Kawano, Ken-Ichi, Yasuhiko Ikeda, Kazunao Kondo, and Kazuo Umemura. Increased cerebral infarction by cyclic flow reductions: studies in the guinea pig MCA thrombosis model. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1578–R1583, 1998.—We have developed a photochemical model of thrombotic middle cerebral artery (MCA) occlusion in the guinea pig for investigating factors contributing to the development of cerebral infarction. In this model, cyclic flow reductions (CFRs) after recanalization of the MCA are a common observation and might contribute to the development of cerebral infarction. We sought to measure the time course of recanalization of the guinea pig MCA after the artery had been occluded by a thrombus. Thrombotic occlusion of the MCA was induced by photochemical reaction between intravenously administered rose bengal (RB) and transluminal green light (19, 33); this is immediately followed by platelet adhesion, aggregation, and formation of an occlusive platelet-rich thrombus in the MCA at the site of endothelial injury (20). Using the thrombotic occlusion model in guinea pigs, we found cyclic flow reductions (CFRs) after recanalization. CFRs may be assumed to cause repeated short periods of ischemia-reperfusion, a phenomenon that is suspected to influence the extent of cerebral tissue damage (13, 14, 18, 21, 24, 30). Therefore, we study the contribution of CFRs to the development of cerebral infarction using the thrombotic occlusion model in the guinea pig MCA.

METHODS

Animal preparation. The experimental protocol was approved by the local committee on ethics of animal experiments and extra care was taken to avoid animal suffering. Male Hartley guinea pigs weighing 300–450 g were anesthetized with 1% isoflurane in a 70% N2O-30% O2 mixture with the use of a face mask. Arterial blood pressure (BP) and heart rate were monitored continuously via a catheter inserted into the femoral artery. Arterial blood gases were analyzed with a gas monitor (model 850, Ciba-Corning). Another catheter was inserted into the jugular vein for injection of RB. Animal body temperature was maintained at 38°C with a heating pad (K-module K-30, Baxter). After a left temporal incision was made, the temporalis muscle was removed with the use of an electric cauterizer. The orbital bone was removed to open a 6-mm-diameter oval window with the use of a dental drill (model LMM-7, Morita, Tokyo, Japan). The main trunk of the MCA was observed under an operating microscope without cutting the dura mater.

MCA occlusion. Photointravascular with green light (wavelength 520–620 nm) was achieved on the dura mater using a xenon lamp (model L-4887, Hamamatsu Photonics, Hamamatsu, Japan) with a heat-absorbing filter and a green filter. The irradiation was directed by a 3-mm-diameter optic fiber mounted on a micromanipulator. The head of the optic fiber was placed on the MCA including the proximal end of the lenticulostriate branch, providing an irradiation dose of 0.636 J/cm2. The blood flow velocity in the MCA was measured with a pen-type pulse-Doppler flow probe (PVD-20, Crystal Bio-Tech) positioned on the MCA 2–3 mm distal to the irradiated segment. When a stable baseline blood flow was established, RB (20 mg/kg body wt) was administered and the green light irradiation was continued for 10, 15, 20, and 30 min after RB. The blood flow in the MCA was monitored for 2 h after RB administration, unless stated otherwise. At the end of the observation period, the animals were sterilized with kanamycin spray (Meiji Seika, Tokyo, Japan) and were allowed to...
 recover from anesthesia. Twenty-four hours after MCA occlusion, the same site was reopened under anesthesia and the MCA blood flow was measured with the pulse-Doppler flow probe for 20 min. In the permanent occlusion model, the MCA was explored with the same procedure to thrombotic occlusion model. The MCA was cauterized with a bipolar coagulator (model 80–1160, Codman & Shurtleff) ~5-mm long at the segment described in the photochemical model. Blood gas and BP were monitored for 2 h under anesthesia, and cessation of MCA blood flow was observed with the pulse-Doppler flow probe. Twenty-four hours after surgery, cessation of the MCA blood flow was reconfirmed.

Recording of blood flow in the MCA. The following parameters were measured for evaluation of MCA blood flow: 1) the occlusion time, defined as the time taken from injection of RB to the first thrombotic occlusion of the MCA; 2) frequency of cyclic flow reductions during the 2-h observation period; and 3) total reflux time during the 2-h observation period, expressed as a percentage of total observation time (2 h). The MCA was considered to be occluded when the flow monitor recorded zero flow.

Determination of infarct volume. Twenty-four hours after MCA occlusion, animals were decapitated under the isoflurane anesthesia. Each brain was cut into seven consecutive coronal slices of 1 mm thickness using a microslicer. Each slice was stained with 1% triphenyltetrazolium chloride and fixed with buffered formaldehyde solution (pH 7.2). For each slice, the area of infarction was measured using a computerized image analysis system (VM-30, Olympus, Tokyo, Japan) and the ratio between the area of infarction and the whole area of the corresponding area was calculated.

Transmission electron microscopy. A separate group of guinea pigs that underwent photochemical reaction (10-min irradiation) or only irradiation without RB were analyzed with transmission electron microscopy. At 24 h after photochemical reaction, guinea pigs were perfused with a transcardiac infusion of 100 ml of saline containing 50 U/ml heparin under constant pressure of 60 mmHg. Then perfusion buffer was switched to 200 ml of 50 mM PBS containing 2% glutaraldehyde and 1% paraformaldehyde. The MCA was carefully isolated and placed in the fixative for at least 24 h, then postfix in sodium PBS containing 1% osmium tetroxide for 2 h. The specimen of the MCA was dehydrated in a series of ethanol solutions, then postfixed in sodium PBS containing 1% osmium tetroxide and 1% uranyl acetate. The specimen was then cut transversely into a 0.1-µm-thick section and stained with uranyl acetate and lead citrate. The section was examined under a transmission electron microscope (JEM1220; Jeol, Tokyo, Japan).


table1. Baseline physiological measurements

<table>
<thead>
<tr>
<th>Irradiation Time</th>
<th>Body Wt, g</th>
<th>Blood pH</th>
<th>Blood P O2, mmHg</th>
<th>Blood P CO2, mmHg</th>
<th>MBP, mmHg</th>
<th>HR, beats/min</th>
<th>MBF, kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombotic occlusion</td>
<td>415 ± 16</td>
<td>7.34 ± 0.01</td>
<td>137.1 ± 6.3</td>
<td>38.7 ± 2.3</td>
<td>40.4 ± 2.2</td>
<td>217.8 ± 9.7</td>
<td>108.0 ± 0.17</td>
</tr>
<tr>
<td>10 min</td>
<td>412 ± 28</td>
<td>7.37 ± 0.02</td>
<td>148.9 ± 5.5</td>
<td>37.2 ± 3.0</td>
<td>35.3 ± 1.6</td>
<td>238.8 ± 5.1</td>
<td>0.68 ± 0.18</td>
</tr>
<tr>
<td>15 min</td>
<td>402 ± 28</td>
<td>7.36 ± 0.01</td>
<td>124.7 ± 9.9</td>
<td>37.9 ± 2.7</td>
<td>37.8 ± 4.2</td>
<td>222.2 ± 8.9</td>
<td>0.62 ± 0.10</td>
</tr>
<tr>
<td>20 min</td>
<td>415 ± 15</td>
<td>7.35 ± 0.03</td>
<td>135.2 ± 10.6</td>
<td>36.4 ± 2.8</td>
<td>37.0 ± 2.7</td>
<td>224.5 ± 9.1</td>
<td>0.63 ± 0.14</td>
</tr>
<tr>
<td>30 min</td>
<td>413 ± 10</td>
<td>7.41 ± 0.02</td>
<td>124.6 ± 11.4</td>
<td>36.4 ± 3.4</td>
<td>34.3 ± 3.4</td>
<td>236.8 ± 7.8</td>
<td>0.90 ± 0.10</td>
</tr>
</tbody>
</table>

Values are measured before middle cerebral artery occlusion and presented as means ± SE (n = 6). There was no significant differences among groups in physiological parameter (ANOVA). MBP, mean arterial blood pressure; HR, heart rate; MBF, mean blood flow in the middle cerebral artery.
in the 30-min irradiation group, additional infarct areas were observed at some distance from the core infarct area. The total infarct volumes measured after 10-, 15-, 20-, and 30-min irradiation were 124.9 ± 11.8, 158.3 ± 10.8, 174.3 ± 17.7, and 205.3 ± 4.7 mm$^3$, respectively, and 157.2 ± 9.4 mm$^3$ for permanent occlusion. The percentage infarct volume for each group is presented in Fig. 5. In the cortex, the infarct volume was increased with the duration of irradiation and the value for the 30-min irradiation group was significantly ($P < 0.01$) larger than the value for 10- or 15-min irradiation groups. There was also a significant ($P < 0.05$) difference between 20-min irradiation and 10-min irradiation. Infarct volume in the basal ganglia was not different among groups. Surprisingly, the infarct volume in the permanent occlusion group was significantly ($P < 0.05$) smaller than that in the 30-min irradiation group.

**DISCUSSION**

In this study, we induced vessel occlusion by photothermal reaction between intravenously administered RB and transluminal green light, which causes endothelial injury followed by platelet adhesion, aggregation, and formation of an occlusive platelet-rich thrombus at
The site of photochemical reaction. This method serves as a nonmechanical approach to induce vessel wall endothelial injury and vessel occlusion. RB is an efficient photosensitizer dye, producing singlet molecular oxygen, \( \text{^1O}_2 \), by "type II" photodynamic energy transfer. Endothelial injury is believed to be caused by singlet oxygen, but involvement of other excited species cannot be ruled out. In this model, endothelium injury was restricted to the irradiated MCA segment. This indicates that singlet oxygen is produced locally, is captured by endothelium, and does not have a chance to interact with distant tissues. In fact, the smooth muscle cells beneath the denuded endothelium were not damaged. Therefore, we exclude the possibility that singlet oxygen could reach the neuronal area and directly contribute to the infarct volume.

The aim of the present study was to use the photochemical technique to establish a guinea pig model of MCA occlusion different from previous models and use this model to study the recanalization of MCA. Most importantly, we were interested in understanding the contribution of CFRs to the progression of cerebral infarction. Recanalization after thrombotic occlusion was observed followed by CFRs in the majority of animals; the time to recanalization was dependent on the duration of irradiation in the presence of RB (photochemical reaction). Thus, for a 10-min irradiation period, recanalization and CFRs were observed ~15 min after thrombotic occlusion, and then the blood flow returned to the baseline value ~60 min after RB injection. In contrast, after a 30-min irradiation, recanalization was observed beyond 60 min after occlusion and the CFRs continued for up to 24 h. We also confirmed the continuous appearance of CFRs during 24 h after thrombotic occlusion with preliminary studies using conscious animals implanted with a laser-Doppler flow probe. Thus the animals continued to show CFRs both during the 24 h after thrombotic occlusion and beyond. These observations suggest that the time to recanalization and the continuation of CFRs depended on the extent of endothelial injury.

CFRs were first described in coronary arteries of dogs by Folts et al. (8), who suggested that they were caused by periodic acute occlusive platelet thrombi. Subsequently, similar phenomena have been reported in various species, including coronary artery in pigs, carotid artery in monkeys, carotid and femoral arteries in dogs, femoral and carotid arteries in rabbits, and popliteal artery in humans. The underlying cause of unstable angina and transient ischemic attacks have been attributed to CFRs in the coronary and carotid arteries, respectively. However, as far as we are aware, CFRs have not been previously described in intracranial arteries.

Under the present experimental conditions, cerebral infarction due to ischemia was observed 24 h after photochemical reaction. Unexpectedly, the infarct volume in permanent occlusion induced by electrocoagulation was smaller than that of the photochemically induced thrombotic occlusion model in the 30-min irradiation group. In 5 of 6 animals receiving 30-min irradiation, CFRs in the MCA continued up to

<table>
<thead>
<tr>
<th>Irradiation Time</th>
<th>Time to Occlusion, min</th>
<th>Frequency of CFRs, /h</th>
<th>Total Reflow Time, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>4.8 ± 0.5</td>
<td>14.5 ± 2.1</td>
<td>59.5 ± 5.0</td>
</tr>
<tr>
<td>15 min</td>
<td>3.7 ± 0.2</td>
<td>11.2 ± 3.1</td>
<td>38.8 ± 10.9</td>
</tr>
<tr>
<td>20 min</td>
<td>4.1 ± 0.6</td>
<td>10.5 ± 3.3</td>
<td>25.6 ± 11.5</td>
</tr>
<tr>
<td>30 min</td>
<td>4.3 ± 0.6</td>
<td>3.3 ± 1.5*</td>
<td>9.3 ± 5.9†</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE (n = 6). *P < 0.05 and †P < 0.01 vs. 10-min irradiation (Tukey-Kramer). CFR, cyclic flow reduction.

![Fig. 3. Transmission electron micrographs of the irradiation segment of the guinea pig middle cerebral artery. Arteries were isolated 24 h after operation. A: artery exposed to green light for 10 min without rose bengal administration. B: artery exposed to green light for 10 min with rose bengal administration. Photochemical reaction denuded the endothelium and platelets adhered on the internal elastic lamina. Bars in the photograph denote 2 µm. SM, smooth muscle cell; E, endothelial cell; IE, internal elastic lamina; P, platelet.](http://ajpregu.physiology.org/)

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24 h after recanalization. This observation plus the results from the permanent occlusion model strongly suggested that long-lasting CFRs enhance cerebral infarct volume. It is possible that other than CFRs contribute to the enhancement of cerebral infarction, such as difference in reperfusion time in each group. However, earlier reports that the infarct volume in the reperfusion injury models did not exceed that of the permanent model make this unlikely (11, 22, 36). Therefore, we concluded that CFRs were the most reasonable explanation to enlarge cerebral infarction. The following explanations may account for the enhanced infarct volume associated with CFRs. First, ischemia-reperfusion is known to cause cerebral damage; repeated ischemia-reperfusion can produce oxygen radicals (14) and release glutamate (28), which can damage the cerebral tissue. Second, CFRs may mobilize microemboli and thus impair microvascular perfusion. The latter may account for the enlarged infarct area away from the core infarct. This is supported by the fact that embolism from the cerebral artery and distal often caused additional infarct (16, 17, 33).

In conclusion, in this study, we show a spontaneous recanalization process and continuous presence of CFRs in the guinea pig MCA after the artery had been occluded by a thrombus. Furthermore, for the first time, we report increased cerebral infarction attributed to long-lasting CFRs. CFRs may be assumed to cause repeated short periods of ischemia-reperfusion, which are suspected to influence the extent of cerebral tissue damage. A similar phenomenon may also operate in human cerebral ischemia.

Perspectives

This study reveals that the MCA occluded by a thrombus necessarily shows CFRs during the recanalization process. Because CFRs were found in the human coronary artery with thrombolytic therapy (4, 10), CFRs could generally be followed by arterial recanalization. This phenomenon could conceivably proceed in human cerebral arteries according to the following assertions. First, in preliminary studies we observed CFRs in the MCA occlusion model using other species, including the rabbit and a primate model. Second, rethrombosis was observed in human cerebral artery (23, 31, 32).

If the presence of CFRs, in part, plays a role in the development of cerebral infarction, as shown in our study, prevention of CFRs would be a reasonable intervention to prevent cerebral infarction. Thus antiplatelet agents may have a role in preventing further cerebral damage even after the therapeutic window for thrombolytic agents. The results of a recent clinical study showing effectiveness of aspirin on cerebral infarction even when administered within 48 h after brain attack supported the efficacy of antithrombotics (6). On the basis of these understandings, we are now working on further characterization of CFRs in the cerebral artery and seeing if antiplatelet agents show prevention of CFRs and thereby reduce infarct volume.

Address for reprint requests: K.-I. Kawano, Dept. of Pharmacology, Hamamatsu Univ. School of Medicine, Hamamatsu 431-3192, Japan.

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REFERENCES


