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Influence of edaravone on growth arrest and DNA damage-inducible protein 34 expression following focal cerebral ischemia-reperfusion in rats

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Comments

This work explores the effect of the new agent, edaravone on GADD34 expression following focal cerebral ischemia-reperfusion in rats. The animal model is new and shows new finding that can be useful for further referencing in antioxidant research.
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ABSTRACT

Objective: To investigate the influence of edaravone on the expression of growth arrest and DNA damage-inducible protein 34 (GADD34).
Methods: A total of 108 healthy male Sprague-Dawley rats were randomly divided into sham operation group, model group and edaravone group (36 cases for each group). Transient focal cerebral ischemia was induced by middle cerebral artery occlusion for 2 h followed by reperfusion in Sprague-Dawley rats. Then, GADD34 expression was measured with immunohistochemistry at different time-points after reperfusion in the peri-infarct regions of all rats.
Results: The GADD34 expression was detected in the peri-infarct regions of rats 1 h after reperfusion, which reached its peak 24 h after reperfusion. And edaravone could significantly down-regulate the GADD34 expression.
Conclusions: Edaravon could down-regulate GADD34 expression, which suggests that edaravone may exert an important function in inhibiting endoplasmic reticulum stress reaction by scavenging free radicals in the upper stream.

KEYWORDS
Edaravone, Cerebral ischemia-reperfusion, Growth arrest and DNA damage-inducible protein 34

1. Introduction

Growth arrest and DNA damage-inducible protein 34 (GADD34) is a kind of cell cycle protein that can be up-regulated under conditions of DNA damage, cell cycle arrest and endoplasmic reticulum (ER) dysfunction, *etc*[1]. Some animal models with cerebral ischemia indicated that GADD34 expression was up-regulated at certain time windows after cerebral ischemia, but there was no consistent conclusion on the dynamic changes of GADD34 expression due to the different animal breeds and models used in the researches[2,3]. Edaravone can scavenge free radicals and is verified to have neuro-protection function in animal experiments. However, its influence on GADD34 expression has been rarely reported in recent years. In this study, edaravone was used as an interventional agent to detect the GADD34 expression changes in peri-infarct regions on ischemic parietal cortex, hoping to provide experimental basis for the prevention and treatment of cerebral infarction.

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2. Materials and methods

2.1. Materials

Edaravone was supplied by Jiangsu Simcere Company, GADD34 antibody was purchased from American Santa Cruz Company, and streptavidin-biotin complex (SABC) immunohistochemical kits and diaminobenzidine stain were brought from Wuhan Boster Biological Technology, Ltd.

2.2. Animal models and grouping

A total of 108 healthy male Sprague-Dawley (SD) rats (230-280 g) aged 10-12 weeks were fed by full-price nutritional fodders at 18-25 °C room temperature, 50%-60% relative humidity and 12 h diurnal cycle of illumination. All rats could eat and drink freely. Then they were randomly divided into sham operation group, model group and edaravone group by random number table (36 cases for each group). Suture-occluded method reported by Paschen *et al.* was adopted to prepare left focal middle cerebral artery (MCA) occlusion models[4]. The occlusion was maintained for 120 min and then unplugged to form reperfusion. After model rats revived, neurological deficit score was conducted based on the level V of Paschen's standard scoring method[4]. Rats with 1-3 scores in the initial neurological deficit score were included, whereas the dead rats, or whose with subarachnoid hemorrhage and without contralateral hemiplegia signs were considered as failed ones. Successful rat models in the same period were selected to supplement the object numbers. And then, the two groups were divided into six subgroups according to the reperfusion times such as reperfusion 1, 3, 6, 12, 24 and 72 h groups (12 cases for each group). At each perfusion corresponding time, the rats were sacrificed.

2.3. Methods

2.3.1. Edaravone group

A volume of 10 mg edaravone injection (Trade name: Edaravone; Batch number: H20031342; Specification: 10 mg/5 mL) was diluted by 5 mL normal saline to prepare 1 mg/mL solution. About 3 mg/kg edaravone injection was injected into the caudal veins immediately after reperfusion.

2.3.2. Model group

A total of 3 mL/kg normal saline was injected into the caudal veins immediately after reperfusion.

2.3.3. Sham operation group

This group was divided into six subgroups according to the above reperfusion corresponding times (six cases for each). In this group, suture occlusions were inserted 8-10 mm in depth so as to maintain the smooth of anterior and posterior MCA. The rest operations were similar to those in other groups.

2.4. Preparation of paraffin sections

At reperfusion corresponding time-point, 10% chloral hydrate was used to narcotize the rats, and then 250 mL 0.9% normal saline was infused from apex cordis. When the solution discharged from the right atrial appendage was clear, 300 mL 4% paraformaldehyde was infused. All rat brains were decollated and the part from antinion to occipital lobe was divided into five equal sections marked as A, B, C, D and E. C section was embedded by paraffin to prepare the consistent slices (4 μm in thickness).

2.5. Immunohistochemical staining

SABC was used. The potency of GADD34 polyclonal antibodies was verified to be 1:100 by the pre-experiment, and the detailed operations were as follows. The slices were routinely deparaffinaged into water, soaked in 3% H_2O_2 at room temperature for 5-10 min, and washed by double distilled water by 2 min×3 times. The antigen was repaired by microwave heating, washed by 0.1 mol/L phosphate buffer solution (PBS) by 2 min×2 times after natural refrigeration; added with normal goat serum blocking buffer, incubated at room temperature for 20 min and the serum was discarded; then added with the primary antibodies of rabbit anti-mice GADD34 polyclonal antibodies (1:100), incubated at 4 °C overnight, washed by 0.1 mol/L PBS by 2 min×3 times; added with biotinylated goat anti-rabbit IgG antibodies (carried with kits with potency 1:100), incubated at 37 °C for 20 min and washed by 0.1 mol/L PBS by 2 min×3 times; added with SABC, incubated at 37 °C for 30 min and washed by 0.1 mol/L PBS by 5 min×4 times; added with diaminobenzidine to develop color; and then dehydrated, transparentized and sealed. The above steps were performed in sequence. Five fields were randomly collected in the peri-infarct regions of ischemic cortex of each rat under microscope (×400), and the mean value was regarded as the estimated value. High-definition pathological image analysis system (HPIAS-1000) was applied to analyze the images of slices and determine the grey value of positive protein expression.

2.6. Statistical data analysis

SPSS 11.5 software was applied for all data analysis. All data were expressed as mean±SD. One-way ANOVA was used for the comparisons of multi-sample means while student's t-test was for comparisons among groups with α=0.05. P<0.05 was considered to be statistically significant.

3. Results

In sham operation group, there were positive GADD34 immunoreactive cell expressions in the left side of the parietal cortex, but ANOVA test showed no significant differences in GADD34 immunoreactive cell expressions at each time point (P>0.05). In model group, 1 h after reperfusion, GADD34 expression increased in peri-infarct regions on ischemic parietal cortex, whose grey values decreased along with the prolonged reperfusion times, indicating that the counts of positive GADD34 cells increased gradually and reached its peak at 24 h, and even 72 h after reperfusion, the positive cells were still visible. However, the GADD34 expression, marked by dynamic changes, were evidently higher in edaravone group 6, 12 and 24 h after reperfusion than in model group (P<0.05), but the differences at other time-points were not statistically significant (P>0.05), as shown in Table 1 and Figures 1 and 2.