Differential Effects of Traumatic Brain Injury on the Cytochrome P450 System: A Perspective into Hepatic and Renal Drug Metabolism

AUINASH KALSOTRA,1 CHERI M. TURMAN,1 PRAMOD K. DASH,2 and HENRY W. STROBEL1

ABSTRACT

Traumatic brain injury is known to cause several secondary effects, one of which is altered drug clearance. Given the fact that patients who sustain TBI are subsequently treated with a variety of pharmacological agents for the purpose of either neuroprotection or physiological support, it is imperative to clarify changes in expression and/or activities of enzymes involved in clearing drugs. The mixed function oxidase system, which consists of cytochrome P450 and cytochrome P450 reductase, plays a vital role in phase I drug metabolism. This paper addresses the issue as to what extent TBI affects the levels and activity of various rat CYP450 subfamilies. Our results show that TBI induces tissue-specific and time-dependent alterations. Total hepatic CYP450 content showed a biphasic response with a decrease seen at 24 h followed by an increase at 2 weeks. CYP450 reductase, in contrast, showed an opposite temporal profile. Immunoblot analyses and marker substrate metabolism demonstrated a clear decrease in hepatic CYP1A levels while a significant increase in kidney was seen at both 24 h and 2 weeks. A dramatic induction of CYP3A was evident at 2 weeks in liver, while no changes were noticed in CYP2B or CYP2D subfamilies. CYP4F subfamily showed induction in kidney only. Collectively, the data reveal the differential effects of TBI on hepatic and renal drug metabolism.

Key words: CYP450 drug substrates; inflammation and injury; leukotriene B₄; tissue-specific response

INTRODUCTION

TRAIUMATIC BRAIN INJURY (TBI), especially closed head injury resulting from hemorrhage, blunt trauma or traffic accidents, has become an increasing problem in medical care and as a result a focus of study (Marion, 1999). TBI often results in a variety of complications affecting neurological, psychological, cardiovascular, pulmonary, vascular and metabolic functions (Clifton et al., 1983; Fisher et al., 1999; McIntosh et al., 1999). More importantly, trauma to the brain leads to a myriad of pathophysiologic processes and therapeutic interventions both of which influence the expression of drug metabolizing enzymes (Boucher et al., 1991; Toler et al., 1993; Mckindley et al., 1997). For instance, antipyrine, pentobarbital and phenytoin are all metabolized faster in brain injured patients when compared to healthy volunteers (Heinemeyer et al., 1986; Boucher et al., 1991; O’mara

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et al., 1995). This suggests that TBI can prompt serious alterations of hepatic and extrahepatic metabolism of various drugs that are routinely given to trauma patients.

Cytochrome P450s (CYP450s), Phase I drug metabolizing enzymes, comprise a superfamily of heme-thiolate proteins that play critical roles in endogenous as well as xenobiotic metabolism (Guengerich, 1991; Anzenbacher and Anzenbacherova, 2001; Nebert and Russell, 2002). The term “Cytochrome P450” originates from the observation that upon binding of carbon monoxide and subsequent reduction, these enzymes exhibit an absorbance maximum at 450 nm (Omura and Sato, 1964). The CYP450-dependent drug metabolism system contains two components, namely CYP450 and NADPH CYP450 reductase. The reductase transfers two electrons to the CYP450 supplying reducing equivalents to the heme iron for reduction of molecular oxygen and product formation during catalysis (Strobel et al., 1995). CYP450s collectively are highly expressed in liver followed by kidney, small intestine, brain and lung (Guengerich and Mason, 1979; Strobel et al., 1997). Currently, >700 CYP450s have been characterized, inclusive of the many different species of organisms that have been studied (Nelson, 1999). CYP450 proteins are conveniently arranged into families and subfamilies on the basis of percent amino acid sequence identity (Nebert and Gonzalez, 1987). Taken together CYP families 1–4 account for more than 99% of all drug metabolism in mammals.

In this study, we have focused on the subfamilies CYP3A, 2D, 1A, and 2B which account for >50%, 30%, 5%, and <1%, respectively, of all human drug metabolism of clinical significance. We have also investigated the CYP4F subfamily, which plays an important role in metabolizing inflammatory mediators in the arachidonic acid cascade (Kikuta et al., 2002; Cui et al., 2003). A list of these subfamilies with their endogenous and drug substrates relevant after brain trauma is compiled in Table 1.

The drugs shown in the table represent important classes such as analgesics, antibiotics, anti-epileptics, anti-depressants, anti-inflammatory, anti-hypertensives, anti-psychotics, anxiolytics, cognitive enhancers, neuro-protectives, thrombolytics and sedatives, which comprise a large portion of the regimen of the pharmacological treatments offered post trauma (Sabel and Stein, 1986; Kraus, 1999).

Of course, if any of the major CYP450 isoforms responsible for metabolizing these drugs change in response to injury, it will cause either lowered efficacy of the drug in the case where the parent drug administered is active, or increased adverse effects when the metabolite formed is toxic. This warrants an active investigation of CYP450 expression and activities post trauma since a variety of drugs are administered to these patients in emergency care as well as during the subsequent recovery programs.

The TBI model in rat closely simulates contusion head injury in humans who have been victims of traffic accidents or have received blunt trauma (Dixon et al., 1991). Few studies have been conducted demonstrating the alterations of CYP450 system after TBI (Toler et al., 1993; Cui et al., 2003). Recently, Poloyac et al. (2001) have reported tissue specific alterations in chlorozoxazone metabolism after brain injury. They observed that injury leads to decreased hepatic CYP2E1 levels and its associated chlorozoxazone metabolizing activity while an opposite response was seen in kidney. In this study, using immunoblot analysis and marker substrate metabolism for specific subfamilies, we have determined the effects of TBI on rat hepatic and renal CYP450s involved in phase I drug metabolism.

**MATERIALS AND METHODS**

**Materials**

Male Long-Evans rats (250g body wt) were purchased from Harlan Laboratories (Indianapolis, IN) for use in this study. The polyclonal rat CYP450 antibodies for immunoblot analyses were Anti-CYP1A1 and Anti-CYP3A2 (Research Diagnostics Inc., Flanders, NJ), Anti-CYP2B2 and Anti-CYP4F5 made in-house (Geng and Strobel, 1998; Kalsotra et al., 2002) and Anti-CYP2D1 (Chemicon International, Temecula, CA). Cytochrome C, erythromycin, NADPH and sodium dithionite were obtained from Sigma chemicals (St. Louis, MO). Imipramine HCL was purchased from Research Biochemicals International (Natrick, MA). Benzphetamine HCL was a gift from The Upjohn Company (Kalamazoo, MI). LTBA, 20-OH LTBA and PGB1 were purchased from Cayman Chemicals (Ann Arbor, MI). AMMC (3-[2-(N,N-diethyl-N-methyl- ammonium)ethyl]-7-methoxy-4-methyl-coumarin) and AHMC (3-[2-(N,N-diethyl-N-methyl-ammonium)ethyl]-7-hydroxy-4-methyl-coumarin), were obtained from Gentest (Woburn, MA). CEC (3-cyano-7-ethoxycoumarin) and CHC (3-cyano-7-hydroxycoumarin) were purchased from Molecular Probes Inc. (Eugene, OR). All other reagents used were of analytical grade or higher.

**Experimental Brain Injury Model**

All protocols were in compliance with NIH’s Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee. A controlled cortical impact device was used to cause brain injury as previously described (Dash et al., 2002). Rats were anesthetized using 4% isoflurane with a 2:1 N2O:O2 mixture and then maintained with a 2% isoflurane/2:1 N2O:O2 mixture via a face mask. Animals were mounted in the injury device stereotaxic frame secured by ear bars.
and an incisor bar. The head was held in a horizontal plane with respect to the interaural line. A midline incision was made, the soft tissues reflected, and bilateral 6-mm craniectomies were made midway between the bregma and lambda with the medial edge of each craniectomy 1 mm lateral to midline. Cortically impacted rats received a single impact at 2-mm deformation with an impact velocity of 6 m/sec at an angle of 30° from the vertical plane. Core body temperature was maintained at 37–38°C by use of a rectal thermometer coupled to a heating pad. Sham rats were anesthetized and received craniectomies but were not cortically impacted. After injury or sham operation, the scalp was sutured and the animal placed in an oxygenated chamber for monitoring during recovery.

**Microsome Preparation**

At 24 h and 2 weeks following injury or sham operation, animals were sacrificed by decapitation. The livers and kidneys were excised and microsomes prepared as previously described (Kalsotra et al., 2002). The total microsomal proteins in each sample were determined by BCA assay using bovine serum albumin (BSA) as the standard.

**CYP450 Spectral Analysis**

Total cytochrome P450 content in different samples was measured by CO difference spectroscopy as described previously (Omura and Sato, 1964). The difference in absorbance at 450 nm relative to that of 490 nm, Beer’s law, and extinction coefficient of 91 mM⁻¹ cm⁻¹ were used to calculate total CYP450 content.

**Immunoblot Analysis**

Microsomal samples were boiled in Laemmli buffer and resolved on 4–15% Tris-glycine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (Laemmli, 1970). Proteins were transferred to nitrocellulose membranes using a semi-dry transfer apparatus. Membranes were blocked overnight with 5% dried non-fat milk followed by an overnight incubation in anti-rat polyclonal primary antibodies against CYP1A1 (1:1000),

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**TABLE 1. ENDOGENOUS AND XENOBIOTIC SUBSTRATES FOR CYTOCHROMES P450 SUBFAMILIES INVESTIGATED IN THIS STUDY**

<table>
<thead>
<tr>
<th>P450 subfamily</th>
<th>Endogenous substrate</th>
<th>Xenobiotic substrates (clinically relevant after brain injury)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A</td>
<td>17β-estradiol,</td>
<td>Acetaminophen&lt;sub&gt;ap&lt;/sub&gt;, Aspirin&lt;sup&gt;an,ai&lt;/sup&gt;, Chlorpromazine&lt;sup&gt;ap&lt;/sup&gt;, Chlorozoxazone&lt;sup&gt;mr&lt;/sup&gt;, Clozapine&lt;sup&gt;ap&lt;/sup&gt;, Haloperidol&lt;sup&gt;ap&lt;/sup&gt;, Huperzine&lt;sup&gt;cs&lt;/sup&gt;, Naproxen&lt;sup&gt;ai&lt;/sup&gt;, Phenacetin&lt;sup&gt;t&lt;/sup&gt;, Riluzole&lt;sup&gt;ph&lt;/sup&gt;, Tacrine&lt;sup&gt;cs&lt;/sup&gt;, Verapamil&lt;sup&gt;ph&lt;/sup&gt;, Warfarin&lt;sup&gt;ai&lt;/sup&gt;, Zotepine&lt;sup&gt;ap&lt;/sup&gt;, Zoxazolamine&lt;sup&gt;mr&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Prostaglandins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td></td>
</tr>
<tr>
<td>CYP2B</td>
<td>Androstenedione,</td>
<td>Benzphetamine&lt;sup&gt;an&lt;/sup&gt;, Bupropion&lt;sup&gt;ad&lt;/sup&gt;, Diazepam&lt;sup&gt;an&lt;/sup&gt;, Mianserin&lt;sup&gt;ce&lt;/sup&gt;, Phenylbutazone&lt;sup&gt;an,ai&lt;/sup&gt;, Phenytoin&lt;sup&gt;ce&lt;/sup&gt;, Valproic acid&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td></td>
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<tr>
<td>CYP2D</td>
<td>Dopamine,</td>
<td>Amitriptyline&lt;sup&gt;ad&lt;/sup&gt;, Chlorpromazine&lt;sup&gt;ap&lt;/sup&gt;, Citalopram&lt;sup&gt;ad&lt;/sup&gt;, Clomipramine&lt;sup&gt;ad&lt;/sup&gt;, Clozapine&lt;sup&gt;ap&lt;/sup&gt;, Codeine&lt;sup&gt;an&lt;/sup&gt;, Dextromethorphan&lt;sup&gt;ph&lt;/sup&gt;, Fluphenazine&lt;sup&gt;t&lt;/sup&gt;, Fluvoxamine&lt;sup&gt;ad&lt;/sup&gt;, Fluoxetine&lt;sup&gt;ad&lt;/sup&gt;, Galantamine&lt;sup&gt;cs&lt;/sup&gt;, Haloperidol&lt;sup&gt;ap&lt;/sup&gt;, Imipramine&lt;sup&gt;ad&lt;/sup&gt;, Mianserin&lt;sup&gt;ce&lt;/sup&gt;, Ondansetron&lt;sup&gt;ad&lt;/sup&gt;, Paroxetine&lt;sup&gt;ad,ax&lt;/sup&gt;, Propranolol&lt;sup&gt;ph&lt;/sup&gt;, Risperidone&lt;sup&gt;ap&lt;/sup&gt;, Sertindole&lt;sup&gt;ap&lt;/sup&gt;, Tranadol&lt;sup&gt;ph&lt;/sup&gt;, Venlafaxine&lt;sup&gt;ap&lt;/sup&gt;, Zotepine&lt;sup&gt;ap&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5-methoxytryptamine</td>
<td></td>
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<tr>
<td></td>
<td>Tyramine</td>
<td></td>
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<tr>
<td>CYP3A</td>
<td>Bile acids,</td>
<td>Acetaminophen&lt;sup&gt;an,ai&lt;/sup&gt;, Alprazolam&lt;sup&gt;ax&lt;/sup&gt;, Amitriptyline&lt;sup&gt;ad&lt;/sup&gt;, Aspirin&lt;sup&gt;an,ai&lt;/sup&gt;, Bromocriptine&lt;sup&gt;ak&lt;/sup&gt;, Buprenorphine&lt;sup&gt;an&lt;/sup&gt;, Buspiron&lt;sup&gt;ad,ax&lt;/sup&gt;, Carbamazepine&lt;sup&gt;ac&lt;/sup&gt;, Citalopram&lt;sup&gt;ad&lt;/sup&gt;, Clarithromycin&lt;sup&gt;ab&lt;/sup&gt;, Clozapine&lt;sup&gt;ap&lt;/sup&gt;, Cyclcobenzaprine&lt;sup&gt;an&lt;/sup&gt;, Cyclosporine&lt;sup&gt;A,ab&lt;/sup&gt;, Dexamethasone&lt;sup&gt;ac,cs&lt;/sup&gt;, Diazepam&lt;sup&gt;at&lt;/sup&gt;, Diltiazem&lt;sup&gt;ah&lt;/sup&gt;, Donepezil&lt;sup&gt;fs&lt;/sup&gt;, Erythromycin&lt;sup&gt;ab&lt;/sup&gt;, Ethylmorphine&lt;sup&gt;an&lt;/sup&gt;, Haloperidol&lt;sup&gt;ap&lt;/sup&gt;, Imipramine&lt;sup&gt;ad&lt;/sup&gt;, Meloxicam&lt;sup&gt;ad&lt;/sup&gt;, Midazolam&lt;sup&gt;fs&lt;/sup&gt;, Nifedipine&lt;sup&gt;ap&lt;/sup&gt;, Ondansetron&lt;sup&gt;ad&lt;/sup&gt;, Prednisone&lt;sup&gt;cs,ad,cs&lt;/sup&gt;, Sertraline&lt;sup&gt;ad&lt;/sup&gt;, Trazodone&lt;sup&gt;ad&lt;/sup&gt;, Verapamil&lt;sup&gt;ph&lt;/sup&gt;, Warfarin&lt;sup&gt;at&lt;/sup&gt;, Zolpidem&lt;sup&gt;at&lt;/sup&gt;, Zotepine&lt;sup&gt;ap&lt;/sup&gt;, Zoxazolamin&lt;sup&gt;mr&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Androstenedione</td>
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<td></td>
<td>Cortisol</td>
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<tr>
<td></td>
<td>Dehydroepiandrosterone</td>
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<tr>
<td></td>
<td>17β-estradiol</td>
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<td></td>
<td>Progestrone,</td>
<td></td>
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<tr>
<td></td>
<td>Testosterone</td>
<td></td>
</tr>
<tr>
<td>CYP4F</td>
<td>Leukotriene B&lt;sub&gt;4&lt;/sub&gt;,</td>
<td></td>
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<tr>
<td></td>
<td>Prostaglandins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid</td>
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</table>

The information in this table has been adapted largely from Anzenbacher and Anzenbacherova, 2001; Nebert et al., 2002; Omiecinski et al., 1999; Rendic et al., 1997; and Zubler et al., 2002. The superscripted letters represent the therapeutic class for the drugs. ab, antibiotic; ac, anti-convulsant; ad, anti-depressant; ae, anti-emetic; ah, anti-hypertensive; ai, anti-inflammatory; an, analgesic; ao, anti-obesity; ap, anti-psychotic; ak, anti-parkinsonian; at, anti-tussive; ax, anxiolytic; cs, cognitive enhancer; cs, corticosteroid; mr, muscle relaxant; np, neuroprotective; s, sedative; t, thrombolytic.
CYP2B2 (1:500), CYP2D1 (1:1000), CYP3A2 (1:1000), and CYP4F5 at 1:250 dilutions. Membranes were then washed and incubated at room temperature with a 1:1000 dilution of anti-rabbit HRP-conjugated secondary antibody (BioRad, Hercules, CA) for 2 h. Immunoreactivity was detected using an HRP chemiluminescence system (Pierce Rockford, IL) and quantified using Molecular Imager FX (BioRad, Hercules, CA).

Activity Assays

**NADPH-CYP450 reductase activity.** The activity was monitored spectrophotometrically at 340 nm following an increase in absorbance with respect to time using a Shimadzu double-beam spectrophotometer (UV-1601 PC). The reaction mixture (1 mL) contained cytochrome C (0.5 mg/mL) in 0.3 M potassium phosphate buffer (pH 7.4), 1 mM EDTA, and 1 mM NADPH. The reaction was initiated by adding 100 μg of microsomal protein and the rate of cytochrome C reduction was calculated using extinction coefficient of 21 mM$^{-1}$ cm$^{-1}$ at 340 nm (Dignam and Strobel, 1977). Reactions were monitored for a period of 60 sec at 25°C.

**CYP1A mediated O-deethylase activity.** The O-deethylase assay for CEC (marker substrate for CYP1A subfamily) was performed following the method described previously with minor modifications (Crespi et al., 1997). The reaction was carried out in 100 mM potassium phosphate buffer (pH 7.4) with final concentrations of 1 mM NADPH, 40 μM CEC, and 0.5–1 mg of microsomal proteins. The relative fluorescence of the product, CHC, was measured on a Perkin Elmer LS-5 Fluorometer with an excitation of 420 nm and emission at 485 nm. Reactions were done at 25°C and monitored for 30 min. The CHC standard curve was prepared with concentrations ranging from 5 to 500 nM to calculate the product formation.

**CYP2B, CYP3A, and multiple CYP mediated N-demethylase activities.** The N-demethylation assays for benzphetamine (marker substrate for CYP2B subfamily), erythromycin (marker substrate for CYP3A subfamily) and imipramine (a substrate for multiple CYP subfamilies) were performed in a volume of 2 mL with 100 mM potassium phosphate buffer (pH 7.4), containing 0.3–0.5 mg of microsomal proteins, and 1 mM substrate. The reaction was initiated by the addition of 1mM NADPH, incubated at 37°C for 10 min and terminated by addition of 600 μL 17.5% perchloric acid. After a brief centrifugation, the supernatant was collected and reacted with 0.2 mL of Nash’s reagent to form diacetyldihydrolutidine as a fluorescent product. The fluorometric measurement EX$_{425}$ and EM$_{515}$ of the product formation was carried out using a standard curve using known amounts of formaldehyde (Lu et al., 1970; Wang and Strobel, 1997).

**CYP2D mediated O-demethylase activity.** The O-demethylase assay for AMMC (marker substrate for CYP2D subfamily) was performed in 100 mM potassium phosphate buffer (pH 7.4) having final concentrations of 1 mM NADPH, 8 μM AMMC, and 0.5–1 mg of hepatic microsomal proteins in 1-mL total volume with reactions carried out at 25°C for 15 min. The relative fluorescence of the product was measured using Perkin Elmer LS-5 Fluorometer with an excitation of 390 nm and emission at 460 nm. The AHMC standard curve was prepared with concentrations ranging from 0.025 to 5 μM and used to calculate product formation.

**CYP4F-mediated LTB$_4$ ω-hydroxylase activity.** The hydroxylation of LTB$_4$ was measured using an high performance liquid chromatography (HPLC) method as described earlier (Kawashima et al., 1997). The reaction mixture containing 340 mM sucrose, 20 mM Hepes buffer (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, 1 mM NADPH, 70 μM LTB$_4$ and 0.3–0.5 mg of microsomal proteins in a total volume of 0.2 mL, was incubated at 37°C for 20 min. The reaction products were extracted with ethyl acetate, dried gently under N$_2$ gas passed over a C$_{18}$ column and measured with an UV detector set at 270 nm. PGB$_1$ served as an extraction control. The quantity of newly formed 20-OH LTB$_4$ was determined from the peak area read from the standard curve (25 nM–1 μM) prepared for the metabolite.

**Statistical Analysis**

Data are presented as mean ± SEM from at least three independent sets of experiments for four animals in each group. Statistical significance for effects of brain injury on CYP450 expression and activities was determined using a two-tailed unpaired Student’s t-test. Multiple groups were compared using one-way ANOVA followed by Tukey’s multiple range test. Statistical differences were considered significant if $p < 0.05$.

**RESULTS**

**TBI Results in Decrease of Hepatic and Renal P450 Content at 24 h Followed by an Increase at 2 Weeks**

The spectral analysis of total CYP450s in liver microsomes revealed a 41% decrease at 24 h but a 78% increase 2 weeks after injury when compared to shams (Fig. 1). In kidney, we observed a 42% decrease in P450 content at 24 h followed by a 17% increase at 2 weeks compared to shams (Fig. 1).
Immunoblot Analysis Reveals a Tissue- and Subfamily-Specific CYP450 Response

CYP1A. The Liver results presented in Figure 2a demonstrate a 54% decrease in CYP1A immunoreactivity at 24 h and a 71% decrease at 2 weeks in comparison to shams. In contrast to liver, CYP1A levels in kidney showed an increase of 24% at 24 h post injury. Two weeks after injury, a threefold increase in CYP1A immunoreactivity was observed in kidney microsomes (Fig. 2A). Thus a complete opposite response was evident in liver and kidney CYP1A levels after brain injury.

CYP2B. No significant changes in CYP2B immunoreactivity were seen in either liver or kidney at 24 h and 2 week time points after injury (Fig. 2B).

CYP2D. There was no significant change in CYP2D immunoreactivity in liver in response to injury; however, a 68% decline in kidney was noted at the 2 week time point (Fig. 2C).

CYP3A. The CYP3As showed the maximal response among all the subfamilies studied. While no change was seen at 24 h in liver, a five-fold induction was observed at the 2-week time point. On the contrary, renal CYP3A levels were unaffected during the course of study (Fig. 2D).

CYP4F. There were no alterations noted in the quantity of liver CYP4F levels during injury response. However, a substantial increase was seen in kidney both 24 h (twofold) and 2 weeks (2.4-fold) following TBI (Fig. 2E).

Effects of TBI on CYP450-Mediated Metabolism Reveals a Tissue- and Subfamily-Specific Response

This study was intended to investigate the alterations in CYP450 mediated metabolism after TBI. CYP450s are known to carry out a myriad of chemical reactions including hydroxylations, epoxidations, N-, S-, and O-dealkylations, N-oxidations, sulfoxidations, dehalogenations and others (Guengerich, 1991). In order to assess subfamily specific activity, we chose marker substrates for each subfamily. Figure 3 shows the chemical structures of the substrates and products used, with specific sites for metabolism and the types of reactions carried out.

CYP450 reductase activity. In order to test the effects of trauma on the activity of CYP450 reductase, we did a 60-sec kinetic analysis of cytochrome C reduction by the hepatic and renal microsomes with NADPH serving as the electron donor. Our results indicate a 37% increase in hepatic reductase activity at 24 h compared to shams (Table 2). At 2 weeks after injury the hepatic activity remained 20% higher than shams (Table 2). Conversely, in the kidney microsomes, the activity recorded at both 24 h and 2 weeks was lower when compared to shams (Table 3).

CYP1A activity. The data presented in Table 2 show that TBI caused a decline in CYP1A catalyzed CEC O-deethylation in liver at 24 h and 2 weeks, a result parallel to that observed in immunoblots. CYP1A activity was reduced by 35% at 24 h and 69% at the 2-week interval. Kidney microsomes showed a slight increase at 2 weeks when compared to their sham counterparts and no major changes were noticed at the 24-h interval (Table 3).

CYP2B activity. The results in Tables 2 and 3 demonstrate that TBI did not bring about any appreciable changes in CYP2B dependent benzphetamine N-demethylation activity in either organ studied. These observations are in accord with the unchanging CYP2B immunoreactivity seen in the Western blots.

CYP2D activity. In parallel to immunoblot results, the formation of the O-demethylated AMMC in liver, a characteristic CYP2D mediated reaction, remained unaltered throughout the course of the study. No activity could be detected in kidney microsomes using a threshold value of 1.5 signal to noise ratio. Therefore, the limit of detection for this fluorometric assay was 0.33 pmol/min/mg.
FIG. 2. Immunoblot analyses of specific CYP450 subfamilies after TBI. 50 μg of microsomal proteins from each sample ($n = 4$) were separated electrophoretically and then transferred on to nitrocellulose membrane. Membranes were blotted and analyzed using specific antibodies as described in Materials and Methods. Ponceau stained membranes are shown as loading controls. 1 = sham, 2 = post-injury 24 h, 3 = post-injury 2 weeks. Bar graphs represent the relative quantitation of the data obtained from three independent sets of experiments. Data are expressed as Mean ± SEM. Statistical analysis was done using one-way ANOVA and differences were considered significant if $p < 0.05$. aSignificant difference from sham group. bSignificant difference from post-injury 24 h group. (A) CYP1A. (B) CYP2B. (C) CYP2D. (D) CYP3A. (E) CYP4F.
**CYP3A activity.** From Tables 2 and 3, it is evident that TBI caused a steady increase in both hepatic and renal erythromycin metabolism over time. In liver microsomes, activity went up by 27% and 91% at 24 h and 2 weeks, respectively. While at 24 h the formation of N-demethylated product by the renal microsomes did not change much, a 54% increase was observed 2 weeks post injury.

**CYP4F activity.** Similar to the western blot data in liver, we did not observe any marked alterations in CYP4F specific LTB$_4$ $\omega$-hydroxylase activity. TBI on the other hand, caused a modest increase in LTB$_4$ metabolism by the kidney microsomes. Increase in renal CYP4F protein levels in response to injury were however much more significant.

**Imipramine N-demethylation activity.** Multiple CYP450s have been reported to metabolize imipramine. Therefore, to examine the effects of injury on a common substrate for several P450 subfamilies, imipramine was
chosen. Results from Table 2 show variable metabolic rates for conversion of imipramine to its N-demethylated product in liver with increases noted over time. The product formation was elevated by 2.5- and 2.8-fold at 24 h and 2 weeks, respectively. Kidney microsomes did not exhibit any dramatic changes; however, a downward trend in activity was evident over time (Table 3).

### Table 2. CYP450 Subfamily and P450 Reductase Activities in Liver Microsomes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity</th>
<th>Sham</th>
<th>24-h post-injury</th>
<th>2-week post-injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>P450 reductase</td>
<td>Cytochrome C reduction (nmol/min/mg protein)</td>
<td>77.6 ± 1.65</td>
<td>106.3 ± 1.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.3 ± 3.37&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP1A</td>
<td>CEC O-deethylation (pmol/min/mg protein)</td>
<td>145 ± 18.8</td>
<td>95.0 ± 13.3</td>
<td>45.6 ± 6.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP2B</td>
<td>Benzphetamine N-demethylation (nmol HCHO/min/mg protein)</td>
<td>10.44 ± 0.69</td>
<td>12.20 ± 0.46</td>
<td>11.09 ± 0.71</td>
</tr>
<tr>
<td>CYP2D</td>
<td>AMMC O-demethylation (nmol/min/mg protein)</td>
<td>11.77 ± 1.09</td>
<td>11.60 ± 1.89</td>
<td>13.60 ± 0.833</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Erythromycin N-demethylation (nmol HCHO/min/mg protein)</td>
<td>0.875 ± 0.007</td>
<td>1.11 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67 ± 0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP4F</td>
<td>LTB4 ω-hydroxylation (pmol/min/mg protein)</td>
<td>32.8 ± 2.45</td>
<td>34.2 ± 3.67</td>
<td>34.6 ± 2.90</td>
</tr>
<tr>
<td>Multiple CYPs</td>
<td>Imipramine N-demethylation (nmol HCHO/min/mg protein)</td>
<td>1.47 ± 0.35</td>
<td>3.74 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.13 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Activity assays were done in triplicates for four animals each. For experimental details, see Materials and Methods. Data are expressed as mean ± SEM. Statistical analysis was done using one-way ANOVA followed by Tukey’s multiple range test and differences were considered significant if \( p < 0.05 \).

<sup>a</sup>Significant difference from sham group.

<sup>b</sup>Significant difference from 24-h post-injury group.

### Discussion

In this study, we show that experimental TBI in rats leads to tissue specific alterations in CYP450 expression and associated metabolic activities. We report that brain trauma results in a significant reduction of liver CYP450 content after 24 h, which rise above basal levels by two...
weeks. Other groups have observed similar down regulation in CYP450 levels after CNS injury and infection (Mckindley et al., 1998; Renton and Nicholson, 2000; Poloyac et al., 2001), but our observation that the CYP450 levels are induced at the 2-week interval is unique. This observation can be explained by the notion that early reduction in CYP450 is due to cytokine effects (Abdel-Razzak et al., 1993) and later increase as a result of protein supplementation (Mckindley et al., 1997). It is widely acknowledged that brain injured patients launch a considerable acute phase response with a concomitant rise in local and systemic cytokines (Mcintosh et al., 1999; Woiciechowsky et al., 2002); therefore, decrease in total CYP450 content at early stages after neurotrauma is not unreasonable. Apart from 450 nm, a peak at 420 nm was also observed. Whether this was a due to a direct effect of injury causing the conversion of catalytically active P450 to inactive P420 or the absorbance of other hemoproteins is not clear at the moment and therefore deserves further investigation.

Also, the study revealed that hepatic and renal CYP450 reductase activities show an opposite trend to each other in response to head injury. Increased activity of reductase in liver might be seen as an effort to reconcile the decrease in total CYP450 content observed at 24 h, since reductase is a common partner for all CYP450 mediated oxidative reactions. A decrease in reductase activity accompanying the corresponding increase in CYP450 levels at the 2-week time point strengthens this notion. The significance of the loss of reductase activity in the kidney is not well understood especially when no apparent changes in total renal CYP450s were visible.

Our results revealed a tissue-specific time-dependent change in CYP1A expression and activity. Down regulation of CYP1A levels and activity in the liver and an up regulation in the kidney implicates a differential response of TBI on the CYP1A subfamily. Such a phenomenon has been previously demonstrated with CYP2E1, where injury to the brain led to decreased chloroxazone metabolism in liver, whereas increased activity was seen in kidney (Poloyac et al., 2001). The mechanism of tissue-specific responses in CYP450 expression upon brain trauma, at the moment, is not clear but its consequence on the subsequent drug metabolism is undoubtedly far reaching.

No significant changes in hepatic or renal CYP2B and hepatic CYP2D subfamily in this study were evident upon injury. Although few reports have demonstrated a decrease in CYP2B and CYP2D levels following inflammatory challenges, considerable differences in the experimental conditions exist. For instance, in the study by Shimamoto et al. (1998), the antibodies used to quantify individual isoforms cross-reacted with multiple CYP450s. In some reports where the authors observed a decrease in CYP2B levels and related activities, phenobarbital was used to induce CYP2B levels before the inflammatory challenge (Abdel-Razzak et al., 1995; Clark et al., 1996). Also, in comparison to our experiments, most of these studies were done on isolated hepatocytes indicating mechanistic differences in in vivo versus in vitro models.

Our results showed that CYP2D subfamily metabolism in kidney was not detected. However, there was expression of CYP2D proteins as evident in the Western blot. This may be due to the selectivity of the representative substrate used. Stresser et al. (2002) have shown the metabolism of AMMC by CYP2D2 with no participation by CYP2D1. There are other isoforms found in rat kidney (CYP2D3, CYP2D4, CYP2D5 and CYP2D18) representing the CYP2D subfamily (Hiroi et al., 1998), which were not a part of the former study (Stresser et al., 2002). It is not known what their level of involvement in AMMC metabolism might be. Therefore, the activity may correlate to CYP2D2 levels alone, while the immunoblot probably represents more of the isoforms in the CYP2D subfamily due to cross-reactivity of the polyclonal anti-CYP2D1 antibody.

A massive induction in hepatic CYP3A levels with related increases in erythromycin metabolism was observed at 2 weeks. The finding holds special significance since the CYP3A subfamily, apart from metabolizing key endogenous substrates, also metabolizes the majority of commercially available drugs (Table 1). These facts lead to the undesired possibility of dosage failure in trauma patients because changes in CYP3A mediated metabolism may lead to extremely elevated or diminished drug levels. Particularly in the case of a drug with a narrow therapeutic window, such modifications may result in serious health risks. The increase in imipramine N-dealkylation seen in this study further strengthens the hypothesis about potential pharmacokinetic alterations in drug metabolism in patients with severe head injuries.

The CYP4F subfamily, which plays an important role in LTβ4 metabolism (Kawashima et al., 1997; Kikuta et al., 2002), was studied to assess the effects of TBI on endogenous metabolic functions of inflammatory origin. LTβ4, a classical neutrophil chemoattractant (Ford-Hutchinson et al., 1980), has been detected at high levels in rat brain after TBI (Xu et al., 1990). Post-traumatic changes are also reported to be marked by the accumulation of polymorphonuclear leukocytes and cerebral edema within 24 h post brain injury (Schoettle et al., 1990). Our laboratory has previously shown that CYP4F expression is reduced in hippocampus at 24 h after injury (Cui et al., 2003). To test whether TBI produces any effects on hepatic or renal CYP4F enzymes, their ex-
pression and activity analysis was carried out. The data indicate that liver CYP4F levels do not alter appreciably; however, a significant up-regulation is evident in the kidney. More recently considerable interest with respect to CYP4F function in the kidney has been generated. The ability of arachidonic acid ω-hydroxylation and formation of a potent vasoconstrictor 20-hydroxyeicosatetraenoic acid, in human kidney (Lasker et al., 2000) suggests an important role for CYP4Fs in renal functions such as regulation of salt/water balance and arterial blood pressure.

Results from this study have demonstrated that TBI has definitive effects on hepatic and renal metabolism-based drug clearance. Analogous alterations in CYP450 related metabolism have been shown with other models of inflammation (Morgan, 1997; Renton, 2001). Many of the effects of these inflammatory prompts on P450 expression are thought to be mediated by cytokines like IL-1, IL-6, TNF-α, and IFN-γ. For instance, the suppressive effects of LPS or BaSO₄ on cytochrome P450 mRNAs can be mimicked by administration of interferon inducers and cytokines such as IL-1, IL-6, and TNF-α, indicating that the suppression of CYP gene products in LPS-induced inflammation are likely mediated by inflammatory cytokines (Morgan, 1991; Morgan et al., 1994; Chen et al., 1995). Also, cytokines can act directly on hepatocytes to cause changes of cytochrome P450 gene expression. The mechanisms of this regulation are not known; however, there is evidence that transcriptional events are likely involved. Chen et al., using a transient expressed reporter system, showed that CYP2C11 promoter sequences are involved in the suppression of 2C11 by IL-1 and IL-6 (Chen et al., 1995). When treated with cytokines such as IL-1, IL-6 or TNF-α, the expressions of CYP2C11 and 2B in rat hepatocytes and 1A1, 1A2, 2C, 3A, and 2E1 in human hepatocytes were repressed (Barker et al., 1992; Abdel-Razzak et al., 1993; Morgan et al., 1994). It is known that brain injury and ischemia are associated with the appearance of different immune mediators in the cerebrospinal fluid and plasma (Ott et al., 1994; Woiciechowsky et al., 2002). Moreover, high cytokine levels in the brain and an elevated intracranial pressure (ICP) can also stimulate neuroimmune pathways like hypothalamic-pituitary-adrenal axis which will aggravate the systemic immune changes resulting from the injury-triggered inflammatory response in the brain (Woiciechowsky et al., 1998).

It has been observed that LPS injection in the brain reduces CYP450 protein levels and activity in both brain and liver (Shimamoto et al., 1998; Renton and Nicholson, 2000). Interestingly, similar doses of LPS given systemically are unable to elicit a reduction in hepatic CYP450s, ruling out the possibility of leakage of LPS from the CNS. This suggests that changes in hepatic metabolism during CNS inflammation are a result of signaling pathways initiated in the brain. Although the TBI model, unlike the LPS model, meets the criterion of not requiring an injection to prompt inflammation, it is not clear whether the response generated is purely local. In fact, recent evidence suggests that TBI leads to broad based tissue specific effects (Fisher et al., 1999; McIntosh et al., 1999; Woiciechowsky et al., 2002). The concept of blood–brain barrier compromise (Chen et al., 1999), role of sympathetic nervous system (Woiciechowsky et al., 1998) and systemic burst of cytokines (Ott et al., 1994) in reinforcing these effects should be therefore investigated much more vigorously.

In conclusion, we have shown that trauma to the brain can have drastic effects on the phase I drug metabolizing enzyme system in rats. Our results have stirred an urgent need to re-evaluate the use of standard drug dosage schedules in neurotrauma patients. Aside from issues of potential toxicity, a major issue regarding drug metabolism is bioavailability. CYP450 catalysis will lower the plasma concentration of a drug and also the concentration of the drug at the target site. A thorough investigation in terms of drug clearance with trauma patients is recommended since this could have major relevance in terms of post-trauma therapy.

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REFERENCES


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