The Effect of Genistein on Intracerebroventricular Streptozotocin-Induced Cognitive Deficits in Male Rat

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A B S T R A C T

Introduction: Intracerebroventricular (ICV) injection of streptozotocin (STZ) causes cognitive impairment in rats. The beneficial effect of genistein (GEN) was investigated on ICV STZ-induced learning, memory, and cognitive impairment in male rats.

Methods: For this purpose, rats were injected with ICV STZ bilaterally, on days 1 and 3 (3 mg/kg). The STZ-injected rats received GEN (1 mg/kg/day, p.o.) starting one day pre-surgery for two weeks. The learning and memory performance was assessed using passive avoidance paradigm, and for spatial cognition evaluation, radial eight-arm maze (RAM) task was used.

Results: It was found out that GEN-treated STZ-injected rats show higher correct choices and lower errors in RAM than vehicle-treated STZ-injected rats. In addition, GEN administration significantly attenuated learning and memory impairment in treated STZ-injected group in passive avoidance test.

Discussion: These results demonstrate the effectiveness of GEN in preventing the cognitive deficits caused by ICV STZ in rats and its potential in the treatment of neurodegenerative diseases such as Alzheimer's disease (AD).

1. Introduction

ICV injection of STZ in rats is followed by long-term and progressive deficits in learning, memory, and cognitive performance in rats that is similar to sporadic kind of AD (SAD), as indicated by behavioral tests including passive avoidance paradigm (1). SAD has been known as a chronic debilitating neurodegenerative disorder characterized by progressive cognitive impairment, memory loss, and behavioral disturbances (2) and is considered as the most common cause of dementia in elderly patients (3). Interventions that could delay SAD onset would have a major public health impact (2). Free radical generation has been associated with cognitive impairment in ICV STZ model of SAD in rats (1,4) and ICV STZ has also been known to impair cholinergic neurotransmission (5).

On the other hand, some naturally occurring polyphenols including genistein have the potential to exhibit neuroprotective effect and to prevent oxidative damage in various pathophysiological conditions due to their capability to penetrate into the brain (6-7). Therefore, this study was undertaken to investigate the possible beneficial effect of GEN administration in ICV STZ-induced model of SAD in the rat using passive avoidance and radial eight-arm maze tasks.

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2. Methods

2.1. Animals

Adult male Wistar rats (Pasteur's Institute, Tehran), weighing 310-350 g at the start of the experiment were housed three to four per cage in a temperature-controlled colony room under light/dark cycle. Animals were given free access to water and kept at 80–85% of their free feeding body weight throughout the experiment. All behavioral experiments were carried out between 11 a.m. and 4 p.m. This study was carried out in accordance with the policies set forth in the Guide for the Care and Use of Laboratory Animals (NIH) and those of the Research Council of Iran University of Medical Sciences (Tehran, Iran).

2.2. Experimental Procedure

Rats (n = 60) were randomly divided into the following groups: 1. Sham-operated group (SH), 2. Sham-operated group (SH + CSF) that received bilateral ICV injection of artificial CSF (ACSF) (10 µl on each side) as the solvent of STZ (Upjohn Chemical, France) and 10% Cremophor (p.o.) (Sigma Chemical, UK) as the vehicle for GEN (Sigma, Germany), 3. GEN-treated sham-operated group (SH + GEN), 4. STZ-injected group (STZ) which received ICV injection of STZ in addition to Cremophor, and 5. GEN-treated STZ group (STZ + GEN), which also received GEN (1 mg/Kg/day; p.o.) one day before surgery for two weeks. For stereotaxic surgery, rats were anesthetized with a combination of ketamin (100 mg/Kg, i.p.) and xylazine (5 mg/Kg, i.p.), placed in a Stoelting stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution, incised on the midline and a burr hole was drilled through the skull 0.8 mm posterior to bregma, 1.4 mm lateral to saggital suture, and 3.4 mm beneath the surface of brain, according to the stereotaxic atlas (10). STZ and GEN-treated STZ groups were given a bilateral ICV injection of STZ (Sigma, St. Louis, USA) (3 mg/kg). STZ was freshly dissolved in cold artificial CSF and at a volume of 10 µl on each side. The injection was repeated on day 3. In the sham group, only artificial CSF (120 mM NaCl; 3 mM KCl; 1.15 mM CaCl2; 0.8 mM MgCl2; 27 mM NaHCO3; and 0.33 mM NaH2PO4 adjusted to pH 7.2) (Merck Chemical, Germany) was ICV injected. Post-operatively, special care was undertaken until spontaneous feeding was restored.

2.3. RAM Task

The effect of ICV STZ injection and administration of GEN was tested in different groups in the radial maze according to the paradigm as described before with some modifications introduced. The apparatus consisted of a 50-cm elevated (off the ground) eight-arm RAM. The maze was made of black-painted wood and located in a sound-attenuated and dimly lit room. The apparatus consisted of eight arms (60 cm long × 10 cm wide × 15 cm high) extending radially from an octagonal central starting platform (35 cm in diameter) and with a recessed food cup at the end of each arm. This cup contained a single food pellet (50 mg) as reinforcer. A plastic cylinder (30 cm in diameter and 20 cm high) was placed on the central platform and a rat was placed inside this cylinder 15 s before the test. Following this interval, the ring was removed and timing began. The central platform at the entrance to arms was also separated by removable guillotine doors in order to confine and block the ability of the rat to enter an arm. The RAM was surrounded by various extra maze cues. Their orientation relative to the maze was kept constant throughout the experiment. The maze was cleaned with diluted ethanol between trials.

Prior to acquisition (pre-surgery), the rats were maintained on a restricted feeding schedule designed to keep their body weight at about 85% of the free-feeding level, and the body weights were maintained at this level throughout the experiment. Rats learned to visit each arm, ate the food pellet, and not to re-enter an arm that had been visited during the same test. Each entry into each arm with all four paws was scored. Behavioral observation was discontinued after 10 min even if the animal did not finish the task. The number of correct choices and of errors was used to assess the performance of the animal in each session. An error was defined as a re-entry into an already visited arm. Rats
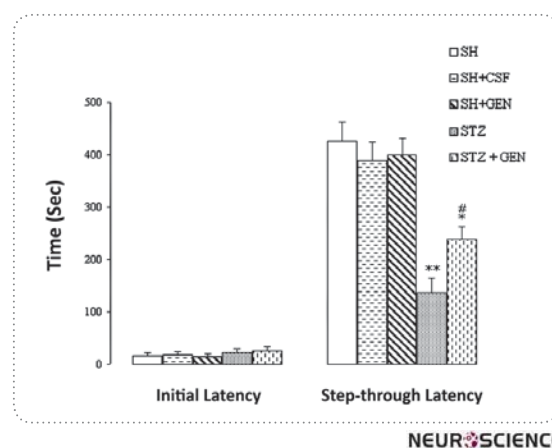

Figure 1. The effect of GEN administration (1 mg/Kg/day, p.o.) on spatial cognition deficit induced by ICV injection of STZ in rats. A total of 60 rats were used for this test. Values are means ± S.E.M. of the number of correct choices or the number of errors. * p<0.05, ** p<0.01, *** p<0.005 (in comparison with SH group); # p<0.05 (STZ + GEN vs. STZ) (Wilcoxon's rank sum test).

that had made seven or more correct choices and either one or no errors during the first eight choices in each of three consecutive sessions were used in the subsequent behavioral experiment. Training was performed at 24-h intervals and rats that had not reached the above criteria within two weeks were excluded. Retention trials were performed at 20th day post-surgery (1st ICV injection of STZ).

2.4. Single Trial Passive Avoidance Test

The apparatus (BPT Co., Tehran) consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (2 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded form the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL up to a maximum of 600 s). This test was conducted after 3 weeks post-surgery.

2.5. Statistical Analysis

All results were expressed as mean ± S.E.M. For the passive avoidance test, nonparametric Kruskal-Wallis test was used which, if significant, was followed by Mann-Whitney U-test for pair-wise comparisons. Data for the 8-arm radial maze task were evaluated by Wilcoxon's rank sum test. In all calculations, a difference at $p < 0.05$ was regarded as significant.

3. Results

Only those rats were investigated which their correctness of ICV injection site were histologically verified at the end of study. The body weight of the rats within the groups was weekly recorded. In this respect, there were no significant differences among the different experimental groups. Regarding serum glucose level, no significant changes were observed in this parameter in STZ and STZ + GEN groups. During the experimental study, the majority of the animals (nearly 83%) well tolerated

the treatments. There was no significant mortality in the STZ-treated group as compared to the sham-operated groups.
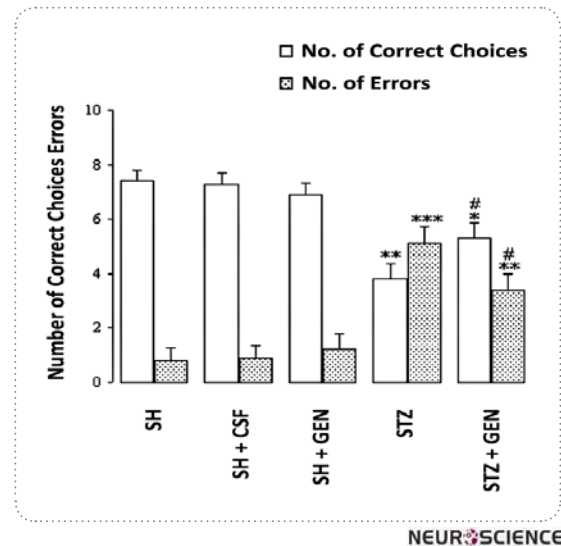

Figure 2. The effect of GEN treatment (1 mg/Kg/day, p.o.) on passive avoidance performance after ICV injection of STZ in rats as indicated by initial and step-through latencies. A total of 60 rats were used for this test. Values are expressed as means ± S.E.M. * $p < 0.01$, ** $p < 0.005$ (in comparison with SH group); # $p < 0.05$ (STZ + GEN vs. STZ) (non-parametric Kruskal-Wallis and Mann-Whitney U-tests

3.1. Effect of GEN on Spatial Cognition Deficit in RAM Task

As shown in Fig. 1, there was no significant difference among the different sham groups. On the other hand, vehicle-treated STZ-injected rats showed a significant deficit in spatial cognition in the 8-arm radial maze task after three weeks as determined by the number of correct choices ($p < 0.01$) and by the number of errors ($p < 0.005$) in comparison with relevant data of SH group. On the other hand, administration of GEN (5 mg/Kg/day) starting 1 day before 1st ICV STZ injection significantly attenuated this cognitive deficit. In this respect, there was a higher number of correct choices ($p < 0.05$) and lower number of errors ($p < 0.05$) in STZ + GEN group as compared to vehicle-treated STZ group in the radial maze task.

3.2. Effect of GEN on Memory Retention Deficit in Passive Avoidance Test

The mean initial latency was not different among the experimental groups. In this regard, the initial latency was 16.8, 19.2, 14.5, 22.1, and 25.3 s in SH, SH+CSF+PG,

SH+GEN, STZ, and STZ + GEN groups respectively. Meanwhile, there was no significant difference among SH, SH+CSF+GEN, and SH+GEN groups regarding STL. On the other hand, the STZ + GEN group exhibited significant reversal of STL (with a mean retention latency of 238.1 s) (p<0.05) as compared to vehicle-treated STZ group, indicating improved acquisition or retention of memory (Fig. 2).

4. Discussion

The results of the present study demonstrated that ICV STZ injection in rats induces a significant learning and memory disturbance in passive avoidance paradigm and a spatial cognitive deficit in RAM task and treatment of rats with GEN (1 mg/kg/day) for 2 weeks could significantly attenuate these abnormalities.

It is a well-established fact that ICV injection of STZ is characterized by a progressive deterioration of learning, memory, and cerebral glucose and energy metabolism and this may provide an appropriate and relevant experimental model of SAD (4,12). In the present study, STZ at a dose of 3 mg/kg was used. This dose has been shown not to cause any change in the peripheral blood glucose level, although this dose induces a significant cognitive impairment in all of the animals (4). The possibility of the effect of increased CSF pressure due to ICV injection was rejected in this study as no behavioral changes reflecting significant increase in intracranial pressure e.g. bulging of eyes were observed. Also, in the sham-operated rats, no apparent signs of raised intracranial pressure were observed. The results from the passive avoidance test showed that the STZ-injected rats reveal significantly reduced retention latencies (STLs), suggesting an impairment in learning and memory processes. In conformity with this, the results from RAM task for the first time showed that ICV STZ animals also exhibit a higher score of errors and lower correct choices, indicating an abnormality in spatial cognitive processes. On the basis of the obtained results, it is suggested that impairment in passive avoidance behavior may reflect poorer acquisition and/or retention of memory after ICV STZ injection. The results from the RAM task may also indicate a spatial cognition deficit in ICV STZ rats.

In this study, treatment of ICV STZ rats with GEN (1 mg/kg/day) starting 1 day before surgery for three weeks caused a significant improvement in learning, memory, and spatial cognitive skills. The beneficial effect of GEN in this study could be attributed to the following potential mechanisms: first, it has been verified that brain damage due to oxidative stress induces the impairment of learning and memory abilities and the development of disturbance in spatial cognitive functions as evaluated by water maze and RAM tasks (13) and naturally occurring polyphenols like GEN have the potential to exhibit neuroprotective effect in the 6-OHDA model of Parkinson's disease and to prevent oxidative damage in various pathophysiological conditions due to their capability to penetrate into the brain (6), secondly, AD is characterized by alterations at the level of various neurotransmitters and related markers and receptors. Out of these, the most severely affected by far is the cholinergic system (14). The cholinergic system is responsible for the storage and retrieval of items in memory and its degradation correlates well with the severity of cognitive and memory impairment. Hence it has been suggested that elevation of the acetylcholine (ACh) level might be helpful in attempts to improve the symptoms of cognitive deficits in AD (15). Loss of cholinergic innervation, as demonstrated by reduced choline acetyltransferase (ChAT) and elevated acetylcholinesterase (AChE) activity is well correlated with the degree of dementia and the severity of the neuropathological hallmarks of AD (16-17).

In conclusion, the present study clearly demonstrated that GEN treatment could significantly prevent the cognitive impairments following ICV STZ and this suggests the therapeutic potential of this compound in aging and age-related neurodegenerative disorders where cognitive impairment are involved.

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