Thromboxane synthetase inhibition in acute focal cerebral ischemia in cats

NAZIH A. MOUFARRIJ, M.D., JOHN R. LITTLE, M.D., VICTOR SKRINSKA, Ph.D., FRED V. LUCAS, M.D., JOHN P. LATCHAW, M.D., ROBERT M. SLUGG, A.B., and RONALD P. LESSER, M.D.

Departments of Neurosurgery and Neurology, and Division of Laboratory Medicine, Cleveland Clinic, Cleveland, Ohio

The purpose of this investigation was to study the effects of a selective thromboxane A2 (TXA2) synthetase inhibitor (TSI) upon the evolution of cerebral infarction in the cat. Adult cats, lightly anesthetized with nitrous oxide, underwent right middle cerebral artery (MCA) occlusion for 4 hours followed by a 2-hour period of reperfusion before sacrifice. Ten cats received 3 mg/kg TSI intravenously immediately before, and 10 cats received 3 mg/kg TSI intravenously immediately after MCA occlusion. Ten cats were used as controls receiving no treatment. The bleeding time was determined at baseline and at the end of each experiment. Electroencephalographic (EEG) recordings were obtained before and after MCA clipping and MCA release, and at hourly intervals thereafter. Regional cerebral blood flow (rCBF) was measured using the xenon-133 (~33Xe) clearance technique before and after MCA occlusion, after MCA reopening, and before terminating each experiment. Thirty minutes before each cat was sacrificed, Evans blue dye and sodium fluorescein were given intravenously. The animals were then perfused with colloidal carbon and the brains removed and evaluated for midline shift, Evans blue dye and sodium fluorescein extravasation, carbon staining, and infarct size. The bleeding time, arterial blood pressure, rCBF changes, brain swelling, and vital dye extravasation were not statistically different between the three treatment groups. The EEG changes, carbon staining, and infarct size differences between the three groups also failed to reach statistical significance, but there was a suggestion that these parameters were adversely affected in the cats pretreated with TSI. Ten additional cats undergoing MCA occlusion and reperfusion were used for pharmacological studies. Five of them received 3 mg/kg TSI intravenously immediately after MCA occlusion, and serial drug and thromboxane B2 (TXB2) levels (a stable metabolite of TXA2) were determined. Another five cats were not treated and serial TXB2 levels were obtained. Production of TXA2 was inhibited by 95% in cats receiving TSI. In conclusion, thromboxane synthetase inhibition failed to modify favorably the evolution of cerebral infarction. When TSI was given before MCA occlusion, cerebral infarction tended to be more extensive.

Key Words: cerebral ischemia · thromboxane synthetase inhibitor · platelet function · lipoxygenase products

Thromboxane A2 (TXA2) is an arachidonic acid derivative produced mainly by the platelet microsomes and is endowed with platelet aggregatory and vasoconstrictor effects. Prostacyclin (PGI2) is another arachidonic acid derivative produced mainly by the endothelial cell. It induces platelet disaggregation and vasodilatation (Fig. 1). Pharmacological manipulation in favor of PGI2 over TXA2 may have beneficial effects on the evolution of cerebral infarction.4,7 The purpose of this study was to define the effect of a selective thromboxane synthetase inhibitor (TSI) on experimental acute focal cerebral ischemia.

Materials and Methods

Forty adult cats were anesthetized with nitrous oxide. A tracheostomy was performed and mechanical ventilation started. Intravenous d-tubocurarine, 1 mg/kg as needed, was used to maintain skeletal muscle paralysis. Electrocardiogram leads were placed for continuous monitoring. A catheter was inserted into the femoral artery and vein for continuous blood pressure monitoring and venous access, respectively. The right carotid artery was cannulated via the lingual artery for cerebral blood flow measurement using the xenon-133 (~33Xe)
intracarotid injection technique. The animal was then placed in a head holder with a rectal probe inserted and a heating pad laid over the trunk to maintain a core temperature close to 37°C. The right middle cerebral artery (MCA) was exposed via a transorbital approach using microsurgical techniques. A miniature aneurysm clip was used to occlude the MCA for 4 hours. The clip was then removed and perfusion reestablished for 2 hours, at the end of which the cats were sacrificed.

The end-tidal pCO\textsubscript{2} was continuously monitored and maintained in the physiological range.* In addition, blood samples were drawn to determine arterial blood gases and hematocrit as needed to ascertain that physiological conditions were achieved.

**Bleeding Time Studies and Thromboxane B\textsubscript{2} Levels**

The bleeding time was determined at baseline and at the end of each experiment. For this purpose, a bleeding time device† was applied to the hindpaws of the animal. At the end of each experiment, 5 cc of arterial blood was drawn immediately before perfusion for measurement of thromboxane B\textsubscript{2} (TXB\textsubscript{2}).

**EEG Studies**

Electroencephalographic (EEG) recordings were obtained via paired midfrontal, posterior frontal, and parietal epidural electrodes implanted through the skull but not penetrating the dura. Another electrode was placed in the midline over the frontal air sinus for use as a reference electrode, and the left temporal muscle was used as the ground. This electrode placement corresponded to the border zone between the anterior cerebral artery and the MCA territories rather than being in the core area of ischemia. The EEG recordings were made on an instrument‡ with recorded amplitudes 20% down at 1 and 70 Hz. Two-minute EEG's were recorded before and after MCA clipping and MCA release, and at hourly intervals thereafter.

**Cerebral Blood Flow Studies**

Regional cerebral blood flow (rCBF) was measured immediately before and after MCA clipping and reperfusion, and just before termination of each experiment. The \textsuperscript{133}Xe clearance technique was used as described in previous reports from our laboratory.\textsuperscript{1}

**Treatment Groups**

Ten cats received a 3-mg/kg intravenous bolus of TSIw immediately after MCA clipping and 10 cats were similarly treated immediately before MCA clipping. The drug, UK-38,485, was dissolved in a solution of normal saline at a concentration of 2 mg/ml. Ten cats served as controls.

**Pathological Studies**

Thirty minutes before each cat was sacrificed, Evans blue dye (1 ml of a 10% solution) and sodium fluorescein (1 ml of a 10% solution) were given intravenously. Subsequently, a midline thoracotomy was carried out and the ascending aorta cannulated, the descending aorta was clamped, and the right atrium was incised. The animals were perfused at a constant pressure of 120 mm Hg with 50 ml of isotonic saline followed by a mixture of 125 ml of colloidal carbon and 125 ml of phosphate-buffered 4% formaldehyde. The brains were removed and placed in fixative solution. The brains

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* CO\textsubscript{2} monitor 130 manufactured by Siemens-Elema, Sweden.
† Simplette-II bleeding time device manufactured by General Diagnostics, Morris Plains, New Jersey.
‡ Model 6 electroencephalograph manufactured by Grass Instrument Co., Quincy, Massachusetts.
§ Thromboxane synthetase inhibitor, UK-38,485 supplied by the Pfizer Co., Groton, Connecticut.
Thromboxane synthetase inhibition in cerebral ischemia

TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cats</th>
<th>Time of Measurement</th>
<th>Systolic Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>baseline</td>
<td>106 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>at 6 hrs</td>
<td>110 ± 17</td>
</tr>
<tr>
<td>pretreated</td>
<td>10</td>
<td>baseline</td>
<td>122 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>at 6 hrs</td>
<td>117 ± 18</td>
</tr>
<tr>
<td>posttreated</td>
<td>10</td>
<td>baseline</td>
<td>115 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>at 6 hrs</td>
<td>106 ± 13</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations. MCA = middle cerebral artery. Comparability of the change from baseline to the 6-hour values: control and pretreated cats: p = 0.27; control and posttreated cats: p = 0.21.

were subsequently studied for midline shift, Evans blue dye and sodium fluorescein extravasation, carbon staining, and infarct size, according to a protocol described in earlier communications from our laboratory.

Pharmacological Studies

Ten cats were used for pharmacological studies only. The MCA was clipped for 4 hours and reopened for 2 hours. Five cats received a 3-mg/kg intravenous bolus of TSI immediately after MCA occlusion. Drug and TXB₂ levels were determined immediately before MCA clipping, 30 minutes after occlusion, 30 minutes before and 30 minutes after MCA release, and just before sacrifice in each animal. The drug levels were determined by high-performance liquid chromatography and the TXB₂ levels were determined by radioimmunoassay. TXB₂ rather than TXA₂ was assayed since it is a stable direct metabolite of the short-lived TXA₂, which has a half-life of 30 seconds (Fig. 1). The other five cats were not treated, and TXB₂ levels were determined following the same schedule. The 10 cats in this segment of the study did not undergo EEG, rCBF, or pathological studies.

Results

Bleeding Time and Thromboxane B₂ Levels

Bleeding time was not significantly affected by the administration of TSI either before or after MCA clipping. However, substantial variability was seen in the three groups. Table 1 reports the numerical results and their statistical significance obtained with the Mann-Whitney rank sum test.

The bar diagram of Fig. 2 illustrates the serum TXB₂ levels in the treatment groups just before termination of the experiment. Thromboxane production was inhibited by close to 95% in the cats receiving TSI either immediately before or immediately after MCA clipping compared with untreated animals.

Arterial Blood Pressure

There was no significant difference in systolic blood pressure over the course of the experiments when the control and pretreated groups were compared. Table 2 summarizes the data, and presents the statistical significance obtained with the Mann-Whitney rank sum test.

Electroencephalography

An EEG index (in percentages) was derived by comparing the average wave amplitude of the ischemic right hemisphere (numerator) to the average wave amplitude of the intact left hemisphere (denominator). The EEG changes with time are plotted for the three groups in Fig. 3. The analysis of variance (ANOVA) method revealed that the drop in the EEG index after MCA clipping was statistically significant (p < 0.001). The control and treated groups, however, had comparable EEG indices at any given time, indicating that the effect of the different treatments was not statistically significant.

Regional Cerebral Blood Flow

The ANOVA method was used to interpret the rCBF data. There was a significant drop in rCBF in all these groups after MCA clipping (p < 0.005). Treatment with TSI, either immediately before or immediately after MCA clipping, failed to significantly alter the rCBF changes when compared with the control group. This is illustrated in Fig. 4, where the almost parallel tracking of the three groups shows the lack of effect of TSI treatment on rCBF changes.
Brain Swelling and Vital Dye Extravasation

Brain swelling in the ischemic right hemisphere was considered present if there was more than 1 mm of difference on a standard cross-sectional measurement between the right and the left sides. The brain swelling and Evans blue dye and fluorescein extravasation results are summarized in Table 3. The three treatment groups did not differ significantly.

Carbon Perfusion

A representative coronal section 3 mm posterior to the temporal lobe tip was examined for carbon filling. The brains of the 30 cats in the three treatment groups were blindly separated into two categories: minimal or significant failure of carbon filling. The former category indicated either normal carbon filling or referred to a few small foci of poor filling; the latter category indicated a larger area of subcortical improper filling or extensive cortical and subcortical regions of impaired filling. Significant failure of carbon filling was present in five control, seven posttreated, and eight pretreated cats. The difference between the control and the pretreated groups achieved the lowest p value (p = 0.20).

Infarct Size

The mean infarct size and standard deviation in the control, pretreated, and posttreated groups are shown in Fig. 5. The p values obtained by comparing the control versus the posttreated groups, the control versus the pretreated groups, and the posttreated versus the pretreated groups are p = 0.85, p = 0.25, and p = 0.30, respectively. Although Fig. 5 suggests that pretreatment with TSI resulted in a larger infarct size when compared to the control group, there was insufficient statistical evidence to firmly support this conclusion.

Pharmacological Studies

The TSI serum levels in five cats after MCA occlusion and a 3-mg/kg intravenous dose of TSI are depicted in Fig. 6. Fifteen minutes after the injection the mean level was 6 μg/ml. It declined during the initial 3 to 6 hours. The concomitant changes in TXB2 serum levels (Fig. 7) reveal a precipitous fall from a pretreatment mean level of 185 ng/ml to mean values between 0.6 and 4 ng/ml at 30 minutes after the administration of TSI, and remained at that level. The plasma TXB2 levels in the five untreated cats did not significantly change with time.
Thromboxane synthetase inhibition in cerebral ischemia

Fig. 6. Serum levels of thromboxane synthetase inhibitor after intravenous administration of 3 mg/kg in five cats with middle cerebral artery (MCA) occlusion.

Fig. 7. Effect of thromboxane synthetase inhibitor (TSI) on the serum thromboxane B2 levels in five cats undergoing middle cerebral artery (MCA) occlusion. A 3-mg/kg intravenous bolus of TSI was given immediately after MCA occlusion. S.D. = standard deviation.

TABLE 3

<table>
<thead>
<tr>
<th>Group &amp; Feature Tested*</th>
<th>No. of Cats</th>
<th>Feature Present</th>
<th>Feature Absent</th>
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<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>brain swelling</td>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Evans blue dye</td>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>TSI pretreatment</td>
<td>10</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>brain swelling</td>
<td></td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Evans blue dye</td>
<td></td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>TSI posttreatment</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>brain swelling</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Evans blue dye</td>
<td></td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

* TSI = thromboxane synthetase inhibitor.

Discussion

The role of platelets in the evolution of cerebral infarction has not been clearly defined. Thromboxane A2, produced in platelet microsomes, increases platelet aggregation and produces vasoconstriction. These actions could be damaging in acute focal cerebral ischemia. This agent is one of the metabolites of arachidonic acid produced by a specific enzymatic complex known as TXA2 synthetase. It is in turn rapidly metabolized to TXB2, its direct stable assayable metabolite. The inhibition of TXA2 production has potential beneficial effects upon the evolution of cerebral infarction. In this study, UK-38,485 (a selective thromboxane synthetase inhibitor) was used. This compound is 3-(1H-imidazole-1-yl-methyl)-2 methyl-1H-indole-1-propanoic acid, with the imidazole ring being the active moiety of the molecule.

In cats receiving TSI, the pharmacological findings demonstrated rapid and sustained suppression of TXB2 production. Studies in other species given a comparable dose revealed similar findings. In the present study, bleeding time failed to show any difference between the treated and control groups despite marked suppression of TXA2 production in cats receiving TSI. This can be explained on the basis of methodological factors. First, the mean bleeding time in humans is associated with a large standard deviation. Second, the great inter-animal variability in terms of thickness of the paw dermis makes statistical differences more difficult to elicit.

The rCBF changes were not affected by treatment with TSI. This suggests that release of TXA2 by platelets in untreated cats did not substantially increase the resistance in collateral arterial channels to the ischemic zone. In addition, the findings of the rCBF and carbon perfusion studies are contrary to the hypothesis that intravascular platelet aggregation induced by TXA2 release obstructs the microcirculation in the ischemic zone and thereby impairs perfusion. Treatment with TSI did not significantly alter systemic arterial blood pressure. Similar findings have been reported in a rabbit model of global cerebral ischemia, in which rCBF was not affected by TSI treatment.

In the present study, pretreatment with TSI tended to produce a larger infarct size (43%) when compared with no treatment (28%) and also with treatment started after MCA occlusion (32%). When the EEG curves were examined, the TSI-pretreated group seemed to
recover less well after reperfusion than did the untreated and the posttreated groups. Although these two observations did not attain statistical significance they are worthwhile noting, because of their possible pathophysiological implications. Indeed, preservation of some degree of platelet function in the setting of cerebral infarction may be protective. Conversely, the severe abolition of platelet aggregation achieved by TXA\textsubscript{2} synthetase inhibition may be detrimental and hence may be unmasking a hitherto unsuspected beneficial role of platelets in cerebral infarction.

One possible mechanism that would explain the increased infarct size found in cats pretreated with TSI is that inhibition of TXA\textsubscript{2} production will lead to redirection of its precursors into the leukotriene and other lipoxigenase products, which may propagate tissue damage. The two fundamental premises of such a mechanism are: 1) the recent demonstration that platelet-derived lipoxigenase by-products may be taken up by other cells, in particular neutrophils;\textsuperscript{9} and 2) neutrophils have been recently implicated in the propagation of infarction in several myocardial infarction models.\textsuperscript{6,7,13} Why results would differ with pretreatment as opposed to posttreatment is unclear.

Acknowledgments

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References

17. Uchida Y, Murao S: Role of prostaglandin I\textsubscript{2} and thromboxane A\textsubscript{2} in recurring reduction of carotid and cerebral blood flow in dogs. Stroke 12:786–792, 1981

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Address reprint requests to: Nazih A. Moufarrij, M.D., Box 294, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44106.