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Biochemical Pharmacology 68 (2004) 791–798
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Effect of acetaminophen on expression and activity of rat liver multidrug resistance-associated protein 2 and *P*-glycoprotein

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Received 6 April 2004; accepted 11 May 2004

Abstract

We evaluated the effect of acetaminophen (APAP), given as a single, 1 g/kg body weight dose, on expression and activity of rat liver multidrug resistance-associated protein 2 (Mrp2) and *P*-glycoprotein (P-gp), two major canalicular drug transporters. The studies were performed 24 h after administration of the drug. APAP induced an increase in plasma membrane content of Mrp2 detected by western blotting, consistent with increased detection of the protein at the canalicular level by immunoflourescence microscopy. In vivo biliary excretion of dinitrophenyl-*S*-glutathione, a well known Mrp2 substrate, was slightly but significantly increased by APAP, agreeing well with upregulation of the transporter. Basal biliary excretion of oxidized glutathione, an endogenous Mrp2 substrate, was also increased by APAP, likely indicating increased hepatic synthesis as a result of APAP-induced oxidative stress followed by accelerated canalicular secretion mediated by Mrp2. APAP also increased the expression of P-gp detected by western blotting and immunofluorescence microscopy as well as the in vivo biliary secretory rate of digoxin, a model P-gp substrate. Because specific APAP-conjugated metabolites are Mrp2 substrates, we postulate that induction of Mrp2 by APAP may represent an adaptive mechanism to accelerate liver disposition of the drug. In addition, increased Mrp2-mediated elimination of oxidized glutathione may be essential in maintaining the redox equilibrium in the hepatocyte under conditions of APAP-induced oxidative stress.

Keywords: Acetaminophen; Mrp2; *P*-Glycoprotein; Bile secretion; Glutathione; Liver toxicity

1. Introduction

Acetaminophen (APAP) is one of the most commonly used analgesic and antipyretic drugs. It is generally accepted as a safe drug when administered within the therapeutic range. However, an overdose can induce severe hepatotoxicity in human and experimental animals [1]. Its toxic effect on rat liver is well documented [2,3] and involves a reversible alteration of hepatic excretory function [4] among other alterations. Primarily, APAP is metabolized in the liver by conjugating enzymes (phase-II metabolism), mainly suffering glucuronidation and sulphation, thus generating the non-toxic metabolites, APAP-glucuronide and APAP-sulphate [1]. Under APAP overloading conditions, these metabolic paths are saturated, and a substantial amount of the drug is metabolized by the CYP450 system. This system oxidizes APAP, generating a reactive metabolite, NAPQI, which can reacts with GSH to form a conjugate. This step is considered to be a detoxification pathway since free NAPQI exerts its toxicity by binding to macromolecules like proteins, lipids and DNA [5]. In consequence, an efficient phase II-mediated metabolism of APAP followed by bile secretion of the conjugated metabolites could be instrumental in neutralizing drug toxicity.

Abbreviations: ABC, ATP-binding cassette; APAP, acetaminophen; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDNB, 1-chloro-2,4-dinitrobenzene; DNP-SG, dinitrophenyl-*S*-glutathione; GSH, reduced glutathione; GSSG, oxidized glutathione; MDR, multidrug resistance protein; Mrp2, multidrug resistance-associated protein 2; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NO, nitric oxide; nrf, nuclear factor erythroid 2-related factor; P-gp, *P*-glycoprotein

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doi:10.1016/j.bcp.2004.05.014

The transport of glucuronide, sulphate and glutathione conjugates into the extracellular space has been characterized as a primary-active, ATP-dependent transport and is mediated by members of the family of ABC transporters known as multidrug resistance-associated protein [6,7]. One of these isoforms, Mrp2 or canalicular multispecific organic anion transporter (cMOAT), mediates the transport of conjugated compounds across the apical membrane domain of liver, tubular renal and intestinal cells [6–10]. It has been proposed that APAP-glucuronide is a Mrp2 substrate since its biliary secretion is severely impaired in Mrp2 congenitally-deficient (TR-) rats [11]. Thus, it is possible that Mrp2 plays a role in protection against APAP toxicity through elimination of the different APAP conjugates. While the effect of an acute dose of APAP on phases I and II liver metabolism is well characterized in human and experimental animals [1,12,13], the effect of the drug on expression and activity of canalicular transporters such as Mrp2 is not known. Because of Mrp2 participation in elimination of some specific APAP metabolites, changes in Mrp2 activity induced by APAP, could lead, for example, to a significant change in drug disposition.

In the present study, we evaluated the effect of APAP, given as a single dose, on expression and activity of canalicular Mrp2 in the rat. To establish whether the effect of APAP was specific or affected other canalicular transporters, we also evaluated the expression and activity of P-gp, an important member of the ABC transporters family involved in secretion of a wide range of lipophilic and cationic drugs into bile [14]. The data indicate increased expression and transport function of both Mrp2 and P-gp. The possibility that these events represent an adaptive mechanism for protection against APAP toxicity is discussed.

2. Materials and methods

2.1. Chemicals

[H^3] digoxin (37.0 Ci/mmol) and OptiPhase liquid scintillation cocktail were purchased from Perkin-Elmer Life Science Products. Digoxin was from ICN Biomedicals Inc., whereas APAP, 3α-hydroxy-steroid dehydrogenase, leupeptin, aprotinin, phenylmethylsulfonyl fluoride, pepstatin A, CDNB, bovine serum albumin, NADPH, GSH, and glutathione reductase were from Sigma Chemical Company. All other chemicals were of analytical grade purity, and used as supplied.

2.2. Animals and experimental protocols

Male Wistar rats (250–290 g) were used throughout. The rats had free access to food and water and were maintained on a 12-h automatically timed light and dark cycle. All procedures involving animals were conducted in accordance with NIH guidelines for the Care and Use of Laboratory Animals.

2.3. Experimental groups

APAP was administered i.p. at a single dose of 1 g/kg body weight (100 mg/ml in 1:5 polyethylene glycol 400:saline solution). The control group received the same volume of vehicle i.p. All studies were performed 24 h after APAP or vehicle administration.

2.4. Basal biliary excretion of glutathione species and bile salts

The rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.), and thus maintained throughout. Body temperature was measured with a rectal probe, and maintained at 37 °C with a heating lamp. The common bile duct was cannulated with polyethylene tubing (PE10). After a 30-min stabilization period, bile was collected for 10 min in pre-weighed tubes containing 0.1 ml of 10% sulfosalicylic acid to minimize oxidation of GSH [15]. Basal bile collected in pre-weighed tubes for additional 10 min was assayed for total bile salts. Bile flow was determined gravimetrically, assuming a density of 1 g/ml. At the end of the bile collection periods, the animals were sacrificed by exsanguination and the livers were perfused for 30 s with cold saline and removed for western blotting and immunofluorescence microscopy studies, and for glutathione species determination. Sera were separated from blood samples to asses serum markers of liver damage. Aliquots of the liver samples were gently frozen in liquid nitrogen and preserved at −80 °C until used for plasma membrane preparation, or frozen in precooled isopentane for immunofluorescence studies. Other portion was homogenized (20% (w/v) in saline solution), two volumes of the homogenate were mixed with one volume of 10% sulfosalicylic acid, centrifuged at 5000 × *g* for 5 min, and the supernatant used in total glutathione and GSSG assays.

2.5. Liver transport of DNP-SG

To evaluate Mrp2 transport activity, rats from both groups were anesthetized and body temperature maintained as described above. The jugular vein and the common bile duct were cannulated with polyethylene tubing (PE50 and PE10, respectively). After a 30-min stabilization period, a single bolus of CDNB (10 μmol/kg in 1:19 dimethylsulfoxide:saline) was administered i.v. Bile was collected at 10-min intervals for 60 min. Transport activity of Mrp2 was estimated by determining the concentration of DNP-SG, the glutathione derivative of CDNB formed in the liver, in bile samples.

2.6. Liver transport of digoxin

To evaluate P-gp transport activity, the jugular vein and the carotid artery as well as the common bile duct from anesthetized rats were cannulated with polyethylene tubing (PE50 and PE10, respectively). After a 30-min stabilization period, a single bolus of digoxin (2.5 μCi; 0.2 mg/kg in 1:1 ethanol:saline) was injected through the venous catheter. Arterial blood samples were taken at 1, 2, 4, 6, 8, 10, 15 and 30 min and immediately centrifuged to separate plasma. Bile was collected at 2-min intervals for the first 10 min of the experiment, at 10-min intervals for the 10–60 min period, and at 15-min intervals for the 60–120 min period. Appropriate volumes of 5% bovine serum albumin in saline solution were administered through the venous catheter to replenish body fluids. At the end of the bile collection period, the livers were perfused for 30 s with saline solution, removed, and used in homogenate preparation (25% (w/v) in saline solution). Bile flow was determined gravimetrically. Samples of bile, plasma, and homogenates were used in digoxin determination.

2.7. Preparation of liver membranes for Western blot analysis

Membrane fraction enriched in plasma membrane was prepared by differential centrifugation. Liver samples were homogenized in 0.3 M sucrose containing 0.1 mM phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, 5 μg/ml aprotinin and 5 μg/ml pepstatin A (50 mg liver per ml buffer). The homogenate was used for membrane preparation as described [16]. Protein concentration was measured using bovine serum albumin as a standard [17].

2.8. Western blot studies

Immunoblotting and subsequent densitometry were performed with mixed plasma membranes as previously described [18] using a monoclonal antibody to human Mrp2 (M_2 III-6, Alexis Biochemicals) and a rabbit polyclonal antibody to human MDR-1 (Santa Cruz Biotechnologies).

2.9. Immunofluorescence microscopy

Liver slices (5 μm) were prepared with a Zeiss Microm HM500 microtome cryostat, air dried for 2 h, and fixed for 10 min with cold acetone (−20 °C). For labeling, tissue sections were incubated overnight with the monoclonal anti-human Mrp2 (1:100) and polyclonal anti-human MDR-1 (1:100) antibodies. Sections were then washed five times with PBS, and incubated with Cy3-conjugated donkey anti-mouse or anti-rabbit IgG (Jackson ImmunoResearch Laboratory Inc.) (1:200) for 2 h. After washing three times with PBS and once with distilled water, slices were air dried and mounted. The images were captured on a Zeiss Axiovert 25 CFL inverted microscope. To ensure comparable staining and image capture performance for APAP and control groups, liver slices were prepared the same day, mounted on the same glass slide, and subjected to the staining procedure and microscopy analysis simultaneously.

2.10. Analytical methods

Total glutathione (GSH + GSSG) and GSSG concentrations in bile and liver homogenate were determined spectrophotometrically by using the recycling method of Tietze [19], as modified by Griffith [15]. The results were expressed as equivalents of GSH. Concentration of GSH in bile and liver homogenates was calculated as the difference between total and oxidized glutathione. Total bile salt concentration in bile samples was determined by using the 3α-hydroxysteroid dehydrogenase procedure [20]. Biliary concentration of DNP-SG was determined spectrophotometrically, as described [18]. Radioactivity corresponding to labeled digoxin was detected in appropriate volumes of bile, plasma, and liver homogenates with addition of 3 ml of liquid scintillation cocktail, and counted in a liquid scintillation analyzer (model 1409, Wallac counter). To determine whether APAP affects the uptake of digoxin by the main tissues involved in its metabolism, the data on plasma concentration of digoxin, plotted against time, were fitted to a typical biexponential equation by the GraphPad Prism program (GraphPad Software Inc.). The kinetic constant representing digoxin uptake was calculated using the same program.

Liver damage induced by APAP was assessed in serum samples by measuring activity of the enzymes AST, ALT and ALP with commercial kits (Boehringer Mannheim). Because increased NO synthesis in response to APAP administration was proposed to be an important mediator of APAP-induced hepatotoxicity [21], we also measured NO serum levels by chemiluminescence reaction with ozone [22].

2.11. Statistical analysis

Data are presented as the means ± S.D. Comparison between groups was performed using the Student's *t*-test. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Effect of APAP on serum markers of liver damage

Table 1 shows significantly increased values of AST and ALT but not of ALP, as previously reported for the same dosage of APAP [23], clearly indicating hepatotoxicity

Table 1
Serum markers of liver damage

	APAP	Control
AST (U/L)	225 ± 50*	39 ± 4
ALT (U/L)	279 ± 60*	98 ± 8
ALP (U/L)	601 ± 70	499 ± 85
NO (μM)	104 ± 31**	32 ± 3

Activity of AST, ALT and ALP, as well as concentration of NO were determined in serum as described in Section 2. Data are means ± S.D. of four animals per group.
* Significantly different from Control group ($P < 0.001$).
** Significantly different from Control group ($P < 0.01$).

induced by the drug. Data on Table 1 also indicates that APAP induced a significant release of NO into blood as its serum concentration exhibited a three-fold increase in response to drug administration.

3.2. Effect of APAP on expression of Mrp2 and P-gp

Expression of Mrp2 and P-gp in liver mixed plasma membranes from control and APAP treated rats was first evaluated by western blotting. Fig. 1 shows that the membrane content of Mrp2 in the APAP group was about 40% higher than in the control group, as detected by densitometry. APAP treatment also increased significantly P-gp expression by 50%. To confirm the presence of increased levels of Mrp2 and P-gp in the canalicular membrane in situ, we performed an immunofluorescence microscopy study. Fig. 2 shows that Mrp2 and P-gp displayed a typical pattern of staining in control rats, with both transporters mainly localized to the canalicular domain. In the APAP treated livers, immunofluorescence detection of both transporters was stronger than in controls, with preserved localization at the canalicular level.

3.3. Effect of APAP on transport activity of Mrp2 and P-gp

To determine the impact of the induction of Mrp2 and P-gp protein levels on their functionality, we studied the ability of the liver to secrete Mrp2 and P-gp model substrates into bile.

Biliary excretion of DNP-SG, a model Mrp2 substrate, was proposed to estimate canalicular Mrp2 activity in vivo [18,24]. This measure was significantly increased in response to APAP (40% over controls) during the first period of bile collection (see Fig. 3A). Though cumulative excretion of DNP-SG was not affected by APAP (see inset in Fig. 3A), the time-course data would indicate increased ability of the livers from APAP animals to eliminate the conjugated metabolite of CDNB at the peak of its biliary secretion. The fact that biliary secretion of DNP-SG was increased only slightly by APAP treatment would suggest that the transport system at the canalicular level might not be saturated in the current experimental conditions. This likely explains why differences between groups reached significance only when a maximal dose of the conjugated xenobiotic was available for transport, i.e. during its excretory peak. Due to its lipophilic nature, CDNB administered systemically is assumed to freely enter the liver cell. Once in the cytosol, CDNB is efficiently converted by glutathione-*S*-transferase in its glutathione conjugate. We observed that hepatic level of GSH, the co-substrate in glutathione-*S*-transferase-mediated reaction, is normal in the APAP group (see Table 2). Previous studies reported preserved [25] or decreased [26] glutathione-*S*-transferase activity towards CDNB in experimental animals in response to APAP administration. In consequence, our data on increased excretion of DNP-SG into bile in APAP animals likely reflects increased activity of Mrp2, rather than exacerbated metabolism of its parent compound,

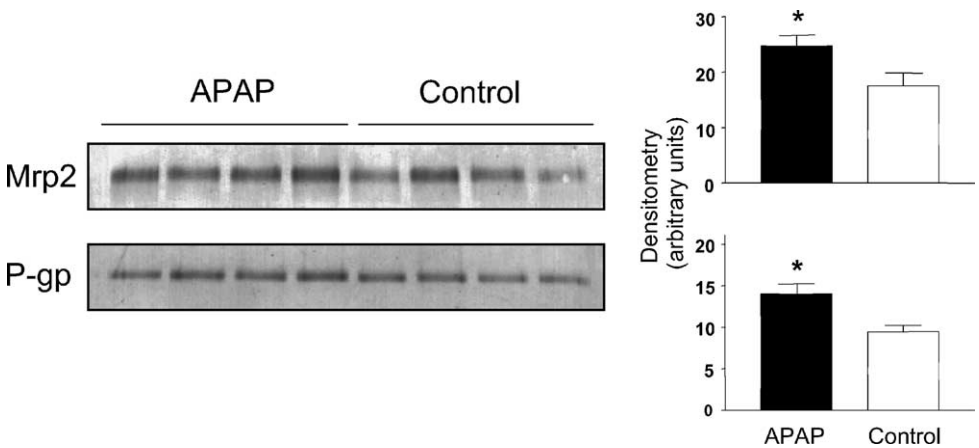

Fig. 1. Expression of Mrp2 and P-gp. Western blot analysis was performed using liver mixed plasma membranes (2 μg per well) prepared from rats treated with APAP and from control rats. Densitometry was performed in four animals per group and expressed in arbitrary units. Data are means ± S.D. (*) Significantly different from control group ($P < 0.05$).

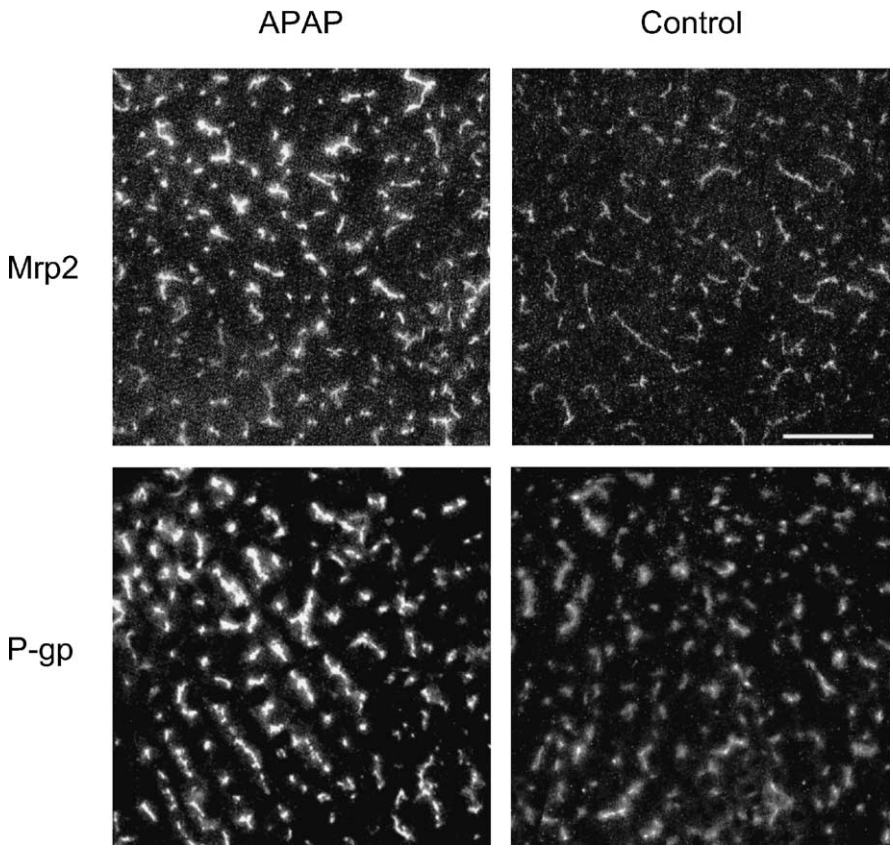

Fig. 2. In situ detection of Mrp2 and *P*-glycoprotein P-gp. Mrp2 and P-gp were detected by immunofluorescence microscopy. Similar pattern of staining was observed in three independent preparations per group. White scale bar represents 40 μm.

agreeing well with increased expression of Mrp2 at the plasma membrane level.

Mrp2 mediates the active transport of oxidized glutathione into bile and may also contribute to the active transport of reduced glutathione at the canalicular level [7,27]. Table 2 shows that biliary secretion of total glutathione was increased in the APAP group (about 50% over controls). The oxidized specie mainly accounts for this increase, as biliary excretion of GSSG was enhanced by about 130% in APAP group whereas GSH excretion was not affected. Because liver content of GSSG was not affected by APAP (see Table 2), increased secretion of this compound would indicate accelerated synthesis at the hepatic level followed by efficient biliary secretion. Glutathione species are considered critical components of biliary secretory function by generating the bile salt-independent fraction of bile flow [28]. Table 2 shows that bile flow was significantly increased by 60% in the APAP group, consistent with accelerated biliary secretion of GSSG. In contrast, biliary secretion of total bile salts, another major component of bile flow generation, was not affected by the drug (see Table 2).

To evaluate the effect of APAP on P-gp transport activity, we examined the in vivo liver transport of digoxin, a model substrate of this transporter [29,30]. Fig. 3C shows changes in biliary excretion of digoxin with time. APAP

Table 2
Bile flow, biliary excretion and liver content of glutathione species and biliary excretion of bile salts

	APAP	Control
Bile Flow (μl/min g liver)	2.78 ± 0.81*	1.77 ± 0.18
Biliary excretion of total glutathione (nmol/min g liver)	11.39 ± 3.86*	7.72 ± 2.68
Biliary excretion of GSSG (nmol/min g liver)	7.76 ± 5.33*	3.32 ± 1.40
Biliary excretion of GSH (nmol/min g liver)	3.63 ± 2.08	4.40 ± 1.95
Liver content of total glutathione (nmol g liver)	9946 ± 3763	10523 ± 3063
Liver content of GSSG (nmol/g liver)	475 ± 215	614 ± 237
Liver content of GSH (nmol/g liver)	9470 ± 3553	9909 ± 2829
Biliary excretion of total bile salts (nmol/min g liver)	45.8 ± 7.1	44.7 ± 8.7

Total glutathione, GSSG and GSH are expressed as equivalents of GSH. Data are means ± S.D. of three to five animals per group.
* Significantly different from control group ($P < 0.05$).

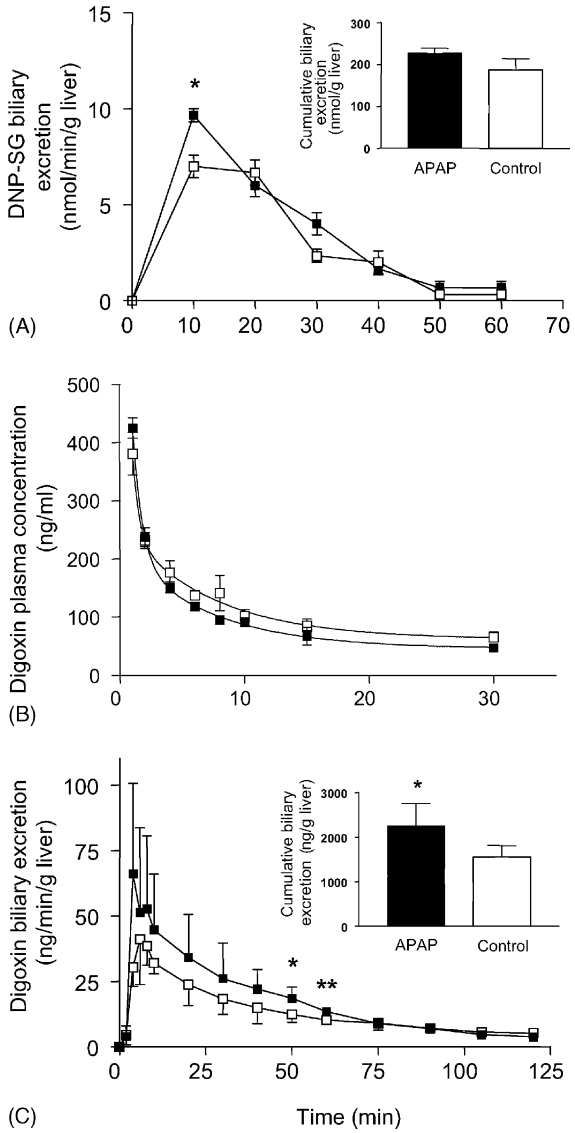

Fig. 3. Biliary excretion of DNP-SG and plasma decay and biliary excretion of digoxin. Biliary excretion of the Mrp2 and P-gp prototypical substrates, DNP-SG and digoxin, are shown in panels A and C, respectively. Insets depict cumulative biliary excretion of both substrates. Changes in plasma levels of digoxin with time are shown in panel B. Controls measures are represented by white squares and APAP-treated rat measures by black squares. Data are means ± S.D. of four animals per group. (∗) Significantly different from control group ($P < 0.05$). (∗∗) Significantly different from control group ($P < 0.01$).

produced an increase in this measure, which reached significance at 50 and 60 min after digoxin administration. The inset in Fig. 3C shows that cumulative excretion of digoxin by 120 min was clearly higher for the APAP group (about 50% over controls). As expected, percent recovery of injected digoxin was significantly higher ($P < 0.05$) for the APAP group (39.4 ± 6.5%) than for control group (27.7 ± 5.8%). Secretion at the canalicular level, rather than cellular uptake, is considered as the rate-limiting step in the overall liver transport of ABC substrates under normal conditions and in consequence, can be estimated by their biliary secretory rate. It is not known however, whether digoxin uptake may be stimulated by APAP, as a potential explanation for increased biliary secretion. The panel B in Fig. 3 shows changes of plasma digoxin concentration with time registered throughout the 30 min period. No substantial difference between groups was observed early after injection of the drug. The kinetic of decay was adjusted using a bi-exponential function to determined de two decay constants. We were particularly interested in the first one, K_1, which represents the rapid distribution in organs with high uptake. We found no difference in K_1 between the APAP (0.935 ± 0.452 min^{-1}) and control group (0.867 ± 0.380 min^{-1}). Because of the experimental model used in the current study, it is not possible to discriminate between the extent of participation of the liver or other organs in digoxin uptake as determined by the kinetic constant. However, these data, together with the fact that liver content of digoxin at the end of the experiment did not increase but rather decreased in response to APAP (283 ± 175 ng and 637 ± 145 ng of digoxin/g liver for APAP and controls respectively, $P < 0.05$) likely indicate increased efficiency for transport of the drug at the canalicular level, thus suggesting increased P-gp activity. This assumption is consistent with the reported increase in P-gp expression detected by western blotting and immunofluorescence microscopy.

4. Discussion

In this study, we evaluated the effect of a single, high dose of APAP on the expression and activity of two major canalicular drug transporters, Mrp2 and P-gp. These transporters play a crucial role in the elimination of a wide variety of endobiotics, drugs, pollutants, etc. Mrp2 is particularly involved in elimination of specific APAP conjugates and in consequence, changes in Mrp2 expression and activity may critically affect APAP liver disposition. Western blot and immunofluorescence microscopy studies revealed increased expression of both transporters in a similar extent. The in vivo biliary excretion of Mrp2 and P-gp prototypical substrates revealed increased transport activity, agreeing well with the increased amount of protein.

Transport of GSSG and glutathione conjugates at the canalicular level is well accepted to occur against a concentration gradient and to be mediated by Mrp2. [7,27,31–33]. Our data on increased biliary excretion of GSSG together with preserved liver concentration, and in consequence, negative liver-to-bile gradient, agreed well with increased Mrp2 activity detected by DNP-SG transport experiments. Changes in biliary excretion of glutathione species in response to treatment with prototypical inducers, in association with preserved intrahepatic levels, were also reported by other authors, and were linked to parallel changes in Mrp2 expression [34]. Our data also show that GSH biliary secretory rate and intrahepatic levels were not affected by APAP 24 h after its administration. An increased biliary GSSG to GSH ratio is considered to be

a sensitive indicator of oxidative stress [32,35]. In normal conditions, this oxidized fraction represents a small portion of total glutathione output, since most of GSSG formed within the cells is efficiently recycled to GSH by the enzyme glutathione reductase. Under oxidative stress conditions, the ability of the cell to reduce GSSG may be overcome, leading to cytosolic accumulation of GSSG. To protect the cell from a shift in the redox equilibrium, some of the disulfide is actively transported out of the hepatocyte, mainly to bile [32]. Though it was demonstrated that NO is an important mediator of APAP-induced hepatotoxicity [21], the mechanisms underlying the specific action of NO under such a condition are unknown. We provide evidence that the accelerated synthesis of NO in APAP animals may initiate oxidative stress events as detected by increased biliary secretion of GSSG. In consequence, it is possible to speculate that the increased expression of Mrp2 in response to APAP administration represents an adaptive mechanism to preserve the intracellular redox equilibrium.

The current data also show accelerated basal formation of bile flow in the APAP group. Hjelle and Klaassen [36] reported increased bile flow in rats 4 h after a single injection of a dose of 600 mg/kg of APAP. Because this effect was observed shortly after APAP administration, it is possible that the choleresis could result from biliary excretion of osmotically active APAP metabolites. Interestingly, we observed that choleresis persisted 24 h after APAP administration. Our data also show that biliary excretion of bile salts, the major contributor to bile flow generation, was not affected by APAP. Whether these latter results reflect preserved expression and activity of the transporter involved in canalicular secretion of bile salts in response to APAP administration is not known. Though we cannot exclude the possibility that alternative osmotically active solutes, such as endogenous bicarbonate or APAP metabolites, contribute to APAP-induced choleresis, it seems likely that increased secretion of GSSG, which is known to be a component of the fraction of bile flow independent of bile acids [28], is at least partially involved.

It was previously reported that repeated administration of increasing doses of APAP to mice for 8 days conferred resistance to an additional, normally supralethal dose of the drug [37]. The authors postulated that this was due in part to down-regulation of specific isoforms of CYP450 protein (e.g. CYP2E1 and CYP1A2) involved in conversion of APAP to NAPQI, the toxic metabolite of the drug. This likely led to increased production of alternative, less toxic metabolites of APAP. This, together with increased efficiency for excretion out of the cell, could be instrumental in decreasing APAP toxicity, and may represent an alternative mechanism to explain drug resistance. Our data on induction of Mrp2 by APAP support this possibility. We also observed that the inducing effect of APAP was not restricted to Mrp2, but also applied to P-gp, another major component of the drug secretory apparatus at the canalicular level. Whether the changes registered in P-gp function could affect APAP liver disposition is uncertain, since at present, no derivatives of APAP were identified as P-gp substrates. Because several substrates of P-gp and Mrp2 are therapeutic drugs [14], it is possible that coadministration of APAP together with these drugs may result in undesirable drug–drug interactions.

Induction of functional Mrp2 and P-gp could be a common response linked to the oxidative stress generated by APAP. It was reported that ethoxyquin and oltipraz, two antioxidant response element ligands, induced Mrp2 expression in the rat model [34] and that human MRP2 can be upregulated by redox-active compounds in MCF-7 cells [38]. Similarly, Ziemann et al. [39] reported that reactive oxygen species mediate mdr1b mRNA and P-gp protein overexpression in primary rat hepatocyte culture under increased redox conditions. Recently, it has been pointed out the importance of Nrf2 in combating oxidative stress during APAP intoxication [40]. Nrf2 is known to bind to the antioxidant response element and to regulate several human and rodent metabolic enzyme genes [41–43]. Chan et al. [40] showed that congenitally-deficient (Nrf−/−) mice were more susceptible to APAP administration than wild type because of an impaired capacity to replenish GSH stores, in concert with decreased detoxification capability. Interestingly, the P-gp gene promoter contains a sequence (–123 to –115) capable to bind either Nrf1 or Nrf2 [44]. Whether Nrf2 regulates P-gp expression during acute APAP administration needs to be explored. The mechanisms mediating Mrp2 induction under the current experimental conditions are far less known.

In summary, we demonstrated increased expression of canalicular Mrp2 and P-gp, as well as their activities, in response to administration of a single, high dose of APAP to rats. Particularly, Mrp2 induction may represent an adaptive mechanism to accelerate liver disposition of the drug and elimination of the excess of GSSG produced as a consequence of APAP-induced oxidative stress.

Acknowledgments

This work was supported by grants from UBA (B036), UNR, CONICET, ANPCyT and Fundación Antorchas. We Thank Dr. Jose Antunes Rodrigues for performing NO determinations.

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