Blood Components Contribute to Rise in Gerbil Brain Levels of Leukotriene-like Immunoreactivity After Ischemia and Reperfusion

Kiyoshi Saito, MD, Lawrence Levine, ScD, and Michael A. Moskowitz, MD

The mean ± SEM concentrations of immunoreactive leukotriene C₄ and D₄ (iLTD₄) and prostaglandin D₂ (iPGD₂) increased from 3.0 ± 1.2 and 0.71 ± 0.33 to 16.3 ± 4.7 and 3.0 ± 1.14 ng/g forebrain, respectively (p < 0.05, iLTD₄; p < 0.01, iPGD₂), in the forebrains of 12 gerbils after 15 minutes of bilateral common carotid artery occlusion and 15 minutes of reperfusion. Removal of blood from ischemic brain of 11 gerbils by intracardiac perfusion with ice-cold saline for 10 minutes decreased iLTD₄ concentrations significantly to 7.0 ± 0.9 (p < 0.05) but did not change iPGD₂ concentrations. Severe granulocytopenia (4.98 ± 1.79 to 0.05 ± 0.03 X 10³/mm³, p < 0.01) in seven gerbils following intraperitoneal injection of 50 mg/kg busulfan was associated with decreased iLTD₄ accumulation in the brain to 3.46 ± 1.36 ng/g forebrain (p < 0.01). Taken together, our results suggest that blood components (most likely leukocytes) are a source of leukotriene-like immunoreactivity in the ischemic and reperfused brain. (Stroke 1988; 19:1395-1398)

Leukotrienes (LTs) possess a variety of actions in mammalian tissues, including constriction of vascular smooth muscle, secretion of mucus, extravasation of plasma protein from the vessel wall, sensitization of nociceptors, activation of polymorphonuclear leukocytes (PMNs), and stimulation of myelopoiesis.¹⁻⁶ LTs have been implicated in the pathogenesis of neurologic diseases including such conditions as brain ischemia, trauma, tumors, subarachnoid hemorrhage, and brain edema.⁷⁻¹²

We have reported large increases of LT and prostaglandin (PG) concentrations in brain following ischemia and recirculation and have found high concentrations of LTs predominantly in gray matter.⁸⁻¹³ Since leukocytes are a potentially important source of LTs, we investigated the effects of granulocytopenia and cardiac perfusion on these elevations.

Materials and Methods

Fifty-four adult male Mongolian gerbils (Meriones unguiculatus; Tumblebrook Farms, West Brookfield, Massachusetts) weighing 40–70 g housed under diurnal lighting conditions and allowed food and water ad libitum were anesthetized during the ischemia and reperfusion periods by diethylether and room air as described.⁸⁻⁹ The anesthetic was maintained throughout the period of ischemia and reperfusion, and no attempt was made to regulate the gas composition. Following tracheostomy through a midline incision, both common carotid arteries (CCAs) were dissected in 12 gerbils and occluded after 15 minutes of occlusion, the clips were removed to allow recirculation for an additional 15 minutes. In 10 sham-operated gerbils, CCAs were dissected but not occluded.

Leukocytopenia was induced in seven gerbils by 50 mg/kg i.p. busulfan (1,4-butanediol dimethanesulfonate) (Fluka Chemical Co., Hauppauge, New York) in 0.5 ml dimethyl sulfoxide (Fisher Scientific Co., Fairlawn, New Jersey) vehicle. Fourteen days later, the CCAs were occluded as described above. Erythrocytes, hemoglobin, hematocrit, leukocytes with differential, and platelets were counted before the injection of busulfan and on the day of the experiment. To wash blood from the circulation, 11 gerbils were perfused through a percutaneous puncture of the left cardiac ventricle with a 25-gauge needle with 50 ml ice-cold saline for 10 minutes following 15 minutes of ischemia and 15 minutes of reperfusion. Drainage was achieved from the right axillary vein. The complete removal of blood was...
confirmed by the presence of a white, colorless brain at dissection.

Following ischemia and reperfusion or sham operation, the gerbils were killed by immersion in liquid nitrogen for 45 seconds and decapitation. The forebrains were removed quickly while still frozen, placed in 2.5 ml cold ethanol, homogenized on dry ice with a Polytron C (Brinkmann Instruments Inc., Westbury, New York), and centrifuged at 10,000 rpm for 20 minutes. Supernatants were dried under nitrogen for 45 seconds and decapitation. The forebrain±SEM. Significant differences between groups were determined by Student's t test. Probability values of <0.05 were considered significant.

Results

As previously reported, forebrain concentrations of LTD4 in the sham-operated group were at the lower limits of assay sensitivity.9 However, in the ischemia and reperfusion group forebrain concentrations of immunoreactive LTD4 and immunoreactive PGD2 increased approximately fivefold compared with the sham-operated group (Figure 1). When brain extracts were separated by high-performance liquid chromatography, immunoreactivity corresponded to LTC4 and LTD4.8 An injection of vehicle alone did not modify these increases (iLTD4, 11 ±3, iPGD2, 7 ±1 ng/g in vehicle-treated group; iLTD4, 13 ±3, iPGD2, 8 ±2 ng/g in untreated group; n = 4–6 per group). However, saline perfusion significantly reduced the increases in LTD4 to 7.0 ±0.9 (p<0.05); iPGD2 concentrations were unchanged.

Fourteen days after busulfan pretreatment, erythrocytes, hematocrit, hemoglobin, platelets, and PMN were significantly decreased (Table 1). Numbers of leukocytes and platelets were reduced by >99%. In these gerbils, iLTD4 and iPGD2 concentrations were decreased by 73% and 54%, respectively (Figure 2, top). iLTD4 concentrations in gerbils with severe granulocytopenia (<50 PMN/mm3) were significantly lower than in those with higher PMN counts (>50/mm3) (Figure 2, bottom). iPGD2 concentrations did not show this correlation.

Discussion

The conclusion from both experiments suggests that an important source of leukotriene-like immunoreactivity in gerbil brain following ischemia and reperfusion derives from the circulation. By using two different methods, we partially circumvented weaknesses inherent in each experimental para-

**TABLE 1. Effect of 50 mg/kg i.p. Busulfan on Peripheral Blood Components in Seven Gerbils**

<table>
<thead>
<tr>
<th>Component</th>
<th>Day 0 (mean ± SEM)</th>
<th>Day 14 (mean ± SEM)</th>
<th>% of Day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (10^12/mm³)</td>
<td>8.1 ± 0.1</td>
<td>4.6 ± 0.3*</td>
<td>57</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>50 ± 1</td>
<td>25 ± 2*</td>
<td>50</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.8 ± 0.2</td>
<td>8.3 ± 0.6*</td>
<td>60</td>
</tr>
<tr>
<td>Leukocytes (10^9/mm³)</td>
<td>8.9 ± 2.1</td>
<td>3.0 ± 0.8†</td>
<td>34</td>
</tr>
<tr>
<td>Polymorphonuclear leukocytes (10^9/mm³)</td>
<td>4.98 ± 1.79</td>
<td>0.5 ± 0.3*</td>
<td>1</td>
</tr>
<tr>
<td>Platelets (10^9/mm³)</td>
<td>1137 ± 146</td>
<td>11 ± 2*</td>
<td>1</td>
</tr>
</tbody>
</table>

*|t|p<0.01, 0.05 different from Day 0, paired t test.
pared with six gerbils with less severe granulocytopenia. By contrast, results from saline perfusion and pancytopenia suggest that a larger fraction of PGD$_2$ derives from brain parenchyma.

Our results support previous suggestions that granulocytes play a role in the injury associated with ischemia and reperfusion. Recent studies in experimental animals have shown that granulocytes accumulate in the postischemic hemisphere within hours of reperfusion and that the severity of ischemia correlates with the extent of accumulation. Similar accumulations have been reported in humans. It has been postulated that granulocytes contribute to reperfusion injury by generating active oxygen species and by generating LTs and PGs, molecules that possess chemotactic properties in addition to their effects on vascular permeability and contractility. Indeed, a role for granulocytes in the pathophysiology of reperfusion injury has been best demonstrated to date in the dog heart.

A relation between LTs and the development of brain edema has been suggested. LTs are detected in pathologic conditions associated with brain edema and 15 minutes of reperfusion decreased concentrations of the immunoreactive leukotriene D$_4$ (LTD$_4$) and immunoreactive prostaglandin D$_2$ (PGD$_2$) in brain (*p<0.05, **p<0.01) compared with five untreated gerbils. Bottom: Concentrations of LTD$_4$ but not PGD$_2$ became significantly lower in five busulfan-treated gerbils with severe granulocytopenia (PMN<50/mm$^3$) (*p<0.05) compared with six gerbils with less severe granulocytopenia (PMN>50/mm$^3$).

In summary, we have demonstrated that blood component(s) contribute to elevation of brain LT concentrations during ischemia and reperfusion.

References


Or capillaries do not produce significant amounts of LT even after addition of arachidonic acid or calcium ionophore. By contrast, results from saline perfusion and pancytopenia suggest that a larger fraction of PGD$_2$ derives from brain parenchyma.

production has not been identified within platelets and since brain LT concentrations decreased with severe leukopenia, granulocytes are the most likely source. Neurons may also contribute since LT can be synthesized in vitro from brain slices. Moreover, immunoreactivity has been visualized in nerve cells synthesized in vitro from brain slices. Moreover, free radicals are generated during cyclooxygenation, and these molecules enhance the vascular leakage of plasma proteins. The relevance of all these observations to brain ischemia can be questioned since brain edema characteristically develops 1-3 hours after the ischemic insult, whereas arachidonate release and LT formation occur within minutes of ischemia and reperfusion. Of course, an initiating role for LTs cannot be excluded now.


**Key Words** • prostaglandins • leukotrienes • cerebral ischemia • gerbils
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