Acetaminophen Inhibits Intestinal P-Glycoprotein Transport Activity

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ABSTRACT: Repeated acetaminophen (AP) administration modulates intestinal P-glycoprotein (P-gp) expression. Whether AP can modulate P-gp activity in a short-term fashion is unknown. We investigated the acute effect of AP on rat intestinal P-gp activity *in vivo* and *in vitro*. In everted intestinal sacs, AP inhibited serosal–mucosal transport of rhodamine 123 (R123), a prototypical P-gp substrate. R123 efflux plotted against R123 concentration adjusted well to a sigmoidal curve. V_{max} decreased 50% in the presence of AP, with no modification in EC50, or slope, ruling out the possibility of inhibition to be competitive. Inhibition by AP was absent at 0°C, consistent with interference of the active transport of R123 by AP. Additionally, AP showed no effect on normal localization of P-gp at the apical membrane of the enterocyte and neither affected paracellular permeability. Consistent with absence of a competitive inhibition, two further strategies strongly suggested that AP is not a P-gp substrate. First, serosal–mucosal transport of AP was not affected by the classical P-gp inhibitors verapamil or Psc 833. Second, AP accumulation was not different between P-gp knock-down and wild-type HepG2 cells. *In vivo* intestinal absorption of digoxin, another substrate of P-gp, was assessed in the presence or absence of AP (100 μM). Portal digoxin concentration was increased by 214%, in average, by AP, as compared with digoxin alone. In conclusion, AP inhibited P-gp activity, increasing intestinal absorption of digoxin, a prototypical substrate. These results suggest that therapeutic efficacy of P-gp substrates can be altered if coadministered with AP. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3830–3837, 2013
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INTRODUCTION

P-glycoprotein (P-gp), also known as multidrug resistance protein-1 (MDR1, ABCB1), belongs to the ATP-binding cassette gene family. P-gp is an ATP-dependent efflux pump that decreases intracellular accumulation of a wide range of cationic, hydrophobic, endogenous, and exogenous compounds.[1] It is constitutively expressed at the apical membrane of mature epithelial cells of different organs, including liver, brain, kidney, and intestinal tract. Owing to this localization, P-gp is one of the most important efflux proteins in the small intestine and colon[2] and influences the pharmacokinetics of many clinical therapeutic drugs.[3,4] P-gp expression limits the intestinal absorption of a diverse range of orally administered xenobiotics.[5,6] In addition, P-gp can be responsible for drug–drug interactions when two or more of its substrates are coadministered, for example as a consequence of competition or induction of transporter activity.[7–10]

Acetaminophen (N-acetyl-para-aminophenol, paracetamol, AP) is a well-known over-the-counter drug. Many formulations contain AP alone or in combination with other drugs because of its analgesic and antipyretic properties. Also, it is usually coadministered with other medicines, by prescription or self-medication. We have demonstrated that the administration of repeated subtoxic, intraperitoneal doses of AP (0.2, 0.3, and 0.6 g/kg, injected in 3 consecutive days) to rats upregulated the expression and activity of P-gp in intestine. This induction altered the pharmacokinetics of digoxin, a prototypical P-gp substrate, decreasing its bioavailability when administered intraluminally.[11] It has been noted that many substrates of P-gp are also inducers of the transporter.[12,13] Although we have described the long-term effect of AP on intestinal P-gp expression in rats, the direct, short-term effect of AP on the activity of P-gp and its functional consequences have not been studied yet.

In this study, we explored whether AP can acutely affect rat intestinal P-gp activity toward prototypical P-gp substrates, using *in vivo* and *in vitro* experimental models. Additionally, it was studied whether AP transport is affected by P-gp inhibitors in intestinal sacs, and if AP accumulation in HepG2 cells is affected after knocking down P-gp, to establish whether AP is a P-gp substrate. Together, the data demonstrate that AP acutely inhibits P-gp transport activity, increases the bioavailability

Abbreviations used: P-gp, P-glycoprotein; AP, acetaminophen; R123, rhodamine 123; Ver, verapamil; Psc, Psc833; PSP, phenolsulfonphthalein.

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of a model P-gp substrate, and that AP itself is not a P-gp substrate.

METHODS AND MATERIALS

Chemicals

[^{3}H] digoxin (37.0 Ci/mmol) and OptiPhase liquid scintillation cocktail were purchased from PerkinElmer Life Science Products (Boston, Massachusetts). Unlabeled digoxin was from obtained from ICN Biomedicals Inc. (Costa Mesa, California). AP, verapamil (Ver), and rhodamine 123 (R123) were obtained from Sigma Chemical Company (St. Louis, Missouri). Psc 833 (Psc) was kindly provided by Novartis (Basel, Switzerland). All other chemicals were of analytical grade purity.

Animals

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 were fasted for 12 h before the studies were performed.

Effect of AP on P-gp Activity *In Vitro*

To test the effect of AP on P-gp activity, rats were anesthetized intraperitoneally (i.p.) with ketamine/xilazine at 100 and 3 mg/kg body weight (b.w.) doses, respectively, and the distal portion of the ileum (20 cm) was removed, gently rinsed with ice-cold saline, and immediately used to test P-gp activity *in vitro*. Intestinal sacs were everted and the serosal compartment was filled with different concentrations of R123 (4.5, 9, 12, 18, and 36 μM) prepared in Krebs–Henseleit (KH) buffer (40 mM glucose, pH 7.4), as a model P-gp substrate. The sacs were incubated in 5 mL KH buffer with or without the addition of AP (100 μM), used as a putative P-gp inhibitor. The mucosal medium was continuously gassed with 95% O_2–5% CO_2 and maintained at 37°C. Aliquots of 100 μL were sampled from this compartment every 5 min along a 40-min period. Then, the sacs were gently dried and weighed. R123 concentration was determined spectrofluorometrically in all samples taken from mucosal medium,[14] and its excretion rate was calculated and normalized per gram of tissue. To test whether AP can modify R123 serosal–mucosal transport in absence of any active process (e.g., P-gp-mediated transport), additional everted intestinal sacs were filled with R123 (18 μM) and incubated in 5 mL KH buffer continuously gassed at 0°C, with or without the addition of AP (100 μM).

Effect of AP on Phenol Red Diffusion *In Vitro*

Phenol red (phenolsulfonphthalein; PSP) is a nonabsorbable marker of use in perfusion studies to test intestinal absorptive capacity.[15,16] To evaluate whether AP can modify the paracellular route, everted intestinal sacs were filled with PSP (1 mM) and incubated in 5 mL KH buffer continuously gassed at 37°C, with or without the addition of AP (100 μM). Aliquots of 100 μL were sampled from the mucosal compartment every 5 min along a 40-min period. Then, the sacs were gently dried and weighed. PSP concentration was determined spectrometrically at 560 nm after the addition of 900 μL of 0.5 N NaOH. PSP excretion rate was calculated and normalized per gram of tissue.

Effect of AP on P-gp Intestinal Expression

To evaluate whether the acute treatment with AP (100 μM) can affect the expression of P-gp at the apical membrane of the enterocytes, brush border membranes (BBMs) were isolated at the end of intestinal sac incubations (40-min period). To prepare BBMs, the mucosal tissue was obtained by scraping,[17] homogenized, and processed as described previously.[18] Protein concentration in membrane preparations was measured using bovine serum albumin as standard.[19] Detection of P-gp was performed on BBMs using a rabbit polyclonal antibody to human MDR1 (Santa Cruz Biotechnology, Santa Cruz, California), as previously described.[20] Densitometry was performed using the Gel Pro Analyzer (Media Cybernetics, Inc., Silver Spring, Maryland) software.

Effect of P-gp Inhibitors on AP Intestinal Transport *In Vitro*

To evaluate whether P-gp inhibitors modulate AP intestinal secretion, everted intestinal sacs were prepared as described above, and the serosal compartment was filled with KH buffer (40 mM glucose, pH 7.4) containing AP (100 μM), as a putative P-gp substrate. The sacs were incubated in 5 mL KH buffer with or without the addition of different P-gp inhibitors (Ver 100 μM or Psc 10 μM). The mucosal content of AP was assayed by HPLC as previously described,[21] and AP excretion rate was calculated and normalized per gram of tissue.

Transport of AP in P-gp Knock-Down HepG2 Cells

To test whether P-gp is involved in AP extrusion, human P-gp was transiently knocked down with P-gp siRNA (h) (sc-29395; Santa Cruz Biotechnology) targeting the human P-gp mRNA. Control siRNA-A (sc-37007; Santa Cruz Biotechnology), a nontargeting siRNA, was used as a negative control. Transfections were performed using Dharmafect4 transfection reagent (Dharmacon, Lafayette, Colorado) as described.[22] The effectiveness of P-gp knock-down was evaluated by western blotting and P-gp expression normalized to β-actin content.[23] For transport studies, HepG2 cells were loaded with AP (10 μM) or with R123 (5 μM), used as a positive control, for 2 h. Retention of AP or R123 into the cells after this period was inversely correlated with its extrusion. To quantify the intracellular content of AP, cells were lysed by sonication. Lysates were subjected to solvent extraction with acetonitrile–dimethyl sulfoxide (1:1) and deproteinized with 10% trichloroacetic acid. AP was measured in supernatants by HPLC as described.[23]

Effect of AP on Intestinal Absorption of Digoxin *In Vivo*

Surgical procedures on rats were performed as previously described,[24] and blood from portal vein was sampled every 5 min, up to 30 min after digoxin administration. A dose of 25.6 nmol/kg b.w. of digoxin (17.0 μmol of [^{3}H]digoxin/mol unlabeled digoxin), a well-known P-gp substrate, was administered directly into duodenum, alone or with AP (100 μM) or Ver (100 μM). Ver was used to visualize the impact of significant inhibition of P-gp activity on portal availability of digoxin. Portal digoxin concentration was determined by liquid scintillation analysis.

Calculations and Statistical Analysis

P-glycoprotein transport activity versus time was adjusted to a classical linear regression curve. The rate (V) of P-gp transport was fitted to a sigmoidal dose-response, Boltzmann nonlinear regression 1, where E_{C50} and x represent half maximal effective concentration and substrate concentration, respectively. This well-characterized sigmoidal function is used to reflect active transport and passive diffusion, as a biphasic dose response.[25]

$$V = V_{\min} + \frac{(V_{\max} - V_{\min})}{1 + \mathrm{Exp}[(\mathrm{Ec}_{50} - x)/\mathrm{slope}]} \quad (1)$$

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.

RESULTS

AP Inhibits Intestinal P-gp Transport Activity

Serosal to mucosal transport of R123, a typical P-gp substrate, was evaluated in everted intestinal sacs in the presence or absence of AP. Figure 1 a shows a prototypical curve reflecting the excretion rate of R123 in everted intestinal sacs, in this particular example after loading 18 μM R123 into the serosal side. P-gp activity was linear with time along the 20–40-min period. It is also shown that, in the presence of AP (100 μM), the rate of R123 excretion decreased significantly with respect to controls (from 0.043 ± 0.004 to 0.002 ± 0.002 nmol/(g min), $p < 0.01$). This experiment was repeated at 0°C, and no differences were detected in the rate of serosal–mucosal transport of R123 between control and AP groups (0.016 ± 0.002 and 0.014 ± 0.002 nmol/(g min), respectively). This result likely indicates that the presence of AP did not affect the transport of R123 independent of P-gp (e.g., passive diffusion) across the intestinal epithelium.

We then studied the inhibitory effect of AP in the presence of different R123 concentrations (4.5 to 36 μM). We found that R123 secretion rate was linear with time in the 20–40-min period for all concentrations of R123 tested, and that this measure progressively increased with substrate concentration (Fig. 1b). We also found that R123 secretion rate adjusted well to a sigmoidal curve when plotted against R123 concentration. The presence of AP significantly decreased V_{max} (from 0.0369 ± 0.0026 to 0.0179 ± 0.009 pmol/(g min), $p < 0.05$), with no modification in Ec_{50} or slope.

To evaluate whether the presence of AP can affect intestinal paracellular permeability in everted sacs, serosal–mucosal transport of PSP, a typical nonabsorbable compound, was studied. Figure 1c shows that PSP diffusion did not change in the presence of AP.

Effect of AP on Intestinal P-gp Expression

Figure 2 shows that acute incubation with AP did not produce significant changes in P-gp expression as detected in BBMs.

Intestinal Transport of AP Is Not Affected by Prototypical P-gp Inhibitors

As an approach to test whether AP behaves as a P-gp substrate, we evaluated its intestinal transport in the presence of two well-known P-gp inhibitors, Ver and Psc, the latter proven to be more specific than Ver.[26] The results show increased AP efflux with time in control conditions, with no significant changes in response to P-gp inhibitors (Fig. 3).

P-gp Knock-Down Does Not Affect AP Transport in HepG2 Cells

To explore in a more direct way whether AP is a P-gp substrate, we performed transport studies in a cell model normally expressing P-gp, in which expression of this transporter was partially silenced. We choose the HepG2 cell line because of the well-characterized P-gp-mediated transport of prototypical substrates and transport assay simplicity.[27–29] Figure 4a shows that the silencing treatment decreased the P-gp expression significantly (−79%; $p < 0.05$), evaluated by western blotting, confirming downregulation of expression of P-gp. Assessment of intracellular accumulation of R123, considered an inverse estimation of extrusion, was used as a positive control. Figure 4b shows a 70% increment in R123 accumulation in knock-down compared with control cells ($p < 0.01$), whereas no significant difference was observed in AP accumulation between groups.

AP Increases Intestinal Absorption of Digoxin

Intestinal P-gp plays a role as a barrier reducing the net absorption of xenobiotics, such as digoxin, from the luminal compartment to the portal circulation,[29] and hence its therapeutic efficacy. We studied the time course of portal digoxin concentration after intraluminal administration of a digoxin bolus. Inhibition of P-gp activity with Ver was used as a positive control. Figure 5 shows that the amount of digoxin absorbed per milliliter of portal blood increased significantly in digoxin + Ver when compared with digoxin group at all time points studied (fourfold in average, $p < 0.01$). The presence of AP also significantly increased portal digoxin concentration. This was particularly evidenced as a twofold increase in average ($p < 0.05$), detected from 20 min onwards.

It is not possible to completely rule out the occurrence of changes in cytochrome P450 3A (CYP3A)-mediated intestinal metabolism of digoxin induced by AP, with eventual impact on its portal detection. However, digoxin bioavailability, *in vitro* activity, and quantitative immunohistochemistry and immunoblotting studies strongly suggest that digoxin disposition after its oral administration correlates with expression of intestinal P-gp rather than with CYP3A activity.[30]

DISCUSSION

P-glycoprotein plays a critical role in regulating the absorption of a wide range of xenobiotics including food contaminants and therapeutic drugs, because of its strategic localization at the BBM of the enterocyte, all along the intestine.[31] AP is one of the most sold over-the-counter drugs and is usually coadministered with other medicines. We explored whether AP can acutely affect P-gp activity with concomitant impact on intestinal absorption of P-gp substrates, eventually coadministered with AP. The data demonstrate that AP was able to inhibit P-gp-mediated transport of R123, a model substrate, as detected

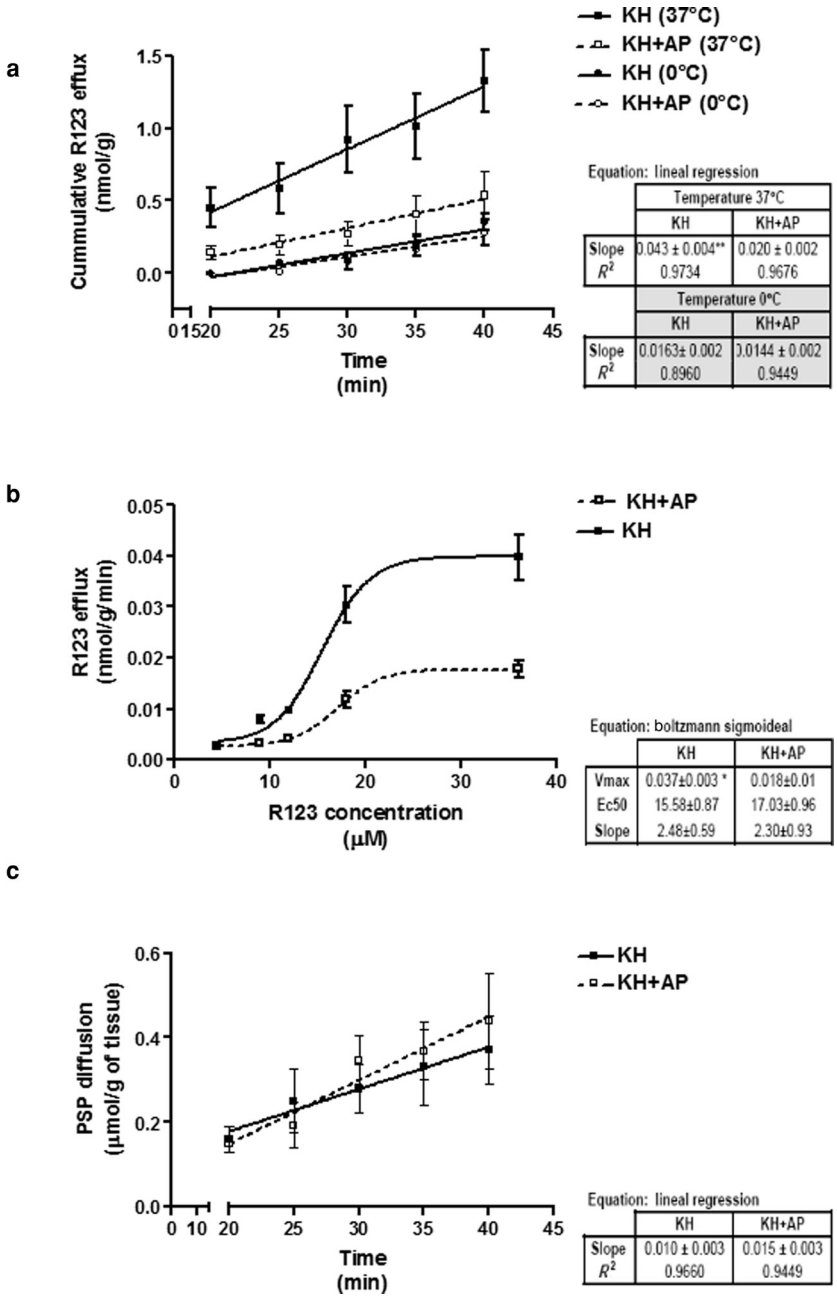

Figure 1. Effect of AP on intestinal P-gp activity *in vitro*. P-gp-mediated R123 efflux was measured using 10-cm everted sacs from rat distal ileum. (a) The sacs were filled (serosal side) with R123 (18 μM) and incubated in KH buffer. The concentration of dye secreted into the outside compartment (mucosal side) was assessed every 5 min for 40 min, in the absence or presence of 100 μM of acetaminophen (KH + AP). The experiment was performed at 37°C and at 0°C. The cumulative excretion rate was adjusted to a classical linear regression curve. Data are means ± SD of six rats per group. **Significantly different from KH + AP (37°C); KH (0°C); and KH + AP (0°C) ($p < 0.01$). (b) The sacs were filled (serosal side) with different concentrations of R123 (4.5, 9, 12, 18, and 36 μM) and incubated in KH buffer. The dye secreted into the outside compartment (mucosal side) was assessed every 5 min for 40 min, in the absence or presence of 100 μM acetaminophen (KH + AP). The kinetic parameters were calculated using the Boltzmann sigmoidal equation. Data are means ± SD of six rats per group. *Significantly different from AP ($p < 0.05$). (c) The sacs were filled (serosal side) with phenol red (PSP, 1 mM). The concentration of dye secreted into the outside compartment (mucosal side) was assessed every 5 min for 40 min, in the absence or presence of 100 μM AP (KH + AP), at 37°C. The cumulative excretion rate was adjusted to a classical linear regression curve. Data are means ± SD of six rats per group.

in rat intestinal everted sacs. Kinetic analysis showed a significant decrease in V_{max}, which rule out the possibility of competitive inhibition to be involved, and consequently, of AP to be a P-gp substrate itself. Two different strategies indeed strongly suggested that AP is not a P-gp substrate. First, AP transport was not affected by P-gp classical inhibitors. Second, intracellular accumulation of AP in a human cell line was not affected after partial silencing of P-gp protein expression.

Although transport of AP-conjugated metabolites has been well characterized and involves Mrps and Bcrp,[32–36]

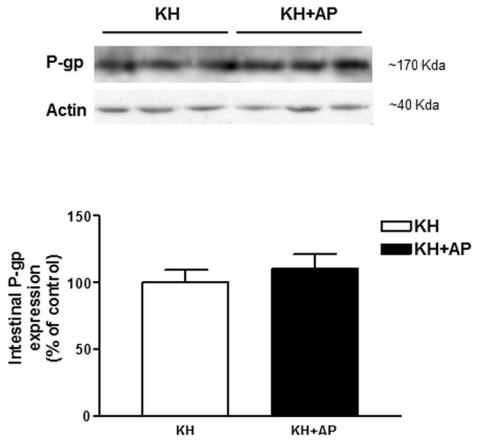

Figure 2. Effect of AP on P-gp expression. At the end of the R123 transport experiment, BBMs were isolated from intestinal sacs. Western blot study of P-gp was performed loading equal amounts of BBM total protein (40 μg) in all lanes. Uniformity of loading and transfer from gel to nitrocellulose membrane were controlled using actin as housekeeping gene. Data on densitometric analysis are expressed as percentage of controls and presented as means ± SD of three experiments per group.

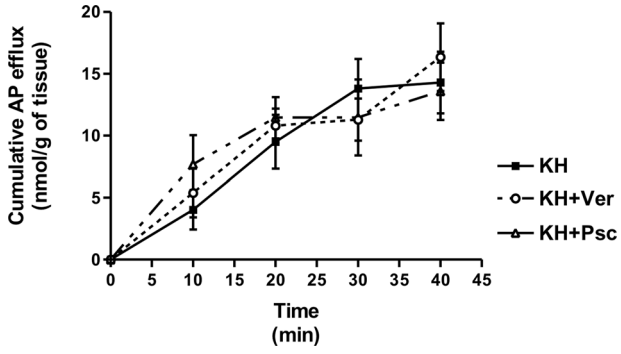

Figure 3. Effect of P-gp inhibitors on AP intestinal transport. Intestinal sacs were filled (serosal side) with 100 μM of AP and incubated in KH buffer. The AP secreted into the outside compartment (mucosal side) was assessed every 10 min up to 40 min, in the absence or presence of the P-gp inhibitors Ver (100 μM) or Psc (10 μM).

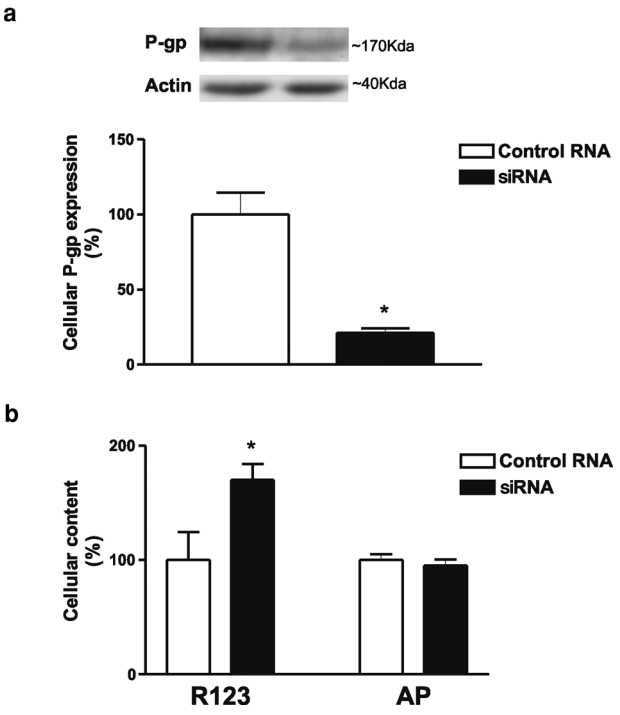

Figure 4. Effect of P-gp knock-down on AP transport. (a) P-gp expression levels were assessed by Western blotting in lysates from HepG2 cells transfected either with control siRNA-A (scrambled siRNA) or with P-gp (h) siRNA. Data are means ± SD of four individual experiments and expressed as percentage of P-gp expression in scrambled siRNA-transfected cells. *Significantly different from RNA control cells ($p < 0.05$). (b) Cells were loaded with R123 (5 μM, 2 h) or AP (10 μM, 2 h), and drug accumulation was determined in cellular lysates. Data are means ± SD of four individual experiments and expressed as percentage of drug accumulated in scrambled siRNA-transfected cells. *Significantly different from RNA control cells ($p < 0.05$).

identification of a transporter recognizing unmetabolized AP as a substrate, either involving cellular uptake or extrusion is lacking. Manov et al.[37] hypothesized that AP behaves as a P-gp substrate because coincubation with Ver increased AP toxicity in HepG2 cells, which was attributed to increased AP intracellular accumulation. Reported adverse effects of Ver include hepatic injury and hepatotoxicity, which was also demonstrated in HepG2 cells.[38,39] It is possible that the exacerbated toxicity observed by Manov et al.[37] resulted from inhibition of P-gp by AP and consequent accumulation of Ver to the extent of reaching toxic levels. Alternatively, AP toxicity depends on intracellular Ca^{2+} concentration,[40–42] which in turn can be modulated by the voltage-operative Ca^{2+} channel blocker, Ver.[43] Irrespective of the explanation for exacerbated cytotoxicity of AP and Ver used in combination, our data strongly suggest that AP is not a substrate for P-gp, either of rat or human origin. Other authors also hypothesized that AP is not transported by P-gp. Faassen et al.[44] suggested that AP is not transported by P-gp in Caco 2 cells as they found no difference between apical–basolateral and basolateral–apical net flux, either at low or high doses of drug. Wang et al.[45] have studied the effect of known P-gp and CYP3A4 inhibitors on the activity of both proteins. In concordance with our results, the authors found that AP, a well-known inhibitor of CYP3A4, was not transported by P-gp, as demonstrated by the absence of ATP hydrolysis in the presence of the drug. They also classified AP as a P-gp noninhibitor, using the extension of Ver inhibition as cutoff level. Because of the criteria used, they could only identify inhibitors exhibiting more potency than Ver. Their report is also compatible with our data in Figure 5, demonstrating that AP is a P-gp inhibitor of potency weaker than Ver.

It is well demonstrated that several compounds inhibit P-gp activity noncompetitively. This is the case of tariquidar,[46] progesterone,[47] vanadate,[48] amoxapine and loxapine,[49] among others. It is speculated that drug–protein interaction would cause P-gp conformational changes that could hinder intramolecular movements necessary for the transportation process as well as transport-associated ATP hydrolysis.[50,51] Whereas our data demonstrate that AP is not a competitive inhibitor of intestinal P-gp, more direct studies are necessary to confirm that inhibition is of the noncompetitive type, and

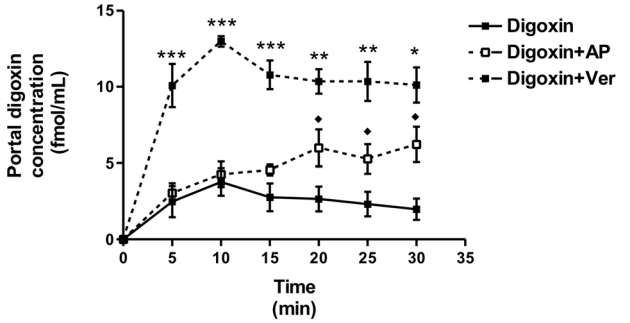

Figure 5. Effect of AP on intestinal absorption of digoxin (Dig). Net intestinal absorption of Dig was estimated by changes in its portal blood concentration over a 30-min period. Dig (25.6 nmol/kg b.w.) was intraduodenally administered, alone or with AP (100 μM) or Ver (100 μM). Data are means ± SD of five rats per group. *; **; ***; Dig + Ver significantly different from Dig and Dig + AP ($p < 0.05$); ($p < 0.01$); ($p < 0.001$), respectively; ♦; Dig + AP significantly different from Dig ($p < 0.05$).

to characterize molecularly the P-gp–AP interaction. In our experimental conditions, AP was incorporated to the luminal side of intestinal sacs to study R123 transport. Whether AP inhibits P-gp from the external side of the enterocyte, or from the cytosolic side, remains unknown. Importantly, our results demonstrate that P-gp was also inhibited by AP *in vivo*, as absorption of digoxin, incorporated to the intestinal lumen, was exacerbated by AP, also incorporated intraluminally.

The intraluminal concentration of AP reached in humans after a single, oral dose of 500 mg has never been assessed. Gastrointestinal fluid formation is a complex, dynamic and fluctuating process.[52] The average gastrointestinal fluid volume was measured in individuals post-mortem, resulting in mean values of 118 mL for the stomach and 212 mL for the small intestine.[53] Total colonic water was estimated by Cummings et al.[54] and found to be 187 mL. AP intraluminal concentration could be theoretically estimated from dilution of 500 mg of AP (equivalent to a single, regular dosage) in overestimated 1000 mL volume of digestive-intestinal contents, thus leading to a concentration of 3.2 mM. This theoretical concentration is at least one order of magnitude higher than the concentration used in the current experiments (100 μM), clearly suggesting that an inhibitory effect could actually occur and influence absorption of P-gp substrates of therapeutic use. Digoxin, sedatives and antidepressants, estrogens, antihypertensive drugs, anti-HIV agents, immunosuppressants, antibiotics and anticancer drugs[55] are just examples of these medicines. It was reported that AP potentiates staurosporine-mediated neuroblastoma cell death (SH-SY5Y).[56] The mechanism of action postulated by these authors is AP-induced generation of reactive oxygen species and decreased intracellular glutathione levels. However, SH-SY5Y cells express P-gp[57,58] and stauroposporine was demonstrated to be a P-gp substrate.[59] According to our results, inhibition of P-gp-mediated extrusion of stauroposporine, leading to increased intracellular concentration, could actually contribute to explain the increased sensitivity of neuroblastoma cells to its action.

In spite of the fact that AP is a weak inhibitor of P-gp, the high concentrations estimatively reached inside the intestinal lumen after its oral incorporation at therapeutic doses, allow us to propose that drug–drug interactions are indeed possible. We found that same doses of AP and Ver produced a rather different impact on digoxin absorption *in vivo* (Fig. 5). Estimation of intraluminal concentration of Ver, as performed above for AP, results in a concentration of about 0.18 mM after usual therapeutic administration of 80 mg,[60–62] a dosage known to produce drug–drug interactions.[63] This theoretical concentration is at least 18-fold lower than that resulting from AP administration. We postulate that the lower intrinsic inhibitory effect of AP respect to Ver could be compensated by the higher intraluminal concentration of AP reached *in vivo*.

CYP3A is the most important subfamily of drug–biotransformation enzymes in humans, metabolizing more than 50% of the drugs of therapeutic use.[64] It represents 70% of the total CYP450 in intestine.[65] CYP3A and P-gp, acting individually or in combination, are major determinants of disposition of a large number of drugs, and in consequence, interactions between substrates, inducers or inhibitors of CYP3A and P-gp are frequent.[66,67] AP is a well-known CYP3A inhibitor.[68] This concept, together with our findings, reinforce the consideration that drug–drug interactions are highly possible in intestine and could significantly influence therapeutic efficacy and safety of CYP3A and P-gp substrates.

We previously demonstrated that repeated administration of AP induces intestinal P-gp expression and activity in rat.[69] This induction produces the opposite effect on digoxin absorption than the acute, inhibitory effect currently observed. Prevalence of either of these opposite effects when AP is administered in combination with P-gp substrates will probably depend on whether AP is chronically or acutely administered and on the relation of doses between AP and the different medicines. An inhibitory effect would be expected if AP is taken only occasionally, whereas prolonged treatments would lead to more uncertain effects, and compensation between upregulation of expression and acute inhibition would be even possible.

CONCLUSION

We have demonstrated a short-term, inhibitory action of AP on intestinal P-gp transport activity, resulting in increased bioavailability of the prototypical P-gp substrate, digoxin. The potential occurrence of drug–drug interactions should be considered when P-gp substrates are coadministered with AP.

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