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# Neuroprotective effects of edaravone on cognitive deficit, oxidative stress and tau hyperphosphorylation induced by intracerebroventricular streptozotocin in rats☆

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ABSTRACT

Oxidative stress is implicated as an important factor in the development of Alzheimer's disease (AD). In the present study, we have investigated the effects of edaravone (9 mg/kg, 3-methyl-1-phenyl-2-pyrazolin-5-one), a free radical scavenger, in a streptozotocin (STZ-3 mg/kg) induced rat model of sporadic AD (sAD). Treatment with edaravone significantly improved STZ-induced cognitive damage as evaluated in Morris water maze and step-down tests and markedly restored changes in malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) adducts, hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide ($H_2O_2$), total superoxide dismutase (T-SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and protein carbonyl (PC) levels. In addition, histomorphological observations confirmed the protective effect of edaravone on neuronal degeneration. Moreover, hyperphosphorylation of tau resulting from intracerebroventricular streptozotocin (ICV-STZ) injection was decreased by the administration of edaravone. These results provide experimental evidence demonstrating preventive effects of edaravone on cognitive dysfunction, oxidative stress and hyperphosphorylation of tau in ICV-STZ rats. Since edaravone has been used for treatment of patients with stroke, it represents a safe and established therapeutic intervention that has the potential for a novel application in the treatment of age-related neurodegenerative disorders associated with cognitive decline, such as AD.



## 1. Introduction

Alzheimer's disease (AD) is an age-related, progressive neurodegenerative disorder. This condition is characterized by deterioration of cognitive function including behavioral impairments and memory deficits and is associated with pathological aggregations of the amyloid β (Aβ) peptide and neurofibrillary tangles of aggregated hyperphosphorylated tau protein in the brain. Although the exact etiology of AD is unknown, there exists considerable evidence implying that abnormal generation of free radicals may underlie the development and accompanying neuronal deterioration of AD (Markesbery, 1999; Su et al., 2008). Excesses in reactive oxygen species (ROS) and free radical generation, as induced by oxidative metabolism imbalance, lead to a range of changes in cellular structure and function (Smith et al., 2010). These changes including protein and DNA injury, energy deficiency, inflammation, mitochondrial dysfunction, tau hyperphosphorylation and Aβ overexpression, all of which play important roles in the acceleration of aging and age-related neurodegenerative disorders (Pratico and Delanty, 2000; Melov et al., 2007; Zawia et al., 2009; Coppieters and Dragunow, 2011). Therefore, free radical scavengers and antioxidants, which have the capacity of neutralizing free radicals, have been proposed as therapeutic agents for delaying, inhibiting or reversing the pathological process of neurodegenerative disorders (Uttara et al., 2009).

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent free radical scavenger and it has been demonstrated that edaravone exerts beneficial effects against oxidative stress in acute ischemic stroke patients (Yoshida et al., 2006). In addition to



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its use in cerebral infarction, edaravone has been found to have neuroprotective effects upon Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD), and in animal models of these conditions as demonstrated in vivo and in vitro (Kikuchi et al., 2011). Taken together, these findings suggest that edaravone may be effective in the treatment of neurodegenerative disorders associated with oxidative stress, such as AD.

Streptozotocin (STZ), a glucosamine compound which, when metabolized, generates a cytotoxic product that induces preferential damage in pancreatic β cells. Interestingly, intracerebroventricular (ICV) administration of a sub-diabetogenic dose of STZ in rats has been shown to induce cognitive dysfunction, impaired intracerebral glucose and energy metabolism, increased oxidative stress, hyperphosphorylation of tau protein and other neuropathological and biochemical changes which are similar to those observed in sporadic Alzheimer's disease (Sharma and Gupta, 2001; Grunblatt et al., 2007; Salkovic-Petrisic and Hoyer, 2007). Therefore, ICV-STZ was applied to establish a valid experimental model of the early pathophysiological alterations in sAD (Salkovic-Petrisic and Hoyer, 2007; Salkovic-Petrisic et al., 2011).

The present study was designed to investigate the effects of edaravone, a potent free radical scavenger, in the intracerebroventricular streptozotocin (ICV-STZ) rat model. Specifically, we focused upon the capacity for edaravone to modulate learning and memory impairments, oxidative stress and tau protein phosphorylation in this rat model of sAD.

## 2. Materials and methods

### 2.1. Animals

Adult male Sprague-Dawley rats weighing 320–350 g (5–7 months) were obtained from the Beijing Vital River Laboratory Animal Technology Co. Ltd in the People's Republic of China. The animals were placed in a quiet room with a 12 h light/dark cycle, an ambient temperature of 23 ± 2 °C and a relative humidity of 56 ± 4%. All surgeries were performed under anesthesia using a 10% chloral hydrate solution. All efforts were made to minimize animal suffering. All animal procedures performed in this work followed guidelines in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals, and approved by the animal care and welfare committee of Harbin Medical University.

### 2.2. Drugs and chemicals

Streptozotocin (STZ) from Sigma Chemical Co., St. Louis, USA was dissolved in artificial cerebrospinal fluid (a-CSF – 2.9 mM KCl, 147 mM NaCl, 1.7 mM $CaCl_2$, 1.6 mM $MgCl_2$, 2.2 mM D-glucose) in a 25 mg/ml solution. This solution was prepared immediately prior to injection. Edaravone (MCI-186, 3-methyl-1-phenyl-2-pyrazolin-5-one) purchased from Calbiochem, Germany was dissolved in 1 N NaOH, diluted with distilled water, and pH-adjusted to 7.4 with 1 N HCl according to the manufacturer's instructions. All bioluminescent assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, Jiangsu, China. The nissl staining kit was purchased from the Beyotime Institute of Biotechnology, Shanghai, China. Total tau, ser396-phosphorylated tau, thr181-phosphorylated tau and β-actin-specific antibodies were purchased from Santa Cruz Biotechnology Inc., USA and the polyclonal 4-HNE antibody was purchased from Abcam Biotechnology Inc., USA. The secondary antibodies were purchased from the Zhongshan Company of Beijing, China. All other chemicals were of analytical reagent grade.

### 2.3. Experimental design

After a week of adaption to colony room conditions the rats were randomly divided into six groups: Group 1-Sham (S): sham operated rats were infused with a-CSF in each ventricle on days 1 and 3, and the animals were administered with distilled water (0.5 ml b.i.d. intraperitoneally) as a vehicle of edaravone for two weeks following the first day after surgery in each group. Group 2-Edaravone + S: sham operated rats were administered with 9 mg/kg b.i.d. i.p. of edaravone. Group 3-Lesion (L): rats were performed with bilaterally ICV infusion of STZ on days 1 and 3, and the rats were treated with the same vehicle as S group. Group 4-Edaravone 1 + L: ICV-STZ rats were immediately administered with edaravone 1 mg/kg b.i.d. i.p. after first STZ infusion for two weeks. Similar as group 4, animals in groups 5 (Edaravone 3 + L) and 6 (Edaravone 9 + L) were infused with ICV-STZ on days 1 and 3, and treated with edaravone at doses of 3 mg/kg and 9 mg/kg respectively for two weeks following the first day after surgery.

All behavioral tests were carried out to evaluate the capacity of learning and memory for each group between day 15 and day 24. On day 25, the rats were sacrificed for assay of T-SOD, MDA, GSH, GPx as a dose dependent study. Based on the above results, the dose of 9 mg/kg was choosed for further study, including the content of content of PC, $H_2O_2$ and hydroxy radical, the content of 4-HNE adducts and phosphorylation levels of tau protein. Fig. 1 shows the treatment schedule design and time intervals for estimations of the various parameters.

### 2.4. Surgical procedure

Animals were anesthetized using a 10% chloral hydrate solution of 3 ml/kg administered intraperitoneally. The head was positioned in a stereotaxic apparatus and skull exposed. Two holes were drilled through the skull for bilateral placement of a microinjector into the lateral cerebral ventricles using the following coordinates: 0.8 mm posterior to bregma; 1.6 mm lateral to the sagittal suture; and 4.0 mm ventral from the surface of the brain (Ishrat et al., 2009b). The STZ solution was infused slowly (1 μl/min) into each ventricle on days 1 and 3. The sham group received the same surgery but infused with an equal volume of a-CSF only.

### 2.5. Memory and learning test

#### 2.5.1. Morris water maze test

Spatial learning and memory were tested with use of a Morris water maze (MWM) (Morris, 1984). The water maze was a circular black tank (150 cm diameter, 60 cm height) with an automatic

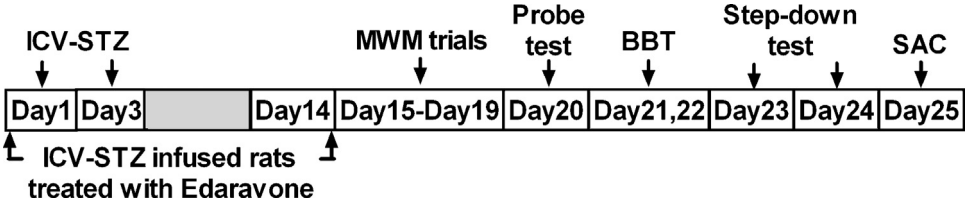


**Fig. 1.** The experimental design treatment schedule and intervals for estimation of various parameters. ICV-STZ = intracerebroventricular streptozotocin, MWM = Morris water maze, BBT = Beam balance test, and SAC = sacrificed.