Development of a Rat Model of Photothrombotic Ischemia and Infarction Within the Caudoputamen

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Background and Purpose—Basal ganglia infarction is typically caused by the occlusion of deep arteries and the formation of relatively small lesions called lacunes. In the present study, a rat model of lacunar infarction was induced by photothrombotic occlusion of the small vessels within the caudate-putamen and subsequently characterized.

Methods—Male Sprague-Dawley rats (n=143) were anesthetized, and Rose Bengal dye (20 mg/kg) was intravenously injected. The left caudoputamen was exposed to cold white light for 5 to 10 minutes via a stereotaxically implanted polymethylmethacrylate optic fiber (0.5–0.75 mm diameter). Neurological and morphological changes were assessed at various times during the following 6 weeks. Local cerebral blood flow was measured 90 minutes after photothrombosis by [14C]-N-isopropyl-p-iodoamphetamine quantitative autoradiography. The time course of blood–brain barrier opening and ischemic brain edema as well as the effects of aspirin and tissue plasminogen activator treatment were also determined.

Results—A virtually round infarct with thrombosed parenchymal vessels surrounded by a layer of selective neuronal death was formed within the caudoputamen; it turned into a cystic cavity (lacune) over 6 weeks. A central zone of markedly reduced blood flow and surrounding oligemic zone were observed 90 minutes after light exposure. Lesion size was proportional to light exposure, and the severity and duration of neurological deficits paralleled infarct size. Early blood–brain barrier opening with edema peaked at day 1. After tissue plasminogen activator treatment, infarction volume and neurological deficits were reduced.

Conclusions—This study describes a new rat model of lacunar infarction by photothrombotic occlusion of the microvessels within the caudoputamen. With this model, infarct size correlates with the severity and duration of the neuropathology and can be varied by altering light exposure. (Stroke. 2009;40;248-253.) Key Words: cerebral blood flow ■ histology ■ infarction ■ neurological deficits ■ photothrombosis

Small infarctions in deep brain structures are typically caused by occlusion of small parenchymal arteries and are referred to as lacunes. In contrast, large cortical infarctions are generally produced by the occlusion of major intracranial arteries such as the middle cerebral artery. Although there are numerous animal models of cerebral ischemia induced by large pial artery occlusion, no entirely satisfactory model of deep small artery occlusion and infarction is available.

The aim of the present study was to establish a reproducible rat model of parenchymal vessel–microvessel occlusion by photothrombosis within the caudoputamen via a stereotaxically implanted plastic optic fiber. Photothrombosis has been widely used to induce ischemia in the cortex but not deep brain structures. To characterize the model, local cerebral blood flow (local CBF) 1.5 hour after lesioning and the time course of histological changes, blood–brain barrier (BBB) disruption, ischemic edema, and behavioral deficits were measured. The potential therapeutic effects of aspirin and tissue plasminogen activator (tPA) were also evaluated.

Materials and Methods

Animal Preparation

Experimental protocols were approved by the University of Michigan Committee on the Use and Care of Animals. Male Sprague-Dawley rats (Charles River Laboratories; Portage, Mich) weighing 270 to 320 grams (n=143) were used. Animals had free access to food and water before surgery. Anesthesia was induced by inhalation of 4% isoflurane in a mixture of nitrous oxide/oxygen mixture (70/30) and maintained by 2% isoflurane administered through a face mask. Rectal temperature was maintained at 37.5°C with the use of a feedback controlled heating pad (Model 74;Yellow Springs Instrument Co). The left femoral vein and artery were cannulated for drug injection and for monitoring blood gases and blood pressure. The rats were then placed in a stereotaxic holder and an optic fiber implanted into the left caudoputamen (stereotaxic coordinates: anterior to bregma=0 mm, left=3 mm, depth=4.5 mm) via a small burr hole. After light exposure, the optic fiber and catheters were removed, and the incisions were closed.

Photothrombosis Procedure

A polymethylmethacrylate optic fiber coated with polyethylene with 0.5 mm or 0.75 mm in diameter (Eska CK-20 or CK-30, respectively,
Morphometry, Histopathology, and Neurological Deficits

In the first part of this study, the time course of lesion volume, histology, and behavioral deficits were examined after photothrombosis. Lesion volumes were measured on cryosections of brains taken at 4 hours, 1 day, and 6 days after photothrombosis with 0.5 mm optic fiber and 10 minutes of light exposure (n=5 per group). Brains were immersed in 25% sucrose for 3 to 4 days at 4°C and embedded in the mixture of 25% sucrose and optimal cutting temperature compound (Sakura Finetek). Twenty-μm coronal frozen sections were taken every 0.2 mm with a cryostat from 2 mm anterior to 2 mm posterior to bregma. These sections were stained with hematoxylin and eosin and used to determine lesion volume with National Institutes of Health Image software.

To assess the dependency of lesion volume on the severity of light exposure, 3 groups (n=5/group) were treated as follows: 0.75 mm optic fiber plus 10 minutes of light; 0.5 mm optic fiber plus 10 minutes of light; and 0.5 mm optic fiber plus 5 minutes of light. Exposure for the controls was 0.5 mm optic fiber plus 10 minutes of light without Rose Bengal infusion (n=5).

For histopathology, rats were reanesthetized with 4% isoflurane and transcardially perfused with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (pH 7.4). The fixed brains were removed and kept in 4% paraformaldehyde for 6 hours. A coronal slab of brain tissue containing the center of the lesion was cut, embedded in epon, and prepared for ultrastructural examination. Electron microscopy was also performed on tissue from the 4-hour group (n=2). The rats underwent transcardiac perfusion with 4% paraformaldehyde and 2% glutaraldehyde. Specimens containing the photothrombosis-induced lesion were excised, embedded in epon, and prepared for ultrastructural examination.

Neurological deficits in the 4 groups (n=6/group) were examined by using forelimb placing, forelimb use asymmetry, and corner turn tests before and 1, 3, 7, 14, 28, and 42 days after photothrombosis. The 3 tests are sensitive to deficits induced by caudoputamenc injury and have been described previously. All tests were performed by a blinded investigator (Y.H.).

Brain Edema and BBB Leakage

The time course of brain edema formation and BBB opening was examined at 1.5 and 4 hours, and 1, 3, 7, and 42 days after light exposure (0.5 mm fiber, 10 minutes, n=6 per group). These animals were anesthetized and injected with Evans Blue solution (2 mL/kg, 2% in saline). After 1 hour, brains were removed and cut coronally (1-mm-thick) on a brain slicing matrix at the level of optic fiber 12,3,5-triphenyl tetrazolium chloride-stained coronal sections of brain to the level of the optic fiber 1 day after photothrombosis (bar=1.0 mm). A clearly demarcated (white) lesion indicative of mitochondrial failure is seen in the center of the caudoputamen around the end of the needle track. In this animal, Evans blue was injected intravenously before sacrifice and an area of blue staining is seen (indicating BBB leakage to Evans blue tagged albumin) within the 2,3,5-triphenyl tetrazolium chloride-demarcated lesion.

Local CBF Measurement

Local CBF was determined at 1.5 hour after photothrombosis (n=8) and in controls (n=3) using [14C]-N-isopropyl-p-iodoamphetamine ([14C]-IMP; American Radiolabeled Chemicals) and quantitative autoradiography. An intravenous bolus of [14C]-IMP (10 μCi) was injected at time zero. Just before this, a withdrawal of blood from the femoral artery was begun at a constant rate (0.2 mL/min); this yields the time-concentration integral of isotope in arterial blood. After 1 minute, the animal was killed by decapitation, and the brain was quickly removed and frozen in 2-methylbutane cooled by dry ice. Three 20-μm-thick coronal sections were taken at 300-μm intervals on a cryostat and heated for autoradiography for quantitative autoradiography. Radioactivity levels were quantified by measuring the optical densities within the regions indicated in Figure 1 with a computerized image analysis system (Model AIS; Imaging Research). The rate of local CBF (mL/100g/min) was calculated using the following equation:

\[ \text{local CBF} = \frac{Q_b(T) \times Fs}{Q_s(T)} \]

where \( Q_b(T) \) is the quantity of [14C]-IMP in the tissue at time T; \( Fs \) is the rate of blood withdrawal from t=0 to t=T; and \( Q_s(T) \) is the quantity of [14C]-IMP present in the withdrawal at time T.
Drug Efficacy
In the last part of the study, rats were treated with aspirin (acetylsalicylic acid, Sigma; 30 mg/kg, dissolved in 1 mL distilled water intraperitoneal injection) 2 hours before photothrombosis; 0.5-mm diameter optic fiber, 10 minutes of light exposure; n=6) or tPA (Alteplase, Genentech, 10 mg/kg, dissolved in 1 mL saline, infused intravenously over 30 minutes started at 0.5 hour after the end of light exposure, n=6). These rats were euthanized at 1 day under 5% isoflurane anesthesia, and their brains were removed and cut coronally at the level of optic fiber implantation. Specific gravity measurements and 2,3,5-triphenyl tetrazolium chloride staining were performed on these specimens as described: water content and size of the mitochondrial lesion, respectively, were calculated from these data. Results were compared to saline-treated (1.0 mL infusion, n=6) and control group (no Rose Bengal, n=6) data. The dosage and timing of aspirin and tPA treatments were determined from published studies of optimal therapeutic effects in rodents.9,10,11 Behavior tests were performed before and 1 day after photothrombosis.

Statistical Analysis
Statistical analysis was performed using repeated-measures ANOVA followed by Bonferroni post hoc test for multiple comparison. The level of statistical significance was set at P<0.05. Values are presented as mean±SEM.

Results
All animals survived the surgery. Blood gases and blood pressure were stable and within the normal range. At 24 hours after light exposure, an oval, lightly stained, ischemic lesion was present in the caudate-putamen around the tip of the optic fiber (Figure 1A–C), which became dead and infarcted (Figure 1D). Four hours after photothrombosis, the histology on the contralateral side was normal (Figure 2A), but that of the periphery of the lesion was abnormal with reversibly (pyknotic and eosinophilic) and reversibly injured neurons and numerous microvacuoles (Figure 2B,C). The latter structures are swollen astrocytic and neuronal processes within the neuropil. Many thrombosed microvessels were seen in the lesion periphery (Figure 2D). Evident at this time was platelet thrombus formation within parenchymal small vessels (Figure 2E,F), dark neurons (Figure 2G), hydropic swelling of astrocytes, especially the end-feet abutting microvessels and neuronal cell bodies (Figure 2E–G), and oligodendrocytes (not shown).

By day 1, neuronal destruction and neuropil microvacuolation became more evident in the center of the lesion (Figure 2H), and neuron immunoreactivity was essentially gone (data not shown). In the lesion periphery, marked neutrophil infiltration was seen at 3 days after photothrombosis (Figure 2I), whereas macrophage infiltration peaked at 2 weeks after photothrombosis (Figure 2J). Reactive astrogliosis (glial fibrillary acidic protein immunoreactive) and new capillary formation was also evident in the lesion periphery at 2 weeks after photothrombosis (Figure 2K). Reactive astrocytes, especially the end-feet abutting microvessels and numerous microvacuoles that give the neuropil a spongy appearance. In the lower left corner, most neuronal nuclei appear normal, and neuropil vacuolation is less prominent. C, Two pyknotic neurons with thin eosinophilic cytoplasm (red neurons; marked by *) in the lesion periphery 4 hours after light exposure. Such neuronal morphology is indicative of irreversible injury. Large vacuoles indicating cytotoxic edema are widespread. D, A small thrombosed blood vessel (~10 μm diameter) 4 hours after ictus. Typical electron micrograph of a large (E) and small microvessel (F) within the periphery at 4 hours after ictus. Both are filled with platelets and are surrounded by swollen perivascular cells, mostly astrocytic end-feet (* in F). G, An example of a shrunk, triangulated dark neuron within the periphery (bar=1.0 μm). It is surrounded by several swollen perineuronal astrocytic processes (*). H, Histology from the lesion center 1 day after ictus showing infarcted tissue and thrombosed small vessels (arrow). I, Marked neutrophil infiltration 3 days after ictus is evident on histology. Prominent macrophage infiltration at 2 weeks postictus (J) and reactive astrocytes with new capillary formation (K) are seen in these examples. L, Glial fibrillary acidic protein immunohistochemistry showing reactive astrocytes in the lesion periphery at 2 weeks. Cystic infarction with a thin glialic wall (arrow) and normal-looking adjacent tissue at low (M) and high (N) magnification. Scale bars: 20 μm in A,B, H, K–M; 10 μm in C,D; 1 μm in E–G; 40 μm in I, J, N.

Brain edema was detectable by 90 minutes, peaked at 1 day, and was resolved by 6 weeks (Figure 3C).

The color-coded maps of local CBF at 1.5 hour after photothrombosis (Figure 4A; 10 minutes of light exposure and a 0.5-mm optic fiber) indicated a large portion of the ipsilateral caudoputamen with very low flow (~10–15 mL/100g/min) compared to contralateral (~80 mL/100g/min). The area of low-flow was similar to the size of a typical lesion and much larger than the needle track (Figure 1C).
The profile of local CBF as a function of distance (0.3 mm intervals) from the center of the lesion shows that blood flow for the photothrombotic group increased progressively from 15% of contralateral near zero to 30% at 0.75 mm and 50% at 2 mm (Figure 4B). In controls, blood flow was reduced in the area where the fiber optic was placed but was greater than in the photothrombotic animals. The difference between the 2 lines is the extent that photothrombosis reduced local CBF and ranged from 15% of contralateral in the center to 40% in the periphery. There was no difference in contralateral blood flows between photothrombotic and control rats (average 80±12 mL/100g/min).

As for the regions of interest marked in Figure 1A, local CBF in the lesion center (site “a”) was 15±7% of that in the contralateral hemisphere (P<0.001). In the periphery of the caudoputamen (site “b”), local CBF was 64±15% of contralateral (location “c”; P<0.01), a level that is not likely to cause ischemic damage. The reduction in flow in the 2 cortical regions (areas “d” and “e”; 95±18% and 91±19% of contralateral, respectively) was statistically insignificant.

Significant neurological deficits were noted after photothrombosis (Figure 5) and were more severe and lasted longer in animals with larger lesions (Figure 3 shows the relationship among optic fiber diameter, duration of light exposure, and lesion size at 1 day). For all 3 tests, the scores peaked at 1 day and then gradually improved (Figure 5). For the medium intensity exposure case (10 minutes of light with a 0.5-mm fiber) significant impairment persisted for 3 days for the forelimb placing test, 1 week for the forelimb use asymmetry test, and 2 weeks for the corner turn test.

Aspirin pretreatment had no effect on lesion volume and brain edema formation 1 day after photothrombosis (Figure 6A,B, respectively). Whereas there was a slight improvement in the corner turn test in the aspirin-treated rats, there was no effect on forelimb placing or forelimb use asymmetry tests (Figure 6C). In contrast, tPA treatment (starting 30 minutes after light exposure) significantly reduced lesion volume.
(Figure 6A) and ameliorated neurological deficits as assessed by all 3 tests (Figure 6C) but did not affect postischemic edema (Figure 6B). We did not detect any macroscopic hemorrhages in rats treated with tPA; occasional hemorrhages at the microscopic level were observed.

Discussion

Watson et al developed a method to induce photothermotic infarction in the cerebral cortex that has the advantage of precise control of the size and location of the infarct, ie, reproducibility. The current study aimed to establish a reproducible model of a deep small infarction in the caudoputamen using a stereotaxically implanted optic fiber and Rose Bengal to cause photothermotic ischemia.

A thin polymethylmethacrylate optic fiber was used in this study to deliver cold light to the caudoputamen. Polymethylmethacrylate optic fibers are suitable for cold lighting because they transmit very little infrared light. In fact, tissue injury and neurological deficits were relatively mild in control rats that underwent optic fiber implantation and light exposure without Rose Bengal dye infusion. Histological examination early after light exposure with Rose Bengal showed an almost spherical infarct around the tip of the fiber optic surrounded by a peripheral area of selective neuronal death and ischemic edema. Thrombotic occlusion of small parenchymal vessels was found in the center and periphery of the lesion, and local CBF was decreased by \( \frac{85}{100} \) in the lesion center, the result of both fiber implantation and photothermotic ischemia. This is, thus, a model of deep, localized brain ischemia that induces subsequent infarction. We did not detect any perifocal hyperemia as has been observed after cortical photothermotic ischemia.

Previous studies on cortical photothermotic ischemia revealed significant enlargement of the ischemic lesion in the early phase after light exposure. In the present study, a significant increase in the size of caudoputamen ischemic lesion occurred over the day after injury. The lesion volume, after subtracting enlargement by edema, approximately doubled between 4 and 24 hours after induction of photothermotic ischemia, which suggests an ischemic penumbra within this lesion. Our finding of a beneficial effect of tPA supports this suggestion of an ischemic penumbra for this model.

For the evaluation of neurological deficits, forelimb placing, forelimb use asymmetry, and corner turn tests were used. These tests have been shown to be very sensitive to
caudoputamen injury in rats. The present findings concur with this. The deficits gradually recovered over 6 weeks after injury. The severity of neurological deficits paralleled the size of ischemic lesion and severity of exposure (light intensity times duration). A reduction in infarct size with tPA was also reflected by an improvement in neurological status as assessed by these three tests.

BBB opening takes place soon after lesioning in this model. Early BBB opening was found in air embolus model of ischemia. 15 In both models, occlusion of small parenchymal vessels is probably an important factor in the early BBB opening, which is different from the delayed BBB opening that often occurs after large artery occlusion. 16 Time course of brain edema and BBB opening was similar to cortical photothrombosis. 17

Lacunar infarction is a common type of ischemia involving deep brain structures and, in most instances, the occlusion of small parenchymal arterioles; it often shows characteristic neurological symptoms. Beneficial effects of tPA treatment on lacunar infarction in humans has been shown. 18 The present results are in accordance with these findings. The current caudate photothrombosis model of lacunar infarction thus appears suitable for future therapeutic and behavioral studies. Previous studies on arterial photothrombotic ischemia revealed a lack of beneficial effect of tPA treatment, 19 indicating that platelet thrombi are not fibrin-stabilized. In the present study on caudoputamen in situ photothrombosis, reduction of infarct size was observed after tPA treatment, suggesting some fibrin stabilization of thrombi produced in the small vessels of caudoputamen. Further studies are needed to clarify possible differences in the thrombotic mechanisms after caudoputamen photothrombosis.

Pretreatment with aspirin has been reported to be effective for reducing infarction volume after common carotid artery occlusion in rodents. 9 In contrast, the effect of aspirin on infarct volume and neurological symptoms was very mild in the present lacunar infarction model. These results indicate different mechanisms of aspirin on the small vessel and large vessel thrombotic occlusion. Because the mechanism of action changes depending on the dosage, further studies on the dosage and time course will be important for the understanding of the pathomechanisms of the basal ganglia infarction caused by photothrombotic occlusion of small vessels.

The current study primarily used a 0.5-mm-diameter plastic optic fiber. The needle tract lesion was significantly smaller than the photothrombotic lesion (Figure 3A). There were no behavioral deficits in rats that just had the fiber optic insertion plus light (no Rose Bengal), and the local CBF decrease did not reach ischemic level except for the area adjacent to the optic fiber. However, several additional mechanisms have been shown to be involved in the tissue injury by brain penetration. Therefore, as for technical improvements, a thinner optic fiber that could transmit enough cold light for lesioning and still retaining enough stiffness for accurate stereotaxic implantation might prove to be advantageous.

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Disclosures

None.

References

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