Arachidonic Acid Metabolite Production Following Focal Cerebral Ischemia: Time Course and Effect of Meclofenamate

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Arachidonic acid metabolites have been implicated in the development of cerebral edema following ischemia. To define the time course of metabolite production, subtemporal craniectomies were performed on 60 male Sprague-Dawley rats (350–400 g). Thirty rats underwent middle cerebral artery occlusion while 30 rats underwent craniectomy alone. Five rats in each of two groups (middle cerebral artery occlusion and sham) were sacrificed at 15 minutes, 1 hour, 4 hours, 1 day, 3 days, and 6 days. The cerebral hemispheres were removed and divided in the midsagittal plane. Each hemisphere was immediately frozen in isopentane cooled in dry ice and stored at \(-70^\circ C\). Tissue prostaglandins \(E_2\) and 6-keto \(F_{1\alpha}\), and leukotrienes \(LT\) \(B_4\) and \(C_4\) were measured by radioimmunoassay. Prostaglandin \(E_2\) and 6-keto prostaglandin \(F_{1\alpha}\) were significantly elevated at 15 minutes in the middle cerebral artery occlusion hemispheres \((p < 0.05)\). Prostaglandins were not significantly elevated after 15 minutes. \(LT\) \(B_4\) and \(C_4\) were never significantly elevated.

Meclofenamate, a nonsteroidal anti-inflammatory agent, was administered to 21 additional rats. Seven controls underwent middle cerebral artery occlusion alone, 7 were given intraperitoneal meclofenamate \((20 \text{ mg/kg})\) 30 minutes prior to middle cerebral artery occlusion, and 7 underwent middle cerebral artery occlusion followed immediately by intraperitoneal meclofenamate \((20 \text{ mg/kg})\). The animals were sacrificed at 15 minutes and similarly studied. There was a significant reduction of prostaglandin \(E_2\) and 6-keto prostaglandin \(F_{1\alpha}\) following pretreatment with meclofenamate \((p < 0.01\) and \(p < 0.05)\). In pretreated rats, leukotrienes were not affected by meclofenamate. Similarly, prostaglandins and leukotrienes did not change when meclofenamate was administered after middle cerebral artery occlusion.

We conclude that cyclo-oxygenase metabolite production begins within 15 minutes of middle cerebral artery occlusion. Treatment with meclofenamate prior to middle cerebral artery occlusion significantly reduced cyclo-

**Materials and Methods**

Increased tissue levels of arachidonic acid (AA) metabolites have been associated with cerebral edema following ischemia and reperfusion, experimental subarachnoid hemorrhage, and experimental concussive brain injury \([5,6,10,11]\). Injection of AA has been associated with increased brain edema \([2,4]\). Increased leukotriene \(LT\) \(C_4\) levels have been demonstrated in intracranial neoplasms that produce vasogenic brain edema \([3]\).

To investigate the role of AA metabolite production secondary to ischemic stroke, two separate experiments were performed. Part I determined the time course of AA metabolite production following focal cerebral ischemia, and Part II determined the effect of meclofenamate administration on tissue levels of AA metabolites when administered before and after focal cerebral ischemia.

**Method I**

Subtemporal craniectomies were performed on 60 male Sprague-Dawley rats \((350–400 \text{ g})\) after injection of general anesthesia with intramuscular ketamine \((4 \text{ mg/kg})\) and rompun \((0.6 \text{ mg/kg})\), according to the guidelines proposed by the University Animal Labora-
Table 1. MCAO Versus Nonoperated and Sham-Operated Hemispheres at 15 Minutes Following Occlusion

<table>
<thead>
<tr>
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<th>MCAO vs non-op (pg/mL/mg)</th>
<th>MCAO vs sham (pg/mL/mg)</th>
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<tbody>
<tr>
<td>PG E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.315 ± 2.407</td>
<td>4.315 ± 2.407</td>
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<tr>
<td>0.642 ± 0.382; p &lt; .01</td>
<td>1.232 ± 0.677; p &lt; .05</td>
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<tr>
<td>6-Keto PG F&lt;sub&gt;15&lt;/sub&gt;</td>
<td>1.246 ± 0.574</td>
<td>p &gt; .05</td>
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<tr>
<td>0.390 ± 0.33; p &lt; .05</td>
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<td></td>
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<tr>
<td>LT C&lt;sub&gt;4&lt;/sub&gt;</td>
<td>p &gt; .05</td>
<td>p &lt; .05</td>
</tr>
<tr>
<td>LT B&lt;sub&gt;4&lt;/sub&gt;</td>
<td>p &gt; .05</td>
<td>p &gt; .05</td>
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</tbody>
</table>

+ Values expressed in pg/mL/mg.
* x ± S.D.

Result I

PG E<sub>2</sub> was significantly elevated in the MCAO-operated hemispheres versus both the sham-operated and MCAO contralateral hemispheres (p < .01 and p < .05, respectively) (Table 1). At 15 minutes, 6-keto PG F<sub>15</sub> was significant in the MCAO operated versus contralateral hemispheres (p < .05). LTs B<sub>4</sub> and C<sub>4</sub> were not significantly elevated in any group. There were no infections and the average weight loss was 20 g/d.

Result II

There was a significant reduction of PG E<sub>2</sub> and 6-keto F<sub>15</sub> following pretreatment with meclofenamate (Table 2). In pretreated animals, however, LTs were not affected. Similarly, PGs and LTs did not change when meclofenamate was administered after MCAO.

Statistical analysis was carried out using two tailed paired Student's t test.

Discussion

Arachidonic acids are polyunsaturated, long-chain fatty acids with 19 to 21 carbons that differ primarily in the number of unconjugated double bonds and the various side chains. Cleavage of AA from phospholipid in the cellular plasma membrane occurs through enzymatic conversion by phospholipase. Free AA can then be metabolized by either cyclo-oxygenase or lipogenase [2]. Further enzymatic conversion yields PGs and LTs, respectively.

Arachidonic acid metabolites have been implicated in the pathogenesis of stroke, however, their exact role remains unclear [5,6,10–12]. Alterations in vascular permeability may be important in the development of cerebral edema after ischemia [7,8,12].

The technique of MCAO employed was a modification of a previously reported model [13]. The left zygoma was removed to facilitate exposure of the foramen ovale and subsequent craniectomy. The animals were primarily on the right side of the mouth. Left hemisphere infarction limited activity and frequency of feedings.

PG E<sub>2</sub> and 6-keto F<sub>15</sub> were increased significantly 15 minutes following MCAO. Previous experiments involving MCAO in cats sampled brain tissue for PGs 3 and 6 hours following occlusion [8,12]. The current experiment failed to demonstrate significant increases in PGs at 1 hour, 4 hours, 1 day, or 3 days following occlusion when two controls were compared to the MCAO-operated hemispheres. This may reflect increased metabolism of PGs following occlusion or may

Table 2. MCAO Following Pretreatment and Posttreatment with Meclofenamate at 15 Minutes Following Occlusion

<table>
<thead>
<tr>
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<th>Control vs pretreatment (pg/mL/mg)</th>
<th>Control vs posttreatment (pg/mL/mg)</th>
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<tbody>
<tr>
<td>PG E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.838 ± 1.505</td>
<td>p &gt; .05</td>
</tr>
<tr>
<td>0.732 ± 0.288; p &lt; .01</td>
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<tr>
<td>6-Keto PG F&lt;sub&gt;15&lt;/sub&gt;</td>
<td>1.505 ± 0.626; p &gt; .05</td>
<td>p &gt; .05</td>
</tr>
<tr>
<td>0.476 ± 0.326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT C&lt;sub&gt;4&lt;/sub&gt;</td>
<td>p &gt; 0.5</td>
<td>p &gt; 0.5</td>
</tr>
<tr>
<td>LT B&lt;sub&gt;4&lt;/sub&gt;</td>
<td>p &gt; 0.5</td>
<td>p &gt; 0.5</td>
</tr>
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</table>

+ Values expressed in pg/mL/mg.
* x ± S.D.
be the result of changes in metabolite levels secondary to dilution by the noninfarcted portion of the hemisphere. Reactive changes in the noninfarcted portions of the hemisphere may have altered PG metabolism as well. However, hemispheric analysis provided a relatively constant sample size and provided adequate tissue for radioimmunoassay.

At 15 minutes following MCAO, 6-keto PG Fiω was significantly elevated when MCAO-operated versus nonoperated hemispheres were compared, but not in MCAO-operated versus sham-operated hemispheres. It is possible that the changes in this metabolite could be due to the ipsilateral craniectomy as opposed to the focal ischemia itself.

Arachidonic acid metabolites have been associated with cerebral edema following focal ischemia; however, in this experiment no specific measurements of edema were made. How this edema is quantified and lessened is the focus of ongoing research at this institution [2].

Leukotrienes were not significantly increased in any group in this model of focal cerebral ischemia. Previous reports of transient cerebral ischemia have shown increased LT levels 15 to 50 minutes following reperfusion [6,10,11]. Inhibition of the lipoxygenase pathway of AA metabolism may occur as a result of permanent cerebral arterial occlusion.

Pre-treatment with meclofenamate, a nonsteroidal anti-inflammatory agent, significantly reduced PG levels in phase II of the experiment, while LTs were unaffected. Imidazole has been shown to decrease PG E2 levels 3 and 6 hours following MCAO when administered 1 hour prior to occlusion [12]. Pretreatment with indomethacin in a model of transient cerebral ischemia resulted in significant decreases in PG E2 and 6-keto F1ω, while LT B4 was increased [6]. While cyclooxygenase inhibition may result in increased substrate availability for the lipoxygenase pathway in transient cerebral ischemia, the present experiment revealed no increase. Meclofenamate may interfere with cyclooxygenase despite its apparent lack of effectiveness against lipoxygenase.

No reduction of AA metabolites was seen when meclofenamate was administered after MCAO. It is possible that this drug requires an instant blood-brain barrier in order to be effective. It would appear that the rate of absorption of meclofenamate into the rat bloodstream is rapid, given the reduced PG levels with MCAO 15 minutes after its administration.

In conclusion, cyclooxygenase metabolite production begins within 15 minutes of MCAO. Treatment with meclofenamate prior to MCAO significantly reduced cyclooxygenase metabolite production, suggesting a protective effect against ischemia-induced elevation of vasoactive PGs. Lipoxygenase metabolite production was not affected by MCAO or pharmacological intervention. The vasoactive properties of PGs may play a role in the pathophysiology of stroke.

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References