An Improved Photochemical Model of Embolic Cerebral Infarction in Rats

Nancy Futrell, MD

To provide further evidence that the multiple cerebral infarcts found in rats following photochemical damage to the carotid artery are caused by emboli and to eliminate the systemic hypotension and heating of the blood reported with the previous photochemical embolic stroke model (rose bengal and a green laser), I have modified the photochemical technique. Brain pathology was studied in 18 Wistar rats following carotid artery irradiation with a red laser (632 nm) at powers ranging from 100 to 800 mW/cm² for 10 or 20 minutes following the injection of the photosensitizing dye Photofrin II. Multiple cerebral arterioles were occluded by platelet aggregates containing frequent erythrocytes and leukocytes, identical to the thrombotic material in the carotid artery but different from the platelet aggregates seen in the carotid artery and the brain in the rose bengal model. Eighty infarcts were distributed randomly throughout the brain ipsilateral to the nonocclusive carotid thrombus. Significant heating (0.5°C or more) of the blood occurred only with laser powers higher (1,600 mW/cm²) or laser irradiations longer (25 minutes) than those used in the improved model of embolic stroke. This model mimics one mechanism of stroke in humans and provides a means to study systematically the morphological evolution of small cerebral infarcts. (Stroke 1991;22:225-232)

Photochemical induction of a nonocclusive thrombus in the carotid artery of a rat produces multiple cerebral infarcts. Defects in that model include transient, severe hypotension and heating of the blood. To eliminate these undesirable components of the model and to establish more directly embolism as the mechanism producing the multiple cerebral infarcts, a new model has been designed.

Although emboli can cause cerebral infarction, it is generally impossible to prove that an embolus was the cause of stroke in a particular patient. The evidence for an embolus is generally presumptive, most often based on the temporal profile (sudden onset of a focal neurologic deficit) in a patient with a potential source of emboli documented by cerebral angiography or echocardiography or on evidence of systemic embolization. Occasionally, autopsy provides additional evidence when the composition of the embolus in the brain is identical to that of a thrombus found at a potential source of embolization. Sometimes at autopsy, markers are found in the occluding material in the brain that not only suggest an embolus, but also a particular source; namely, calcium (embolus from a cardiac valve) and cholesterol (embolus from the carotid artery).

Models of embolic stroke have been produced in animals by the injection of numerous materials, including autologous blood clot or paraffin emboli, into the carotid artery, leaving little doubt of the mechanism. Embolism is the presumed mechanism of stroke following photochemical (laser and dye) damage to the carotid artery in rats. This assumption was based on several observations, including the finding of a nonocclusive thrombus in the carotid artery and presumed emboli, all composed of platelets, in distal arterioles in the brain. Additional evidence for embolism could be obtained by using a technique that produces a thrombus that is not composed entirely of platelets. Photofrin II (Quadralogic, Vancouver, B.C.), a mixture of hematoporphyrin ethers (HPE), a photosensitizing agent that produces a platelet thrombus that also contains red blood cells (RBCs) and white blood cells (WBCs), was selected.

Materials and Methods

Thirty-three male Wistar rats (21 experimental, four for measurement of physiological variables, and eight controls) weighing 275-450 g were used; all were anesthetized with 12 ml/kg i.p. chloral hydrate, 4.5 mg%. The neck was opened in the midline, and the right common carotid artery was exposed by separating the strap muscles. The vagus nerve was

From the Department of Neurology, Henry Ford Hospital, Detroit, Mich.

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Address for correspondence: Nancy Futrell, MD, Department of Neurology, K-11, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202-2689.

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The head was removed from each rat and placed in 10% neutral buffered formalin for at least 2 hours at room temperature. The brain was removed from the skull and placed in formalin at room temperature for at least 24 hours before being grossly sectioned into 2-mm-thick coronal blocks. Tissues were processed and embedded in paraffin, and two sections 7 μm thick from the posterior surface of each block were stained with hematoxylin and eosin. Following transcardiac perfusion, the carotid arteries of the rats sacrificed at 30 minutes and 2 hours were removed. After processing, serial cross sections 5 μm thick from the posterior surface of each block were stained with hematoxylin and eosin. The eight control rats (four with HPE injection followed by 0.6×1 cm, was placed under the vessel perpendicular to it and a second piece of aluminum foil, 0.6×1 cm, was placed under the vessel and accessible to the laser beam. A piece of aluminum foil, 3x3 cm with an opening of 5x5 mm, was placed over the carotid artery. This serves to expose a 5 mm length of the vessel to laser irradiation and to protect surrounding tissues from photochemical damage.

In the experimental rats, 12.5 mg/kg HPE was injected over 1 minute into the tail vein. The right carotid artery was irradiated with red light at 632 nm for 10–20 minutes with a laser power of 100–800 mW/cm² (Table 1). Rectal temperature was maintained between 35.5 and 37.0°C during the procedure. The animals were sacrificed (one 30 minutes, two 2 hours, and 18 1–7 days after irradiation) by transcardiac perfusion with 250 ml normal saline followed by 250 ml of 10% neutral buffered formalin at a pressure of 100 mm Hg as measured by a mercury manometer. The head was removed from each rat and placed in 10% neutral buffered formalin for at least 2 hours at room temperature. The brain was removed from the skull and placed in formalin at room temperature for at least 24 hours before being grossly sectioned into 2-mm-thick coronal blocks. Tissues were processed and embedded in paraffin, and two sections 7 μm thick from the posterior surface of each block were stained with hematoxylin and eosin. Following transcardiac perfusion, the carotid arteries of the rats sacrificed at 30 minutes and 2 hours were removed. After processing, serial cross sections 5 μm thick were cut and stained with hematoxylin and eosin. The eight control rats (four with HPE injection followed by carotid artery exposure and four with carotid artery exposure and laser at 200 mW/cm² for 20 or 30 minutes only) were sacrificed the day after the procedure and processed as above.

Physiological variables were measured in four rats. Blood pressure was monitored in two through a femoral arterial catheter during and for 2 minutes following the injection of 12.5 mg/kg HPE into a tail vein over 1 minute. The other two rats had a micro-

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### Table 1. Brain Pathology in Control and Experimental Rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>Laser Power (mW/cm²)</th>
<th>Laser Duration (min)</th>
<th>Laser Dye (mg/kg)</th>
<th>Infarct Location</th>
<th>Total Infarcts</th>
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<td></td>
<td></td>
<td></td>
<td>Basal ganglia</td>
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<td>Hippocampus</td>
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<tr>
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<td>35</td>
<td>12</td>
<td>22</td>
<td>4</td>
<td>80</td>
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</tbody>
</table>

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dissected from the carotid artery. With the vessel gently elevated, the strap muscles were sutured together so the carotid artery would remain exposed and accessible to the laser beam. A piece of aluminum foil, 0.6×1 cm, was placed under the vessel perpendicular to it and a second piece of aluminum foil, 3x3 cm with an opening of 5x5 mm, was placed over the carotid artery. This serves to expose a 5 mm length of the vessel to laser irradiation and to protect surrounding tissues from photochemical damage.

In the experimental rats, 12.5 mg/kg HPE was injected over 1 minute into the tail vein. The right carotid artery was irradiated with red light at 632 nm for 10–20 minutes with a laser power of 100–800 mW/cm² (Table 1). Rectal temperature was maintained between 35.5 and 37.0°C during the procedure. The animals were sacrificed (one 30 minutes, two 2 hours, and 18 1–7 days after irradiation) by transcardiac perfusion with 250 ml normal saline followed by 250 ml of 10% neutral buffered formalin at a pressure of 100 mm Hg as measured by a mercury manometer. The head was removed from each rat and placed in 10% neutral buffered formalin for at least 2 hours at room temperature. The brain was removed from the skull and placed in formalin at room temperature for at least 24 hours before being grossly sectioned into 2-mm-thick coronal blocks. Tissues were processed and embedded in paraffin, and two sections 7 μm thick from the posterior surface of each block were stained with hematoxylin and eosin. Following transcardiac perfusion, the carotid arteries of the rats sacrificed at 30 minutes and 2 hours were removed. After processing, serial cross sections 5 μm thick were cut and stained with hematoxylin and eosin. The eight control rats (four with HPE injection followed by carotid artery exposure and four with carotid artery exposure and laser at 200 mW/cm² for 20 or 30 minutes only) were sacrificed the day after the procedure and processed as above.

Physiological variables were measured in four rats. Blood pressure was monitored in two through a femoral arterial catheter during and for 2 minutes following the injection of 12.5 mg/kg HPE into a tail vein over 1 minute. The other two rats had a micro-
Results

A nonocclusive thrombus composed mostly of platelets with some RBCs and WBCs was seen in the right carotid artery of the experimental rats sacrificed 30 minutes or 2 hours after irradiation (Figure 1, left). In several areas the superficial portion of the thrombus appeared fragmented, and a tethered thrombus was identified (Figure 1, right). Numerous cerebral arterioles were occluded with platelet thrombi containing some RBCs and WBCs, appearing identical in composition to the thrombus in the carotid artery (Figure 2, left). Occluded arterioles were frequently seen in association with cerebral infarcts. In three experimental rats occlusions of two or more neighboring cortical arterioles were seen (Figure 2, right).

Multiple cerebral infarcts were present in the right side of the brain, ipsilateral to the nonocclusive carotid artery thrombus (Table 1) in the 18 experimental rats sacrificed 1–7 days after irradiation; there were no infarcts on the contralateral side. While most infarcts were oval (Figure 3, left) or round (Figure 3, right) with a diameter of 200–1,200 \( \mu m \), there were two larger infarcts, one in the dorsolateral caudate (1,500×600 \( \mu m \)) (Figure 4, top) and the other in the cortex (longest dimension 1,600 \( \mu m \)); this second infarct was irregularly shaped and was associated with multiple occluded vessels (Figure 4, bottom). There were no infarcts in the control animals (Table 1). Only the 18 experimental rats sacrificed 1–7 days after irradiation are included in Table 1; the three rats sacrificed ≤2 hours after irradiation were not included in the table since such early infarcts may not be reliably detected.
FIGURE 2. Left: Cortical arteriole is occluded with presumed embolus composed mainly of platelets with a few leukocytes in rat 1 day after irradiation (compare with thrombus in Figure 1, left). Scale marker 50 μm. Right: Five neighboring cortical arterioles are occluded 2 hours after irradiation. Scale marker 500 μm.

There was no change in blood pressure either during or in the 2 minutes following the injection of HPE. The first rat with rectal and carotid artery temperature monitoring had a rectal temperature of 36°C throughout the irradiation and a carotid artery temperature alternating between 36.1°C and 36.2°C on the digital monitor prior to irradiation. During the first 25 minutes of irradiation at 400 mW/cm², the temperature of the blood in the carotid artery remained stable at 36.3°C but increased gradually during the last 5 minutes of irradiation to 36.6°C. During irradiation of the tip of the probe, the temperature increased to 37°C. The second rat had a rectal temperature of 37°C and a carotid artery temperature of 37.2°C prior to irradiation. During irradiation at 400 mW/cm² the carotid artery temperature increased to 37.3°C. It increased further to 37.5°C during irradiation at 800 mW/cm² and to 38.0°C during irradiation at 1,600 mW/cm².

Discussion

This new technique reliably produces multiple cerebral infarcts in rats at laser powers and times equal to or greater than 200 mW/cm² for 20 minutes or 400 mW/cm² for 10 minutes while eliminating the heating of the blood and the systemic hypotension that occur in the rose bengal model. Irradiation at 200 mW/cm² for 20 minutes produced 37 infarcts in six rats (6.2 infarcts/animal), an improvement over the 44 infarcts in 13 rats (3.4 infarcts/animal) in the rose bengal model.

The evidence for emboli as the cause of multiple cerebral infarcts following photochemical damage to the carotid artery of rats has been strengthened by these new findings. The tethered thrombus (Figure 1, right) suggests that the thrombus in the carotid artery can release embolic fragments. Further support for this notion is provided by reports of emboli seen following photochemical damage to blood vessels using HPE12 and other photochemical techniques.13-15 The possibility of platelets aggregating in the blood stream (rather than on the endothelium, with subsequent embolization) is not likely because photochemically induced platelet aggregation is not seen in the absence of endothelium.16 Further, the same investigators found platelet aggregates deposited on dam-
aged endothelium in the area of irradiation but not on adjacent normal endothelium, as would be expected if platelets were directly injured by the photochemical insult.17

Comparing the composition of the occluding material in the brain arterioles and the nonocclusive thrombus in the carotid artery in the rose bengal and the HPE models provides further evidence that the occluding material in the brain arterioles originated in the carotid artery. In the rose bengal model, platelet thrombi and presumed platelet emboli were found in the carotid artery and brain arterioles. In the HPE model, the platelet thrombi and emboli contained frequent RBCs and WBCs (Figure 1 and Figure 2, left). Since platelet thrombi in humans generally contain some RBCs and WBCs,6,18,19 the HPE model may produce thrombi and emboli that simulate more closely those occurring in humans.

The reason for differences in the composition of thrombi produced by the rose bengal and the HPE techniques is unknown. Although the HPE photochemical model has been extensively studied since it is being used as therapy for several types of neoplasms20–22 and for the ablation of atherosclerotic plaques,23,24 the exact mechanism of photosensitization by porphyrins is not completely understood.25 Phototherapy with porphyrins produces damage to the endothelium,26 most likely via the production of singlet oxygen27,28 and/or free radicals.29 The postulated mechanism of the rose bengal model also includes singlet oxygen.30 Since these mechanisms are complicated and multifactorial,28,31 there may be a difference in mechanisms that accounts for the different composition of thrombi in the two models.

A major difference between the two techniques of embolic production of cerebral infarcts is the significant thermal effect produced by the rose bengal method,32 which uses much higher laser energies9 than the HPE method. In the rose bengal model, the temperature of the blood just distal to the site of irradiation in the carotid artery was raised to 39°C.1 Since WBCs and RBCs can be damaged by heat and by high laser powers,33,34 WBCs and RBCs might have been present initially in the forming thrombus.
in the carotid artery, only to be destroyed by the high laser power and the heat. This heating of the blood could also explain the effects on the blood-brain barrier found by investigators who used a variation of the rose bengal embolic stroke model\textsuperscript{35} with a much higher (2.5 times) average laser power.

There is less thermal effect in the HPE model since lower laser powers are used. This is possible because HPE can be used with red light (632 nm), which has a higher tissue penetration than the green laser\textsuperscript{36} (514.5 nm) used with rose bengal. Although slight direct tissue heating was detected in these experiments and has been reported using HPE phototherapy, there was no significant heating of the flowing blood in these experiments. Experiments are ongoing with a laser power of 200 mW/cm\textsuperscript{2} for 20 minutes since these are irradiation parameters that do not heat the blood and reliably produce infarcts.

Large and irregularly shaped infarcts are probably not produced by a single platelet embolus, but rather by several emboli occluding nearby vessels (Figures 2, right and 4, bottom). This could occur if multiple emboli lodged randomly in neighboring vessels. Another explanation is the division of platelet emboli, which I have observed (Figure 5) and has been reported in the literature\textsuperscript{6} in the retina of a patient with the new onset of unilateral amaurosis, with platelet emboli dividing at arteriolar bifurcations.
Concern has been raised about the lack of relevance of animal models of stroke to human disease, with an emphasis on the need to "adopt models that more closely approximate the clinical situation" in humans. This model was developed specifically to simulate one method of stroke production in humans. A nonocclusive thrombus is formed in the carotid artery and platelet emboli are carried to the brain by the flowing blood, simulating one mechanism of stroke in humans. The small cerebral infarcts that are produced are probably the brain correlates of retinal infarction and may be an important cause of disease in humans. The variability of infarct number and the randomness of infarct location occurs in other models of embolic stroke and also mimics embolic stroke in humans.

Advantages of this model include the lack of invasion of the carotid artery required by other models of embolic stroke and the possibility of spontaneous reperfusion occurring with this model (presently under study). Disadvantages include the need for anesthesia and the difficulty of using a model with the morphologic evolution of small embolic strokes.

FIGURE 5. Schematic drawings of platelet embolus in small arteriole. Center: As embolus contacts bifurcation it bulges and eventually splits. Right: Two emboli then course distal to bifurcation.

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References


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N Futrell


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