·Original Article·

Correlation of free radical level and apoptosis after intracerebral hemorrhage in rats

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Abstract: Objective To investigate the correlation of perihematomal free radical level and neuronal apoptosis following the intracerebral hemorrhage (ICH). **Methods** Animals were randomly divided into 4 groups: sham operation group, model group, 1 mg/kg edaravone group, and 3 mg/kg edaravone group. Each group was then divided into seven subgroups, in which the rats were correspondingly killed at 6 h, 12 h, 24 h, 48 h, 72 h, 7 d or 14 d (n = 1 in each subgroup of the sham group, and n = 6 in each subgroup of the other 3 groups). By Horseley-Clarke technique, autoblood (80 μL) were administered into the left caudate putamen of SD rats in a double administration-withdrawal way. Rats in the sham group were needled in but not administered with autoblood. The ICH model was then evaluated by Bederson's scale. Around the hematoma, the levels of malonaldehyde (MDA) and hydroxyl radical were tested by spectrophotometer, and the process of apoptosis was tested by terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) method. **Results** (1) ICH significantly increased the levels of MDA and hydroxyl radicals. Significant differences in MDA and hydroxyl radical contents were observed among the four groups. (2) In the sham group, a small number of TUNEL-positive cells were found. In the other three groups, the TUNEL-positive cells were observed at 6 h, increased significantly at 24 h, and reached peak level at 3 d, then fell profoundly at 7 d, but remained detectable at 14 d. (3) The positive correlation existed between apoptosis and free radical level (r = 0.2003), and existed between apoptosis and MDA content (r = 0.6563) in the brain. **Conclusion** Post-hemorrhagic apoptosis was related to the production of free radicals, indicating that the elevated free radicals following the ICH could induce neuron and glial cell apoptosis.

Keywords: intracerebral hemorrhage; free radical; apoptosis; TUNEL; edaravone

1 Introduction

Nerve cell apoptosis after cerebral hemorrhage has been demonstrated in many researches. Qureshi *et al*.[1] discovered apoptosis cell in tissues surrounding the hematoma of intracerebral hemorrhage (ICH) patients early at 24 h after the onset, and the apoptosis phenomenon remained for 5 d. According to Xue *et al*.[2], in the model of rat autologous arterial blood ICH, apoptosis cells could be detected 4 h after the surgery, and remained for 4 weeks. The correlation of free radical and cell apoptosis has also been demonstrated by other researches[3-8]. A great amount of free radicals are produced as results of surrounding tissue ischemia, erythrocyte fragmentation, ferric ion release, and inflammatory reaction after cerebral hemorrhage. However, no report on the correlation of free radical and cell apoptosis after cerebral hemorrhage is available currently.

In this study, cerebral hemorrhage model was established via autoblood injection, then the rat model received free radical scavenger of different doses. Spectrophotometer was taken to detect the contents of malonaldehyde (MDA) and hydroxyl radicals. Terminal deoxynucleotidyltransferase-

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mediated dUTP-biotin nick end labeling (TUNEL) staining was used to examine the number of apoptotic cells in tissues around hematoma. Correlation of free radical level and apoptosis after cerebral hemorrhage was analyzed based on the above inspection.

2 Material and methods

2.1 Animals and materials Healthy male Sprague-Dawley (SD) rats, weighting about 300 g, were purchased from Chinese Academy of Science and raised in clean environment. The apparatuses included Jiangwan type 2 animal cranium stereotaxic apparatus (The Second Military Medical University, China), desk-top low temperature high-speed centrifuge (Heraeus Company, Germany), and 756MC ultraviolet-visible spectrophotometer (The Third Analytical Apparatus Factory, Shanghai, China). Agents including hydroxyl radical determination kit, MDA determination kit (The First Branch Institute of Nanjing Jiancheng Bioengineering Institute, China), *in situ* apoptosis determination kit (Roche Company, Switzerland), and DAB staining kit (Wuhan Boshide Company, China) were used.

2.2 Establishment of cerebral hemorrhage model Rat ICH model was established by twice blood injection / needle withdrawal according to Deinsberger W[9] and Zhou ZH *et al.*[10]. After weighing, rats were anesthetized using 10% chloral hydrate (0.4 g/kg, i.p.) and then fixed in prone position on a rat stereotaxis instrument[11], with the upper incisor sulcus level 2.6-mm lower than the biauricular line. After routine sterilization, about 10 mm length of longitudinal incision was cut along the middle line of the scalp and then the periost was cut open, and the anterior fontanel was exposed by blunt dissection. The stereotaxic apparatus was adjusted to set the injection point at caudate putamen. The pointed-end of microinjection apparatus was located at 0.5 mm in the front of the anterior fontanel (3 mm from the middle line), and the needle entered 6 mm into the brain. An eyehole with diameter of 0.5 mm was drilled with a micro-gimlet. Autologous anti-clotting blood (80 μL) was obtained from rat femoral vein with a 1-mL syringe, which was then entered 6 mm into the brain along the drilled eyehole (to the position of rat caudate putamen). The initial injection volume was 20 μL, and then the needle was retained in the position for 2 min and the subsequent 60 μL was injected slowly (finishing the injection within 2 min in a well-distributed manner). Retain the needle for about 2 min, withdraw the needle for 2 mm and retain the needle for another 2 min, and finally remove the syringe slowly out of the brain. In the sham group, only needle entered into the caudate putamen without blood injection, and the syringe was withdrawn after 6-min retaining. The skull wound was sealed with bone wax, and the scalp was sewed up, followed by local iodine sterilization. The animals were placed in warm condition until consciousness.

2.3 Animal grouping and chemical administration Rats were randomly divided into sham group, model group, 1 mg/kg edaravone group and 3 mg/kg edaravone group. Each group was separately divided into seven subgroups (n = 1 in each subgroup of the sham group, and n = 6 in each subgroup of the other 3 groups), in which the animals were correspondingly killed at 6 h, 12 h, 24 h, 48 h, 72 h, 7 d or 14 d after model establishment. For edaravone treated groups, edaravone was intraperitoneally injected 1 h after model was established (for 3 mg/kg group, edaravone was administered in the form of stock solution; for 1 mg/kg group, the stock solution was diluted to the same volume before administration), once per 24 h until the animals were killed. In the sham group and the model group, equal amount of saline was intraperitoneally injected instead of edaravone. The rat brains were collected after rats were killed.

2.4 Preparation of pathological sections At designated time points, overdose of chloral hydrate were employed for anesthesia, and then the chest of rat was opened immediately to expose the heart. A pipe was inserted into the aortic root through the left ventricle, and a small mouth was opened at the right auricular as the exit of perfusion solution. The saline solution (100 mL, 4 °C) was rapidly perfused, and then the perfusion speed was reduced until the effluent turned clear. The rats were decapitated to obtain the brain tissues. A coronal cut was made with the pin hole as the center. The nasal side tissues, which were prepared for free radical content determination, were placed in liquid nitrogen for 30 min before storage at −80 °C in centrifuge tubes; the caudal tissues, which were prepared for apoptosis detection, were soaked in 10% neutral formalin solution for fixation before routine dehydration and wax embedment, and finally cut into serial sections at 5 μm thickness by a slicing machine.

2.5 TUNEL staining The TUNEL staining strictly followed the procedure introduced in the apoptosis kit. Positive staining was determined when nucleolus appeared dark yellow or

brown under a light microscope. Reaction sediments distributed around the nucleolus as concentrated lump; few cells appeared even yellow light staining, which was considered as necrotic cells and excluded during statistical analysis. With the help of image analytical system, TUNEL-positive cells in regions surrounding the hematoma were counted under a 400× visual field. Three unrepeated visual fields were observed and the total number was calculated.

2.6 Determination of the contents of MDA and hydroxyl radicals The procedure strictly followed the instruction in the MDA and hydroxyl radical detection kit. Thio-barbituric acid (TBA) method was employed for MDA detection. TBA and MDA can react to yield a red product, which presents a maximum absorption peak at 532 nm. Fenton reaction was employed for hydroxyl radical detection, with Gress agent as the developer. The red products, which were paralleled with the hydroxyl radicals in amount, were detected at the wave length of 550 nm.

2.7 Statistical analysis Data are expressed as mean±SEM. SPSS11.0 statistical software was used for analysis. Statistical group comparisons were carried out by ANOVA or *t*-test. Correlations between the number of apoptosis cells and the contents of hydroxyl radicals or MDA were analyzed. The limit was set at $r = 0.5$: $r > 0.5$ demonstrated that hydroxyl radical and MDA contents influenced cell apoptosis, while $r < 0.5$ demonstrated that hydroxyl radical and MDA contents produced no significant influence on apoptosis; $r > 0.7$ demonstrated that hydroxyl radical and MDA contents produced a significant influence on apoptosis. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Cell apoptosis In the sham group, few TUNEL-positive cells were detected around the pin hole. The number slightly increased with time ($P > 0.05$). In the model group and the two edaravone groups, TUNEL-positive cells were detected at 6 h, continued to increase until 12 h, increased significantly at 24 h, and reached the peak at 3 d (Fig. 1, 2). The number of TUNEL-positive cells was reduced significantly at 7 d, but remained detectable in the perihematomal region at 14 d. ICH significantly increased the number of apoptotic cells ($P < 0.01$ *vs* sham group). At the time points of 24 h, 48 h and 72 h, the numbers of TUNEL-positive cells in the two edaravone groups were reduced as compared with the model group ($P < 0.05$). Also, significant difference was determined in the number of TUNEL-positive cells between the edaravone group (1 mg/kg or 3 mg/kg) and the model group at different time points. At 48 h and 72 h, edaravone at 3 mg/kg was more effective in reducing apoptotic cells than 1 mg/kg edaravone ($P < 0.05$, Fig. 2).

3.2 Content of MDA in brain tissues In the model group, the MDA content in tissues surrounding hematoma increased significantly at different time points compared with that in the sham group. After treatment with 1 mg/kg or 3 mg/kg edaravone, the MDA content decreased obviously at different time points ($P < 0.05$ or $P < 0.01$ *vs* model group), although still higher than that in the sham group ($P < 0.05$ or $P < 0.01$). At 6 h, 12 h, 24 h, 48 h and 72 h, the MDA content in the 1 mg/kg edaravone group was significantly higher than that in the 3 mg/kg edaravone group ($P < 0.05$ or $P < 0.01$).

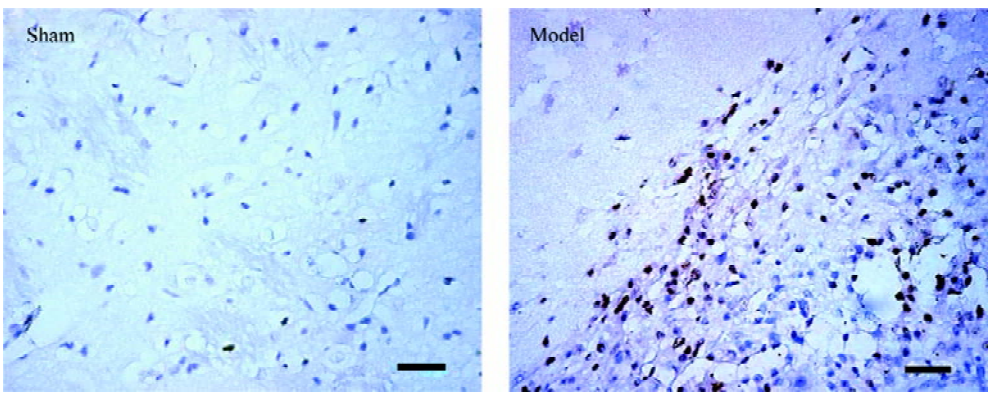

Fig. 1 TUNEL staining for apoptotic cells (staining at 24 h for sham group, and staining at 72 h for model group). Few positive cells (the nucleolus presented dark yellow or brown) were detected at the basal ganglia region adjacent to the needle entering district in the sham group. In the model group, a large number of TUNEL-positive cells were detected in and surrounding the hematoma. Scale bar, 160 μm. (TUNEL staining, hematoxylin after TUNEL)

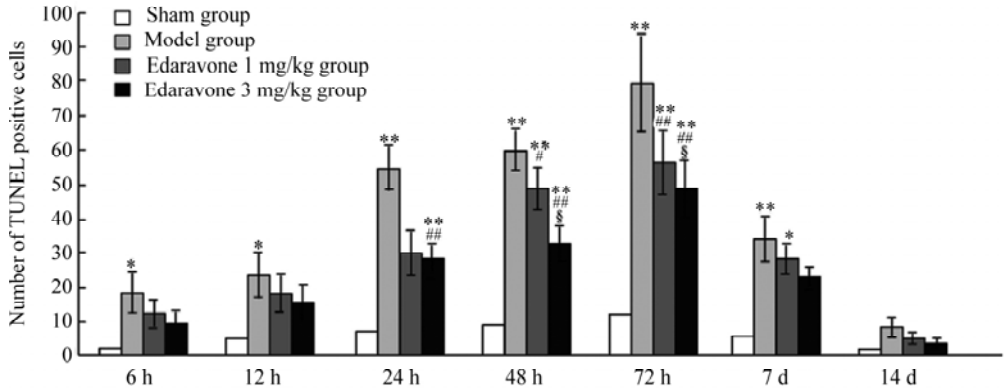

Fig. 2 Comparison of TUNEL-positive cell numbers at different time points. Compared with the sham group, ICH significantly increased the number of apoptotic cells. At the time points of 24 h, 48 h and 72 h, the numbers of TUNEL-positive cells in the edaravone groups were reduced compared with that in the model group. Edaravone at 3 mg/kg was more effective in reducing the apoptotic cells as compared with 1 mg/kg edaravone. *$P < 0.05$, **$P < 0.01$ *vs* sham group; #$P < 0.05$, ##$P < 0.01$ *vs* model group; §$P < 0.05$ *vs* 1 mg/kg edaravone group.

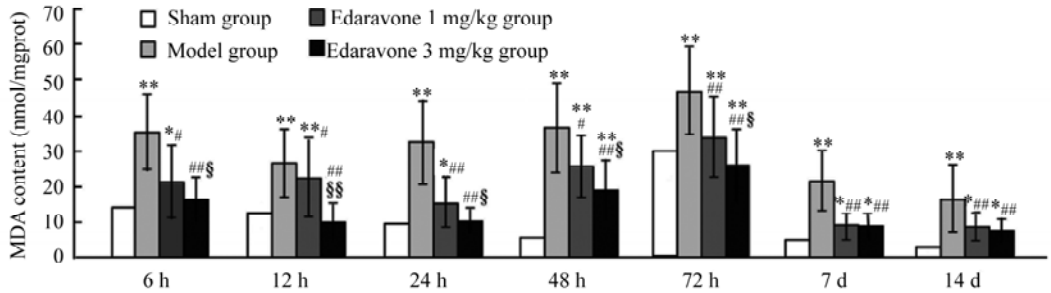

Fig. 3 Perihematomal MDA content at different time points. After ICH treatment, MDA content in tissues surrounding hematoma was increased compared with that in the sham group. After treatment with edaravone, the MDA content was significantly decreased at different time points as compared with the model group, and 3 mg/kg edaravone was more effective than 1 mg/kg edaravone. *$P < 0.05$, **$P < 0.01$ *vs* sham group; #$P < 0.05$, ##$P < 0.01$ *vs* model group; §$P < 0.05$, §§$P < 0.01$ *vs* 1 mg/kg edaravone group.

These results suggested that edaravone could decrease the MDA content in tissues surrounding hematoma, and 3 mg/kg edaravone was more effective than 1 mg/kg edaravone (Fig. 3).

3.3 The content of hydroxy radicals in brain tissues In the model group, the hydroxy radical content in tissues surrounding hematoma was significantly increased at different time points compared with that in the sham group. After edaravone treatment (1 mg/kg or 3 mg/kg), the hydroxy radical content in tissues surrounding haematoma was decreased at different time points ($P < 0.05$ or $P < 0.01$ *vs* model group), but still higher than that in the sham group at some time points ($P < 0.05$). The hydroxy radical content in the 1 mg/kg edaravone group seemed higher than that in the 3 mg/kg edaravone group in tendency, but no significance was found between the two edaravone groups. The results disclosed that edaravone could decrease the hydroxy radical content in tissues surrounding hematoma (Fig. 4).

3.4 Correlation analysis of content of MDA and apoptosis in brain tissues Correlation analysis was conducted between the number of apoptotic cells and the MDA content in brain tissues at different time points. Positive correlation was found at five time points: 6 h, 24 h, 48 h, 72 h and 7 d ($P < 0.05$, $t > 0$). MDA produced a dominant influence on cell apoptosis at 24 h, 48 h and 72 h ($t > 0.7$), and produced an important influence at 6 h and 7 d ($t > 0.5$, Table 1). The results showed that apoptosis cell could increase along with the enhancement of MDA content at these time points.

With the exclusion of time factor, relatively strong positive correlation was determined between MDA content and cell apoptosis ($r = 0.6563$), and the MDA content in brain tissues produced an important influence on cell apoptosis ($t > 0.5$, Fig. 5).

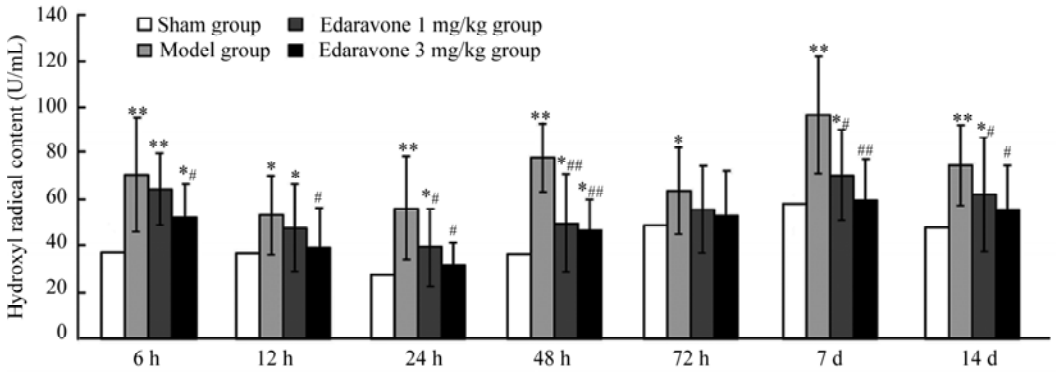

Fig. 4 Perihematomal hydroxyl radical content at different time points. In the model group, the hydroxy radical content was significantly increased at different time points compared with that in the sham group. In two edaravone groups, the hydroxy radical content was decreased at different time points as compared with the model group. It seemed higher in 1 mg/kg edaravone group than that in 3 mg/kg edaravone group in tendency, but no significance was observed. *$P < 0.05$, **$P < 0.01$ *vs* sham group; #$P < 0.05$, ##$P < 0.01$ *vs* model group.

Table 1 Correlation analysis of MDA content and cell apoptosis in the brain tissue

Correlation coefficient	Time after model establishment			
	6 h	24 h	48 h	7 d
r	0.5402	0.8030	0.7356	0.8068

Table 2 Correlation analysis of hydroxyl radical content and cell apoptosis in the brain tissue

Correlation coefficient	Time after model establishment			
	6 h	24 h	48 h	7 d
r	0.5878	0.4828	0.5856	0.4163

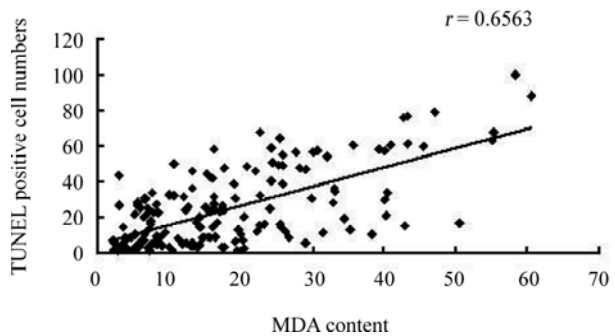

Fig. 5 Linear-regression curve of TUNEL-positive cell number and MDA content. Relatively strong positive correlation was determined between MDA content and cell apoptosis.

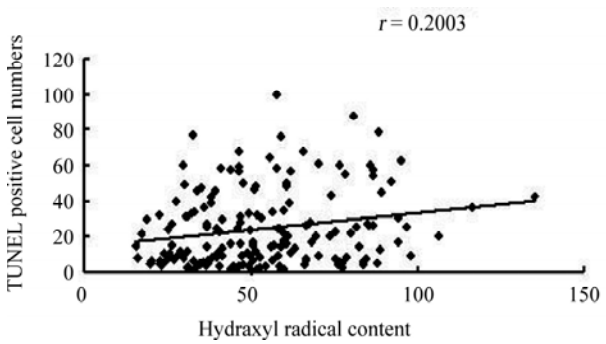

Fig. 6 Linear-regression curve of TUNEL-positive cell number and hydroxyl radical content. Positive correlation was confirmed between cell apoptosis and hydroxyl radical content.

3.5 Correlation analysis of apoptosis and hydroxyl radical content in the brain tissues Correlation analysis was conducted between the number of apoptosis cells and the hydroxyl radical content in brain tissues at different time points. Positive correlation was found at 4 time points: 6 h, 24 h, 48 h and 7 d ($P < 0.05$, $t > 0$). Hydroxyl radicals productivity produced an important influence on cell apoptosis at 6 h and 48 h ($0.5 < t < 0.7$), and produced some influences at 24 h and 7 d ($0 < t < 0.5$, Table 2). The results showed that apoptosis cell could increase along with the enhancement of hydroxyl radical content at these time points.

With the exclusion of time factor, positive correlation was confirmed between cell apoptosis and hydroxyl radical content ($r = 0.2003$), and the hydroxyl radical content in the brain tissues produced some influences on cell apoptosis ($0 < t < 0.5$, Fig. 6).

4 Discussion

The present study demonstrated that ICH caused cell apoptosis at perihematomal regions in the brain, and significantly increased the levels of MDA and hydroxyl radicals. Correlation analysis showed that post-hemorrhagic apoptosis

was related to the production of free radical levels, indicating that the elevated free radicals following the ICH was involved in the apoptosis of neurons and glial cells. Our results also demonstrated that edaravone, a recently developed potent radical scavenger, had neural protective effects against ICH injuries by reducing the free radicals and apoptotic cells in SD rats.

Secondary brain injury following the cerebral hemorrhage exerted significant influence on the prognosis of cerebral hemorrhage, during which the oxidation and anti-oxidation balance of free radicals was broken, and a large number of free radicals were produced, bringing damage to the brain tissues. Free radicals cause damage to cells by reacting with various cellular components, such as lipids, proteins, and nucleic acids. Wang YF *et al*.[12] observed that H_2O_2 exposure impaired the viability of neurons, reduced mitochondria potential, and decreased long-term potentiation in the CA1 region of the hippocampus. There is evidence that free radical is involved in the development of seizures under pathological conditions and it has been implicated in the seizure-induced neuronal death[13-15]. Some current hypothesis of neuronal degeneration in Parkinson's disease have been proposed, also including formation of free radicals, as well as oxidative stress, mitochondrial dysfunction, excitotoxicity, trophic factor deficiency, inflammatory processes, genetic factors, toxic action of nitric oxide, apoptosis, and so on[16]. Human and animal studies have provided evidence that apoptosis is a prominent form of cell death associated with ICH in the perihematomal region.

In the present study, we evaluated the neuronal apoptosis, as well as changes of perihematomal free radical level following the ICH. We found that ICH increased the number of apoptotic cells, and elevated the levels of MDA and hydroxyl radicals. Kuzuya *et al*. demonstrated that the generation of reactive oxygen species (ROS) correlated positively with the percentage of dead cells in canine cardiac myocytes cultured under hypoxia–reoxygenation conditions. The correlation analysis of our study showed that post-hemorrhagic apoptosis was positively related to the production of free radicals. The result is in accordance with previous researches. We believe that the oxidative stress produced by free radical contributes to ICH-induced early brain injury through activation of apoptosis signaling. Oxidative stress from ROS has been shown to trigger cytochrome c release, which is often followed by DNA damage and cell death[17]. When cytochrome c is released from the mitochondria into the cytosol as a result of increased mitochondrial permeability, it activates the initiator caspase-9, which then cleaves and activates caspase-3, finally leading to apoptotic cell death[18].

Edaravone is a free radical scavenger, particularly for hydroxyl radicals. It has been shown to have neuroprotective effect against cerebral ischemia, and it has been approved for treatment of cerebral infarction in Japan since 2001. There are some other evidences that free radical scavengers can protect against ICH-induced injury. Tejima E *et al*.[19] found that hemoglobin-induced matrix metalloproteinase-9 (MMP-9) could be reduced by the free radical scavengers U83836E and MMP-9, and might be an important factor in the pathogenesis of hemorrhagic brain edema. Some evidences showed that upstream and downstream caspases were upregulated after hemoglobin-induced neurotoxicity *in vitro*, but only an antioxidant approach with a potent free radical scavenger significantly improved neuronal survival[20]. Prophylactic administration of hydroxyl radical scavenger AVS [(±)-N,N'-propylenedinicotinamide, also called nicaraven] could improve cerebral blood flow and cerebral glucose utilization in the brain of rat subjected to the experimental subarachnoid hemorrhage (SAH), and can be a good pharmacological treatment for prevention of delayed ischemic neurological deficits following the SAH[21]. AVS also significantly ameliorated performances on Beam Balance and decreased blood-brain barrier permeability changes when administered after experimental SAH in rats[22]. The present study showed that edaravone decreased the hydroxyl radical and MDA levels in a dose-dependent manner. Edaravone at 3 mg/kg almost completely eliminated the ICH-induced increase of hydroxyl radicals at several time points, thus exerting protective effects against ICH-induced cell apoptosis in rat model.

In summary, the present study demonstrated that the post-hemorrhagic apoptosis was related to the production of free radicals following the ICH. Edaravone was a potent neural protective agent against ICH injury in rat model, and the protection could be achieved by eliminating the production of perihematomal free radicals induced by ICH.

References:

[1] Qureshi AI, Suri MF, Ostrow PT, Kim SH, Ali Z, Shatla AA, *et al*. Apoptosis as a form of cell death in intracerebral hemorrhage.

Neurosurgery 2003, 52: 1041-1048.

[2] Xue M, Del Bigio MR. Intracerebral injection of autologous whole blood in rats: time course of inflammation and cell death. Neurosci Lett 2000, 283: 230-232.

[3] Viña J, Lloret A, Vallés SL, Borrás C, Badía MC, Pallardó FV, *et al*. Mitochondrial oxidant signalling in Alzheimer's disease. J Alzheimers Dis 2007, 11: 175-181.

[4] Saeed SA, Shad KF, Saleem T, Javed F, Khan MU. Some new prospects in the understanding of the molecular basis of the pathogenesis of stroke. Exp Brain Res 2007, 182: 1-10.

[5] Han J, Goldstein LA, Gastman BR, Rabinovitz A, Wang GQ, Fang B, *et al*. Differential involvement of Bax and Bak in TRAIL-mediated apoptosis of leukemic T cells. Leukemia 2004, 18: 1671-1680.

[6] Nutt LK, Gogvadze V, Uthaisang W, Mirnikjoo B, McConkey DJ, Orrenius S. Indirect effects of Bax and Bak initiate the mitochondrial alterations that lead to cytochrome c release during arsenic trioxide-induced apoptosis. Cancer Biol Ther 2005, 4: 459-467.

[7] Cox AG, Pullar JM, Hughes G, Ledgerwood EC, Hampton MB. Oxidation of mitochondrial peroxiredoxin 3 during the initiation of receptor-mediated apoptosis. Free Radic Biol Med 2008, 44: 1001-1009.

[8] Kandasamy K, Srinivasula SM, Alnemri ES, Thompson CB, Korsmeyer SJ, Bryant JL, *et al*. Involvement of proapoptotic molecules Bax and Bak in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced mitochondrial disruption and apoptosis: differential regulation of cytochrome c and Smac/DIABLO release. Cancer Res 2003, 63: 1712-1721.

[9] Deinsberger W, Vogel J, Kuschinsky W, Auer LM, Böker DK. Experimental intracerebral hemorrhage: description of a double injection model in rats. Neurol Res 1996, 18: 475-477.

[10] Zhou ZH, Qu F, He X, Wang YS. An improved rat autoblood cerebral hemorrhage model: twice blood injection/needle withdrawal method. Chin J Clin Neurosci 2004, 12: 406-408. (Chinese, English abstract)

[11] Bao XM, Shu SZ. Rat Brain Stereotaxic Atlas. Beijing: People's Health Publishing Company, 1991, 39-40.

[12] Wang YF, Li CC, Cai JX. Aniracetam attenuates H_2O_2-induced deficiency of neuron viability, mitochondria potential and hippocampal long-term potentiation of mice *in vitro*. Neurosci Bull 2006, 22: 274-280.

[13] Folbergrová J, He QP, Li PA, Smith ML, Siesjö BK. The effect of α-phenyl-N-tert-butyl nitrone on bioenergetic state in substantia nigra following flurothyl-induced status epilepticus in rats. Neurosci Lett 1999, 266: 121-124.

[14] Gupta RC, Milatovic D, Dettbarn WD. Depletion of energy metabolites following acetylcholinesterase inhibitor-induced status epilepticus: protection by antioxidants. Neurotoxicology 2001, 22: 271-282.

[15] Kovács R, Schuchmann S, Gabriel S, Kann O, Kardos J, Heinemann U. Free radical-mediated cell damage after experimental status epilepticus in hippocampal slice cultures. J Neurophysiol, 2002, 88: 2909-2918.

[16] Yuan H, Zheng JC, Liu P, Zhang SF, Xu JY, Bai LM. Pathogenesis of Parkinson's disease: oxidative stress, environmental impact factors and inflammatory processes. Neurosci Bull 2007, 23: 125-130.

[17] Matz PG, Fujimura M, Lewen A, Morita-Fujimura Y, Chan PH. Increased cytochrome c-mediated DNA fragmentation and cell death in manganese-superoxide ismutase-deficient mice after exposure to subarachnoid hemolysate. Stroke 2001, 32: 506-515.

[18] Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. Cell Death Differ 2006, 13: 1423-1433.

[19] Tejima E, Zhao BQ, Tsuji K, Rosell A, van Leyen K, Gonzalez RG, *et al*. Astrocytic induction of matrix metalloproteinase-9 and edema in brain hemorrhage. J Cereb Blood Flow Metab 2007, 27: 460-468.

[20] Wang X, Mori T, Sumii T, Lo EH. Hemoglobin-induced cytotoxicity in rat cerebral cortical neurons: caspase activation and oxidative stress. Stroke 2002, 33: 1882-1888.

[21] Yamamoto S, Teng W, Nishizawa S, Kakiuchi T, Tsukada H. Improvement in cerebral blood flow and metabolism following subarachnoid hemorrhage in response to prophylactic administration of the hydroxyl radical scavenger, AVS, (+/-)-N,N'-propylenedinicotinamide: a positron emission tomography study in rats. J Neurosurg 2000, 92: 1009-1015.

[22] Imperatore C, Germanò A, d'Avella D, Tomasello F, Costa G. Effects of the radical scavenger AVS on behavioral and BBB changes after experimental subarachnoid hemorrhage. Life Sci 2000, 66: 779-790.

大鼠脑出血模型中神经细胞凋亡与自由基水平的相关性

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摘要：目的 研究脑出血后血肿周围组织不同自由基水平对于细胞凋亡的影响。**方法** 成年SD大鼠随机分为4组：假手术组、模型组、1 mg/kg依达拉奉组、3 mg/kg依达拉奉组，各组又根据造模后处死动物的不同时间(6 h, 12 h, 24 h, 48 h, 72 h, 7 d, 14 d)分为七个亚组，假手术组中每个亚组1只大鼠，其余三组中每个亚组6只大鼠。左尾壳核立体定向注入自体血80 μL，制作大鼠脑出血动物模型。分光光度计法检测血肿周围丙二醛(malonaldehyde, MDA)及羟自由基含量，末端转移酶标记技术(terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling, TUNEL)检测血肿组织周围细胞凋亡数，并分析血肿周围组织自由基水平和凋亡相关性。**结果** (1)模型组羟自由基及MDA含量较假手术组明显增加，四组间进行统计学分析，具有显著性差异。(2)模型组和两种剂量的依达拉奉组均于6 h即可观察到TUNEL阳性细胞，24 h明显增加，72 h时达到高峰，7 d时明显减少，14 d时于血肿周边仍可见少量阳性细胞。(3)凋亡细胞数与脑组织产生自由基能力($r = 0.2003$)及MDA含量($r = 0.6563$)具有相关性。**结论** 脑出血后细胞凋亡数和自由基水平变化趋势相同，二者具有相关性，提示自由基可能参与诱导出血后神经元及胶质细胞凋亡。

关键词：脑出血；自由基；凋亡；末端转移酶标记技术；依达拉奉