Inflammatory Cytokines, but Not Bile Acids, Regulate Expression of Murine Hepatic Anion Transporters in Endotoxemia

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ABSTRACT
Endotoxin-mediated cholestasis stems from impaired hepatobiliary transport of bile acids and organic anions due to altered expression and activity of transporters, including Oatp, Mrp, Ntcp, and Bsep. However, the mechanisms by which the Oatp and Mrp genes are down-regulated are largely unknown. Using in vivo and in vitro murine models of inflammation, we examined the role of cytokines and bile acids in regulating Oatp and Mrp. Endotoxin (lipopolysaccharide, LPS), interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, cholic acid, taurocholate, or taurodeoxycholate was administered in vivo to mice or in vitro to Hepa 1-6 mouse hepatoma cells. Mrp, Oatp, and Bsep mRNA levels were measured by reverse transcription-polymerase chain reaction. Mrp efflux activity was measured using 5-carboxyfluorescein. In vivo, LPS treatment profoundly suppressed hepatic mRNA levels of Mrp2, Mrp3, Oatp1, Oatp2, and Bsep to 15, 60, 44, 30, and 32% of controls, respectively (p < 0.05), but did not significantly alter Mrp1 expression. IL-6 or IL-1β administration suppressed Mrp2, Oatp1, Oatp2, and Bsep mRNA levels to 20 to 60% controls (p < 0.05). TNF-α administration affected mRNA levels of Mrp2, Mrp3, and Oatp2 but not Oatp1 or Oatp. Bile acid treatment increased the in vivo expression of Bsep but not Mrp or Oatp. Likewise, significantly lower mRNA levels of Mrp2 with a corresponding decrease in cellular efflux of 5-carboxyfluorescein was seen in vitro in IL-6- and IL-1β-treated Hepa 1-6 cells, whereas bile acids did not have significant effects. In conclusion, cytokines are key mediators in regulating hepatic expression of anion transporters in inflammatory cholestasis, whereas bile acids likely play a minor role.

A number of liver diseases are characterized by disturbances in the hepatobiliary transport of endogenous and exogenous compounds. Primarily, these changes have been attributed to altered expression and activity of hepatic transport proteins that are involved in the uptake and excretion of organic compounds (Trauner et al., 1998). Contribution and down-regulation of key hepatic bile salt transporters such as the sodium-dependent taurocholate transporter (Ntcp) has been elegantly characterized in various models of liver disease. However, members of the Mrp and Oatp families of organic anion transporters, located on the basolateral and canalicular membranes of hepatocytes, are also involved in maintaining liver homeostasis. These transporters participate in the uptake and efflux of many endogenous organic anions, including the bile acids. Indeed, inherited defects in Mrp2 (also termed canalicular multispecific organic anion transporter) result in Dubin-Johnson syndrome, a congenital disease associated with chronic hyperbilirubinemia and jaundice (Paulusma et al., 1997). Organic anion transporters also play a key role in the hepatic uptake and excretion of numerous xenobiotics. For the Oatp transporters, a wide range of substrates have been identified, including bromosulphophthalein, estrone-3-sulfate, and taurocholate (Meier et al., 1997). Substrates for the Mrp transporters include numerous anionic drugs such as vincristine, daunorubicin, and etoposide (Cole et al., 1994) as well as their glucuronide, glutathione, and sulfate conjugates (Leier et al., 1994; König et al., 1999). Furthermore, both families are often involved in the secretion of these compounds; that is, many anionic drugs are taken up into hepatocytes by Oatp, conjugated by phase II enzymes, and then excreted into bile via Mrp (Cui et al., 1999). Hence, changes in the expression and activity of the Oatp and Mrp transporters are capable of influencing the pharmacokinetics of many clinically important drugs.

Hepatic and cholestatic diseases often result in the accumulation of toxic compounds and metabolites that can lead to eventual liver failure (Trauner et al., 1998). Endotoxin administration to rodents imposes a sepsis model of cholestasis that is also associated with dramatic changes in the expression of several hepatic transporters, including Ntcp, the bile salt export pump (Bsep), the canalicular organic anion trans-

ABBREVIATIONS: Mrp, multidrug resistance-associated protein; Oatp, organic anion-transporting polypeptide; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; DMEM, Dulbecco’s modified Eagle’s medium; 5-CF, 5-carboxyfluorescein; 5-CFDA, 5-carboxyfluorescein diacetate; RT-PCR, reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; rRNA, ribosomal RNA; OD, optical density.
porter, Mrp2, and P-glycoprotein (Trauner et al., 1997; Piquette-Miller et al., 1998; Vos et al., 1998; Lund et al., 1999; Hartmann et al., 2001). Nevertheless, underlying mechanisms involved in endotoxin-imposed regulation of organic anion transporters are still relatively unknown. Inflammatory cytokines and bile acids are felt to be the principle mediators of LPS-induced cholestasis (Denson et al., 2000), but it is unclear whether activity of cytokines and/or bile acids is responsible for observed changes in expression of the hepatic anion transporters. Also the question arises whether these mediators act independently or in concert. Hence, we examined the effects of individual cytokines and bile acids on the expression and functional activity of murine organic anion transporters in vitro and in vivo. Examination of Bsep, mRNA levels of which are reportedly suppressed after administration of LPS or cytokines, was included as a control. Novel findings from this study demonstrate that inflammation-inducing stimuli, including endotoxin (LPS), turpentine, IL-1β, and IL-6 elicit an in vivo down-regulation of Mrp2, Oatpl, and Oatp2 expression in mouse liver, whereby IL-1β and IL-6 likely act as principle mediators. In comparison, acute administration of bile salts and acids did not impose significant effects on these transporters. These findings have important implications in the prediction of overall drug disposition during infectious and inflammatory disease and in identifying potential drug-disease interactions.

Materials and Methods

Animals and in Vivo Animal Studies. Animal studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care. Eight-week-old male CD-1 mice (25–35 g) were supplied by Charles River (St. Constant, QC, Canada). In acute inflammation studies, animals (n = 4/group) were injected either with 200 µl of s.c. turpentine oil (Wiler Fine Chemicals, London, ON, Canada) or 5 mg/kg i.p. LPS (from Escherichia coli serotype 055:B5 (Sigma-Aldrich, St. Louis, MO). Control mice received saline buffer. In the cytokine studies, animals (n = 4) received different doses of IL-1β (1,000 and 10,000 U i.p.), IL-6 (1,000, 2,500, and 10,000 U i.p.), TNF-α (2,500, 10,000, and 25,000 U i.p.), or a combined dose of all three cytokines (2,500 U of IL-1β, IL-6, and TNF-α). In the bile acid studies, animals (n = 4) received equal doses (1.5 µmol/30 g of body weight i.p.) of cholic acid, taurocholate (sodium salt), or taurodeoxycholate (sodium salt). To establish whether cytokine and bile acid combinations would elicit further changes, mice (n = 4) received combined doses of cholic acid (1.5 µmol) and IL-6 (2,500 U). Murine IL-1β (bioactivity 2.8 × 10^4 U/µg), IL-6 (8 × 10^4 U/µg), TNF-α (6.25 × 10^5 U/µg), and bile acids were purchased from Sigma-Aldrich. For acute inflammation and cytokine studies, animals were sacrificed 6 h after treatment (unless otherwise indicated). Increased hepatic levels of serum amyloid A mRNA were used to confirm the hepatic levels of serum amyloid A mRNA were used to confirm the inflammatory response in LPS- and turpentine-treated animals. For bile acid studies, animals were fasted for 16 h immediately after treatment and sacrificed at 24 h. Livers were excised, rapidly frozen in liquid nitrogen, and stored at −80°C.

In Vitro Studies. Murine Hepa 1-6 hepatoma cells (kindly provided by G. Kirby, University of Guelph, Guelph, ON, Canada) were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Burlington, ON, Canada) containing 4.5 g/l D-glucose, 10% (v/v) fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). Hepa 1-6 cells were plated in 100-mm Petri dishes (Starstedt, St. Leonard, QC, Canada), incubated in a incubator (Forima Scientific, Marietta, OH) in a 5% CO2 atmosphere at 37°C. Cells were grown to confluence and treated with different concentrations of cytokines (IL-1β, IL-6, TNF-α; 1 or 10 ng/ml in DMEM) or bile acids (taurocholate, cholic acid; 25 or 100 µM in DMEM, pH 7.4). Controls were treated with DMEM. Total RNA was harvested at 6- and 24-h time points (n = 4–6).

The accumulation of 5-carboxyfluorescein (5-CF) was examined in Hepa 1-6 cells 24 h after treatment as described previously (Lee and Piquette-Miller, 2001). The nonfluorescent ester 5-carboxyfluorescein diacetate 5-CFDA (Sigma-Aldrich) is rapidly and passively taken up into cells where it is converted by intracellular esterases to the fluorescent moiety 5-CF, which is a specific substrate of the Mrp transporters (Draper et al., 1997). Briefly, cells were grown in six-well culture dishes (Costar, Cambridge, MA) to confluence. Culture medium was removed and replaced with serum-free DMEM containing 5 µM 5-CFDA (Sigma-Aldrich) for 15 min at 37°C. After 5-CFDA preincubation, the media were replaced with fresh, serum-free DMEM, and 5-CF was allowed to efflux (in the presence or absence of 400 µM indomethacin) for 5 min, and cells were rapidly washed three times with ice-cold phosphate-buffered saline containing 150 mM NaCl, 4 mM Na2HPO4, and 1.5 mM KH2PO4 and lysed with 1% (v/v) Triton X-100 in phosphate-buffered saline (2 ml/well). Intracellular retained 5-CF was measured by fluorometry at excitation wavelength 492 nm and emission wavelength 518 nm using a Shimadzu RF5000U fluorometer (Mandel Scientific, Guelph, ON, Canada). Fluorometric values were normalized to cellular protein content in lysates as determined by the Bradford protein assay (Bio-Rad, Hercules, CA). For examination of Mrp-mediated transport, we calculated the amount of intracellular 5-CF remaining after 5-min efflux as a percentage of intracellular 5-CF remaining in control (nontreated) cells. A 2- to 3-fold increase in intracellular 5-CF was seen in controls in the presence of the Mrp inhibitor indomethacin. All values represent means of three measurements (n = 3), performed in triplicate on separate experimental days. Similar values were obtained between culture days.

RT-PCR Analysis of mRNA. Total RNA was extracted from livers and from cells using the QuickPrep RNA extraction kit (Amersham Biosciences, Piscataway, NJ) following manufacturer’s instructions. A quantitative RT-PCR assay was used based on methods reported previously (Hartmann et al., 2001; Sukhai et al., 2001). The primer sequences used for PCR amplification are listed in Table 1. Briefly, reverse transcription of 0.5 µg of RNA was performed using the First Strand cDNA Synthesis kit (MBI Fermentas, Flamborough, ON, Canada) in a total volume of 20 µl following manufacturer’s instructions. Standard PCR curves were generated for each PCR product to establish linearity of the RT-PCR reaction and determine optimal template concentrations. One to 2 µl of reverse transcription product was used for amplification of specific DNA sequences in the presence of 1 mM MgCl2, 200 µM dNTP, and 50 pmol of each primer in a total volume of 100 µl using a GeneAmp 2400 thermocycler (PerkinElmer, Mississauga, ON, Canada). The reaction was initiated by addition of 2.5 units of Taq polymerase (MBI Fermentas) and amplification proceeded through 25 to 26 cycles. A total of 33 to 35 cycles was required for detection of Mrp1. PCR products were separated by electrophoresis on 2% agarose gels, stained with SYBR Gold nucleic acid stain (Molecular Probes, Eugene, OR), and visualized by UV. The DNA band sizes (300 base pairs for each gene product) were confirmed using Gene Ruler 100-bp DNA ladder (Invitrogen). Optical densities were normalized to 18S ribosomal RNA (rRNA) band intensities. Results obtained from RT-PCR were routinely confirmed on Northern blots.

Western Blots. Mrp2 protein expression was compared in crude membrane fractions isolated from control and treated mice on Westerns (Lee and Piquette-Miller, 2001). Briefly, 20 µg of protein sample was separated on SDS-polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes (Amersham Biosciences). After blocking, the blots were washed and incubated with M2III-6 (ID Labs Inc., London, ON, Canada), a monoclonal antibody capable of detecting Mrp2. The bound antibody and optical density were quantitated using Digital Science 1D Image Analysis software (Eastman Kodak, Rochester, NY). Western blot analysis with MRp1,
TABLE 1
PCR primers used for mouse Mrp1, Mrp2, Oatp1, and Oatp2

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which is specific for the Mrp1 protein, did not detect measurable levels of Mrp1. Antibodies that have been developed for rat and human Oatp1 and Oatp2 do not cross-react with the mouse Oatp proteins (A. Wolkoff, personal communication).

Data Analysis. Optical densities of bands obtained from denaturing gels and agarose gels (PCR products) were quantitated using DC120 camera and DS12D Scientific Imaging software (Eastman Kodak). Levels of mRNA expression are reported as percentages of normalized values, compared with control values. Normalized values were calculated as ratios: (OD Mrp mRNA)/(OD 18S rRNA) and (OD Oatp mRNA)/(OD 18S rRNA), respectively. Statistical analysis was performed using both the Student’s t test (Excel; Microsoft, Redmond, WA) and analysis of variance (GraphPad Prism; GraphPad Software, San Diego, CA).

Results
In Vivo Effects of Inflammatory Stimuli. The effects of LPS and turpentine-induced acute inflammation on the hepatic expression of Mrp, Oatp, and Bsep are depicted in Fig. 1. At 6 h after LPS treatment (Fig. 1A), we saw a substantial down-regulation of hepatic mRNA levels of Mrp2, Mrp3, Oatp1, Oatp2, and Bsep (15–60% of controls). The mRNA levels of these transporters began to return to normal after 24 h (70–75% controls). Significant changes in Mrp1 (95 ± 15% controls) were not seen in the LPS-treated mice. Likewise, we observed significant reductions in the mRNA levels of Mrp2, Oatp1, Oatp2, and Bsep (35–70% of controls) 6 h after turpentine treatment (Fig. 1B). Levels were still reduced by 20 to 30% at 24 h, but this reduction was not significant (data not shown). Western blots of liver protein samples obtained from mice 24 h after treatment with turpentine or LPS detected corresponding reductions of 65 to 80% in the immunodetectable expression of Mrp2 (Fig. 1C).

The effects of cytokine administration on the hepatic expression of these transporters are depicted in Fig. 2. As shown in Fig. 2A, administration of IL-1β to mice elicited significant dose-dependent decreases in mRNA levels of the transporters examined. Pronounced reductions in mRNA levels of Oatp1, Oatp2, and Bsep (20–42% of control values) were seen in the IL-1β-treated mice. Compared with controls, mRNA levels of Mrp2 were reduced to approximately 70% of control values. Likewise, significantly lower immunodetectable levels of Mrp2 (36 ± 15% of control values) were detected in the livers isolated at 24 h from IL-1β-treated mice. Whereas mRNA levels of Mrp3 were not significantly affected, we detected lower mRNA levels of Mrp1 in the IL-1β-treated mice (10,000 U: 52 ± 17% controls in samples containing detectable expression).

Treatment of mice with IL-6 similarly resulted in prominent reductions in the mRNA expression of several transporters (Fig. 2B). A significant dose-dependent suppression of Mrp2 was seen in IL-6-treated mice. Likewise, a corresponding reduction in immunodetectable levels of Mrp2 was observed at 24 h in mice receiving 10,000 U of IL-6 (optical density, 65 ± 5.5% control values; p < 0.05). Expression of Oatp1, Oatp2, and Bsep mRNA was also reduced in the IL-6-treated mice in a dose-dependent manner to 20 to 55% of control values (p < 0.01). Similar to that seen after IL-6 administration, mRNA levels of Mrp3 were not significantly affected; however, lower levels of Mrp1 mRNA levels were seen in the IL-6-treated mice (10,000 U: 32 ± 9.6% controls in samples containing detectable expression).

The overall profile of changes in transporter mRNA expression was quite different in mice receiving TNF-α (Fig. 2C). A pronounced reduction in the mRNA levels of Mrp3 mRNA was seen in the TNF-α-treated mice. Although TNF-α-treated mice also expressed approximately 30% lower mRNA levels of Mrp2 and Oatp2, the reduction in their mRNA levels was relatively mild compared with that seen after IL-1β or IL-6 treatment. Furthermore, significant effects were not seen at lower (1000 and 2500 U) doses of TNF-α (data not shown). Levels of Mrp1, Oatp1, and Bsep mRNA were not altered by TNF-α administration. Compared with controls, immunodetectable levels of the Mrp2 gene product were not significantly different in the TNF-α-treated mice.

Administration of all three cytokines in combination did not impose further suppression in the hepatic expression of these genes. Mice administered combined doses of IL-6, IL-1β, and TNF-α (2500 U of each) expressed much lower levels of Mrp2 mRNA (45 ± 3.5% controls), similar to that seen in mice treated with 2500 U of IL-6 alone (37 ± 11% controls). However, levels of Mrp1 (108 ± 17% controls) and Oatp1 (83 ± 11% controls) were not significantly different between treated and controls.

In Vivo Effects of Bile Acid Administration. As shown in Fig. 3, administration of conjugated and unconjugated bile acids did not alter the expression of Mrp1, Mrp2, Oatp1, and Oatp2 mRNA to any remarkable extent. Expression levels of Bsep were unaffected by cholic acid and taurocholate but markedly increased by taurodeoxycholate treatment. Although slight increases in Mrp2 mRNA levels were seen in
the cholic acid-treated mice, these increases did not reach significance. On the other hand, significant decreases in mRNA levels of Mrp2 (65 ± 13% controls) and Oatp1 (68 ± 15% controls) were seen in mice given a combination dose of cholic acid and IL-6, similar to levels observed in mice dosed with 2500 U of IL-6 alone.

In Vitro Studies. Regulation of the organic anion transporters was also examined in the Hepa 1-6 cells, a cell line that phenotypically resembles mouse hepatocytes and has functional responses to cytokines (Darlington et al., 1980; Fardel et al., 1993). RT-PCR analysis detected quantifiable expression of the liver transport proteins Mrp1, Mrp2, Mrp3, mdr1a, mdr1b, and mdr2 in the Hepa 1-6 cells. Very low levels of Bsep mRNA were also observed, but Oatp1 and Oatp2 could not be detected (data not shown).

Similar to that observed in vivo, significantly diminished levels of Mrp2 mRNA were detected in the 10 ng/ml IL-1β- and IL-6-treated Hepa 1-6 cells, whereas lower, nonsignificant reductions were seen in the TNF-α-treated cells (Fig. 4). Although a dose-dependent increase in Mrp1 mRNA levels
was seen in the IL-1β-treated cells (1 ng/ml, 127 ± 8% controls; 10 ng/ml, 165 ± 11% controls; p < 0.05), expression of Mrp3 was not significantly altered. Moreover, significant changes in mRNA levels of Mrp3 and Mrp1 were not detected in the TNF-α- and IL-6-treated cells. Incubation of Hepa 1-6 cells with the bile acids, taurocholate and cholic acid, imposed changes in mRNA levels of Mrp1 but not Mrp2 (Table 2). The cellular response to bile acids in Hepa 1-6 cells was confirmed by induction of SHP (small heterodimer partner) mRNA levels in the taurocholate- and cholic acid-treated cells (188 ± 12% controls; p < 0.05).

Due to inherent difficulties in establishing transport assays from mouse hepatocytes, Hepa 1-6 cells were chosen as an in vitro model to examine the effects of cytokines and bile acids on the functionality of the Mrp transporters. Substantial intracellular accumulation of 5-CF was seen in Hepa 1-6 control cells after preincubation with 5-CFDA, with more than 75% of 5-CF effluxed within the first 5 min (data not shown). In controls the percentage of 5-CF remaining in cells after a 5-min efflux was substantially increased by the addition of the Mrp2 inhibitor indomethacin. Compared with controls, significantly greater intracellular amounts of 5-CF were seen in the IL-6-, IL-1β-, TNF-α-, and LPS (10 and 50 ng/ml)-treated Hepa 1-6 cells after the 5-min efflux period (Fig. 5A), indicating a reduction in total efflux activity of the Mrp transporters. On the other hand, treatment of cells with the bile acids cholic acid or taurocholate did not impose changes in the intracellular accumulation or efflux of 5-CF (Fig. 5B).

Discussion

Results from our in vivo studies clearly demonstrate that hepatic expression of the Mrp and Oatp anion transporters are suppressed during an acute inflammatory response. Using in vivo and in vitro models of experimental inflammation and administering individual cytokines, we attempted to delineate the mechanisms through which this suppression occurs. Expression of Mrp2, Oatp1, and Oatp2 mRNA was down-regulated upon exposure to LPS (Fig. 1A), a bacterial endotoxin that generates septic cholestasis, as well as after subcutaneous administration of turpentine (Fig. 1B), a chemical irritant that evokes a local aseptic inflammatory response. These models of acute inflammation have been well characterized with regard to cytokine stimulation and their effects on hepatic acute phase protein production. Although inflammation induced by both turpentine and LPS stimulate the systemic release of IL-6 and IL-1β, there is additional involvement of IL-1α and TNF-α in LPS-induced inflammation (Fautuzzi and Dinarello, 1996). Indeed, it is believed that TNF-α likely plays a prominent role in down-regulation of bile salt transport and cholestasis in endotoxemia (Whiting et al., 1995). However, because we observed substantial reductions in the mRNA expression of Mrp1, Mrp2, Oatp1, and Oatp2 after induction of inflammation with turpentine, a model that is not associated with TNF-α induction nor accumulation of bile acids, our data suggest a more pronounced role of IL-6 and IL-1β. Likewise, a down-regulation of the hepatic expression of the efflux transporter P-glycoprotein has been observed in mice and rats after induction of inflammation with either turpentine, LPS, IL-6, or IL-1β (Piquette-Miller et al., 1998; Hartmann et al., 2001).

It is generally believed that biochemical pathways underlying regulatory effects of inflammation on hepatic production of the acute phase proteins are primarily evoked by the proinflammatory cytokines. Indeed, results obtained from our cytokine-dosed mice indicated a prominent role of IL-6, IL-1β, and TNF-α in regulating the expression of several of these hepatic transporters. Dramatic reductions of 30 to 80% were seen in the mRNA levels of Mrp, Bsep, Oatp1, and Oatp2 in both the IL-6- and IL-1β-treated mice. The pronounced down-regulation of organic anion transporters in the IL-6-treated mice is consistent with other studies that have implicated IL-6 in the transcriptional regulation of transporters (Green et al., 1994; Sukhai et al., 2000, 2001) and drug-metabolizing enzymes found in liver (Morgan, 1997). Furthermore, a reduced expression of Mrp2 has been recently reported in IL-6-treated rats (Kim et al., 2000). On the other hand, we saw much less pronounced changes in mRNA expression of the hepatic anion transporters in the TNF-α-
treated mice, even upon administrating doses up to 25,000 units.

Similar to findings reported by others (Trauner et al., 1997; Kubitz et al., 1999; Lee et al., 2000; Tang et al., 2000), we observed dramatically lower levels of Mrp2 mRNA (15% of controls) and protein (18.5% of controls) in the livers of LPS-treated mice. Interestingly, although the expression of Mrp2 was significantly reduced in the cytokine- and turpentine-treated mice, this suppression was much less pronounced than that seen after LPS administration. Furthermore, administration of all three cytokines or cytokine-bile acid combinations did not impose reductions further than that seen with IL-6 alone (data not shown). Hence, this may indicate contribution of other endogenous mediators in Mrp2 down-regulation.

Novel in vivo findings from this study indicate an inflam-

![Fig. 4. Effect of proinflammatory cytokines on Mrp mRNA expression in vitro in Hepa 1-6 cells. Effect on Mrp1 (A) and Mrp2 (B). Cells (n = 4 plates/group) were incubated with 1 or 10 ng/ml IL-1β, IL-6, or TNF-α, harvested at 6 h, and mRNA quantitated by RT-PCR, as described under Materials and Methods. Bars represent mean values ± S.E.M. (percentage) of normalized ODs, compared with controls (*, p < 0.05).]

![TABLE 2
In vitro effects of bile acids on transporter expression in Hepa1-6 cells
Values are expressed as mean ± S.E.M. (n = 4).](image)
mation- and cytokine-mediated down-regulation of Oatp1 and Oatp2. Effects of inflammation on hepatic Oatp2 expression have not been reported; however, this is consistent with previous observations of reduced hepatic anion uptake in endotoxemic rats (Lund et al., 1999). Lund et al. (1999), who observed a decrease in protein expression of Oatp1, postulated that TNF-α was not the principle mediator involved in suppression of Oatp1 expression during endotoxemia. This was confirmed by our data, indicating a small but insignificant effect of TNF-α on Oatp1 mRNA levels. Although levels of Oatp2 were significantly lower after TNF-α treatment, the down-regulation was much more pronounced in IL-6- and IL-1β-treated mice. Likewise, in agreement with our observations of reduced Oatp1 mRNA in turpentine- or LPS-treated mice, administration of either IL-1β or IL-6 imposed pronounced reductions of 60 to 70% in the mRNA levels of Oatp1.

Results from our studies in Hepa 1-6 cells indicated that in vitro treatments with cytokines alter the cellular expression of organic anion transporters. Consistent with our in vivo findings, exposure of Hepa 1-6 cells to IL-1β, IL-6, or TNF-α-imposed reductions in the mRNA levels of Mrp2 with the most pronounced changes occurring after IL-6 treatment (Fig. 4A). Cytokine treatments were also associated with a corresponding decrease in the 5-min efflux of 5-CF (Fig. 5A). Because 5-CF is primarily removed from normal hepatocytes and Hepa 1-6 cells via Mrp2, this implies that Mrp2 mRNA changes are associated with suppression in Mrp2 efflux activity. Changes at the post-transcriptional level such as protein stability could also be involved. An induction of Mrp1 mRNA expression was seen also in IL-1β-treated Hepa 1-6 cells, similar to that which has also been reported in human hepatoma cells (Ikegami et al., 2000; Lee and Piquette-Miller, 2001). Likewise, neither Mrp-mediated efflux of 5-CF nor mRNA levels were significantly altered by treatment with bile acids.

Our results clearly indicated that short-term administration of bile acids did not elicit the typical alterations in transporter mRNA levels that were seen in inflammation. It has been suggested previously that the accumulation of bile salts and bile acids could contribute to the down-regulation of hepatic anion transporters during cholestasis (Denson et al.,

Fig. 5. Effects of proinflammatory cytokines, LPS, and bile acids on cellular efflux of 5-CF from Hepa 1-6 cells. Cells were incubated for 24 h with the indicated concentrations of IL-1β, IL-6, TNF-α, LPS (10 or 50 ng/ml), cholic acid, or taurocholate. Efflux of 5-CF was measured as described under Materials and Methods. Bars represent mean values ± S.E.M (percentage) of n = 3 measurements (*, p < 0.05).
To clarify the role of these bile acids during acute inflammatory conditions, we examined the impact of the conjugated and unconjugated bile acids in the presence and absence of cytokines such as IL-6. It has been established that continuous or chronic administration of bile acids induces liver inflammation and activates cytokine release from Kupffer cells (Miyake et al., 2000). Hence, we used an acute administration regimen that results in physiologically relevant concentrations of these bile acids and has been demonstrated to impose significant effects on the hepatic expression of several cytochromes P450 (Paolini et al., 1999). Recent studies indicate that the bile acids modulate gene expression in the liver through signaling pathways involving the nuclear hormone receptors of the NR1 family such as the farnesoid X receptor (Lu et al., 2000; Schuetz, 2001). Indeed, activation of these pathways by chronic administration of bile acids (Sinal et al., 2000; Fickert et al., 2001) or acute administration of the hepatotoxic bile acid lithocholic acid has been demonstrated to induce levels of Mrp2, Oatp2, and Bsep (Ananthanarayanan et al., 2001; Kast et al., 2001; Staudinger et al., 2001). Of note, we saw an induction in Bsep mRNA levels (160% controls) in mice treated with taurodeoxycholate, consistent with reports indicating that hydrophobic bile salt activate the Bsep promoter (Asamoto et al., 2001; Plass et al., 2002). However, neither our in vivo nor in vitro data demonstrated a significant contribution of bile acids to the inflammation-mediated down-regulation of Mrp2, Oatp1, or Oatp2. Hence, it is likely that the bile acids play a greater role in conditions that impose chronic inflammation of the liver.

In conclusion, our in vitro and in vivo findings indicate that induction of inflammation and treatment with cytokines, but not with bile acids, affect the expression and functional activity of multiple organic anion transporters in liver. The net efflux of organic anions is determined by the expression levels and functional activities of several Mrp and Oatp proteins present in the cell, and the interplay of these carriers governs the overall cellular influx and excretion kinetics of their substrates. Future studies will further delineate the contribution of individual Oatp and Mrp isoforms to hepatic uptake and elimination of anionic substrate drugs in vivo and in vitro, as well as delineating the molecular pathways involved in their down-regulation. The relevance of these findings to humans has yet to be established. However, previous studies in human hepatoma cell lines have also demonstrated cytokine-mediated changes in the expression and activity of the Mrp transporters (Lee and Piquette-Miller, 2001). Because the Oatp and Mrp proteins mediate the hepatic uptake and efflux of a broad range of anionic and even cationic drug substrates, this implies that the hepatobiliary transport of these substrates may be significantly reduced in patients during inflammatory conditions, possibly resulting in reduced hepatic elimination and increased systemic drug concentrations in these patients.

References


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