Characterization of Photothrombotic Cerebral Infarction Model At Sensorimotor Area of Functional Map in Rat

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Summary

Purpose: The purpose of this study is to induce a photothrombotic cerebral infarction (PTCI) model with sensorimotor dysfunction in rats with a cortical functional map and to find the time course of neurological and morphological changes.

Methods: Using sixteen rats, a PTCI was produced by using an intravenous injection of rose bengal dye (20mg/kg) and exposed to cold light for 20 min at a position of 1 mm anterior to bregma and 3.5 mm right lateral of midline, with the help of a cortical functional map. Serial changes were evaluated in magnetic resonance images, in neurology by Rota-rod test (RRT) and Dynamic Plantar Aesthesiometer (DPA), and in pathology over time from day 1 through 14.

Results: The brain infarction was successfully induced in all 16 rats without a single death. Infarction volume was reduced gradually over time from 147 mm³ to 100, 47, 35, and 27 mm³ on days 1, 3, 7, 11, and 14 respectively. By RRT evaluation, significant increased neurological dysfunction from 41% to 49% was observed in 2 weeks. A DPA examination revealed a dysfunction of contralateral forelimb from 181% to 163% within 2 weeks. Hemorrhage and liquefaction were observed by MRI and histopathology.

Conclusions: Sensorimotor dysfunction continued for 2 weeks in the PTCI model by targeting relevant cortex areas in the rat brain guided by a cortical functional map.

Key words: Photothrombotic cerebral infarction model, Cortical functional map, Rota-rod test, Dynamic plantar aesthesiometer, Sensorimotor cortex

Sonuçları Üzgün Olan Sensorimotor Alanında Fototrombotik Serebral İnfarkt Karakterizasyonu

Özet

Amaç: Bu çalışmanın amacı bir kortikal fonksiyonel harita ile üçanda sensorimotor bozukluğun фототромботик сerebral инфаркт modelinde (PTCI) oluşturmak ve nörolojik ve morfolojik değişiklikleri bir zaman seyrince araştırmaktır.

Yöntemler: PTCI kortikal fonksiyonel harita yardımı ile 16 üçanda intravenöz yolla verilen rose Bengal boyası (20mg/Kg dozunda) ve bregmanın 1mm önü orta-hattın 3,5mm sağ odaklanarak 20dakika soguk ışık kaynağı ile ekspoze edilerek oluşturuldu. Manyetik rezonans görüntüleme ile seri olarak değişiklikler değerlendirildi. Nöroloji Rota-rod testi (RRT) ve Dinamik Planter Estesiometre (DPA) ile ve patoloji 1 ila 14 gün arası araştırıldı.

Sonuçlar: Tek bir ölüm olmadan beyin infarktüsleri tüm üçanlarda (n=16) başarı ile oluşturuldu. İnfart hacmi başlangıç 147 mm³ degerinden sırasıyla 1., 3., 7., ve 14. günlerde 100, 47, 35, 27 mm³ olarak giderek azalan degerlerde ölçüldü. RRT değerlendirilmesinde 2 hafta
INTRODUCTION

Stroke is a severe acute neurological disorder causing irreversible damage of the brain tissue with motor, sensory, and cognitive impairments. The major reasons for stroke are: i) a decreased or completely blocked blood flow to an area of brain and ii) cerebral hemorrhage. Stroke is one of the leading causes of long-term disability, morbidity, and mortality in the world. Due to the aging of the population, the socioeconomic importance of stroke is growing. The increased stroke prevalence has drawn the attention of researchers to using good animal models to study the pathophysiology, molecular mechanisms, diagnosis, and therapy of stroke disease.

Several established animal models for cerebral infarction, such as middle cerebral artery (MCA) ligation model, carotid artery occlusion model, MCA embolization model, photothermogenic stroke model, chemical agent injection model, and cardiac arrest model are available in the literature.

Photothrombotic cerebral infarction (PTCI) model was first introduced more than 2 decades ago. This model involves the intravenous injection of a photosensitizing dye and applying a light on a skull, which is exposed through an incising scalp. The PTCI model has been increasingly used by many researchers compared to the MCA occlusion model, because this model is less invasive, highly reproducible, easy to produce, and easy to aim at the desired lesion.

To improve the usefulness of the PTCI model, it is important to localize the light on the area using a special functioning cortex. For example, to make a constant and reproducible model of sensorimotor dysfunction, the light should be applied and localized correctly on the sensorimotor cortex. There is a map book available on rat's cerebral cortical function. However, the book used thick coronal slices to mark the functions. It is difficult to localize the area for infarction at sensorimotor cortex with the map book. Therefore for the present study, we should redraw a cortical functional map using the coronal slice maps.

The purpose of this study is to induce the PTCI model with a sensorimotor dysfunction in a rat with a cortical functional map, to find the time course of neurological and morphological changes occurring in the brain infarction through functional evaluation, MRI and histopathology, and to interpret the MRI findings by matching with histopathology at the corresponding region.

MATERIAL AND METHODS

Rats were maintained in accordance to the guidelines set by the Institutional Animal Ethics Committee at Chung-Ang University. This study was approved by the committee (CAUMD 11-0020). Before using the rats for experiment, they adapted to the environment for one week in a room with 12-hour light-dark cycle at 21-24 °C temperature, and fed with standard rat food and tap water ad libitum. Sixteen male Sprague Dawley rats (Daehan Biolink, Eumseong, Korea) weighing 250-300g each were used for creating a PTCI model. Six rats were used for MRI scans and functional evaluation were performed.
consecutively on days 1, 3, 7, 11, and 14, and 10 rats for MRI scans and pathologic evaluation were performed taking 2 rats on each day of sampling for days 1, 3, 7, 11, and 14 after creating a PTCI model. The rats were sacrificed and their brains were fixed for histopathological studies.

Mapping functional areas of rat brain

We created a cortical functional map with the help of Paxinos and Watson's atlas book, "The Rat Brain in Stereotaxic Coordinates". In the book, cortical functional areas were marked at coronal plane images of the rats' brains. By using AutoCAD® (Autodesk Inc., San Rafael, CA, USA), the marked points were translocated to the surface image of the rats' brains viewed from vertex (Fig. 1).

**Fig 1:** Cortical map of functional area in rats' brains viewed from vertex. Seven-mm diameter circle focusing on 1 mm anterior to bregma and 3.5 mm at right lateral of the midline representing the right sensorimotor cortex

**Induction of PTCI**

Photothrombosis in the targeted tissue of the rats' brains was induced by slightly modifying existing standard methods. Rats were starved for food overnight, and were anesthetized with isoflurane (Ilung Pharmaceutical Co., Seoul, Korea) 5% for induction and 1.5-2% during surgical preparation. Rats were placed in a prone position on a stereotactic head holder. The scalp was incised in the midline and the periosteum was kept aside to expose the desired area of the skull. Rose bengal (Rose bengal sodium salt; Sigma–Aldrich Co., Saint Louis, MO, USA) solution of 10mg/ml concentration in 0.9 % saline was injected into each rat's tail vein at a dose of 20 mg/kg body weight. A light generator (Fiber-Lite MI-150; Dolan Jenner Co., Lancaster, SC, USA) with a halogen bulb (Ushio EKE 150W/21V; Ushio, Tokyo, Japan), infra-red filter, a fiber optic cable, and applicator was used to produce cold light (3,200 K in color temperature) of 400-670 nm wave length from 7 mm
diameter aperture applicator. The target area that was stereotactically focused on 1 mm anterior to the bregma and 3.5 mm at the right lateral of the midline to induce topographically defined photothrombotic lesions in the right sensorimotor cortex was illuminated for 20 min (Fig. 1). After removing the light source, the incisions were sutured. During surgical procedure, the body temperature of the rat was maintained between 36°C and 37°C by a thermostatically controlled heating blanket (Homeothermic blanket control unit; Harvard Apparatus, Holliston, MA, USA) and the rectal temperature was monitored continuously.

Magnetic resonance images (MRI)

In vivo MRI of the rats' brains in prone position was performed using a 3.0 T clinical MRI scanner (Achieva; Philips Healthcare, Eindhoven, The Netherlands) and wrist radiofrequency coil (Sense Wrist-4; Philips Healthcare). Rats were anesthetized by using an intramuscular injection of a mixture of Ketara® (Ketamine hydrochloride; Yuhan, Seoul, Korea) at 100 mg/kg body weight and Rompun® (Xylazine hydrochloride; Bayer Korea, Ansan, Korea) at 10 mg/kg. The MR protocol includes T1 weighted images (turbo spin echo technique, TR 838 msec, TE 28 msec, field of view 50 x 50 mm, slice thickness 1 mm, matrix size 172 x 176, number of excitation 4, resolution 291 x 284 x 1000 µm) and T2 weighted images (turbo spin echo technique, TR 2741 msec, TE 80 msec and other parameters similar to T1 weighted image) in coronal plane. MR images were analyzed by two independent experts using a picture archiving and communication system (PACS, Maroview version 5.4; Marotech, Seoul, Korea) on the infarct itself, any change in the surrounding brain, and swellings on T1 and T2 WI. Cerebral infarction in volume was quantitatively assessed by a summation of the areas of infarction (high signal) in coronal plane of T2WI with Image J software (National Institute of Health, Bethesda, MD, USA).

Motor coordination and balance tests

a. Rota-rod test

Motor and sensory function after induction of cerebral infarction was evaluated by placing rats on a moving Rota-rod cylinder and measuring the duration of contact on the rod by using a Rota-rod device (Ugo Basil, Comerio, Italy). The rats were trained 5 times a day on the Rota-rod for 5 days before inducing photothermolysis. Initially, the Rota-rod cylinder was set to rotate at 4 rpm speed for the first 30 seconds and the speed was gradually increased in 9 steps with an acceleration of 4 rpm for every 30 seconds and a constant speed of 40 rpm was maintained. The duration of contact on the Rota-rod cylinder was measured 5 times in seconds by an automatic sensor before and 1, 3, 7, 11, and 14 days after photothermolysis in the rats. Only three readings were considered out of a total of five measurements because the maximum and minimum readings were omitted to reduce statistical error.

b. Measurement of foot pressure by dynamic plantar aesthesiometer

The effect of cerebral infarction on sensorimotor function was evaluated by using a Dynamic Plantar Aesthesiometer (DPA, Ugo Basile, Comerio, Italy) which is an advanced form of von Frey's hair testing. The level of touch sensitivity due to cerebral damage was detected by different von Frey hairs of 20 microfilaments in each set pressed against the foot skin of a rat on plantar surface until the paw is withdrawn and the weight force can be automatically recorded in grams. The rats were placed on a testing arena with a wire mesh floor. The DPA device was positioned beneath the rat, so that the filaments were raised to contact the foot and the weight force was gradually increased from zero grams to a maximum force of 50g in a 20 sec time.
with an increment of 2.5g per second until the rat withdrew its foot\textsuperscript{21}. The rats adapted to the DPA device five times a day for 5 days before inducing PTCI. The weight force in grams at the time of paw withdrawal was measured before PTCI and 1, 3, 7, 11, and 14 days after infarction in rats and the average percentage of 3 readings of post and pre data was recorded \[\frac{(\text{Post data} / \text{pre data}) \times 100}{\text{Post data}}\]. Only three readings were considered out of a total of five measurements because the maximum and minimum readings were omitted to reduce statistical error.

Tissue extraction

The animals were euthanized at different time points by cardiac perfusion of tissue fixative, using 4% paraformaldehyde. First, the animals were anesthetized by intramuscular injection of a mixture of Ketara\textsuperscript{®} (ketamine hydrochloride; Yuhan, Seoul, Korea) and Rompun\textsuperscript{®} (xylazine hydrochloride; Bayer Korea, Ansan, Korea) at 100 mg/kg and 10 mg/kg doses, respectively. A thoracoabdominal incision was performed to expose the heart and a 16G needle was inserted into the left ventricle to the ascending aorta and 250-280 ml of cold saline solution containing 2 U/ml heparin (heparin sodium; Green Cross Co., Yongin, Korea) was infused using an infusion pump (Master Flex; Cole-Parmer, Barrington, IL, USA) at 1 ml/g body weight and at a rate of 16.5 ml/min. Through an incision in the right atrium, the blood was discharged freely; thus, the discharge changed in color from bloody to clear. A similar amount of tissue fixative, 4% paraformaldehyde (pH 7.4), was infused immediately with the same rate of perfusion. The brain was excised and fixed in 4% paraformaldehyde. The excised brain was cut into 2 in the coronal plane of the brain at the center of the infarction area using a rat brain matrix (RBM-4000C; ASI Instruments, Warrin, MI, USA) and the slices were re-fixed in the same fixative for 24 hours before making paraffin blocks.

Histological analysis of brain infarction

Tissue sections of 5 µm thickness were made from paraffin blocks using microtome. The sections were stained with hematoxylin and eosin. A histological evaluation was performed by a pathologist using an optical microscope (Olympus BX51; Olympus Co., Tokyo, Japan) through supports of MRI findings matched with HE staining slides of the corresponding rat from day 1 through day 14 after photothrombosis. Inflammatory cells (polymorphonuclear leukocyte, PMN) and macrophages infiltration, hemorrhage, angiogenesis, and gliosis were observed in the tissue sections and the degree of severity was assessed as mild (+), moderate (++), or remarkable (+++). The presence (O) or absence (-) of tissue degeneration or liquefaction necrosis was also represented.

Data analysis

Data was analyzed using SPSS V 18.0 (PASW statistics 18; IBM, Chicago, IL, USA) and the difference between each group was evaluated by applying the Wilcoxon signed rank test, with a p value of less than 0.05 considered as significantly different. All data were expressed as the mean ± error of mean.

RESULTS

Assessment of brain infarction by magnetic resonance imaging (MRI)

The brain infarction was successfully induced in all 16 rats without a single death. High signal intensity of infarction was located at the cortex of the right frontoparietal lobe and more remarkable on T2WI than T1WI (Fig. 2A). On T2WI, the area of infarct was 6.9 mm ± 0.5 in diameter and 3.2 mm ± 0.3 in depth on the coronal view of the brain on day 1. The high signal intensity of infarction was highest on day 1, reduced through days 3 and 7, and then developed iso-signal intensity on days 11 and 14. A low signal intensity rim was noted on days 3, 7, and 11, especially at the inside along the border
between infarction and the surrounding normal brain tissue, which might be hemorrhage or neo vessels. Small areas with bright and high signal intensity on T2WI and low signal intensity on T1WI at the border were noted on days 11 and 14, which might be gliosis or liquefaction of infarcted tissue with cerebrospinal fluid. Cytotoxic edema with midline shift was remarkable on day 1, reduced through day 3, and then normalized on days 7 through 14.

Using Image J software, the infarction area of all slices was summed up to obtain the total infarction volume of 147 mm$^3$ ± 20 (100%) on the 1st day, 100 mm$^3$ ± 20 (68%) on the 3rd day, 47 mm$^3$ ± 14 (32%) on the 7th day, 35 mm$^3$ ± 10 (24%) on the 11th day and 27 mm$^3$ ± 9 (18%) on the 14th day after an induction of cerebral infarction on T2WI (Fig 2B). A gradual reduction in the cerebral infarction volume occurred spontaneously over time without any treatment.

Motor coordination and balance

a. Rota-rod test

Average duration of contact to assess the motor coordination and balance for 6 normal rats on Rota-rod was 300 s ± 18 (100%), however the duration in infarcted rats was 124 s ± 14 (41%), 151 s ± 21 (50%), 122 s ± 10 (41%), 172 s ± 19 (57%) and 146 s ± 18 (49%) on 1, 3, 7, 11 and 14th day respectively after infarction (Fig. 3). Neurological dysfunction and deterioration of motor function in rats PTCI model resulted significantly with a rapid decline of 41% within one day, and this persisted for 14 days (p<0.05).

b. Dynamic plantar foot pressure

Paw withdrawal weight threshold was automatically recorded by von Frey filaments using the Dynamic Plantar Aesthesiometer (DPA) and the percent weight changed with brain infarction of the rats was plotted (Fig. 4). Plantar responses were different for each paw in normal rats before phototherombosis. Basically (fundamentally), forepaws (13.5 g ± 1.7) were more sensitive than hindpaws (22.7 g ± 4.1). Before inducing phototherombosis, the average stimulating weight with the left forepaw of 6 rats was 12.3 g ± 0.8 (100%). Upon inducing phototherombosis in the right hemisphere of the rats' brains, the stimulating weight with the left forepaw was dramatically increased to 22.3 g ± 1.2 (181%) on day 1, and maintained at 19.6 g ± 0.7 (159%) on day 3, 21.3 g ± 0.5 (173%) on day 7, 21.7 g ± 0.9 (176%) on day 11, and 20.1 g ± 0.6 (163%) on day 14 (Fig. 4), which indicates a significant sensorimotor dysfunction (p < 0.05) for 2 weeks. However in the case of the right forepaw, the weight was 14.7 g ± 1.1 (100%) before phototherombosis, whereas the weights were 15.4 g ± 1.0 (105%), 14.0 g ± 0.7 (95%), 16.9 g ± 0.4 (115%), 17.0 g ± 1.2 (116%), and 15.0 g ± 0.9 (102%) after 1, 3, 7, 11, and 14 days of brain infarction, respectively (p > 0.05). The paw withdrawal weight thresholds with the left hindpaw before (23.4 g ± 1.9 - 100%) and 1, 3, 7, 11, and 14 days (26.7 g ± 1.8 - 114%, 24.9 g ± 0.6 -106%, 25.9 g ± 1.7 - 111%, 24.1 g ± 0.9 - 103% and 24.9 g ± 1.7 - 106%) after inducing PTCI did not change significantly (p > 0.05). The results were very similar with the right hindpaw, before (22.0 g ± 1.6 - 100%) and 1, 3, 7, 11, and 14 days (19.7 g ± 2.0 - 90%, 19.7 g ± 0.9 - 90%, 21.8 g ± 1.8 - 99%, 21.6 g ± 1.1 - 98% and 23.6 g ± 1.8 - 107%) after infarction. There was no significant difference found in the right forelimb and both hindlimbs (p > 0.05) in the PTCI model. The data showed that infarction in the right hemisphere of the brain could cause significant sensorimotor dysfunction in the left forepaw for 2 weeks, while the other 3 paws remained within normal limits.

Histological analysis of cerebral infarction

MRI signals of rats' brains from day 1 through day 14 after phototherombosis matched with the microscopic findings of HE-stained brain tissue slices of the
corresponding rat (Fig. 5). The low signal intensity rim on T1 and T2WI on days 3, 7, and 11 on the inside along the border between infarction and surrounding normal brain tissue were hemorrhage. Small areas with bright and high signal intensity on T2WI and low signal intensity on T1WI at the border on days 11 and 14 were liquefied area with cerebrospinal fluid confirmed by the HE staining of the corresponding plane.

PMN infiltrations were remarkable at the area of infarction on day 1 (+++) and reduced through day 7 (+). Macrophage infiltrations were seen on day 3 (++), increased remarkably (+++) on day 7, and then reduced (+/-) on day 11. Hemorrhages were developed on day 3 (++), were remarkable on day 7 (+++), and then sustained through day 14 (++). Angiogenesis was developed in the periphery of infarction on day 3 (+), increased on day 7 (++), and then became remarkable on day 14 (+++). Gliosis with active proliferation of glial cells was seen on day 11 (+), and remarkable on day 14 (+++). Liquefaction of the necrotic tissue created an empty space on days 11 and 14 (Table 1).

Fig 2: Serial changes in the cerebral cortical infarction on magnetic resonance images over time from day 1 through 14. Series of T1 and T2 weighted magnetic resonance images in one rat. a White arrows indicate infarction, black arrow heads hemorrhage, and white arrow head gliosis and liquefaction. b Changes of infarction volume in six rats are shown using Image J software during a two-week period.
Fig 3: Assessment of a sensorimotor dysfunction using a Rota-rod test after photothrombotic cerebral infarction in 6 rats over time. There is significant sensorimotor dysfunction and this continued for 2 weeks \( (p < 0.05) \) after inflicted injury on the right sensorimotor cortex.

Fig 4: Assessment of sensorimotor dysfunction by recording the paw withdrawal rate \( (\%) \) using a Dynamic Plantar Aesthesiometer (DPA) after photothrombosis. There is a significant sensorimotor dysfunction in the left forelimb after photothrombosis and this continued for 2 weeks \( (p < 0.05) \); however, the other limbs were not affected \( (p > 0.05) \) after inflicting injury on the right sensorimotor cortex.
**Table 1** Histopathologic analyses with hematoxylin-eosin staining of cerebral infarction over time (day 1 through day 14) in Sprague Dawley rats after modeling the photothrombotic cerebral infarction model

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DISCUSSION

In the PTCI model, the intravenous injection of a photosensitive dye, Rose Bengal (C_{20}H_{4}Cl_{4}I_{4}O_{5}, Molar mass = 973.67g/mol) into the body causes photooxidation in the illuminated area due to a generation of free radicals, which in turn damages the endothelial cells, resulting in thrombosis in the illuminated vessels, and ultimately leading to infarction and brain ischemia\(^{25,21}\). By adjusting the light intensity, the size of infarction and the degree of neurological dysfunction can be controlled\(^{16,11}\).

The PTCI model, unlike other conventional cerebral infarction models in rats, has many advantages: less invasive, highly reproducible, easy to produce, and easy to aim desired lesion. The size and location of the infarct lesion could not be controlled in the MCA occlusion model of infarction but the PTCI model allows modulation. Additionally, ipsilateral ICA or MCA can be saved and is not occluded. Through the artery, the intra-arterial administration of drug or cells can be tried for its special purposes to understand the effect of a treatment. However, the PTCI model is different from the cerebral infarction that is developed in human beings in its cause, course, and morphometry. Because the transition from infarction to normal brain is abrupt, the ischemic penumbra zone is narrower in the PTCI model than other models. One other disadvantage is that a deep region, such as the basal ganglia, cannot be targeted because of the penetrating limitation of the depth of light. To overcome this problem, a lacunar infarction model of rats was created through a photothrombotic occlusion of small vessels within the caudoputamen\(^{11}\), but the craniectomy and insertion of a needle-optic fiber in the brain are needed, and as such, the advantages of the PTCI model disappear.

As there has not been a convenient map of the cortical function in rats, previous investigators have applied light at different foci from each other to create a PTCI model for a rat's sensorimotor dysfunction: focused at posterior 2.5 mm to bregma, 3.0 mm lateral of midline\(^{8}\), anterior 0.5 mm, 4.5 mm lateral\(^{14}\), and anterior 1.5 mm, 2.5 mm lateral\(^{17}\). As a result, the degree of sensorimotor dysfunction was not constant and not effective to test certain drugs for treatment and cells to improve dysfunction. Using the map of a rat's brain, the optimal point for the sensorimotor cortex may be anterior 1 mm to bregma, lateral 3.5 mm to midline (Fig. 1). In the present study, a consistent impairment of sensorimotor function was induced successfully by cold light from 7 mm aperture.

In several investigations with a focal ischemic stroke model, the degree of neurological dysfunction was assessed by sensorimotor functional tests, such as the Rota-rod test, motor scoring, Single pellet reaching task (SPRT), and Morris water maze (MWM) test, among others\(^{3,5,7}\). However these tests have limitations because the results obtained are not consistent and reproducible when the neurological dysfunction is at a low level. In the present study, the Rota-rod test, which is a motor functional test, yielded reproducible results with a dramatic decline in the motor function (Fig. 3) because the sensorimotor cortex was selectively targeted with the help of a cortical functional map (Fig. 1). We used the DPA to evaluate sensory and motor functions in the PTCI model for the first time. Each limb can be tested by this method. Injury in the right sensorimotor cortex region could cause dysfunction in the left forelimb alone, but not in the other 3 limbs (Fig. 4). The left hindlimb was not affected. Findings were statistically significant. The extent of damage and rate of recovery can be evaluated by functional
tests, which may help to design future treatment strategies in stroke models.

Several papers in which MRI was used for the PTCI model have been reported on the MRI findings of the PTCI. In the present study, several MRI findings were newly observed and confirmed by using the HE staining of the same plane in a corresponding rat. Hemorrhage and liquefaction necrosis in conjunction with infarction were observed and might be typically seen only in a PTCI model. Hemorrhage was located inside or at the border of infarction on the MRI on days 3, 7, and 11. The reason for hemorrhage in the PTCI model is not known; however, the shift in angiogenic processes might cause the newly formed vessels and collateral vessels to be leaky in structure. Another possibility is that the damage of vascular endothelium by free radicals may come to break down to bleed. To interpret MRI of PTCI, a low signal intensity rim should be considered a hemorrhage. Meanwhile, liquefaction necrosis created a bright and high signal on T2WI and a low signal on T1WI. It is probably that the liquefaction of the necrotic tissue and the absorption of infarction debris created fluid-filled empty spaces on days 11 and 14. These findings were first mentioned in our study using the PTCI model.

One limitation of this study is that the accuracy of the cortical functional map was made with the assistance of a book reference. The book had used a fresh brain from a male 290 g Wistar rat, not a Sprague Dawley rat as we had used. Thus, the map may be not perfect for this study using a Sprague Dawley rat. However, if the Wistar rat can be used or a correct map can be made for the Sprague Dawley rat, this PTCI model may be more useful. In addition, observations were limited to 2 weeks and the number of rats used was small. Further long term follow-up studies with a larger number of rats are necessary to evaluate the time course of the sensorimotor dysfunction.

In conclusion, cerebral infarction was successfully induced in rats by using a cold light from a halogen lamp for 20 min through an optic fiber with 7 mm diameter applicator focusing on 1 mm anterior to bregma and 3.5 mm at the right lateral of the midline, which could result in significant motor and sensory dysfunctions.

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

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