) [7] whereas 1 day of dexamethasone treatment increased P-gp expression more than 2.5-fold in liver and lung. This increase of P-gp levels in liver by dexamethasone contrasts with previous in vitro studies that have shown an early and conspicuous increase of P-gp mRNA levels in cultured rat hepatocytes while dexamethasone-treated cells repressed expression of the *mdr1b* gene [13] and delayed increase

of mdr mRNA [15]. However, dexamethasone has been shown to increase the mdr1a and mdr1b proteins in a mouse hepatoma cell line [14] and mdr1b levels in the rat hepatoma cell line H35 [16]. MDR1 mRNA levels were also reported to be elevated in a dexamethasone-treated human hepatoma cell line but not in a non-hepatoma cell line suggesting that the hormonal regulation of *mdr* gene expression is gene and cell type specific [14]. A more recent study reported that administration of dexamethasone to male rats caused a five-fold increase in P-gp expression and a three-fold increase in mdr2 mRNA [17]. In contrast to these results, administration of dexamethasone to female rats decreased P-gp expression and mdr2 mRNA by 60% and 30%, respectively, suggesting that the gender response to the drug could result from effects on a hormonal regulatory pathway. These measurements were performed after 3–4 days of intraperitoneal administration of dexamethasone at 80 mg kg^{-1} day^{-1}. In the present study, both Western blots and photoaffinity labeling experiments strongly suggest that a much lower dose of dexamethasone (1 mg kg^{-1} day^{-1}) was sufficient to induce a 2.7-fold increase in mdr2 expression in liver after only 1 day of treatment. We have to mention that we cannot rule out the possibility that dexamethasone may induce spgp [24]. However, Western blot analysis performed in the present study strongly suggests that mdr2 is modulated by dexamethasone rather than spgp. This dose corresponds to the concentration used in the vincristine-adriamycin-dexamethasone regimen (40 mg day^{-1}) as well as to the initial doses (10–100 mg) for neurologic syndromes [25,26]. Since various liver functions have been demonstrated to be strongly modulated by corticosteroid hormones and P-gp expression could be regulated by a hormonal regulatory pathway [17], the reported variation in the effect of dexamethasone on P-gp expression may be caused by differences between the in vivo and in vitro responses to the drug.

In the present study, lung was the only tissue other than liver where P-gp expression was increased by dexamethasone. P-gp was previously detected in luminal membranes of the vascular endothelium isolated from lung [27] and its presence has also been reported in bronchial cells [28]. In this tissue, glucocorticoids have been shown to increase the accumulation of surfactant apoprotein in adult rat lung compartments after dexamethasone treatments [29]. The increase of P-gp levels in lung suggest that it may be involved in the regulation of the lipid and protein components of surfactants which are hydrophobic molecules. Furthermore dexamethasone, which is commonly used for anti-inflammatory therapy in asthma and in interstitial lung disease, affects various lung processes [30,31]. It was suggested that dexamethasone inhibition of lung epithelial cell apoptosis induced by interferon-γ and Fas may be one mechanism by which it suppresses the inflammatory response [32]. In other cells, the anti-inflammatory activity of dexamethasone was associated with inhibition of cytokine-stimulated collagenase and stromelysin synthesis [33]. Other matrix metalloproteases have also been reported to be involved in this anti-inflammatory process of dexamethasone [34,35]. It is unclear to what extent any of these processes affected by dexamethasone is associated with the modulation of P-gp expression.

P-gp expression in many tissues was unaffected by dexamethasone (testis, heart, muscle, spleen, intestine and stomach). However, a decrease in P-gp levels following dexamethasone treatment was detected in renal BBM. Although dexamethasone is a potent corticosteroid the mechanism that may explain the decrease in P-gp levels detected in specific tissues remains unknown. The anti-inflammatory, immunosuppressant, anti-neoplastic and anti-emetic properties of this drug suggest that it has many targets. In renal BBM, we have seen a decrease in alkaline phosphatase activity. Alterations in the activity of this enzyme by dexamethasone were also found in intestinal BBM [36]. These results suggest that dexamethasone does not cause a general degradation of the renal BBM since two other membrane markers (aminopeptidase M and γ-glutamyltranspeptidase) were unaffected by the drug.

In contrast to the effects of dexamethasone on P-gp activity and expression, the effect of dexamethasone on cMOAT is totally unknown. Using Western blotting analysis, we evaluated the effect of the drug on the expression of this transporter. Surprisingly, a rapid increase in cMOAT levels was detected in liver and renal BBM. The mechanisms involved in this modulation of cMOAT by dexamethasone remain unclear. Physiologically important substrates for this transporter include glutathione *S*-conjugates such as leukotriene C4, bilirubin glucuronides and glutathione disulfide [10], which may be affected by dexamethasone. In fact, dexamethasone was shown to bind to a subclass of glutathione *S*-transferases in rat liver [37] and to decrease bilirubin levels in patients with metastatic colorectal cancer [38]. The increased cMOAT levels could thus be related to variations in the content of its substrates. In addition, our results suggest that the cMOAT response to dexamethasone takes place in two steps. One possibility, as often seen with steroid hormones, could involve a primary response which rapidly induces cMOAT expression and a secondary response which reduces its expression even before the end of the treatment.

In conclusion, there was a rapid modulation of P-gp and cMOAT expression in normal tissues which was reversible. Since dexamethasone is widely administered for different conditions, 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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