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RESEARCH ARTICLE

# Mefenamic Acid Attenuates Intracerebroventricular Streptozotocin-Induced Cognitive Deficits in the Rat: A Behavioral Analysis

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**ABSTRACT**

Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats is followed by long-term and progressive deficits in learning, memory, and cognitive performance in rats which is somewhat similar to sporadic Alzheimer's disease (SAD). Epidemiological studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) could delay or slow the clinical expression of SAD. Therefore, the beneficial effect of mefenamic acid (MA) was investigated on ICV STZ-induced learning, memory, and cognitive impairment in male rats. For this purpose, rats were injected bilaterally with ICV STZ, on days 1 and 3 (3 mg/kg). The STZ-injected rats received MA (30 mg/kg/day, i.p.) starting from day 5 post-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. It was found out that MA-treated STZ-injected rats showed higher correct choices and lower errors in RAM than vehicle-treated STZ-injected rats. In addition, MA administration significantly attenuated compared to and memory impairment in STZ-injected group in passive avoidance test. These results demonstrate MA efficacy against cognitive deficits as well as learning and memory impairment caused by ICV injection of STZ in rats and its potential in the treatment of some neurodegenerative disorders including SAD.

**Keywords:** *Mefenamic acid, Streptozotocin; Learning, Memory, Spatial cognition Rat*

Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats is followed by long-term and progressive deficits in learning, memory, and cognitive performance that is similar to sporadic Alzheimer's disease (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]. Due to primary disturbance in neuronal insulin and insulin receptor signal transduction, drastic abnormalities in cerebral glucose and energy metabolism have been demonstrated in experimental models of SAD [4]. On this basis, interventions that could delay SAD onset would have a major public health impact [2]. Many epidemiological studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) could delay or slow the clinical expression of SAD [5-8]. However, until now there has not been any strong evidence from completed randomized controlled trials to indicate the applicability of anti-inflammatory treatment for this purpose [9]. Among these compounds, mefenamic acid (MA) has exhibited anti-inflammatory [6] and neuroprotective properties [10-11]. Meanwhile, it has recently been shown that MA improves learning and memory impairment in an A-beta amyloid (1-42)-infused Alzheimer's disease rat model [12]. Therefore, this study was undertaken to investigate the possible beneficial effect of MA administration in ICV STZ-induced model of SAD in the rat using passive avoidance and radial eight-arm maze tasks.

## MATERIALS AND METHODS

### *Animals*

Adult male Wistar rats (Pasteur's Institute, Tehran), weighing 320-360 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

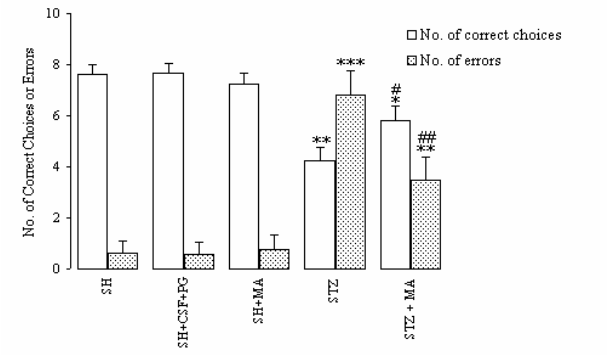


Fig 1. The effect of mefenamic acid administration (30 mg/Kg/day, i.p.) on spatial cognition deficit induced by ICV injection of STZ (twice with a 2-day interval) in rats. A total of 60 rats were used for this test. Values are means ± S.E.M. of the number of correct choices or the number of errors. * $p$<0.05, ** $p$<0.01, *** $p$<0.005 (in comparison with SH group); # $p$<0.05, ## $p$<0.01 (STZ + MA vs. STZ) (Wilcoxon's rank sum test).

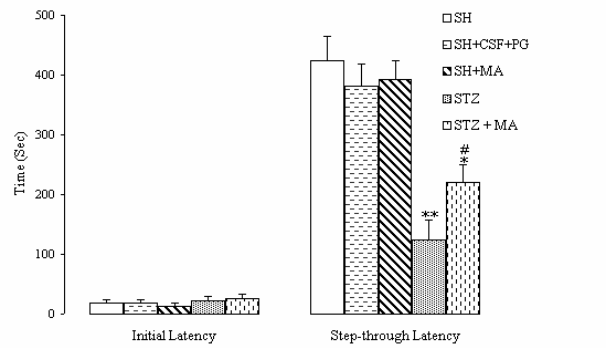


Fig 2. The effect of mefenamic acid treatment (30 mg/Kg/day, i.p.) on passive avoidance performance after ICV injection of STZ in rats as indicated by initial and step-through latencies. A total of 60 rats were used for this test. Values are expressed as mean Values are expressed as means ± S.E.M. * p<0.01, ** p<0.005 (in comparison with SH group); # p<0.05 (STZ + MA vs. STZ) (non-parametric Kruskal-Wallis and Mann-Whitney U-tests)

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 (National Institutes of Health) and those of the Research Council of Iran University of Medical Sciences (Tehran, Iran).

### *Experimental procedure*

Rats (n = 60) were randomly divided into the following groups (in equal size): 1. Sham-operated group (SH), 2. Sham-operated group (SH + CSF + PG) that received bilateral ICV injection of artificial CSF (ACSF) (10 µl on each side) as the solvent of STZ (Upjohn Chemical, France) and propylene glycol (Merck Chemical, Germany) (PG, i.p.) as the vehicle for MA (Alhavy Chemical, Iran), 3. MA-treated sham-operated group (SH + MA), 4. STZ-injected group (STZ) which received ICV injection of STZ in addition to PG (0.5 ml; i.p.), and 5. MA-treated STZ group (STZ + MA), which also received MA (30 mg/Kg/day; i.p.) from day 5 post-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 [13]. STZ and MA-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. Postoperatively, special care was undertaken until spontaneous feeding was restored.

### *Radial arm maze (RAM) task*

The effect of ICV STZ injection and intraperitoneal administration of MA was tested in different groups in the radial maze according to the paradigm as described before [14] 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 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 19th day post-surgery (1st ICV injection of STZ).

### *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 16 days following 1st MA administration.

### *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.

## RESULTS

The body weight of the rats within the groups was recorded every other day. There was no significant difference among the different experimental groups. Regarding serum glucose level, no significant changes were observed in this parameter in STZ and STZ + MA groups after 1 and 2 weeks. During the experimental study, the majority of the animals (nearly 92%) well tolerated the treatments. There was no significant mortality in the STZ-treated group as compared to the sham-operated groups.

### *Effect of mefenamic acid 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 two 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, two-week administration of MA (30 mg/Kg/day) starting 5 days after 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 ($p<0.01$) in STZ + MA group as compared to vehicle-treated STZ group in the radial maze task.

### *Effect of mefenamic acid 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 17.8, 19.2, 13.1, 22.1, and 25.1 s in SH, SH+CSF+PG, SH+M, STZ, and STZ + MA groups respectively. Meanwhile, there was no significant difference among SH, SH+CSF+MA, and SH+MA groups regarding STL. On the other hand, the STZ + MA group exhibited significant reversal of STL (with a mean retention latency of 219.5 ± 29.7 s) ($p<0.05$) as compared to vehicle-treated STZ group, indicating improved acquisition or retention of memory (Fig. 2).

## 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 MA (30 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, 15]. 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 animals [15]. 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 MA (30 mg/kg/day) starting 5 days after 1st STZ injection for two weeks caused a significant improvement in learning, memory, and spatial cognitive skills. The results of a study on the efficacy of MA on A-beta amyloid (1-42)-infused Alzheimer's disease rat model has shown its usefulness against induced learning and memory impairment [12]. The beneficial effect of MA in this study could be attributed to the following potential mechanisms: 1) 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 [16]. On the other hand, non-steroidal anti-inflammatory drugs including aspirin and MA directly and dose-dependently exhibit neuroprotective and nitric oxide radicals-scavenging activity [11] and there are strong evidence for the fact that inflammatory processes are associated with the pathophysiology of Alzheimer's disease and that treatment with NSAIDS reduces the risk for Alzheimer's disease [5]. Since free radical generation is also associated with cognitive impairment in ICV STZ model of SAD in rats [15], therefore, MA in this way could attenuate the observed behavioral deficits in this study. Furthermore, it has recently been demonstrated that ICV STZ in rats could also lead to increased expression of beta amyloid in the rat brain which itself may enhance inflammatory processes within the central nervous system [17], 2) treatment of adult rats with ICV STZ provides an animal model of neuronal dysfunction that is characterized by a decrease in both the neuronal metabolism of glucose and the formation of energy [4]. Since fenamates exert protective effect on neurons under abnormal conditions including ischemia (which is accompanied by glucose/oxygen deprivation) and/or excitotoxic conditions (through influencing glutamate receptor-mediated currents in a way that excitotoxic insults are attenuated [10], this property can also explain the beneficial effect of MA in this study, and 3) ICV injection of STZ, which inhibits insulin receptor function, develops progressive behavioral deficits as well as biochemical changes and neuronal degeneration in rats that is very similar to SAD [3]. It has been suggested that ICV STZ may cause neuronal damage independent of its action on glucose metabolism [18]. In this respect, STZ induces specific damage to axons and myelin in some brain regions including the fornix, anterior hippocampus and periventricular area that are essential for learning and spatial memory. Therefore, it seems likely that STZ does not induce learning deficits only by impairing glucose utilization [19] through an action on brain insulin receptor function [20]. Although the mechanism of action of STZ on myelin is not yet known, it probably involves the induction of oxidative stress, to which myelin is particularly vulnerable [18]. Evidence of lipid peroxidation in whole brain homogenates was provided by the finding of an increase in malondialdehyde and a decrease in glutathione 3 weeks after ICV injection of STZ [15]. It is possible that the protective effect of mefenamic acid in this study has been through its effects on these processes. Further research studies are underway to clarify the related mechanisms.

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

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