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Acetaminophen-induced stimulation of MDR1 expression and activity in rat intestine and in LS 174T human intestinal cell line

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ABSTRACT

The well-known analgesic and antipyretic drug N-acetyl-*p*-aminophenol (acetaminophen; APAP) has been previously reported to affect MDR1 expression in rat liver. In this study, we have investigated the effect of subtoxic doses of APAP on MDR1 expression and activity in rat intestine and human intestinal cells. Administration of APAP at increasing doses of 0.2, 0.3, and 0.6 g/kg b.w., i.p. over three consecutive days, induced MDR1 expression in rat duodenum (+240%) and ileum (+160%) as detected by western blotting. This was accompanied by preserved localization of the protein at the surface of the villus, as detected by confocal immunofluorescence microscopy. MDR1 activity was increased by 50% in APAP treated rats, as evaluated by serosal to mucosal secretion of rhodamine 123 in everted intestinal sacs. Treatment with APAP also decreased by 65% the portal vein concentrations of digoxin found in anesthetized rats after intraduodenal administration of this drug, which is consistent with an APAP-induced increased efficacy of intestinal barrier for digoxin net absorption. Exposure of LS 174T human colon adenocarcinoma cells to subtoxic APAP concentration (5 mM) induced an increase in MDR1 mRNA expression (+46%), which was accompanied with an enhanced ability (+78%) to reduce intracellular content of rhodamine 123. Taken together these data suggest the existence of APAP-induced stimulation of MDR1 transcription in the intestinal epithelium. These findings are of clinical relevance, as co-administration of APAP with other MDR1 substrates could indirectly inhibit the net intestinal absorption of these drugs, leading to changes in their pharmacokinetics and therapeutic efficacy.

1. Introduction

P-glycoprotein or multidrug resistance protein-1 (MDR1, gene symbol ABCB1) is a 170 kDa protein which was first described in cancer cells resistant to chemotherapeutic drugs [1]. It is a member of the ATP-Binding Cassette (ABC) gene family. In rodents, there are two orthologues to this protein, Mdr1a and Mdr1b. As in the present study we have investigated both human MDR1 and rat Mdr1a expression and activity, for simplicity reasons the nomenclature MDR1 was used in both cases. MDR1 is an efflux pump whose activity directly dependent on ATP hydrolysis results in a decreased intracellular accumulation of a wide range of cationic, hydrophobic, both endogenous and xenobiotic compounds.

MDR1 is constitutively expressed at the apical membrane of mature epithelial cells of different organs, including liver, brain, adrenal gland, kidney and intestinal tract. Owing to this tissue distribution, MDR1 plays an important role in the pharmacokinetics of many clinically relevant therapeutic drugs [2,3]. In the small intestine and colon, MDR1 is one of the most important efflux proteins [4]. A role for intestinal MDR1 in modulating bioavailability of several orally administered drugs, with consequences on their pharmacological potency has been demonstrated [5]. Because these drugs may act as substrates, inhibitors, or inducers of MDR1 [6,7], drug–drug interactions may occur when they are co-administered clinically.

N-acetyl-*p*-aminophenol (acetaminophen, APAP) is one of the most sold over the counter analgesic and antipyretic drugs. Its administration to rats, either at single toxic (1 g/kg b.w., i.p.) or repeated subtoxic (0.2, 0.3, and 0.6 g/kg, i.p., injected in three consecutive days) doses, up-regulated the expression and activity of MDR1 and multidrug resistance-associated proteins Mrp2 and Mrp3 in liver, respectively [8,9]. Because APAP-glucuronide is a

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major metabolite in rats and a common substrate for both Mrp2 and Mrp3, induction of these transporters resulted in significant changes in APAP pharmacokinetics and liver toxicity [10]. Interestingly, Manov et al. have described the induction of MDR1 by APAP also in two human liver cell lines, HepG2 and Hep3B [11], suggesting sensitivity of human ABCB1 gene to APAP in a similar fashion as described for rodents.

Further studies have confirmed changes in the expression of MDR1 by APAP in human liver. Indeed, Barnes et al. described the induction of MDR1 in liver biopsies from patients acutely intoxicated with APAP, which would suggest a parallel increased activity [12]. These authors speculated that prevention of hepatocyte damage through increased efflux of potentially toxic compounds is mediated in part by ABC transporters, together with decreased expression of uptake transporters, induction of detoxification enzymes, and proliferation of hepatocytes, may represent a compensatory response.

While the effect of APAP on modulation of MDR1 in the liver has been well characterized, the effect of APAP on expression of intestinal MDR1 or its functional consequences is poorly understood. Due to the frequent co-administration of APAP with other therapeutic drugs, and the important role of intestinal MDR1 in limiting their absorption, any effect of APAP on MDR1 activity might result in drug–drug interactions. In this study we explored if treatment with repeated, subtoxic doses of APAP, induces the expression and activity of intestinal MDR1 in the rat. Additionally we explored the effect of subtoxic concentrations of APAP on MDR1 expression and activity in the human intestinal cell line LS 174T. The data indicate that APAP up-regulated intestinal MDR1, with concomitant increase in its transport activity. As a consequence, intestinal absorption of intraduodenally administered digoxin, a well-known MDR1 substrate, was significantly decreased. A similar effect on the human ABCB1 gene was confirmed in LS 174T cells.

2. Methods and materials

2.1. Chemicals

[^{3}H]digoxin (37.0 Ci/mmol) and OptiPhase liquid scintillation cocktail were purchased from PerkinElmer Life Science Products (Boston, MA). Unlabeled digoxin was from ICN Biomedicals Inc. (Costa Mesa, CA). APAP, verapamil, rhodamine 123, urethane, dimethyl sulfoxide (DMSO), leupeptin, aprotinin, phenylmethylsulfonyl fluoride (PMSF) and pepstatin A were from Sigma Chemical Company (St. Louis, MO). All other chemicals were of analytical grade purity.

2.2. Experiments in rats

2.2.1. Animals and treatments

Male Wistar rats (250–290 g) 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 (Institutional Animal Care and Use Committee Guidebook, 2nd ed., 2002). The rats included in APAP group received APAP dissolved (100 mg/ml) in carboxymethylcellulose solution (1%, w/v) administered i.p. at three increasing doses (0.2, 0.3, and 0.6 g/kg b.w.) once per day over three consecutive days. Control group received only the same volume of vehicle, which was also administered i.p. for three consecutive days. Unless otherwise stated, all studies were performed 24 h after the last dose of APAP or the vehicle.

2.2.2. Specimen collection and brush border membrane preparation

Rats were anesthetized with urethane (1 g/kg b.w., i.p.) and two segments of the small intestine were removed. The proximal segment was selected as the first 20 cm starting from the pylorus, whereas the distal portion was selected as the last 20 cm close to the ileocecal valve. They were carefully rinsed with ice-cold saline, and a small fraction was immediately frozen in isopentane pre-cooled with liquid nitrogen and kept at −80 °C until used in immunofluorescence microscopy studies. The rest of the segments were immediately used in the isolation of brush border membranes (BBM). To obtain BBM, the mucosal tissue was obtained by scraping [13], homogenized, and processed as described previously [14]. Protein concentration in membrane preparations was measured using bovine serum albumin as standard [15].

2.2.3. Western blot

Detection of MDR1 was performed on BBM using a rabbit polyclonal antibody to human MDR1 (Santa Cruz Biotechnologies, Santa Cruz, CA), as previously described [8]. Densitometry was performed using the Gel Pro Analyzer (Media Cybernetics, Inc., Silver Spring, MD) software.

2.2.4. Immunofluorescence microscopy

Intestinal slices (5 μm) were prepared with a Zeiss Microm HM5000 microtome cryostat, air dried for 1 h, and fixed for 10 min with cold acetone (−20 °C). Tissue sections were incubated overnight with the anti-MDR1 antibody (1:100), followed by treatment with Cy3-conjugated donkey anti-IgG (Jackson ImmunoResearch Laboratory, Inc., West Grove, PA). For detection of the nucleus, the slices were incubated with 1.5 μM 4′,6-diamidino-2-phenylindole (DAPI, Molecular Probes, CA) for 5 min, just before the last washing. All confocal studies were performed in a Nikon C1 Plus microscope (Tokyo, Japan).

2.2.5. Assessment of MDR1 activity in intestinal sacs

In different sets of rats, the distal portion of the ileum was removed, gently rinsed with ice-cold saline, and immediately used to test MDR1 activity in vitro as described below. Intestinal sacs were everted and the serosal to mucosal transport of rhodamine 123, used here as a model MDR1 substrate, was evaluated at 37 °C. Segments (10 cm) of the everted sacs were filled with Krebs–Henseleit buffer (40 mM glucose, pH 7.4) previously gassed with O_2/CO_2 (95:5) and containing 15 μM rhodamine 123 (serosal compartment). They were incubated in 5 ml of the same buffer, with or without the addition to the mucosal compartment of 100 μM verapamil, used here as MDR1 inhibitor. The mucosal medium was continuously gassed with 95% O_2–5% CO_2 and aliquots of 100 μl were sampled every 5 min for a 40-min period. Then, the sacs were gently dried with filter paper and weighed. Rhodamine 123 concentration was determined spectrofluorometrically (excitation wavelength = 488 nm; emission wavelength = 550 nm) in samples of mucosal medium [16].

2.2.6. Assessment of intestinal absorption of digoxin

To study the functional repercussion of enhanced MDR1 expression, in vivo intestinal absorption of digoxin, a well-known substrate of this protein, was determined. Rats were anesthetized with urethane (1 g/kg b.w., i.p.). Body temperature was controlled with a rectal probe, and maintained at 37 °C with a heating lamp. A dose of 25.6 nmol/kg b.w. of digoxin (17.0 μmol of [^{3}H]digoxin/mol of unlabeled digoxin) was administered directly into the duodenum using a P50 cannula. To study the portal content of digoxin, a PE10 tubing was inserted into the portal vein and secured with an adhesive agent. Blood from portal vein was sampled every 5 min for 30 min after digoxin administration and

the serum was used to determine digoxin concentrations by liquid scintillation analysis.

2.3. Experiments in human cell lines

2.3.1. Human cell cultures and treatments

LS 174T (CL-188) cell line generated from colorectal adenocarcinoma and HepG2 generated from hepatocellular carcinoma (passage 7–15, ATCC, Rockville, MD, USA) were used. Cells were plated at 75 cm^2 flasks (TPP, Trasadingen, Switzerland) and seeded in manufacturer recommended medium. They were supplemented with 10% fetal calf serum (TDI S.A., Madrid, Spain), 1% antibiotic–antimycotic solution (Sigma–Aldrich, Madrid, Spain) and incubated at 37 °C under a 5% CO_2/95% humidified atmosphere. When a 60–70% confluence was reached, the cells were replicated or used in the experiments.

2.3.2. APAP cytotoxicity assessment

To establish a range of subtoxic concentrations of APAP, the effect of 12 different concentrations of the drug (0–40 mM) were tested on cell viability. Cells were seeded in 96-well multidishes (TPP, Trasadingen) at a density of 12,500 cells/well. Twenty-four hours after they were attached, the different concentrations of APAP were added to the culture medium. We used a stock solution of 756 mg APAP/ml DMSO, this concentrated solution allowed us to add non-toxic concentration of DMSO to the culture media. After 48 h exposure, the cell viability was estimated using CellTiter 96 AQueous Non-radioactive cell proliferation assay (Promega, Biotech Ibérico, Madrid, Spain). An evaluation of the percentage of viable cells vs. APAP concentration was firstly performed to choose the appropriate subtoxic concentration of APAP (between 0 and 40 mM). We found that concentrations of 10 mM for LS 174T cells, and 2.5 mM for HepG2 cells, were the highest ones still preserving a 100% cell viability. Accordingly, subtoxic doses of 5 mM for LS 174T cells and 1 mM for HepG2 cells were used in further studies of MDR1 expression and function. After a confluence of 60–70% was reached in both cell lines, the culture medium was replaced with medium containing the selected subtoxic concentrations of APAP. Control cells received only the vehicle (DMSO). After 48 h of incubation, the cells were trypsinized and used as described below.

2.3.3. Real-time quantitative PCR

The cells were immediately immersed in the RNAlater RNA stabilization reagent (Ambion, BioNova Científica, Madrid) and stored at −80 °C. The mRNA levels were determined by real-time quantitative PCR as previously described [17]. The MDR1 primer oligonucleotide sequences (5′–3′) were as follows: forward primer: GCC TAC TTG GTG GCA CAT AAA C; reverse primer: GCA CCA AAG ACA ACA GCT GAA A. The results of mRNA abundance for target gene in each sample were normalized on the basis of its GAPDH mRNA content (forward primer: GTG CAG TGC CAG CCT CGT; reverse primer: CTG TGC CGT TGA ACT TGC CGT).

2.3.4. Transport studies

The cells were resuspended in 200 μl of culture medium containing 2 μM of rhodamine 123 and incubated at 37 °C for 30 min. Then, 2 ml of culture medium with or without verapamil (25 μM) were added to each incubation, and the mixture was immediately used in assessment of rhodamine 123 cellular content (T0), or further incubated at 37 °C for 30 min and used in rhodamine 123 cellular determination (T30). Rhodamine 123 was measured in a Becton Dickinson FAC sort Cytometer (BDEuropa, Madrid, Spain), using the 488 nm argon laser and FL1 channel. The efflux was estimated as the decrease in intracellular rhodamine 123 after 30 min (T0–T30) and was expressed as percentage of the initial concentration at T0. MDR1 activity was calculated as the difference between the percent of efflux in the absence (total efflux) and the presence (MDR1-independent efflux) of verapamil.

2.4. Statistical analysis

Data are presented as the means ± SD. Comparison between groups was performed using the Student's *t* test or one-way ANOVA followed by Bonferroni's test (when more than two groups were compared). Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Effect of APAP on MDR1 expression in rat intestine

Because the level of MDR1 expression varies along the small intestine [18], we studied the expression of this protein in two segments of this organ. The proximal segment of the small intestine has the lowest constitutive expression levels of MDR1, whereas the distal segment has the highest expression levels. Fig. 1 shows western blot detection of MDR1 in BBM isolated from different regions of the intestine of Control and APAP-treated rats. APAP induced an increase in MDR1 expression of 240 and 160% over Controls for proximal and distal segments, respectively. To investigate whether this was accompanied by changes in subtissular distribution, immunofluorescence microscopy study was carried out. Fig. 2 shows that MDR1 was mainly localized at the surface of the intestinal villus (red fluorescence). Localization of the cell nuclei (blue fluorescence) was also shown for reference. The images show that MDR1 subtissular and subcellular distribution were preserved after APAP treatment at both regions of the intestine, indicating that the expression of MDR1 was restricted to the apical membrane of the enterocyte both in Control and APAP-treated animals. Although quantitative analysis of these images was not carried out, stronger red fluorescence signal was seen in samples from rats treated with APAP.

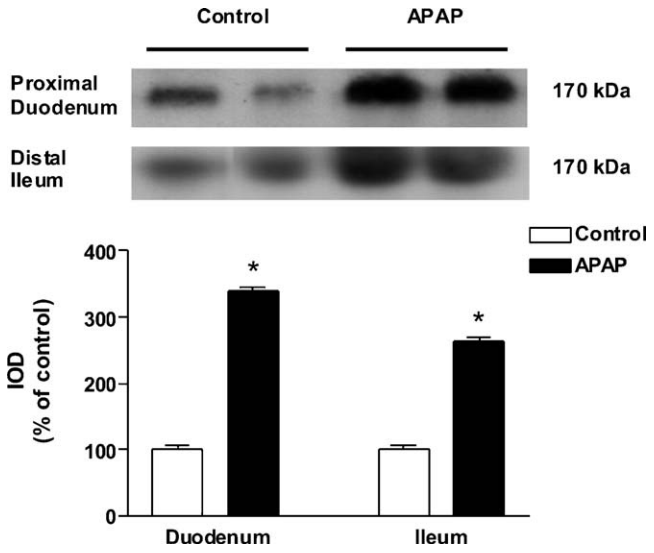

Fig. 1. Effect of APAP on MDR1 expression. Western blot study of MDR1 was performed using brush border membranes prepared from two 20-cm segments (duodenum and ileum) of small intestine collected from Control and APAP treated rats. Equal amounts of total protein (40 μg) were loaded in all lanes. Uniformity of loading and transfer from gel to nitrocellulose membrane was controlled with Ponceau S. Integrated optic density (IOD) calculated from densitometric analysis are expressed as percentage of Controls and presented as means ± SD of four rats per group. *$P < 0.0001$ as compared with Controls.

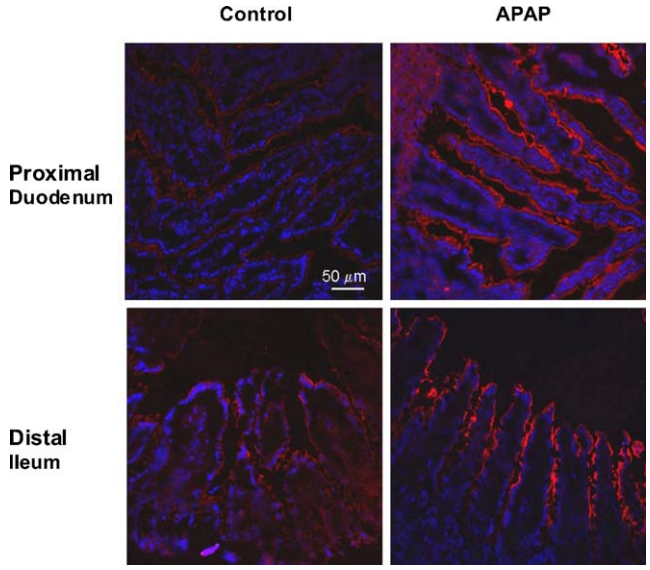

Fig. 2. Effect of APAP on MDR1 localization. Confocal immunofluorescense microscopy of intestinal villi from Control (left panels) and APAP treated (right panels) rats. Studies were carried out using two 20-cm segments (duodenum and ileum) of small intestine. MDR1 was labeled as red and enterocyte nuclei as blue fluorescence. Similar patterns of staining were observed in three independent preparations per group.

3.2. Effect of APAP on MDR1 activity in rat intestine

To determine the functional impact of the increment of MDR1 protein levels by APAP, we first studied its transport activity in vitro. We evaluated the secretion of rhodamine 123, a typical MDR1 substrate, into the mucosal compartment of intestinal sacs prepared from distal ileum. The addition of rhodamine 123 to the serosal compartment resulted in progressive recovery of the dye with time in the mucosal side for all groups studied (Fig. 3). This transport activity was increased in APAP-treated rats (+50% at 40 min, $P < 0.05$) (Fig. 3). It is known that rhodamine 123 may be

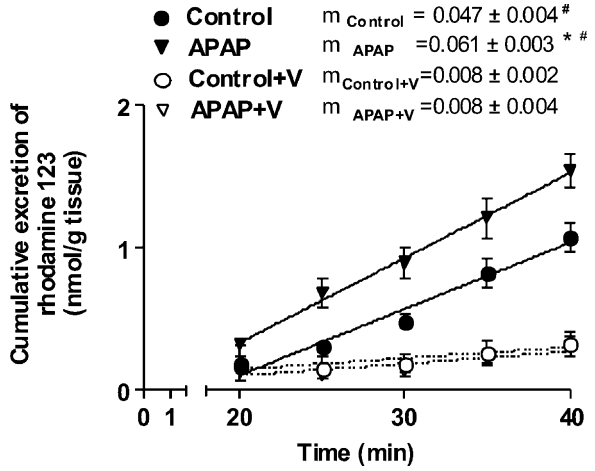

Fig. 3. Effect of APAP on MDR1 activity. MDR1-mediated rhodamine 123 efflux was measured in vitro using 10-cm everted sacs from distal ileum of Control and APAP treated rats. The sacs were filled with 15 μM rhodamine 123 (serosal side). The dye secreted into the outside compartment (mucosal side) was assessed every 5 min for 40 min, in the presence or absence of 100 μM verapamil (V). Data are means ± SD of four rats per group. The excreted amounts of rhodamine 123 into the mucosal side were plotted against the incubation time and the mucosal efflux rates were estimated from the slope (m) of the linear regression, and expressed in nmol of rhodamine 123 transported per g of tissue per min. *, different from Control ($P < 0.001$). #, different from C + V and APAP + V ($P < 0.001$).

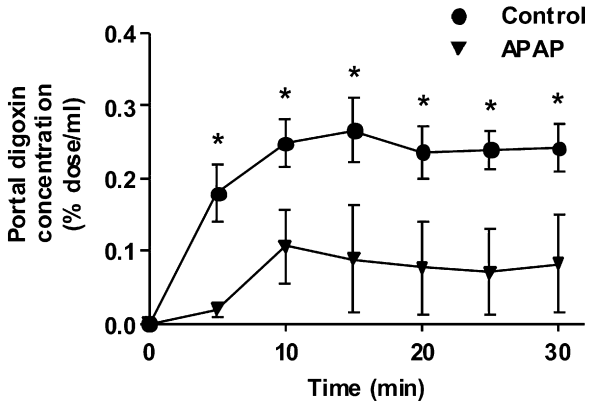

Fig. 4. Effect of APAP on intestinal barrier for digoxin. Net intestinal absorption of digoxin was indirectly determined by changes in portal blood concentrations over 30 min after intraduodenal administration of radiolabeled digoxin (25.6 nmol/kg b.w.) in anesthetized Control and APAP-treated rats. Data are means ± SD of four rats per group.*, different from Control ($P < 0.05$).

transported by other ABC members present in the intestinal epithelium. To further confirm the specific participation of MDR1 in rhodamine 123 transport, we repeated these same experiments, but in the presence of 100 μM verapamil, a MDR1 inhibitor. The results demonstrate that transport activity was decreased by verapamil to 20 and 30% of the activity in absence of the inhibitor, in APAP and Control groups, respectively, at the end of the incubation period (Fig. 3). As an estimation of the efficiency of transport, the recovery into the mucosal side was plotted against the incubation time and the mucosal efflux rates were calculated from the slope (m) of the linear regression. This measure was increased in animals treated with APAP (30%) when compared to controls and consistently reduced in both groups when measured in the presence of verapamil (Fig. 3).

Intestinal MDR1 plays a role as a barrier reducing the net absorption of xenobiotics and drugs reaching the intraluminal space. This strongly affects bioavailability of MDR1 substrates, such as digoxin [19], and hence its therapeutic applications. To test whether APAP treatment enhances this particular function in vivo, the time-course of changes in portal digoxin concentration after intraluminal incorporation of a drug bolus was determined. The percentage of the dose of digoxin absorbed per ml of portal blood increased with time in both groups and reached steady state values from 10 min onwards (Fig. 4). A notable 65% decrease in digoxin absorbed was observed for the APAP group when compared with the Control group (Fig. 4).

3.3. Effect of APAP on MDR1 expression and activity in human cells

LS 174T cells were selected to explore the potential effect of APAP as a modulator of human ABCB1 gene in intestine. The effect of APAP was comparatively analyzed with the HepG2 cell line since these cells, derived from human hepatoblastoma, were previously found to respond to APAP exhibiting an increase in the expression of MDR1 [11]. As described above, we first selected subtoxic concentrations of APAP to be used in these experiments. The subtoxic concentrations was chosen as the last but one which presents a 100% of cell viability. Thus, the values were 5 mM for LS 174T cells and 1 mM for HepG2 cells (data not shown). Incubation with subtoxic concentrations of APAP for 48 h induced a significant increase in the relative abundance of MDR1 mRNA both in LS 174T (Fig. 5A) and HepG2 (Fig. 5B) cells. These increases were of 46 and 70% when compared to controls for intestinal and liver cell lines, respectively.

Finally, we have studied whether the increase in the expression of MDR1 mRNA has functional consequences. With this aim MDR1

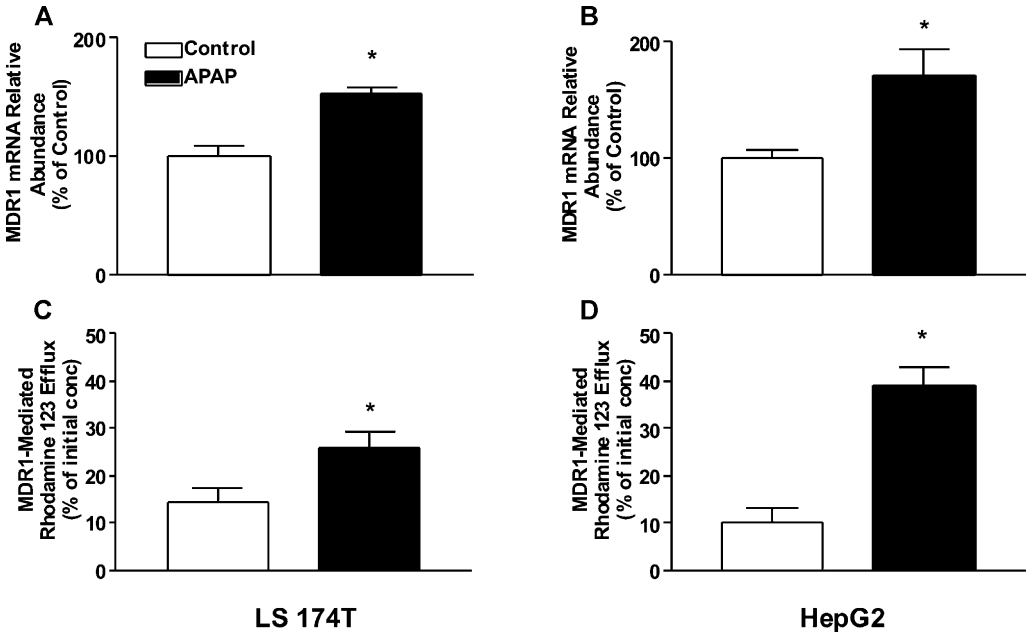

Fig. 5. Effect of APAP on expression and activity of MDR1 in LS 174T (A and C) and HepG2 (B and D) cells. MDR1 mRNA levels (A and B) was measured by real-time quantitative RT-PCR. MDR1 activity (C and D) was determined by the ability to extrude rhodamine 123 added to the incubation medium at 2 μM in the absence or the presence of 25 μM verapamil. Studies were carried out after 48 h of incubation with subtoxic concentrations of APAP (5 mM for LS 174T and 1 mM for HepG2) or vehicle (DMSO, Control). The MDR1 mRNA abundance was determined relative to GAPDH mRNA content and expressed as percentage of Control. Data are means ± SD of three individual experiments per group. *, different from Control ($P < 0.05$).

activity was evaluated by the ability of the cells to carry out rhodamine 123 efflux. In both cell lines, exposure to subtoxic concentrations of APAP resulted in significant increases in the transport activity when compared to Controls, which were of 78% and 160% for LS 174T (Fig. 5C) and HepG2 (Fig. 5D) cells, respectively.

4. Discussion

Since MDR1 modulation plays a critical role in regulating the absorption of many chemicals including therapeutic drugs, it is of pharmacological and toxicological relevance understanding the factors able to affect its expression. APAP is one of the most sold over the counter drugs and is usually co-administered with other medicines. We have previously demonstrated that a single, 1 g/kg b.w., dose of APAP increases MDR1 expression and activity in rat liver [8]. In this study we evaluated the effect of repeated administration of subtoxic doses of APAP on the expression and activity of intestinal MDR1 and its potential drug–drug interaction consequences.

Under physiological circumstances, MDR1 expression varies along the small intestine; its expression increases from duodenum to ileum [18]. The data of the present study indicated that APAP induces intestinal MDR1 expression in both regions of the rat intestine. Also, the immunofluorescence microscopy studies demonstrated that this induction occurred together with preservation of the normal subtissular and subcellular localization of MDR1 in this organ [20], i.e., restricted to the apical region of the enterocyte. We further tested whether induction of MDR1 had functional consequences, particularly at the distal ileum. We demonstrate increased transport activity detected in in vitro experiments using a typical MDR1 substrate, rhodamine 123. It is known that rhodamine 123 is also a substrate for rodent breast cancer resistance protein (Bcrp or Abcg2) [21], which is expressed in rodent intestine with a similar pattern of distribution as MDR1 [22]. To further confirm participation of MDR1 in rhodamine 123 transport, we repeated the same transport experiments but in the presence of verapamil, a MDR1 but not a Bcrp inhibitor [23]. The data confirm induction of specific activity of MDR1 by APAP treatment, which paralleled the increased content of MDR1 protein, as detected by western blotting, and was consistent with the preserved localization at the apical surface of the villus, as detected by immunofluorescence confocal microscopy.

The absorption of the majority of drugs is initiated in the duodenum, where MDR1 presents the lowest constitutive expression. The significant induction of MDR1 by APAP particularly in this portion of the intestine, may contribute to substantially decrease the absorption of MDR1 substrates [5,24,25]. To test this possibility, we performed additional experiments studying the in vivo intestinal absorption of digoxin, a therapeutic drug and model MDR1 substrate. Analysis of changes in portal digoxin concentration with time demonstrated a decrease in intestinal absorption of this drug after its intraluminal incorporation to APAP rats. Taken together, these data suggest a decreased intestinal absorption of digoxin in APAP rats, mainly as a result of an increase in selective intestinal chemical barrier function. The proximal region of the intestine is also the primary site for reabsorption of drugs excreted in bile. Induction of MDR1 in this same region may thus decrease entero-hepatic recirculation of MDR1 substrates taken up and secreted by the liver.

Apart from digoxin, several other therapeutic drugs are demonstrated to be MDR1 substrates. Sedatives and antidepressants (midazolam, alprazolam and amitriptyline), estrogens, antihypertensive drugs (atenolol and losartan), anti-HIV agents (indinavir, ritonavir and saquinavir), immunosuppressants (cyclosporine and tacrolimus) and anticancer drugs (imatinib and irinotecan), are just examples of an extensive list of MDR1 substrates of medical use [7], that could be eventually co-administered with APAP. Whether drug–drug interactions between APAP and other MDR1 substrates occur in humans is uncertain. Because these same drugs frequently present narrow therapeutic indices, if interactions occur in the practice, they

would lead to undermedication rather than to overmedication and toxicity, which would make the interactions even more difficult to detect.

To further explore if APAP is capable of modulating the expression of human MDR1 gene as demonstrated in rats, we studied the effect of subtoxic concentrations of APAP in a human intestinal cell line, LS 174T, generated from colorectal adenocarcinoma [26]. These cells were considered appropriate to study MDR1 induction by other compounds [27,28]. Previously, Manov et al. have described that MDR1 expression is increased by APAP in a dose dependent manner in the HepG2 liver cell line [11]. We thus explored the effect of APAP also in HepG2 cells, taken as a positive control. We first selected safe subtoxic concentration of APAP to be used in both cell line cultures. We found that MDR1 mRNA was indeed increased by APAP in HepG2 cells. More importantly, APAP induced significantly the expression of MDR1 mRNA in LS 174T cells. Additional experiments were performed to evaluate its impact on transport activity using rhodamine 123 in combination with verapamil. Increased secretion of the model substrate by these cells was likely a consequence of increased synthesis of MDR1 protein. Taken together, the data would indicate transcriptional regulation of human MDR1 by APAP. These results suggest similar regulation of intestinal Mdr1/MDR1 by APAP in rats and humans.

The mechanism by which APAP up-regulates intestinal MDR1 is unknown. Several nuclear receptors (NR) have been involved in MDR1 regulation in the intestine. Pregnane X receptor (PXR) was the most studied and was responsible for MDR1 induction by rifampicin [29] and levothyroxine in intestinal cell lines [30]. Also this NR mediates MDR1 induction in inflammatory bowel diseases [31]. Also, in the intestine, constitutive androstane receptor (CAR) binds to a nuclear response element located in the −7.8 kbp enhancer element of MDR1, and transactivates its expression [32]. Tachibana et al. described this same transactivation by vitamin-D receptor (VDR) [33]. On the other hand, APAP has been found to interact directly or indirectly with several nuclear receptors to exert its hepatotoxicity, including CAR, PXR and NF-E2-related factor 2 (Nrf2) [34]. The indirect action might involve participation of oxidative stress or generation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). Our previous results, using an identical protocol of administration as that used here, demonstrated that the induction of Mrp2 and Mrp3 in rat liver may occur in absence of any apparent manifestation of oxidative stress [10]. Accordingly, we hypothesize a direct interaction of APAP or its metabolites with one or more of the receptors involved in regulation of MDR1 in rat intestine or LS 174T cells under the current subtoxic protocol of APAP administration.

In conclusion, the present study demonstrates for the first time increases in expression and activity of MDR1 in response to subtoxic doses of APAP in rat small intestine and human intestinal cell line LS 174T. In vivo experiments demonstrate modifications of intestinal absorption of digoxin when administered intraluminaly to APAP treated rats, suggesting the possibility of drug–drug interaction when APAP is co-administered with other MDR1 substrates.

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