Inhibition of Thromboxane A₂ Production Does Not Improve Post-Ischemic Brain Hypoperfusion in the Dog

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SUMMARY In a canine model of global brain ischemia, six dogs received a selective thromboxane A₂ synthetase inhibitor, UK 38,485 (dazmagrel) before the ischemic event; six received a saline placebo. Cerebral blood flow (CBF), systolic and diastolic arterial pressure, cardiac output, pH, PaCO₂, PaO₂, and arterial and jugular-vein thromboxane B₂ (a stable metabolite of thromboxane A₂) and 6-keto PGF₁α (a stable metabolite of prostacyclin) were measured at baseline, after release of aortic and venae caval occlusion and at intervals up to 120 min thereafter. Treated animals showed nearly complete post-ischemic inhibition of thromboxane B₂ production; control animals showed increases in jugular venous thromboxane B₂. Arterial and jugular venous levels of 6-keto PGF₁α were significantly higher in treated animals at most post-ischemic intervals. CBF in both groups was similar to baseline values at time 0, then declined similarly in both groups by 30 min to ≈35% of baseline values where it remained thereafter. There were no significant differences in other variables at any interval. We conclude that inhibition of thromboxane A₂ production does not alter post-ischemic brain hypoperfusion.

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ONLY 30% of patients hemodynamically resuscitated from in-hospital cardiac arrest are ultimately discharged from the hospital alert, oriented, and responding appropriately.¹ Among patients who are comatose after resuscitation, only 13% regain independent function.² A sustained decrease in cerebral blood flow after global brain ischemia has been implicated in the progression of neurological deficits after resuscitation. The post-ischemic changes that potentially are involved in the progression of those deficits recently have been summarized.³,⁴ Agents that increase cerebral blood flow during that period have been associated with improved neurological outcome in primates and dogs.⁵–⁸

Metabolites of arachidonic acid, released from vascular endothelium and blood cellular elements, may be among the factors that produce post-ischemic hypoperfusion.⁹ Calcium is increased in intracellular fluid during cerebral ischemia,¹⁰ and the enzyme phospholipase A₂, which releases arachidonic acid from cell membranes, is activated by increased calcium levels.¹¹ Arachidonic acid is the substrate from which leukotrienes are synthesized by lipoxygenase and related enzymes and from which prostaglandins, such as prostacyclin (PGI₂) and thromboxane A₂ (TXA₂), are produced through cyclo-oxygenase pathways.¹² Potentially neurotoxic mediators, such as superoxides, may also be released in association with the increased metabolism of arachidonic acid.¹³–¹⁴ PGI₂ and TXA₂ have opposing effects on the microvasculature, PGI₂ producing vasodilation and platelet disaggregation, TXA₂ producing vasoconstriction and platelet aggregation.¹⁵ Infusion of indomethacin to inhibit cyclo-oxygenase and PGI₂ to produce cerebral vasodilation has been shown to promote post-ischemic neurologic recovery in rats and dogs.⁹,¹³

Further evidence that arachidonic acid may play a central role in post-ischemic brain hypoperfusion is the increased excretion of thromboxane B₂ (TXB₂), the stable metabolite of TXA₂, following ischemic stroke in man; that increased excretion correlates with the severity of the neurological insult.¹⁶ Recently, potent, highly selective inhibitors of thromboxane synthetase, the enzyme that produces TXA₂ from the cyclic endoperoxide products of cyclo-oxygenase, have been developed and tested in animals and man.¹⁷,¹⁸ In addition to inhibiting the production of TXA₂, thromboxane synthetase inhibitors also lead to an increased production of PGI₂.¹⁷

We undertook a study of global brain ischemia in a canine model to determine whether the thromboxane synthetase inhibitor UK 38,485 (dazmagrel) decreases the release of TXA₂ and increases the release of PGI₂, thereby increasing cerebral blood flow following global brain ischemia.

Methods

This study was approved by the animal care committee at Wake Forest University Medical Center, Bowman Gray School of Medicine. Twelve mongrel dogs weighing 15–25 kg were anesthetized with thiopental, 8–10 mg/kg intravenously, followed by succinylcholine 1.5 mg/kg. Each animal was endotracheally intubated and ventilated at an FiO₂ of 0.4 and a tidal volume of 15 ml/kg at a rate adjusted to produce a PaCO₂ of 35–45 mm Hg. Halothane 1.0% in oxygen provided maintenance anesthesia for the remainder of the preparation.
A right femoral arterial catheter was placed percutaneously and connected, via a Statham P-23Db transducer, to a Grass model 79D oscillograph. A 7.5 Fr pulmonary artery catheter was passed percutaneously through the right external jugular vein using the Seldinger technique. A left external jugular catheter was passed in a retrograde direction (i.e., cephalad) until resistance was encountered, at which time the catheter was withdrawn 2.0 mm and secured. The animal was then placed in the left lateral decubitus position and a thoracotomy performed in the fifth interspace. The azygos vein was isolated and ligated. The superior and inferior venae cavae were dissected free and snares were passed circumferentially and loosely secured. The pericardium was incised and umbilical tape was passed circumferentially around the aorta at a point immediately proximal to the origin of the brachiocephalic artery. The thoracotomy wound was loosely approximated and the animal was turned to the prone sphinx position. The temporalis muscles were dissected from the cranium using a periosteal elevator. A cadmium telluride gamma detector was placed over each posterior parietal area.

Data Collected

The following data were collected at intervals to be specified below: cerebral blood flow (CBF), PaCO₂, systolic and diastolic arterial pressure, cardiac output, pH, PaCO₂, PaO₂, central venous pressure, and heart rate. Blood samples were collected from the jugular vein and the systemic arterial catheter for radioimmunoassay of TxB₂ and 6-keto PGF₁α, the stable metabolites of TxA₂ and PGI₁, respectively.

Sampling Intervals

Baseline data were collected after the animal had been surgically prepared and halothane had been discontinued for 30 min, during which time the animal was ventilated with a 60:40 mixture of nitrous oxide and oxygen. Immediately after collection of baseline systemic hemodynamic and CBF data, the animal was then assigned to 1 of 2 groups: control animals (n = 6) received 5.0 ml of saline intravenously; treated animals (n = 6) received 1.5 ml/kg of UK 38,485 intravenously in 5.0 ml of saline. The animal was then ventilated with 100% oxygen for 1 min, following which the aorta and venae cavae were occluded. Occlusion was maintained for 10 min. After 9 min and 30 sec of occlusion, sodium bicarbonate 1 mEq/kg was administered intravenously through a peripheral catheter. No other resuscitative drugs were administered. Following occlusion, ventilation was varied to maintain PaCO₂ between 35 and 45 mm Hg and PaO₂ greater than 80 mm Hg; pH was permitted to vary, i.e., no further NaHCO₃ was administered. Hemodynamic and CBF data were collected at time 0 (immediately after release of occlusion) and at times 30, 60, 90, and 120 min following release of occlusion. Blood samples were obtained for determination of TxB₂ and 6-keto PGF₁α at time 0 and at times 10, 30, and 60 min.

Cerebral Blood Flow Measurements

All CBF measurements were performed using ¹³³Xenon, dissolved in sterile saline and injected rapidly intravenously in a dose of 5.0 mCi. CBF was calculated from the clearance curve recorded by each gamma detector using the initial slope index method. The CBF data were displayed on a portable CBF computer with a colorgraphic display during accumulation to eliminate atypical Xenon clearance curves. Data were stored in a Vax 730 computer for later analysis.

Radioimmunoassay Techniques

Simultaneous 4.0-ml samples of blood were withdrawn from the aortic and jugular-vein catheters into sterile disposable syringes and immediately transferred to test tubes containing 0.5% EDTA and indomethacin (25 µg/ml whole blood) and maintained on ice at 4°C until centrifuged at 1500 g for 15 min. The plasma was aspirated and placed in polypropylene test tubes, which were then labelled and stored at −20°C. Immunoreactive TxB₂ and immunoreactive 6-keto PGF₁α were assayed directly in plasma by standard double-antibody radioimmunoassay techniques. Cross reactivities and sensitivity limits of these antibodies and reagent sources have been described.

Statistical Methods

All data were analyzed by the SAS statistical program using analysis of variance of repeated measures. Individual differences between groups for the primary variables TxB₂, 6-keto PGF₁α and CBF were confirmed using the Student’s t-test: One-tailed tests were applied to comparisons of TxB₂ levels between groups; all other tests were two-tailed.

Results

In control animals, immunoreactive TxB₂, measured in jugular venous blood was increased following release of aortocaval occlusion and was still increased at time 60 min post-ischemia (fig. 1). In contrast, treated animals had no increase in immunoreactive TxB₂ levels in jugular venous blood, and the difference between jugular venous TxB₂ levels in the two groups was significant at all intervals following occlusion. Arterial levels of TxB₂ tended to be lower than jugular venous levels in control animals at all time intervals (table 1). In contrast, arterial levels of TxB₂ in treated animals were similar to baseline at time 0 and progressively decreased thereafter. At no interval were arterial levels significantly different from jugular venous levels in treated animals.

Immunoreactive 6-keto PGF₁α levels in jugular venous blood increased in control animals following release of aortocaval occlusion (fig. 2). In treated animals, levels were higher than in control animals at times 0, 10, 30, and 60 min post-ischemia, and those differences attained statistical significance at times 0 and 10 min. Arterial levels of immunoreactive 6-keto PGF₁α were significantly higher in treated animals than in control animals at all intervals following release of occlusion. There were no significant differences between arterial
and jugular venous levels of 6-keto PGF₁α within either group at any time interval.

Systolic and diastolic blood pressure, PaCO₂, cardiac output, central venous pressure, and heart rate were similar in both groups of animals at all intervals before and after release of occlusion. Systolic and diastolic blood pressures quickly returned to control levels in all animals, usually within 60 sec following release of the aortocaval occlusion. PaO₂ was similar in both groups at all intervals and consistently exceeded 80 mm Hg in all animals. pH ranged from 7.20 to 7.42 following occlusion and was similar between groups.

CBF (fig. 3) was similar to baseline in both groups immediately after release of occlusion; it then decreased to levels approximately 35% of baseline values at times 30, 60, and 120 min. There were no significant differences between the groups.

Discussion

The canine model of global cerebral ischemia has been used extensively to test the ability of pharmaco-logic agents to modify post-ischemic hypoperfusion and to protect against post-ischemic neuronal damage. The model used in this study differs in several respects from previously reported models in other studies. First, we ligated the azygos vein before reversibly occluding the superior and inferior venae cavae and aorta. Second, by using pulmonary artery pressure measurements to adjust filling of the cardiopulmonary circuit during the period of systemic ischemia, we avoided excessive cardiac dilatation during the course of the occlusion and presumably preserved stable coronary artery filling pressures by maintaining adequate preload. Third, we measured CBF by measuring the clearance of 133Xenon. Since measurement of CBF by this technique requires at least 10 min of data collection, we did not detect the transient post-ischemic hypoperfusion that had been reported by other investigators.

We measured jugular venous levels of immunoreactive arachidonate metabolites rather than levels in the sagittal sinus or at the confluence of the sagittal and lateral sinuses because we found in preliminary experi-

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial TxB₂ (ng/ml plasma)</td>
<td>1</td>
<td>0.62±0.10</td>
<td>1.42±0.48</td>
<td>1.60±0.20*</td>
<td>2.71±0.83†</td>
<td>2.14±0.49†</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.32±0.65</td>
<td>1.50±0.64</td>
<td>0.35±0.13</td>
<td>0.30±0.08</td>
<td>0.27±0.13</td>
<td>—</td>
</tr>
<tr>
<td>Arterial 6-keto PGF₁α (ng/ml plasma)</td>
<td>1</td>
<td>0.10±0.02</td>
<td>0.68±0.05</td>
<td>0.54±0.11</td>
<td>0.30±0.09</td>
<td>0.16±0.05</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.18±0.04</td>
<td>1.04±0.09†</td>
<td>0.84±0.11†</td>
<td>0.66±0.14†</td>
<td>0.49±0.16†</td>
<td>—</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>1</td>
<td>125±12</td>
<td>127±10.0</td>
<td>—</td>
<td>109±6.7</td>
<td>119±7.3</td>
<td>133±13.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>133±9.0</td>
<td>127±10.0</td>
<td>—</td>
<td>117±6.7</td>
<td>129±6.8</td>
<td>154±11.0</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>1</td>
<td>75±9.6</td>
<td>79±5.4</td>
<td>—</td>
<td>70±3.7</td>
<td>76±4.8</td>
<td>97±10.1</td>
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<tr>
<td></td>
<td>2</td>
<td>84±7.7</td>
<td>84±6.5</td>
<td>—</td>
<td>71±5.5</td>
<td>83±5.4</td>
<td>96±4.7</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>1</td>
<td>39.8±1.7</td>
<td>49.3±3.1</td>
<td>—</td>
<td>38.4±1.9</td>
<td>43.5±1.6</td>
<td>36.0±2.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>37.2±3.1</td>
<td>40.1±3.9</td>
<td>—</td>
<td>38.8±4.2</td>
<td>39.8±3.6</td>
<td>44.0±6.6</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>1</td>
<td>2.6±0.3</td>
<td>2.4±0.3</td>
<td>—</td>
<td>1.8±0.2</td>
<td>1.9±0.2</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.3±0.2</td>
<td>2.0±0.1</td>
<td>—</td>
<td>1.7±0.1</td>
<td>2.0±0.2</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>1</td>
<td>10.5±4.9</td>
<td>9.5±3.8</td>
<td>—</td>
<td>5.7±1.3</td>
<td>4.5±1.0</td>
<td>8.0±2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.7±1.4</td>
<td>9.7±2.4</td>
<td>—</td>
<td>3.7±0.6</td>
<td>6.3±1.1</td>
<td>10.2±3.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>1</td>
<td>144±9.1</td>
<td>134±13.9</td>
<td>—</td>
<td>151±16.1</td>
<td>145±13.8</td>
<td>160±17.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>150±17.3</td>
<td>140±9.5</td>
<td>—</td>
<td>140±14.1</td>
<td>149±10.7</td>
<td>127±19.8</td>
</tr>
</tbody>
</table>

Group 1 = control; Group 2 = UK 38,485. *p < 0.001; †p < 0.05; ‡p < 0.01, all Group 1 versus Group 2.
ments that the combination of heparin, used to prevent clotting in the sagittal sinus, and UK 38,485 produced uncontrollable bleeding from the cranial dissection site. Consequently, although the majority of canine CBF drains through the external jugular vein,27 there may have been extracerebral contamination of our jugular venous samples. In addition, the baseline levels of TxB₂ were somewhat elevated, probably reflecting the release of TxA₂, that accompanies vascular catheterization. We have no explanation for the small differences between groups in baseline levels of TxB₂ and 6-keto PGF₁α, although we speculate that variability may have reflected catheter-induced activation.

These data suggest that TxA₂ and PGI₁ are released in large quantities following global cerebral ischemia, and that the release of TxA₂ may be greater from the cerebral than from the systemic circulation. However, they do not examine the question of the origin, i.e., blood vessel or brain tissue, of the increased plasma levels of TxB₂ and 6-keto PGF₁α. We speculate that the origin is the vessel wall or intraluminal cellular elements. Thus, plasma measurements of the circulating vasoactive compounds may be more pertinent to a study of the hypoperfusion state than would be tissue levels.

Further, these data suggest that inhibiting the conversion of cyclic endoperoxides to TxA₂ increases the synthesis of PGI₁, in post-ischemic blood vessels. These findings are consistent with those in the study of Maguire and Wallis in rats,28 but in our study inhibition of the synthesis of TxA₂, did not modify the state of cerebral hypoperfusion seen following global ischemia. Failure of thromboxane synthetase inhibition to improve perfusion is consistent with data in cats treated with UK 38,485 before and after an acute focal cerebral ischemic lesion,28 and with those in a rabbit model of global cerebral ischemia.29 Logically, inhibiting the production of a potent cerebral vasocostructor in conjunction with increasing synthesis of a potent cerebral vasodilator should improve cerebral blood flow in the post-ischemic period.

On first examination, the failure of thromboxane synthetase inhibition to improve CBF in this model suggests 1) that TxA₂ is not an important factor in the production of the global hypoperfusion seen after global cerebral ischemia, and 2) that further increases in physiologic levels of prostacyclin produced by the inhibition of thromboxane synthetase are insufficient to dilate the cerebral vessels. These data appear to contradict those from the study of Hallenbeck et al. in which the administration of indomethacin (which would inhibit production of both TxA₂ and PGI₁) in conjunction with the intravenous infusion of PGI₁, ameliorated post-ischemic cerebral hypoperfusion.15 It is possible that the pharmacologic doses of prostacyclin used in that study produced levels in the cerebral vessels that were greater than those produced in our study.

However, cyclo-oxygenase inhibition would also decrease other potential vasoconstricting products of the cyclo-oxygenase pathway. In fact, a possible explanation for the failure of thromboxane synthetase inhibition to ameliorate post-ischemic hypoperfusion may be that inhibition of thromboxane synthesis not only redirects arachidonic metabolism toward the production of PGI₁ but also increases the amount of substrate available for metabolism to other eicosanoids. The substrata for thromboxane synthetase, the cyclic prostaglandin endoperoxides, also cause vasoconstriction and platelet aggregation.30 31 Accumulation of cyclic endoperoxides may explain the surprisingly limited effect of thromboxane synthetase in this as in other studies.31 Antagonists of thromboxane and cyclic endoperoxide receptors may produce more useful pharmacologic effects than inhibitors of thromboxane synthetase.30 In addition, just as the quantity of the prostacyclin metabolite present in jugular venous blood increased following inhibition of thromboxane synthetase, it is possible that lipoxigenase products were similarly increased.28 The role, if any, of lipoxigenase-derived metabolites of arachidionate has not been well characterized in the post-ischemic state.

Another possible explanation for the failure of thromboxane synthetase inhibition to ameliorate post-ischemic cerebral vasocostriction may be that increased levels of TxA₂ are not an etiologic component of post-ischemic cerebral hypoperfusion. Further studies are necessary to determine whether other interventions, such as nimodipine, which clearly increase CBF following global cerebral ischemia, are associated with decreased cerebral venous release of TxB₂. The release of TxB₂ and 6-keto PGF₁α may serve as useful markers of the extent of vascular injury following global cerebral ischemia.

In summary, inhibiting the production of TxA₂ failed to alter the marked reduction in CBF that followed a 10-min period of global cerebral ischemia in dogs. Cerebral blood flow remained low despite evidence of markedly decreased production of TxA₂ and reciprocally increased production of PGI₁.

Acknowledgment

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References


![Cerebral blood flow in dogs after 10 minutes of aorto caval occlusion.](http://stroke.ahajournals.org/DownloadedFrom)
Inhibition of thromboxane A2 production does not improve post-ischemic brain hypoperfusion in the dog.

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