Review paper

Expression of cytochromes P450 4F4 and 4F5 in infection and injury models of inflammation


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Abstract

Lipopolysaccharide (LPS) treatment of rats suppresses CYP 4F4 and 4F5 expression by 50 and 40%, respectively, in a direct fashion occurring in the liver. This contention is borne out by essentially parallel dose-dependent changes observed upon treatment of rat hepatocyte cultures with LPS. An alternate avenue of triggering the inflammatory cascade is traumatic brain injury by controlled cortical impact. Such injury brings about a dramatic change in the expression of CYP 4F4 and 4F5 mRNA which reaches its greatest effect 24 h after impact compared with sham-operated but uninjured controls. At time points after 24 h the expression of both isoforms increases dramatically reaching highest levels at 2 weeks post-injury. These changes in mRNA expression are mirrored by changes in protein expression. The results are consistent with the notion that immediately after injury, concentrations of leukotriene and prostaglandin mediators are elevated by decreased CYP 4F concentrations. As time after injury increases those conditions reverse. Increased CYP 4F expression leads to diminished concentrations of leukotriene and prostaglandin mediators and then to recovery and repair.

Keywords: Regulation of CYP 4F expression; Inflammation and P450 expression; Brain cytochrome P450 distribution; Lipopolysaccharide and CYP 4F subfamily expression; CYP 4F subfamily expression; CYP 4F subfamily suppression by inflammation

1. Introduction

The cytochromes P450 constitute a superfamily of enzymes which play a major role in the activation and/or inactivation of most of the therapeutic agents in use today. The cytochromes P450 share significant sequence homology and, it is now becoming apparent, significant three-dimensional structural similarity. Moreover, catalytic abilities seem to overlap in general but with demonstrable substrate preference seen between families, subfamilies and individual forms. It is well known that many cytochromes P450 are induced by particular therapeutic agents and carcinogens or toxic compounds. More recently, it has become clearer that levels of many of the cytochromes P450 are affected by infection and injury. The effects of mediators of inflammation on the expression of cytochromes P450 in animal model systems have been summarized in a recent review by Morgan [1]. Inflammation or administration of cytokines IL-1, IL-6 or tumor necrosis factor (TNF) caused a decline in enzyme activity for cytochromes P450 1A1, 2C11, 2C12, 2E1 and 3A2 [1]. The decreases in catalytic activity was accompanied in most cases by commensurate decreases in enzyme protein and in mRNA levels. On the other hand, arthritic animals evinced an increased level of hepatic CYP 3A protein and an increased progesterone 6β-hydroxylation activity [2]. Sewer et al. [3] reported that CYP 4A1, 4A2 and 4A3 expression in liver was induced after treatment of rats with lipopolysaccharide (LPS), a well-known prompt of the inflammatory cascade. Sewer et al. [4] found that CYP 2E1 expression in Fischer 344 rat liver and kidney was increased after LPS treatment in contrast to earlier observations. Thus, it seems that the response of cytochromes P450 may be affected by species, strain, inflammatory agent or model of inflammation studied.
The cytochrome P450 4F subfamily has been shown to be present in rats [5–7] humans [8–15] mice [16,17] and fish [18]. Rat CYP 4F4 and CYP 4F5 have been shown to catalyze the clinically important drugs imipramine [19] and chlorpromazine [20] and thus could also influence drug effectiveness during infection and injury. In addition, the CYP 4F subfamily has been shown to catalyze the metabolism of leukotriene B4 and prostaglandins A1 and E1 prompts for the inflammatory cascade causing immigration of cells, fluids and ions to the site of injury or infection [7,8,10,21–23]. Hydroxylation of the leukotriene or prostaglandin signals obviates their role in promoting inflammation; thus, the CYP 4Fs seem to serve a role in regulating the concentration of inflammatory prompts. The CYP 4F subfamily is implicated in additional metabolic steps wherein some of the more distant metabolites of arachidonic acid serve prominent vasoactive roles (Fig. 1).

Since a role for CYP 4Fs in regulating the concentration of active prompts of the inflammatory cascade is indicated, the question of what influences the expression levels of CYP 4F arises. We have addressed this question using the LPS-treated rat model system and the controlled traumatic brain injury (TBI) rat model. Since LPS is injected intraperitoneally, this model is a systemic model. TBI is a direct brain injury (TBI) rat model. Since LPS is injected intraperitoneally, this model is a systemic model. TBI is a direct controlled minor impact delivered to the exposed brain cortex under carefully monitored conditions [24,25]. The TBI model in rat closely simulates closed head injury in humans who have been in traffic accidents or blunt trauma. Closed head injury is often associated with secondary injury, which arises due to the excessive release of neurotransmitters, influx of ions and production of inflammatory mediators [26–28]. Analysis of brain tissue following impact in the absence of a secondary injury-prompted inflammatory cascade in the absence of a systemically introduced prompt.

2. Materials and methods

2.1. Materials

All chemicals utilized in the experiments reported here, where not specifically defined, were of reagent grade quality or higher.

2.2. Animal treatments

Six- to eight-week-old male Fisher 344 rats (Harlan, Indianapolis, IN) were used. The animals were allowed free access to food and water at all times. Chromatographically purified Escherichia coli LPS, serotype 0127:B8 (Sigma, St. Louis, MO), was dissolved in sterile saline to a final concentration of 1.0 mg/ml by sonication and injected intraperitoneally at a dose of 1.0 mg/kg body weight. BaSO4 was suspended in phosphate-buffered saline and injected intraperitoneally at a concentration of 5.0 g/kg body weight. Control animals received an equivalent volume of sterile saline. The animals were killed 24 h after treatments by CO2 asphyxiation. The livers were removed for RNA or microsome preparation. These procedures were approved by Animal Care and Use Committees of both Emory University and The University of Texas Medical School at Houston.

2.3. Hepatocyte isolation, culture and treatments

Rat hepatocytes were isolated by in situ collagenase perfusion [29]. The viability of hepatocyte preparations was between 70% and 85% (trypan blue exclusion) and the yield was 200–400 × 10⁶ viable cells/liver. The hepatocytes were plated in Weymouth’s medium containing 0.15 mM insulin on 60 mm culture dishes coated with Matrigel at a density of 3.5 × 10⁶ cells per dish. Medium was changed to remove dead cells 4 h after the plating and every 48 h thereafter.

Cells were cultured for 5 days before beginning treatments to allow for recovery of stable expression of cytochromes P450. The cells were treated on day 5 by changing to medium containing specific concentrations of LPS (Sigma) or vehicle for 24 h.

2.4. RNA

Total RNA was prepared according to method of Chomczynski and Sacchi [30].

2.5. Northern blot analysis

Ten to twenty micrograms of total RNA was fractionated on a 1.3% agarose gel in the presence of 5% formaldehyde and transferred to a charged nylon membrane (Schleicher and Schuel, Keene, NH) in 20 × SSC overnight. The hybridization was carried out at 65 °C for 30 min, then in 0.1 × SSC and 0.1% SDS at 65 °C for 30 min. The amount of probe bound to the filter was quantitated with a PhosphoImager (Parkard, Meriden, CT). The Northern blots were normalized to the content of β-actin mRNA with a cDNA probe.

The probes for CYP 4F4 and CYP 4F5 were generated by PCR using gene-specific primers. The sequence for CYP 4F4 and CYP 4F5 forward primer was the same (5'-GCT CTA GAA CTA GTG GAT C-3') and the specificity of the probe was achieved by unique reverse primers with CYP 4F4 primer being 5'-CTC GAG TGT GAG GGA AAT CAC A-3' and CYP 4F5 primer being 5'-CTC GAG TGC CTT CTT CAT TGC CTT-3', respectively. The full-length CYP 2C11 cDNA was used as probe to detect CYP 2C11 mRNA content. The probes were labeled with [α-32P]dCTP using a random primer labeling kit (Stratagene, La Jolla, CA).

2.6. Protein preparation and Western blotting

Tissues were homogenized (20 strokes) in 10 volumes of a buffer containing 10 mM Tris pH 7.4, 1 mM EDTA,
0.5 mM DTT and a cocktail of protease inhibitors (1 mM PMSF, 1 μg/ml leupeptin and 0.7 μg/ml of pepstatin) using a motorized Teflon glass homogenizer. Homogenate was briefly centrifuged at 10,000 rpm for 15 min and supernatant was collected. The amount of total protein in each sample was determined by BCA assay using bovine serum albumin (BSA) as the standard. Samples were boiled in Laemmli buffer and resolved on 7.5% Tris–glycine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Proteins were transferred to nitrocellulose membranes using a semi-dry transfer apparatus. Membranes were blocked overnight with 5% dried non-fat milk followed by a 2 h incubation in 1:250 dilution of polyclonal primary antibody against 4F5 (which is specific for 4Fs but does not distinguish among the isoforms). Membranes were then washed and incubated at room temperature with HRP-conjugated secondary antibody for 1 h. Immunoreactivity was detected using a HRP chemiluminescence system (Pierce, Rockford, IL).

2.7. Experimental brain injury model

The model for injury was that of a controlled cortical impact which is the most widely used brain trauma model [24,25]. Briefly, the rats were anesthetized with a 4% isoflurane in a 2:1 N2O/O2 mixture. All protocols were conducted in compliance with guidelines set forth in the NIH Guide for the Care and use of Laboratory Animals and approved by The University of Texas Health Science Center’s Institutional Animal Care and Use Committee. Following endotracheal intubation, rats were mechanically ventilated with 2% isoflurane in a 2:1 N2O/O2 mixture. Animals were mounted in a frame with the ventral surface downward. Bilateral craniotomies were performed midway between the bregma and the lambda with the edges of craniotomies 1 mm lateral to the midline, one at the site of impact, the other contralateral to the impact site. Cortically impacted rats received a single, unilateral impact at 6 m/s, 2.5 mm deformation. Two controls were employed. One is the contralateral hemisphere. The other is the brain of a sham-operated but unimpacted rat. Histopathological assessments were conducted to define extent of injury [24]. Core body temperature was monitored continuously by a rectal thermistor probe and maintained at 37–38 °C. After injury, the scalp was sutured and closed and the animal extubated to minimize anesthetic effects and to allow rapid recovery. The impact apparatus for controlled cortical impact injury [24] consists of a small (1.975 cm) bore, double-acting stroke-constrained, pneumatic cylinder with a 5.0 cm stroke. The cylinder is rigidly mounted perpendicular to the cortex on a cross bar. The lower rod end has an impactor tip attached (i.e. that part of the shaft which actually contacts the exposed dura mater). The upper rod end is attached to the transducer core of a linear variable differential transformer (LVDT). The velocity of the impactor shaft is controlled by gas pressure. Impact velocity is directly measured by the LVDT (Shaevitz model 500 HR), which produces an analog signal that is recorded by a PC-based data acquisition (R.C. Electronics) for analysis of time and displacement parameters of the impactor.

3. Results

3.1. Effects of LPS treatment of rats on CYP 4F4 and CYP 4F5 expression in liver

As shown in Fig. 1, both the leukotriene and prostaglandin signals of the inflammatory cascade arise from arachidonic acid through the actions of lipoxygenase and cyclooxygenase, respectively. These two prompts are hydroxylated to

![Metabolism flow chart](image)

**Fig. 1.** Metabolism flow chart for conversion of arachidonic acid to prostaglandins and leukotrienes and further metabolism.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP 4F4</th>
<th>CYP 4F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 10</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>LPS</td>
<td>50 ± 2</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>BaSO4</td>
<td>60 ± 3</td>
<td>70 ± 5</td>
</tr>
</tbody>
</table>

Data represent the mean ± standard error of four or five individual rats in each treatment group wherein 15 μg total RNA was resolved on a 1.3% denaturing agarose gel, transferred to nitrocellulose membrane and hybridized with 32P-labelled CYP 4F4 or CYP 4F5 probes.
products inactive in the promotion of the inflammatory cascade by members of the cytochrome P450 4F subfamily and CYP 2D18. Injury or infection leading to an inflammatory response involves the increase in leukotriene and prostaglandin prompts of the inflammatory cascade. The processes leading to an increase in concentration of leukotriene and prostaglandin prompts would include both an increase in synthesis through lipoxygenase and cyclooxygenase activity and concentration, as well as a possible diminution in degradation of the prompts. In order to test the hypothesis that injury or infection alters CYP 4F formation, we utilized LPS and BaSO4 treatment as models of inflammation. LPS injection simulates aspects of bacterial infection, whereas BaSO4 is a physical irritant. As shown in Table 1, maximum suppression of CYP 4F4 expression (50% of control levels) occurred at LPS concentrations between 1 and 10 ng/ml medium. The CYP 4F5 suppression pattern was similar. By comparison, suppression of CYP 2C11 expression occurred at 0.01 ng/ml of LPS, a 100-fold lower concentration than that required for suppression of CYP 4F4 or CYP 4F5 expression.

3.3. Effects of traumatic brain injury on expression of CYP 4F4 and CYP 4F5 in brain

LPS and BaSO4 injection serve as a useful and well-documented model for inflammation. By its very nature, intraperitoneal injection of any substance including compounds triggering inflammation lead to systemic responses. In order to utilize a model for inflammation whose genesis is local rather than systemic, we have turned to traumatic brain injury as a more focal trigger for the inflammatory cascade and its effects on the expression of CYP 4F4 and CYP 4F5 in brain. The expression of CYP 4F4 and CYP 4F5 in brain following phenobarbital or clofibrate injection

### Table 2

<table>
<thead>
<tr>
<th>LPS (µg/ml)</th>
<th>CYP 4F4</th>
<th>CYP 4F5</th>
<th>CYP 2C11</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 2</td>
<td>100 ± 5</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>0.01</td>
<td>95 ± 4</td>
<td>105 ± 4</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>0.1</td>
<td>94 ± 4</td>
<td>100 ± 4</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>1.0</td>
<td>48 ± 4</td>
<td>54 ± 4</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>10.0</td>
<td>52 ± 2</td>
<td>55 ± 2</td>
<td>18 ± 2</td>
</tr>
</tbody>
</table>

Hepatocytes were cultured on Matrigel for 5 days and treated with LPS for 24 h. RNA harvested from the cells was resolved by Northern blot technique, quantitized using PhosphoImager and normalized to the β-actin content of each sample. Data represent mean ± standard error of five culture plates for each treatment group.

In order to determine whether the effects of LPS-suppression of CYP 4F4 and CYP 4F5 expression were direct effects on hepatic cells or required participation of other cells or cellular products, we examined the response of rat hepatocytes maintained in culture for 5 days to a 24-h exposure to LPS. A five-day culture period resulted in a stable level of expression of cytochrome P450 which could then be used to examine the effects of LPS concentration on cytochrome P450 expression. As shown in Table 2, maximum suppression of CYP 4F4 expression (50% of control levels) occurred at LPS concentrations between 1 and 10 ng/ml medium. The CYP 4F5 suppression pattern was similar. By comparison, suppression of CYP 2C11 expression occurred at 0.01 ng/ml of LPS, a 100-fold lower concentration than that required for suppression of CYP 4F4 or CYP 4F5 expression.

**Fig. 2. Time course of changes in CYP 4F4 and CYP 4F5 expression in the hippocampus following traumatic brain injury.** Rats were treated as described in Materials and methods. Total RNA (20 µg) was resolved on a 1.3% denaturing agarose gel, transferred to a nitrocellulose membrane, analyzed by semi-quantitative Northern blot analysis and compared to sham-treated controls.
was shown earlier by Kawashima et al. [7]. In the use of TBI, extensive efforts were made to control all variables except the injury itself and, therefore, effects on expression are measured over time against a sham-operated but uninjured control. As shown in initial experiments in Fig. 2, both CYP 4F4 and CYP 4F5 decline from an elevated level of expression in the hippocampus at 1 h post-injury to a low at 24 h post-injury. The expression of both forms increases thereafter to reach elevated levels at 3 days and 2 weeks after injury. CYP 4F4 is 25% above sham-operated at 2 weeks post-injury compared to 20% below sham at 24 h post-injury. CYP 4F5 reaches an elevation of 50% above sham at 2 weeks post-injury. A similar pattern of fall and rise occurs in frontal cortex, though the pattern of the two forms do not seem to follow one another as closely as in the hippocampus (data not shown).

3.4. Effects of traumatic brain injury on expression of P450 4F protein expression

While the data of Fig. 2 show that mRNA expression is affected by brain injury in a time-after-injury fashion, it is important to examine the effects of injury on P450 4F protein. As shown in the Western blot data of Fig. 3, CYP P450 4F protein in the hippocampus declines dramatically 24 h post-injury in comparison with sham-operated controls, consistent with what is observed in the mRNA expression studies.

4. Discussion

The ability of LPS and BaSO₄ to suppress CYP 4F4 and CYP 4F5 expression appears qualitatively similar although the response to LPS is quantitatively greater. There are interesting differences in the possible mechanism. BaSO₄ is believed to work by causing a sterile peritonitis after ingestion of this particulate irritant by macrophages [31] or by Kupffer cells which encounter the BaSO₄ particles through blood circulation [32]. The BaSO₄ effects seem macrophage or Kupffer cell-mediated, whereas LPS may directly affect P450 as evidenced by effects in hepatocytes culture. Thus, the mechanism through which LPS and BaSO₄ operate may be different or may share certain mediators such as the cytokines to a greater or lesser degree.

Of interest in this light is the evidence that the effects on CYP 4F4 and CYP 4F5 expression seen after in vivo treatment of rats with LPS were also seen in hepatocyte culture. This has been observed by others who showed that CYP 1A1, 1A2, 2C, 2F1 and 3A in human hepatocytes were repressed by cytokine treatment and CYP 2C11 and CYP 2B in rat hepatocytes were also repressed by cytokine treatment [33–37]. It is possible that these direct effects may be receptor-mediated, a notion supported by demonstrations that the LPS receptor CD14 is expressed on hepatocytes and is up-regulated by LPS and cytokines [38,39]. Further, the greater sensitivity of CYP 2C11 to LPS may suggest different or additional regulatory modalities control CYP 4F expression and CYP 2C11 expression.

TBI initiates complex biochemical cascades, some of which induce the expression of genes whose protein products are thought to contribute to production of pro-inflammatory eicosanoids including leukotrienes and prostaglandins. Cytochrome P450 4Fs catalyze ω-hydroxylation of LTB₄, PGE₁ and PGA₁ thereby inactivating these inflammatory mediators. Our initial studies of TBI as a prompt for the inflammatory cascade show a clear decrease in hippocampal mRNA expression of CYP 4F4 and CYP 4F5, which is confirmed by Western blot analysis of P450 proteins with greatest reduction occurring 24 h after injury. This effect corresponds with the occurrence of maximum effects on cyclooxygenase −2 expression at 24 h after injury. The effect on cyclooxygenase 2, however, is a stimulation of expression [40]; the mRNA expression increases five-fold 24 h after injury. An increase in cyclooxygenase expression generating inflammatory cascade prompts at the time of a decrease in CYP 4F forms which decrease the activity of those prompts for the inflammatory cascade is consistent with the notion of coordinated reciprocal effects on both the synthesis and degradation pathways for inflammatory cascade prompts (see Fig. 1).

While these results fit within a reasonable frame for the response of the inflammatory cascade to injury or infection, continuing investigations to deconvolute mechanism of initiation of the response and the manner of regulation of cytochromes of P450 are required. Although traumatic brain injury meets the criterion of not requiring an injection of a
chemical prompt of inflammation, it is not clear that the response is purely local. For instance, it must be determined whether what is seen in the hippocampus is reflected temporarily in other parts of the brain as well as on the contralateral side. Further, others have reported that brain injury has effects on the expression and activities of cytochromes P450 in liver [41]. While the time frame of these effects remains to be elucidated, it seems premature to conclude that TBI induces purely local effects. These issues are now being addressed.

Acknowledgements

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References


