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Neurobehavioural deficits correlate with the cerebral infarction volume of stroke animals: A comparative study on ischaemia-reperfusion and photothrombosis models

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ABSTRACT

The study investigated the correlation between infarction areas and behavioural deficits in middle cerebral artery occlusion (MCAO) and photothrombosis stroke models. In the MCAO model, a 0.38 mm-diameter silicone-coated thread was introduced through the left external carotid artery and advanced 18 mm via the internal carotid artery to the origin of middle cerebral artery of male Sprague–Dawley rats weighing 300–350 g. The thread was removed for reperfusion after occlusion for 0.5, 1 or 2 h. In the photothrombosis model, after a mid-line incision on the scalp, a focused light (10,000 lux, 6 mm-diameter) was delivered 1 mm anterior to the bregma and 3 mm left of the midline for 5, 10 or 20 min. During the first 2 min of irradiation, Rose Bengal dye (30 mg/kg) was injected intravenously. Twenty four hours post-surgery, the animals were subjected to neurological scoring and behavioural performances, and were sacrificed for macroscopic and microscopic examinations of brain injury. Total infarction volumes in the MCAO model rats increased in an occlusion time-dependent manner, while the infarction volumes in photothrombosis model rats plateaued relatively quickly with no time-dependent increase. The MCAO model displayed neurological scores and behavioural deficits that correlated well with infarction volumes, while relatively poor correlation between infarction volume and neurobehavioural abnormalities was evident in the photothrombosis model. The results indicate the suitability of the MCAO model for studies on preventive or therapeutic compounds related to functional recovery, although the photothrombosis model might be useful to generate focused lesions leading to the location-related behavioural changes.

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1. Introduction

Stroke is the third leading cause of death in the United States. With 700,000 cases per year, a person dies of stroke every 3 min (Carmichael, 2005). Stroke is also the leading cause of adult disability; in the 76% of those who survive their stroke, 50% have a hemiparesis, 26% are dependent in activities of daily living, and 26% are forced into a nursing home. These statistics have propelled the search for neuroprotective therapies to reduce cell death and infarct volume after stroke. Most strokes (80%) are ischaemic and the majority of ischaemic strokes result from an occlusion of a major cerebral artery by a thrombosis or an embolism, which leads to loss of blood flow in a specific region (Green et al., 2003). The remaining strokes are hemorrhagic, where a blood vessel bursts either in the brain or on its surface.

Cerebral ischaemia is a deficiency of the blood supply to a part of the brain, which produces a cerebral infarction (Garcia et al., 1993). There is a long list of symptoms and signs of stroke that are determined by the size and location of the infarction (Rogers and Hunter, 1997). These range from neck stiffness, nausea and headache to blurred vision, vertigo, dizziness and convulsions. There are also a wide range of motor and sensorimotor deficits, including tremor, lack of coordination and partial paralysis. In addition, higher cortical dysfunction is also manifested as amnesia, dementia and delirium as well as language and speech disturbances, and patients surviving a stroke may be mentally and physically disabled.

There are several main ischaemia models, and these can be predominantly grouped into global and focal ischaemia models. Global models concern cardiac arrest producing selective necrosis, while focal models are more relevant to acute ischaemic stroke producing brain infarction (Hunter et al., 1995). Many different models of focal cerebral ischaemia have been used to study potential therapeutics and pathological progression over time. Methods of producing ischaemia utilizing middle cerebral artery occlusion (MCAO) include direct surgical arterial occlusion by intraluminal suture (Tamura et al., 1981a). A photothrombosis model uses a different method of producing ischaemia, which involves direct thrombosis of cortical microvessels generated by singlet oxygen activation of the thrombotic cascade, produced via a photochemical reaction induced by the activation of intravenous Rose Bengal with intense light (Watson et al., 1985). This model produces a circumscribed region of ischaemia whose location can be tailored. In addition, it is extremely easy to implement, and can be instituted rapidly and repetitively (Van Bruggen et al., 1992; Kharlamov et al., 1993; Pierpaoli et al., 1993; Lanens et al., 1995; De Ryck et al., 2000).

Each model has been characterized by using conventional histochemical and neurological techniques (Tamura et al., 1981b; Dietrich et al., 1987a). However, the relationship between treatment duration, infarction area and consequential neurological impairment has not been established. Therefore, we carried out a comparative study on MCAO and photothrombosis rat models to explore the correlation between morphological findings and neurobehavioural deficits according to the treatment duration.

2. Materials and methods

2.1. Animals

Nine-week-old male Sprague–Dawley rats weighing 300–350 g were purchased from a commercial breeder (Daehan Biolink, Eumseong, Korea). They were housed in an environmentally controlled room with constant temperature (23 ± 3 °C), relative humidity (50 ± 10%), and 12-h light cycle. Animals were fed a standard commercial rodent chow (Daehan Biolink). All animal experiments were in accordance with the Standard Operation Procedures of Laboratory Animal and approved by Institutional Animal Care and Use Committee of Laboratory Animal Research Center, Chungbuk National University.

2.2. Surgeries

2.2.1. MCAO

Rats used for the MCAO model were divided into 4 groups depending on the occlusion time: sham control ($n = 6$), 0.5 ($n = 15$), 1 ($n = 15$) and 2 h ($n = 15$). The occlusion times were adopted to induce minimal, moderate and severe visible infarctions 24 h later as demonstrated in our recent report (Park et al., in press). All the animals were subjected to behavioural tests 24 h after the onset of occlusion and were then sacrificed for the morphological analyses.

Silcone-coated thread was prepared based on the method described by Schmid-Elsaesser et al. (1998). Briefly, polyethylene tubing (Intramedic, Batavia, IL, USA) with a nominal internal diameter of 0.38 mm was filled with silicone (Koreseal, Busan, Korea). A 3/0 monofilament nylon suture (Ailee, Busan, Korea) was inserted 5 mm into one end of the polyethylene tubing. Care was taken throughout the procedure to ensure that bubbles did not form in the silicone. After 24 h, the tubing and encased silicone was cut using a razor blade at a point 0.5 mm beyond the tip of the suture. Immediately prior to surgery, the suture was removed from the tubing, leaving a uniform coating of silicone bonded to the distal 5 mm.

Focal cerebral ischaemia was produced as described previously (Longa et al., 1989) with slight modification. Briefly, a midline incision was made on the ventral side of the neck, exposing the left common carotid artery, which was freed from surrounding tissues. The external carotid artery was ligated and dissected. Silicone-coated thread was introduced through the external carotid artery and advanced 18 mm via the internal carotid artery up to the origin of the middle cerebral artery. After occlusion was achieved, the silicone-coated thread was secured in place by ligature and the incision was sutured. In the sham-operated group, only the filament was introduced into the external carotid artery but was not advanced. During the operation, the animals were anaesthetized by isoflurane via a nose cone and the rectal body temperature was maintained at 37 °C using a temperature-regulated heating pad. The silicone thread was removed for reperfusion after 0.5, 1, or 2-h occlusion ($n = 15$/group). Anaesthesia was maintained during the surgery (approximately 10 min) and each occlusion time; 40, 70 or 130 min.