

## A Model of Focal Cortical Infarction in Rat : Minimally Invasive Craniotomy

XUE Jing , GAO Pei-yi , AN Yi-hua , SUN Chong-ran , LI Jin , HUANG Hua

**[ Abstract ] Objective** To develop a stable model of focal cerebral infarction in rat to study the curative effect of neural stem cells transplantation. **Methods** Thirty-seven rats were selected which were divided into two groups in random, experimental group and control group. The focal infarction model was developed by the ligation of the left middle cerebral artery followed by the ligation of the ipsilateral common carotid artery and the temporary clip occlusion of the contralateral common carotid artery for 1.5 h. The operation adopted minimally invasive craniotomy through temporal bone. The model was evaluated by examining the neurologic deficits, ink perfusion, TTC staining and Magnetic Resonance imaging. **Results** All the rats were in good condition after the operation, the mortality rate was 6.25% after 4 weeks. Ink perfusion and TTC staining confirmed that the ischemia was confined to the cortex. The areas of infarction measured 83.52 mm<sup>3</sup> by Magnetic Resonance imaging after 4 weeks. **Conclusion** A stable focal cerebral infarction model can be achieved by minimally invasive craniotomy. It is superior for its homogeneity of infarction volume and site, and its low mortality. It can be used for the study of transplantation of neural stem cells.

**[ Key words ]** cerebral infarction; animal model; middle cerebral artery; rat  
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**[ 摘要 ] 目的** 建立一种稳定的大鼠脑局灶性梗死模型,以用于脑梗死后神经干细胞移植的长期观察。**方法** 37 只大鼠随机分为实验组和对照组。微创法经颞骨局部钻孔开颅,采用直接结扎大脑中动脉终段,同时永久结扎同侧颈总动脉,暂时性夹闭对侧颈总动脉的方法制备大鼠脑梗死模型。通过大鼠脑梗死后的神经功能评分、墨汁灌注、TTC 染色、MRI 成像结果对该模型进行评价。**结果** 大鼠术后状态良好,实验组大鼠观察 4 周后死亡率仅为 6.25%。大鼠神经功能评分均为 1 分,墨汁灌注及 TTC 染色观察梗死范围局限于皮层,4 周后 MRI 成像测量梗死体积稳定,平均为 83.52 mm<sup>3</sup>。**结论** 该模型对大鼠创伤小,梗死灶的位置和体积恒定,长期存活率高,为脑梗死后神经干细胞移植的研究提供了一种理想的动物模型。

**[ 关键词 ]** 脑梗死;动物模型;大脑中动脉;大鼠

CLC Number: R743.3 Document code: A Article ID: 1006-9771(2006)01-0011-03

**[ Citation index ]** XUE Jing, GAO Pei-yi, AN Yi-hua, et al. A model of focal cortical infarction in rat: minimally invasive craniotomy[J]. Chin J Rehabil Theory Practice, 2006, 12(1): 11-13.

Considerable progress has been made in treating cerebral infarction through neural stem cells transplantation in rat. Current understanding of migration of transplanted cells in the host brain has been derived mainly from regional measurements of labeled grafted cells using histological and immunohistological methods. However, these methods require the invasive analysis of brain sections postmortem and do not allow dynamic assessment of migration of grafted cells. Therefore, it is the key point how to track the transplanted cells in vivo. Magnetic resonance imaging (MRI) could offer a noninvasive method for studying the fate of magnetically labeled cells and it is possible to make a breakthrough in such area. Here, using dynamic monitoring by MR imaging, the grafted cells should be observed for a long time in host

brain, so the selection of the animal model of cerebral infarction is very important. Requisition for such study included: ① the model is stable and the volume of infarction is relatively constant; ② the volume should be small, which avoided the scattering of the transplanted cells' migration and that will affect the observation of MR imaging; ③ the rate of intracerebral hemorrhage should be low, because oxyhaemoglobin may transform into hemosiderin, and the signal intensity of hemosiderin is the same as superparamagnetic particle within labeled cells; ④ the model needs to survive for a long time (>2 months) for monitoring the migration of the transplanted cells, which require that the ability of drinking water and taking food is not affected after operation.

Our teams aimed directly at the above requisition and developed the focal infarction model by ligation of the left middle cerebral (MCA) and the left common carotid arteries (CCA) and temporary clip occlusion of the right common carotid for one and a half hours. Then histology analysis and magnetic resonance imaging were performed, so we can establish a kind of animal model fitting to such studies.

### 1 Material and Methods

**1.1 Animals and grouping** All experiments were

Foundation Projects: The study is supported by Beijing Natural Science Foundation (No. 7062031; 7032009); National Natural Science Foundation of China (No. 3037042).

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approved by the Animal Ethics Committee of the Capital University of Medical Sciences. Thirty-seven male Sprague-Dawley rats weighting 280 ~ 300 g were obtained 7 ~ 10 d prior to the study and housed in the vivarium with free access to water and food in a 12-hour-light and darkness cycle. They were divided into two groups in random: control group (nine animals) and experimental group (twenty-eight animals).

Two groups of animal underwent different procedures. The animals of control group (sham operation group) underwent isolation of both the left MCA and bilateral CCAs without ligation. The animals of experimental group underwent ligation of the left MCA and left CCA and temporary clip occlusion of the right CCA for one and a half hours.

## 1.2 Methods

**1.2.1 Ligation of left MCA** The rats were anesthetized with Chloral Hydrate (400 mg/kg body weight, i. p.). Skin of the temporal-parietal region was shaved and a 1.5 cm scalp incision was made at the anterior 1/3 between the left eye and the left ear. The temporalis was separated in the plane of its fiber bundles and retracted in order to expose the zygoma and squamosal bone. Using microsurgical techniques, a burr hole, 2 mm in diameter, was made with a dental drill 1 mm rostral to the anterior junction of the zygoma and the squamosal bone. The drill pit was constantly cooled with saline at ambient temperature and care was taken to preserve a thin bone layer (that was later removed gently with forceps) at the depth of the hole in order to avoid thermal and physical injury to the cerebral cortex. The dura mater was carefully pierced with a scalpel. The end piece of the left MCA was exposed which was proximal to the inferior cerebral vein as described by Chen, et al<sup>[1]</sup>. The exposed MCA was isolated and ligated with a square knot using a 10-0 suture. Then the craniotomy was covered with a small piece of gelfoam, the temporalis and overlying skin were allowed to fall back and were sutured separated.

**1.2.2 Ligation of CCAs** The CCAs were isolated via a ventral midline cervical incision and both the CCAs were isolated. For experimental groups, the left CCA was ligated with 4-0 suture and the right CCA was clipped temporarily with a pair of non-traumatic micro-aneurysm clips for one and a half hours.

**1.2.3 Neurologic deficit** Neurologic deficit evaluation was conducted at 24 h after operation. The neurologic findings were scored on a four-point grade scale: no observable deficit, 0; forelimb flexion, 1; forelimb flexion and decreased resistance to lateral push, 2; forelimb flexion, decreased resistance to lateral push, and unilateral circling in three successive trials, 3; score 3 plus a decreased in consciousness, 4. Any other behavioral changes were also observed,

but not scored.

**1.2.4 Ink perfusion** Five minutes after operation, nine rats ( $n=3$  for the control group and  $n=6$  for the experimental group respectively) received intracardiac perfusion of 2 ml ink. The brain was removed from the skull two minutes later and observed the extent of ink perfusion.

**1.2.5 Histology analysis** Twenty-four hours after the left side MCA ligation, the rats ( $n=3$  for the control group and  $n=8$  for the experimental group) received intracardiac perfusion of 100 ml normal saline, and then 250 ml 4% paraformaldehyde in 0.1 M PBS (pH7.4) under deep anesthesia. The brain was removed from the skull and was put into the refrigerator at -15 °C for 20 ~ 30 min. For morphometric study, 2-mm-thick coronal sections were cut using a rat brain matrix. Total six coronal sections were prepared for estimation of the degree of infarct damage. Then brain slices were incubated in 2% 2,3,5-triphenyl tetrazolium chloride (TTC) for 30 min at 37 °C.

**1.2.6 Intercerebral hemorrhage observation** Intercerebral hemorrhage was evaluated at the time of the brain cutting. The location and extent of hemorrhage was recorded. Direct visualization of the brain surface (for subarachnoid hemorrhage) and careful study of sliced section (for intracerebral hemorrhage) were used to evaluate the extent of hemorrhage. The exact extent of the hemorrhage, however, was not quantified.

**1.2.7 MRI** MRI of animals were performed at 3 T clinical MR (Signa, GE medical system) by using a 5-inch received surface coil. Single sagittal, coronal, and transversal images were obtained by a fast gradient-echo sequence to localize the subsequent T2-weighted transversal images. The sequence parameters were: repetition time (TR) = 3100 ms, effective echo time (TE) = 122 ms, field of view (FOV) = 12 cm, matrix = 512 × 512, slice thickness = 2 mm, and slice separation = 1 mm. Then the volume of cerebral infarction is measured.

## 2 Results

After the dura mater was tored, the left MCA was seen clearly which was vertical to the inferior cerebral vein and had branches (Figure 2.1 in C3). When ligated with 10-0 suture, the distal end of the left MCA had no blood engorging (Figure 2.2 in C3). The animal models were well and could drink water and take food normally after operation. Four weeks later, there was no mortality in control group, and we noted 1 death in a total of 16 rats in experimental group.

Neurologic deficit<sup>[2]</sup> scores were observed in the two groups 24 h after operation. No abnormal neurological signs were detected in the control group rats. In contrast, the contralateral forelimb flexion was ob-

served in the experimental group. And the animals showed no significant difference in neurologic behavior.

Ink perfusion shows no perfusion distal to the ligation of left MCA in experimental group, so the ischemic parenchyma showed light pink and the other normal parenchyma is homogeneously black (Figure 2.3 in C3).

TTC staining<sup>[3]</sup> confirmed that the infarction was confined to the cortex with clear margin (Figure 2.4 in C3). The infarction tissue was white and the normal tissue was red. There was no evidence of intercerebral hemorrhage in the cerebral parenchyma and subarachnoid spaces in all animals.

MR scanning showed that the infarction tissue was hyperintensity on T<sub>2</sub>-weighted imaging. It localized to the cortex with well-defined margin and the ipsilateral ventricle was dilated (Figure 2.5 in C3). The volume of infarction measured 83.52 mm<sup>2</sup> by MR imaging after 4 weeks in experimental group. There was no abnormal signal intensity in the parenchyma of the control group.

### 3 Discussion

To our knowledge, there are four methods to develop a focal ischemia model in rats: by craniotomy, by thromboembolism, by a nylon thread, and by photochemical reaction<sup>[4-7]</sup>. There are advantages and disadvantages of each method. The criteria for selection aimed directly at the specified objective. Our interest focuses on the radiological tracking of the transplanted neural stem cells, that is, observing the migration and the distribution of the superparamagnetic particle labeled transplanted neural stem cells by MR scanning. So we choose the method of craniotomy for the preparation of the animal model, i. e., directly ligating the MCA to block its blood flow and decreasing the collateral circulation. Our study confirmed the stability of the infarction volume and the high long-term survival rate of this kind of model, which are critical to the further experiments.

The advantages of this model are as follows: ① the craniotomy method provides the possibility of occluding the MCA directly which in turn ensures the better stability comparing with the thromboembolism and thread method; ② we improved Chen ST's method by treating the bilateral common carotid arteries to reduce the collateral circulation, so as to acquire a more stable infarction volume; ③ the minimally invasive operative approach by temporal bone brings about the minimal injury to the animal comparing with the other two kinds of operative route: by fossa or

bitalis and by zygomatic arch<sup>[8]</sup>. Our method did not affect the normal postoperative food intake and water drinking thus ensured the long-term observation; ④ the direct ligation of MCA is preferable to the electric coagulation because the former has less injury to the cortex and it leads to the similar pathological change of human ischemic stroke. The limitation of this method is that the exposure and the ligation of the MCA require some surgical skills.

The TTC staining 4 weeks after the operation showed a malacia and atrophy in the infarction site which hinder the measurement of the infarction volume, but the infarction area can be clearly demonstrated with MR imaging, and its volume can be easily measured. These results bring about the question of expanding the application of MR imaging in the animal experiments. The application of magnetic resonance imaging has brought the advantage of in vivo monitoring of pathological changes for longitudinal studies, and leads therefore to a higher efficiency in animal experiments, such as the observation of chronic infarction lesion and the comparison of the infarction volume between the pre- and post-thrombolytic therapy. For the MR imaging can provide a serial observation within the same individual, can it be more superior to the histological methods?

In short, our study demonstrated that this kind of focal ischemic model by minimally invasive craniotomy provided a ideal model for the study of transplantation of NSCs because of the minimal injury, stable infarction volume and high survival rate.

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(Received date: 2005-09-16)



Fig.2.1 Middle cerebral artery after dura mater reflection

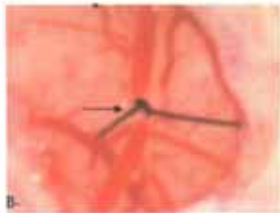


Fig.2.2 Ligation of MCA with nylon thread



Fig.2.3 Lateral views of the brain specimen show the ischemic site



Fig.2.4 TTC staining

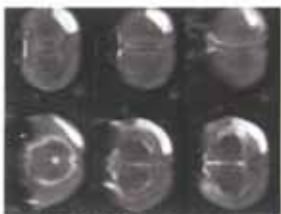


Fig.2.5 MR T2-weighted imaging

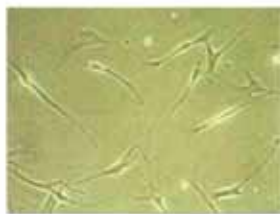


图3.1 原代培养13 d时骨髓基质细胞 (相差, 50 ×)



图3.2 原代培养3 d时脑血管内皮细胞 (相差, 50 ×)



图3.3 共培养组5 d时体外微血管网形成