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The regulation of cytochrome P450 2E1 during LPS-induced inflammation in the rat

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Abstract

It is well known that inflammatory and infectious conditions differentially regulate cytochrome P450 (P450)-mediated drug metabolism in the liver. We have previously outlined a potential pathway for the downregulation in hepatic cytochrome P450 following LPS-mediated inflammation in the CNS (Abdulla, D., Goralski, K.B., Garcia Del Busto Cano, E., Renton, K.W., 2005. The signal transduction pathways involved in hepatic cytochrome P450 regulation in the rat during an LPS-induced model of CNS inflammation. Drug Metab. Dispos). The purpose of this study was to outline the effects of LPS-induced peripheral and central nervous system inflammation on hepatic cytochrome P450 2E1 (CYP2E1) in vivo, an enzyme that plays an important role in various physiological and pathological states. We report an increase in hepatic mRNA expression of CYP2E1 that occurred as early as 2–3 h following either the intraperitoneal (i.p.) injection of 5 mg/kg LPS or i.c.v. administration of 25 μg of LPS. This increase in CYP2E1 mRNA expression was sustained for 24 h. In sharp contrast to the increase in hepatic CYP2E1 mRNA, we observed a significant reduction in the catalytic activity of this enzyme 24 h following either the i.c.v. or i.p. administration of LPS. Cycloheximide or actinomycin-D did not change the LPS-mediated downregulation in hepatic CYP2E1 catalytic activity. Our results support the idea that LPS acts at two different levels to regulate hepatic CYP2E1: a transcriptional level to increase CYP2E1 mRNA expression and a post-transcriptional level to regulate CYP2E1 protein and activity.

Keywords: Lipopolysaccharide; CYP2E1; Cycloheximide; Actinomycin-D; Inflammation; In vivo regulation; HNF1α

It is well known that the cytochrome P450 family of enzymes are differentially regulated during conditions of inflammation and infection (Renton, 2001; Morgan et al., 2002). The administration of lipopolysaccharide (LPS) in the rat induces a potent acute phase response (APR) characterized by an increased production in pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6 (Renton and Nicholson, 2000; Roe et al., 2001). The activity and/or protein levels of CYP1A1/2, CYP2B1/2, CYP2D5, and CYP3A1 have been shown to be downregulated following conditions of inflammation induced by LPS or cytokine administration in the rat (Barker et al., 1992; Barker et al., 1994; Pan et al., 2000; Renton and Nicholson, 2000; Morgan, 2001; Nicholson and Renton, 2001; Renton, 2001; Morgan et al., 2002; Nicholson and Renton, 2002; Garcia Del Busto Cano and Renton, 2003). The transcription factor NF-κB is known to play a dominant role in the regulation of various P450 isoforms such as CYP1A1, CYP2C11, and CYP2D5 (Iber et al., 2000; Ke et al., 2001; Abdulla et al., 2005). On the other hand, the regulation of CYP2B can occur at the level of the gene and the protein (Agrawal and Shapiro, 1996).

CYP2E1 is constitutively expressed in the liver but can also be found in extrahepatic organs such as the kidneys, lungs, and the central nervous system (CNS) (Roe et al., 2001; Kelicen and Tindberg, 2004). The transcription factor hepatocyte nuclear factor-1 (HNF-1) is thought to play a role in the transcriptional control of constitutive CYP2E1 in the liver (Ueno and Gonzalez, 1990). CYP2E1 plays an important toxicological

Abbreviations: LPS, lipopolysaccharide; CNS, central nervous system; P450, cytochrome P450; TNFα, tumor necrosis factor alpha; IL-1β, interleukin-1β; IFNγ, interferon gamma; NF-κB, nuclear factor-kappa B; Ap-1, activator protein-1; i.c.v., intracerebroventricular; i.p., intraperitoneal; CZX, chlorzoxazone; AD, actinomycin-D; CHX, cycloheximide; HNF, hepatic nuclear factor.

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role in various physiological disease-induced states such as alcohol induced liver injury, diabetes, and non-alcoholic steatohepatitis, and its induction can increase the risk of developing cancer due to its role in the production of reactive oxygen species (Xu and Pasco, 1998; Leclercq et al., 2000; Caro and Cederbaum, 2004). At birth and during conditions of starvation, a rapid induction in the expression and protein levels of CYP2E1 occurs (Simi and Ingelman-Sundberg, 1999). CYP2E1 can also be regulated at the level of the protein, where substrates are known to bind to the heme group of the enzyme and prevent its proteosomal degradation (Tindberg et al., 2004). It is thought that the majority of CYP2E1 regulation occurs at the level of the protein (Hu et al., 1995).

The effects of inflammatory and infectious states on CYP2E1 have been examined; however, little is known regarding the mechanisms of regulation of this isoform during such conditions. It has been shown that the expression and activity of CYP2E1 are downregulated in a rat hepatoma cell line following the administration of pro-inflammatory cytokines, leading to a loss in catalytic activity (Hakkola et al., 2003). When cytokines and/or LPS are administered to rats i.p., a downregulation in the activity and protein levels of CYP2E1 is observed (Renton and Nicholson, 2000). In contrast to its regulation in the liver, CYP2E1 expression is upregulated both in vitro and in vivo in the brain following LPS administration (Tindberg et al., 1996). The objective of this work was to determine the mechanisms that regulate CYP2E1 during LPS-mediated peripheral and CNS inflammatory models. We observed an increase in mRNA expression of CYP2E1 and a decrease in enzyme activity following either the i.c.v. or i.p. administration of LPS, indicating that LPS is acting in two separate mechanisms to regulate hepatic CYP2E1. Using inhibitors of transcription and translation, we present evidence that hepatic CYP2E1 regulation during conditions of LPS-induced inflammation in the rat occurs mainly at the post-transcriptional level.

Materials and methods

Reagents. All laboratory reagents were purchased from Sigma (St. Louis, MO) with the exceptions noted in the text. Gel purified *Escherichia coli* lipopolysaccharide (LPS) of serotype 0127:B8 (Sigma, St Louis, USA) was utilized in the experiments as outlined below.

Animals and treatment. Male Sprague-Dawley rats (125–150 g) were obtained from Charles River Laboratories (Quebec, Canada) and were housed on corncob bedding for a period of 5 days on a 12-h light/dark cycle. All animal procedures were performed according to the Dalhousie University Committee on Laboratory Animals following the guidelines established by the Canadian Council on Animal Care. Rats were allowed ad libitum access to food and water prior to and following the experimental procedure. Intracerebroventricular (i.c.v.) injections into the lateral ventricle were performed using a KOPF® stereotaxic instrument. The coordinates utilized relative to bregma were 1.7 mm lateral and 4.7 mm below the skull surface. On the day of the experiment, rats were anesthetized using enflurane and maintained on a 4% level of the anaesthetic during the surgery. We have previously performed dose-responses for LPS administration i.c.v. and have shown that a dose of 25 μg LPS (made in pyrogen-free saline and injected in a volume of 5 μl) is sufficient to induce CNS inflammation with observable CNS and peripheral effects (Renton et al., 1999; Renton and Nicholson, 2000). To induce systemic inflammation, animals were injected intraperitoneally with a dose of 5 mg kg^{-1} LPS (in 100 μl saline). This dose has been previously shown to cause a downregulation in P450 levels (Rockich and Blouin, 1999). In experiments to examine the effects of LPS intravenously (i.v.), rats were administered either saline or 25 μg LPS via the tail vein as previously described (Abdulla et al., 2005). When rats were dosed with LPS and either CHX or AD, the following schedule was applied: at the 0 h time point, rats were administered LPS (either i.c.v. or i.p.), and 20 h following this, rats were divided in groups based on actinomycin-D (AD at a dose of 7 mg kg^{-1}) administration, cycloheximide (CHX at a dose of 10 mg kg^{-1}) administration, or appropriate vehicle. All rats in this dosing schedule were sacrificed at 24 h following the initial LPS administration. These doses for AD and CHX were chosen based upon previous reports outlining that the use of these antibiotics at the specified doses for 6 h was sufficient to cause an inhibition in the P450 isoform CYP3A (Kim et al., 2001). The results for the CHX and AD experiments were obtained 24 h following LPS administration by either route because both the upregulation in CYP2E1 mRNA and the downregulation in CYP2E1 activity can be observed at this time point. All experiments utilized 4–6 rats per treatment.

Microsomal fraction preparation and CYP2E1 metabolism assay. Liver microsomes were isolated at 2 and 24 h following i.c.v. and i.p. injections and at 24 h following the i.v. administration of LPS as described previously (Renton and Nicholson, 2000) and suspended in a glycerol-phosphate buffer (50 mM KH_2PO_4 buffer, pH 7.4, containing 20% glycerol and 0.4% KCl). Liver microsomal fractions were stored at −80 °C until usage. Protein concentrations were determined according to a modified Lowry protocol (Lowry et al., 1951).

The formation of 6-hydroxychlorzoxazone (6-OH CZX) from chlorzoxazone (CZX) was used as a specific marker for rat CYP2E1 activity (Kharasch and Thummel, 1993). Incubation mixtures containing 1 mg of total microsomal protein were incubated with 100 μM of CZX in 50 mM of KH_2PO_4 buffer, pH 7.4. The reaction mixtures were preincubated in a 37 °C water bath with a shaker set at 100 oscillations/min for a period of 2 min. Reactions were initiated by the addition of the cofactor NADPH (1 mM) and incubated at 37 °C for a period of 10 min. Reactions were terminated by the addition of 5 ml of dichloromethane at which point 2000 ng of the internal standard pentoxifylline was added to each sample. The samples were centrifuged at 2700 rpm for a period of 10 min, and the lower organic phase containing the metabolites and internal standard was transferred into a clean glass tube and evaporated to dryness using a nitrogen evaporator. The metabolite and internal standard residues were then reconstituted in 125 μl of HPLC mobile phase (22% acetonitrile in 0.5% H_3PO_4, v/v), and 50 μl was separated on a reverse phase C-18 (25 cm × 4.5 mm I.D.) analytical column (Beckman Coulter, Fullerton, CA, USA) attached to a Waters 2690 Separations Module. The mobile phase flow rate for separation was set at 1.0 ml min^{-1}, with detection by UV absorption at 287 nm (Waters 2487 Dual λ absorption unit). The approximate retention times were 6.7 min for 6-OH CZX, 7.8 min for pentoxifylline, and 21.5 min for CZX.

RNA extraction and Northern blot analysis. Liver samples (~ 100 mg) were obtained for RNA isolation at 2, 4, 6, 9, and 24 h following the i.c.v. injection of 25 μg of LPS and at 3 and 24 h following the i.p. injection of 5 mg kg^{-1} LPS. Liver RNA was also isolated at 3 and 6 h following the i.v. administration of LPS. Total liver RNA was extracted using the TriZol® method according to manufacturer's instructions, and quality was determined using 260/280 nm ratios. 10 μg of total RNA was electrophoresed on a 1.1% formaldehyde gel and transferred onto an immobolin–NY + membrane (Millipore Corporation, MA, USA) overnight and fixed to the membrane by UV cross-linking and heating for 1 h at 65 °C. Blots were prehybridized for 1 h in 10 ml of Sigma Perfecthyb™ Plus (Sigma, St Lois, MS) after which the [^{32}P]dCTP (Perkin and Elmer, Canada) labeled probes (RmT Random Primer Labelling kit, Stratagene, USA) were added to a specific activity of 1×10^7 cpm. Blots were exposed to a storage phosphor screen (Amersham Biosciences, NJ, USA) for 16–24 h and scanned using a phospher imager (Amersham Biosciences, NJ, USA). Bands were quantified using ImageQuant 5.2™ software (Amersham Biosciences, NJ, USA). A CYP2E1 (Hellmold et al., 1995) probe was constructed from forward and reverse primers (CYP2E1 FWD 5′ CTG ATT GGC TGC GCA CCC TGC 3′, CYP2E1 REV 5′ GAA CAG GTC GGC CAC AGT CAC 3′, primer sequences verified using an NCBI BLAST search) using a TOPO TA Cloning® kit (Invitrogen, Ontario, Canada) according to the manufacturer's instructions.