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Changes in local cerebral blood flow in photochemically induced thrombotic occlusion model in rats

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Abstract

We demonstrated earlier that in a photochemically induced thrombotic occlusion model, a reperfusion-like phenomenon may be involved in the progress of brain damage. Therefore, we now investigated changes in local cerebral blood flow in a photochemical model compared with changes in a thermocoagulated occlusion model, using autoradiography. At 5 min, and 3, 6 and 24 h after middle cerebral artery occlusion, local cerebral blood flow was measured by intravenous injection of 4-iodo[*N*-methyl-^{14}C]antipyrine (20 μCi). In the ischemic core zone, the reduction in blood flow was similar in the two models. However, blood flow in the ischemic border zone in the photochemical model decreased transiently in the third hour after ischemia and then increased again, while the blood flow in a thermocoagulated model continued to decrease. Time courses of brain damage formation in both models were no different up to 24 h. These findings suggest that the transient reduction in cerebral blood flow in the third hour following ischemia may contribute to a reperfusion-like phenomenon in a photochemical model.

Keywords: Cerebral ischemia; Middle cerebral artery occlusion; Cerebral blood flow, local

1. Introduction

A photochemically induced thrombotic occlusion model of the rat middle cerebral artery is a well-characterized model of focal cerebral ischemia. The neuroprotective effects of several compounds have been tested using this model (Umemura et al., 1993, 1994a,b, 1995a,b,c,d, 1996, 1997; Matsuno et al., 1993; Kawai et al., 1995; Kaku et al., 1997; Takamatsu et al., 1998a,b). The most unique aspect of this model is that it has the characteristics of both permanent ischemia and ischemia–reperfusion. Reperfusion-like events may be involved in the progress of brain damage in photochemical models (Takamatsu et al., 1998b). We found that some oxygen radical scavengers attenuated brain damage following photochemically induced thrombotic occlusion of the rat middle cerebral artery, and these compounds did not show neuroprotective effects in a thermocoagulated occlusion model (Takamatsu et al., 1998b).

It is generally considered that reoxygenation during reperfusion provides oxygen as a substrate for numerous enzymatic oxidation reactions that produce reactive oxidants (Chan, 1994, 1996). It is also thought that reperfusion disrupts the blood–brain barrier and exacerbates edema formation (Yang and Betz, 1994). In fact, the brain edema formation following photochemically induced thrombotic occlusion was greater than that following thermocoagulated occlusion (Takamatsu et al., 1998b).

From the above, we hypothesized that reperfusion-like events may occur in the photochemical model, and that the period of occlusion may be the third hour after ischemia, because a short-acting hydroxyl radical scavenger, EPC-K_1, was effective when its administration was started 3 h after ischemia (Takamatsu et al., 1998a). In the present study, to test our hypothesis, autoradiographic studies were per-

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formed for the assessment of local cerebral blood flow in a photochemical and thermocoagulated model.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (Japan SLC, Japan) weighing 280–300 g were used. All experiments were performed in accordance with the institutional guidelines of The Medical and Pharmacological Research Center Foundation, Central Research Laboratory of Hamamatsu Photonics (Hamamatsu, Japan) and Hamamatsu University School of Medicine.

The animals were anesthetized with 4% halothane and anesthesia was maintained with 2% halothane in an oxygen 30% and room air 70% mixture. The left middle cerebral artery was occluded via the transorbital approach under an operating microscope. Briefly, a vertical incision was made between the left orbit and the external auditory canal. The temporalis muscle was reflected, and a subtemporal craniotomy was performed without removing the zygomatic arch. The main trunk of the middle cerebral artery and olfactory tract were observed through the dura mater. In the photochemical model (Umemura et al., 1993), photoillumination with green light (wave length, 540 nm) was done using a xenon lamp (model L-4887, Hamamatsu Photonics, Japan) over the main trunk of the middle cerebral artery at the olfactory tract through the dura mater. Photoillumination was performed for 10 min after intravenous injection of rose bengal (20 mg/kg). The middle cerebral artery was occluded via thermocoagulation (Tamura et al., 1981) using a microbipolar electrocoagulator (MICRO-3D, Mizuho, Japan). The middle cerebral artery from the proximal to the olfactory tract to the inferior cerebral vein and the lenticulostriate arteries were permanently occluded and transected to avoid recanalization. After occlusion of the middle cerebral artery, the temporalis muscle and skin were closed in layers and anesthesia was discontinued. During the operation, the body temperature of the animals was maintained at 37.5°C with a heating pad (K-module model K-20, American Pharmaseal, USA). The surgical procedure was carried out within 20 min for the photochemical model or within 10 min for the thermocoagulation model.

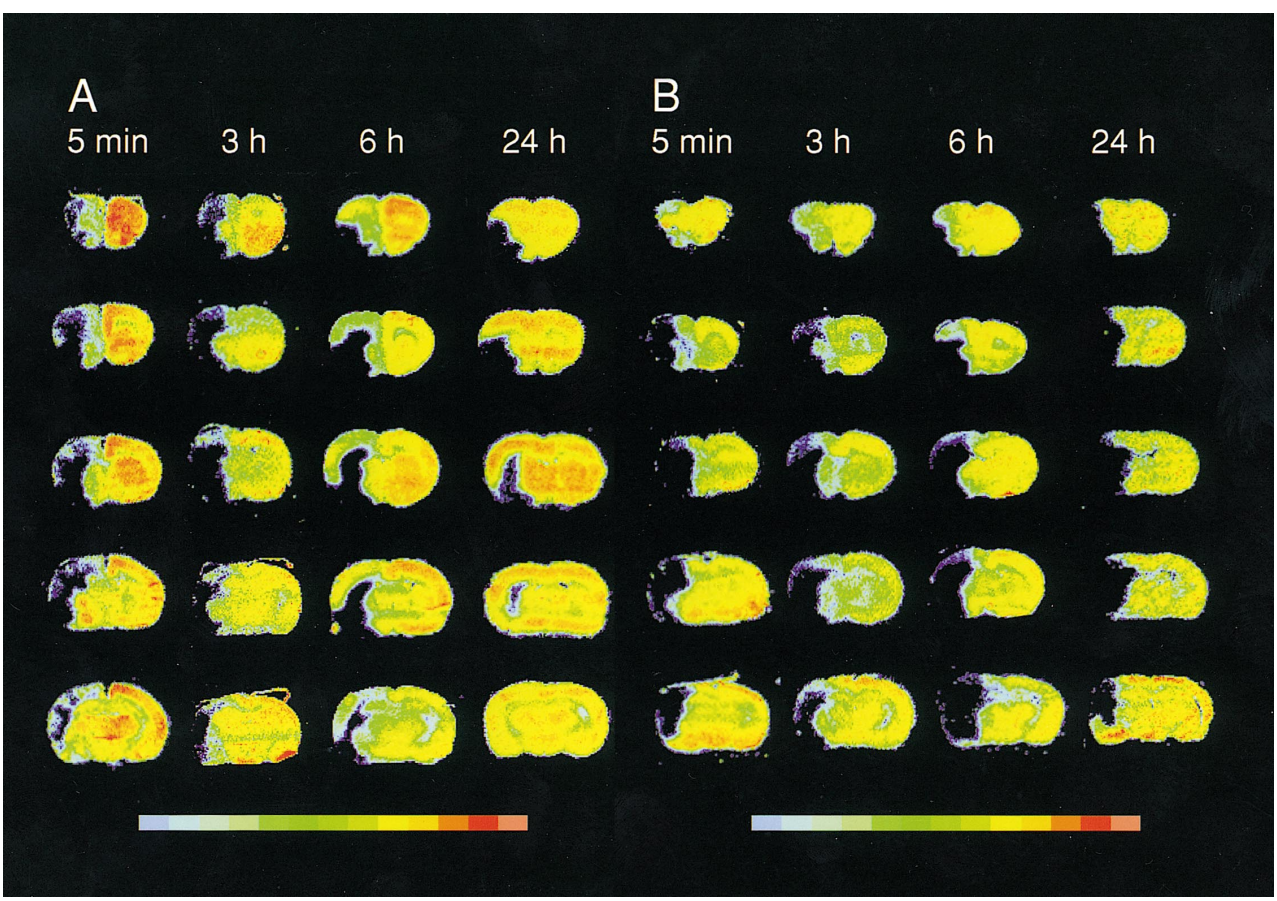

Fig. 1. Typical images of cerebral blood flow following photochemically induced thrombotic occlusion (A) and thermocoagulated occlusion (B) of the rat middle cerebral artery. At 5 min, 3, 6, or 24 h after ischemia, 4-iodo[*N*-methyl-^{14}C]antipyrine (20 μCi) was intravenously injected, the brains were removed and coronal slices (20-μm thickness) were cut. After 10 days of exposure to an imaging plate, images of cerebral blood flow were analyzed with a Fuji BAS system.

2.2. Autoradiographic studies

At 5 min, and 3, 6 and 24 h after the middle cerebral artery occlusion, the animals were anesthetized with 4% halothane in an oxygen 30% and room air 70% mixture. Thirty seconds after intravenous injection of 20 μCi of 4-iodo[*N*-methyl-^{14}C]antipyrine (specific activity 40 μCi/mmol, New England Nuclear, Boston, MA, USA), the animals were decapitated and their brains were removed. The brains were frozen in −50°C 2-methylbutane and mounted on tissue holders with a chilled embedding medium (Sakura Finetechnical, Tokyo, Japan). The brains were cut into 20-μm thick coronal sections with a cryostat (CR-520, Nakagawa Seisakusho, Tokyo, Japan) maintained at −20°C. A series of sections was taken at the level of the anterior commisure according to the atlas of Paxinos and Watson (1998). These sections were exposed to an imaging plate (Fuji Film, Tokyo, Japan) for 10 days. Radioactivity was converted into digitalized imaging data via a Fuji Bass system (Fuji Film). Five regions of interest (2 mm^2) were placed on a section; bregma + 0.7 mm (see Fig. 2A). These regions of interest were copied and pasted on to the contralateral hemisphere. The densities of all regions of interest and background on the imaging data were measured and the ratio to the contralateral hemisphere was calculated.

2.3. Time course of brain damage

3, 6, 12, and 24 h after middle cerebral artery occlusion, the animals were anesthetized with pentobarbital sodium. The brain was fixed by transcardial perfusion with a 10% formalin neutral buffer solution, pH 7.4, following saline perfusion at 100 mm Hg. The brains were then removed and six preselected coronal sections (from the anterior 3.5–13.5-mm long and 2-mm thick) were made using a brain matrix (RBS-02, Neurosience, Japan). Each section was embedded in paraffin wax, and 10-μm thick sections were cut and stained with hematoxylin and eosin. For each animal, the sum of the area of brain damage (Osborne et al., 1987) and the sum of the whole area of the cerebrum in six sections were calculated using a computerized image analysis system. After correction for swelling (Swanson et al., 1990), the brain damage in each animal was calculated as the ratio of the area of brain damage to the total area of the cerebrum.

2.4. Statistical analysis

The data are presented as the means ± S.D. Statistical analysis was performed using the analysis of variance

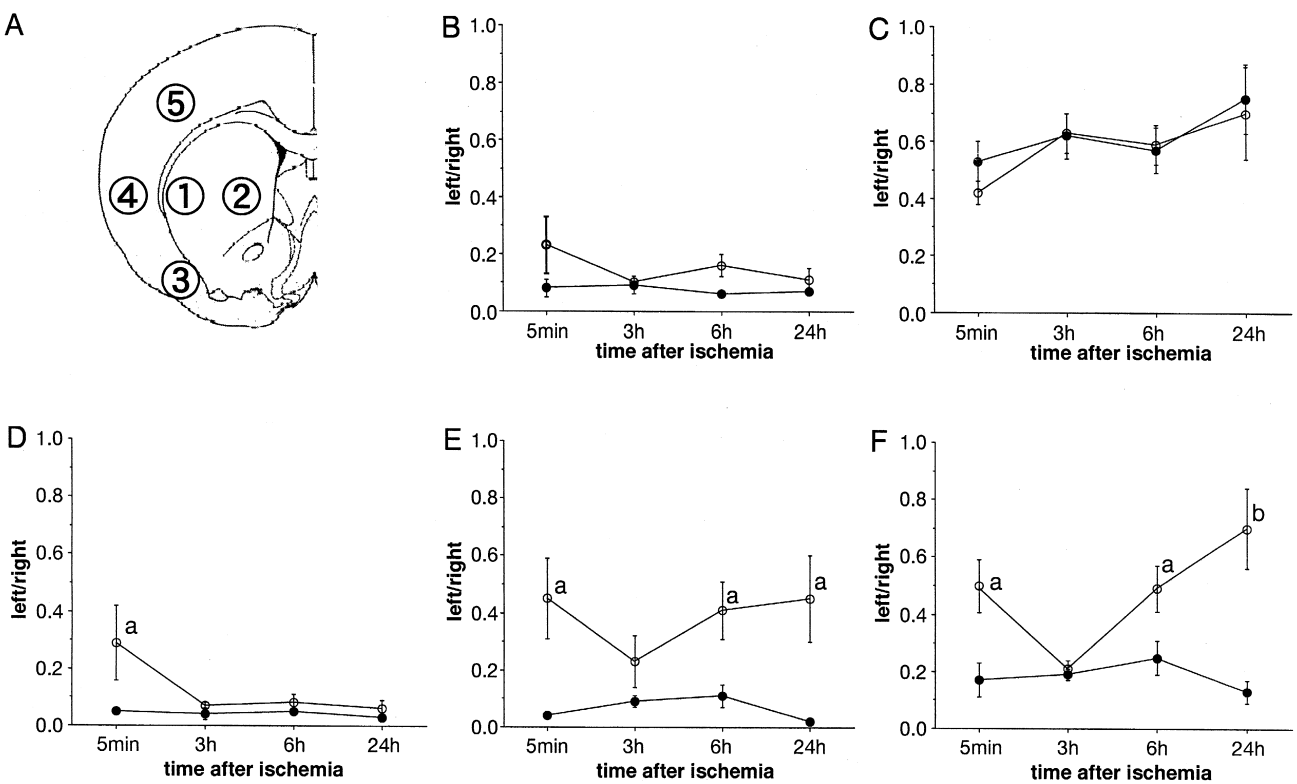

Fig. 2. The time course of cerebral blood flow changes following photochemically induced thrombotic occlusion (open circle) and thermocoagulated occlusion (closed circle) of the rat middle cerebral artery. (A) Placement of regions of interest; (B) results of analysis in region 1; (C) results of analysis in region 2; (D) results of analysis in region 3; (E) results of analysis in region 4; (F) results of analysis in region 5. Data represent the ratio of the ipsilateral hemisphere/contralateral hemisphere (left/right). Each point represents the mean for five animals, and the bars indicate the S.D. $^{a}P < 0.05$, $^{b}P < 0.01$ vs. the thermocoagulated occlusion model.

(ANOVA). Comparison of two groups was made with an unpaired Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

Physiological variables following both middle cerebral artery occlusion operations were within the normal range.

A typical image of cerebral blood flow is shown in Fig. 1. The middle cerebral artery occlusion reduced blood flow in the cerebral cortex and striatum.

In the striatum, cerebral blood flow was reduced by about 85% in the exterior part (Fig. 2B) and about 40% in the interior part (Fig. 2C) up to 24 h following middle cerebral artery occlusion. These reductions were no different between the two models.

Thermocoagulated occlusion reduced blood flow by about 90% in the cortical area in regions of interest 3 and 4, and by about 80% in region of interest 5. These reductions continued up to 24 h (Fig. 2D).

In regions of interest 3, 4 and 5, the reduction in blood flow in the photochemical model 5 min after ischemia was significantly less than that in the thermocoagulated model. Then, the blood flow decreased further 3 h after ischemia and was as low as that in the thermocoagulated model (Fig. 2D, E, F). In regions of interest 4 and 5, the blood flow increased again and returned to the level of the contralateral areas after 6 and 24 h (Fig. 2E, F).

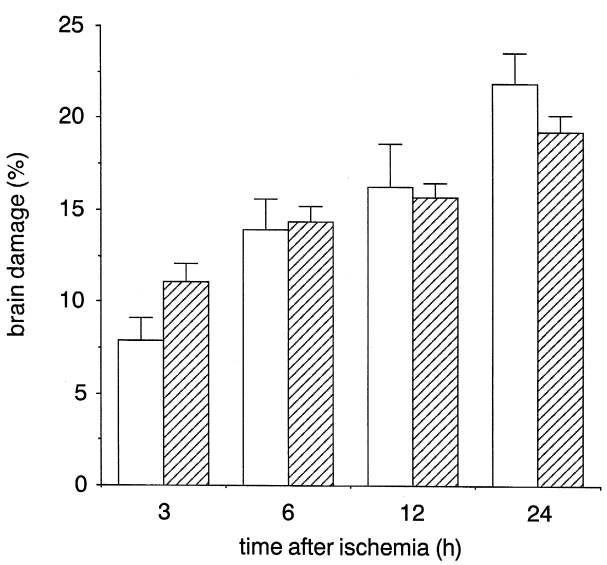

Fig. 3. The time course of brain damage formation following photochemically induced thrombotic occlusion (open column) and thermocoagulated occlusion (hatched column) of the rat middle cerebral artery. Each column represents the mean for seven to eight animals, and the bars indicate the S.D.

The brain damage in both models was no different at any time point examined within 24 h after ischemia (Fig. 3).

4. Discussion

Our previous studies demonstrated that reperfusion-like events contribute to brain damage formation in a photochemically induced thrombotic middle cerebral artery occlusion model. This conclusion was supported by evidence that the neuroprotective properties of radical scavengers were detectable in the photochemical model, but not in a thermocoagulation one. Furthermore, the therapeutic time-window of a short-acting radical scavenger suggested that the appearance time of reperfusion-like events may be the third hour after ischemia. In the present study, to test our hypothesis, autoradiography was used for the assessment of local cerebral blood flow in both photochemical and thermocoagulated model.

In the thermocoagulated model, local cerebral blood flow reduction continued constantly up to 24 h after ischemia. In the photochemical model, although the striatal blood flow reduction was similar to that in the thermocoagulated one, the cortical blood flow reduction was quite different. Unexpectedly, in the photochemical model, it took about 3 h for the cerebral blood flow to fall to the same degree as in the thermocoagulated model. These observations suggest that a thrombus may first occlude the main tract of the middle cerebral artery immediately after occlusion, and then diffuse toward the distal part within 3 h. After the third hour, as expected, the cortical blood flow in the ischemic border zone in the photochemical model increased. This suggests that the diffused thrombus was enzymatically dissolved from the third hour following ischemia.

It has generally been reported that the area of neuronal damage depends on the extent of blood flow reduction and the duration of ischemia (Crowell et al., 1981, Jones et al., 1981). The time courses of cortical blood flow reductions in the photochemical model and the thermocoagulated model were quite different. However, the time courses of brain damage formation in the two models were almost similar. Because it is well confirmed that reoxygenation during reperfusion provides oxygen as a substrate for numerous enzymatic oxidation reactions that produce reactive oxidants (Chan, 1994, 1996), the brain damage following photochemical exposure may be made up of both ischemic damage and reperfusion injury.

In conclusion, autoradiographic study clearly demonstrated that in a photochemical model in rats, the change in cerebral blood flow indicated a reperfusion-like phenomenon. Therefore, the photochemical model has characteristics of both permanent ischemia and ischemia–reperfusion.

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