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Chronic Intracerebroventricular Administration of Dimethyl Sulfoxide Attenuates Streptozotocin-iduced Memory Loss in Rats

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Abstract

Background: The memory impairment, obtained from intracerebroventricular (i.c.v.) infusion of streptozotocin in rats through activation of oxidative stress, is accepted as sporadic Alzheimer's disease model in most experimental studies. Dimethyl sulfoxide (DMSO) as a solvent is widely used in animal studies to have antioxidant effects as well. However, no report is available about DMSO effect on oxidative stress-induced cognition deficit i.e. Alzheimer's disease. The present work was designed to assess the effect of chronic treatment of DMSO on STZ-treated rats.

Materials and Methods: STZ (3 mg/ kg; i.c.v.; bilateral with 10 µl volume in either side; days 1 and 3) using a single-day version of Morris water maze. The DMSO (2.5, 5 and 10 %v/v in saline), started from the first day, was infused for 14 days.

Results: The chronic administration of DMSO 10% improved the distance to hidden platform ($P<0.01$) in training sessions and time spent in the target quadrant in probe tests ($P<0.01$). Neither STZ nor DMSO had any intervention on velocity and visuo-motor coordination in the visible version of MWM.

Conclusion: Totally, the results suggest that DMSO may be appropriate as adjuvant therapies for the prevention of memory impairment in the experimental models of Alzheimer's disease. Therefore, use of DMSO as a solvent in Alzheimer's disease animal studies should be considered having beneficial effects on cognitive function.

Keywords: Alzheimer's disease; Dimethyl sulfoxide; Morris water maze; Streptozotocin

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Introduction

Medicine and health care have had positive points in most aspects of human life and allowed people experience aging. Epidemiological consequence of such development in increase of life expectancy is the progressive occurrence in neurodegenerative disorders including Alzheimer's disease (AD). Continuing loss of memory, attention and behavioral disturbances are the major signs of AD (1). Oxidative stress refers to some imbalance between free radical production and disability in neutralization of these radicals in cells (2-3). So far, lots of experimental evidence have supported oxidative stress hypothesis in pathogenesis of AD (4-5). The oxidative stress caused by free radicals is the common path of effective factors which contribute to AD incidence (6-7). Hence, many researchers have proposed the therapeutic and preventive potential of antioxidants in AD (8-10).

Transgenic animals (11), intracerebroventricular (i.c.v.) infusion of beta-amyloid (9), and i.c.v. administration of scopolamine (12) are experimental models of AD which have revealed their own

particular advantages and disadvantages. In addition, the i.c.v. infusion of streptozotocin (STZ) causes progressive memory loss corresponding to sporadic Alzheimer's disease (SAD); the most common type of AD (13, 14). It has been indicated in behavioral tasks like passive avoidance learning (15), Morris Water Maze (MWM) (16) and radial arm maze (17). Considering the fact that experimental evidence has attributed STZ mechanism in induction of AD-related cognitive dysfunctions to the activation of oxidants production paths that damage cholinergic neurons, henceforth i.c.v. infusion of STZ as the most appropriate animal models have been suggested to evaluate the preventative potential of antioxidant agents in AD development (14, 18, 19). So far, various researchers have investigated and endorsed preventative and therapeutic effect of some antioxidants in this model (20- 23).

The natural product dimethyl sulfoxide (DMSO) [(CH3)2SO] is extracted from wood pulp during paper manufacturing process. It is an amphipatic molecule which can be simply dissolved in organic and inorganic media. So, such a physico-chemical property of DMSO has made it a widespread used solvent in biological studies. Moreover, it has some biological properties such as inhibition of calcium influx (24) and platelet aggregated blockade (25-26). Additionally, numerous beneficial clinical evidence about DMSO is reported including effective reducing of brain edema (27), inhibition of inflammation process (28), relief of pain (29), effectively treating the herpes zoster (30), preventing the tissue necrosis caused by extravasation of chemotherapy drugs (31), attenuation of pulmonary infiltration and improving arterial blood gas in pulmonary amyloidosis (32), lengthening survival in patients with colon carcinoma (33), and even improving psychiatric disorders (34). In spite of the wide-ranging therapeutic potentials of DMSO, in 1978, FDA agreed on the intravesical DMSO administration only for the treatment of interstitial cystitis that is the main treatment to date (35). In the recent years, it has been found that DMSO via scavenging hydroxyl free radical is a notable antioxidant (36-37). In accordance with oxidative stress in pathogenesis of neurodegenerative disease, particularly AD, as far as we know, no study has investigated the involvement of DMSO on memory performance. So, in this study, we sought to is evaluate preventive effect of chronic dministration of DMSO on STZ-induced memory deficit in rats.

Materials and Methods

Animals

40 adult male Wistar rats (250-300g), which were free to access to food and water, were selected. Every four rats were located in a cage and kept under standard housing conditions with room temperature of 25±2 °C and a 12-hour light/dark cycle. These animal experiments were performed based on recommendations from the Declaration of Helsinki; and the internationally established principles for the care and use of laboratory animals.

Surgery and bilateral implantation of guide cannulae

The rats were anesthetized by i.p. infusion of Ketamine Ketamine (100mg/kg) and Xylazine (2.5 mg/kg) mixture, and their heads were placed in stereotax apparatus. Located in a site (AP= 0.8 mm; ML= ±1.4 mm; DV= 3.6 mm) based on the rat brain atlas (38), each guide cannula was positioned with a 0.5 mm distance from the lateral ventricles level. Then, the two small screws were located in the skull and were fixed to the canuulae by dental cement.

Microinjection procedure

Infusion solutions in this study consist of normal saline, STZ (Sigma-Aldrich, USA) dissolved in normal saline, and DMSO (Sigma-Aldrich, USA). All solutions were injected into the lateral ventricles. According to the experimental protocol, the infusion was done bilaterally (STZ/saline) and/or unilaterally (DMSO/saline) through injection needles which were connected to Hamilton microsyring by PE pipe. The needle was long enough to be located in the middle part of lateral ventricles after it moved beyond the tip of guide cannula. The infusion process lasted 2 to 3 minutes. One minute after infusion, the needle was removed very slowly from the site.

Assessment of memory and visuo-motor coordination by MWM

MWM is a black circular pool with 140 cm diameter and 55 cm height which is filled with water (20 ±1 °C) to a depth of 25 cm. This pool is divided into four virtual quadrants called north-east (NE), south-east (SE), south-west (SW), and north-west (NW). The animal movement and position were tracked by a camera that was located above the pool. The animals were marked by an LED display inside a ping-pong ball that was held on the rats back by a rubber jacket. The camera signal was digitalized with 1000 Hz sampling rate and fed to a computerized tracking system that monitored and stored the position and movement of the rat. In order to measure spatial memory, we used a single day invisible version of MWM. In this method a circular Plexiglas platform with 11 cm diameter is located in the center of the SW quadrant (target quadrant) 2 cm under the water surface. The single training session incorporated in 8 trials with four different starting places that were regularly distributed the outer limits of the pool (39, 40). During each trial, each animal was given 60 seconds to escape on the hidden

platform, and it was permitted to stay on the platform for 20 seconds. Then, the animal rested for 30 seconds outside the maze. The next trial was repeated the same way for eight times. Immediately after the last trial, the rats were returned to their cages. The next day, they were brought to the laboratory for retrieval testing (probe trial). In the probe trial, the animal was given 60 seconds to swim in the maze without any platform. The time spent in the target quadrant, the swimming velocity, and the numbers of entrance to the target quadrant during 60 seconds were recorded for the statistical analysis. Furthermore, the mean of the distance to hidden platform and swimming velocity of the eight trails was calculated for the statistical analysis. In the invisible version of MWM, the animal was able to realize the exact location of the hidden circular platform by making a multiple connection between extra maze visual cues such as the bookshelf, the computer, the camera, the clock or even the door and the window.

After the probe trial, it was the visible version of MWM that was performed for all of the rats in order to evaluate the probable interference of treatment on the visuo-motor coordination and motivation. The platform, in this test, was located 1 cm above the water surface. This platform was also wrapped by aluminum foil easily seen by the rats. This task consists of four trials in each of which the animal was randomly released next to the wall of the pool from one of the four geographical directions. The escape latency, the time it takes to find the platform, was measured for the statistical analysis.

Experimental protocol

Therapeutic period was 14 days in all rats. They were treated on the first and the third days by STZ (3 mg/ kg; i.c.v.; bilateral with 10 µl volume in either side) or saline as a placebo (i.c.v.; bilateral with 10 µl volume in either side). All of the STZ or saline recipient animals in the first and the third days were infused for 14 days with DMSO (2.5, 5 and 10 %v/v in saline) or saline with 2 µl volume in the right side. So that, six groups were examined in this investigation as follows;

Sal-Sal (control) = Rats infused with saline on days 1 and 3 and treated with saline for 14 days. (n=7)

STZ-Sal = Rats infused with STZ on days 1 and 3 and treated with saline for 14 days. (n=7)

STZ-DMSO2.5%, STZ-DMSO5%, STZ-DMSO10%, Sal-DMSO10% = Rats infused with STZ on days 1 and 3 and treated with DMSO 2.5, 5 or 10% for 14 days, respectively. (n=6, 7, 8)

Sal- DMSO10% = Rats infused with saline on days 1 and 3 and treated with DMSO10% for 14 days. (n=5)

On the day 15, without any treatment, the rats were trained to find the hidden platform in the invisible version of MWM. After 24 hours, the probe trial was performed to assess spatial memory. Finally, all of the animals were tested in the visible version of MWM. The whole experiment was performed in the morning between 9 and 12. *

Statistical analysis

Collected data were evaluated by Kolmogorov – Smirnov and Bartlett's tests in order to examine the normal distribution and the differences of standard deviations among the experimental groups, respectively. Determining statistically significant differences in distance to hidden platform, visible platform, and the time spent in the target quadrant a one-way ANOVA was employed The significant, result was followed by Tukey-Kramer post-hoc test for paired comparison. Data for swimming velocity in training sessions and probe tests were analyzed by Kruskal-Wallis test. In addition, the distance to platform and time spent in the target quadrant in control and Sal-DMSO10% was analyzed by using unpaired student *t* test. The results are presented as Mean±S.E.M. Also, $P<0.05$ was considered as a statistical significant level.

Results

There was no significant difference in the animals' weight during the 14 day period among the studied groups. Moreover, no animal died during the experiment.

The effect of STZ with or without DMSO on acquisition of spatial information has been shown in Figure 1. One-way ANOVA analysis showed that there was a significant difference in the distance to the hidden platform of Sal-Sal group and the other groups in the training sessions [$F_{3, 34}=6.15$; $P=0.001$].

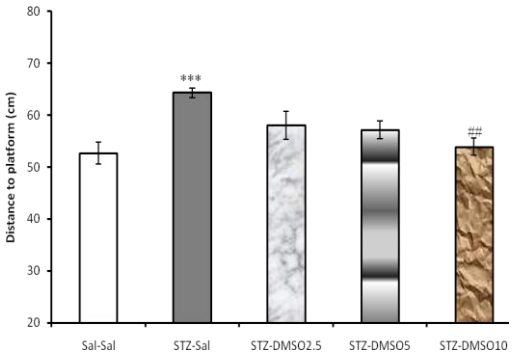

Figure 1. The effect of chronic i.c.v. administration of DMSO on acquisition of spatial memory in the STZ-treated rats. The columns represent mean±S.E.M of distance to hidden platform during 8 trials in training session**s**. The result of Tukey-Kramer multiple comparisons post test was presented in below of the figure (*** $P<0.001$ vs Sal-Sal group; ## $P<0.01$ vs STZ- Sal group).

The result of Tukey-Kramer post-hoc test showed a significant difference existed between Sal-Sal group and STZ-Sal group (P<0.001). This means that the central administration of STZ was able to cause impairment of acquisition in spatial memory. In addition, post-test results indicate a significant difference between the STZ-Sal group and the STZ-DMSO10 group in the distance to hidden platform (P<0.01). However, compared to the Sal-Sal group, this difference was not observed in the STZ-DMSO2.5 and STZ-DMSO5 groups (P>0.05). Indeed, chronic treatment of animals with DMSO10% prevents STZ-induced acquisition of memory impairment.

The effect of i.c.v. STZ with or without DMSO on retrieval of spatial information has been shown in Figure 2. The one-way ANOVA analysis also showed that there was a significant difference in the time spent in target quadrant of Sal-Sal group and the other groups in the probe trial of the invisible version of MWM test [F3,34=11.69; P=0.001]. Based on the Tukey-Kramer post-hoc test, there was a significant difference between Sal-Sal and STZ-Sal groups (P<0.001), Sal-Sal and STZ-DMSO2.5 groups (P<0. 01), and Sal-Sal and Sal-DMSO5 groups (P<0.001), but not seen between Sal-Sal and the STZ-DMSO10 groups. In addition, a significant increase in time spent in the target quadrant was observed in the STZ-DMSO10 group compared with the STZ-Sal group (P<0.01). This means that the chronic i.c.v. infusion of animals with DMSO10% prevents STZ-induced retrieval of memory impairment. The number of entrance into the target quadrant in probe trials paralleled the time spent in the target quadrant, therefore, was not shown separately.

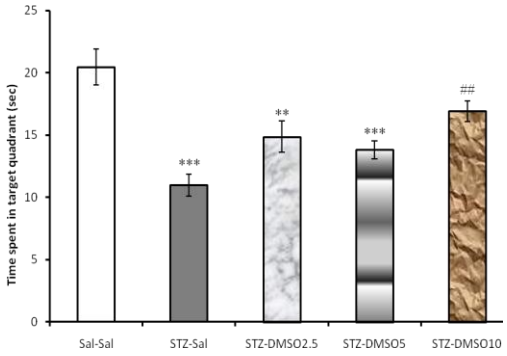

Figure 2. Twenty-four hours after training session, retrieval of spatial memory was tested in probe trial. The columns represent mean±S.E.M of time spent in target quadrant during the 60 s probe test. The result of Tukey-Kramer multiple comparisons post test was presented in below of the figure (*** P<0.001and ** P<0.01 vs Sal-Sal group; ## P<0.01 vs STZ- Sal group).

The effect of STZ with or without DMSO on motor performance has been shown in Figure 3. Statistical analysis of the swimming velocity in all groups, both in the training sessions and the probe tests on Bartlett's test revealed difference. Therefore, non-parametric test was used to analyze animal swimming velocity.

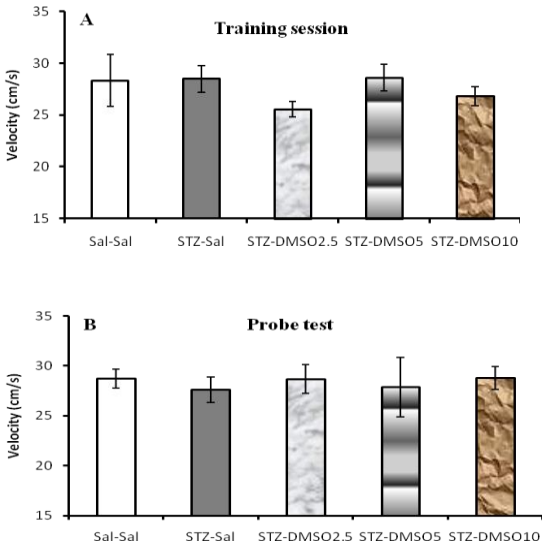

Figure 3. The effect of i.c.v. administration of STZ and/or DMSO on the motor activity. The columns represent mean±S.E.M. of swimming velocity of during 8 trials in training session (A) and the 60 s probe test (B).

One-way Kruskal-Wallis ANOVA showed that there was no significant difference in the swimming velocity among the groups in the training sessions [(Fig. 3A) (KW=1.46; P=0.97)] and the probe tests [(Fig. 3B) (KW=4.01; P=0.4)]. The effect of STZ with or without DMSO on visuo-motor coordination was assessed immediately after probe trial in visible version of MWM. The STZ and/or DMSO treated groups showed no difference in escape latency to find visible platform compared to the control (Sal-Sal) group [(Figure 4) (F4, 34=0.19; P=0.94)].

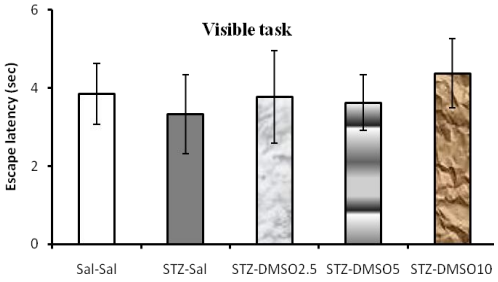

Figure 4. The effect of i.c.v. administration of STZ and/or DMSO on visuo-motor coordination of the animals. The columns represent mean±S.E.M. of escape latency to find the visible platform of during 4 trials in visible version of MWM task. The STZ and/or DMSO treated rats showed no significant differences in escape latency compared to the control (Sal-Sal) group.

Effective dose of DMSO (i.e. 10%) that improves memory impairment in STZ-treated rats were studied on acquisition and retrieval of spatial memory in STZ-free rats. One-way ANOVA analysis showed that there was no significant difference in the distance to the hidden platform [(Figure 5A) (P=0.65)] and time spent in the target quadrant [(Fig. 5B) (P=0.98)] of Sal-Sal group and the Sal-DMSO10% groups in the training sessions and the probe tests, (P=0.65).

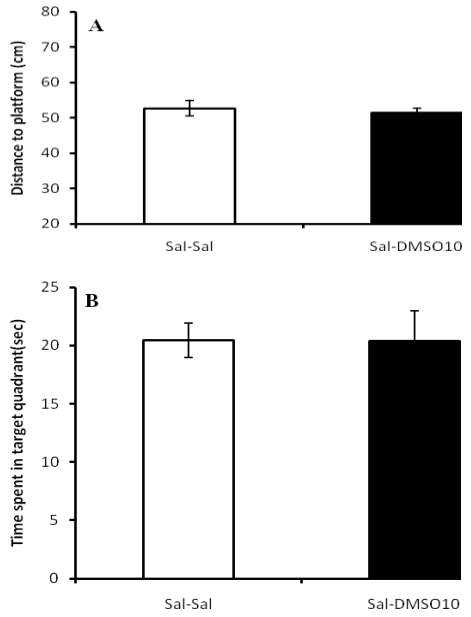

Figure 5. The effect of Chronic i.c.v. infusion of DMSO 10% on acquisition and retrieval of spatial memory in STZ-free rats. The columns represent mean±S.E.M of distance to hidden platform during 8 trials in training sessions (A) and time spent in target quadrant during the 60 s probe test (B).

Discussion

The main findings of this study are: summarized in 5 points 1) After two weeks, i.c.v. administration of STZ on the first and the third days caused deficit in acquisition and retrieval of spatial memory in MWM (2). The two-week i.c.v. DMSO 10% administration in the STZ-treated rats prevented the STZ-induced memory acquisition and retrieval impairment. 3) The two-week i.c.v. DMSO 10% administration in the STZ-free rats had no effect on the spatial memory in MWM. 4) Neither DMSO nor STZ had effect on swimming velocity in invisible task of MWM. 5) i.c.v. administration of STZ and/or DMSO had no effect on escape latency to find the visible platform in non-spatial version of MWM.

The evidence we obtained found here, clearly indicates that chronic i.c.v. infusion of DMSO10% prevents the impairment effect of STZ on acquisition and retrieval of spatial memory in single day invisible version of MWM, which involve place memory. Preventing effect of DMSO in STZ-treated rats was observed in both the distance swum in order to locate the hidden platform and time spent in target quadrant using visual environment cues. Evaluation of the rats during training session and probe trial, it is clear the chronic i.c.v. administration of DMSO has no effect on locomotor performance. Indeed, DMSO treated groups did not differ in escape latency to find the visible platform during non-spatial memory task, namely the observed DMSO effects could not be attributed to the non-mnemonic factors such as motivation or visuo-motor coordination.

Our findings illustrate that i.c.v. administration of STZ caused acquisition and retrieval impairment of spatial information after two weeks. However, most of the studies in which STZ was used to induce animal model of AD reported that i.c.v.-STZ infusion caused memory deficit after three weeks (20, 22). So far, various experimental models of AD such as lesion of forebrain cholinergic neuron by i.c.v. injection of Ig-G-saporin (41), transgenic animals (11), i.c.v. and intrahippocampal infusion of β-amyloid (42), aged animals (43) and antagonization of cholinergic pathways in the fore brain by anticholinergic agents like scopolamine (12). have been used. However, i.c.v.-STZ administration of STZ in rats causes progressive and long-term learning, memory and cognitive performance deficit which are very similar to SAD (13, 14). Moreover, the STZ model has been considered to be the most appropriate model for evaluating therapeutic and preventive effects of drugs with unknown functions (18, 19).

Some findings in this research have shown that chronic i.c.v administration of DMSO had no effect on memory function in STZ-free animals, thought. Although, in many researches, DMSO has mainly been used as a vehicle and locally infused in some areas of central nervous system without interference in food intake (44), reinforcement behavior (45) and sleep-wake pattern (46), this is the first report highlights the existence of some cognition effect of i.c.v. DMSO in STZ-treated and -free rats.

DMSO has mainly been used as a solvent due to its dissolving of hydrophilic and hydrophobic substances, although scavenger of hydroxyl free radicals is considered one of the most important features of this agent (37) Considering the oxidative reaction interference in pathogenesis of AD (4, 5), it seems that in the SAD model some improving effect of DMSO in our study can be attributed to its antioxidant effect. This is the first report presented for the improving potential of DMSO on STZ-induced memory deficit. However, many other studies have found that chronic treatment of the antioxidant agents such as alpha lipoic acid (20),

pioglitazone (21) and quercetin (22) have considerable preventing and improving effects on the cognitive impairment related to STZ. Moreover, it has been reported that by the blockade of hydroxyl radical-mediated oxidative damage, the DMSO has been protected against the toxic effects of the 1-methyl-4-phenylpyridinium in substantia nigra neurons (47).

There has already been evidence of the relationship between reduced cerebral blood flow and memory impairment in the incidence of AD (48). Furthermore, it has been proposed that cerebral hypoperfusion be a sign of AD development (49). Since DMSO causes increase in cerebral blood flow (50), then we can partly attribute improving effect of DMSO in STZ-treated rats to its hyperperfusion effect. In the same way, by bilateral occlusion of common carotid arteries in rats and induction of cerebral hypoperfusion that followed by memory deficit, Farkas and et al (51) showed that 5-day treatment with dizoxide dissolved in DMSO and DMSO alone could attenuate generation of the cognition disorder. In addition, in this brain hypoperfusion model, it has been found that combination of DMSO and fructose 1, 6-diphosphate improves spatial memory resulting in brain hypoperfusion (52).

Stimulation of the inflammatory process is another proposed hypothesis in the pathogenesis of the AD. McGeer et al (53) meta-analyzed 17 epidemiological studies and concluded that anti-inflammatory agents have a protective effect in the AD incidence. Considering that anti-inflammatory effects of DMSO have been confirmed in many studies (17, 33), we can probably attribute the DMSO benefit on spatial memory in SAD model to its anti-inflammatory properties.

There are two points with regard to DMSO interference in the STZ-induced AD model: first, while using the DMSO in the experimental memory deficit models, the researchers should be careful so as not to misinterpret the result. Second, DMSO, as solvent of hydrophilic and hydrophobic substances, has frequently been used in the animal studies, and then we suggest that administrating DMSO as an adjuvant with therapeutic drugs in experimental models of AD have potential effects on prevention or even treatment of this disease.

Limitations

In this study we have not measured the oxidative stress parameters such as malondialdehyde and glutathione activity in the animal's brains. If this is done, provides valuable information about the mechanism of the memory improvement of DMSO.

Conclusion

In general, this study showed that after two weeks of i.c.v. STZ infusion, acquisition and retrieval of spatial memory in single-day testing version of MWM was impaired, and 14 days treating the animals with DMSO 10% caused some prevention in development of memory deficit. We concluded that anti-inflammation, hyper perfusion, and anti-oxidant effects of DMSO might be the probable memory-improving mechanisms. However, the exact mechanisms of the DMSO to improve memory should be studied in future.

Conflict of interest

The author declare no conflicts of interest.

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