Modification of focal cerebral ischemia by prostacyclin and indomethacin

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The object of this investigation was to study the effects of prostacyclin (PGI₂), with and without indomethacin, upon the evolution of cerebral infarction in the cat. Thirty-five fasted adult cats, lightly anesthetized with nitrous oxide, underwent right middle cerebral artery (MCA) occlusion. Eleven cats received an intracarotid infusion of 10 mg/ml PGI₂ in buffered saline, pH 10.5, at a rate of 0.01 ml/kg/min (100 ng/kg/min), 10 cats received the same infusion plus a single dose of intravenous indomethacin (4 mg/kg) in buffered saline, and 11 cats received intracarotid buffered saline, pH 10.5, at a rate of 0.01 ml/kg/min, without therapeutic agents. Treatment with PGI₂ was started upon MCA occlusion and continued for 6 hours, whereas indomethacin was given immediately prior to occlusion. Thirty minutes before perfusion, the animals were given fluorescein and Evans blue by intravenous injection. The cats were perfusion-fixed in vivo with carbon and buffered formalin 6 hours after MCA occlusion. Another five cats received tritium-labeled PGI₂, and peripheral venous samples were collected and assayed for PGI₂ and its alpha-keto metabolite. Mean arterial pressure was stable in treated animals during 6 hours of MCA occlusion, while untreated cats had significant (α = 0.05) progressive hypertension during that period. The regional cerebral blood flow (rCBF), measured by the intracarotid xenon-133 clearance method, decreased markedly in all animals immediately upon MCA occlusion. Untreated animals had a significant progressive improvement in rCBF during the occlusion period (α = 0.005), while treated animals had no such improvement. Quantitative electroencephalographic changes, gross edema, areas of fluorescein extravasation, and microscopic morphology (edema and infarct size) were not significantly different in the three groups. Prostacyclin appeared to reduce the extravasation of Evans blue dye. Systemic PGI₂ levels were significant despite intracarotid administration. The authors conclude that 1) intracarotid PGI₂ has a protective effect against the breakdown of the blood-brain barrier to protein-bound dyes seen in ischemic edema; 2) the systemic hemodynamic influence of PGI₂, in the presence of impaired autoregulation, may compromise rCBF in the ischemic zone and offset any direct beneficial effects; and 3) indomethacin fails to modify the effects of PGI₂.

Key Words • blood-brain barrier • cerebral blood flow • cerebral ischemia • indomethacin • prostacyclin

Prostacyclin (PGI₂) is a prostaglandin with a short half-life, synthesized in large quantities in blood vessel walls, including large and small cerebral arteries. It is a potent vasodilator and inhibitor of platelet aggregation. Another short-lived prostaglandin, thromboxane A₂ (TXA₂), is synthesized in the platelet itself, and possesses vasoconstricting and platelet-aggregating properties. Both compounds exert their effect via the intracellular messenger, cyclic adenosine monophosphate (cAMP), with PGI₂ increasing intracellular cAMP concentration, and TXA₂ decreasing it. Under physiological conditions, a precise balance exists between the effects of PGI₂ and TXA₂. It has recently been suggested that this PGI₂-TXA₂ homeostasis may be disturbed in the ischemic brain. This may contribute to the state of microcirculatory impairment, and in turn may aggravate ischemic damage. Proponents of this hypothesis have proposed that exogenously administered PGI₂ may be beneficial in reversing ischemic injury. Furthermore, the prostaglandin synthesis inhibitor, indomethacin, may amplify these beneficial effects of PGI₂ by limiting the endogenous synthesis of TXA₂.

The objective of this investigation was to study the effects of PGI₂ upon the evolution of cerebral infarc-
Modification of experimental cerebral ischemia

tion after right MCA occlusion in the cat, and to
determine whether these effects can be modified by
indomethacin.

Materials and Methods

Experimental Groups

Thirty-seven fasted adult cats, lightly anesthetized
with nitrous oxide, underwent right middle cerebral
artery (MCA) occlusion via the transorbital approach.
A small catheter was inserted into the right carotid
artery through the lingual artery, and secured with
a ligature. Eleven cats received a continuous intracarotid
infusion of 0.9% saline buffered to pH 10.5 with
Na₂CO₃ and containing 10 mg/ml of PGI₂ given at a
rate of 0.01 ml/kg/min (100 ng/kg/min); 10 cats
received the same infusion plus a single dose of intra
venous indomethacin (4 mg/kg) freshly suspended in
2 ml of buffered saline; and 11 cats received intra
carotid buffered saline, pH 10.5, at a rate of 0.01 ml/
kg/min, without therapeutic agents. Treatment with
PGI₂ was started upon MCA occlusion and continued
for 6 hours, whereas indomethacin was given imme-
diately prior to occlusion.

Protocol

Prostacyclin solutions were prepared immediately
prior to the experiment from a more concentrated
solution (1.0 mg/ml). The infused material was kept
on ice throughout the treatment period. The infused
solution was periodically assayed for PGI₂ concen-
tration, using high-pressure liquid chromatography
(HPLC).32 No significant breakdown of PGI₂ was
noted under these conditions. Throughout the exper-
iment, arterial blood pressure, pulse rate, and the
electroencephalogram (EEG) were monitored contin-
uously. The EEG electrodes were placed in the border
zone between the anterior cerebral artery and MCA
territories, and not in the core of ischemia.18,21
Regional cerebral blood flow (rCBF) was measured by
the xenon-133 (¹³³Xe) clearance technique, after
right intracarotid injection of 200 mCi of ¹³³Xe in 0.5
ml normal saline.30 A collimated 1.5-cm sodium iodide
crystal, recessed 5.0 cm, was applied to the skull
overlying the right Sylvian cortex after retraction of
the scalp and temporalis muscle to avoid extracranial
contamination. These rCBF measurements were per-
formed immediately before and immediately after
MCA occlusion, and at 3 and 6 hours after occlusion.

Thirty minutes before the end of the ischemic pe-
riod, Evans blue and sodium fluorescein (0.5 ml of a
10% solution of each) were given intravenously. Intra-
arterial perfusion of a carbon fixative was carried out
at the end of the 6-hour ischemic period. A midline
thoracotomy was performed, and a large cannula was
passed through a left ventriculostomy into the ascend-
ning aorta and secured with a ligature. The descend-
ing aorta was clamped and the right atrium incised. The
right MCA was reopened by removing the aneurysm
clip in order to improve delivery of the carbon fixative
solution to the ischemic tissue. The animals were then
perfused with 50 ml of isotonic saline followed by a
mixture of colloidal carbon (125 ml) and phosphate-
buffered 4% formaldehyde (125 ml) at a constant
pressure of 120 mm Hg. The brain of each cat was
removed, sliced coronally, and placed in a fixative
solution for 48 hours.

Studies

The brain slices were photographed, and the pre-
ence or absence of fluorescein or Evans blue staining
was noted. The shift of the midline structures, if any,
was measured. The distribution of carbon staining
was graded on a scale of 0 to 3, according to a
previously described system.5,23 Grade 0 indicated
normal carbon filling in cortical and subcortical gray
matter, while Grade 3 referred to extensive cortical
and subcortical regions of impaired carbon filling.

Thin semi-serial coronal sections were prepared
from paraffin-embedded slides of both hemispheres,
stained with hematoxylin and eosin and periodic acid
Schiff stains, and examined with the light microscope.
Ischemic neuronal alterations were graded blindly by
a single investigator according to a previously estab-
lished classification (Grades 1, 2, or 3).17,23 The cross-
sectional area of gray matter, where moderate and
severe neuronal alterations (that is, Grades 2 and 3)
predominated, was determined using planimetric
measurements on photographs of coronal sections of
the right cerebral hemispheres 3 mm posterior to the
temporal lobe tip. The percentage of gray matter
surface area where moderate and severe ischemic
neuronal alterations predominated was also deter-
mined (ischemic gray area/total gray area × 100).

Five additional animals received an intracarotid
PGI₂ infusion containing a known fraction of tritium-
labeled PGI₂. The total PGI₂ concentration in the
infused solution and the rate of infusion were identical
to those used in the treated animals. Peripheral ve-
 nous samples were collected at 15-minute intervals
and assayed for tritium-labeled PGI₂ using a chemi-
al assay developed in our laboratory.23 The assay
technique combines HPLC for separation of PGI₂
from its metabolites and radiochemical techniques
to achieve the great degree of sensitivity required for
detecting blood levels.

Data Analysis

Hemodynamic data (blood pressure and pulse)
were compared between the treated and untreated
groups to verify initial comparability (t-test) and to
detect changes over time (regression analysis and
paired t-test). The rCBF, EEG, and infarct size data
were analyzed using the Wilcoxon rank sum test. This
nonparametric test does not assume normal distribu-
tion of the data and detects differences based on the
ranks of the values rather than the values themselves.

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Results

Mean arterial blood pressures and mean pulse rates for the three animal groups are presented in Fig. 1. The PGI₂-treated animals and untreated animals showed a significant ($\alpha = 0.05$) decreasing trend in pulse rate over the 6-hour occlusion period. This trend was less pronounced in the PGI₂-treated group. No such decrease in pulse rate was observed in the animals receiving indomethacin and PGI₂. The arterial blood pressure showed a significant ($\alpha = 0.05$) increasing trend in the untreated animals but not in either of the treated groups.

The results of the $^{133}$Xe rCBF determinations are illustrated in Fig. 2. There was a pronounced decrease in rCBF in all animals upon occlusion of the MCA. This was followed by a smaller progressive improvement in rCBF throughout the period of ischemia. This improvement in rCBF was more prominent and achieved statistical significance only in the untreated group ($p = 0.005$).

The EEG data were analyzed quantitatively, comparing right and left amplitudes, and analyzing voltage changes during the course of ischemia. All animals exhibited a decrease in amplitude on the right side after MCA occlusion. This worsened gradually, reaching a plateau after 3 to 4 hours. Some animals showed an improvement in EEG during the 6-hour ischemic period. Taking each group as a whole, there were no significant differences between groups for each postocclusion period. Moreover, intragroup variabilities were great.

Gross swelling and carbon perfusion data are summarized in Table 1. There appeared to be no differ-
Modification of experimental cerebral ischemia

TABLE 1
Gross swelling and carbon perfusion data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Untreated Group</th>
<th>Treated Groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>no. of cats</td>
<td>PGI2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>midline rt-to-lt shift</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>0-0.5 mm</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.5-1 mm</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>1-1.5 mm</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>&gt; 1.5 mm</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>carbon perfusion*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no pallor</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Grade 1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Grade 3</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

* Grade 1 = mild carbon filling; Grade 2 = moderate carbon filling defect; Grade 3 = severe carbon filling defect.

TABLE 2
Extravasation of dyes

<table>
<thead>
<tr>
<th>Degree of Extravasation</th>
<th>Untreated Group</th>
<th>Treated Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGI2</td>
<td>PGI2 &amp; Indo-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methacin</td>
</tr>
<tr>
<td>fluorescein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
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<td>0</td>
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<tr>
<td>moderate</td>
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<td>3</td>
</tr>
<tr>
<td>marked</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Evans blue dye</td>
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<td></td>
</tr>
<tr>
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<td>2</td>
<td>7</td>
</tr>
<tr>
<td>moderate</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>marked</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>total cats</td>
<td>11</td>
<td>11</td>
</tr>
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</table>

TABLE 3
Infarct size in untreated and treated animals *

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Infarct Index (%)</th>
<th>No. of Cats</th>
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<tbody>
<tr>
<td>untreated</td>
<td>47</td>
<td>11</td>
</tr>
<tr>
<td>PGI2</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>PGI2 &amp; indomethacin</td>
<td>54</td>
<td>10</td>
</tr>
</tbody>
</table>

* Infarct index = ischemic gray area/total gray area x 100.

ence in gross swelling or obstruction to carbon filling among the three groups. The majority of animals in all three groups showed diffuse extravasation of fluorescein after 6 hours of MCA occlusion (Table 2). The fluorescein staining was present in both gray and white matter in the right MCA territory. Nine animals in the untreated group showed some degree of Evans blue staining in the right hemisphere, while only four animals showed such staining in either treatment group (Table 2). The Evans blue staining was usually limited to gray matter areas.

Severe ischemic neuronal alterations were present in the caudate nucleus and/or cortex supplied by the right MCA of 10 untreated animals, 11 treated with PGI2, and nine treated with PGI2 and indomethacin. The mean percentage of gray matter surface area with moderate or severe neuronal alterations was not statistically different among the three groups (Table 3).

Figure 3 shows PGI2 concentrations in femoral venous blood during right intracarotid infusion of a tritium-labeled PGI2 solution (100 ng/kg/min) in five additional animals. The right MCA was clipped at the onset of the infusion. Significant peripheral PGI2 concentrations were detected within 5 minutes of the onset of infusion. A plateau was approached within 60 minutes.

Discussion

During the course of focal cerebral ischemia, a state of progressive microcirculatory impairment develops. Carbon perfusion studies demonstrate patchy nonperfused areas within the ischemic zone. These have been interpreted as a "no-reflow" phenomenon analogous to that seen in global ischemia. However, more recent investigations of erythrocyte and plasma transit time, and studies of collateral artery resistance, all indicate a progressive increase in microcirculatory resistance but persistence of microcirculatory flow during focal ischemia, despite the apparent lack of flow on carbon perfusion. Several mechanisms have been proposed to account for this phenomenon, including edema, vasospasm, and intravascular coagulation. While the significance of this microcirculatory impairment in causing or aggravating ischemic damage remains speculative, maintenance of microcirculation with mannitol appeared to decrease ischemic injury in animal studies.

Several indirect lines of evidence suggest that an imbalance in PGI2-TXA2 homeostasis may play a role in ischemic microcirculatory impairment. Free fatty acids, including arachidonic acid, are released in great quantities into the ischemic brain of various species. Under conditions of incomplete ischemia, or during reperfusion after complete ischemia, there is also accumulation of cyclo-oxygenase products of arachidonic acid metabolism, including vasoactive prostaglandins. No such accumulation occurs during complete ischemia, presumably because the enzyme cyclo-oxygenase requires small amounts of oxygen. Furthermore, heparin, indomethacin, and PGI2 were found to prevent circulatory deficits during reperfusion after generalized compression ischemia, and to promote postischemic neuronal recovery after transient multifocal ischemia in dogs.

The animal model of acute focal cerebral ischemia used in this investigation has been widely used by us and others. The events that follow experimental occlusion of the MCA in cats appear to resemble the neurological and pathological changes in acute major artery occlusion in humans.

In the present study, we chose intracarotid admin-
istration of PGI\(_2\) to insure maximal delivery of this short-lived compound to the ischemic zone via collateral channels and to minimize its systemic side effects. The dosage used was in the same range as that employed by others in various animal models,\(^{11,12}\) and was, in our experience, the highest intracarotid dose not accompanied by hypotension in the cat.

Prostacyclin had a significant effect on the hemodynamic response during focal ischemia. The PGI\(_2\)-treated animals maintained stable mean arterial pressure throughout 6 hours of MCA occlusion, while untreated animals showed a significant progressive increase in arterial pressure during that period. A decrease in pulse rate was observed in all animals during the ischemic period, but untreated animals exhibited more pronounced bradycardia. Since intracranial pressure was not monitored in this study, we cannot rule out an improvement in intracranial pressure in animals receiving PGI\(_2\) as the cause for these hemodynamic changes. However, direct hemodynamic effects of PGI\(_2\) appear a more likely cause in light of what is known about the systemic effects of this agent (diffuse vasodilation).\(^{28}\) Despite intracarotid infusion, our pharmacokinetic data indicate the presence of detectable peripheral levels of PGI\(_2\) with this regimen.

Untreated animals had significant progressive improvement in rCBF during the 6-hour occlusion period. This may be due to the progressive increase in arterial pressure, to the recruitment of new collateral channels, or to hemodiluting effects of the intracarotid buffer infusion. Animals treated with PGI\(_2\), and PGI\(_2\) and indomethacin, showed no significant improvement in rCBF during the occlusion period.

Electroencephalographic changes in the ischemic border zone were not significantly different in the three groups. The findings in our study were similar to those of Hossman and Schuerer,\(^{14}\) who also described a decrease in amplitude during focal ischemia rather than slow-wave activity.

Alterations of the blood-brain barrier permeability to vital dyes during focal ischemia in this experimental model have been described previously.\(^{19}\) There appears to be early leakage of fluorescein into the ischemic zone. Since fluorescein exists in the plasma primarily in an unbound state, this is consistent with the breakdown of the blood-brain barrier to small molecules. Three to 6 hours after MCA occlusion, there is leakage of the protein-bound Evans blue dye into ischemic gray matter, suggesting a breakdown of the blood-brain barrier to large molecules. Current concepts of ischemic edema describe a "primary phase," associated predominantly with membrane pump failure, and a "secondary phase" with a lower rCBF threshold, coinciding with frank membrane disruption and breakdown of the blood-brain barrier to large molecules.\(^{19}\) The leakage of Evans blue dye coincides with the onset of the "secondary phase" of ischemic edema.

While fluorescein leakage was present in essentially all treated and untreated animals in this study, there appeared to be some protection against Evans blue leakage in animals receiving PGI\(_2\). Iannotti, et al.,\(^{15}\) suggested a role for prostaglandins in the late phase of ischemic edema in the gerbil, but not the early phase. Our findings are consistent with this hypothesis.

Progressive impairment of microcirculatory filling with carbon perfusion has been demonstrated previously in experimental models of acute focal cerebral ischemia.\(^{22}\) This was initially thought to represent a state of so-called "no-reflow;"\(^{92}\) subsequent studies have indicated, however, that the flow persists despite an increase in microcirculatory resistance.\(^{30}\) Carbon-filling defects after 6 hours of MCA occlusion were similar in all treatment groups.

Light and electron microscopic studies in fixative-perfused animals have clearly demonstrated the morphological changes in ischemic neurons, allowing the recognition and grading of such changes.\(^{5,7,8,23}\) These changes are distinct from the so-called "dark neurons" which are thought to result from inadequate fixation.\(^{4}\) In the present investigation, the tissue studied was well fixed by in vivo perfusion and later immersion. Ischemic changes were observed in all treated and untreated animals and were consistent with previous descriptions. The mean size of infarcts was not significantly different in the three groups.

The current investigation does not rule out a role for the PGI\(_2\)-TXA\(_2\) mechanism in focal cerebral ischemia. However, it demonstrates that within the limitations of our model and the number of animals used, high doses of intracarotid PGI\(_2\) provided no beneficial effects on the electrical and morphological changes of focal cerebral ischemia. Furthermore, there is a possible compromise of rCBF in PGI\(_2\)-treated animals. Dye extravasation studies were, however, consistent with a possible protective effect of PGI\(_2\) on the blood-brain barrier. Indomethacin failed to modify these effects of PGI\(_2\).

Several hypotheses can be proposed to explain the lack of improvement of ischemic injury by PGI\(_2\). While PGI\(_2\) inhibits platelet synthesis of TXA\(_2\), there may be other sources of TXA\(_2\) and other prostaglandins involved in microcirculatory impairment.\(^{30}\) Also, since PGI\(_2\) and TXA\(_2\) probably act on platelets and vascular walls via biochemically distinct receptors rather than by simple competition at a single receptor, excessive PGI\(_2\) may not be capable of completely reversing TXA\(_2\)-induced vasoconstriction and platelet aggregation. This is suggested by the study of Hallenbeck and Furlow,\(^{11}\) where PGI\(_2\) or indomethacin alone failed to prevent microcirculatory impairment when administered after the onset of global ischemia in dogs, but a combination of PGI\(_2\) and indomethacin was beneficial. This is not borne out by our study, however, since indomethacin was found to provide no additional benefit in our model.
Modification of experimental cerebral ischemia

An alternative hypothesis is that endogenous PGI₂ released by the brain vasculature during ischemia provides the maximal beneficial effect this agent can contribute. Any additional PGI₂ may not be capable of overcoming other restrictions on microcirculation, such as edema, vasospasm, or intravascular coagulation. On the other hand, systemic hemodynamic effects in the presence of impaired autoregulation may reduce collateral blood flow and offset any direct beneficial effects PGI₂ may have on microcirculation or ischemic edema. As in all animal investigations, species variability may place additional restrictions on useful therapeutic conclusions.

Acknowledgment

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References

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