Brain Edema and Blood–Brain Barrier Opening After Photothrombotic Ischemia in Rat

Toshihiko Kuroiwa, Guohua Xi, Ya Hua, Tavarekere N. Nagaraja, Joseph D. Fenstermacher, and Richard F. Keep

Abstract We have examined the time course of brain edema and the blood–brain barrier opening in rat after basal ganglia ischemia induced by photothrombotic occlusion of the small vessels within the caudate–putamen. Male SD rats were anesthetized, and Rose Bengal dye was intravenously injected. The left caudo-putamen was exposed to cold white light for 5–10 min via a stereotaxically implanted optic fiber. Ischemic brain edema and the blood–brain barrier, as well as the histological changes, were assessed at various times during the following 6 weeks. Local cerebral blood flow was measured 90 min after photothrombosis by quantitative autoradiography. A round infarct with thrombosed parenchymal vessels surrounded by a layer of selective neuronal death was formed within the caudo-putamen. The ischemic lesion turned into a lacune over a period of 6 weeks. A central zone of markedly reduced blood flow and a surrounding oligemic zone were observed 90 min after light exposure. Early blood–brain barrier opening with edema was observed as early as 4 h after photothrombosis, peaked at day 1, and disappeared at 7 days after photothrombosis. In a model of lacunar infarction, we observed an early and transient brain edema and blood–brain opening after onset of ischemia.

Keywords Photothrombosis • Caudo-putamen • Histology Cerebral blood flow • Blood–brain barrier and brain edema

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Introduction

Small infarctions in deep brain structures are typically caused by occlusion of small parenchymal arteries and are referred to as lacunes. Stroke-prone spontaneous hypertensive (SHRSP) rat has been shown to develop small infarcts in the basal ganglia, but the unpredictable onset, size, and location of the ischemic lesions in this model make it difficult to use in mechanistic and therapeutic studies [3, 13]. Photothrombosis has been widely used to induce ischemia in the cortex, but not deep brain structures [1, 11]. We have developed an animal model of photothrombotic ischemia within the caudo-putamen in rat, and examined the time course of brain edema and blood–brain barrier opening after basal ganglia photothrombosis [5].

Materials and Methods

Male SD rats were anesthetized, and Rose Bengal dye (20 mg/kg) was intravenously injected. The left caudo-putamen was exposed to cold white light for 5–10 min via a stereotaxically implanted optic fiber (0.5 mm diameter, Eska CK-20; Mitsubishi Rayon, Tokyo, Japan; Fig. 1a, b) [5, 9]. For histopathology, rats were re-anesthetized with 4 % isoflurane and transcardially perfused with 4 % paraformaldehyde in 0.1 mol/l phosphate-buffered saline at various times from 4 h to 6 weeks following photothrombosis. The fixed brains were removed and kept in 4 % paraformaldehyde for 6 h. A coronal slab of brain tissue containing the center of the lesion was cut, embedded in paraffin, sectioned, and prepared for hematoxylin and eosin staining. Electron microscopy was also performed on tissue from the 4 h group. The rats underwent transcardiac perfusion with 4 % glutaraldehyde. Specimens containing the photothrombosis-induced lesion were excised, embedded in epon, and prepared for

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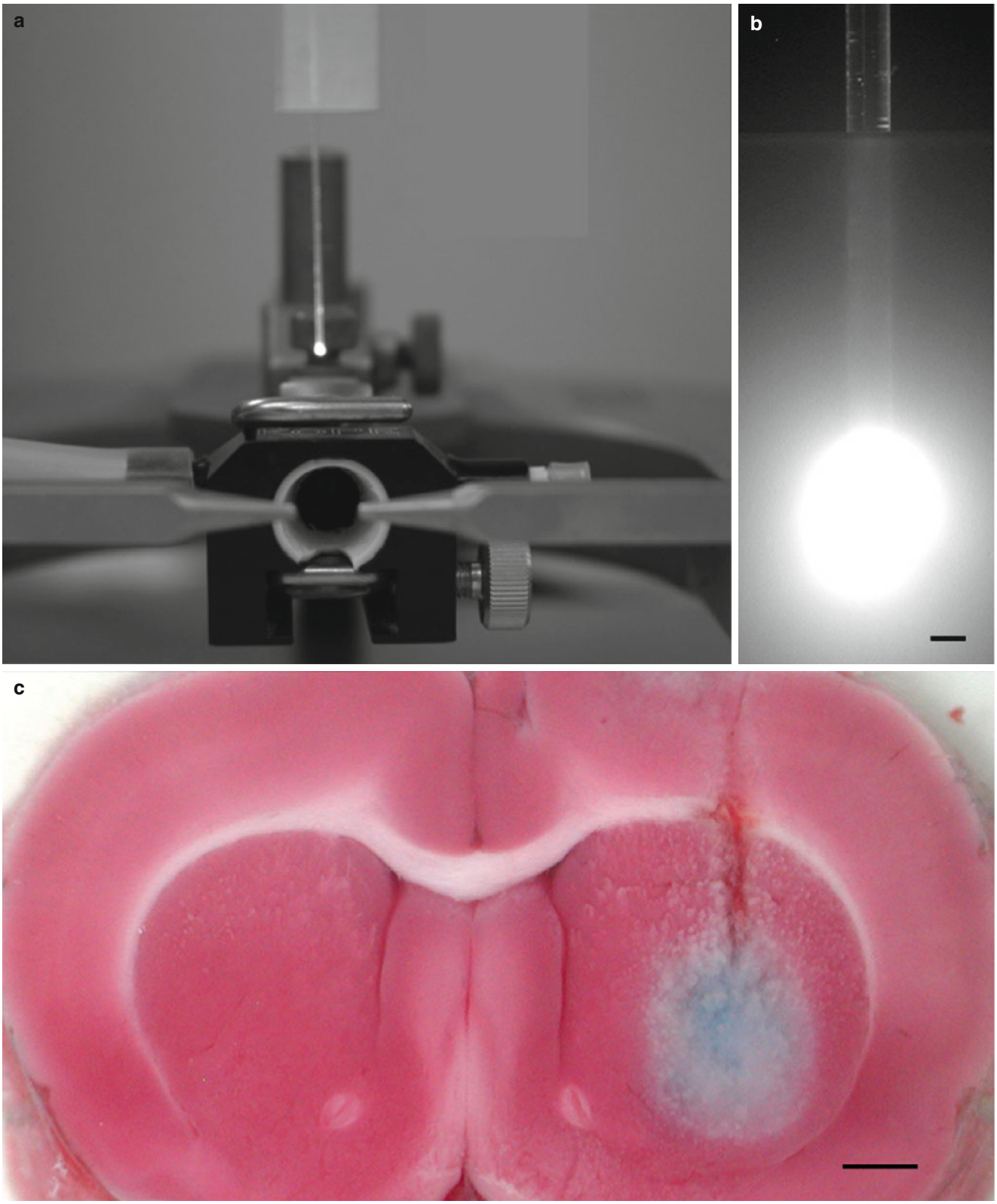

Fig. 1 (**a**) A stereotaxic device with a polymethylmethacrylate optic fiber coated with polyethylene measuring 0.5 mm in diameter (Eska CK-20, Mitsubishi Rayon, Tokyo, Japan) (**b**) Optic fiber with sharp beveled edge connected to a light source (bar = 0.5 mm) (**c**) Typical TTC-stained coronal section of brain at the level of the optic fiber 1 day after photothrombosis (bar = 1.0 mm). A clearly demarcated (*white*) lesion indicative of infarction is seen in the center of the caudo-putamen around the end of the needle track. In this animal, Evans Blue was injected i.v. before sacrifice and an area of *blue* staining is seen (indicating blood–brain barrier leakage to Evans Blue-tagged albumin) within the 2,3,5-triphenyl tetrazolium chloride (TTC)-demarcated lesion

ultrastructural examination. For the assessment of brain edema and blood–brain barrier permeability, animals were anesthetized and injected with Evans Blue solution. After 1 h, brains were removed and cut coronally on a brain-slicing matrix at the level of optic fiber implantation. Tissue samples of the photothrombotic lesion and corresponding site in the contralateral caudate were excised and dropped into a kerosene/monobromobenzene gradient column for specific gravity measurement, and the water content was calculated [2, 7]. An adjacent section was stained with 2,3,5-triphenyl tetrazolium chloride (TTC) to visualize the area of infarction and Evans Blue leakage (Fig. 1c) [6]. Local cerebral blood flow was measured 90 min after photothrombosis by quantitative autoradiography [8]. Ultrastructural assessment of blood–brain barrier change was performed in the 4 h group. One milliliter of saline with 25 mg horseradish peroxide (HRP; Sigma Type II) was injected i.v. into the animals 30 min before the animals were perfused with the fixative. Fixed brains were processed for enzyme histochemical reactions [10].

Results

A round infarct with thrombosed parenchymal vessels surrounded by a layer of selective neuronal death was formed within the caudo-putamen around the tip of the optic fiber (Fig. 2a). The ischemic lesion turned into a cystic cavity (lacune) over a period of 6 weeks. Four hours after photothrombosis, the histology of the periphery of the lesion was abnormal with pyknotic and eosinophilic neurons and numerous microvacuoles. The latter structures are swollen astrocytic and neuronal processes within the neuropil. Platelet thrombus formation within parenchymal small vessels, dark neurons, and hydropic swelling of astrocytes and oligodendrocytes were evident at this time (Fig. 2b, c). Multiple HRP vesicles were present in the endothelia of small vessels in the lesion in the 4 h group (Fig. 2d, e). By day 1, neuronal destruction and neuropilar microvacuolation became more evident in the center of the lesion. At the lesion periphery, marked neutrophil infiltration was seen 3 days after photothrombosis, whereas macrophage infiltration peaked at

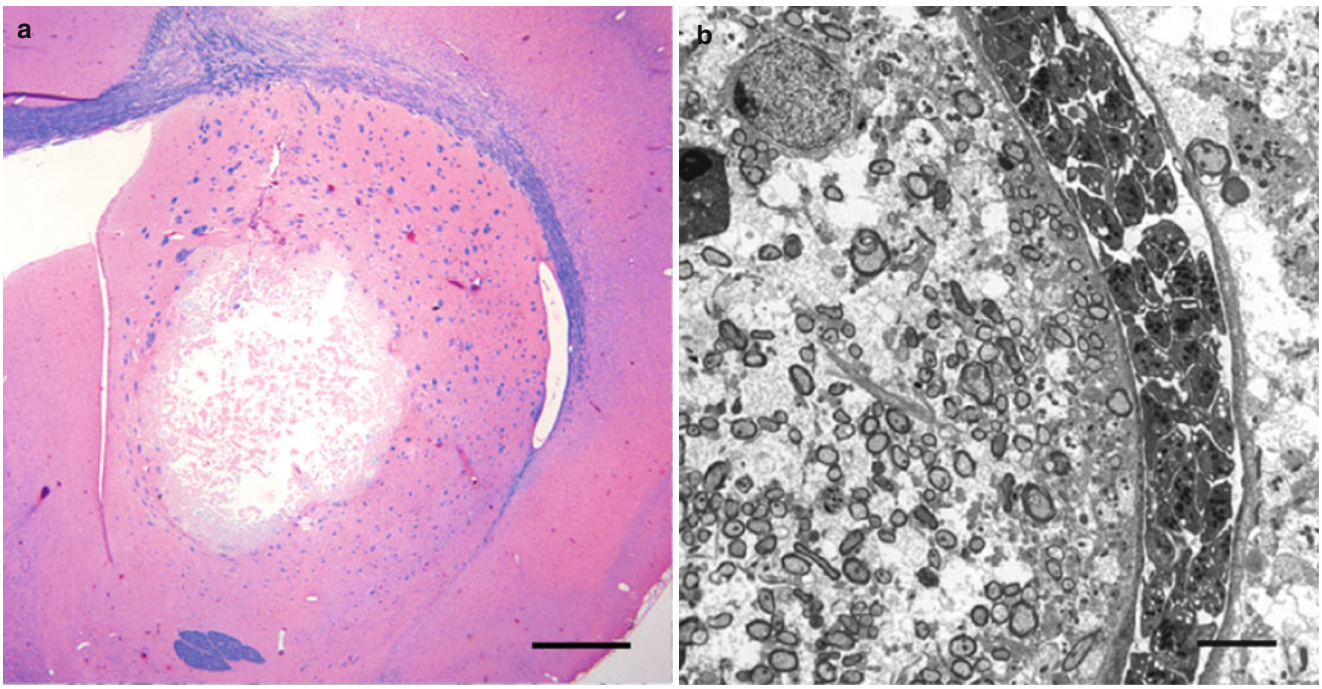

Fig. 2 (**a**) Typical lesion 1 day after photothrombosis (10 min exposure; Luxol Fast Blue and Eosin staining; scale bar = 1.0 mm). There is a round lesion in the center of the caudo-putamen. The area of central infarction was surrounded by a thin layer of selective neuronal death. (**b**) Typical electron micrograph of a small microvessel within the periphery at 4 h after light exposure. The vessel is filled with platelets and is surrounded by swollen perivascular cells, mostly astrocytic end-feet (bar = 10 μm). (**c**) An example of a triangulated ischemic neuron within the ischemic periphery (bar = 10 μm). The neuron is surrounded by several swollen perineuronal astrocytic processes. (**d**) Horseradish peroxide (HRP) vesicles detected in the endothelium of microvessels in the ischemic region, indicating increased blood–brain barrier permeability (bar = 1 μm). (**e**) Higher magnification of the capillary endothelium with HRP vesicles (bar = 0.1 μm)

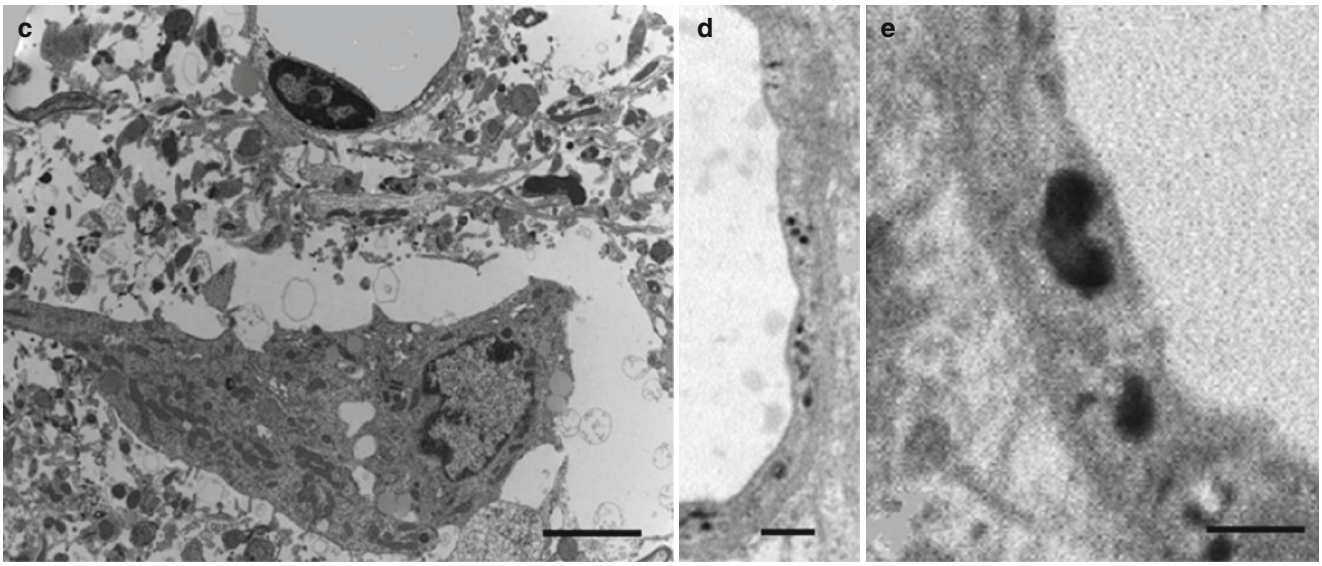

Fig. 2 (continued)

2 weeks after photothrombosis. Reactive astrocytosis and new capillary formation were also evident in the lesion periphery at 2 weeks. A small cyst with a thin layer of gliosis was observed 6 weeks after light exposure. The color-coded maps of lCBF at 1.5 h after photothrombosis indicated a large portion of the ipsilateral caudo-putamen with very low flow (approximately 10–15 ml/100 g/min) compared with contralateral (approximately 80 ml/100 g/min). Evans Blue leakage was observed at 1.5 h in 3 out of 6 animals, 4 h in 6 out of 6 animals, 1 day in 6 out of 6 animals, 4 days in 4 out of 6, 7 days in 1 out of 6 animals after photothrombosis, but not at all at 6 weeks. Brain edema was detectable by 1.5 h, peaked at 1 day, and was resolved by 6 weeks (Fig. 3).

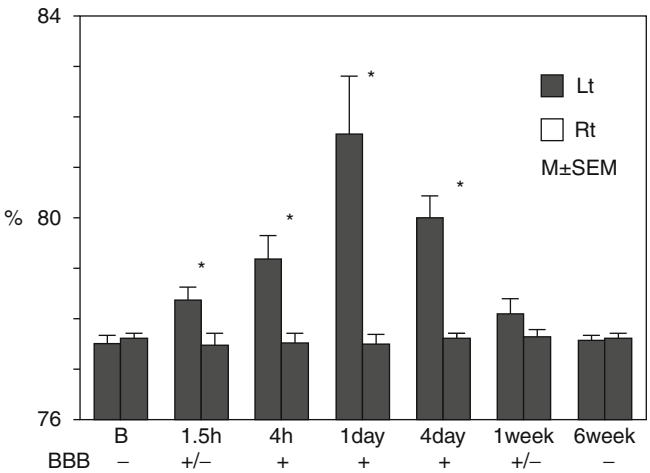

Fig. 3 Time course of tissue water content and blood–brain barrier permeability change after photothrombosis. Water content (%, vertical axis) was measured in the left and right caudate. $*P<0.05$ versus contralateral. Evans Blue leakage was observed at 1.5 h, 4 h, 1 day, 4 days, and 7 days after photothrombosis, but not at 6 weeks. Brain edema was detectable by 1.5 h, peaked at 1 day, and was resolved by 6 weeks

Conclusion

Watson et al. developed a method of inducing photothrombotic infarction in the cerebral cortex that has the advantage of precise control of the size and location of the infarct [11]. Current study aimed to establish a reproducible model of a deep small infarction in the caudo-putamen using a stereotaxically implanted optic fiber. Polymethylmethacrylate optic fibers are suitable for cold lighting because they transmit very little infrared light. Histological examination early after light exposure after Rose Bengal dye infusion 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 lCBF was decreased by about 85 % in the lesion center. This is, thus, a model of deep, localized brain ischemia that induces subsequent infarction. Blood–brain barrier opening takes place shortly after lesioning in this model. Early BBB opening was found in an air embolus model of ischemia [12]. In both models, occlusion of small parenchymal vessels is probably an important factor in early BBB opening, which is different from the delayed BBB opening that often occurs after large artery occlusion [4]. In summary, an animal model of photothrombotic caudo-putamen infarction with histological changes similar to those found in lacunar infarcts has been developed.

Opening of the BBB and ischemic edema were found at an early stage after onset. This model can be employed to test differences in the effects of potential therapeutic agents between large and small vessels (lacunar) ischemia or among sites of ischemic injury.

Conflict of Interest We declare that we have no conflict of interest.

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