Effect of Indomethacin and a Free Radical Scavenger on Cerebral Blood Flow and Edema After Cerebral Artery Occlusion in Cats

Hiroo Johshita, MD, DMSc, Takao Asano, MD, DMSc, Tetsu Hanamura, MD, and Kintomo Takakura, MD, DMSc

Using the middle cerebral artery occlusion model in cats, we evaluated the possible role of the cyclooxygenase pathway in alterations of local cerebral blood flow and the development of cortical edema following prolonged ischemia or recirculation. We divided 57 cats into three groups, and each cat received saline (control), indomethacin, or the free radical scavenger ONO-3144. Each group was subdivided into prolonged ischemia (4 hours of occlusion: PI) and recirculation (2 hours of occlusion followed by 2 hours of recirculation: RC) subgroups. We compared local cerebral blood flow and cortical specific gravity between the PI and RC subgroups of the control and drug-treated groups. In the PI subgroup, indomethacin did not influence the time course of local cerebral blood flow but significantly worsened the decrease in cortical specific gravity. On the other hand, indomethacin significantly improved postischemic hypoperfusion and ameliorated the decrease in cortical specific gravity in the RC subgroup. The effects of ONO-3144 were similar to those of indomethacin, except that ONO-3144 did not affect cortical specific gravity in the PI subgroup. Indomethacin inhibits cyclooxygenase activity, whereas ONO-3144 scavenges the oxygen-centered radical released in the conversion of prostaglandin G₂ to prostaglandin H₂. Thus, prostaglandins do not seem to play a major role in the occurrence of brain edema due to prolonged regional ischemia. By contrast, oxygen-centered radicals released from the cyclooxygenase pathway appear to be at least partially responsible for the occurrence of recirculation-induced edema and postischemic hypoperfusion. (Stroke 1989;24:788-794)

The mechanism underlying ischemic brain edema has not yet been fully elucidated, despite its serious consequences on the course of ischemic brain injury. During the acute stage of prolonged focal ischemia, edema develops mainly in the gray matter 1-2 hours after middle cerebral artery (MCA) occlusion in experimental animal models. Further, acute restoration of cerebral blood flow (CBF) amplifies edema formation if the reduction of CBF persists with a certain degree and duration. Since the restoration of blood flow is essential for the ultimate recovery of living tissue from ischemia, elucidation of the pathophysiologic mechanism underlying ischemic brain edema during as well as after insult is of prime importance. It has been reported that cerebral ischemia and recirculation cause the release of free fatty acids, especially arachidonic acid, and lead to the synthesis of highly bioactive eicosanoids. In particular, effects of the cyclooxygenase-derived products such as prostaglandins (PGs) and thromboxane on cerebral ischemia have been extensively investigated, and they have been causally related to the pathophysiologic mechanisms associated with ischemic brain injury. However, relatively little attention has been paid to the effect of oxidizing radicals derived from the cyclooxygenase pathway.

We investigated the role of the cyclooxygenase pathway in edema formation, using the cyclooxygenase inhibitor indomethacin and another nonsteroidal anti-inflammatory drug, ONO-3144 (2-aminomethyl-4-tert-butyl-6-propionylphenol hydrochloride, ONO Pharmaceutical Co. Ltd., Osaka, Japan) on the cat.

From the Department of Neurosurgery, Saitama Medical Center, Saitama Medical School, Kawagoe (H.J., T.A.) and the Department of Neurosurgery, University of Tokyo Hospital, Tokyo (T.H., K.T.), Japan.

Address for correspondence: Hiroo Johshita, MD, Assistant Professor, Department of Neurosurgery, Kawagoe Medical Center, Saitama Medical School, 1981 Kamoda, Kawagoe, Saitama 350, Japan.

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MCA occlusion model of prolonged ischemia or recirculation. ONO-3144, a phenol derivative, scavenges oxygen-centered radicals generated from PGG, in a manner similar to that of MK-447 and enhances prostaglandin endoperoxide synthetase activity. As a radical scavenger, ONO-3144 also inhibits thromboxane synthetase and lipid peroxidation, such as H2O2-induced hemolysis. Thus, indomethacin and ONO-3144 have in common the action to suppress the generation of radicals while acting differently regarding the synthesis of PGs. Using these two drugs, we tried to discriminate the pathophysiologic effects of cyclooxygenase products from those of radicals on brain edema formed during and after focal cerebral ischemia.

Materials and Methods

We used the cat MCA occlusion model as previously described. We divided 57 cats into three experimental groups receiving saline, indomethacin, or ONO-3144 and further subdivided the groups into those experiencing prolonged ischemia (PI) and recirculation (RC). In the PI subgroups, the MCA was occluded for 4 hours; in the RC subgroups, the clip was removed 2 hours after MCA occlusion and the brain was recirculated for 2 hours.

Adult cats of either sex were anesthetized by halothane inhalation and tracheostomized. The femoral artery and vein were catheterized to monitor systemic arterial blood pressure (SABP), to sample blood, and to administer saline or drugs. The left MCA and internal cerebral artery (ICA) were exposed by the transorbital approach, and six hydrogen-sensitive platinum electrodes were inserted into the cortex through small burr holes at predetermined positions within the MCA territory using an operating microscope. Thereafter, controlled ventilation was initiated with a gas mixture of 70% N2O and 30% O2, following the administration of 2 mg/kg i.v. gallamine triethiodide. The O2 and CO2 concentrations of the expired gas were continuously monitored using a gas analyzer (San-Ei, 1H21A expired gas monitor, Tokyo, Japan), and the rectal temperature was kept at 37.5±0.5°C using a heating lamp and warming blanket. Cortical local CBF (ICBF) was measured using the hydrogen clearance method.

After the first measurement of ICBF, the initial loading dose of saline, indomethacin, or ONO-3144 was administered, followed by the infusion of an equal amount of saline or drug using an infusion pump (approximately 25 ml/4 hr) 30 minutes before MCA occlusion. Indomethacin was dissolved in a phosphate buffer of pH 9.0, which was later adjusted to 7.4 before administration. ONO-3144 was dissolved in physiological saline. The control-PI (n=11) and control-RC (n=11) cats received a continuous infusion of saline. The indomethacin-treated cats (PI, n=8; RC, n=9) received a 3-mg/kg bolus injection of indomethacin followed by a saline infusion. In the ONO-3144 treated cats (PI, n=9; RC, n=9) a 1-mg/kg bolus injection and a continuous infusion of 0.1 mg/kg/min ONO-3144 were administered. Following the second measurement of ICBF, the previously exposed MCA and ICA were occluded using small clips (Zen clip, Ohwa Tsusho, Tokyo, Japan), which were released 2 hours later in the RC subgroups. After arterial occlusion, ICBF was measured at 10, 30, 60, 90, 120, 150, 180, and 210 minutes in the PI subgroups and at 10, 30, 60, 90, 130, 150, 180, and 210 minutes in the RC subgroups. In both PI and RC subgroups the cats were killed using a bolus injection of saturated KCl solution at 240 minutes. Duplicate samples of gray matter (10–20 mg each) were excised at each electrode location, and the specific gravity of each sample was measured using the microgravimetric technique. The specific gravities of standard and stock solutions used in this technique were determined using a densimeter (SS-D-200, Shibayama Scientific Co. Ltd., Tokyo, Japan). The mean specific gravities of the duplicate samples was used to represent the cortical specific gravity (coSG)-topographically correlated to each electrode. In each cat, several cortical samples were also obtained from the MCA territory of the contralateral hemisphere for the measurement of specific gravity.

For statistical evaluation, the data for coSG and ICBF were analyzed by nonparametric analysis using the Kruskal-Wallis test. The differences between groups were estimated according to Dunn’s procedure. The data for the physiologic parameters were examined by analysis of variance. p<0.05 (two-tailed hypothesis) was considered to indicate significance. Values are presented as mean±SEM.

Results

No significant differences in SABP or blood gases were found (Table 1) between groups. Mean±SEM ICBF of all the electrodes in the control, indomethacin, and ONO-3144 groups before saline or drug administration were 92.2±3.2 (n=105), 98.6±3.3 (n=100), and 96.6±3.5 (n=106) ml/100 g/min, respectively. To examine the topographic relation between the severity of ischemia and the ensuing edema, the coSG of each electrode site was plotted against the corresponding ICBF value during MCA occlusion (the clip flow). As shown in Figure 1, an apparent decrease of coSG occurred when the clip flow was <20–25 ml/100 g/min in the control-PI subgroup. A similar ischemic threshold for edema formation was found in the control-RC subgroup, although the severity of the coSG decrease was more pronounced than in the control-PI subgroup.

Since the ICBF threshold for edema formation was 20–25 ml/100 g/min in both the control-PI and control-RC subgroups, the electrode sites were divided into three groups according to their corresponding clip flows: severely (<15 ml/100 g/min), moderately (15–30 ml/100 g/min), and slightly (>30 ml/100 g/min) ischemic cortical areas. The time course of ICBF in each cortical area in each sub-
### TABLE 1. Physiologic Variables for Cats Subjected to Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Group/Variable</th>
<th>Before</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
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<td><strong>Prolonged ischemia</strong></td>
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<td>145.5±4.7</td>
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<td>145.0±5.3</td>
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<td>26.6±1.36</td>
<td>26.5±1.10</td>
</tr>
<tr>
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<td>144.5±6.00</td>
<td>139.5±4.9</td>
<td>134.2±3.8</td>
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<tr>
<td>SABP</td>
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<td>136.7±5.5</td>
<td>133.3±6.1</td>
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<td>ONO-3144 (n=9)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>SABP</td>
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<td>167.9±11.4</td>
<td>—</td>
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<tr>
<td>pH</td>
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<td>128.7±5.8</td>
<td>—</td>
<td>126.2±5.5</td>
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</tbody>
</table>

Data are mean±SEM. SABP, systemic arterial blood pressure.

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**Figure 1.** Scatter diagrams showing relation between mean local cerebral blood flow (ICBF) during middle cerebral artery (MCA) occlusion (clip flow) and topographically correlated cortical specific gravity (Co-SG) in control (saline-treated) subgroups of cats. Note that in subgroups with both prolonged ischemia and recirculation, apparent decrease in Co-SG occurred at clip flow of 20–25 ml/100 g/min. Co-SG of contralateral hemisphere (n=11); bar indicates±SEM.
group are shown in Figure 2. In the PI subgroups, the time course of ICBF was not affected by indomethacin or ONO-3144 administration. In the control-RC subgroup, recirculation was accompanied by an immediate restoration of ICBF. In the moderately and severely ischemic cortical areas, however, ICBF rapidly and progressively deteriorated, which represented the occurrence of post-ischemic hypoperfusion. In both the indomethacin and ONO-3144 groups, the recovery of ICBF immediately after recirculation was more pronounced than in the control-RC subgroup, and the ICBF after recirculation was generally maintained at significantly higher levels than in the control-RC subgroup. No significant difference was observed between the indomethacin and ONO-3144 groups.

According to the intensity of ischemia, mean coSG of the severely, moderately, and slightly ischemic cortical areas of each group were compared (Figure 3). In the PI subgroups, a significant difference in coSG was found between the moderately ischemic areas of the control and indomethacin groups. In contrast, no significant difference was observed in the moderately ischemic areas of the control and ONO-3144 groups. In the control-RC subgroup, no significant difference was observed in any of the ischemic areas.

**Figure 2.** Graph of time courses of local cerebral blood flow (ICBF) of severely (○, 0–15 ml/100 g/min), moderately (△, 15–30 ml/100 g/min), and slightly (●, >30 ml/100 g/min) ischemic cortical areas in each subgroup of cats. At each time, values were compared between drug-treated and saline-treated (control) subgroups. *p<0.05, **p<0.01 different from control. Numerals to the right of each graph indicate number of cortical samples.

**Figure 3.** Bar graph. Mean±SEM cortical specific gravities (Co-S.G.) of severely, moderately, and slightly ischemic cortical areas of cats with prolonged ischemia and recirculation. Numerals in parentheses indicate number of cortical samples. Open bars, control; hatched bars, indomethacin; dotted bars, ONO-3144. *p<0.05, **p<0.01 different from control. MCA, middle cerebral artery.
acsin groups, indicating that indomethacin worsened cortical edema. The ONO-3144 PI subgroup showed a higher mean Co-SG value in this flow range than the control-PI subgroup; however, the difference did not reach the level of significance. Mean Co-SG of the severely ischemic area in the control-RC subgroup was significantly lower than that of the control-PI subgroup, whereas mean Co-SG of the moderately and slightly ischemic areas were not significantly different. Between the control and the two drug-treated RC subgroups, highly significant differences in Co-SG were found in the severely ischemic areas. In the moderately and slightly ischemic areas, the differences in mean Co-SG between the RC subgroups did not reach the level of significance.

To evaluate the effects of indomethacin and ONO-3144 on ischemic edema in toto, the frequency distribution of the values of Co-SG was examined in each group (Figure 4). The class intervals were determined according to Sturges's formula. In the control-PI subgroup, the distribution of Co-SG values was unimodal. On the other hand, in the control-RC subgroup the distribution became more flattened and was split into two peaks (bimodal). This difference in configurations between the control-PI and control-RC subgroups was significant (Kolmogorov-Smirnov two-sample test, $p<0.05$).

Although a significant difference in configurations was not observed between the control-PI and drug-treated PI subgroups, the distribution of Co-SG values between the indomethacin PI and the ONO-3144 PI subgroups was significantly different ($p<0.025$). There were no significant differences between the RC subgroups.

Discussion

In our study, the ischemic threshold of edema formation in the cat cerebral cortex exposed to regional, prolonged ischemia was 20–25 ml/100 g/min, which compares well with values obtained in previous studies. The edema occurring within the initial several hours in focal, prolonged ischemia is intracellular and is designated as the cytotoxic type. This ischemic threshold for edema formation is considerably higher than the threshold for energy failure of the cell membrane, which occurs at a CBF of around 6–8 ml/100 g/min. A rise of intracellular osmolality, the release of neurotransmitters due to impaired synaptic transmission, or the electrical failure of the cell membrane has been suggested for this higher threshold. In our parallel study, however, the brain concentrations of PGE2 and PGF2α significantly increased following 2–4 hours of prolonged ischemia in an ischemic range similar to that of edema formation as observed
in our present study. This higher threshold might indicate a relation between ischemic edema formation and the activation of the arachidonic acid cascade in focal ischemia.

As shown in previous studies, pretreatment with indomethacin significantly worsened cortical edema following 4 hours of prolonged ischemia. Since ICBF was not significantly different between groups, edema formation during prolonged ischemia cannot be ascribed to the effects of indomethacin on cerebral circulation. Several possibilities may account for this phenomenon. First, the possible increase of free arachidonic acid due to blockade of the cyclooxygenase pathway may be related to the worsening of brain edema, although the relative ineffectiveness of steroids on ischemic brain edema does not support this possibility. Second, indomethacin may have diminished the concentrations of cytoprotective PGs, such as PGI2. However, in similar experimental models, beneficial effects of PGI2 on ischemic brain edema remain equivocal.

Third, cyclooxygenase inhibition may result in the diversion of free arachidonic acid from the cyclooxygenase to the lipoxygenase pathways. With regard to the last possibility, it is of interest that ONO-3144 did not enhance edema formation. In the actual measurement of tissue eicosanoid concentrations in the same experimental condition is required to substantiate the role of oxygen-centered radical on the cyclooxygenase pathway.

The mean coSG in the control-RC subgroup was significantly lower than that in the control-PI group, although the ICBF threshold for edema formation was almost the same in the two subgroups. Since it seems clear that cortical edema was worsened by recirculation, it may be appropriate to designate this as recirculation-induced edema. Nevertheless, the bimodal configuration of the frequency distribution of coSG values observed in the control-RC subgroup suggests that recirculation exerted both beneficial and detrimental effects.

With regard to the change in ICBF following recirculation, pronounced postischemic hyoperfusion was noted in the control-RC subgroup. Indomethacin and ONO-3144 exerted no significant influence on the time course of ICBF during MCA occlusion, but they significantly increased ICBF after recirculation. Since transient ischemia prominently activates the cyclooxygenase as well as the lipoxygenase pathways, pharmacologic manipulation of these pathways could have led to the amelioration of postischemic hyoperfusion. Further, indomethacin and ONO-3144 showed nearly equivalent effects on brain edema following recirculation. Because the increase of postischemic ICBF occurred in parallel with the amelioration of recirculation-induced edema, the drug effect on cortical edema is not likely to be due to the possible increase of pressure head in the cerebral arteries following recirculation. It appears that postischemic hyoperfusion and recirculation-induced edema are closely related phenomena.

Considering the difference in the effects of indomethacin and ONO-3144 on the cyclooxygenase pathway and the similarity of their effects on postischemic ICBF and edema, it is tempting to speculate that the cyclooxygenase products, but the radicals generated in the arachidionate cascade trigger these phenomena. Previously reported results with cyclooxygenase inhibitors and a radical scavenger are consonant to the above surmise.

It should be noted that ONO-3144 also possesses an inhibitory effect on thromboxane A2 synthetase, which may have influenced our results. However, a synthetic thromboxane antagonist reportedly showed no beneficial effect in an experimental model similar to ours. Therefore, the mechanism underlying the amelioration of recirculation-induced edema by ONO-3144 seems to reside in the scavenging of oxygen-centered radical, which is in the spectrum of drug actions of phenol derivatives. Obviously, actual measurement of tissue eicosanoid concentrations in the same experimental condition is required to substantiate the role of oxygen-centered radical path on the cyclooxygenase pathway.

From a therapeutic view point, the use of free radical scavengers definitely merits further investigation because they may exert beneficial effects on brain edema due to both prolonged ischemia and to recirculation.

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**Key Words** • brain edema • free radicals • indomethacin • cats.
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