· Original Article in English ·

Changes of IGF-1 and its receptor in 3-day-old premature rats with chronic hypoxic-ischemic brain damage

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Abstract: Objective This study examined the expression of insulin-like growth factor-1 (IGF-1) and its receptor in 3-day-old premature rats with chronic hypoxic ischemic brain damage (HIBD) and investigated the role of IGF-1 in the pathogenesis of this disease. **Methods** Ninety 3-day-old Sprague-Dawley rats were randomly assigned into a Control group (n =40) and a Hypoxic-ischemic group (HI, n = 50). HI was induced through bilateral common carotid artery ligation. The Control group was only sham-operated. Hematoxylin and eosin staining, TUNEL immunofluorescence staining and immunohistochemistry ways were used to investigate the expression of IGF-1 and its receptor, morphological changes of brain tissues and cell apoptosis of brain white matter. **Results** The expression of IGF-1 decreased in 3-5 days after HI, but that of its receptor increased in the HI group. The expression changes were most significant at corpus callosum and periventricular white matter and recovered progressively in 7-14 days after HI. After 7 days of HI, the brain white matter presented with morphological changes such as rarefaction, liquefaction and lateral ventricular enlargement. Apoptotic cells in deep white matter increased after HI, and peaked at 48 hrs. **Conclusions** IGF-1 may play an important role in the pathogenesis of chronic HIBD in 3-day-old premature rats. This study provides an experimental basis for the prevention and treatment of HIBD in premature infants. [**Chin J Contemp Pediatr, 2005, 7(3):193 - 197**]

Key words: Insulin-like growth factor I; Hypoxia-ischemia, brain; Rat, premature

3 日龄未成熟大鼠慢性缺氧缺血脑损伤 IGF-1 及其受体的变化

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[**摘 要**] **目的** 探讨胰岛素样生长因子-1 (IGF-1)在 3 日龄未成熟大鼠慢性缺氧缺血脑损伤发病机制中的作用。**方法** 90 只 3 日龄 SD 未成熟大鼠随机分为对照组(n=40)和慢性缺氧缺血(HI,n=50)组。对照组仅切开颈部皮肤,HI 组结扎双侧颈总动脉造成新生大鼠慢性缺氧缺血脑损伤,采用组织切片苏木素 - 伊红染色和免疫组化等方法,观察未成熟大鼠脑损伤时少突胶质细胞营养因子 IGF-1 和受体的表达、脑组织病理变化和白质细胞凋亡。**结果** 3 日龄未成熟大鼠慢性缺氧缺血脑损伤后 IGF-1 及其受体呈现动态变化。HI 组在术后 3 ~ 5 d(生后 6 ~ 8 d) IGF-1 表达阳性的细胞减少,IGF-1 受体表达却有增高趋势,变化以胼胝体和脑室周围白质部位明显,术后 7 ~ 14 d(生后 10 ~ 17d)时逐渐恢复。同时脑白质出现液化疏松、脑室扩大等形态学病理变化,凋亡细胞计数在损伤后增多,以 48 h 最为显著。**结论** 提示 IGF-1 在 3 日龄未成熟大鼠慢性缺氧缺血脑损伤的发病机制中具有重要作用,为早产儿脑损伤的防治提供了实验依据。 [**中国当代儿科杂志,2005, 7(3):193 - 197**]

[**关 键 词**] 胰岛素样生长因子-1;缺氧缺血,脑;大鼠,未成熟

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Recently, the survival rate of premature infants has increased significantly, but the sequela of premature infants especially the neurologic sequela is still very common. Brain injury of premature infants has already become the main form of neonatal brain injury. Premature brain injury is characterized by white matter injury and periventricular hemorrhage infarction. The incidence of white matter injury is 3% - 4% in preterm infants with a body weight <1 500 g, and 4% -10% in infants born at a gestational age of between 33- 35

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weeks and surviving for more than 3 days[1]. The highest incidence (20.7%) was observed in infants born at a gestational age of 27 weeks. Oligodendrocyte is a major target cell of white matter injury. The development of the nervous system in 7-day-old rats equals that in full-term or near full-term infants[2]. Therefore a 7-day-old postnatal rat model through common carotid artery ligation along with hypoxemia exposure characterized by injury of unilateral cortex and hippocampus neurons is a classic model for studying hypoxic-ischemic encephalopathy (HIE) in full-term infants[2]. The brain white matter in 7-day-old rats has almost fully developed and myelinization has occurred, which decreases the susceptibility to injuries; therefore this model is not suitable to be used in the study of white matter injury for the premature infant. Craig and colleagues[3] reported that the developmental window of white matter maturation in 2-3-day-old neonatal rats coincides with cerebral white matter development in the premature infant. A lot of studies have indicated that chronic cerebral hypoxia and ischemia preferentially results in white matter injury[4,5]. Therefore, in this study, a 3-day-old rat model of chronic cerebral hypoxic-ischemic white matter injury was established through bilateral common carotid artery ligation to investigate the expression of insulin-like growth factor-1 (IGF-1) and its receptor.

Subjects and methods

Subjects and grouping

Ninety 3-day-old Sprague-Dawley rats without gender limitation and weighing 7.5-12 g were obtained from the Animal Center, Shanghai Medical College, Fudan University (serial No, 03091, license No. 02-22-2). They were randomly assigned into a Control group (n =40) and a hypoxic-ischemic group (HI, n = 50).

Animal model

Three-day-old SD rats were anesthetized with ether, and bilateral common carotid arteries were isolated. Bilateral arteries were ligated with 9-0 thread in the HI group, whereas incision was closed and arteries were left untouched in the Control group. The rats were kept in 37℃ until temperature and movement recovered. Thereafter, rats were fed in cages. Ten rats in the HI group died during or after operation because of intolerance to surgery interference. The rest of the rats were sacrificed at 1, 2, 3, 7 and 14 days after operation (8 rats in each group at each time point).

Preparation of brain sections

After perfusion and fixation, the brain tissues were imbedded in paraffin, thereafter continuous coronal sections (8 μm thick) were made.

Detection of apoptotic cells

After being formalin fixed and paraffin embedded, brain coronal sections were stained by terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate biotin nick end labelling (TUNEL) to identify apoptotic cells. TUNEL staining, visualized by a fluorescent dye (FITC) conjugated to dUTP, was performed according to the manufacturer′s protocol (Roche Molecular Biochemicals). Three sections of each sample were selected, the number of apoptotic cells in 400 × high power field was counted at 6 randomly selected areas of deep white matter on each section, and the mean was calculated.

Immunohistochemistry staining of IGF-1 and its receptor

The procedures were processed according to the protocol recommended for IGF-1 and its receptor immunohistochemistry kit. Sections were deparaffinised and digested, then immunostained with a standard streptavidin-biotin-peroxidase procedure and diaminobenzidine (DAB) color reaction. IGF-1 and its receptor positive cells in cortex, hippocampus (CA2 and CA3 regions), corpus callosum and deep white matter (mainly in apsula interna) were counted under a light microscope. The positive cells number in 400 × high power field was calculated with the IMS cell image analysis system (Shenteng Information and Technique Corp, Shanghai, China).

Statistical analysis

All data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Statistical analysis was conducted using SPSS 11.5 for windows software. The t-test was used to assess differences between two means.

Results

The expression of IGF-1 and its receptor

IGF-1 was widely distributed in brain tissues in the Control group. The number of IGF-1 positive cells at each brain region in the HI group 3 and 5 days after ligation of common carotid arteries (6 and 8 days postnatal) decreased, and was most significant at corpus callosum and periventricular white matter. It recovered gradually at 10 and 17 days postnatal (Figures 1 and 2).

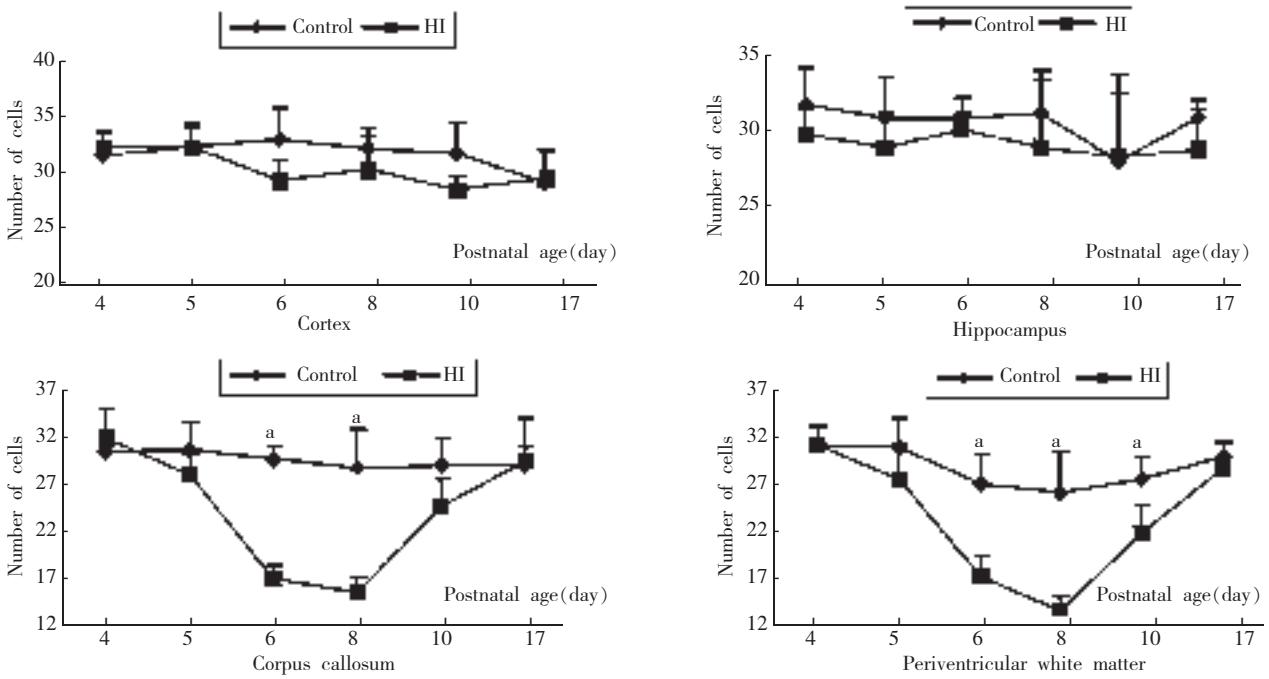

Figure 1　Expression of IGF-1 at each brain region in the Control and HI groups (×400)

a Compared with Control group, $P < 0.01$

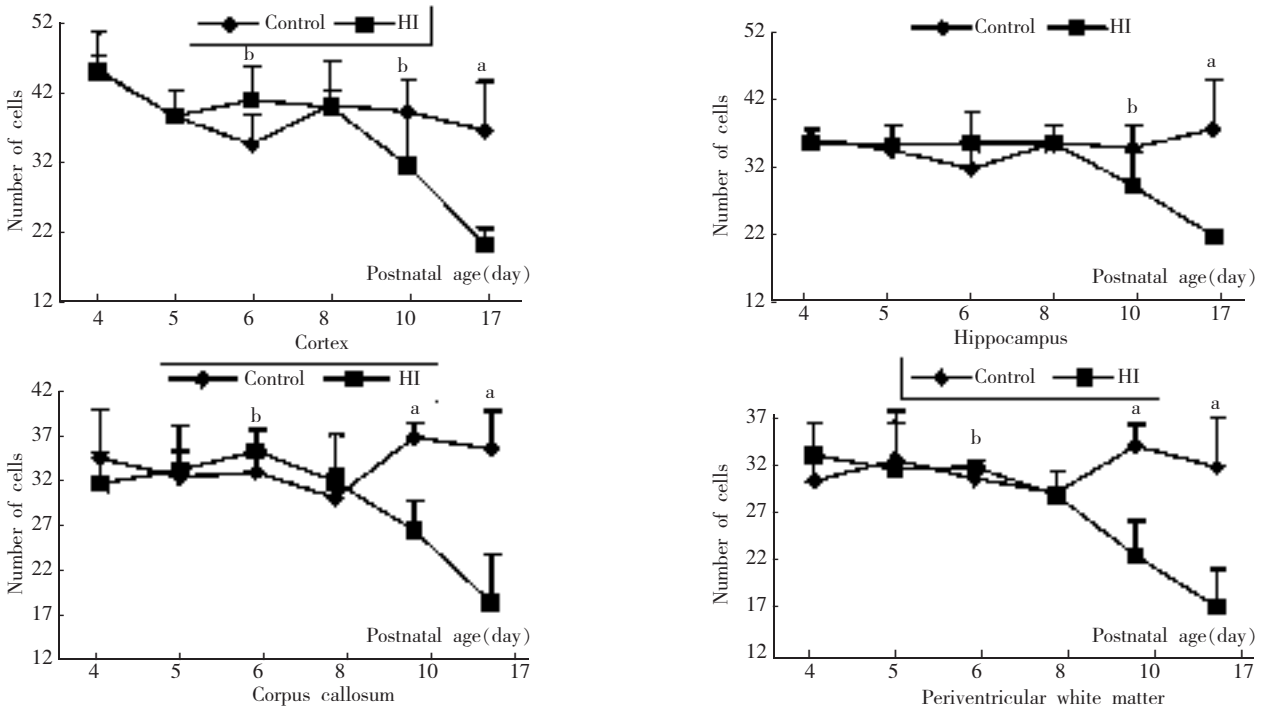

Figure 3　Expression of IGF-1 receptor at each brain region in the Control and HI groups (×400)

a Compared with the Control group, $P < 0.01$; b Compared with the Control group, $P < 0.05$

The number of IGF-1 receptor positive cells in the HI group 3 and 5 days after ligation of common carotid arteries (6 and 8 days postnatal) increased slightly at each brain region and decreased at 10 and 17 days postnatal. The difference compared with the Control group increased with the day age (Figure 3).

Morphological changes of brain tissues

Brain injury in the HI group 1-5 days after ligation of common carotid arteries was relatively mild, but became severe on the 7th day. The diffused rarefaction and foci of liquefaction necrosis at subcortex white matter and periventricular white matter were observed in 13 of the 16 rats (Figure 4), and the cerebral cortex lesion was relatively mild. On the 14th days after ligation, the white matter damage of rats in the HI group had become more severe, with obvious sub-

cortex and periventricular white matter rarefaction, thinning of corpus callosum as well as formation of large amount of foam cells. Bilateral lateral ventricular enlargement with irregular appearance was noticed in 50% (8/16) rats (Figure 5). Three rats presented with bland liquefaction necrosis in cortex, and one with complete absence of hippocampus.

Apoptotic cells in deep white matter

TUNEL-positive apoptotic cells were indicated by the presence of green or yellow-green fluorescence in the nucleus of cells (Figure 6). The number of apoptotic cells peaked at 48 hours after HI (Table 1).

Table 1 Number of apoptosis cells in deep white matter at different time after HI in rats (n = 8)

Group	24 hrs	48 hrs	72 hrs	1 week	2 weeks
Control	3.17 ±0.75	5.00 ±2.58	4.29 ±0.77	3.00 ±1.51	2.22 ±0.97
HI	8.62 ±1.66	14.62 ±4.01	10.82 ±3.16	6.60 ±2.51	2.30 ±1.00
t	8.46	5.71	5.68	3.48	0.16
P	<0.001	<0.001	<0.001	0.004	0.87

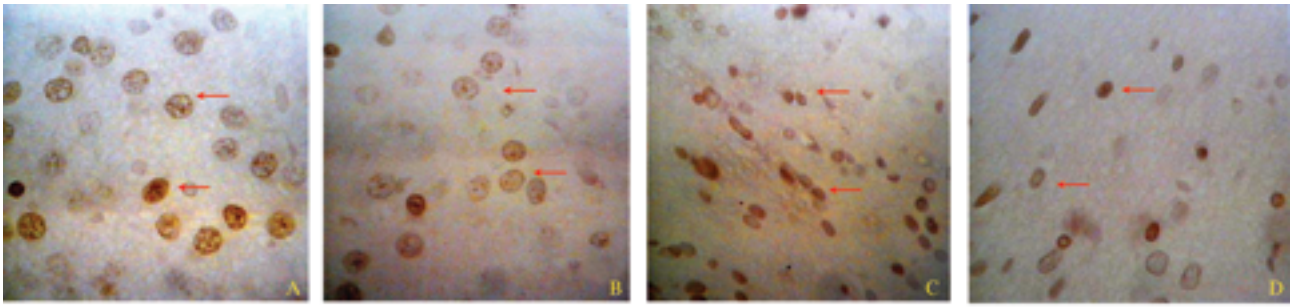

Figure 2 Expression of IGF-1 in brain white matter in rats at postnatal age 6 days (IGF-1 ×400)
A: Control group, periventricular white matter; B: HI group, periventricular white matter; C: Control group, corpus callosum; D: HI group, corpus callosum.

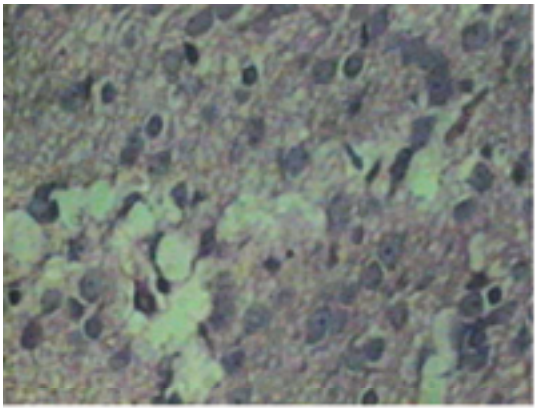

Figure 4 Liquefaction in deep white matter (×400)

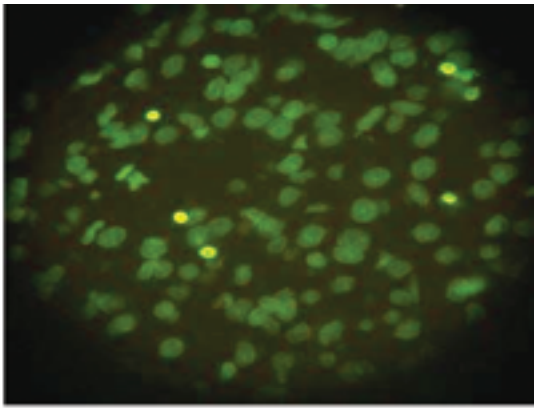

Figure 6 Apoptotic cells in deep white matter (TUNEL ×400)

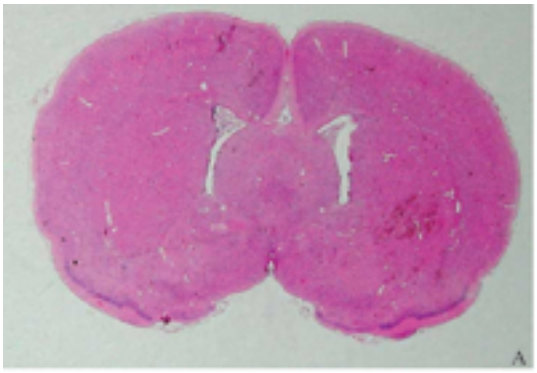

Figure 5 Morphometric changes in the rat lateral ventricle A: Control group; B: HI group

Discussion

IGF-1 is a single-chain polypeptide of 70 amino acids, which is a representative oligodendrocyte-derived neurotrophic factors and possess insulin like anabolism functions as well as growth promotive peptide functions[6]. It was reported that IGF-1 and its recep-

tors are widely distributed in brain tissues. The IGF receptor and IGF-binding protein-3,-4,-5 and -6 mRNA were expressed in oligodendrocyte, which can induce differentitation of oligodendrocyte and myelination, and promote survival of oligodendrocyte or progenitors and neurons. IGF-1 is one of the essential growth factors for cerebral development[7,8]. But there are few reports about the changes of IGF-1 expression following white matter injury.

It was reported that in the unilateral ischemic brain injury model both the IGF-1 mRNA expression and the free IGF-1 concentration increased. These changes may be related to the protective effect of IGF-1 against ischemic neurons necrosis and colloid cell proliferation[9-10]. Lee and colleagues[11] reported that in the unilateral carotid artery ligation induced neonatal rat brain ischemia model the IGF-1 mRNA expression level in the post-ischemic brain decreased, which was significantly lower than controls at 24 hours, and that the IGF-1 gene expression in astrocytes was gradually activated after 72 hours. They thought that the rapid decrease of brain IGF-1 gene expression may promote brain neuron necrosis and myelin sheath damage in the perinatal period. Injury of nervous system may increase the immuno-reactivity of IGF-1 and expression level of IGF-1 mRNA. However, brain ischemia may induce a series of biochemical disorders which lead to neuron injury and necrosis; endogenous nerve protection mechanism will be activated by stimulus and inhibit damage; but the balance between injury and protection is determined by the injured neurocytes. When neurocytes were stimulated by ischemia, and if the damage caused by stimulus was not serious enough to cause substantial changes, neurocytes necessarily compensated in order to accommodate to the changes of environment. The basis level of neurotrophic factor mRNA is positively related to the ability of neuron against ischemic injury. The severity of ischemic injury is related to the expression level of mRNA. Severe injury inhibits its expression. This study indicated that chronic ischemic-hypoxic injury in immature brain mainly resulted in white matter injury. The expression of neurotrophic factor and its receptor showed a separated pattern. Moreover, the time of IGF-1 and related receptor expression changes was not coincident with that of peak apoptosis. The possible reason for this change manner may be that massive apoptosis and necrosis of brain tissue cause a decompensation, which leads to the changes of IGF-1 neurotrophic factor and related receptor changes. In general, results of this study indicated that there was a dynamic change of white matter IGF-1 and related receptors expression during chronic HIBD in 3-day-old immature rats. These changes were in accordance with the brain white matter injury. Based on these findings it is speculated that IGF-1 may play a role in the pathogenesis of brain injury in premature infants. However further studies are needed to clarify the detailed mechanism.

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