·Original Article in English·

Growth Associated Protein-43 mRNA Expression in Neonatal Rats with Hypoxic-Ischemic Brain Damaged

Yu-Chun HUANG[1], *Ying XIN*[2], *Yu-Kun HAN*[2], *Hong GAO*[3]

1. *Department of Neonatology, Dalian Children's Hospital, Dalian, Liaoning* 116012, *China*; 2. *Department of Pediatrics, Second Hospital Affiliated to China Medical University, Shenyang* 110004, *China*

Abstract: Objective To study the mRNA expression of growth associated protein-43 (GAP-43) in neonatal rats with hypoxic-ischemic brain damage (HIBD) and the influence of basic fibroblast growth factor (bFGF) on GAP-43 mRNA. **Methods** Twenty normal Wistar rats were used as the normal group, and another 54 7-day-old Wistar rats were randomly assigned into the sham operation group, HIBD group and bFGF treatment group. Immediately after HIBD models were made, 4 U/g of bFGF was injected each day in the treated group, and in the HIBD group an equal amount of NS was injected ip each day. The expression of GAP-43 was examined by RT-PCR in the three groups on day 1, day 3 and day 7 after HIBD. And the expression of GAP-43 in the normal group on the 2nd, 5th, 7th and 14th days after birth was also examined. **Results** The expression of GAP-43 mRNA on the 2nd day after birth was lower and it increased later to a peak on the 7th day in the normal rats. Compared with the sham operation group, GAP-43 mRNA expression on both sides of the brain in the HIBD group decreased rapidly on the 1st day after damage. In the bFGF treatment group, GAP-43 mRNA expression on the 3rd day after damage increased markedly compared with that in the HIBD group of the same age and that in the 8-day old treatment group, and it still remained at high level on day 7 after damage. **Conclusions** The expression of GAP-43 mRNA in the brain of neonatal rats may change with age. It is related to the development and maturation of the brain. bFGF may have some positive effects on the restoration of brain function after HIBD.

[**Chin J Contemp Pediatr, 2003, 5(2): 86-89**]

Key words: Growth associated protein-43; Fibroblst growth factor, basic; Cerebral ischemia; Cerebral anoxia; Animal; Newborn

生长相关蛋白-43在缺氧缺血性脑损伤新生大鼠脑内表达的变化及意义

黄玉春，辛颖，韩玉昆，高红 大连市儿童医院新生儿科，辽宁 大连 116012

[**摘 要**] **目的** 探讨缺氧缺血性脑损伤(HIBD)后脑内神经突起外生及突触重建标志蛋白——生长相关蛋白-43(GAP-43)mRNA表达的变化及碱性成纤维细胞生长因子(bFGF)对其表达的影响。**方法** 取20只正常Wistar大鼠为正常对照组，另取54只7日龄大鼠随机分为假手术组、HIBD组和bFGF治疗组。HIBD模型参照Rice法制成。治疗组于损伤后腹腔注射bFGF，每天4 U/g，直至处死；HIBD组腹腔注射等量生理盐水。采用逆转录-聚合酶链式反应(RT-PCR)技术测定3组大鼠损伤后1 d，3 d，7 d(即8，10，14日龄)以及正常大鼠2 d，5 d，7 d及14 d脑内GAP-43 mRNA的表达。**结果** 正常大鼠生后2天GAP-43 mRNA表达较低(0.16±0.19)，以后逐渐增强，7 d高峰表达，至14 d仍有较高水平表达(1.0±0.70)。HIBD后1 d双侧脑组织GAP-43 mRNA表达较假手术组明显减低。bFGF治疗3 d后，与同组治疗后1天及同日龄HIBD组比较，双侧脑组织GAP-43 mRNA表达显著增加，至7 d仍维持较高表达。**结论** 新生大鼠生后脑内GAP-43mRNA表达呈时间依赖性变化，与脑发育及功能成熟过程密切相关，bFGF对脑损伤后细胞增殖及功能重建有一定促进作用。

[**中国当代儿科杂志，2003，5(2)：86-89**]

[Received] August 21, 2002; [Revised] January 5, 2003
[Fundation Item] Supported by a national grant program (No. 39970777)
[Introduction to the First Author] Yu-Chun HUANG(1963-), Female, MD, Director of the Department of Neonatology.
[Correspondence Author] Yu-Chun HUANG, Department of Neonatology, Dalian Children's Hospital, Dalian, Liaoning 116012, China (E-mail: Huangyuch@163.com)

[关 键 词] 生长相关蛋白-43;脑缺氧;脑缺血;成纤维细胞生长因子,碱性;动物;新生
[中图分类号] R-332 [文献标识码] A [文章编号] 1008-8830(2003)02-0086-04

Hypoxic-ischemic brain damage (HIBD) is one of the common diseases which result in death and disability in the newborn. At present, the prevention and treatment for sequelae of moderate and severe HIBD are difficult in clinical practice. In recent years, issues on the restoration of brain function after damage have become a hot topic in brain research. Growth associated protein-43 (GAP-43) is an acidic fast-transported membrane-bound protein, the expression of which in the central nervous system is regarded as the indicator of nerve axon outgrowth and synaptogenesis, and it is associated with the forthcoming functional maturation of the developing brain and the restoration and plasticity of the mature brain synapse[1,2]. We utilized the technique of RT-PCR to study the change of GAP-43 mRNA expression in brains of neonatal rats after HIBD and the effects on its the expression of basic fibroblast growth factor (bFGF) in order to discuss the effects on the brain function and development of neonatal rats after HIBD and the effects of bFGF on the restoration of brain function, and propound therapeutic guidelines.

Materials and methods

Materials

Wistar rats were offered by the Experimental Animal Center of the Second Hospital of China Medical University. The primer of GAP-43, whose sequence was GAP-43-1 5'-ATGCTGTGCTGTATGAGAAGAA-3', GAP-43-2 5'-GCCCTTCTTCTCCTCAGAAGGT-3', was synthesized by Ta Ka Ra Biotechnology (Dalian) Co., Ltd., and the length of the amplified product was 345 bp. The inner control was glyceraldehyde phosphate dehydrogenase (GAPDH) of 528 bp, whose primer was synthesized by Shanghai Institute of Cell Biology. Trizol™ total RNA extraction kit was obtained from Ta Ka Ra Biotechnology (Dalian) Co. Ltd. Reverse transcriptase and other enzymes and relevant reagents necessary for PCR amplification were all products of Ta Ka Ra Biotechnology (Dalian) Co. Ltd.

Animal grouping and preparation of HIBD models

Twenty normal newborn rats were used as the control group, and five of them were respectively sacrificed 2, 5, 7 and 14 days after birth and then their brains were used to determine the expression of GAP-43 mRNA. According to the rule of body weight (BW) equilibrium, 54 7-day-old neonatal rats were randomly divided into the sham operation group, HIBD group and bFGF treatment groups. The ischemic brain models were made according to the Rice[3] method; ie. the left common carotid artery of the rat was ligated and severed to cause brain ischemia. Then they were put in the hypoxia cabin with an oxygen concentration of (9±1)% for 1.5 h to form the models of HIBD. The rats of the treatment group were rapidly given bFGF (4 U/g each day, ip); the HIBD group, an equal amount of normal saline. The sham operation group had the left common carotid artery separated without ligation and hypoxia. The rats of the three groups were respectively sacrificed on the 1st (8 days old), 3rd (10 days old) and 7th day (14 days old) and the brain was removed to determine GAP-43 mRNA expression.

Storage of brain specimen

Both cerebral hemispheres were removed rapidly and put in pretreated Eppendorf tubes without RNase and stored in a −70℃ refrigerator after being deep-frozen in liquid nitrogen to determine GAP-43 mRNA.

GAP-43 mRNA determination

We utilized TRIZOL one-step extraction to extract the total RNA. Then the product was amplified by RT-PCR, and separated by agarose gel electrophoresis, and stained with EB. The absorption value was determined by the Kodak gel imaging system, and the relative content of GAP-43 mRNA was obtained by using GAPDH as the inner control.

Statistical processing

SPSS software was used to perform ANOVA among multi-groups and Student's t test between two groups. The results were expressed with $\bar{x} \pm s$.

Results

GAP-43 mRNA expression in brains of normal neonatal rats on different days after birth

GAP-43 mRNA expression in the brain of normal neonatal 2-day-old rats was lower, and it increased obviously on day 5 after birth; the peak expression of GAP-43 mRNA occurred on day 7 after birth, and it remained at high level on the 14th day after birth (See Table 1).

Effects on GAP-43 mRNA expression of bFGF in the brain of HIBD rats

GAP-43 mRNA expression on both cerebral hemispheres in the HIBD group decreased apparently one day after damage (8-day old) compared with that in the sham operation group ($P<0.05$). It showed the trend of increase in left brains (damaged sides) on day 3 after damage (10-day old), and remained at low level on the 7th day after damage (14-day old). The GAP-43 mRNA expressions on both cerebral hemispheres of the 10-day and 14-day HIBD groups were not significantly different from those of the sham operation group ($P>0.05$). The GAP-43 mRNA expression one day after damage in both cerebral hemispheres of the treatment group was also at a lower level compared with that in the sham operation group ($P<0.05$), but it was markedly higher on both sides of the brain on the 3rd day after damage compared with those in the HIBD group of the same age and those in the 8-day treatment group ($P<0.05$), and it remained at high level on day 7 after damage. On the same side of the cerebral hemisphere, there was no significant difference among the HIBD groups of different ages ($P>0.05$). The difference of GAP-43 mRNA on both hemispheres of the HIBD or treatment groups were not significant ($P>0.05$) (See Table 2).

Table 1 GAP-43 mRNA expression in normal neonatal rats

Days(d)	GAP-43 mRNA
2	0.16±0.19
5	0.87±0.58[a]
7	1.45±0.73[a,b]
14	1.00±0.70[a]

Note: a vs 2 d $P<0.05$; b vs 5 d $P<0.05$

Table 2 Comparisons of GAP-43 mRNA expressions ($n=6$, $\bar{x}\pm s$)

Groups	1 d after damage (8 d)		3 d after damage (10 d)		7 d after damage (14 d)	
	Left	Right	Left	Right	Left	Right
Sham operation	1.47±0.43		0.89±0.37		0.91±0.32	
HIBD	0.63±0.30[a]	0.56±0.10[a]	0.77±0.33	0.59±0.23	0.64±0.32	0.61±0.19
Treatment	0.50±0.36[a]	0.58±0.16[a]	1.06±0.19[b,c]	1.10±0.56[b,c]	0.92±0.14	1.33±0.81[b,c]

Note: a vs the sham operation group of the same age $P<0.05$; b vs the same group of 8-day-old $P<0.05$; c vs the HIBD group of the same age $P<0.05$

Discussion

Hypoxic-ischemic brain damage (HIBD) is a common neonatal CNS disorder with sequelas. In recent years, scientists have studied reduction of necrosis and apoptosis of neurons as well as neuronal protection in order to find a way of preventing brain damage; and they have propound therapeutic guidelines for HIBD. The researches on restoration and reconstruction of brain function after HIBD have become increasingly concerned by people.

GAP-43 is the principal phosphoprotein localized in motile growth cones of nerves, which shows the expression of high density with the development of[4] nerve axons. Its expression in the central nervous system is regarded as the indication of nerve axon outgrowth and synaptogenesis[2]. The studies based on immunohistochemistry methods indicated that GAP-43 protein expression emerged in brains of rats after birth, peaked at the 7th day after birth, and diminished sharply or vanished after 7 days[5]. Our research identified that GAP-43 mRNA expression in the brain of neonatal rats after birth took on certain

regularity, and that its expression on the 1st to 2nd day after birth was temporarily lower and increased apparently 5 days after birth and peaked on the 7th day after birth. It remained at a relatively higher level on the day 14 after birth. We deduced that brains of newborn rats after birth were still in the stage of continual development and functional maturation, and the synaptogenesis was still active. Our results were not in accordance with the research of protein location but they might be related to the reason that the time points of protein expression were not consistent with those of mRNA expression.

Researches on the expressions of GAP-43 protein and mRNA in the developing brains after HIBD are seldom reported. In our research, we found that the GAP-43 mRNA expression in brains of 7-day-old HIBD rats decreased dramatically, and such a state of low expression lasted to the 7th day after damage. It implied that HIBD retarded the process of functional maturation of the developing brain. And that the right brain suffering from simple damage of hypoxia showed the same changes also implied that the function damages were extensive and severe.

bFGF, with powerful effects of promoting split and proliferation of cells, is one kind of neurotrophic factors with multiple biological activities. In recent years, a myriades of researches have showed that therapy with bFGF soon after HIBD has the strong ability to diminish the damage area and afford evident protective effects on HIBD sufferers[6]. In our research it was found that when the therapy with bFGF was utilized early after HIBD, the inhibited state of GAP-43 mRNA expression in brains after HIBD could be improved obviously 3 days after administration of bFGF, and the higher expression remained until 7 days after damage. These results implied that the administration of bFGF could prominently improve the stagnant state of brain development after HIBD, and possess some positive effects on the restoration and reconstruction of brain function after damage.

[**References**]

[1] Kanazir S, Ruzdijic S, Vukosavic S, et al. GAP-43 mRNA expression in early development of human nervous system [J]. Molecular Brain Research, 1996, 38(1): 145-155.

[2] Kruger L, Bendotti C, Rivolta R, et al. Distribution of GAP-43 mRNA in the adult rat brain [J]. J Comparat Neurgy, 1993, 333 (3): 417-434.

[3] Rice JE, Vannucci RC, Brierley JB, et al. The influence of immaturity on hypoxic-ischemic brain damage in the rat [J]. Ann Neural, 1981, 9(2): 131-141.

[4] Meiri KF, Johnson MI, Wilard M. Distribution and phosphorylation of the growth associated protein GAP-43 in regenerating sympathetic neurons in culture [J]. J Neurosci, 1988, 8(7): 2571-2581.

[5] McGuire CG, Snipes GJ, and Norden JJ. Light-microscopic immunolocalization of the growth and plasticity-associated protein GAP-43 in the developing rat brain [J]. Dev Brain Res, 1988, 41(1-2): 277-291.

[6] Bethel A, Kirsch JR, Koehler RC, et al. Intravenous basic fibroblast growth factor decreases brain injury resulting from focal ischemia in cats [J]. Stroke, 1997, 28(3): 609-615.

(Edited by Yan YU)