LETTER TO THE EDITOR

A Modified Model of Middle Cerebral Artery Electrocoagulation in Mice

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Ischemic stroke is a devastating disease that represents the second leading cause of death worldwide [1]. As ischemic stroke in patients usually results from a thrombotic or embolic occlusion in a major cerebral artery, most often the middle cerebral artery (MCA), experimental focal cerebral ischemia models have been

developed to mimic human stroke and serve as an indispensable tool in the stroke research field [2].

Two procedures, microsurgical direct occlusion of the MCA by electrocoagulation (electrocoagulation model) and intraluminal thread occlusion of the MCA (intraluminal occlusion model), are

Figure 1 The left middle cerebral artery occlusion sites in the conventional (CEM) and modified electrocoagulation models (MEM) in mice. CEM and MEM indicate, respectively, the occlusion sites in CEM and MEM.

Figure 2 The representative images of 2,3,5-triphenyltetrazolium chloride (TTC)-stained brain sections (A), cerebral infarct volumes (B), and neurobehavioral outcomes (C, Body Asymmetry Test; D, Beam Test) at 24 and 72 h after electrocoagulation occlusion of the left middle cerebral artery in the CEM and MEM. CEM, the conventional electrocoagulation model; MEM, the modified electrocoagulation model; $n = 6-11$. ** $P < 0.01$ versus CEM-24 h and CEM-72 h.

now widely used methods of MCA occlusion in mice [3]. The size of infarction was more consistent in the electrocoagulation model than that in the intraluminal occlusion model [2,3]. However, the size of infarction in the former model was much smaller than that in the latter model, thus affecting the assessment of neurobehavioral outcomes and limiting the use of the electrocoagulation model in stroke research [2-6]. The aim of this study was to modify the conventional electrocoagulation model in an attempt to create another stable and reproducible focal cerebral ischemia model in mice, which could display larger infarct sizes and more profound neurobehavioral changes after cerebral ischemia.

Male C57BL/6 mice weighing 18-22 g were purchased from Sino-British SIPPR/BK Laboratory Animal Ltd., Shanghai, China. All animals received humane care, and the experimental procedures were in compliance with the institutional animal care guidelines. Mice were anesthetized by intraperitoneal injection of 0.1 mL 3.5% chloral hydrate per 10 g body weight. A skin incision was made between the ear and the orbit on the left side. After splitting of the temporalis muscle, a burr hole was drilled at the junction of the zygomatic arch and the squamous bone, through which the stem of the left MCA was exposed and occluded by electrocoagulation. Surgery was performed under a microscope (Zhenjiang Zhongtian Optical Instrument Co. Ltd., Jiangsu, China). In the conventional electrocoagulation model (CEM), the

left MCA is occluded between its cortical branches and the lateral striate arteries [5,6] (Figure 1). In our modified electrocoagulation model (MEM), the left MCA was occluded by electrocoagulation distal to the lateral striate arteries (Figure 1). Body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ using a thermal blanket throughout the surgical procedure. Behavioral tests including Body Asymmetry Test and Beam Test were performed at 24 and 72 h after MCA occlusion with the examiner blind to the experimental groups, as detailed in previous publications [7,8]. The animals were then euthanized and the brains were stained with 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St. Louis, MO, USA) to determine the infarct volume as previously reported [9,10]. Data are expressed as mean $\pm$ SEM and analyzed with ANOVA. Statistical significance was set at $P < 0.05$.

In the present work, the cerebral infarct volume in the conventional electrocoagulation model (Figure 2B) was similar to that in the previous reports [5,6], while it was increased by about twofold in the MEM (24 h: $37.6 \pm 1.26\%$ vs. $16.2 \pm 0.96\%$; 72 h: $34.2 \pm 0.83\%$ vs. $17.5 \pm 0.78\%$; $P < 0.01$; Figure 2A,B). In addition, compared to the conventional model, the modified model exhibited a more profound behavioral outcome after cerebral ischemia in both Beam Test (24 h: 19.5 ± 0.42 second vs. 11.9 ± 0.62 second; 72 h: 18.8 ± 0.59 second vs. 9.82 ± 0.24 second; $P < 0.01$; Figure 2C) and Body Asymmetry Test (24 h:

$90.0 \pm 3.81\%$ vs. $27.5 \pm 7.50\%$; 72 h: $91.7 \pm 3.07\%$ vs. $25.0 \pm 6.71\%$; $P < 0.01$; Figure 2D). The standard error of the mean (SEM) values of all parameters determined in the present work were relatively small (Figure 2B–D), which might indicate that the MEM introduced in our work was a stable and reproducible cerebral ischemia model in mice. This has been confirmed in our several studies (data not shown). These results demonstrated that the MEM was able to show significantly larger infarct size and more profound behavioral changes after cerebral ischemia, as compared to the conventional model.

Above all, the MEM possesses two advantages over the conventional model: (1) it is able to show larger cerebral infarct volumes after cerebral ischemia so as to produce more obvious symptoms and help better mimic human stroke events and (2) it is able to show more profound behavioral changes after cerebral ischemia, which may be of great use in evaluating long-term neurobehav-

ioral outcomes after ischemic stroke. These advantages might help expand the use of electrocoagulation model in stroke research.

In conclusion, this study demonstrated that the MEM described herein is a stable and reproducible cerebral ischemia model in mice, able to show larger infarct volumes and more profound neurobehavioral changes after cerebral ischemia compared to the conventional model.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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