International Journal of Pharmaceutics 428 (2012) 33–38

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Suppression of efflux transporters in the intestines of endotoxin-treated rats

Mikio Tomita, Yusuke Takizawa*, Atsushi Kanbayashi, Hiroyuki Murata, Ayako Tanaka, Mariko Nakaike, Megumi Hatanaka, Tomomi Kai, Masahiro Hayashi

Department of Drug Absorption and Pharmacokinetics, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

ARTICLE INFO

Article history:
Received 28 December 2011
Received in revised form 3 February 2012
Accepted 17 February 2012
Available online 25 February 2012

Keywords:
Lipopolysaccharide
mdr
mrp2
Rhodamine123
5-CFDA
Ileum

ABSTRACT

Infection and inflammation suppress the expression and activity of several drug transporters in the liver. In the intestine, P-glycoprotein (PGP/mdr1) and the multidrug resistance-associated protein 2 (MRP2) are important barriers to the absorption of many clinically important drugs. The protein expression and activity of these transporters were examined during inflammation induced by lipopolysaccharide (LPS). The transport of rhodamine123 (Rho123) and 5-carboxyfluorescein (5-CF) was determined in isolated ileal segments from endotoxin-treated or control rats in the presence or absence of inhibitors. The reverse transcription-polymerase chain reaction was used to measure mRNA levels. Compared with the controls, the mRNA levels of mdr1a and mrp2 were significantly decreased by approximately 50% in the ilea of the LPS-treated rats. Corresponding reductions in the basolateral-apical efflux of Rho123 and 5-CF were observed, resulting in significant increases in the apical-basolateral absorption of these compounds. Neither the permeability of fluorescein isothiocyanate labeled dextran 4000 (FD-4), a paracellular marker, nor membrane resistance was altered. These results indicate that endotoxin-induced inflammation reduces the intestinal expression and activity of PGP and MRP2 in rats, which eliciting corresponding changes in the intestinal transport of their substrates. Hence, infection and inflammatory diseases may induce variability in drug bioavailability through alterations in the intestinal expression and activity of drug transporters.

1. Introduction

Inflammation is a complex immunological response that is a component of many disease states and so it is important for the effects of inflammation to be examined in the field of clinical therapeutics. Acute inflammatory reactions are initiated by a wide variety of pathological stimuli, including infection, tissue damage, trauma, and cellular stress and result in the release of pro-inflammatory cytokines and the modulation of the expression of many hepatic proteins. Numerous clinical reports have described that drug biotransformation reactions are altered during infection and inflammation due to the downregulation of the expression of several drug efflux transporters, which is caused by the inflammatory response elicited (Hartmann et al., 2001, 2002; Slaviero et al., 2003). The concomitant roles (i.e., removing xenobiotics from cells) and close cellular localization of efflux transporters indicate that these proteins function as a protective mechanism that limits the systemic access of xenobiotics, which probably contributes to the high inter-individual variability that is observed for numerous drugs.

The ATP-dependent drug efflux transporter P-glycoprotein (PGP), which is encoded by the multidrug resistance gene (MDR1 in humans; mdr1a, mdr1b in rodents), is responsible for the active excretion of a wide variety of lipophilic cationic drugs from the liver, kidneys, and intestine. Multidrug resistance-associated protein 2 (MRP2) is involved in the extrusion of lipophilic anions and their glutathione, glucuronic acid, and sulfate conjugates.

A variety of pharmaceutical and chemical agents, immune mediators, and disease states affect drug disposition modulation by transport mechanisms. The expression levels of PGP and MRP2 are reduced in animal livers during infection-induced inflammation (Piquette-Miller et al., 1998; Tang et al., 2000; Payen et al., 2002). In vivo and in vitro studies have shown that interleukin-1β (IL-1β) and other pro-inflammatory cytokines released during the inflammatory response are primarily involved in mediating this down-regulation (Tomita et al., 2010a,b). Although the molecular mechanism of this phenomenon has not yet been elucidated, it is possible that these proteins share common regulatory pathways. Indeed, the pregnane X receptor (PXR) has been shown to regulate a gene network of drug transporters including MDR1 and MRP2 (Synold et al., 2001) in the liver and intestine.

In addition, a study using several protein markers showed that the intestine exhibited a similar response to the liver during the

* Corresponding author at: Department of Drug Absorption and Pharmacokinetics, School of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. Tel.: +81 42 676 3168; fax: +81 42 676 3142.
E-mail address: takizawa@toyaku.ac.jp (Y. Takizawa).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2012.02.027

acute phase (Molment et al., 1993). However, the effect of inflammation on transport proteins in the intestine has received little attention, despite the fact that the intestine is the first major barrier to xenobiotic absorption and that the modulation of drug transport affects oral bioavailability. We conducted the present study using an animal model of inflammation, to ascertain the effects of PGP and MRP2 protein expression and activity on drug transport in the intestine. Our results indicate that the intestine suppresses xenobiotic transport during infection and inflammation in a similar manner to the liver.

2. Materials and methods

2.1. Materials

Lipopolysaccharides, fluorescein isothiocyanate labeled dextran 4000 (FD-4), rhodamine123 (Rho123), verapamil, 5-carboxyfluorescein diacetate (5-CFDA), and MK571 were purchased from Sigma Co. Ltd. or Funakoshi Co. Ltd. All other reagents were of analytical grade or better.

2.2. Animal and experimental design

Male Wistar rats (250–275 g) were purchased from SLC (Hamamatsu, Shizuoka, Japan), and all studies were conducted in accordance with the guidelines of the Tokyo University of Pharmacy and Life Sciences. The rats were i.p. injected with 5 mg/kg endotoxin (LPS from *Escherichia coli* serotype O55:B5; Sigma-Aldrich, St. Louis, MO) dissolved in 0.5 ml of sterile saline, and the controls received an i.p. injection of 0.5 ml of sterile saline. The rats were housed under controlled environmental conditions (temperature, $23 \pm 1\,^{\circ}C$; humidity, $55 \pm 5\%$) and were allowed free access to a commercial food diet and water. The animals were sacrificed 8 h after the injection of LPS or saline, and then a 20-cm segment of the ileum was excised, the lumen was rinsed with ice-cold Ringer's solution, and the intestine was opened along the mesenteric border. Sections of the ileum were immediately mounted in Ussing chambers (0.64-cm^2 surface area) and allowed to equilibrate for 15 min with oxygenated Ringer's buffer. Enterocytes were harvested from the remaining tissue by scraping the luminal side and were frozen at $-80\,^{\circ}C$ for subsequent RNA isolation.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of mRNA

Total RNA was extracted from the intestine segment mucosal scrapings using the Amersham QuickPrep Total RNA extraction kit (GE Healthcare Life Sciences), and single-stranded cDNA was synthesized from 3 μg of RNA using the First Strand cDNA synthesis kit (TAKARA BIO Inc), according to manufacturer's protocols. Serial dilutions (20- to 16,000-fold) of RT product were used to generate standard curves for PCR, and the optimal amounts of the template were determined from the linear portions of these curves (data not shown). A standard curve for RT-PCR was produced for each set of RNA samples analyzed, and all RT-PCR standard curves were highly reproducible. Selected RT-PCR results were also confirmed on Northern blots.

The amounts of RT product (cDNA template) used were as follows: 25 ng (mdr1a, mrp2), 32 ng (PXR), 50 ng (IL-1β), or 10 ng (GAPDH). The cDNA templates were amplified in the presence of 1.5 mM $MgCl_2$, 200 μg deoxynucleoside-5′-triphosphate, and 50 pmol of the forward and reverse primers in a total volume of 100 μmol using a GeneAmp 2400 Thermocycler (Scientific Support Inc). The reaction was initiated by the addition of 2.5 U of *Taq* polymerase (MBI Fermentas), and amplification proceeded through 22 cycles for GAPDH, 25 cycles for PXR, and 30 cycles for mdr1a and mrp2. The PCR products were separated by electrophoresis on 2% agarose gels, stained with SYBR Gold nucleic acid stain (Invitrogen), and visualized under ultraviolet light. The size of the DNA band was confirmed using the Gene Ruler 100-bp DNA ladder (Fermentas Life Sciences). Optical densities were normalized to GAPDH band intensities. The PCR primers used, which were obtained from the DNA Synthesis Center (SIGMA GENOSYS), are reported in Table 1.

Table 1
Sequences of the primers used for real-time RT-PCR.

Gene		Sequence (5′–3′)	Amplicon size
GAPDH	Forward	TGA GGT GAC CGC ATC TTC TTG	102bp
	Reverse	TGG TAA CCA GGC GTC CGA TA	
IL-1β	Forward	GGC GGT TCA AGG CAT AAC AG	101bp
	Reverse	CAG TTG AGT TCA GGG ACA GTT GC	
mdr1a	Forward	AGG TCA GTT CAT TCG CTC CTG A	111bp
	Reverse	TCA AGC CCT CCG TGC TGT AG	
mrp2	Forward	CTG GTG GAT AGC GCC AAT G	113bp
	Reverse	AGG ATC GAT GAG GTC ACC ATG	
PXR	Forward	AGA AGA CGG CAG CAT CTG GA	113bp
	Reverse	TGA CGC CCT TGA ACA TGT AGG	

2.4. Ussing chamber studies

The procedures for the intestinal Ussing chamber studies were the same as those reported previously (Tomita et al., 2010a,b). Briefly, the rat gut was opened along the mesenteric border, and 1–3-cm sections of the jejunum, ileum, and colon were excised. The intestine was visually inspected prior to excision, and Peyer's patches were excluded from the sections. The assembled diffusion chambers were placed in a 38 °C heating block, connected to a 95% O_2/5% CO_2 airlift, and filled with 1.0 ml of Ringer's buffer (141 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM H_2CO_3, 0.4 mM NaH_2PO_4, and 1.6 mM K_2HPO_4; pH 7.4) at 38 °C. Buffers of equal isotonicity containing 10 mM mannitol in the donor compartment and 8 mM glucose and 2 mM mannitol in the receiver compartment were used. The Ussing chambers and the airlift/heating block were purchased from TOYOBO Inc. (Tokyo, Japan).

2.5. Determination of PGP-mediated transport (Iida et al., 2005)

The PGP transport studies were initiated by the addition of rhodamine123 (Rho123) to either the apical or basolateral chamber to give a final concentration of 1 μM in the donor compartment. Fluorescein isothiocyanate labeled dextran 4000 (FD-4) was added to the donor side to serve as a paracellular permeability marker. For the inhibition studies, 10 μM verapamil were added to the apical chamber prior to the addition of Rho123. Duplicate samples of 100 μM were taken from the receiver chamber at 15, 30, 45, and 60 min and analyzed with a fluorescence spectrophotometer at excitation and emission wavelengths of 485 and 546 nm, respectively, and were replaced with fresh buffer. Samples were taken from the donor chamber at the start and end of the transport study.

2.6. Determination of MPR2-mediated transport

A previously described 5-carboxyfluorescein diacetate (5-CFDA) efflux assay was used to study the functional activity of MRP (Lee and Piquette-Miller, 2001). In this assay, the non-fluorescent 5-CFDA passively and rapidly diffuses into cells, where it is converted to the fluorescent anion 5-CF by intracellular esterases. 5-CF is effluxed from cells by the MRP family of transporters and is not a substrate of PGP or the human organic anion transporter. 5-CFDA was dissolved in ethanol and diluted in Ringer's buffer to a final

concentration of 50 μM (in 1% ethanol). The directional transport of 5-CF was monitored in both the B to A and A to B directions, with 100 μL samples taken at 15, 30, 45, and 60 min, in the presence or absence of the MRP2 inhibitor MK571 (100 μM). Fresh buffer was added at each sampling time point. The fluorescence of 5-CF was measured using a SpectraMAX Gemini XS plate reader (Molecular Devices) with excitation and emission wavelengths of 490 and 520 nm, respectively.

2.7. Assessment of tissue viability and integrity

The membrane permeation of fluorescein isothiocyanate labeled dextran 4000 (FD-4), a paracellular permeability marker, was monitored in the control and treatment groups for the duration of the experiment (60 min) to assess tissue tight junction integrity. FD-4 (1 mM) was added to the donor side and measured at the receiver side by fluorescence analysis (LS Plate manager 2001, WAKO) with excitation and emission wavelengths of 492 and 515 nm, respectively, at 15, 30, 45, and 60 min.

The membrane resistance (Rm) of the rat ileum was calculated from the membrane potential difference measured under the load of a small external current (0.1 mA and 0.01 mA) according to Ohm's law (Nagira et al., 2006). Morphological examination of the intestinal tissue was achieved by immediately placing a 1-cm section of intestine into 10–15 mL formalin (10% formaldehyde solution) fixative. The fixed section was then blocked with paraffin wax, before being sectioned into 6-μm thick slices and stained with eosin and hematoxylin (Tomita et al., 2009).

2.8. Statistical analysis

All results are expressed as the mean value ± standard error (mean ± S.E.). The statistical significance of the differences between two groups was analyzed using Dunnett's test, Tukey's test, or the Student's *t*-test. *p* values less than 0.05 were considered to be significantly different.

3. Results

3.1. Induction of acute inflammation in rats

Although the rats treated with LPS displayed pronounced exudate formation around the eyes and nostrils and severe diarrhea, no mortality occurred after the treatment. IL-1β mRNA expression, which is used as a marker of inflammation, was induced 3- to 14-fold in the whole intestine at 8 h after LPS treatment, and was most strongly induced in the colon (Fig. 1a). In the jejunum and ileum, the IL-1β mRNA expression level had a tendency to increase (100 ± 25% to 862 ± 388% and 100 ± 33% to 299 ± 101%), although the differences failed to reach the 5% level of statistical significance ($0.05 < p < 0.1$).

Similar cell viability and membrane integrity were seen in ileal segments obtained from the LPS-treated and control rats. The directional transport of FD-4 remained unchanged, and LPS treatment did not increase FD-4 permeability in the ileal segments mounted on Ussing chambers (Fig. 1b). The level of membrane resistance (Rm) did not significantly decrease over the experimental duration and was similar between the LPS-treatment and control groups (Fig. 1c). Slight changes in morphological characteristics were seen in the histological sections from the LPS-treated animals, including loss of tissue color and enlarged spacing between adjacent villi (data not shown). Nevertheless, similar cell viability and integrity were seen in the LPS-treated and control samples.

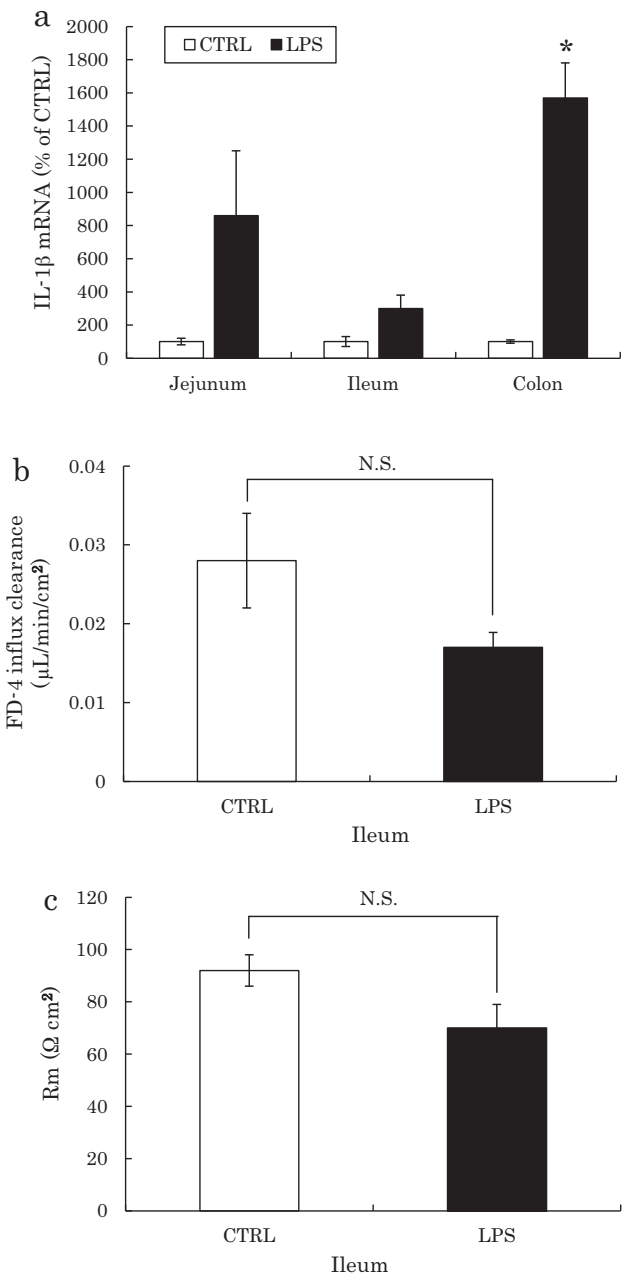

Fig. 1. (a), (b) and (c) show the expression of IL-1β mRNA in intestinal segments, FD-4 permeability in the mucosal to serosal direction in the ileum, and Rm in the ileum, respectively. Values (mean ± S.E.M., $n = 6–8$) are shown as percentages of the respective control values. The closed and open columns represent the LPS-treated and control rats, respectively. The intensities of the IL-1β genes were normalized to that of GAPDH. *, $p < 0.05$. N.S.: not significant.

3.2. Gene expression

The levels of mdr1a mRNA were consistently and significantly reduced in both the jejunal and ileal regions to approximately 31 ± 7.3% and 40 ± 3.2% of the control level, respectively, in the LPS-treated animals (Fig. 2a). The levels of mdr1a mRNA also tended to be lower in the colons of LPS-treated animals; however, this change did not reach significance (Fig. 2a). The expression of mrp2 mRNA was also suppressed in the ilea of the LPS-treated animals to 59.8 ± 8.7% of the control value (Fig. 2b). Likewise, PXR mRNA expression was suppressed to 63.7 ± 3.9% of the control value in response to LPS treatment.

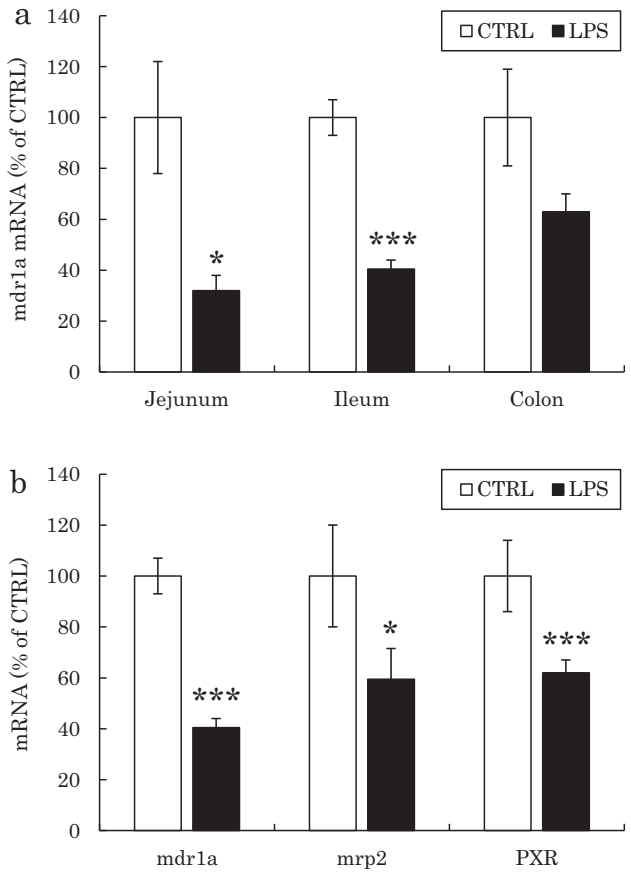

Fig. 2. (a) and (b) show the expression level of mdr1a mRNA in intestinal segments, and those of mdr1a, mrp2, and PXR mRNA in the ileum, respectively. The closed and open columns represent the LPS-treated and control rats, respectively. The optical densities of the mdr1a, mrp2, and PXR PCR products were measured and normalized to that of GAPDH. Values (mean ± S.E.M., $n = 6–8$) are reported as percentages of the control values (*, $p < 0.05$, ***, $p < 0.005$).

3.3. Rho123 transport by PGP in the ileum

In the LPS-treated rats, the efflux clearance of Rho123 in the serosal to mucosal direction was reduced to 40% of the control value (Fig. 3). The addition of the PGP-specific inhibitor verapamil to the mucosal side of segments isolated from the control rats

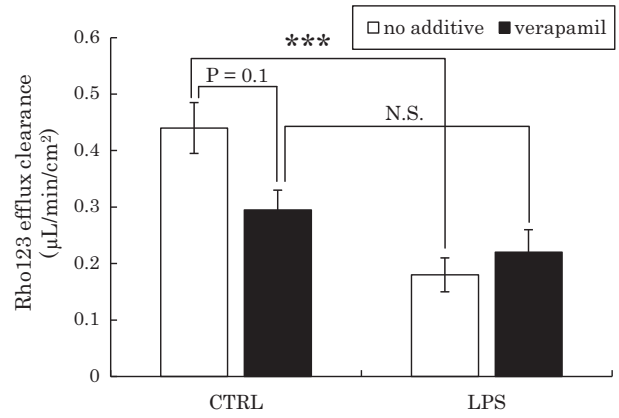

Fig. 3. PGP-mediated efflux clearance of Rho123 in the ileum. The values (mean ± S.E.M., $n = 6–8$ per groups) are reported as percentages of the control values. The open and closed columns represent the no additive and verapamil groups, respectively. ***, $p < 0.005$. N.S.: not significant.

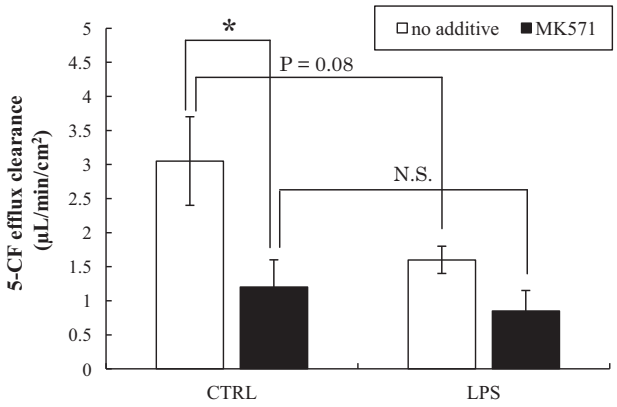

Fig. 4. MRP-mediated efflux clearance of 5-CF in the ileum. The values (mean ± S.E.M., $n = 6–8$ per groups) are reported as percentages of the control values. The open and closed columns represent the no additive and MK571 groups, respectively. *, $p < 0.05$. N.S.: not significant.

reduced transport to 60% of the control level, whereas the addition of the inhibitor to the mucosal side of segments isolated from the LPS-treated rats did not inhibit Rho123 transport further. The Rho123 transport in the mucosal to serosal direction, which represents net absorption, was increased from 100 ± 18% in the control to 230 ± 40% in the LPS-treated animals (data not shown).

3.4. 5-Carboxyfluorescein transport by MRP2 in the ileum

MRP activity, as shown by the efflux clearance of 5-CF in the serosal to mucosal direction, was reduced to 50 ± 7% of the control values ($p = 0.08$) in the LPS-treated animals (Fig. 4). Adding the MRP-specific inhibitor MK571 to intestinal segments isolated from the control rats reduced the efflux to 37 ± 14% (Fig. 4). In contrast, the addition of the inhibitor to the mucosal side of segments isolated from the LPS-treated rats tended to decrease 5-CF efflux, although the differences failed to reach the 5% level of statistical significance ($0.05 < p < 0.1$) (Fig. 4). 5-CF transport in the mucosal to serosal direction (net absorption) was significantly increased from 100% in the controls to 190% in the LPS-treated rats ($p < 0.05$) (data not shown).

4. Discussion

The suppression of ABC transporters' expression, the suppression of ABC transporters' function and unaltered paracellular membrane permeability in the intestine are already recognized at 24, 48 and 72 h after LPS administration (Kalitsky-Szirtes et al., 2004; Moriguchi et al., 2007). On the other hand, we have been focusing on the changes in the expression and function in the early phase after LPS administration (Tomita et al., 2004; 2010). In the present study, we investigated the levels of intestinal P-gp expression and activity of P-gp in the small intestine at 8 h using a rat model induced by LPS.

Previous studies have demonstrated that inflammation reduces the hepatic expression and activity of the drug efflux transporters mdr1 and mrp2 (Piquette-Miller et al., 1998; Tang et al., 2000). This suppression is primarily mediated by pro-inflammatory cytokines, particularly IL-1β (Tomita et al., 2010a,b). Whether inflammation-mediated changes in mdr1 or mrp2 expression occur in epithelial tissues such as the intestine is unknown. However, there is increasing evidence that the intestinal mucosa responds to endotoxins in a similar manner to the well-characterized response observed during the hepatic acute phase response (Molment et al., 1993). Endotoxins are known to stimulate the production of IL-1β in the liver (Tomita et al., 2010a,b). Indeed, we observed an increase in the

expression of IL-1β mRNA in the intestinal segments of LPS-treated rats, which is consistent with the results reported by Molment et al. (1993).

We found that LPS-induced inflammation downregulated the intestinal mRNA expression of mdr1 and mrp2, as was previously reported in the liver (Piquette-Miller et al., 1998; Tang et al., 2000; Goralski et al., 2003). Although the levels of mdr1a mRNA were reduced throughout the whole intestinal regions of the LPS-treated rats, mdr1b expression was low and was not significantly altered by LPS-treatment. Low and often undetectable expression of mdr1b in the intestine has been reported previously (Salphati and Benet, 1998), and investigations in knockout mice have clearly shown that mdr1a is the major determinant of PGP-mediated drug efflux in the intestine (Stephens et al., 2002).

In accordance with the changes in mRNA expression, we found that LPS treatment induced significant reductions in PGP- and MRP2-mediated transport in the intestine, whereas the permeability of FD-4 was unchanged. Compared with the controls, significant reductions in the serosal to mucosal (S to M) efflux of model substrates of PGP and MRP (Rho123 and 5-CF, respectively) were observed in the intestinal segments isolated from the LPS-treated rats. In these animals, the residual S to M transport was not reduced further by the addition of specific inhibitors such as verapamil or MK571, suggesting that the remaining transport reflects passive diffusion. Hence, it is likely that the expression and activity of PGP and MRP2 were not completely repressed, indicating that other post-translational factors may also be involved. The elevated mucosal to serosal (M to S) flux of Rho123 and 5-CF suggests that a net increase in the bioavailability of PGP and MRP2 substrates occurs in vivo during the inflammatory response.

Our protein expression and activity data regarding PGP/mdr1a and MRP2 agree with each other, leading us to believe that the reductions in both PGP and MRP activity are the result of a mechanism regulating acute inflammation. Furthermore, these studies indicate that statistically significant alterations in intestinal drug absorption are predicted by altered drug transporter levels. Since tissue viability (as measured by Rm) and FD-4 permeability were not significantly different between the LPS-treated and control animals, it is unlikely that alterations in membrane integrity are responsible for the observed changes in the expression and activity of PGP and MRP2. It is important to consider the potential contribution of other transporters and metabolic enzymes. Although 5-CF efflux reflects total MRP activity, the levels of mrp1 and mrp3 were not significantly affected, and the mRNA expression of other MRP isoforms was not detectable after LPS treatment, indicating that changes in 5-CF transport reflect changes in MRP2 activity.

From these studies, significant and concurrent reductions in the expression and activity of PGP/mdr1a and mrp2 in the intestinal tissue were found during acute inflammation. Hence, a coordinated system that regulates multiple drug transporters genes is activated in response to inflammatory stimuli. The activation of the PXR nuclear receptor has been reported to induce the expression of MDR1 (Geick et al., 2001) and MRP2 (Kast et al., 2002) in both the liver and intestine (Staudinger et al., 2001). Furthermore, the negative regulation of PXR has been reported to occur via an IL-1β-mediated mechanism in human hepatocytes (Pascussi et al., 2000). This phenomenon has not been examined in vivo or in intestinal tissue. Hence our results, demonstrating a significant reduction in PXR mRNA levels in LPS-treated animals in conjunction with the suppression of mdr1 and mrp2, suggest the involvement or coregulation of PXR during inflammation. Further studies examining the involvement of PXR in the basal expression and negative regulation of these genes are currently being performed in PXR knockout and wild-type animal models of inflammation (Jekerle et al., 2003).

Intestinal efflux transporters contribute to drug clearance, including drug secretion into bile and the direct exsorption of drugs into the intestinal lumen. Hence, inflammatory stimuli are likely to induce changes in the bioavailability and clearance of numerous drugs that are substrates of PGP and MRP2, thus increasing the possibility of adverse drug reactions or therapeutic failure.

Our findings indicate that the intestine is changed during an inflammatory response. Alterations in drug transport in the intestine, as well as the liver, should therefore be considered when predicting drug disposition during inflammation. Increased and variable drug absorption is likely to occur during inflammation, and proper precautions must be taken when determining the appropriate dose regimen for a particular drug therapy. The phenomena described in this study are useful for predicting therapeutic efficacy and understanding drug–disease interactions.

References

Geick, A., Eichelbaum, M., Burk, P., 2001. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. J. Biol. Chem. 276, 14581–14587.

Goralski, K., Hartmann, G., Piquette-Miller, M., Renton, K.W., 2003. Downregulation ofmdr1a expression in the brain and liver during CNS inflammation alters the in vivo disposition of digoxin. Br. J. Pharmacol. 139, 35–48.

Hartmann, G., Kim, H., Piquette-Miller, M., 2001. Regulation of the hepatic multidrug resistance gene expression by endotoxin and inflammatory cytokines in mice. Int. Immunopharmacol. 1, 189–199.

Hartmann, G., Cheung, A., Piquette-Miller, M., 2002. Inflammatory cytokines, but not bile acids, regulate expression of murine hepatic anion transporters in endotoxemia. J. Pharmacol. Exp. Ther. 303, 273–281.

Iida, A., Tomita, M., Hayashi, M., 2005. Regional differences in P-glycoprotein function in rat intestine. Drug Metab. Pharmacokinet. 20, 100–106.

Jekerle, V., Hartmann, G., Piquette-Miller, M., 2003. Interleukin downregulates expression of the pregnane X receptor (PXR) in mice. Clin. Pharmacol. Ther. 73, 60–63.

Kalitsky-Szirtes, J., Shayeganpour, A., Brocks, D.R., Piquette-Miller, M., 2004. Suppression of drug-metabolizing enzymes and efflux transporters in the intestine of endotoxin-treated rats. Drug Metab. Dispos. 32, 20–27.

Kast, H.R., Goodwin, B., Tarr, P.T., Jones, S.A., Anisfeld, A.M., Stoltz, C.M., Tontonoz, P., Kliewer, S., Willson, T.M., Edwards, P.A., 2002. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J. Biol. Chem. 277, 2908–2915.

Lee, G., Piquette-Miller, M., 2001. Influence of IL-6 on MDR and MRP-mediated in human hepatoma cells. Can. J. Physiol. Pharmacol. 79, 876–884.

Molment, E.P., Ziambaras, T., Perlmutter, D.H., 1993. Evidence for an acute phase response in human intestinal epithelial cells. J. Biol. Chem. 268, 14116–14124.

Moriguchi, J., Kato, R., Nakagawa, M., Hirotani, Y., Ijiri, Y., Tanaka, K., 2007. Effects of lipopolysaccharide on intestinal P-glycoprotein expression and activity. Eur. J. Pharmacol. 565, 220–224.

Nagira, M., Tomita, M., Mizuno, S., Kumata, M., Ayabe, T., Hayashi, M., 2006. Ischemia/reperfusion injury in the monolayers of human intestinal epithelial cell line Caco-2 and its recovery by antioxidant. Drug Metab. Pharmacokinet. 21, 230–237.

Pascussi, J., Gerbal-Chaloin, S., Pichard-Gracia, L., Daujat, M., Fabre, J., Maurel, P., Vilarem, M.J., 2000. Interleukin negatively regulates the expression of PXR and CAR in primary human hepatocytes. Biochem. Biophys. Res. Commun. 274, 707–713.

Payen, L., Spafel, L., Courtois, A., Vernhet, L., Guillouzo, A., Fardel, O., 2002. The drug efflux pump MRP2: regulation of expression in physiopathological situations and by endogenous and exogenous compounds. Cell Biol. Toxicol. 18, 221–233.

Piquette-Miller, M., Pak, A., Kim, H., Anari, R., Shahzamani, A., 1998. Decreased expression and activity of P-glycoprotein in rat liver during acute inflammation. Pharm. Res. 15, 706–711.

Salphati, L., Benet, L.Z., 1998. Modulation of PGP expression by CYP 3A induces in male and female rat liver. Biochem. Pharmacol. 55, 387–395.

Slaviero, K., Clarke, S., Rivory, L., 2003. Inflammatory response: an unrecognized source of variability in the pharmacokinetics and pharmacodynamics of cancer chemotherapy. Lancet Oncol. 4, 224–232.

Staudinger, J., Goodwin, B., Jones, S., Hawkins-Brown, D., Mackenzie, K., LaTour, A., Liu, Y., Klaassen, C., Brown, K., Reinhard, J., 2001. The nuclear receptor PXR is a lithocolic acid sensor that protects against liver toxicity. Proc. Natl. Acad. Sci. U. S. A. 98, 3369–3374.

Stephens, R., O'Neill, C., Bennett, J., Humphrey, M., Henry, B., Rowland, M., Warhurst, G., 2002. Resolution of PGP and non-PGP effects on drug permeability using intestinal tissues from mdr1a (−/−) mice. Br. J. Pharmacol. 135, 2038–2046.

Synold, T., Dussault, I., Forman, B., 2001. The orphan nuclear receptor SXR coordinately regulated drug metabolism and efflux. Nat. Med. 7, 584–590.

Tang, W., Yi, C., Kalitsky, J., Piquette-Miller, M., 2000. Endotoxin downregulates hepatic expression of PGP and MRP2 in 2-acetylaminofluorene-treated rats. Mol. Cell Biol. Res. Commun. 4, 90–97.

Tomita, M., Ohkubo, R., Hayashi, M., 2004. Lipopolysaccharide transport system across colonic epithelial cells in normal and infective rat. Drug Metab. Pharmacokinet. 19, 33–40.

Tomita, M., Kishimoto, H., Takizawa, Y., Hayashi, M., 2009. Effect of intestinal ischemia/reperfusion on P-glycoprotein mediated biliary and renal excretion of rhodaimne123 in rat. Drug Metab. Pharmacokinet. 24, 428–437.

Tomita, M., Watanabe, A., Fujinaga, I., Yamakawa, T., Hayashi, M., 2010a. Nonlinear intestinal absorption of methylprednisolone caused by absorptive and secretory transporters. Int. J. Pharm. 387, 1–6.

Tomita, M., Kanbayashi, A., Murata, H., Tanaka, A., Nakaike, M., Hatanaka, M., Hayahsi, M., 2010b. Effect of lipopolysaccharide on P-glycoprotein-mediated intestinal and biliary excretion of rhodamine123 in rats. Int. J. Pharm. 392, 35–41.