· Original Article in English ·

Enhanced neurogenesis in neonatal rats after hypoxic-ischemic brain damage

Shu-Zhen MENG[1], *Xiao-Hua HAN*[1], *Yu-Kun HAN*[2]

1. *Department of Pediatrics, Second Affiliated Hospital, China Medical University, Shenyang* 110004, *China*;
2. *Department of Neonatology, Maternal and Child Health Hospital of Shenzhen, Shenzhen, Guangdong* 518133, *China*

Abstract: **Objective** Many studies have demonstrated that neurogenesis is enhanced after cerebral ischemia in the adult rats. However, little is known about neurogenesis in the brain of neonatal rats after hypoxia-ischemia (HI). This study investigated neurogenesis in neonatal rats 1 and 4 weeks after HI. **Methods** Twenty-four seven-day-old Wistar rats were randomly assigned into Control group ($n=8$) and Experimental group ($n=16$). HI was induced by ligating the right common carotid artery combined with hypoxia exposure (8% oxygen in nitrogen) in the Experimental group. In the Control group, the right common carotid artery was isolated but not ligated and there was no exposure to hypoxia. MR imaging was performed 24 hrs after HI to confirm the formation of infarct. Bromodeoxyuridine (BrdU) was injected intraperitonally daily between 2-6 days after operation or HI to label newly generated cells in both groups. Neurogenesis was examined by immunofluorescence assay 1 and 4 weeks after HI. **Results** Subventricular zone (SVZ) was obviously enlarged in the ischemic hemisphere but not in the contralateral hemisphere in the Experimental group 1 or 4 weeks after HI. The number of BrdU positive cells in the SVZ of the ischemic hemisphere in the Experimental group increased significantly compared with that in the Control group or that in the contralateral hemisphere 1 week after HI (both $P<0.05$). After 4 weeks of HI the number of BrdU positive cells in the ischemic hemisphere decreased compared with that 1 week after HI, but still remained significantly higher than that in the Control group ($P<0.05$). The number of BrdU positive cells in the subgranular zone (SGZ) of the ischemic hemisphere increased 1 week after HI, being significantly higher than that in the Control group ($P<0.05$). After 4 weeks of HI the number of BrdU positive cells in the SGZ of the ischemic hemisphere decreased compared with that 1 week after HI, but still was significantly higher than that in the Control group ($P<0.05$). Some scattered BrdU positive cells were observed in the striatum or cortex of the ischemic hemisphere, particularly in peri-infarct 1 or 4 weeks after HI. **Conclusions** Similar to the brain of adult rats, neurogenesis is enhanced in the brain of neonatal rats following HI. This result suggests that immature brain may have the capacity for self-repair.

[**Chin J Contemp Pediatr**, **2005**, **7**(**5**):**393 – 397**]

Key words: Hypoxia-ischemia, brain; Neurogenesis; Rats, newborn

新生鼠脑缺氧缺血损伤后神经细胞再生增加

孟淑珍, 韩晓华, 韩玉昆　中国医科大学附属第二医院儿科, 辽宁 沈阳, 110004

[**摘　要**] **目的**　许多研究已证实成年鼠脑缺血后神经细胞再生增加,但新生鼠脑缺氧缺血后神经细胞再生如何尚不太清楚。本文旨在调查新生鼠脑缺氧缺血后神经再生情况。**方法**　24 只 7 日龄新生鼠分为对照组($n=8$)和缺氧缺血组($n=16$),缺氧缺血组于缺氧后 24 h 行 MR 扫描以证实脑梗塞灶产生。术后或缺氧后第 2 ~ 6 天每日腹腔注射 1 次 BrdU 标记新生的细胞,应用免疫荧光法检查缺血缺氧后 1 周和 4 周时神经再生情况。**结果**　缺氧缺血后 1 周或 4 周时缺血侧脑室管膜下区(SVZ)明显增宽。缺血侧 SVZ 的 BrdU 阳性细胞数在缺氧缺血后 1 周时显著高于对照组和非缺血侧($P<0.05$),缺氧缺血后 4 周时较 1 周时下降,但仍显著高于对照组($P<0.05$)。缺血侧海马齿状回颗粒细胞层下区(SGZ)的 BrdU 阳性细胞数在缺氧缺血后 1 周增高,明显高于对照组($P<0.05$),缺氧缺血后 4 周时较 1 周时减少,但仍显著高于对照组($P<0.05$)。缺氧缺血后 1 周或 4 周时在皮质和纹状体梗塞坏死灶周围可见散在分布的 BrdU 阳性细胞。**结论**　新生鼠与成鼠类似,脑缺氧缺血后神经再生增强,提示不成熟脑具有一定自身修复能力。

[**中国当代儿科杂志,2005, 7(5):393 – 397**]

[**关　键　词**] 缺氧缺血,脑;神经再生;大鼠,新生

[Received] June 13, 2005; [Revised] August 1, 2005

[Biography] Shu-Zhen MENG (1962-), Female, Medical doctor, Associate professor, Specializing in hypoxic-ischemic brain damage (Email: shuzhenmeng@yahoo.com).

[中图分类号] R-33 [文献标识码] A [文章编号] 1008－8830(2005)05－0393－05

An increasing number of studies shows that neurogenesis occurs in some regions of adult mammalian brains, including the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus [1-2]. Some physiological and pathological events, such as a rich environment of exposure, running, learning, epilepsy and ischemia, can stimulate neurogenesis in the regions. Recent studies have demonstrated that neurogenesis in the SVZ and SGZ is enhanced following focal or global ischemia in the adult brains and some of these studies have shown that newborn cells are observed in the peri-infarct of the cortex or striatum [3-9]. How- ever, there are few reports on the study of neurogenesis in the neonatal rats after hypoxia-ischemia (HI) [10, 11]. In this paper neurogenesis in the brain of neonatal rats was investigated 1 and 4 weeks after HI.

Materials and methods

Experimental animals and grouping

Twenty-four seven-day-old Wistar rats, with body weights of 11-12 grams, were randomly assigned into Control group ($n = 8$) and Experimental group ($n = 16$). The Experimental group was subdivided into 1-week group and 4-week group according to the post-HI period (both $n = 8$).

Preparation of animal model

The cerebral HI model was produced by ligating the right common carotid artery under ether anesthesia. Two hours after recovering from surgery, the rats were exposed to hypoxia (humidified 8% oxygen in nitrogen) for 1.5 hours. In the Control group, the right common carotid artery was isolated without ligation and there was no exposure to hypoxia. Body temperature was maintained at 37-38 ℃ during HI using a heating lamp.

Magnetic resonance (MR) imaging

Brains of neonatal rats were imaged at 24 hours after HI to confirm the cerebral ischemic damage with an MR imaging system equipped with a 9.4T/21-cm horizontal bore magnet (Magnex) and an Advance console (Bruker, Germany). The rat was placed in the small chamber and the head was restrained with foam under anesthesia and then the small chamber was put into the magnet. Diffuse weighted image (DWI) and T2 weighted imaging (T2WI) were acquired with a field of view of 2.5 cm^2 and a data matrix of 256 × 128 for five 1.5-mm-thick slices through the cerebrum. Apparent diffuse coeffcient (ADC) and T2 maps were then acquired from DWI and T2WI.

Bromodeoxyuridine (BrdU) injection

Both group of rats were intraperitoneally injected with BrdU (Oxford Biotechnology, UK) (50 mg/kg) daily between 2-6 days after surgery or HI.

Brain tissue processing

The animals were sacrificed 1 week or 4 weeks after surgery or HI. The brains were removed and cut into 2 blocks at -1.8 mm from bregma, then cryoprotected with sucrose and stored at －80 ℃. Thirty five-mm thick coronal sections at the level of the SVZ (－0.4 mm from bregma) and the SGZ (－3.1 mm from bregma) were cut with a cryostat and four consective sections in the each region were selected for BrdU labeling.

BrdU labeling

Sections were pretreated with 2 M HCl for 2 hours at room temperature to denature the DNA. They were then incubated in blocking solution (3% goat serum/0.3% Triton X-100/1% BSA in PBS) for 1.5 hours at room temperature. Finally sections were incubated with a monoclonal rat anti-BrdU antibody (1∶200, Serotec, UK) overnight at 4 ℃ and then with goat anti-rat Cy3 conjugated affinity purified secondary antibody (1∶200, Chemicon Int.) for 1 hour at room temperature.

BrdU positive cells counting

BrdU labeling was observed under a fluorescence microscope and the images were saved to a computer by Sigma Imaging Analysis System. The number of BrdU labeled positive cells were assessed semi-quantitatively by counting the total number of BrdU positive cells within the SVZ and SGZ under 200 magnifications. Four labeled sections in each region were counted.

Statistical analysis

Data were expressed as $\bar{x} \pm s$. Differences between two hemispheres or two groups were compared using a t-test. Differences were considered significant at $P < 0.05$.

Results

MR imaging

ADC values

In the Control group there was no abnormal signal in both hemispheres. In the Experimental group the ADC values decreased significantly in the cortex, stria-

tum or /and hippocampus, and thalamus of the ischemic cerebral hemisphere compared with those in the non-ischemic hemisphere 24 hours after HI (Figure1).

T2 values

In the Control group there was no abnormal signal in both hemispheres. In the Experimental group T2 value markedly increased in the cortex, striatum or/ and hippocampus, and thalamus of the ischemic cerebral hemisphere compared with those in the non-ischemic hemisphere 24 hours after HI (Figure 1).

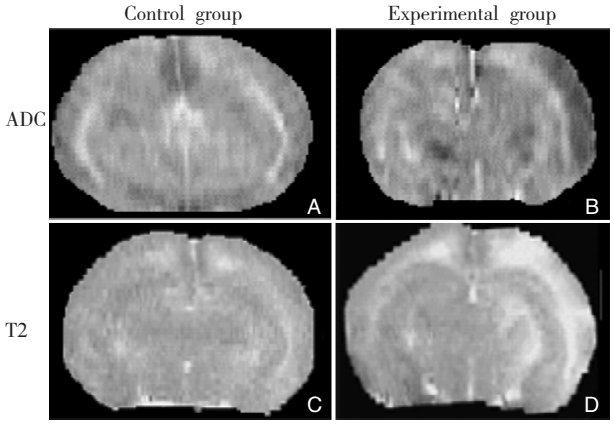

Figure 1 Changes of MRI 24 hrs after HI in neonatal rats. Twenty-four hours after HI, ADC values decreased significantly (B) and T2 values markedly increased (D) in the cortex and striatum of the ixchemic cerebral hemisphere, as compared with those in control rats (A and C).

BrdU labeling

In the Control group, no BrdU positive cell was observed in the cortex and striatum in both hemispheres, whereas in the lateral rostal SVZ and the SGZ there were some scattered BrdU positive cells. There was no difference of BrdU positive cells in the SVZ and SGZ between 1-week and 4-week groups.

In the Experimental group, the SVZ was obviously enlarged in the ischemic cerebral hemisphere but not in the non-ischemic hemisphere 1 and 4 weeks after HI. The number of BrdU positive cells in the SVZ increased in both hemispheres, especially in the lateral rostal SVZ. Compared with that in the Control group the increase in the number of BrdU positive cells was significant in the ischemic hemisphere ($P=0.0004$), but not significant in the non-ischemic hemisphere ($P>0.05$). The number of BrdU positive cells in the ischemic hemisphere was higher than that in the Control group. In addition, the number of BrdU positive cells within the SVZ in the ischemic hemisphere was higher than in the non-ischemic hemisphere ($P=0.031$). Four weeks after HI the number of BrdU positive cells within the SVZ was reduced in both hemispheres compared with that in 1 week after HI, but was still higher in the ischemic hemisphere than that in the Control group ($P=0.004$) (Figure 2).

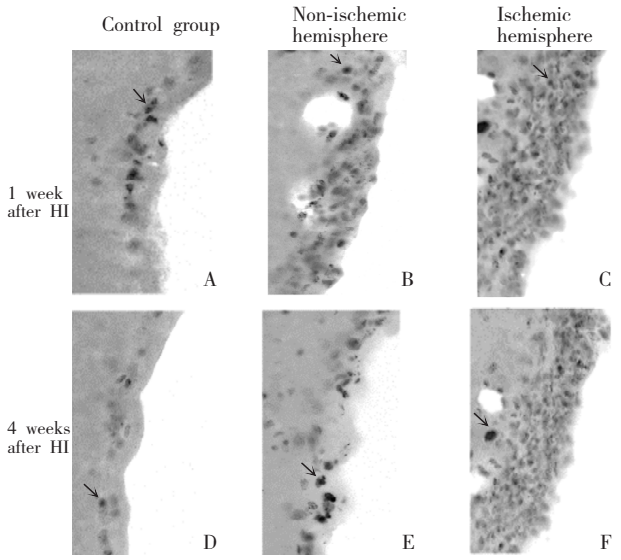

Figure 2 Micrographs of BrdU staining in SVZ of neonatal rats after HI (×20). One week after HI, the number of BrdU positive cells in the SVZ increased in both hemispheres (B, C), especially in the ischemic hemispheres (C), as compared with the Control group (A). Four weeks after HI, the number of BrdU positive cells in the SVZ was reduced in both hemispheres (E, F) compared with that in 1 week after HI, but was still higher than that in the Control group (D).

The number of BrdU positive cells in the SGZ also increased in both sides 1 week after HI. Compared with that in the Control group the number of BrdU positive cells within the SGZ in the ischemic hemisphere increased significantly ($P=0.037$) but did not in the non-ischemic hemisphere ($P>0.05$). There was no significant difference of BrdU positive cells in the SGZ between the ischemic and non-ischemic sides. The number of BrdU positive cells within the SGZ in the ischemic hemisphere decreased 4 weeks after HI compared with that 1 week after HI, but remained significantly higher in the ischemic hemisphere than that in the Control group ($P=0.048$) (Figure 3).

Some scattered BrdU positive cells were observed in the cortex and striatum of the ischemic hemisphere, especially in the peri-infarct, 1 and 4 weeks after HI. There was no obvious difference of the BrdU positive cells in the peri-infarct between 1 week and 4 weeks after HI. There were no BrdU positive cells in the cortex and striatum of the non-ischemic hemisphere.

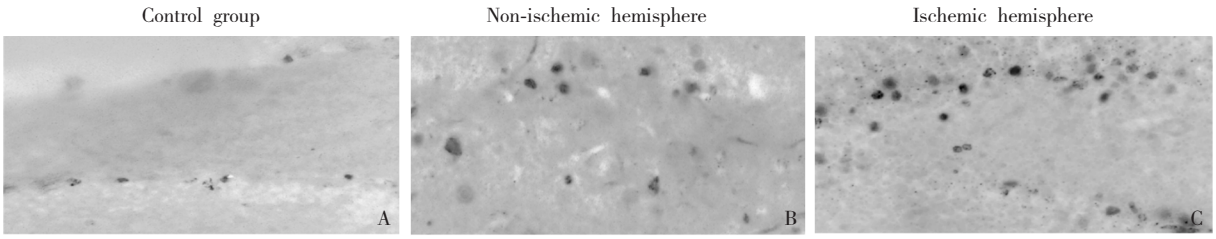

Figure 3 Micrographs of BrdU staining in SGZ of neonatal rats 1 week after HI (×20). The number of BrdU positive cells significantly increased in the SGZ of the ischemic hemisphere (B) but not in the non-ischemic hemisphere (C), as compared with the Control group (A).

Discussion

BrdU is thymidine analog that can incorporate into the DNA of dividing cells during DNA synthesis in S-phase. Thus, BrdU was used as a marker of newborn cells in this study. In some studies, BrdU was intraperioneally injected daily after surgery or HI and immunofluorenecence labeling was used to identify BrdU positive cells [3, 10]. It has been reported that BrdU has teratological effects on the body, brain and on the behavior of fetus and infant rats [12]. However these side effects were not observed in this study.

There are many reports on the studies of neurogenesis after ischemia in adult rats. Jin et al [3] reported that the number of new generated cells in the SVZ and SGZ of both hemispheres obviously increased 7-14 days after focal cerebral ischemia in the adult rat, and that in the SGZ of ischemic hemisphere were more significantly increased. These newborn cells were decreased markedly 2-3 weeks after ischemia. Parent et al [8] have also reported that the number of new born cells in the SVZ of both hemispheres increased significantly in the adult rats 10 days after focal cerebral ischemia, and a more significant increase was noted in the ischemic side. The present study showed that the number of BrdU positive cells in the SVZ and SGZ of both hemispheres increased 1 week after HI, and that the increase of BrdU positive cells in the ischemic hemisphere was significant. The BrdU positive cells in the SVZ and SGZ of both hemispheres were reduced 4 weeks after HI compared with those 1 week after HI. The results were similar to the features of neurogenesis seen in the SVZ and SGZ of the adult rat after cerebral ischemia. The study of focal cerebral ischemia in the adult rat also showed that BrdU positive cells were observed in peri-infarct of the cortex and striatum 2 weeks after ischemia, some of which expressed specific markers of neuron such as microtubule associated protein-2 (Map2) and Neuon-Specific nuclear protein (NeuN) 4-5 weeks after ischemia [8-9]. Some researchers have used doublecortin as a marker of migrating immature neurons and observed chains of neuroblast from SVZ migrating toward peri-infarct of the striatum or/and cortex. This result suggests that these newborn cells may originate from SVZ [8,10,13-14]. However, Jiang et al [15] thought that these newborn cells in the cortex might originate from the proliferation of the neural stem cells inside the cortex after ischemia. In the present study some scattered BrdU positive cells were also observed in peri-infarct of the cortex and striatum 1 and 4 week after HI and the phenotype of these newborn cells is being further investigated.

There has been few studies on neurogenesis after cerebral HI in immature rats. In the model of brain HI injury in 10-day-old rats, Plane et al [10] observed that the number of BrdU positive cells in the SVZ of both hemispheres increased 1 week after HI, and that the increase in the number of BrdU positive cells in the SVZ of the ischemic hemisphere was significant. The number of BrdU positive cells in the SVZ of both sides were reduced 3 weeks after HI compared with that 1 week after HI. The number of BrdU positive cells in peri-infarc of the striatum in the ischemic hemisphere increased 2 weeks after HI. The results were similar to those observed in the present study. The study on transient cerebral hypoxia in neonatal rats also showed that BrdU positive cells in the SVZ significantly increased 21 days after hypoxia, and BrdU positive cells along posterior periventricle tended to migrate towards the hippocampus and expressed markers of neuron, but BrdU positive cells in the hippocampus or granular layer did not change [11].

Presently there is no report about whether the capability of neurogenesis is stronger in neonatal rats than in adult rats. Yagita et al [4] investigated neurogenesis in both young and old rats after cerebral ischemia. The result showed that although neurogenesis in the SGZ increased in both groups, aging accelerated the reduction in new generated cells after ischemia. It

is speculated that neonatal rats have stronger capability of neurogenesis than adult rats, but it needs to be further investigated.

In summary, neurogenesis in neonatal rats increases after HI, as it also does in adult rats. This suggests that an immature brain has the capability for self-repair. The result provides a promise for repair of hypoxic-ischemic brain damage.

[**References**]

[1] Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J. Identification of a neurnal stem cell in the adult mammalian central nervous system [J]. Cell, 1999, 96(1): 25-34.
[2] Suhonen JO, Peterson DA, Ray J, Gage FH. Differentiation of adult hippocampus- derived progenitors into olfactory neurons in vivo [J]. Nature 1996, 383 (6601): 624-627.
[3] Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, et al. Neurogenesis in dentate subgranualr zone and rostral subventricular zone after focal cerebral ischemia in the rat [J]. Proc Natl Acad Sci U S A, 2001, 98(8): 4710-4715.
[4] Yagita Y, Kitagawa K, Ohtsuki T, Takasawa K, Miyata T, Okano H, et al. Neurogenesis by progenitor cells in the ischemic adult rat hippocampus [J]. Stroke, 2001, 32(8): 1890-1896.
[5] Kee NJ, Preston E, Wojtowicz JM. Enhanced neurogenesis after transient global ischemia in the dentate gyrus of the rat [J]. Exp Brain Res, 2001, 136(3): 313-320.
[6] Iwai M, Sato K, Omori N, Nagano I, Manabe Y, Shoji M, Abe K. Three steps of neural stem cells development in gerbil dentate gyrus after transient ischemia [J]. J Cereb Blood Flow Matab, 2002, 22(4): 411-419.
[7] Zhou J-P, Gu Z, Ma R, Han Q-Y, Zhao J, Shi T. The observation of neurogenesis in the subventricular zone in rats after transient focal cerebral ischemia (in Chinese) [J]. Acta Univ Med Nanjing (Natural Science), 2003, 23(4): 335-339.
[8] Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke [J]. Ann Neurol, 2002, 52(6): 802-813.
[9] Jiang W, Gu W, Brannstrom T, Rosqvist R, Wester P. Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion [J]. Stroke, 2001, 32(5): 1201-1207.
[10] Plane JM, Liu R, Wang TW, Silverstein FS, Parent JM. Neonatal hypoxic-ischemic injury increases forebarin subventricular zone neurogenesis in the mouse [J]. Neurobiol Dis, 2004, 16(3): 585-595.
[11] Daval JL, Pourie G, Grojean S, Lievre V, Strazielle C, Blaise S, et al. Neonatal hypoxia triggers transient apoptosis followed by neurogenesis in the rat CA1 hippocampus [J]. Pediatr Res, 2004, 55(4): 561-567.
[12] Kolb B, Pedersen B, Ballermann M, Gibb R, Whishaw IQ. Embryonic and postnatal injections of bromodeoxyuridine produce age-dependent morphological and behavioral abnormalities [J]. J Neurosci, 1999, 19(6): 2337-2346.
[13] Jin K, Sun Y, Xie L, Peel A, Mao XO, Batteur S, et al., Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum [J]. Mol Cell Neurosci, 2003, 24(1): 171-189.
[14] Zhang R, Zhang Z, Wang L, Wang L, Gousev A, Zhang L, et al. Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats [J]. J Cereb Blood Flow Metab, 2004, 24(4): 441-448.
[15] Gu W, Brannstrom T, Wester P. Cortical neurogenesis in adult rats after reversible photothrombotic stroke [J]. J Cereb Blood Flow Metab, 2000, 20(8): 1166-1173.

(Edited by Le ZHONG)