Effect of Lipopolysaccharide on the Xenobiotic-Induced Expression and Activity of Hepatic Cytochrome P450 in Mice

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Infection-associated inflammation can alter the expression levels and functions of cytochrome P450s (CYPs). CYP gene expression is regulated by the activation of several nuclear receptors, including pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR). These receptors can be activated by xenobiotics, including medicines. Here, to study the xenobiotic-induced fluctuations in CYP during inflammation, we examined the effect of lipopolysaccharide (LPS) treatment on the level of mRNAs encoding hepatic CYPs induced by xenobiotic-activated nuclear receptors, in mice. Both the mRNA induction of Cyp genes and the metabolic activities of CYP proteins were examined. LPS treatment caused a significant decrease in the induced expression of the mRNAs for Cyp3a11, 2c29, 2c55, and 1a2, but not for Cyp2b10. To assess the CYP enzymatic activities, CYP3A-mediated testosterone 6α-hydroxylation and the intrinsic clearance (CLin) of nifedipine in liver microsomes were measured in mice treated with the xenobiotic pregnenolone-16α-carbonitrile (PCN) with or without LPS administration. Both assays revealed that the CYP3A activity, which was induced by PCN, declined significantly after LPS treatment, and this decline correlated with the Cyp3a11 mRNA level. In addition, we found that the mRNAs for interleukin (IL)-1β and tumor necrosis factor (TNF)α were increased after treatment with LPS plus xenobiotics. Our findings demonstrated that LPS treatment reduces the PXR- and AhR-mediated, and possibly CAR-mediated Cyp gene expression and further suggest that these decreases are dependent on inflammatory cytokines in the liver.

Key words cytochrome P450; induction; lipopolysaccharide; hepatic metabolism; inflammatory cytokine; transcriptional regulation

The cytochrome P450 (CYP) enzyme superfamily is responsible for the oxidative metabolism of numerous xenobiotics, including medications. CYP expression levels and enzyme activity can be altered as a result of infection-associated inflammation, and such fluctuations in CYP activity could be responsible for some unexpected failures of drug therapies and adverse side effects, including life-threatening ones. For example, during the influenza pandemic in Washington State, U.S.A., in the 1980s, severe side effects, including convulsions and cardiac conduction anomalies, occurred in children who were receiving theophylline as a prophylactic treatment for asthma. This particular incident was probably owing to the decrease theophylline clearance by hepatic CYP enzymes in children infected with influenza, caused by the influenza-infection-related downregulation of CYP gene expression. This incident alerted clinicians to the potential for adverse effects of an otherwise beneficial medicine in patients suffering from another disease.

Several reports have shown that the hepatic drug-metabolizing capability is reduced during infection and inflammation in human, rat, and mouse. Morgan and colleagues reported that the mRNA levels and enzymatic activities of CYPs are downregulated in mice treated with lipopolysaccharide (LPS), a toxic component of Gram-negative bacterial cell walls. LPS activates lymphocytes, macrophages, and neutrophils, and it initiates the synthesis of pro-inflammatory cytokines, including tumor necrosis factor (TNF)α, interleukin (IL)-1β, and IL-6. Other studies showed that the expression of these cytokines is correlated with changes in CYP gene expression and CYP enzymatic activities, during infection and inflammation. In most of these studies, a decrease in CYP protein expression was succeeded or accompanied by a decrease in its mRNA, implicating transcription regulation as a primary mechanism. These findings underscore the importance of understanding the transcriptional mechanisms of the CYPs.

The basal and/or inducible CYP expression is affected by several factors, including gender, age, diet, hormones, and xenobiotics. In particular, it is well known that the inducible expression of the Cyp gene is regulated by the binding of xenobiotics to nuclear receptors, such as the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), and steroid xenobiotic receptor (SXR) (the ortholog of the murine pregnane X receptor (PXR)). For example, pregnenolone-16α-carbonitrile (PCN), a specific agonist of mouse PXR, binds to PXR, causing its translocation from the cytoplasm to the nucleus. PXR then heterodimerizes with retinoid X receptor (RXR), and the heterodimer binds to xenobiotic responsive elements (XRE) in the promoter regions of its target genes, such as Cyp3a11, to initiate their transcription. Similarly, CAR heterodimerizes with RXR, to transcriptionally activate Cyp2b10 gene expression in response to phenobarbital and 1,4-bis-[3,5-dichloropyridin-2-yl]oxy]-benzene (TCPOBOP). In addition, the expression of Cyp1a2 genes can be induced by AhR, which dimerizes with the AhR nuclear translocator in response to many polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (B[a]P).

Here we used an animal model of inflammation, LPS-treated mice, and focused on the xenobiotic-inducible Cyp gene expressions and CYP enzyme activities in the mouse.
liver. We first used this model to determine if LPS alters the PCN-inducible expression of Cyp3a11. The activity of CYP3A under the same conditions was investigated using a testosterone 6β-hydroxylation assay and the intrinsic clearance (CL\textsubscript{int}) of nifedipine. In addition to Cyp3a11, we evaluated the fluctuation of other Cyp mRNAs, Cyp1a2, Cyp2b10, Cyp2e29, and Cyp2c55 in the presence of their inducers, TCPOBOP or B(α)P, with and without LPS administration. Finally, we examined the levels of inflammatory cytokines during the LPS-induced fluctuation in induced CYPs.

Our results indicated that LPS downregulates the inducible expression of several Cyp genes, and that inflammatory cytokines may play a role in this process. These findings help elucidate the relationship between Cyp gene induction and inflammation.

**MATERIALS AND METHODS**

**Chemicals** LPS (0128:B12 from *Escherichia coli*), PCN, TCPOBOP, and B(α)P were purchased from Sigma-Aldrich (MO, U.S.A.), β-Nicotinamide-adenine dinucleotide phosphate (NADPH), glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Oriental Yeast (Tokyo, Japan). Testosterone, 6β-hydroxytestosterone, and nifedipine were purchased from Wako (Osaka, Japan). Other chemicals used were of super-fine or HPLC grade.

**Animals** Eight-week-old male Balb/c mice weighing about 22 g were purchased from SLC (Shizuoka, Japan). Mice were fed standard rodent chow and given water ad libitum. All animals were treated in accordance with the Guiding Principles of the “Care and Use of Laboratory Animals Code,” approved by Mukogawa Women’s University.

**Administration of LPS and CYP Inducers** To investigate the effects of LPS on PCN-inducible Cyp3a11 mRNA expression and the enzymatic activity of the protein, we used 1 mg/kg LPS dissolved in saline to induce inflammation.18 LPS was administered by intraperitoneal (i.p.) injection for 2 days, and the control mice received saline. On the first day, the mice were treated with PCN (50 mg/kg, dissolved in corn oil, i.p.), a known inducer of Cyp3a11 mRNA via the activation of PXR,23 3 h after LPS treatment. Control mice received corn oil alone. For the experiments on Cyp2b10, Cyp2c29, and Cyp2c55 expression, TCPOBOP (3 mg/kg, in corn oil, i.p.) was used as a CAR activator,23,26 and for AhR-controlled Cyp1a2 gene expression, B(α)P (100 mg/kg, in olive oil, i.p.) was used.25,27 On the second day, the mice were sacrificed 3 h after the LPS treatment, and the liver was excised and used for total RNA isolation and microsomal preparation.

**Isolation of Total RNA and Reverse Transcription (RT)** Total RNA was isolated from mouse liver using Sepasol RNA I Super (Nacalai Tesque, Kyoto, Japan) according to the manufacturer’s protocol. The RNA concentration was determined spectrophotometrically by measuring the absorbance at 260 nm, and adjusted to 77 µg/mL. The total RNA was stored at −80°C. cDNA was synthesized from total RNA using the PrimeScript RT reagent Kit (TaKaRa Bio, Shiga, Japan) according to the manufacturer’s protocol. Briefly, 1 µg of total RNA from each sample was resuspended in 20 µL of reaction buffer with PrimeScript RT enzyme, and the mixture was incubated for 15 min at 37°C. Reverse transcription was stopped by denaturing the enzyme at 85°C.

**Real-Time Polymerase Chain Reaction (PCR)** The mRNA expression of the genes of interest was measured by real-time PCR using an ABI PRISM 7000 (Applied Biosystems, CA, U.S.A.). Real-time PCR was conducted using 10 µM (forward and reverse) gene-specific primers (listed in Table 1) and SYBR Premix Ex TaqII (TaKaRa Bio), with 2 µL cDNA/sample as the template. As an internal positive control, glyceraldehyde-3-phosphate dehydrogenase (Gapdh) mRNA was also amplified. The number of cycles and annealing temperature were optimized for each primer pair. For all the reactions, the initial denaturation was 30 s at 95°C. The other PCR conditions were as follows: Gapdh and Cyp3a11, 40 cycles at 95°C for 5 s and 62°C for 32 s; for Cyp1a2, 40 cycles at 95°C for 5 s and 55°C for 30 s; for Cyp2b10, 40 cycles at 95°C for 5 s and 60°C for 30 s; for Cyp2c29, 40 cycles at 95°C for 5 s and 54°C for 30 s; for Cyp2c55, 40 cycles at 95°C for 5 s and 46°C for 30 s; for TNFa, 40 cycles at 95°C for 5 s and 57.8°C for 1 min; for IL-1β, 40 cycles at 95°C for 5 s and 70°C for 1 min. All experiments were conducted in duplicate using the same sample. The mRNA expressions of the genes of interest were calculated from a relative standard curve and normalized to that of Gapdh in the same sample. Results are expressed as the relative fold induction of the target gene mRNA compared with that of a control sample. The specificity of the PCR product was routinely monitored by checking the product melting curves (dissociation curves) for each reaction.

**Preparation of Liver Microsomes** All the procedures below were conducted at 4°C. The excised liver was homogenized with a polytron homogenizer in 100 mM sodium phosphate buffer (pH 7.4) containing 3.3 mM magnesium chloride, and then centrifuged at 9000 × g for 10 min. The supernatant was collected and centrifuged at 105000 × g for 1 h. The microsomal pellet was resuspended in the same buffer and centrifuged again at 105000 × g for 1 h. The resulting microsomal pellet was resuspended in the same buffer and stored at

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Table 1. Primers for Amplification of the Listed Genes by Real-Time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’ to 3’)</th>
<th>Reverse (5’ to 3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp1a2</td>
<td>CAGTATCCAAAGACATCAACAAG</td>
<td>TGTGTACGTTGAGATCTCCAG</td>
<td>47</td>
</tr>
<tr>
<td>Cyp2b10</td>
<td>ACCCCACGTTCTCTTCCA</td>
<td>CAGCACGGCGCAAGAAGTGA</td>
<td>48</td>
</tr>
<tr>
<td>Cyp2c29</td>
<td>AAGTTTGGGGCTTTTTTACG</td>
<td>AGCTTTGTTCCTCTAGTCTT</td>
<td>49</td>
</tr>
<tr>
<td>Cyp2c55</td>
<td>CTTAGAATCTTCGTGTAATGACTACAG</td>
<td>GGACTGCTAATCGAATCTCTG</td>
<td>50</td>
</tr>
<tr>
<td>Cyp3a11</td>
<td>CAGCTTGCGTCCTCTTACCC</td>
<td>TCAAAACCCCCCATTTTT</td>
<td>51</td>
</tr>
<tr>
<td>TNFa</td>
<td>GACAAGGCTGCCGCAGTACG</td>
<td>TTTGGGCGAGGCGGTCTTTCG</td>
<td>49</td>
</tr>
<tr>
<td>IL-1β</td>
<td>CAACCAACAAAGTATTTCTCCATG</td>
<td>GTACCAACTCTCCAGCTGCA</td>
<td>49</td>
</tr>
<tr>
<td>Gapdh</td>
<td>AACTTTGGCATTTGGAAGGG</td>
<td>GGATGCGAGGGATGTTCTT</td>
<td>52</td>
</tr>
</tbody>
</table>
Assays for Microsomal CYP3A Activity in Hepatic Microsomes: The metabolisms of testosterone and nifedipine were assayed as indicators of hepatic CYP3A activity, using high-performance liquid chromatography (HPLC).

Testosterone 6β-hydroxylation was assayed as follows. The reaction mixture (0.5 mL, containing 0.5 mg/mL microsomal protein, 1.3 mM β-NADPH, 3.3 mM glucose 6-phosphate, 0.4 U/mL glucose 6-phosphate dehydrogenase, 3.3 mM magnesium chloride, and 50 μM testosterone in 100 mM sodium phosphate buffer (pH 7.4)) was incubated at 37°C for 15 min, and the reaction was stopped by adding tert-butyl methyl ether. Corticosterone was added to the reaction mixture as an internal standard, and the sample was centrifuged at 2500×g for 3 min at 4°C. The organic layer was isolated, and the solvent was evaporated under a nitrogen gas stream. The residue was dissolved in 150 μL of solvent A, consisting of methanol, acetonitrile, and H2O (39:60:1, v/v/v), injected into the HPLC apparatus, and analyzed as previously described by Ashino et al. The HPLC system consisted of a model L-2130 liquid chromatograph pump, a model L-2455 diode array detector, a model L-2200 auto sampler, a model L-column oven (all from Hitachi High Technologies Co., Tokyo, Japan), and a 4.6×150 mm COSMOSIL 5C18-MS-II packed column (Nacalai Tesque). The mobile phase consisted of solvent A and methanol, acetonitrile, and H2O (80:18:2, v/v/v; solvent B). A linear gradient from 70% solvent A to 30% solvent B was run for 30 min at 1 mL/min. The eluate was monitored at 254 nm.

The metabolism of nifedipine, at an initial concentration of 10 μM, was evaluated by the same method, except for the internal standard (testosterone) and the incubation time (10 min). The concentrations of 6β-hydroxytestosterone and nifedipine were quantified by comparison with standard curves, using the peak area ratio method. To determine C_{L_{in}} in vitro, the slope (−k) of the linear regression of the log concentration versus incubation time was used as follows:

$$C_{L_{in}} = k \times \frac{\text{milliliters incubation mixture}}{\text{milligrams microsomes}}$$

The velocity of the testosterone conversion to 6β-hydroxytestosterone was measured under the initial rate conditions and expressed as nmol/min/mg protein.

Statistical Analysis: All results are presented as the mean±S.E. The results from the various experimental groups and their corresponding controls were compared using one-way analysis of variance followed by the Tukey–Kramer post hoc test. The differences were considered significant when p<0.05.

RESULTS

Effects of LPS on the Expression of Cyp mRNAs after Treatment with Several CYP Inducers: To investigate whether LPS affects the transcription of Cyp genes induced by ligands for individual nuclear receptors, i.e., PCN, TCPOBOP, and B[a]P, we conducted real-time PCR experiments. Figure 1 shows the effect of LPS treatment on the PCN-induced Cyp3a11 mRNA expression. PCN treatment alone enhanced Cyp3a11 mRNA expression in mouse liver 6-fold (gray bar).

Fig. 1. Effect of LPS on the Expression of Cyp3a11 mRNA in PCN-Treated Mice

Mice were injected with saline or LPS (1 mg/kg, i.p.) once per day, on 2 consecutive days. Three hours after the first LPS administration, the mice were treated with corn oil or PCN (50 mg/kg, i.p.). The livers were excised and total RNA was extracted 3 h after the second LPS administration. The Cyp3a11 mRNA levels were determined using real-time PCR and normalized to the Gapdh mRNA level in the same sample. Values represent the mean±S.E. for each group (n=8–9) relative to the control group. **p<0.01, compared with the control group. *p<0.05, compared with the PCN group.

Fig. 2. Effect of LPS on the Expression of Cyp2b10, 2c29, and 2c55 mRNA in TCPOBOP-Treated Mice

Mice were injected with saline or LPS (1 mg/kg, i.p.) once per day, on 2 consecutive days. Three hours after the first LPS administration, the mice were treated with corn oil or TCPOBOP (3 mg/kg, i.p.). The livers were excised and total RNA was extracted 3 h after the second LPS administration. Cyp2b10, 2c29, and 2c55 mRNA levels were determined using real-time PCR and normalized to the Gapdh mRNA level in the same sample. Values represent the mean±S.E. for each group (n=3–7) relative to the control group. **p<0.01, compared with the control group. *p<0.05, **p<0.01, compared with the TCPOBOP group, Tukey–Kramer’s analysis. Note that the dimension of the vertical axis for Cyp2c29 is different from that for Cyp2b10 and 2c55.

Fig. 3. Effect of LPS on the Expression of Cyp1a2 mRNA in B(a) P-Treated Mice

Mice were injected with saline or LPS (1 mg/kg, i.p.) once per day, on 2 consecutive days. Three hours after the first LPS administration, the mice were treated with olive oil or B(a)P (100 mg/kg, i.p.). The livers were excised and total RNA was extracted 3 h after the second LPS administration. The Cyp1a2 mRNA levels were determined using real-time PCR and normalized to the Gapdh mRNA level in the same sample. Values represent the mean±S.E. for each group (n=3–7) relative to the control group. **p<0.01 compared with the control group, +p<0.01 compared with the B(a)P group, Tukey–Kramer’s analysis.
However, the administration of LPS to PCN-treated mice decreased the PCN-induced expression of Cyp3a11 mRNA by about 25% (black bar).

Similar experiments were conducted to examine the expression of Cyp2b10, Cyp2c29, and Cyp2c55 (Fig. 2). These three genes are upregulated by CAR, and TCPBOBOP is one of CAR’s specific ligands. As expected, TCPBOBOP treatment increased the mRNA expression of Cyp2b10 (160-fold), Cyp2c29 (6-fold), and Cyp2c55 (2700-fold). However, treatment with LPS reduced the TCPBOBOP-induced Cyp2c29 mRNA level by 55% and that of Cyp2c55 by 80%. Interestingly, LPS treatment did not downregulate the TCPBOBOP-induced level of Cyp2b10 mRNA.

Finally, we examined the expression of Cyp1a2 as a representative Cyp gene regulated by AhR. Since B(a)P is a specific ligand for AhR, we conducted a similar experiment to those above to reveal the influence of LPS treatment on the Cyp1a2 gene expression. As shown in Fig. 3, B(a)P treatment resulted in a 12-fold increase in the Cyp1a2 mRNA level (gray bar), which was decreased by 80% by LPS administration (black bar).

Overall, except in the case of Cyp2b10, the Cyp genes we tested showed similar responses in their induced expression when the mice were treated with LPS.

**Effects of LPS on the Enzymatic Activity of CYP3A in PCN-Treated Mice**

Since LPS treatment attenuated the PCN-induced transcription of Cyp3a11, we examined its effects on the CYP3A activity in mouse liver microsomes. For this purpose, we assessed testosterone 6β-hydroxylase activity and the CL\text{int}\_\beta of nifedipine, which are well-known assays of CYP3A enzyme activity.

Table 2 shows the effects of LPS on 6β-hydroxylase activity and the hepatic CL\text{int}\_\beta of nifedipine under the conditions of PCN treatment alone or PCN plus LPS. The PCN-only condition induced about a 3-fold increase in 6β-hydroxylase activity compared to controls. However, the administration of LPS with PCN significantly decreased the induced activity of this enzyme, by about 40%. Similar results were obtained for nifedipine: PCN alone increased the CL\text{int}\_\beta of nifedipine by 5.2-fold, but the additional LPS treatment decreased the induction by 30%.

These results suggested that the CYP3A activity was correlated with the level of Cyp3a11 mRNA expression under these experimental conditions. Fig. 4 shows the correlation profiles for the CYP3A activity and Cyp3a11 mRNA levels. Relatively good correlations were observed (R^2=0.732 for testosterone 6β-hydroxylase activity and R^2=0.659 for the CL\text{int}\_\beta of nifedipine).

**Effects of LPS on the Expression of Hepatic Cytokines**

LPS is an inflammation-inducing compound that works by inducing endotoxic shock and stimulating immune cells to release inflammatory cytokines (e.g., TNFα and IL-1β) into the blood circulation. These cytokines are involved in the downregulation of hepatic Cyp mRNAs. To help clarify the mechanism of the LPS-induced effects on Cyp gene expression, we assessed the expression of TNFα and IL-1β mRNAs in the experimental situations described above.

We used a real-time PCR technique to determine the expression levels of TNFα and IL-1β mRNAs after mice were treated with PCN, TCPBOBOP, or B(a)P, alone and with LPS (Fig. 5). As shown in Fig. 5, treatment with the individual inducers alone had no effect on the TNFα or IL-1β mRNA expression. In contrast, LPS treatment with PCN significantly increased the TNFα and IL-1β mRNA expressions, 10-fold compared with their respective controls (Fig. 5A). We observed a similar phenomenon with TCPBOBOP treatment (Fig. 5B), except that the LPS administration increased the expression of TNFα mRNA 4.5-fold, and that of IL-1β 4-fold. B(a)P treatment elicited very similar results; LPS increased the expression of TNFα mRNA 5-fold and that of IL-1β 4-fold (Fig. 5C).
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These results clearly show that under the condition of the forced expression of Cyp genes, LPS treatment significantly increased the hepatic mRNA expression of the inflammatory cytokines TNFα and IL-1β.

DISCUSSION

Disease states, such as bacterial infection, rheumatoid arthritis, and cancer, lead to fluctuations in Cyp gene expressions and the enzyme activities of their products. Here we sought to examine the mechanisms underlying the fluctuations in Cyp gene expressions under an inflammatory physiological state, using LPS-treated mice as a model of inflammation. In this study, we investigated the effects of LPS on the PCN-, TCPOBOP-, and B(a)P-induced expression of several Cyp mRNAs in mouse liver. The xenobiotics PCN, TCPOBOP, and B(a)P are agonists for PXR, CAR, and AhR, respectively, which are critical transcriptional regulators for inducing Cyp gene expression. Among the CYP enzymes we studied, Cyp3a11 was representative of PXR-regulated Cyp genes; Cyp2b10, Cyp2c29, and Cyp2c55 were representative of CAR-regulated ones; and Cyp1a2 was a representative AhR-regulated gene. As shown in Figures 1–3, the three ligands for individual nuclear receptors induced increases of 6–2700-fold in the Cyp mRNA levels, compared to their respective control. Treatment with LPS, however, decreased the induction by...
about 25–80%, with the exception of Cyp2b10 (Fig. 3). Thus, we found that LPS significantly downregulated the xenobiotic-induced Cyp gene expressions in mouse liver, which is almost consistent with the findings of previous studies examining constitutive Cyp gene expression.34)

To learn whether the CYP enzymes are regulated at the transcriptional or translational level, we investigated the correlation between Cyp gene expression levels and their products’ enzymatic activities. To assay the activities, we measured the testosterone 6β-hydroxylation and the CL\text{int} of nifedipine in liver microsomes, \textit{in vitro}. These two chemicals were chosen as representative medicines that are metabolized by CYP3A, which metabolizes more than 50% of the xenobiotics in the human liver.35) As we expected, LPS significantly suppressed the PCN-induced CYP3A activity, and the CYP3A enzyme activity was correlated with the Cyp3a11 mRNA level (Fig. 4, Table 2). Similarly, Gharavi and El-Kadi reported that LPS represses the β-naphthoflavone-mediated induction of CYP1A enzyme activity.36)

Based on experiments using cell culture systems, several mechanisms have been proposed to explain the suppression of CYP gene expression during inflammation. In particular, because many cytokines are involved in the regulation of CYP genes, and because the cytokines affect each other’s expression, it is very difficult to determine which cytokine(s) regulates the expression of which CYP gene.77) In the present study, we paid considerable attention to the inflammatory cytokines TNFα and IL-1β, because they can downregulate the expression of multiple CYP genes,13–16) suggesting that they could be key regulators of this process. Therefore, we assessed the TNFα and IL-1β mRNA levels in mice treated with LPS plus PCN, TCPOBOP, or B(α)P. In spite of the presence of CYP inducers, LPS treatment resulted in significant increases in the TNFα and IL-1β mRNAs (Fig. 5).

Among the components of cytokine-related signal-transduction pathways, nuclear factor kappa B (NF-κB), a well-known regulator of inflammation, also influences Cyp expression levels through its interactions with nuclear receptors.37) Gu et al. reported that p65, a subunit of NF-κB, prevents the PXR-RXR heterodimer from binding to its consensus sequences in primary human hepatocytes.38) Tian et al. reported that the AhR and NF-κB signaling pathways are mutually inhibitory, in that p65 makes a complex with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-activated AhR, and inhibits the transcription of AhR target genes, such as Cypla2, in a murine hepatoma cell line.39–41) These previous findings suggested that the activation of NF-κB by LPS could trigger Cyp gene expression. Since we observed increased TNFα and IL-1β mRNA levels, the activation of NF-κB by these cytokines is likely to be involved in the LPS-induced downregulation of the induced expression levels of Cyp3a11 and Cyp1a2 that we observed \textit{in vivo}.

It is interesting that LPS treatment influenced the mRNA levels of Cyp2c29 and 2c55 but not Cyp2b10 in this study, even though all these genes are known to be mainly transcriptionally regulated by CAR. Many reports have suggested that NF-κB plays a role in the regulation of CAR. Some have concluded that exogenous LPS can also downregulate CAR target genes through the suppression of glucocorticoid receptor-mediated CAR transactivation under the control of NF-κB during inflammation.42–44) Thus, the LPS-induced downregulation of the Cyp2c29 and 2c55 mRNA levels under TCPOBOP treatment might occur through the activation of NF-κB in the CAR-dependent pathway. Ferrari et al. reported different regulatory mechanisms for the Cyp2b10 gene and its transcripts in rat hepatocytes exposed to LPS.45) They revealed that LPS treatment suppressed the CYP2B1 enzyme in an nitric oxide (NO)-dependent manner, although the Cyp2b10 mRNA level was transiently upregulated at the early stage of LPS treatment. So far in our experiments, we found only that LPS did not suppress Cyp2b10 gene expression after treatment with the Cyp2b10 inducer, TCPOBOP. However, the study of Ferrari et al. suggests useful ideas for our future study, aimed at understanding how Cyp2b10 gene regulation differs from that of Cyp2c29 and 2c55. As the CYP2 family enzymes are very diverse and many details of their transcriptional and translational mechanisms remain unclear,46) the above findings provide just one possible explanation for Cyp2b10’s anomalous behavior. Clarifying the reason for the different behavior of Cyp2b10 is one of our primary research goals.

In conclusion, we showed that LPS-treated mice showed significantly suppressed xenobiotic-induced CYP enzyme transcription and activity, with the exception of Cyp2b10. Since CYPs are critical for the metabolism of xenobiotics, elucidating the mechanism of CYP regulation during inflammation should improve our ability to predict drug pharmacokinetics. Our current findings contribute to the understanding of how drug metabolism is altered by particular disease states, which should lead to more appropriate drug-treatment choices and dosing, as well as better patient outcomes.

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REFERENCES

10) Koj A. Initiation of acute phase response and synthesis of cyto-


