Hepatic Flavin-Containing Monoxygenase Gene Regulation in Different Mouse Inflammation Models

Jun Zhang, Madhusudana R. Chaluvadi, Rob Reddy, Meike S. Motika, Terrilyn A. Richardson, John R. Cashman, and Edward T. Morgan

Human BioMolecular Research Institute, San Diego, California (J.Z., R.R., M.S.M., J.R.C.); and Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia (M.R.C., T.A.R., E.T.M.)

Received October 30, 2008; accepted December 16, 2008

ABSTRACT:
The objective of the study was to investigate the regulation of hepatic flavin-containing monoxygenases (Fmo) Fmo1, Fmo3, Fmo4, and Fmo5 in three different mouse models of inflammation, including treatment with Citrobacter rodentium, lipopolysaccharide (LPS), and dextran sulfate sodium (DSS). Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was used to evaluate the steady-state mRNA levels for the various Fmo isoforms in these mouse models of inflammation during different treatment time courses. Fmo3 mRNA was most significantly down-regulated in C. rodentium-treated female mice. Fmo1, Fmo3, and Fmo5 mRNAs were also found to be down-regulated in LPS models of inflammation. The significant down-regulation of hepatic FMO3 protein during C. rodentium treatment was confirmed with Western blot analysis of liver microsomes from treated animals. Toll-like receptor (TLR) 4 is known to be responsible for LPS signaling in association with several proteins. To investigate whether TLR4 was responsible for regulation of Fmo genes in both LPS and C. rodentium animal models, Fmo mRNA levels in female wild-type (C3H/HeOu) and TLR4 mutant (C3H/HeJ) mice were compared in both inflammatory models by real-time RT-PCR. The results showed that Fmo3 down-regulation during C. rodentium infection is independent of TLR4. Whereas TLR4 is likely to play only a partial role in Fmo1 gene regulation in LPS-treated animals, our results show that the down-regulation of Fmo3 and Fmo5 in this model is TLR4-dependent. Unlike cytochrome P450 regulation measured in the same mouse strains, Fmo3 expression was largely refractory to down-regulation in the DSS model of inflammatory colitis.

Inflammation is a defensive response to microbial invasion or physical, chemical, and/or UV irradiation damages, as well as other disease states. Inflammation responses are mediated by cytokines and chemokines to activate a variety of inflammatory signaling receptors and adaptor molecules, and trigger multiple protein kinase cascades that lead to diverse transcriptional and translational regulation of downstream functional genes. During inflammation, the expression, activity, and functions of many hepatic drug-metabolizing enzymes and drug transporters have been found to be regulated and to have exerted profound effects on the metabolism, distribution, and elimination of many drugs (see reviews by Renton, 2001, 2004; Morgan et al., 2008). These include Phase I and Phase II enzymes, and drug transporters, most of which are down-regulated during inflammation, affecting pharmacokinetics and leading to potentially unpredictable drug toxicity. Thus, in addition to drug-drug interactions, drug-disease interactions can modify individuals’ responses to drugs via disease-mediated effects on drug metabolism and pharmacokinetics.

Many different inflammation models have been developed to mimic inflammation-related disease states. Microbial infection is one of the leading causes of inflammation, and treatment with bacterial lipopolysaccharide (LPS) represents a classic inflammatory model for severe systemic infection caused by endotoxemia. LPS stimulates monocytes and macrophages to release early proinflammatory cytokines such as tumor necrosis factor-α and interleukin-1β, which initiate an inflammatory cascade. The majority of the biological effects of LPS have been attributed to its activation of Toll-like receptor 4 (TLR4) (Poltorak et al., 1998). Live bacterial infection of mice with the murine pathogen Citrobacter rodentium is a model of human enteropathogenic Escherichia coli infection (Schauer and Falkow, 1993). C. rodentium colonizes the colons of infected mice and elicits colonic inflammation and pathological changes resembling inflammatory bowel disease. Dextran sulfate sodium (DSS) (Masubuchi and Horie, 2004) and 2,4,6-trinitrobenzene sulfonic acid (Weidenbach et al., 2000) treatments are well established chemical models of colonic inflammation designed to mimic ulcerative colitis. Examining gene regulation in different models provides opportunities to compare and differentiate the common and specific responses to different inflammatory stimuli. Regulation of cytochrome P450 (P450) enzymes has been charac-
terized extensively in the above-noted inflammation models. In both the 2,4,6-trinitrobenzene sulfonic acid and the DSS model, colitis induced in rats was associated with a decrease in P450-dependent metabolism (Weidenbach et al., 2000; Masubuchi and Horie, 2004). Studies in rodents (in vivo and in vitro) have shown decreases of hepatic P450 mRNA and protein expression, as well as corresponding enzyme activity after treatment with bacterial LPS (Warren et al., 1999; Siewert et al., 2000; Ferrari et al., 2001; Ashino et al., 2004; Richardson and Morgan, 2005). In a C. rodentium infection study, several P450 mRNAs were decreased, and a few were up-regulated (Richardson and Morgan, 2005; Richardson et al., 2006). Comparing the pattern of hepatic P450 regulation in live infections as well as LPS and chemically induced inflammatory responses suggests that although a general trend of metabolic enzyme down-regulation is shared among these inflammation models, isoform-specific regulatory factors are certainly involved. The significance of such regulation in clinical drug use in humans is not fully understood. One well recognized case is the dramatic loss in CYP1A2 during an influenza virus infection that led to severe toxicity by theophylline in affected children (Kraemer et al., 1982). It is believed that the impact of such drug-disease interaction will depend on the specific drug metabolism pathways, genetic polymorphisms of the biotransformation enzymes, the specific type of inflammation response involved, and animal species being studied.

Flavin-containing monoxygenases (FMOs) represent another enzyme family that also plays significant roles in the hepatic metabolism of xenobiotics and endogenous substrates in humans and other mammals. Although FMOs are not known to be regulated by drugs (Cashman, 2003; Motika et al., 2007), down-regulation of hepatic Fmo1 in LPS-treated rats has been reported (Park et al., 1999; Ryu et al., 2004a). However, there is a significant gap in knowledge about FMO regulation during inflammation in general. In this study, we examined the effect of inflammation on hepatic Fmo mRNA levels in LPS, DSS, and C. rodentium–treated mice. The most significant regulation of Fmo1 and Fmo3 at the mRNA level in C. rodentium model was confirmed at protein levels using Western blot analysis. In addition, we compared TLR4-deficient mice with control mice in these inflammation models to explore the effect of TLR4 on Fmo regulation.

Materials and Methods

Chemicals, Animals, and Treatment. The female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) treated with C. rodentium (American Type Culture Collection, Manassas, VA) and their pair-fed controls were the same mice described previously (Chaluvasi et al., 2008a). Infection was achieved by allowing the mice to drink a 20% sucrose solution containing C. rodentium for 24 h at a nominal bacterial concentration calculated to result in an average dose of 7 × 108 cells/mouse. The average doses for each group were determined retrospectively by plating on MacConkey agar, and they were 8, 7, 6, and 6 × 108 bacteria/mouse for the 7-, 10-, 15-, and 24-day groups, respectively. Control (pair-fed) mice received only 20% sucrose in the drinking water. The amount of food consumed by the infected group was calculated, and the same amount of food was offered to the pair-fed control group the next day. Mice from each group were killed at 7, 10, 15, and 24 days after infection. Livers were collected, rinsed in cold 1.15% potassium chloride, and stored at −80°C until RNA or microsome preparation was initiated.

The female C3H/HeOu (HeOu) and TLR4-mutant C3H/HeJ (HeJ) mice (The Jackson Laboratory) infected with C. rodentium and their pair-fed controls were the same mice described previously (Richardson et al., 2006). HeOu and HeJ mice derive from the same C3H background strain; therefore, they are genetically very similar except for a naturally occurring spontaneous mutation in the HeJ mice inactivating TLR4 gene. These mice received a dose of 2 × 108 C. rodentium or saline by oral gavage and were killed 6 days after infection. Livers were collected as described above.

For induction of colitis with DSS, 10-week-old female HeOu or HeJ mice were administered 3% DSS in their drinking water for 5 or 7 days, then killed, and their livers removed for analysis. Control animals drank water and were allowed food ad libitum because it was observed that DSS consumption did not affect food consumption. In the experiment to study the acute effects of LPS injection, ad libitum-fed, 10-week-old female HeOu or HeJ mice were given a single intraperitoneal injection of either 1 mg/kg E. coli, LPS, or saline. The animals were killed 24 h later, and livers were collected as described above. The treatment groups studied and numbers of animals are summarized in Table 1.

Western Immunoblotting. Liver microsomes from control and treated mice were prepared as described previously (Richardson et al., 2006). FMO1 and FMO3 protein levels in mouse liver microsomes were determined by SDS-polyacrylamide gel electrophoresis followed by Western blotting. Polyclonal goat antibodies recognizing mouse FMO1 and FMO3 were provided by Professor Ernest Hodgson (North Carolina State University, Raleigh, NC). In brief, 30 μg (for FMO1 detection) and 60 μg (for FMO3 detection) of liver microsomal protein from five C. rodentium–infected and four control mice were fractionated by 10% SDS-polyacrylamide gel electrophoresis under denaturing conditions as described by Laemmli (1970). Proteins were transferred onto polyvinylidene difluoride membranes (Millipore Corporation, Billerica, MA), and equal protein loading and transfer were verified by Ponceau S staining of the membrane. Membranes were blocked in 5% milk in phosphate-buffered saline/Tween 20 (0.2%), and a 1:1000 dilution of the primary antibodies against FMO1 or FMO3 was applied for 1 h. After thorough washing, a 1:10,000 dilution of horseradish peroxidase-coupled rabbit anti-goat antibodies (Pierce, Rockford, IL) was applied for 1 h. Bound antibodies were detected using SuperSignal Western Substrates (Pierce), FMO quantification in each microsomal sample was achieved by densitometric analysis using Kodak Molecular Imaging software (Eastman Kodak Company, Rochester, NY).

Reverse Transcription. Liver RNA was prepared from control and treated mice as described previously (Richardson et al., 2006). Each RNA sample was reverse-transcribed to cDNA using 1 μg of RNA and Superscript III RT kit

### Table 1

<table>
<thead>
<tr>
<th>Infection Model</th>
<th>Mouse Strain</th>
<th>Treatment Period</th>
<th>Control (n =)</th>
<th>Treated (n =)</th>
<th>Fmo Isoform Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. rodentium</td>
<td>C57BL/6</td>
<td>7 days</td>
<td>6</td>
<td>5</td>
<td>Fmo1, Fmo3, Fmo4, Fmo5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 days</td>
<td>5</td>
<td>6</td>
<td>Fmo1, Fmo3, Fmo4, Fmo5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 days</td>
<td>6</td>
<td>6</td>
<td>Fmo1, Fmo3, Fmo4, Fmo5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 days</td>
<td>6</td>
<td>6</td>
<td>Fmo3</td>
</tr>
<tr>
<td>LPS</td>
<td>HeOu</td>
<td>6 days</td>
<td>5</td>
<td>4</td>
<td>Fmo3</td>
</tr>
<tr>
<td></td>
<td>HeOu</td>
<td>1 day</td>
<td>5</td>
<td>4</td>
<td>Fmo3</td>
</tr>
<tr>
<td>DSS</td>
<td>HeOu</td>
<td>5 days</td>
<td>6</td>
<td>6</td>
<td>Fmo1, Fmo3, Fmo4, Fmo5</td>
</tr>
<tr>
<td></td>
<td>HeOu</td>
<td>7 days</td>
<td>5</td>
<td>6</td>
<td>Fmo1, Fmo3, Fmo4, Fmo5</td>
</tr>
<tr>
<td></td>
<td>HeOu</td>
<td>7 days</td>
<td>6</td>
<td>6</td>
<td>Fmo1, Fmo3, Fmo4, Fmo5</td>
</tr>
</tbody>
</table>

Number of samples from control and treated mice, and Fmo isoforms analyzed in each respective group in the following figures are summarized. Infection models are abbreviated as C. rodentium for C. rodentium–treated mice, LPS for bacterial lipopolysaccharide-infected mice, and DSS for dextran sulfate sodium-treated mice.
(Invitrogen, Carlsbad, CA) including random hexamers and oligo(dT)s in a 20-μl total reaction volume following manufacturer’s instruction.

**Real-Time Polymerase Chain Reaction.** Sequences for mouse Fmo1 (MMU87456), Fmo2 (AF184981), Fmo3 (MMU87147), Fmo4 (AF461145), and Fmo5 (MMU90535) were aligned to identify gene-specific sequences for primer synthesis. Primers designed for amplification of specific Fmo isoforms are listed in Table 2. Comparison of all the Fmo primers with Fmo6 and reported pseudogene clusters (Hernandez et al., 2004) revealed that mF3f193 has 95% alignment with mouse Fmo6. However, the reverse primer mF3r419 showed no alignment with relative genes, ensuring that the amplified polymerase chain reaction (PCR) products produced by the primer pairs were from a single template. All the other primers showed no alignment to these loci. All the quantitative PCR reactions were run in 96-well PCR plates using an iQ5 Thermal Cycler (Bio-Rad, Hercules, CA). The PCR reactions were prepared in 15-μl volumes in duplicate for each sample using the following components: 1 X SYBR super mix (Bio-Rad), gene-specific primer pairs (10 pmol), cDNA (10 ng), and H2O. The quantitative PCR was run at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 58°C for 30 s. Duplicate no-template controls were included on the same plate for each gene analyzed. At the end of the PCR cycling steps, product melting curves were inspected and confirmed to have a single amplification peak.

**Quantification of Relative mRNA Level by a Standard Curve Method.** A pool of cDNA from control C57BL/6 mice (C. rodentium experiment, control day 7, n = 6) (Table 1) was used to generate a standard series (no dilution, 10-, 100-, 1000-, and 10,000-fold dilution) used for all the gene analyses. Standard curves were developed by plotting Ct value against log of -fold dilutions. To normalize the intersample variation in quality inherently associated with RNA preparation, the transcription level of housekeeping gene Gapdh was quantified for all the samples. The value obtained from each target gene was then normalized by Gapdh value to calculate the relative Fmo mRNA levels in comparison with the corresponding control groups.

**Statistical Analysis.** For both FMO protein level analysis and Fmo mRNA relative level analysis, treated mouse groups (C. rodentium, LPS, and DSS) were compared with the corresponding control mice group using unpaired t test analysis using GraphPad Software Inc. (La Jolla, CA) Prism Programs (version 3.00). The level of statistical significance was set at P < 0.05.

**Results**

**Effect of C. rodentium Infection on Fmo mRNA Expression.** Real-time reverse transcription-PCR analysis revealed down-regulation of hepatic Fmo1, Fmo3, and Fmo4 mRNAs in C. rodentium-treated C57BL/6 female mice. Fmo1 mRNA was slightly suppressed at 7 days of treatment; however, this did not achieve statistical significance. The mRNA level for Fmo1 decreased modestly at day 10 post-treatment to 42%, and started to increase toward control level at day 15 post-treatment to 64% of control animals (Fig. 1A).

C. rodentium treatment was associated with significant decreases in the mRNA level for Fmo3 at all the time points studied. In the day 7 and day 10 treatment groups, Fmo3 levels were 5 and 0.6% of control, respectively. At day 15 and day 24 post-C. rodentium treatment, the Fmo3 mRNA level increased back to 13 and 27%, respectively. However, the mRNA level for Fmo3 at 24 days post-treatment was still significantly lower than the control group (Fig. 1B).

In C. rodentium-treated mice, the expression of Fmo4 seemed to follow a similar trend to that of Fmo1, but because of a relatively large variability in Fmo4 expression, the difference was not statistically significant even at the lowest point we observed (27%, P = 0.06) (Fig. 1C). In contrast to the other Fmos, the mRNA level of Fmo5 did not change significantly over the 7- to 15-day monitoring period post-C. rodentium treatment (Fig. 1D).

**Effect of C. rodentium Infection on FMO Protein Regulation.** Through Western blot analysis, we also evaluated protein levels of FMO1 and FMO3 in hepatic microsomes prepared from the corresponding animals examined above (Fig. 2). C. rodentium infection significantly down-regulated FMO3 protein expression in mouse liver to 65% of control at day 7 after infection, and FMO3 proteins were further down-regulated to 14% of control on day 10 postinfection. Thereafter, the protein expression increased to 22 and 36% of control on days 15 and 24, respectively (Fig. 2A). The FMO3 protein level regulation followed the same trend as the mRNA level identified from real-time PCR described above. The effect of C. rodentium infection on FMO1 protein expression was less dramatic. Nevertheless, significant down-regulation of FMO1 protein occurred at days 10 and 24 to 56 and 78% of control, respectively (Fig. 2B). In general, the FMO1 protein regulation pattern also agreed with the mRNA regulation.

**Role of TLR4 on Fmo3 Regulation in C. rodentium Infection.** TLR4, in association with several proteins, is responsible for LPS signaling (Hoshino et al., 1999), and mice lacking TLR4 have been used as an essential and convenient tool to examine the role of TLR4 in infection models (Richardson et al., 2006). To investigate the functional involvement of TLR4 in Fmo regulation during inflammation response, we used two mouse strains (i.e., HeOu and HeJ) whose genetic backgrounds are similar except that HeJ strain lacks TLR4 while HeOu contains the TLR4 gene. The Fmo3 mRNA level was significantly down-regulated during C. rodentium infection in both HeOu and HeJ mice to 26 and 41% of control, respectively (Fig. 3).

**Regulation of Hepatic Fmos in the LPS Model of Bacterial Sepsis.** A single time point (i.e., 24 h post-treatment) was chosen to examine the effect of acute LPS treatment on Fmo regulation because this is an optimal time to monitor LPS-mediated hepatic gene regulation based on our past experience with P450 studies. The effects of a single dose of E. coli LPS on hepatic expression of Fmo mRNAs are shown in Fig. 4. Fmo1, Fmo3, and Fmo5 mRNAs were found to be significantly down-regulated in the livers of HeOu mice. In the HeJ strain, which lacks TLR4, Fmo3 and Fmo5 mRNAs were unaffected by LPS treatment (Fig. 4, B and D), whereas a small but significant reduction in Fmo1 mRNA was observed (Fig. 4A). Fmo4 mRNA was significantly up-regulated in HeJ but not in HeOu mice treated with LPS (Fig. 4C).

**Effect of DSS Treatment on Fmo mRNA Regulation.** Distinct from the C. rodentium and LPS inflammation responses, no significant differences were found in the mRNA levels for Fmo1, Fmo3, and Fmo4 in HeOu or HeJ mice treated for 5 days with DSS, a chemically induced model of ulcerative colitis (Fig. 5). This was also true at 7 days of treatment (data not shown). Only Fmo5 was found to be up-regulated by 2-fold in HeJ-treated mice. This only occurred after 5
Discussion

Although FMO expression in mammalian species is known to be species-, tissue-, age-, and gender-dependent, and the association of defective FMO3 mutations in humans with the primary form of trimethylaminuria, a fish odor syndrome associated with poorly metabolized trimethylamine, has been clearly shown at the genetic level, the physiological mechanisms controlling and regulating FMO expression are not well understood (Cashman, 2003; Motika et al., 2007). Evidence exists for the regulation of FMOs by sex hormones (Dannan et al., 1986; Lemoine et al., 1991; Falls et al., 1995, 1997; Coecke et al., 1998) and glucocorticoids in rodents (Dixit and Roche,
Transient trimethylaminuria has been reported to be associated with menstruation in women (Cashman, 2003; Shimizu et al., 2007). FMO3 activity alteration has also been associated with severe hepatic diseases in both human patients and rodent models (Nakajima et al., 1998; Mitchell et al., 1999). In this study, we examined the hepatic mRNA and protein regulation of four FMO isoforms in different inflammation mouse models. A general trend of down-regulation of Fmos in both the C. rodentium-infected and LPS-treated mice, but not the DSS-treated mice, was observed. The regulation of each Fmo isoform also showed a different profile.

Fmo1 Regulation. In the C. rodentium model, down-regulation of Fmo1 mRNA correlated well with the time course of bacterial colonization of the colon, which was significant at 7 days postinfection, reached its peak at 10 days, and then declined to negligible counts at days 15 and 24 (Chaluvadi et al., 2008a). This temporal profile is similar to that of the regulation of many P450 mRNAs studied in these same mice, as well as of the plasma profile of cytokine including interleukin-6, tumor necrosis factor-α, and interferon-γ (Chaluvadi et al., 2008a). The Fmo1 protein level followed the same time course as the down-regulation of mRNA, indicating a pretranslational physiologically relevant regulation. Acute LPS treatment also decreased Fmo1 mRNA. Attenuated Fmo1 down-regulation in the TLR4-deficient HeJ strain indicated that Fmo1 mRNA down-regulation in the LPS model is at least partially TLR4-mediated. However, although the reduction of Fmo1 mRNA in HeJ strain was attenuated in HeJ strain post-LPS treatment, the difference with the control group was significant, indicating that there is also a TLR4-independent component in LPS-induced Fmo1 regulation.

Fmo3 Regulation. Fmo3 mRNA was the most significantly decreased Fmo isoform after C. rodentium infection. The gradual recovery of Fmo3 mRNA in the C. rodentium-treated mouse was noticeably slower than what we observed previously for P450 mRNAs, most of which were back to normal at 15 days (Chaluvadi et al., 2008a). Even at day 24 when bacterial infection was completely cleared and no pathological symptoms were apparent for these animals, the levels of Fmo3 mRNA (13%) and protein (36%) were still far from normal. Down-regulation of Fmo3 is likely to be pretranslational based on the parallel protein level regulation. C. rodentium infection-mediated Fmo3 down-regulation is TLR4-independent, whereas regulation of Fmo3 after LPS infection is largely TLR4-mediated. Comparing between the experimental animal strains, HeOu mice are much more sensitive to the pathogenic effects of C. rodentium infection than C57BL/6 mice, and the P450 down-regulation was associated with significant morbidity in the animals in our previous study (Richardson and Morgan, 2005). It is interesting that the down-regulation of Fmo3 was much more profound in the C57BL/6 strain, one that showed less pathogenesis, than in the HeOu mice. This was not true of the most profoundly down-regulated P450 studied, the CYP45As, which were more affected in the HeOu mice (Richardson and Morgan, 2005; Chaluvadi et al., 2008a). These results suggest that regulatory factors distinct from that involved in P450 regulation are required for the down-regulation of Fmo3 during infection and the restoration of Fmo3 transcription post-C. rodentium infection of mice.

Fmo4 and Fmo5 Regulation. The variability of expression of Fmo4 in the C57BL/6 mice indicates that no significant effects could be detected in the C. rodentium model, although the overall pattern was similar to that of Fmo1. In the LPS model, a slight increase of Fmo4 mRNA was observed in the HeJ strain. It is difficult to assess the role of TLR4 in this effect because a similar but nonsignificant trend was observed in the HeOu mice. Fmo5 mRNAs did not change significantly in the C. rodentium model. However, LPS treatment caused TLR4-dependent down-regulation of Fmo5.

Inflammation Model-Specific Fmo Regulation. A clear difference between the C. rodentium model of colonic inflammation, which down-regulated Fmo1, Fmo3, and Fmo4 in C57BL/6 mice, and the DSS model, where no Fmo was affected in HeOu mice, was observed. A comparison of basal Fmo mRNA levels among control mice of the three experimental strains revealed that the C57BL/6 strain expressed 30 to 40% less Fmo1, Fmo3, and Fmo5 (P < 0.05) mRNAs compared with the HeOu and/or HeJ strains. Basal levels of Fmo4 mRNA in HeOu and HeJ were 2- to 3-fold higher than that in C57BL/6, respectively (P < 0.01, data not shown). No significant differences in the basal levels of Fmo mRNAs were observed between HeOu and HeJ strains. Because Fmo3 was down-regulated by C. rodentium infection in both strains, we believe the basal level Fmo differences among the strains are not the reason for the significant differences observed comparing C. rodentium infection and DSS treatment. Livers of DSS-treated mice showed dramatic effects on P450 enzymes similar to those in C. rodentium-treated mice, thus confirming acute colitis during DSS treatment (Chaluvadi et al., 2008b). Based on these differences, we hypothesize that general disruption of intestinal microflora and the release of endotoxins after DSS treatment are not sufficient to trigger Fmo regulation. The effect of gastrointestinal infection with C. rodentium on hepatic Fmos is not likely to be solely because of the colonic inflammation it evokes, but that factors unique to the infecting organism are important.

Inflammation-mediated Fmo1 and Fmo3 regulations observed here are probably controlled at the level of gene transcription or mRNA stability. The down-regulation of Fmo3 was largely independent of TLR4, in agreement with our previous work on P450 regulation in this model (Richardson and Morgan, 2005). Several transcription factors, including CCAAT enhancer-binding protein-α and -β and hepatic nuclear factor (HNF)-1α and HNF-3, have been shown to play an essential function in sepsis-associated decreases in the transcription of a number of hepatic genes in mice and have been suggested to have similar roles in other inflammatory states (Haaxma et al., 2003). HNF-1α and HNF-4α, among several transcription factors, have been suggested to be involved in FMO1 and FMO3 regulation in humans (Luo and Hines, 2001; Hines et al., 2003; Klick and Hines, 2007). Comparison of human FMO3 promoter elements with other mammalian species revealed striking differences (Klick et al., 2008). A number of regulatory elements identified in the human FMO3 promoter region, including the CCAAT enhancer-binding protein-β element, are absent in the specific region examined, suggesting the gene is possibly under very different regulatory mechanisms in humans and
mice. In rats treated with LPS, FMO enzyme activity and FMO1 contents in liver were decreased, and this was at least partially prevented by treatment with inhibitor of inducible nitric oxide synthase (Park et al., 1999). A later study showed that the decrease in FMO activity is caused by a cGMP-independent destabilizing effect of nitric oxide on FMO mRNA that results in a decreased half-life of the mRNA (Ryu et al., 2004a,b). Whether the effects of inflammation on FMO mRNA levels in our study are associated with any of the transcriptional and/or post-transcriptional factors identified from other model systems remains to be explored further.

Many of the effects of inflammation and infection of hepatic P450 expression are thought to be the result of proinflammatory cytokines because purified cytokines can regulate P450 expression in rodents in vivo, as well as in hepatocyte cultures (Aitken et al., 2006). Although little is known about the abilities of cytokines to regulate FMO expression, it is reasonable to speculate that some of the in vivo

**Fig. 4.** Effect of LPS treatment on hepatic Fmo mRNA regulation. Hepatic Fmo1 (A), Fmo3 (B), Fmo4 (C), and Fmo5 (D) mRNA levels were measured in female HeJ and HeOu mice 24 h after injection with 1 mg/kg LPS or saline (control groups). Number of mice for each group is as listed in Table 1. Mean levels are graphed, and error bars represent S.E.M. Statistically significant differences between the control and LPS-treated (LPS) mice are identified by *, P < 0.05, **, P < 0.01, and ***, P < 0.001.

**Fig. 5.** Effect of DSS treatment on hepatic Fmo mRNA regulation. Hepatic Fmo1 (A), Fmo3 (B), Fmo4 (C), and Fmo5 (D) mRNA levels in DSS-treated female HeJ and HeOu mice after 5 days are shown in comparison with the corresponding control mouse groups. Number of mice for each group is as listed in Table 1. Mean levels are graphed, and error bars represent S.E.M. Statistically significant differences between the control and DSS-treated (DSS) mice are identified by **, P < 0.01.
effects that we report in this study are caused by proinflammatory cytokines acting on the hepatocytes. Research is also needed to clarify the role of hormones in the expression of FMO, and to explore whether hormone level fluctuation resulting from inflammation-induced stress might be involved in alteration of FMO levels.

Conclusions

The results of this study showed isoform-specific down-regulation of mouse liver Fmo mRNAs during inflammation mediated by in vivo infection and LPS treatment, with Fmo3 being most significantly down-regulated. The suppression of Fmo3 in infected mice was much slower to recover than was P450 expression shown in previous studies. In contrast to P450s, Fmo expression in wild-type mice was not significantly affected in the DSS model of colonic inflammation. Overall, mouse Fmo mRNA down-regulation in the LPS model of sepsis was TLR4-dependent, whereas the down-regulation of Fmo3 during C. rodentium infection was TLR4-independent. Nevertheless, other mechanisms must also be involved because even in mice lacking the TLR4 a slight decrease in Fmo1 mRNA levels was observed after LPS treatment. Further research is in progress to clarify the mechanism of inflammation-mediated down-regulation of Fmos. Our work suggests that the pharmacokinetics of drugs cleared by FMO enzymes in mice, especially FMO3, is likely to be altered during inflammation and infection, and provides strong evidence that the isoforms and drugs affected are likely to be disease model-dependent.

Acknowledgments. We thank Professor Ernest Hodgson (North Carolina State University, Raleigh, NC) for providing polyclonal goat antibodies to mouse FMO1 and FMO3.

References


Chaluvadi MR, Nyagode BA, Kinloch RD, and Morgan ET (2008b) TLR4-dependent and -independent regulation of hepatic cytochrome P450 in mice with chemically induced inflammation and infection. Research is also needed to clarify the role of hormones in the expression of FMO, and to explore whether hormone level fluctuation resulting from inflammation-induced stress might be involved in alteration of FMO levels.

Conclusions

The results of this study showed isoform-specific down-regulation of mouse liver Fmo mRNAs during inflammation mediated by in vivo infection and LPS treatment, with Fmo3 being most significantly down-regulated. The suppression of Fmo3 in infected mice was much slower to recover than was P450 expression shown in previous studies. In contrast to P450s, Fmo expression in wild-type mice was not significantly affected in the DSS model of colonic inflammation. Overall, mouse Fmo mRNA down-regulation in the LPS model of sepsis was TLR4-dependent, whereas the down-regulation of Fmo3 during C. rodentium infection was TLR4-independent. Nevertheless, other mechanisms must also be involved because even in mice lacking the TLR4 a slight decrease in Fmo1 mRNA levels was observed after LPS treatment. Further research is in progress to clarify the mechanism of inflammation-mediated down-regulation of Fmos. Our work suggests that the pharmacokinetics of drugs cleared by FMO enzymes in mice, especially FMO3, is likely to be altered during inflammation and infection, and provides strong evidence that the isoforms and drugs affected are likely to be disease model-dependent.

Acknowledgments. We thank Professor Ernest Hodgson (North Carolina State University, Raleigh, NC) for providing polyclonal goat antibodies to mouse FMO1 and FMO3.

References


Chaluvadi MR, Nyagode BA, Kinloch RD, and Morgan ET (2008b) TLR4-dependent and -independent regulation of hepatic cytochrome P450 in mice with chemically induced inflammation and infection. Research is also needed to clarify the role of hormones in the expression of FMO, and to explore whether hormone level fluctuation resulting from inflammation-induced stress might be involved in alteration of FMO levels.

Conclusions

The results of this study showed isoform-specific down-regulation of mouse liver Fmo mRNAs during inflammation mediated by in vivo infection and LPS treatment, with Fmo3 being most significantly down-regulated. The suppression of Fmo3 in infected mice was much slower to recover than was P450 expression shown in previous studies. In contrast to P450s, Fmo expression in wild-type mice was not significantly affected in the DSS model of colonic inflammation. Overall, mouse Fmo mRNA down-regulation in the LPS model of sepsis was TLR4-dependent, whereas the down-regulation of Fmo3 during C. rodentium infection was TLR4-independent. Nevertheless, other mechanisms must also be involved because even in mice lacking the TLR4 a slight decrease in Fmo1 mRNA levels was observed after LPS treatment. Further research is in progress to clarify the mechanism of inflammation-mediated down-regulation of Fmos. Our work suggests that the pharmacokinetics of drugs cleared by FMO enzymes in mice, especially FMO3, is likely to be altered during inflammation and infection, and provides strong evidence that the isoforms and drugs affected are likely to be disease model-dependent.

Acknowledgments. We thank Professor Ernest Hodgson (North Carolina State University, Raleigh, NC) for providing polyclonal goat antibodies to mouse FMO1 and FMO3.

References


Chaluvadi MR, Nyagode BA, Kinloch RD, and Morgan ET (2008b) TLR4-dependent and -independent regulation of hepatic cytochrome P450 in mice with chemically induced inflammation and infection. Research is also needed to clarify the role of hormones in the expression of FMO, and to explore whether hormone level fluctuation resulting from inflammation-induced stress might be involved in alteration of FMO levels.

Conclusions

The results of this study showed isoform-specific down-regulation of mouse liver Fmo mRNAs during inflammation mediated by in vivo infection and LPS treatment, with Fmo3 being most significantly down-regulated. The suppression of Fmo3 in infected mice was much slower to recover than was P450 expression shown in previous studies. In contrast to P450s, Fmo expression in wild-type mice was not significantly affected in the DSS model of colonic inflammation. Overall, mouse Fmo mRNA down-regulation in the LPS model of sepsis was TLR4-dependent, whereas the down-regulation of Fmo3 during C. rodentium infection was TLR4-independent. Nevertheless, other mechanisms must also be involved because even in mice lacking the TLR4 a slight decrease in Fmo1 mRNA levels was observed after LPS treatment. Further research is in progress to clarify the mechanism of inflammation-mediated down-regulation of Fmos. Our work suggests that the pharmacokinetics of drugs cleared by FMO enzymes in mice, especially FMO3, is likely to be altered during inflammation and infection, and provides strong evidence that the isoforms and drugs affected are likely to be disease model-dependent.

Acknowledgments. We thank Professor Ernest Hodgson (North Carolina State University, Raleigh, NC) for providing polyclonal goat antibodies to mouse FMO1 and FMO3.

References


Chaluvadi MR, Nyagode BA, Kinloch RD, and Morgan ET (2008b) TLR4-dependent and -independent regulation of hepatic cytochrome P450 in mice with chemically induced inflammation and infection. Research is also needed to clarify the role of hormones in the expression of FMO, and to explore whether hormone level fluctuation resulting from inflammation-induced stress might be involved in alteration of FMO levels.

Conclusions

The results of this study showed isoform-specific down-regulation of mouse liver Fmo mRNAs during inflammation mediated by in vivo infection and LPS treatment, with Fmo3 being most significantly down-regulated. The suppression of Fmo3 in infected mice was much slower to recover than was P450 expression shown in previous studies. In contrast to P450s, Fmo expression in wild-type mice was not significantly affected in the DSS model of colonic inflammation. Overall, mouse Fmo mRNA down-regulation in the LPS model of sepsis was TLR4-dependent, whereas the down-regulation of Fmo3 during C. rodentium infection was TLR4-independent. Nevertheless, other mechanisms must also be involved because even in mice lacking the TLR4 a slight decrease in Fmo1 mRNA levels was observed after LPS treatment. Further research is in progress to clarify the mechanism of inflammation-mediated down-regulation of Fmos. Our work suggests that the pharmacokinetics of drugs cleared by FMO enzymes in mice, especially FMO3, is likely to be altered during inflammation and infection, and provides strong evidence that the isoforms and drugs affected are likely to be disease model-dependent.

Acknowledgments. We thank Professor Ernest Hodgson (North Carolina State University, Raleigh, NC) for providing polyclonal goat antibodies to mouse FMO1 and FMO3.