Effects of Indomethacin on rCBF During and After Focal Cerebral Ischemia in the Cat

Shuku Shigeno, M.D.,* Emanuel Fritschka, M.D.,† Taku Shigeno, M.D.,‡ and Mario Brock, M.D.‡

SUMMARY The effect of indomethacin on rCBF was studied in cats anesthetized with Nembutal either under resting conditions or with temporary middle cerebral artery (MCA) occlusion. rCBF was measured by the microsphere method. In control animals (n = 3), indomethacin (4 mg/kg, i.v.) significantly reduced rCBF by about 25% in both cortex (from 36 ± 4 to 26 ± 2 ml/100 g/min, p < 0.001) and white matter (from 36 ± 4 to 26 ± 2 ml/100 g/min, p < 0.001). After MCA occlusion rCBF was markedly decreased in the sylvian region ipsilateral to occlusion (ischemic core) (from 38 ± 4 to 14 ± 2 ml/100 g/min in cortex, 4 animals). Although pretreatment with indomethacin (4 mg/kg) (4 animals) 30 min prior to occlusion did not alter rCBF during ischemia, a marked enhancement of reactive hyperemia was observed in the ischemic core immediately upon reperfusion following 2 h occlusion (54 ± 11 untreated vs 95 ± 13 treated, p < 0.05). In the delayed postischemic period, namely 2 h after recirculation, rCBF still remained to be higher in the animals treated with indomethacin (40 ± 6 untreated vs 96 ± 9 treated, p < 0.001). Such an effect of indomethacin for ameliorating postischemic blood flow in both the immediate and delayed period was less prominent in the adjacent area (penumbra) ipsilateral to occlusion. In the contralateral hemisphere, indomethacin caused slight reduction in rCBF during ischemia. An altered relationship between the actions of PGI2 and TXA2 has been proposed to occur at the blood-endothelium interface during reperfusion after ischemia in which a disproportionate synthesis of TXA2 might be suppressed by indomethacin, whereas indomethacin dominantly suppresses PGI2 synthesis under normal conditions.

AN ALTERED RELATIONSHIP between the actions of several prostaglandins (PGs) such as prostacyclin (PGI2) and thromboxane A2 (TXA2) may occur in pathological situations, whereas a balanced interaction is maintained under normal conditions. In addition to the general agreement that PGI2 is produced in vascular endothelia, and TXA2 in platelets, there is increasing evidence that TXA2 synthesis can also occur in the vascular endothelium, where a dominant but opposing role of PGI2 with respect to TXA2 has been documented. Thus previous results which have shown a decrease in basal cerebral blood flow with indomethacin treatment might be indicative of preferential suppression of PGI2 synthesis at the vascular endothelium, although indomethacin is thought to inhibit more or less all the products of cyclooxygenase.19 In cerebral ischemia, a significant accumulation of cyclooxygenase products is known to occur, particularly when the ischemia is either transient or incomplete.20-22 Although the pathogenetic role of accumulation of PGs in cerebral ischemia remains unclear, the improvement shown in cerebral microcirculation with indomethacin treatment following global cerebral ischemia suggests a reversal of roles: a relative dominance in TXA2 level during this pathological situation.23-26 Accordingly the present study was aimed at exploring whether the above relationship can be altered by treatment with indomethacin in cats with focal cerebral ischemia.

Material and methods

Adult mongrel cats weighing 3 to 4 kg were anesthetized with Nembutal® (30 mg/kg), paralyzed with Flaxedil®, and artificially ventilated with nitrous oxide/oxygen gas mixture via a cuffed endotracheal tube. Following catheterization to one femoral vein and two femoral arteries, one of the arterial catheters being introduced to the left cardiac ventricle, the animal was fastened on the stereotaxic frame in a sphinx like position. Systemic blood pressure and cardiac ventricular pressure were continuously monitored. Arterial blood gases were intermittently determined and maintained within normal limits throughout the experiment. Rectal temperature was continuously measured and kept between 36° and 38°C by an electrically heated blanket. Regional cerebral blood flow (rCBF) was measured by the microsphere method, using microspheres 15 μm in diameter and labelled with scandium-46, strontium-85, cerium-141 and iodine-125. Preparation of the microspheres and calculation of rCBF values have been described in detail elsewhere.27 In three animals rCBF was determined in a resting state before and 30 min after intravenous injection of 4 mg/kg of indomethacin (Sigma®). The solution of indomethacin was prepared just prior to the experiment by dissolving 20 mg of indomethacin in 10 ml of a 0.5% sodium bicarbonate solution followed by addition of 10 ml of a phosphate buffer (Na 149.2, K 4.2, Cl 139.6, Phosphate 8.2 mM/L), thus yielding a 1 mg/ml solution. The pH value was 7.45 ± 0.01 and the appropriate volume was delivered by an infusion pump (Braun®) over 10 min. After the experiment, brain tissues weighing 100 to 200 mg were rapidly dissected from 9 regions of the cortex and subjacent white matter...
in the cerebral hemisphere and the wet weight was
determined after storing tissues in preweighed tightly-
sealed test tubes. A paired comparison by Student's t-
test was made using the rCBF values against tissue wet
weight before and after treatment.

In eight animals, MCA was occluded and subse-
quently reperfused with (n = 4) or without (n = 4)
indomethacin pretreatment. Occlusion of MCA was
performed by a Yasargil type aneurysm clip through a
transorbital approach, and recirculation lasting 2 hours
was achieved by removing the clip 2 hours after occlu-
sion. Indomethacin (4 mg/kg) was given 30 min prior
to the first determination of rCBF which was per-
duced after the exposure of MCA but without occlu-
sion. Immediately after the first rCBF measurement,
the MCA was occluded. RCBF was determined subse-
duently 5 min after occlusion, and at 5 min and 2 hours
upon recirculation. Occlusion and recirculation of
MCA was clearly visible and successful under opera-
tive microscope in all animals. Brain tissues from both
cortex and white matter were collected from 4 regions
in the territory of MCA (ischemic core) and 4 regions
adjacent to the core (penumbra) as in figure 1. Brain
tissues in the hemisphere contralateral to occlusion
were also sampled in the same way for interhemis-
pheric comparison. The perfusion territory of MCA
was previously determined and found to be consistent
on the angioarchitectural basis by transventricular
intraval perfusion of carbon black after modification of

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Areas of brain tissue sampling. Four brain tissues
from cortex and white matter were obtained from each core and
penumbral area in the hemisphere ipsilateral to occlusion as
well as from corresponding regions in the contralateral hemi-
sphere.

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Perfusion territory of the MCA revealed as poor
filling of carbon black which was infused through the heart
while the animal was alive; a modification of the single dye
passage technique. Note the clear boundary between the supra-
sylvian and the marginal gyrus ipsilateral to occlusion.

the single dye passage technique (28) (fig. 2). Data were
pooled from each group of animals and analysed either
by unpaired Student's t-test between the treated and
untreated groups or by analysis of variance test (29) to
compare sequential changes in rCBF within the group.

### Results

#### Effect of Indomethacin on Resting Blood Flow

There were no significant changes in blood pressure
or blood gases before and after the administration of
indomethacin (table 1). A significant reduction in
rCBF by around 25% was observed after the adminis-
tration of indomethacin in both cortex and white matter
of the normal brain (table 2).

#### Changes of rCBF during and after Ischemia

Values of blood gases and blood pressure at each
time of measurement of CBF in both treated and un-
treated groups did not yield significant level of statisti-
cal difference as shown in table 3. Changes in cortical
CBF before, 5 min after occlusion (ischemia), 5 min
(hyperemia) and 2 h upon recirculation (delayed) are
summarized in figure 3. RCBF before occlusion was
lower throughout the brain in the treated group than in
the untreated group, again showing the effect of indomethacin in decreasing CBF under resting conditions. However, in the core region ipsilateral to occlusion, the pre-occlusion values were lower than in other regions in both groups which might have been caused by some operative insult in exposing the MCA. Or, since the flow value was calculated against tissue wet weight and the tissues in the ischemic core developed massive water increase in this study, the CBF values in the core could be lower than those in the other regions without increase in water content. In the untreated group, a marked flow reduction in the core was followed by immediate hyperemia exceeding the contralateral flow upon recirculation and returned toward the pre-occlusion value 2 h later. In the penumbra the reduction in rCBF during ischemia and subsequent hyperemia were less marked. In the contralateral hemisphere, sequential values of rCBF during occlusion and upon recirculation stayed unchanged in the corresponding regions to core and penumbra, although there was tendency of slight reduction in rCBF in the area corresponding to core.

By contrast in the animals treated with indomethacin, striking and sustained increases in CBF were observed in the ipsilateral ischemic core in both the immediate and delayed period upon recirculation. The decrease in rCBF during ischemia in the core did not differ significantly irrespective of the indomethacin treatment. In the penumbral region ipsilateral to occlusion, such an effect for enhancing hyperemia was less marked. Contralaterally, indomethacin only displayed an effect of slight reduction in rCBF throughout the ischemia and recirculation period without developing any hyperemic stage.

CBF changes in the white matter were similar to those in the cortex (fig. 4), where a significant and sustained increase of flow in the ischemic core following indomethacin treatment was also observed in the postischemic period. Occlusion and recirculation of MCA did not influence contralateral flow, where indomethacin had no effect, either.

**Table 2**

<table>
<thead>
<tr>
<th>Effect of Indomethacin (4 mg/kg) on Resting rCBF</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>44 ± 6</td>
<td>32 ± 3*</td>
</tr>
<tr>
<td>White matter</td>
<td>36 ± 4</td>
<td>26 ± 2*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. MABP = mean arterial blood pressure. No statistical significance was obtained either within or between groups.

**Table 3**

<table>
<thead>
<tr>
<th>Physiological Variables before, 5 min after MCA Occlusion (ischemia), 5 min (hyperemia) and 2 h (delayed) upon Recirculation with or without Indomethacin Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (n = 4)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>MABP</td>
</tr>
<tr>
<td>PO2</td>
</tr>
<tr>
<td>PCO2</td>
</tr>
<tr>
<td>pH</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. MABP = mean arterial blood pressure. No statistical significance was obtained either within or between groups.

**Discussion**

Since the effect of indomethacin on resting CBF was first reported by Pickard et al., there have been a number of reports which have shown CBF reduction by indomethacin in both normal and hypercapnic situations. In accordance with those reports, the present study in cats under barbiturate anesthesia showed a decrease in resting CBF. However, others have shown a total lack of effect on CBF. As to the microsphere method, Busija & Heistad did not observe any effect of indomethacin, while other workers observed CBF reduction. Explanation for these conflicting results is difficult but might have been caused by differences in the methods employed for CBF measurement, animal species or anesthetics.

Gaudet & Levine first reported a marked accumulation of PGs upon reperfusion following global cerebral ischemia in the gerbil. The PGs which accumulated in the postischemic period were PGE2, PGF2α, and 6-keto-PGF1α. Pretreatment with indomethacin almost completely inhibited this accumulation. Thereafter similar results on the massive accumulation of PGs in cerebral ischemia were also obtained. Using reversible global ischemia in the rat, Shohami et al. reported an accumulation of PGE2, 6-keto-F2α, and TXB2 during ischemia and upon reperfusion. Of interest to note was a further accumulation of TXB2 upon reperfusion, whereas other PGs tended to be decreased. Pretreatment with indomethacin (4 mg/kg) prior to the induction of ischemia ameliorated the increases of these PGs, particularly of TXB2, after recirculation.

The biosynthesis of prostaglandins is primarily determined by substrate availability as long as the minimum level of tissue oxygen is maintained. Kagström et al. assumed that high concentrations of arachidonic acid which accumulated during the ischemic period might trigger a burst of PG synthesis as soon as the tissue is reoxygenated. For the activity of cyclooxygenase, however, the presence of endoperoxides is
paradoxically required. It may also be assumed that hydroperoxides formed in the lipoxygenase pathway (HPETEs) which is probably involved in cerebral ischemia take part in the elucidation of cyclooxygenase activity. Once these hydroperoxides are formed and further converted to hydroxyacids by peroxydases, the active oxygen liberated concomitantly in this process is known to suppress the synthesis of PGI, but not TXA, leading to imbalance between these opposing vasoactive substances in pathological situations.

The effect of indomethacin in ameliorating impaired postschismic reperfusion was first documented by Furlow and Hallenbeck. Pretreatment with indomethacin 4 mg/kg one hour prior to ischemia improved the delayed hyperfusion. In the present study we could demonstrate that indomethacin increased postschismic flow either in the immediate period upon reperfusion or in the delayed period. The reactive hyperemia was much more enhanced and the flow still remained to be higher in the delayed period than in the untreated controls. Although the degree of interaction between PGI,

![Figure 3. Changes in rCBF in the cortex ipsilateral or contralateral to MCA occlusion with (closed bars) or without indomethacin treatment (open bars). RCBF was measured before, 5 min after MCA occlusion (ischemia), 5 min (hyperemia) and 2 h (delayed) upon reperfusion.](http://stroke.ahajournals.org/)

![Figure 4. Changes in rCBF in the white matter ipsilateral or contralateral to MCA occlusion with (closed bar) or without (open bar) indomethacin treatment. RCBF was measured before, 5 min after MCA occlusion (ischemia), 5 min (hyperemia) and 2 h (delayed) upon reperfusion. Note a marked increase in blood flow upon reperfusion in the ischemic core of the treated animals as observed in the cortex (upper left). Asterisks indicate statistical significance between untreated and treated groups by Student's t-test at each time of CBF measurement (*p < 0.05, **p < 0.01, ***p < 0.001). Circles on the top of the bars indicate statistical significance between changes in CBF during and after MCA occlusion as compared to the pre-occlusion value by Scheffe test in each treated and untreated group (°p < 0.05, °°p < 0.01).](http://stroke.ahajournals.org/)
CBF in normal subjects, but increase in patients with recent stroke.

It has been generally agreed that TXA₂ and PGI₁ interact at the blood-endothelium interface, with the site of synthesis being in the platelets for the former and vascular endothelia in the latter. Recently, however, evidence of the production of TXA₂ in vascular endothelia has arisen which suggests that the ratio of PGI₁ synthesis to that of TXA₂ is greater under normal conditions. Therefore the effect of indomethacin for decreasing resting CBF can be understood as a result from a greater suppression of PGI₁ synthesis. By contrast, following cerebral ischemia disproportionate synthesis of TXA₂ might occur, at least as judged by the amelioration of postischemic flow in the present study. As stated previously, arachidonic acid accumulated during the ischemic period is involved in the subsequent cascades after incorporation of oxygen either for cyclooxygenase reaction or for lipoxygenase reaction. Since the prostacyclin synthetase is selectively inhibited by peroxidase-derived products, possibly oxygen radicals in both pathways, disproportional synthesis of TXA₂ is readily suspected. However, evidence for this hypothesis is still indirect on account of the rather non-specific inhibition of all prostaglandin synthesis by indomethacin, or the possibility that indomethacin may not be a pure cyclooxygenase inhibitor. The recent discoveries of more specific thromboxane antagonist, either of synthetase or of receptor may be of value in order to clarify the mechanisms involved. In this regard, the resolution of experimental vasospasms by a TXA₂ synthetase inhibitor also suggests a disproportionate synthesis of TXA₂ in pathological situations.

In summary, although there is strong evidence of disproportionate synthesis of TXA₂ in cerebral ischemia, the significance of PGs in the postischemic pathophysiology such as edema formation and ischemic brain damage remains unclear. Furthermore, whether the augmented reactive hyperemia caused by indomethacin is of actual benifit to neuronal recovery is open to question.

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