Are Blood Platelets Involved in the Pathogenesis of Ischemic Brain Edema in Gerbils?

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Edema formation following severe permanent or temporary cerebral ischemia in gerbils with an artificially reduced platelet count was investigated. Acute focal cerebral ischemia was produced by extracranial carotid ligation, and the local cerebral blood flow was estimated using the hydrogen clearance method. Brain tissue water and sodium and potassium contents were taken as indexes of brain edema. The platelet count was reduced in some gerbils by intravenous injection of neuraminidase. After 60 minutes of ischemia, a marked increase in tissue water and sodium contents accompanied by a decrease in potassium content was observed in untreated gerbils. However, gerbils with a reduced platelet count revealed similar but significantly smaller changes in all the measured parameters. Restoration of blood flow after 60 minutes of ischemia resulted in further accumulation of water and sodium and in depletion of potassium in both groups. These changes were significantly smaller in the gerbils with a reduced platelet count. It is concluded that platelets, activated by cerebral ischemia, may be involved in the development of ischemic brain edema in gerbils. (Stroke 1988;19:486–489)

Cerebral ischemia is regularly accompanied by brain edema, which further aggravates ischemic damage. There is abundant experimental evidence to suggest that the edema associated with cerebral ischemia has both cytotoxic and vasogenic features, but the mechanisms underlying the genesis and propagation of ischemic brain edema are not yet fully understood.

It has been demonstrated that one effect of experimental acute focal ischemia is to induce activation of platelets. Platelet activation involves specific reactions, such as the liberation of arachidonic acid from membrane phospholipids, aggregation of platelets, and the release of numerous biologically active substances stored in the platelet granules. Since some of these released substances (e.g., serotonin, cationic proteins, vascular permeability factor, and platelet activating factor) have been shown to alter the vascular permeability, it is possible that platelets may contribute to the evolution of ischemic brain edema. To clarify the effects of a reduced platelet count on the development of ischemic brain edema, experiments were undertaken in gerbils.

Materials and Methods

Adult Mongolian gerbils (Meriones unguiculatus) weighing 80–90 g were maintained on standard laboratory chow and water ad libitum. Three days before the experiment, the skin overlying the calvarium was removed under light pentobarbital anesthesia, and open-tip-type platinized platinum electrodes (0.25 mm diam.) were implanted into the gray matter of both parietal cortices through small drill holes in the skull and were anchored rigidly in place with dental cement. On the day of the experiment, previously prepared gerbils were anesthetized with 30 mg/kg i.p. pentobarbital sodium, and spontaneous respiration was maintained throughout.

The left femoral artery was exposed and catheterized with a PE-10 polyethylene catheter for blood sampling and arterial blood pressure monitoring. Focal cerebral ischemia was produced by clipping both a common carotid artery and the contralateral external carotid artery as described in detail elsewhere. Constant body temperature was maintained with a heating pad.

Platelet count was reduced in some gerbils by treatment with neuraminidase according to Smith and White. Neuraminidase (type V, Sigma Chemical Co., St. Louis, Missouri) at a dose of 4 units/kg i.v. was administered 72, 48, and 24 hours before the experiment.

Brain water content was measured by the wet–dry method. The gerbils were decapitated and the brains were removed promptly. Each cerebral hemisphere was carefully separated from the brainstem and weighed immediately (wet weight). The dry weight was measured after complete dehydration at 90°C for 4 days. The difference between the wet and dry weights of the sample was taken as the water content and was expressed as percent of the wet weight.

The dehydrated cerebral hemispheres were homogenized with 10 ml 0.75 M nitric acid and incubated at 4°C for 3 days. The sodium and potassium contents were measured by atomic absorption spectrometry, and the results were expressed in milliequivalents per kilogram dry weight.

Regional cerebral blood flow (rCBF) was measured in both parietal cortices simultaneously by the hydro-
Ken clearance method. Hydrogen clearance curves were obtained by inhalation of hydrogen gas for 10 seconds, and rCBF was calculated from the initial 1–4 minutes of the washout curves.

Red blood cells (RBC), white blood cells (WBC), platelets (PLT), hematocrit (Hct), and hemoglobin (Hgb) were estimated in 100 μl of anticoagulated arterial blood by means of an automatic blood cell counter (Ortho Instruments Corp.).

**Experimental Protocols**

*Ischemia without recirculation.* Steady-state measurements of rCBF and hematologic parameters were made in eight untreated and eight pretreated (reduced PLT) gerbils. Thereafter, all gerbils underwent carotid occlusion (ischemic group). rCBF was measured 5 and 60 minutes after the occlusion. Gerbils with rCBF of >20 ml/100 g/min or <5 ml/100 g/min during ischemia were discarded. Gerbils were decapitated 1 hour after occlusion.

*Ischemia with recirculation.* In another series, an experimental protocol similar to that in the ischemic group was employed (seven untreated gerbils, eight reduced PLT gerbils), except blood flow was restored by removal of the clips for 15 additional minutes after 60 minutes of ischemia.

Ten control gerbils were treated identically except for carotid occlusion.

The data were analyzed by standard statistical methods (G test, F test), and the unpaired t test was used to compare differences between the means.

**Results**

The platelet count in reduced PLT gerbils was reduced to 14.2 ± 4.0% of the control values. Other hematologic parameters were not significantly affected (Table 1), and no signs of any adverse reaction to the neuraminidase pretreatment were observed.

Blood flow in the parietal cortices of untreated and reduced PLT gerbils before ischemia averaged 43.3 ± 7.4 and 44.1 ± 9.1 ml/100 gm/min, respectively. There were no marked differences between the left and right hemispheres. The changes in rCBF after carotid occlusion as well as during recirculation are summarized in Table 2. No significant differences in rCBF between untreated and reduced PLT gerbils were observed in any experimental situation.

During 60 minutes of ischemia in untreated gerbils, a significant amount of water accumulated in the ischemic hemisphere (80.11 ± 0.45%) compared with the controls (78.49 ± 0.46%). Recirculation for 15 minutes resulted in a further increase in water content in the ipsilateral hemisphere (Figure 1). In reduced PLT gerbils, the ipsilateral hemisphere accumulated significantly less water during ischemia as well as during recirculation compared with that in untreated gerbils (Figure 1).

Water accumulation in the ischemic hemisphere was accompanied by a marked increase in sodium content (Figure 2) and by a decrease in potassium content (Figure 3). The changes in untreated gerbils were significantly greater than those in reduced PLT gerbils.

**Discussion**

The participation of platelets in the pathogenesis of ischemic cerebrovascular diseases is experimentally and clinically well documented. It has been shown that platelets play an important role in atherogenesis and in microcirculatory flow disturbances, but their contribution to the evolution of brain edema associated with ischemia has not yet been satisfactorily evaluated.

To analyze the possible relations between platelet activity and the development of ischemic brain edema, we employed a model of acute focal cerebral ischemia in gerbils. This model is widely used, and its suitability as a model of ischemic brain edema has been suggested, despite the fact that blood flow in the ischemic hemisphere is unpredictable. Since the formation of ischemic brain edema depends strongly on the residual blood flow in the ischemic area, we...

### Table 1. Effect of Neuraminidase Pretreatment on Basic Hematologic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 10)</th>
<th>Pretreated (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood platelets (10^3/μl)</td>
<td>945 ± 150</td>
<td>134 ± 38*</td>
</tr>
<tr>
<td>White blood cells (10^3/μl)</td>
<td>8.5 ± 1.8</td>
<td>9.3 ± 1.6</td>
</tr>
<tr>
<td>Red blood cells (10^6/μl)</td>
<td>7.71 ± 0.62</td>
<td>7.59 ± 0.75</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.6 ± 3.3</td>
<td>12.2 ± 1.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47.0 ± 3.3</td>
<td>46.1 ± 5.3</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

* p<0.001.

### Table 2. Changes in Regional Cerebral Blood Flow After Extracranial Carotid Ligation in Untreated Gerbils and Gerbils Pretreated With Neuraminidase to Reduce Platelet Count

<table>
<thead>
<tr>
<th></th>
<th>Steady state</th>
<th>Ischemia 1 min</th>
<th>Ischemia 60 min</th>
<th>Recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>C</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>Ischemia without recirculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>44.3 ± 6.8</td>
<td>44.5 ± 6.5</td>
<td>12.1 ± 3.1</td>
<td>39.3 ± 6.3</td>
</tr>
<tr>
<td>Reduced PLT</td>
<td>42.8 ± 8.2</td>
<td>42.3 ± 4.9</td>
<td>12.0 ± 3.0</td>
<td>41.2 ± 9.1</td>
</tr>
<tr>
<td>Ischemia with recirculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>42.2 ± 8.0</td>
<td>43.3 ± 9.6</td>
<td>10.4 ± 2.2</td>
<td>37.5 ± 8.1</td>
</tr>
<tr>
<td>Reduced PLT</td>
<td>45.6 ± 11.0</td>
<td>45.8 ± 10.2</td>
<td>13.4 ± 3.0</td>
<td>43.8 ± 10.3</td>
</tr>
</tbody>
</table>

Data are mean ± SD (ml/100 g/min). I, ipsilateral to ligation; C, contralateral to ligation; PLT, platelet count.
selected only gerbils in which the rCBF after extracranial ligation of the carotid artery was in the range of 5–20 ml/100 g/min.

The involvement of platelets in various physiologic and pathologic processes has been investigated mostly by using pharmacologic inhibitors of platelet functions. However, the inhibitory actions of the majority of substances are not specific to platelet activity and may also affect other systems and influence blood pressure, cerebral blood flow, and biosynthesis of prostaglandins. Since changes in these parameters can substantially affect the development of edema, we used another approach, that is, elimination of platelets from the systemic circulation. Reduction of platelet count by neuraminidase pretreatment has been reported to be effective and safe. Applying this procedure, we were able to achieve an 85.8% reduction in platelet count in gerbils without any detectable side effects.

The mechanisms underlying the development of ischemic brain edema are not yet fully understood. It has been assumed that the intracellular accumulation of fluid is due to disturbance of cellular osmoregulation caused by energy failure developing in ischemia. Other authors have emphasized the role of lactate accumulation, alterations in membrane phospholipids, changes in neurotransmitter metabolism, and changes in Na,K-ATPase activity. Recently, Iannotti et al have demonstrated that prostaglandins released by ischemia can induce disruption of the cell membrane.

In our study, gerbils with a reduced platelet count had significantly reduced water accumulation and changes in sodium-potassium equilibrium after ischemic insult. Our results suggest that platelets may also contribute to some extent to the development of ischemic brain edema. The design of our experiments did not permit a precise evaluation of the mechanism of platelet contribution. However, we speculate that platelet participation may be mediated by various substances released from the platelets in response to ischemia. Among these substances, prostanoids, serotonin, cationic proteins, and platelet activating factor could be of particular importance. The platelet-borne prostanoids may be involved in edema formation by affecting endothelial cells, neurotransmitter uptake, and Na,K-ATPase activity. Platelet-derived serotonin causes endothelial gap formation between cells, which could lead to edema formation. Cationic proteins released from platelet α-granules influence the vascular
permeability either directly by an ionic mechanism\textsuperscript{13} or indirectly by degranulation of mast cells in the vicinity of blood vessels.\textsuperscript{14} Finally, several reports have indicated that platelet activating factor can also increase the vascular permeability.\textsuperscript{25,26} It remains to be determined which substance is the main mediator of platelet participation in the pathogenesis of ischemic brain edema.

In conclusion, we found that a reduction in platelet count resulted in a partial suppression of edema formation in both ischemic and recirculated gerbil brains. Our data indicate the involvement of platelets in the development of ischemic brain edema and suggest a possible rationale for antiplatelet therapy of ischemic cerebrovascular diseases.

References


Key Words • brain edema • cerebral ischemia • gerbils • platelets
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