· Original Article in English ·

Effects of inosine on neuronal apoptosis and the expression of cytochrome C mRNA following hypoxic-ischemic brain damage in neonatal rats

DENG Yong-Hong, KUANG Shou-Jin, HEI Ming-Yan, TIAN Lang

Department of Pediatrics, Third Xiangya Hospital of Central South University, Changsha 410013, *China*

Abstract: Objective It has been reported that neuronal apoptosis plays a critical role in pathology of hypoxic-ischemic encephalopathy (HIE). Cytochrome C (CytC) is an important apoptotic protease activating factor. Inosine might have a neuroprotective effect against cerebral ischemia reperfusion injury by inhibiting the neuronal apoptosis and the expression of CytC mRNA in adult rats. This study examined the effects of inosine on neuronal apoptosis and CytC mRNA expression following hypoxic-ischemic brain damage (HIBD) in order to investigate the neuroprotectivity of inosine against cerebral ischemia injury in neonatal rats and the possible mechanism. **Methods** A total of 140 healthy 7-day-old Sprague-Dawley rat pups were randomly assigned into Control ($n = 40$), HIBD ($n = 50$) and Inosine treatment groups ($n = 50$). HIBD rat models were established by ligating the left common carotid artery, followed by 8% O_2 hypoxia exposure for 2 hrs in the HIBD and Inosine treatment groups. The Control group was not subjected to hypoxia-ischemia (HI). The Inosine treatment and the HIBD groups were randomly divided into 5 sub-groups sacrificed at 6 and 12 hrs, and 1, 3 and 7 days post- HI ($n = 10$ each). The Control group rats were sacrificed at the corresponding time points ($n = 8$ each). Inosine was administered to the Inosine treatment group by intraperitoneal injection immediately after HIBD at the dosage of 100 mg/kg twice daily for 7 days. TUNEL staining and *in situ* hybridization method was used to detect neuronal apoptosis and CytC mRNA expression respectively. **Results** Few apoptotic cells and CytC mRNA positive cells were found in brain tissues of the Control group. In the HIBD group, the number of apoptotic cells and the CytC mRNA expression in the cortical and hippocampal gyrum CA1 areas increased 6 hrs after HI, peaking at 1 day after HI and then decreased gradually. Until the 7th day, the number of apoptotic cells and the CytC mRNA expression in the cortical and hippocampal gyrum CA1 areas in the HIBD group remained significantly higher than in the Control group. Inosine treatment decreased the apoptotic cells and the CytC mRNA expression in both areas from 6 hrs to 7 days after HI compared with the HIBD group. The linear correlation analysis demonstrated that the number of apoptotic cells was positively correlated to the CytC mRNA expression in neonatal rats with HIBD ($r = 0.88$, $P < 0.01$). **Conclusions** Inosine can reduce the number of apoptotic cells and down-regulate the expression of CytC mRNA following HIBD in neonatal rats. The decreased number of apoptotic cells was positively correlated to the decreased CytC mRNA expression after inosine treatment, suggesting that inosine offered neuroprotectivity against HIBD possibly through inhibiting the CytC mRNA expression and resulting in a decrease of cell apoptosis. [**Chin J Contemp Pediatr, 2006, 8 (4): 266 – 271**]

Key words: Hypoxia-ischemia, brain; Inosine; Apoptosis; Cytochrome C; Neonatal rats

肌苷对新生大鼠缺氧缺血性脑损伤神经细胞凋亡和细胞色素 C 基因表达的影响

邓永红, 旷寿金, 黑明燕, 田朗　中南大学湘雅三医院儿科, 湖南 长沙 410013

[**摘　要**] **目的**　目前认为神经细胞凋亡在新生儿缺氧缺血性脑病(hypoxic-ischemic encephalopathy, HIE)的病理过程中起重要作用,细胞色素 C(CytC) 是重要的促凋亡蛋白因子之一。近年研究发现,肌苷对成年大鼠脑缺血损伤有保护作用,肌苷可降低 CytC mRNA 的表达从而抑制神经细胞凋亡。本实验通过观察肌苷对新生大鼠缺氧缺血性脑损伤(HIBD)后神经细胞凋亡和细胞色素 C 基因表达的影响,以初步探讨肌苷对 HIBD 新生大鼠脑保护作用和可能的机制。**方法**　健康 7 日龄 SD 大鼠 140 只被随机分为 3 组:正常对照组($n = 40$),肌苷治疗组($n = 50$)和 HIBD 组($n = 50$),其中肌苷治疗组和 HIBD 组分别再随机分为缺氧缺血(HI)后 6 h, 12 h, 1 d, 3 d, 7 d

[Received] March 1, 2006; [Revised] May 23, 2006
[Biography] DENG Yong-Hong, Female, Attending doctor, Master, Specializing in neonatology.
[Correspondence Author] KUANG Shou-Jin, Professor, Department of Pediatrics, Third Xiangya Hospital of Central South University, Changsha 410013, China (Email: kuang_shja@sohu.com).

5 个亚组(各亚组 $n=10$)。通过分离、结扎左颈总动脉和 8% 低氧暴露制备 HIBD 动物模型。正常对照组不进行缺氧缺血处理,按肌苷治疗组和 HIBD 组各相同时间点随机分为 5 个亚组(各亚组 $n=8$)。肌苷治疗组于模型制备后即刻开始腹腔注射肌苷注射液 100 mg/kg,每天 2 次,连续 7 d。以 TUNEL 法检测神经元凋亡情况,原位杂交技术测定 CytC mRNA 表达情况。**结果** 正常对照组皮质区和海马区可见少许凋亡细胞和 CytC 阳性细胞,HI 后 6 h HIBD 组皮质区和 CA1 区凋亡细胞和 CytC 阳性细胞即见增多,于 HI 后 1 d 达高峰,之后逐渐下降,HI 后 7 d 凋亡细胞和 CytC 阳性细胞数仍明显高于正常对照组,各时间点与正常对照组相比较,差异有显著性意义($P < 0.05$)。经肌苷治疗后凋亡细胞数和神经细胞 CytC mRNA 表达均减少,各时间点与 HIBD 组相应时间点比较,差异有显著性意义($P < 0.05$)。直线相关分析显示 HIBD 后,凋亡细胞数与 CytC mRNA 表达呈显著正相关($r=0.88$, $P < 0.01$)。**结论** HI 损伤后给予肌苷干预能减少 HI 导致的神经细胞凋亡,下调 CytC mRNA 表达。肌苷治疗后,新生 HIBD 大鼠的凋亡细胞数减少与 CytC mRNA 表达下调呈显著正相关,提示肌苷可能通过抑制 CytC mRNA 表达从而起到减少细胞凋亡、保护神经元的作用。 [中国当代儿科杂志,2006,8(4):266 - 271]

[关 键 词] 缺氧缺血,脑;肌苷;凋亡;细胞色素 C;新生大鼠
[中图分类号] R - 33 [文献标识码] A [文章编号] 1008 - 8830(2006)04 - 0266 - 06

Neonatal hypoxic-ischemic encephalopathy (HIE) caused by perinatal asphyxia is a major cause of acute mortality and chronic neurological disability in survivors. More and more studies have indicated that apoptosis plays an important role in the hypoxic-ischemic pathological changes of immature brain [1,2]. Cytochrome C (CytC) is one of the main apoptotic protease activating factors secreted by mitochodria and it plays a key role in the cell apoptosis process. Previous studies have suggested that inosine has protective effects against brain ischemic injury in adult rats [3,4]. During the adult rat brain ischemic reperfusion injury, inosine can decrease the CytC mRNA expression and then inhibit the neuronal apoptosis [5]. This study prepared the HIE model and examined the effects of inosine on neuronal apoptosis and CytC mRNA expression in neonatal rats, in order to provide an experimental basis for the clinical use of inosine in the treatment of HIE.

Materials and methods

Establishment of animal model and experimental animal grouping

A total of 140 healthy 7-day-old Sprague-Dawley (SD) rats were provided by Hunan Agriculture University Animal Center (clear grade, body weight 10-15 g, gender unlimited). These rats were randomly assigned into 3 groups: Control group ($n=40$), Inosine treatment group ($n=50$) and HIBD group ($n=50$). HIBD was induced by the classic Rice-Vannucci method [6], i. e. ligating the left common carotid artery, followed by 8% O_2 hypoxia exposure for 2 hours (temperature was controlled at 36 ± 1°C) in the Inosine treatment and HIBD groups. The Control group was not subjected to hypoxia-ischemia (HI). The Inosine treatment and HIBD groups were randomly divided into 5 sub-groups sacrificed at 6 and 12 hrs, and 1, 3 and 7 days post-HI ($n=10$ each). The Control group rats were sacrificed at the corresponding time points ($n=8$ each). Inosine venous infusion solution was administered to the Inosine treatment group by intraperitoneal injection immediately after HIBD at the dosage of 100 mg/kg twice daily for 7 days.

Sample collection and tissue section

Samples were collected from 8 randomly chosen rats at planned time points from each group. After they were anesthetized by inhalational ether, the thorax cavity of the rats was opened and the heart was exposed. Cool sterilized normal saline was infused into the left ventricle. Simultaneously, their right atrial auricle was cut and the blood flowed out until the color of lung and liver changed into white and the fluid flowed out from right atrial auricle changed into clear water. Thereafter, 30-40 mL fixation solution (4% paraform/0.1M PBS) was infused. The rats were sacrificed and the brains removed. The brain specimens were preserved in the 4% paraform/0.1M PBS fixation solution for 30 minutes, and then were soaked in sterilized 30% sucrose/0.1M PBS until they sank to the bottom. Constant freezing slicing (-20°C) was carried out. The brain specimens were taken out from the sucrose solution and were then embedded with distilled water. The left and right brain was distinguished by cutting an incision at the left frontotemporal lobe. Continuous coronal slicing was performed at parahippocampal gyrus level (25 μm thick). The sections were placed onto polyysine (contains 0.1% DEPC) treated glass slides and kept under room temperature.

Detection of neuronal apoptosis

Neuronal apoptosis was detected by using terminal deoxynucleotidyl Transferase Biotin-dUTP nick end labeling (TUNEL) technique. The TUNEL kits were provided by Beijing Zhongshan Jingqiao Bio-Technique Co Ltd. Apoptosis was observed under a light micro-

scope. The cells with intra-nuclear brown stain particles were TUNEL positive cells, i. e. apoptotic cells.

Detection of CytC mRNA

CytC mRNA was detected by *in situ* hybridization. The kit was provided by the Wuhan Boster Bio-Engineering Company. CytC positive cells were observed under a light microscope. The cells with intra-plasma brown stain particles were CytC positive cells.

Data processing and statistical analysis

Positive cells in brain tissue sections (3 slides each) at each time point of each group ($n=8$) were counted under a high power lens (40 ×). Four view fields were randomly selected from the cortical and hippocampal gyrum CA1 areas. Cells distinguishable were counted and the average value was calculated. Statistic analysis was performed using SPSS13. 0 software. All experimental data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Multi-sample mean comparison between groups was carried out using ANOVA analysis. The correlation between the variables was analyzed using linear correlation analysis.

Results

Death of the animals

No animal died in the Control group. In the HIBD group, 10 rats died (1 died 12 hrs, 7 died 1 day and 2 died 2 days after HI), with a death rate of 20%. In the Inosine treatment group, 3 rats died 1 day after HI, with a death rate of 6%.

TUNEL detection results

Few TUNEL positive cells (apoptotic cells) were observed in the cortical and hippocampal gyrum CA1 areas in the Control group. In the HIBD group, the apoptotic cells in the cortical and CA1 areas increased 6 hrs after HI. Brown particles could be noticed in the nuclei of TUNEL positive cells. With the HI time increasing, the number of apoptotic cells increased progressively, peaking 1 day after HI and then decreased gradually. Until the 7th day, the number of TUNEL positive cells in the cortical and hippocampal gyrum CA1 areas in the HIBD group remained higher than in the Control group. The number of apoptotic cells was significantly decreased in the Inosine treatment group from 6 hrs to 7 days after HI compared with the HIBD group. See Table 1 and Figure 1.

CytC mRNA expression

Few CytC mRNA positive cells were found in the brain tissues of the Control group. In the HIBD group, the CytC mRNA expression in the cortical and hippocampal gyrum CA1 areas increased 6 hrs after HI. Brown particles could be noticed in the plasma of positive cells. With the HI time increasing, the CytC mRNA expression increased progressively, peaking 1 day after HI and then decreased gradually. Until the 7th day, the CytC mRNA expression in the cortical and hippocampal gyrum CA1 areas in the HIBD group remained higher than in the Control group. The CytC mRNA expression level was significantly lower in the Inosine treatment group from 6 hrs to 7 days after HI than in the HIBD group. See Table 2 and Figure 2.

Table 1 Number of apoptotic cells ($\bar{x} \pm s$, $n=8$)

Group	Apoptotic cells in the cortex	Apoptotic cells in the hippocampus CAI
Control		
6 h	3.00 ± 1.31	1.13 ± 1.13
12 h	2.50 ± 0.53	1.13 ± 0.64
1 d	2.50 ± 0.76	1.25 ± 0.71
3 d	2.75 ± 1.04	1.13 ± 0.83
7 d	2.63 ± 0.92	1.00 ± 0.76
HIBD		
6 h	29.25 ± 2.82[a]	23.00 ± 2.07[a]
12 h	44.00 ± 3.55[a]	35.88 ± 1.96[a]
1 d	62.50 ± 5.58[a]	56.63 ± 1.51[a]
3 d	36.50 ± 6.50[a]	33.13 ± 2.17[a]
7 d	17.00 ± 2.51[a]	16.00 ± 1.51[a]
Inosine treatment		
6 h	19.38 ± 1.85[a, b]	14.88 ± 1.81[a, b]
12 h	34.38 ± 1.85[a, b]	21.75 ± 1.49[a, b]
1 d	46.88 ± 2.53[a, b]	37.88 ± 1.81[a, b]
3 d	9.63 ± 1.92[a, b]	8.25 ± 1.98[a, b]
7 d	3.25 ± 1.67[b]	2.63 ± 1.30[b]

a vs the Control group, $P<0.05$; b vs the HIBD group, $P<0.05$

Table 2 CytC mRNA expression ($\bar{x} \pm s$, $n=8$)

Group	CytC mRNA expression in the cortex	CytC mRNA expression in the hippocampus CAI
Control		
6 h	9.75 ± 1.58	8.63 ± 1.69
12 h	9.50 ± 1.69	8.63 ± 1.41
1 d	9.63 ± 1.41	8.50 ± 1.41
3 d	9.50 ± 1.31	8.50 ± 1.41
7 d	9.63 ± 1.19	8.38 ± 1.30
HIBD		
6 h	60.63 ± 1.60[a]	35.50 ± 0.93[a]
12 h	80.25 ± 2.87[a]	56.25 ± 1.49[a]
1 d	115.50 ± 3.82[a]	82.13 ± 1.81[a]
3 d	92.63 ± 2.56[a]	62.50 ± 1.93[a]
7 d	31.25 ± 1.75[a]	28.50 ± 1.60[a]
Inosine treatment		
6 h	43.13 ± 1.64[a, b]	19.50 ± 1.60[a, b]
12 h	62.38 ± 2.20[a, b]	38.00 ± 2.00[a, b]
1 d	78.75 ± 2.71[a, b]	58.75 ± 2.25[a, b]
3 d	58.13 ± 2.36[a, b]	38.13 ± 1.81[a, b]
7 d	13.63 ± 1.19[a, b]	12.75 ± 1.67[a, b]

a vs the Control group, $P<0.05$; b vs the HIBD group, $P<0.05$

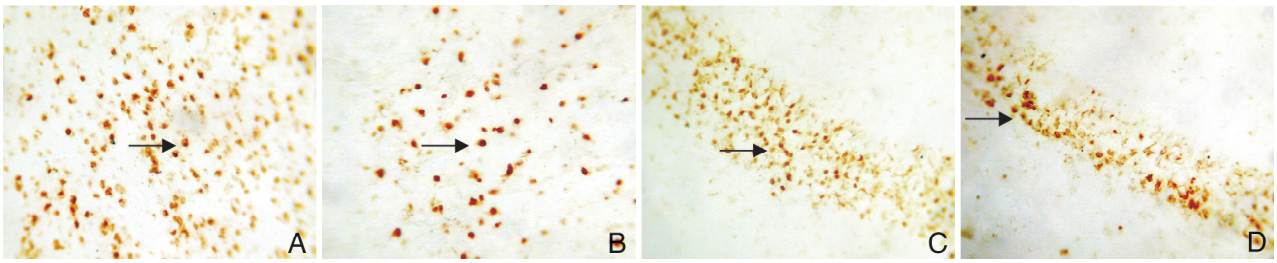

Figure 1　TUNEL detection results 1 day after HI（TUNEL ×200）　**A**: Many TUNEL positive cells in the left cortex were found in the HIBD group. **B**: TUNEL positive cells in the left cortex in the Inosine treatment group decreased compared that the HIBD group. **C**: Many TUNEL positive cells in the left hippocampus CA1 were found in the HIBD group. **D**: TUNEL positive cells in the left hippocampus CA1 decreased in the Inosine treatment group.

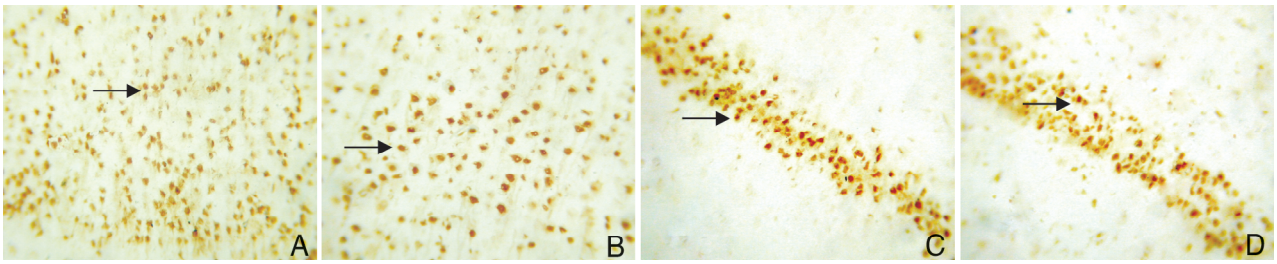

Figure 2　CytC mRNA expression 1 day after HI（*in situ* hybridization ×200）　**A**: Many CytC positive cells in the left cortex were found in the HIBD group. **B**: CytC positive cells in the left cortex in the Inosine treatment group decreased compared that the HIBD group. **C**: Many CytC positive cells in the left hippocampus CA1 were found in the HIBD group. **D**: CytC positive cells in the left hippocampus CA1 decreased in the Inosine treatment group.

Correlation between the apoptotic cell number and CytC mRNA expression

The linear correlation analysis demonstrated that the apoptotic cell number was positively correlated to the CytC mRNA expression in neonatal rats with HIBD ($r = 0.88$, $P < 0.01$). See Figure 3.

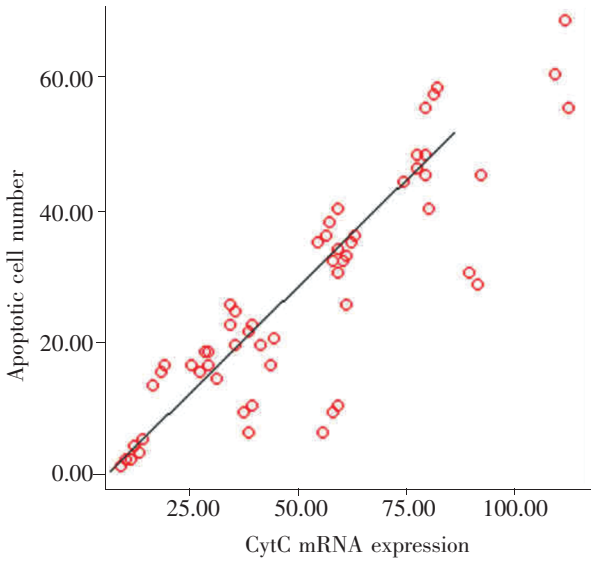

Figure 3　The correlation between the apoptotic cell number and the CytC mRNA expression

Discussion

Cell death could be divided into 2 models, i. e. necrosis and apoptosis. Studies have indicated that during the pathological process of HIBD there is not only acute edema and necrosis of neurons but also cell apoptosis [1]. Cell apoptosis is predominant in immature brain injury [7], which may be related to the much higher content of caspase enzyme in immature brain compared with adult brain tissue [8], as well as the higher expression of apoptotic protease activating factor-1 (Apaf-1), apoptosis-related gene Bcl-2 and Bax [9-11]. In this study, it was observed that the apoptosis reached its peak 1 day after HI and decreased gradually thereafter, but still remained higher than normal controls on the 7th day. These results suggested that apoptosis lasted for a long time. It was also noticed that the apoptotic cell number of CA1 and cortical areas reached a peak at the same time, which was in accordance with previous study results [12]. This suggests that the CA1 and cortical areas are susceptible to ischemic injuries. This study also found that death of experimental animals mainly occurred on the 1st day after HI, which may be related to the massive neuronal apoptosis 1 day after HI. These findings indicated that the clinical severity is related to the number of apoptot-

ic cells. It is suggested that the best timing of inhibiting apoptosis is within 1 day after HI. This means that early interference treatment for HIE is important.

CytC is a fundamental element of mitochondrial respiratory chain. It was reported that CytC not only participates in cellular aerobic respiration but also plays important roles in activating caspase and inducing cell apoptosis [13-15]. Apoptosis signals stimulate the releasing of CytC from mitochondria to plasma, which combines with Apaf-1. CytC/Apaf-1 compound activates caspase-9, which activates caspase-3 and initiates cell apoptosis. Liu [16] reported that CytC could activate caspase-3 and induce apoptosis in *in vitro* apoptosis system (containing cell organelles fragments plasma) experiments. During the cell apoptosis, plasma CytC concentration increased while the mitochondria CytC concentration decreased [17]. Further studies indicated that when cells were stimulated by apoptosis signals, apoptosis promoting factors could up-regulate the CytC mRNA expression. Nuclear gene encoded pre-CytC increased, which entered into mitochondrial membrane space and increased the synthesis of mature CytC. Finally the mitochondria increased the mature CytC relieving. This is a positive feedback circle until all of the mature CytC in mitochondria is released into plasma [18]. This study found that the CytC mRNA expression in the injured brain tissue increased 6 hrs after HI along with an increase of the TUNEL positive cell number, and both of them reached a peak 1 day after HI. It was also found that the apoptosis cell number was positively related to the CytC mRNA expression. These findings indicated that HIBD could induce CytC mRNA expression and CytC releasing. With the HI time increasing, plasma CytC accumulated progressively and caused cell apoptosis.

Inosine is a kind of purine substance with very low molecular weight (268.2). Recently, more and more research has focused on the neuroprotective effect of inosine. Adenosine is a well known endogenous protective factor synthesized following HIE. Adenosine exerts its neuroprotective effect by changing into inosine [19-20]. Adenosine induces ischemic neuroglia cells to secrete CytC, promotes the expression of apoptosis factor Bax, inhibits the expression of anti-apoptosis factor Bcl-2 and then activates caspase enzyme, which finally induces cell apoptosis. Inosine can promote the transportation and transforming of adenosine into cells, eliminates its function and therefore exerts its anti-apoptosis function [21]. This study found that the CytC mRNA expression and the number of neuronal apoptosis in the Inosine treatment group were significantly reduced compared with that in the HIBD group. Moreover, the decreased number of neuronal apoptosis was statistically positively related with the decreased CytC mRNA expression. These results indicated that inosine has protective effects on the brain in neonatal rats following HIBD and this effect may be related to anti-apoptosis function. In one way, it can directly decrease the CytC mRNA expression and then inhibit apoptosis. On the other hand, exogenous inosine provides raw materials for the synthesis of ATP, which enables the metabolism of cells under hypoxic-ischemic status and increases the intracellular ATP level. It then recovers the mitochondria membrane potential and permeability. This prevents mitochondria from releasing CytC and inhibits the cell apoptosis.

It is concluded that inosine treatment can decrease the neuronal apoptosis caused by HI, and that it can also decrease the CytC mRNA expression in cortical and CA1 areas. After inosine treatment, the decreased number of cell apoptosis was positively related with the decreased CytC mRNA expression. These results indicate that inosine can decrease cell apoptosis and offer neuroprotectivity against HI insults through inhibiting the CytC mRNA expression in neonatal rats.

[References]

[1] Nakajima W, Ishida A, Lange MS, Gabrielson KL, Wilson MA, Martin LJ, et al. Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat [J]. J Neurosci, 2000, 20(21): 7994-8004.

[2] Benjelloun N, Joly LM, Palmier B, Plotkine M, Charriaut-Marlangue C. Apoptotic mitochondrial pathway in neurones and astrocytes after neonatal hypoxia-ischaemia in the rat brain [J]. Neuropathol Appl Neurobiol, 2003, 29(4): 350-360.

[3] Bocklinger K, Tomaselli B, Heftberger V, Podhraski V, Bandtlow C, Baier-Bitterlich G. Purine nucleosides support the neurite outgrowth of primary rat cerebellar granule cells after hypoxia [J]. Eur J Cell Biol, 2004, 83(2): 51-54.

[4] Shen H, Chen GJ, Harvey BK, Bickford PC, Wang Y. Inosine reduces ischemic brain injury in rats [J]. Stroke, 2005, 36(3): 654-659.

[5] Zhang H, Wang Y, Gong WW, Sun GL, Guo YL. Effects of inosine on the apoptosis and the expression of cytochrome C mRNA in neurons after cerebral ischemia and reperfusion in rats (in Chinese) [J]. Chin J Geriatr, 2004, 23(9): 652-655.

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

[7] Johnston MY, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic-ischemic injury in the developing brain [J]. Pediatr Res, 2001, 49(6): 735-741.

[8] Hu BR, Liu CL, Ouyang Y, Blomgren K, Siesjo BK. Involvement of caspase-3 in cell death after hypoxia-ischemia declines during brain maturation [J]. Cerebr Blood Flow Metab, 2000, 20: 1294-1300.

[9] Ota K, Yakovlev AG, Itaya A, Kameoka M, Tanaka Y, Yoshihara K. Alteration of apoptotic protease-activating factor-1 (APAF-1)-dependent apoptotic pathway during development of rat brain and liver [J]. J Biochem, 2002, 131(1): 131 -135.
[10] Hagberg H. Mitochondrial impairment in the developing brain after hypoxia-ischemia [J]. Bioenerg Biomembr, 2004, 36(4): 369-373.
[11] Vekrellis K, McCarthy MJ, Watson A, Whitfield J, Rubin LL, Ham J. Bax promotes neuronal cell death and is downregulated during the development of the nervous system [J]. Development, 1997, 124(6): 1239 -1249.
[12] Kuang SJ, Hei MY, Yin P. Expression of cleaved caspase-3 in neonatal rats with hypoxic-ischemic brain damage (in Chinese) [J]. Chin J Contemp Pediatr, 2003, 5(4): 331-334.
[13] Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al. Cytochrome C and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade [J]. Cell, 1997, 91(4): 479-489.
[14] Eldering E, Mackus WJ, Derks IA, Evers LM, Beuling E, Teeling P, et al. Apoptosis via the B cell antigen receptor requires Bax translocation and involves mitochondrial depolarization, cytochrome C release, and caspase-9 activation [J]. Eur J Immunol, 2004, 34(7): 1950-1960.
[15] Hao Z, Duncan GS, Chang CC, Elia A, Fang M, Wakeham A, et al. Specific ablation of the apoptotic functions of cytochrome C reveals a differential requirement for cytochrome C and Apaf-1 in apoptosis [J]. Cell, 2005, 121(4): 579-591.
[16] Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome C [J]. Cell, 1996, 86(1): 147-157.
[17] Kluck RM, Bossy-Wezel E, Green DR, Newmeyer DD. The release of cytochrome C from mitochondria: a primary site for Bcl-2 regulation of apoptosis [J]. Science, 1997, 275(5303): 1132-1136.
[18] Chandra D, Liu JW, Tang DG. Early mitochondrial activation and cytochrome C up-regulation during apoptosis [J]. Biol Chem, 2002, 277(52): 50842-50854.
[19] Hasko G, Sitkovsky M V, Szabo C. Immunomodulatory and neuroprotective effects of inosine [J]. Trends Pharmacol Sci, 2004, 25(3): 152-157.
[20] Haun SE, Segeleon JE, Trapp VL, Clotz MA, Horrocks LA. Inosine mediates the protective effect of adenosine in rat astrocyte cultures subjected to combined glucose-oxygen deprivation [J]. J Neurochem, 1996, 67(5): 2051-2059.
[21] Imura T, Shimohama S. Opposing effects of adenosine on the survial of glial cells exposed to chemical ischemia [J]. Neurosci Res, 2000, 62(4): 539-546.

(Edited by DENG Fang-Ming)