Mechanisms of Down-Regulation of CYP2E1 Expression by Inflammatory Cytokines in Rat Hepatoma Cells

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ABSTRACT

CYP2E1 is one of the major cytochrome P450 forms whose expression is strongly inhibited by inflammatory cytokines in humans and rodents. In the present study, we have used the Fao rat hepatoma cell line that constitutively expresses CYP2E1 enzyme to investigate mechanisms of cytokine action. The cells were treated with interleukin (IL)-1β, tumor necrosis factor-α (TNFα), or IL-6 for 24 or 72 h, and the expression of CYP2E1 was monitored at the transcriptional, mRNA, and protein levels. All three cytokines decreased the CYP2E1 mRNA levels after 24 h, and the effect was even stronger after 72 h. In contrast, significant inhibition of CYP2E1 protein was seen only after 72 h. In transfection assays using a CYP2E1 5’ −3685 to +29–luciferase construct, it was found that IL-6 inhibited gene transcription after 24 h, but a similar effect by IL-1β and TNFα was registered only after 72 h. Using 5’ deletions of the CYP2E1 5’-reporter construct a responsive region for the IL-6 effect was located to −669 to −507 base pairs in the CYP2E1 5’-flanking region. Interestingly, IL-1β, but not TNFα, was found to reduce hepatocyte nuclear factor (HNF)-1α binding to the CYP2E1 promoter. However, the transactivation function of HNF-1α was found to be impaired in Fao cells. In mouse primary hepatocytes, IL-1β decreased HNF-1α-mediated transactivation. In conclusion, our data indicate that inflammatory cytokines inhibit CYP2E1 expression by multiple mechanisms, including control of HNF-1α function and regulation of other transcriptional factors acting on the CYP2E1 5’-upstream regulatory region. In addition, regulation of factors of importance for the CYP2E1 mRNA stability may be involved.

Infections and inflammatory stimuli cause a reduction of drug elimination and metabolism of other xenobiotics (Morgan, 1997; Renton, 2001). The major part of the reduction is believed to be consequence of decreased cytochrome P450 (P450) enzyme levels and activities in liver. Suppression of the major xenobiotic-metabolizing P450 enzymes by different types of infectious diseases or by administration of endotoxin or other types of inflammatory substances to animals, humans, and to cell culture has been observed in numerous studies (Morgan, 1997; Renton, 2001).

Several mechanisms have been proposed to be involved in down-regulation of P450 enzymes by inflammation. The major hypothesis emphasizes the role of inflammatory cytokines such as interleukin (IL)-6, IL-1, and tumor necrosis factor-α (TNFα), which mediate the induction of acute-phase proteins in liver (Morgan, 1997; Renton, 2001). Indeed, administration of cytokines such as IL-1β, TNFα, IL-6, or interferon-α is able to mimic the effects of infections or endotoxin treatment (Singh et al., 1982; Wright and Morgan, 1991; Abdel-Razzak et al., 1993). Furthermore, use of null mice for certain cytokines or cytokine receptors has been shown to abolish or modify P450 response to inflammatory stimuli (Warren et al., 1999; Siewert et al., 2000).

Also the role of nitric oxide has been extensively studied (Khatsenko et al., 1993; Carlson and Billings, 1996). The generation of nitric oxide by inducible nitric-oxide synthase increases during endotoxemia. Nitric oxide is able to react with heme-containing proteins such as P450 enzymes and can decrease P450-mediated activities. Furthermore, nitric oxide may be able to decrease mRNA and protein of at least some forms of cytochrome P450 in the early phase of endotoxemia. However, Morgan and coworkers have shown that endotoxin down-regulates P450 mRNA and protein also in the absence of nitric oxide (Sewer and Morgan, 1998; Sewer et al., 2000). Therefore, several mechanisms, including inflammatory cytokines and nitric oxide, may contribute to down-regulation of P450s at various stages and types of inflammation.

CYP2E1 is one of the major P450 forms both in human and rat liver that has been shown to be sensitive to inflammatory

ABBREVIATIONS: P450, cytochrome P450; IL, interleukin; TNFα, tumor necrosis factor-α; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; ANOVA, analysis of variance; bp, base pair(s).
stimulus and cytokines both in vivo and in primary hepatocytes (Abdel-Razzak et al., 1993; Morgan et al., 1994; Sinhdu et al., 1996; Shedlovsky et al., 2000). The suppression of CYP2E1 has been detected at the level of mRNA, protein, and catalytic activity and also the systemic elimination of chlorzoxazone, a probe drug for CYP2E1, was decreased in the acute-phase endotoxin response in rats (Rockich and Blouin, 1999).

In the present study, we have used the Fao rat hepatoma cell line model to study the mechanisms of action of inflammatory cytokines IL-1β, TNFα, and IL-6 on expression of CYP2E1. We show that all these major inflammatory cytokines down-regulate CYP2E1 in Fao cells and that multiple mechanisms are involved in the process.

### Materials and Methods

**Chemicals.** Recombinant human cytokines were from the following sources: IL-1β (Roche Diagnostics, Mannheim, Germany), TNFα (Sigma-Aldrich, St. Louis, MO), and IL-6 (Sigma-Aldrich).

**Cell Culture.** The Fao rat hepatoma cell line was provided by Dr. Mary Weiss (The Pasteur Institute, Paris, France). The Fao cell line is a differentiated derivative of the H4IIEC3 cell line and expresses a wide spectrum of liver-specific proteins (Deschatrette and Weiss, 1974; Herbst et al., 1991). This cell line expresses some cytochrome P450 forms and has been used to study the regulation and properties of CYP2E1 (De Waziers et al., 1995; Zhukov and Ingelman-Sundberg, 1999). The cells were cultured in F-12 Coon’s modification medium (Sigma-Aldrich) containing 5% fetal calf serum (Invitrogen). The medium was changed and the serum was withdrawn 24 h before the experiments. The cytokines were diluted with the cell culture medium and were added at final concentrations of 4 ng/ml IL-1β, 0.5 ng/ml TNFα, and 70 ng/ml IL-6. The concentrations were chosen based on previous information in the literature of the effective concentrations in cell culture (Abdel-Razzak et al., 1993).

Mouse primary hepatocytes were isolated from DBA/2 mouse liver as described previously (Salonpää et al., 1994). Briefly, the mouse liver was perfused with collagenase solution (Worthington Biochemicals, Lakewood, NJ), and the liver cells were collected. After filtration and centrifugation, the isolated hepatocytes were dispersed in Williams’s medium E (Sigma-Aldrich) containing 20 ng/ml dexamethasone (Sigma-Aldrich), insulin-transferrin-sodium selenite media supplement (Sigma-Aldrich) (5 mg/l insulin, 5 mg/l transferrin, and 5 μg/l sodium selenate), 10 μg/ml gentamycin (Invitrogen), and 10% fetal bovine serum (Invitrogen) at a density of 300,000 cells/one well in 12-well plate. The cultures were maintained at 37°C in humidified incubator for 2 to 3 h, after which nonattached cells were discarded by aspiration, and the medium was replaced with fresh serum-free William’s medium E (Sigma-Aldrich) containing 20 ng/ml dexamethasone. The enhanced chemiluminescence method was used for protein detection. The Fao cells were washed once with buffer containing 10 mM HEPES, 0.25 M sucrose, pH 7.5 and harvested to the same buffer. The cell suspension was sonicated and centrifuged at 13,000g for 10 min. The pellet was discarded and 15 μg of the 13,000g supernatant protein was separated by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose filter. After completion of transfer, the membrane was dried, resoaked in 50 mM Tris pH 7.5, 0.2 M NaCl, 0.05% Tween 20, blocked in 5% nonfat milk in NaCl/Tris/Tween for 1 h, incubated for 1 h with CYP2E1 antisera, and then 1 h with protein A-conjugated horseradish peroxidase. The enhanced chemiluminescence method was used for protein visualization (Amersham Biosciences AB, Upsala, Sweden).

**Preparation of Nuclear Extracts.** For preparation of nuclear extracts, the Fao cells were harvested to phosphate-buffered saline, spun down at 1,800g for 10 min, and resuspended in hypotonic buffer (10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, and 0.2 mM PMSF). The cells were pelleted and resuspended again to the hypotonic buffer and homogenized using a glass homogenizer. The nuclei were spun down at 3,300g for 15 min and suspended with low-salt buffer (20 mM HEPES pH 7.9, 25% glycerol, 1.5 mM MgCl2, 10 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, and 0.2 mM PMSF). KCl (2.5 M) was added drop-wise to a final concentration of 0.4 M, and nuclear proteins were extracted by gentle shaking for 30 min. The nuclei were pelleted at 25,000g for 30 min and the nuclear extract was aliquoted and stored at −70°C. All steps were carried out at 4°C.

**Electrophoretic Mobility Shift Assay.** The DNA-bound protein is separated on electrophoresis and transferred to a nitrocellulose filter. The filters were hybridized with [32P]ATP. Labeled probe (7 fmol) was incubated with 4.5 μg of nuclear extract in a protein extract in a final volume of 20 μl in a buffer containing 10 mM Tris-HCl pH 7.6, 8 mM HEPES, 14% glycerol, 1 mM EDTA, 0.6 mM MgCl2, 125 mM KCl, 1 mM DTT, 0.1 mM PMSF, and 1 μg of poly(dI-dC)·poly(dI-dC). For competition, 25- to 100-fold molar excess of unlabeled probe or mutation probe (5'-TGAAATGATAGCCAACTGCA-3') containing the HNF-1α binding site of rat CYP2E1 promoter was labeled with T4 polynucleotide kinase (Amersham Biosciences AB) using [γ-32P]ATP. Labelled probe (7 fmol) was incubated with 4.5 μg of nuclear extract in a protein extract in a final volume of 20 μl in a buffer containing 10 mM Tris-HCl pH 7.6, 8 mM HEPES, 14% glycerol, 1 mM EDTA, 0.6 mM MgCl2, 125 mM KCl, 1 mM DTT, 0.1 mM PMSF, and 1 μg of poly(dI-dC)·poly(dI-dC). For competition, 25- to 100-fold molar excess of unlabeled probe or mutation probe (5'-TGAAATGATAGCCAACTGCA-3') was used. The identity of the protein components in the retarded complexes was confirmed with antibody specific against HNF-1α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

**Statistical Analysis.** Student’s t test was used for comparison between two groups. Comparison of several groups was done with one-way ANOVA followed by least significant difference post hoc test.

**Results**

**Inflammatory Cytokines Inhibit CYP2E1 Expression in Fao Cells.** Regulation of CYP2E1 by the inflammatory cytokines IL-1β, TNFα, and IL-6 was studied in the rat hepatoma cell line Fao. This cell line constitutively expresses...
CYP2E1 and is therefore a suitable model for this kind of experiment. The cells were maintained in serum-free media and treated with the cytokines for 24 or 72 h until harvested and analyzed for CYP2E1 mRNA and protein contents by RNA and immunoblotting, respectively.

All three cytokines studied caused a decrease of the CYP2E1 mRNA content after 24 h treatment. After 72-h treatment, the effect was even stronger (Fig. 1). In contrast, the CYP2E1 protein was not decreased after 24-h treatment but only after 72-h treatment (Fig. 2).

Transcriptional Regulation Is Involved in Inhibition of CYP2E1 Expression Caused by IL-1β, TNFα, and IL-6. The involvement of transcriptional regulation in decreased expression on CYP2E1 by the inflammatory cytokines was next studied. A −3685 to +28 CYP2E1 5′-flanking region luciferase reporter construct was transfected into the Fao cells, and the cells were treated with IL-1β, TNFα, or IL-6 for 24 or 72 h. Using this reporter construct, it was found that only IL-6 inhibited transcription during the first 24-h treatment, whereas at 72 h significant inhibition was seen after treatment with all cytokines (Fig. 3).

Regulation of HNF-1α by Cytokines. HNF-1α plays a major role in activation of CYP2E1 transcription in the rat liver (Liu and Gonzalez, 1995) and is among the several liver-enriched transcription factors that are expressed in the Fao cells (Herbst et al., 1991). The effect of IL-1β and TNFα on HNF-1α binding to CYP2E1 promoter was studied next.

Fao cells were treated with IL-1β or TNFα for 72 h or left untreated. The cells were harvested and nuclear extract was prepared and consequently binding of HNF-1α to CYP2E1 promoter HNF-1α element was measured by electrophoretic mobility shift assay. Two specific bands were detected that were competed away by 25- to 100-fold excess of unlabeled HNF-1α oligonucleotides. However, mutation of the nucleotides known to be important for HNF-1α binding abolished competition. The correct identity of the bands was confirmed by supershifting with HNF-1α-specific antibody. IL-1β treatment clearly reduced HNF-1α binding to CYP2E1 promoter element, whereas TNFα had no effect (Fig. 4).

HNF-1α Does Not Transactivate CYP2E1 in Fao Cells. We have previously shown that the major segments mediating the transcriptional activation of CYP2E1 gene in Fao cell lie within the proximal 500 bp of the 5′-flanking region (Hu et al., 1999). To analyze the function of the proximal promoter and search for targets of the cytokine effect, we prepared a series of CYP2E1 5′-flanking region-luciferase reporter plasmids and transfected these constructs into Fao cells (Fig. 5). The highest activation of transcription was produced by the longest −507 to +28 construct, i.e., 70-fold activation compared with the no promoter containing pGL3-basic plasmid. 5′ deletion to −183 caused 2.2-fold decrease of the luciferase activity, indicating localization of activating elements within the region between −507 and −183. Surprisingly, further 5′ deletion to −41 bp had

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**Fig. 1.** Effect of cytokine treatment on CYP2E1 mRNA expression in Fao cells. a, cells were treated with IL-1β, TNFα, or IL-6 or left untreated for 24 or 72 h. Total RNA (20 μg) was subjected to electrophoreses, blotted, and hybridized with rat CYP2E1 and β-actin probes. b, densitometric quantitation of the mRNA blot. The values (means of two samples) are normalized against control level in each time point. Difference to the untreated cells, ***, p < 0.001 (one-way ANOVA). The experiment was repeated twice and all three independent experiments gave similar results.

**Fig. 2.** Effect of cytokine treatment on CYP2E1 protein expression in Fao cells. a, cells were treated with IL-1β, TNFα, or IL-6 or left untreated for 24 or 72 h. The 13,000g supernatant fraction (15 μg) was separated by SDS-polyacrylamide gel electrophoresis and blotted. Blots were developed for immunoreactivity using anti-CYP2E1 sera; 0.5 μg (first lane) and 1 μg (last lane) of rat liver microsomal protein was used as reference. b, densitometric quantitation of the immunoblot. The values (means of two samples) are normalized against control level in each time point. Difference to the untreated cells, ***, p < 0.001 (one-way ANOVA). The experiment was repeated once and both independent experiments gave similar results.
only minor effect to the luciferase activity. Therefore, −41 to +28, TATA box-containing, minimal construct was responsible for a significant proportion of the total activation of CYP2E1 transcription in Fao cells and produced 48-fold higher luciferase activity than the no promoter containing pGL3-Basic construct. Transcription in Fao cells and produced 48-fold higher luciferase activity than the no promoter containing pGL3-Basic construct.

Deletion of the HNF-1α binding site, believed to be the major activator of the CYP2E1 transcription in rat liver, did not reduce the transcription but had a slight opposite effect. To further analyze the function of HNF-1α in the Fao cell line, we constructed a collection of reporter plasmids for this purpose. The HNF-1α element was linked directly to the CYP2E1 core promoter and a more general failure of HNF-1α transcription was a phenomenon specific for CYP2E1 promoter alone. IL-1β decreased transcription of HNF-1α-containing construct by 40% but had no statistically significant effect on CYP2E1 core promoter alone. IL-1β decreased transcription of HNF-1α-containing construct by 40% but had no statistically significant effect on CYP2E1 core promoter alone (Fig. 7).

Localization of the Cytokine-Regulated Elements in the CYP2E1 Promoter. To further characterize the mechanisms involved in depression of CYP2E1 transcription, a series of reporter constructs with different lengths of 5′ deletions were transfected to Fao cells and the cells were transfected with different cytokines. The results of this deletion analysis are summarized in Figs. 8 and 9. IL-1β and TNFα had very similar effects on the luciferase activity produced by the different constructs, suggesting that they both regulate CYP2E1 transcription through an analogous mechanism. For both cytokines, the major effect was localized at the very proximal promoter but some additional effect was seen using the longer constructs. In contrast, it was found that IL-6 did not affect the proximal promoter but acted through a more distal regulatory element located between −669 and −507 bp.

**Discussion**

The inflammatory cytokines IL-1β, TNFα, and IL-6 were found to suppress CYP2E1 expression in Fao rat hepatoma
cell line in a similar manner as observed previously in primary hepatocytes (Abdel-Razzak et al., 1993). The Fao cell line therefore seemed to be a useful model system for the study of CYP2E1 regulation by inflammatory cytokines. Several potential mechanisms for CYP2E1 suppression were revealed. IL-6 was shown to inhibit CYP2E1 expression at the transcriptional level. Late transcriptional suppression by IL-1β and TNFα was preceded by early decrease in the CYP2E1 mRNA levels. IL-1β was found to down-regulate HNF-1α binding to the CYP2E1 promoter. However, the function of HNF-1α was apparently impaired in the Fao cells compared with H4IIE cells, and the effect on HNF-1α by IL-1β could not explain the inhibition of CYP2E1 expression found in the Fao cells, but may well be an important mechanism for the IL-1β-dependent transcriptional control of the hepatic CYP2E1 gene in vivo. Indeed, IL-1β was able to decrease HNF-1α-mediated activation of transcription in mouse primary hepatocyte culture system.

The overall regulation of CYP2E1 expression is very complex and involves, as emphasized, multiple mechanisms at different levels. The constitutive level of hepatic CYP2E1 is regulated transcriptionally by liver-enriched transcription factors, especially by HNF-1α (Ueno and Gonzalez, 1990). Pathophysiological conditions such as diabetes regulate CYP2E1 expression by affecting RNA stability (Song et al., 1987; De Waziers et al., 1995). On the other hand, exogenous compounds mainly regulate CYP2E1 at post-translational level by stabilizing the protein (Roberts et al., 1995). There is little evidence for transcriptional induction of CYP2E1 by xenobiotics and only few examples of such physiological conditions exist. These include starvation (Johansson et al., 1990) and interleukin-4 stimulation (Lagadic-Gossmann et al., 2000), but the exact mechanism of induction is still unclear.

The Fao cell line is one of the very rare hepatoma cell lines constitutively expressing CYP2E1, and it has been used in several studies to investigate the CYP2E1 regulation (De Waziers et al., 1995; Simi and Ingelman-Sundberg, 1999; Zhukov and Ingelman-Sundberg, 1999). In the current study, however, we show that there are important differences in the regulatory mechanisms of CYP2E1 between the Fao cells and the rat liver. HNF-1α is clearly involved in activation of CYP2E1 transcription in the liver (Ueno and Gonzalez, 1990; Liu and Gonzalez, 1995). However, we found that HNF-1α failed to activate CYP2E1 transcription in the Fao cells and that other factors contribute to the transcriptional activity in the Fao cells. The functional defect of HNF-1α in the Fao may explain the relative low expression level of CYP2E1 in these cells.

**Fig. 5.** Transcriptional activity of CYP2E1 5′-luciferase and albumin 5′-luciferase constructs transfected to Fao cells. The cells were harvested and luciferase activities measured 24 h after transfection. The activities produced by the studied constructs were normalized against cotransfected control plasmid (pRL-TK) activities. The values represent means + S.D. of four individual samples.

**Fig. 6.** Transcriptional activity of albumin 5′-luciferase constructs transfected to H4IIE cells. The cells were harvested and luciferase activities measured 24 h after transfection. The activities produced by the studied constructs were normalized against cotransfected control plasmid (pRL-TK) activities. The values are presented as fold activation of the no promoter containing pGL3-basic construct. The values represent means + S.D. of four individual samples.

**Fig. 7.** Transcriptional activity of CYP2E1 5′-luciferase constructs transfected to mouse primary hepatocytes. After transfection the cells were treated for 24 h with IL-1β or left untreated after which the cells were harvested and the luciferase activities were measured. The activities produced by the studied constructs were normalized against cotransfected control plasmid (pRL-TK) activities. The values are presented as CYP2E1 5′-luciferase/pRL-TK ratio. The values represent means + S.D. of 12 individual samples in four separate experiments (three samples in each experiment). Difference to the untreated cells, **, p < 0.01 (Student’s t test).
were treated for 72 h with IL-1β/NFκB/CYP2E1. It has previously been shown to be reduced by LPS and HNF-1α, or necessary coactivators could be absent. HNF-1α (Hansen and Crabtree, 1993) is expressed in Fao cells, able to bind at a specific DNA binding site and therefore also presents normal dimerization function. However, the Fao cells could lack the dimerization cofactor HNF-1α (DeoH), which does not change DNA binding characteristics of HNF-1α, but affects transcriptional activation (Hansen and Crabtree, 1993) or necessary coactivators could be absent. HNF-1α binding to its cognate response elements has previously been shown to be reduced by LPS and HNF-1α is probably involved in inflammatory repression of some genes such as ntcp and CYP27A (Trauner et al., 1998; Memon et al., 2001). Here, we show that IL-1β is able to decrease HNF-1α binding to the CYP2E1 promoter. This finding is in agreement with a recent investigation showing decreased HNF-1α binding to the CYP2E1 promoter after lipopolysaccharide treatment of rats (Roe et al., 2001). The functional consequence of decreased HNF-1α binding could not be studied in Fao cells. Instead, we show in mouse primary hepatocytes that IL-1β treatment also affects transactivation by HNF-1α, suggesting that HNF-1α inhibition is one of the mechanisms involved in CYP2E1 suppression by inflammation and is mediated by IL-1β.

Although IL-1β-mediated HNF-1α down-regulation may be implicated in decreased CYP2E1 expression in liver, additional mechanisms clearly exist and predominate in HNF-1α-impaired Fao cell line. IL-1β and TNFα had very similar effect on the CYP2E1 transcription. They both affected even very short promoter constructs of only up to −40 bp, but additional effects were seen with longer constructs up to −3685 bp, suggesting contribution of multiple transcription factors. Nevertheless, the effect on CYP2E1 transcription was not detectable after 24-h treatment when CYP2E1 mRNA was already decreased. The primary mechanism of CYP2E1 mRNA down-regulation is thus post-transcriptional or alternatively could require elements not present in the 3685-bp construct used. The attempts to analyze involvement of putative post-transcriptional mechanism by measuring the mRNA half-life were unsuccessful because of the mRNA concentrations rapidly dropped to concentrations lower than the sensitivity limit of the assay.

IL-6 efficiently decreased CYP2E1 protein and mRNA. In contrast to IL-1β and TNFα, the CYP2E1 mRNA decrease was accompanied by an early decrease in transcriptional activity, suggesting predominance of transcriptional inhibition by IL-6. The sequence mediating the IL-6 effect was localized to −669 to −507 bp of the CYP2E1 5′-upstream regulatory region. According to our previous studies, this region does not contain activating elements but instead is target for repressors (Hu et al., 1999). IL-6 could affect binding of one these factors or induce binding of new repressing proteins. The major transcription factors mediating IL-6 effects include NF-IL-6 (C/EBPβ) and STAT3 (Akira, 1997). The MatInspector professional search (http://transfac.gbf.de/)

Fig. 8. Effect of IL-1β and TNFα on the transcriptional activity of the CYP2E1 5′-luciferase constructs in Fao cells. After transfection the cells were treated for 72 h with IL-1β or TNFα or left untreated after which the cells were harvested and the luciferase activities were measured and normalized against cotransfected control plasmid (pRL-TK) activities. The values are mean-inhibition ± S.D. in three to six separate experiments and are presented as percentage of the control (untreated cells) activity. Difference to the untreated cells, **, p < 0.01; ***, p < 0.001 (one-way ANOVA).

Fig. 9. Effect of IL-6 on the transcriptional activity of the CYP2E1 5′-luciferase constructs in Fao cells. After transfection the cells were treated for 72 h with IL-6 or left untreated after which the cells were harvested and the luciferase activities were measured and normalized against cotransfected control plasmid (pRL-TK) activities. The values are mean-inhibition ± S.D. in three to seven separate experiments and are presented as percentage of the control (untreated cells) activity. Difference to the untreated cells, *, p < 0.05; **, p < 0.01; ***, p < 0.001 (Student’s t test).
TRANSFAC (Wingender et al., 2000) failed, however, to reveal any NF-IL-6 or STAT3 binding sites at −669 to −507 area of CYP2E1 5′-flanking region, suggesting that other factors mediate the IL-6 effect on CYP2E1 expression. The previous information in the literature did not suggest involvement of IL-6 in the reduction of HNF-1α binding by inflammation (Green et al., 1996; Trauner et al., 1998) and our transfection studies suggested that the mechanism of IL-6 action is mediated by sequences upstream from the CYP2E1 HNF-1α site. Therefore, the effect of IL-6 on HNF-1α binding to the CYP2E1 promoter was not studied.

The reduction of P450-mediated drug metabolism during inflammation has been well established (Shedlofsky et al., 1994; Morgan, 1997). The involvement of inflammatory cytokines in this process is also well recognized. Yet, the molecular mechanisms mediating the effects of inflammatory cytokines on P450 enzymes are still poorly understood. Recently, it was shown that nuclear factor-κB was involved in suppression of rat CYP2C11 transcription by binding to the transcription start site (Iber et al., 2000). In the current study, we show that CYP2E1 is regulated by inflammatory cytokines by a number of mechanisms. Different cytokines differentially affect individual P450 forms and even the same cytokine may act on various signal transduction pathways. Therefore, it is plausible that a number of mechanisms, both direct and indirect, are involved in the down-regulation of P450 enzymes by inflammatory cytokines and thus no single mechanism may explain suppression of all P450 forms. In fact, each P450 gene may have a unique way of regulation by inflammatory cytokines.

In conclusion, our results indicate that the CYP2E1 down-regulation by inflammation is mediated by several cytokines and by multiple regulatory pathways. These include the control of transcription factors such as HNF-1α known to mediate CYP2E1 constitutive expression, effects through other transcriptional factors acting on specific elements in the CYP2E1 5′-upstream regulatory region, and possibly regulation of factors of importance for the CYP2E1 mRNA stability. This is in agreement with recent studies using null mice showing that the absence of individual cytokines modulate but do not abolish the effect of endotoxin on CYP2E1 expression (Warren et al., 1999; Siewert et al., 2000).

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