Tissue-Specific Alterations in the 6-Hydroxylation of Chlorzoxazone Following Traumatic Brain Injury in the Rat

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ABSTRACT:
Interest in the non-neuronal alterations following traumatic brain injury (TBI) has led to research evaluating hepatic metabolism following injury. Several models of injury demonstrate tissue-specific alterations in cytochrome P450 activity. This study examined tissue-specific alterations in cytochrome P450-mediated hydroxylation in the rat model of TBI. Male Sprague-Dawley rats received anesthesia alone, craniotomy, or craniotomy plus TBI. Rats were sacrificed at 24 and 48 h. Liver, kidney, and brain cortex microsomes were isolated. Total liver P450 content, 6-hydroxylchlorzoxazone formation rate, and CYP2E1 protein were evaluated. In liver microsomes, spectral P450 was decreased to 86 ± 5% (p < 0.05) of control at 24 h following injury, and 6-hydroxylchlorzoxazone formation rate decreased to 74 ± 18% of control (p < 0.05) at 48 h following injury. In kidney microsomes, 6-hydroxylchlorzoxazone formation rate was increased to 154% of control (p < 0.01) at 24 h following injury. 6-Hydroxylchlorzoxazone formation rate was unaffected by TBI in brain cortical microsomes. The CYP2E1 inhibitor, 4-methylpyrazole, inhibited the formation of 6-hydroxylchlorzoxazone in brain, kidney, and liver microsomes. These data demonstrate that tissue-specific alterations in 6-hydroxylchlorzoxazone formation rate occur following TBI.

Patients suffering traumatic brain injury (TBI) undergo a host of pathophysiologic processes and therapeutic interventions, both of which influence the expression of drug-metabolizing enzymes (Toler et al., 1993). Pentobarbital (Heinemeyer et al., 1986), phenytoin (O’Mara et al., 1995), and antipyrine (Boucher et al., 1991) all demonstrate increased clearance in TBI patients as compared with historical or healthy volunteer controls. TBI patients develop significant activation of the acute phase response with increases in both local and systemic cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor. If the cytokine effect predominates during TBI, decreased hepatic metabolism would be predicted (Sewer et al., 1996; Rockich and Blouin, 1999); however, clinical studies demonstrate increased hepatic metabolism. One explanation of this discrepancy is provided in a study by McKindley et al. (1997), who suggested that early reductions in drug metabolism may be due to cytokine effects and later increased metabolism is associated with protein supplementation. One P450 isoform that is significantly altered by traumatic and immunologic insults is CYP2E1. CYP2E1 is best known for its role in chemical detoxification/activation, fatty acid metabolism, metabolism of acetone to gluconeogenic intermediates, and free radical production. Both generalized traumatic and immunogenic insults have demonstrated tissue-specific alterations in this isoform’s expression (Griffeth et al., 1984; Renton and Nicholson, 2000). Sewer et al. (1997) demonstrated down-regulation of CYP2E1 in the liver and up-regulation in kidney microsomes following inflammatory insults. The evidence of tissue-specific regulation of CYP2E1 by inflammatory insults led us to evaluate the effects of traumatic brain injury on microsomal chlorzoxazone metabolism as an index of CYP2E1 activity in liver, kidney, and brain.

Experimental Procedures

Animals. Adult male Sprague-Dawley rats (250–300 g) were obtained from Harlan Industries (Indianapolis, IN). Rats were housed in pairs by treatment group with a 12-h light/dark cycle with free access to food and water. The University of Kentucky animal care and use committee approved all animal procedures.

Study Design. A total of 36 rats for liver and kidney analysis (n = 6 per treatment per time point) and 27 rats (n = 9 per treatment at 24 h only) for brain analysis were assigned to isoflurane anesthesia control (control), craniotomy (CX), or craniotomy plus TBI treatment groups. Rats were sacrificed at either 24 h following treatment or 48 h following treatment for liver and kidney analysis. For brain microsomal analysis, rats were evaluated at 24 h following treatment. The effect of 4-methylpyrazole (4MP), a CYP2E1 inhibitor, was completed in control rat liver, kidney, and brain microsomes.

Injury Model. The apparatus and surgical procedures used for the administration of the cortical contusion were completed as previously described (Baldwin et al., 1996). Animals randomized to the brain injury group received a 2-mm depth injury via a pneumatically controlled impactor device with a 5-mm impactor rod tip. The injury site was closed with Surgicel (Johnson & Johnson, Arlington, TX) and sealed with dental acrylic.

Microsomal Methods and Spectral Analysis. Animals were fasted the night before sacrifice. For liver and kidney tissue, microsomes were obtained...
as previously described (Rockich and Blouin, 1999). For brain analysis, in vivo perfusion via the left ventricle was completed at the time of sacrifice. The injured hemisphere of the frontal and parietal cortex was dissected, and microsomes were prepared as described by Tindberg and Ingelman-Sundberg (1996). Total protein content was measured by the method of Lowry et al. (1951) and liver P450 concentrations as described by Omura and Sato (1964).

6-Hydroxylation of Chlorzoxazone. Incubation conditions were optimized for liver, kidney, and brain microsomes. Liver and kidney microsomes (400 μg) were incubated for 20 min with 400 μM chlorzoxazone and NADPH-regenerating system (containing 1 mM NADP, 10 mM glucose 6-phosphate, and 2 IU of glucose-6-phosphate dehydrogenase) at 37°C. Conditions for brain microsomes include 250 μg of total protein, 1500 μM chlorzoxazone, and 0.5 mM NADPH in a total of 1-mL reaction solution incubated at 37°C for 80 min. All reactions were stopped with 50 μL of 42.5% o-phosphoric acid followed by the addition of umbelliferone as the internal standard. High-performance liquid chromatography analysis of chlorzoxazone metabolite formation rate was determined as described by Tindberg and Ingelman-Sundberg (1996) with minor modifications. The limit of UV and electrochemical detection was 19.6 and 0.6 pmol, respectively.

Western Blot Analysis. Microsomal proteins were separated via SDS-polyacrylamide gel electrophoresis with a 10% gel. Proteins were transferred to nitrocellulose, blocked with 5% milk, and probed with goat anti-rat CYP2E1 polyclonal antibody (GENTEST, Woburn, MA) followed by alkaline phosphatase conjugate monoclonal anti-goat IgG. Enhanced chemiluminescence was completed with CDP-Star with Nitroblock II substrate (Tropix, Bedford, MA) and developed via autoradiography. Densitometry demonstrated linearity with protein concentration.

Statistical Analysis. All comparisons were made via a one way analysis of variance with Tukey’s post hoc analysis, p < 0.05.

Results

Effect of Traumatic Brain Injury on Total Liver P450 and Liver CYP2E1 Activity and Protein. Spectral P450 content demonstrated a small but significant decrease at 24 h to 89 ± 8% and 86 ± 5% of control (p < 0.05) in the CX and TBI groups, respectively. No significant changes were observed at 48 h in the CX and TBI groups versus control (see Table 1). In contrast, CYP2E1 mediated 6-hydroxychlorzoxazone rate was not altered in the liver of TBI rats at 24 h but was significantly reduced at 48 h following brain injury (74 ± 18% of control; p < 0.05). CYP2E1 protein evaluated at 48 h following injury paralleled the observed values for activity; however, no significant differences were observed. Liver 6-hydroxychlorzoxazone was inhibited by 90% by 1mM 4MP (Fig. 1).

Effect of Traumatic Brain Injury on Kidney 6-Hydroxylation of Chlorzoxazone. The formation rate of 6-hydroxychlorzoxazone in the kidney was approximately 6% of the activity observed in liver microsomes (Table 1). TBI increased the kidney microsomal 6-hydroxychlorzoxazone formation rate to 154% of anesthesia control values 24 h following injury (p < 0.05); however, no differences were observed between the CX group and either the control or TBI groups.

Discussion

The data presented in this study represent the first evaluation of multiple tissue cytochrome P450 activity following TBI in the rat. Specifically, this study demonstrates that the 6-hydroxychlorzoxazone formation rate is reduced in liver, increased in kidney microsomes, and unchanged in brain cortex microsomes following TBI. In addition, this activity was reduced by the CYP2E1 inhibitor 4MP in all tissues evaluated. Previous studies in our laboratory demonstrate decreased hepatic CYP3A and -2C11 mRNA without significant alterations in protein or activity 24 and 48 h following percussive brain injury in the rat (Toler et al., 1993). Consistent with the current

Table 1: Liver total P450; liver, kidney, and brain microsomal CYP2E1 activity and protein at 24 and 48 h

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>24 h</th>
<th>48 h</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CX</td>
</tr>
<tr>
<td>Total P450 (nmol/mg)</td>
<td>0.77 ± 0.07</td>
<td>0.69 ± 0.06*</td>
</tr>
<tr>
<td>Liver 6OH-CHZ formation rate (nmol/mg)/min</td>
<td>1.68 ± 0.18</td>
<td>1.76 ± 0.33</td>
</tr>
<tr>
<td>Kidney 6OH-CHZ formation rate (nmol/mg)/min</td>
<td>0.081 ± 0.031</td>
<td>0.106 ± 0.011</td>
</tr>
<tr>
<td>Cortex 6OH-CHZ formation rate (pmol/mg)/min</td>
<td>1.22 ± 0.69</td>
<td>1.32 ± 0.48</td>
</tr>
<tr>
<td>Liver CYP2E1 protein content (%)</td>
<td>Control CX TBI</td>
<td>Control CX TBI</td>
</tr>
<tr>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>100 ± 28.8</td>
<td>102.4 ± 32.6</td>
<td>117.0 ± 18.4</td>
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6OH-CHZ, 6-hydroxychlorzoxazone; N.A., not assessed.

* Statistically significant differences from control (one way analysis of variance with Tukey’s post hoc analysis, p < 0.05).
results are studies in several animal models of generalized traumatic injury that demonstrate similar reductions in P450 expression (Griffeth et al., 1984). These similar observations in various injury models suggest that a common mechanism may underlie the observed P450 down-regulation. A more recent study by Renton and Nicholson (2000) demonstrates that liver metabolism of ethoxyresorufin and chlorzoxazone, as well as liver CYP2E1 protein content, is decreased following intracerebroventricular injection of lipopolysaccharide. The current study and results from Renton’s laboratory collectively support the hypothesis that neurotraumatic and neuroinflammatory events produce systemic alterations that mediate changes in hepatic metabolism.

In contrast to the observations in the liver, kidney 6-hydroxychlorzoxazone formation rate was increased following TBI. This paradoxical increase in kidney hydroxylation of chlorzoxazone is similar to increased CYP2E1 mRNA, protein, and activity observed following intraperitoneal irritant administration reported by Sewer et al. (1997). Similarly, a study by Hanioka et al. (1997) demonstrated that 1,1-dichloroethane administration increased the formation rate of 6-hydroxychlorzoxazone in rat kidney microsomes. Collectively, these studies demonstrate tissue-specific alterations in 6-hydroxychlorzoxazone formation rate following chemical, immunologic, and traumatic insults.

In summary, traumatic brain injury produces a cascade of pathophysiologic events that alter the function of organs distant from the site of injury. The current study presents evidence of tissue-specific alterations in P450 activity following TBI. The alterations in kidney and liver parallel changes in 6-hydroxychlorzoxazone formation rate seen following immunologic and chemical insults, suggesting that a common mechanism may underlie these alterations.

References
Heinemeyer G, Roots I and Dennhardt R (1986) Monitoring of pentobarbital plasma levels in critical care patients suffering from increased intracranial pressure. Ther Drug Monit 8:145–150.