Original Article

Anti-inflammatory Effects of Memantine in Carrageenan-induced Paw Edema Model in Rats

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Abstract

Background: The anti-inflammatory effect of memantine (MEM) was investigated using carrageenan-induced hind paw edema model in rats. **Materials and Methods:** Thirty male Wistar rats were randomly assigned to five groups (n = 6). Group 1 received 0.1 mL of 1% carrageenan at the right hind paw. Group 2 received dexamethasone (10 mg/kg) and Groups 3, 4, and 5 received 5, 10, and 20 mg/kg MEM intraperitoneally (ip), 20 min after injection of carrageenan into the right hind paw, respectively. Animals' paw thickness was measured at 0, 1, 2, 3, 4, and 5 h after carrageenan injection. Then, animals were euthanized and myeloperoxidase (MPO) and malondialdehyde (MDA) levels were measured in the paw tissues. The tissue samples were further examined histopathologically using light microscopy. One-way ANOVA and Tukey post hoc test was used to compare the mean values between the groups. **Results:** Treating with MEM at all doses significantly decreased hind paw thickness at 2 ($P < 0.05$ and $P < 0.01$ at MEM 10 mg/kg and MEM 5 and 20 mg/kg, respectively), 3 ($P < 0.001$), and 4 ($P < 0.001$ at 5 mg/kg and $P < 0.01$ at MEM 10 and 20 mg/kg) hours after carrageenan injection in comparison to the carrageenan group. There was a significant ($P < 0.05$ and $P < 0.001$, respectively) reduction in MPO activity and MDA levels in MEM-treated groups when compared with the carrageenan group. **Conclusion:** This study showed that MEM decreased paw edema, leukocyte infiltration, MPO activity, and MDA levels, and MEM can be considered as an effective anti-inflammatory agent.

Keywords: *Carrageenan, inflammation, lipid peroxidation, memantine, myeloperoxidase*

Introduction

Inflammation is a normal adaptive physiological response of the immune system to the invasion of pathogenic agents and/or tissue injury.[1] Although inflammation is a defensive response, hyperactivation of the immune system causes secondary complications in the body.[2] *N*-methyl-d-aspartate receptors (NMDARs) are one of the subclasses of ionotropic glutamate receptors that play a role in many neurophysiological functions such as learning, neurogenesis, and synaptic plasticity.[3] In addition to central nervous system, these receptors are present in various peripheral tissues.[4] Some studies have suggested NMDARs' participation in inflammatory processes.[5,6] It has been proposed that activation of NMDARs is directly associated with the onset of the inflammatory cascade which results in cellular generation of free radicals.[6] NMDARs express in leukocytes such as neutrophils, monocytes, and macrophages. Activation of NMDARs modulates migration of these cells and increases secretion of their products. Considering that, blockade of NMDARs may lead to suppression of inflammatory response. It has been reported that some of the NMDAR antagonists possess anti-inflammatory activities. Some studies have shown that ketamine attenuates pro-inflammatory mediators and regulates inflammatory response.[2,7,8] MK-801 (dizocilpine), an uncompetitive NMDAR antagonist, downregulates expression of inflammatory genes.[9] Moreover, administration of dizocilpine decreased cardiovascular inflammation following Mg^{2+} deficiency in rats.[6] Another NMDAR antagonist, dextromethorphan, attenuated lung inflammation induced by heat in rats.[10] However, many NMDAR antagonists such as dizocilpine could not be used in human patients because they have shown highly undesirable adverse effects in clinical trials.[11]

Memantine (MEM) is an uncompetitive NMDAR antagonist that is clinically well tolerated.[12] MEM is used for the treatment of Alzheimer's disease and its suppressive effects on neuroinflammation have been reported in some studies.[13-16] It has been

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demonstrated that MEM attenuated neuroinflammation in streptozotocin (STZ)-induced memory impairment in rats.[13] A study by Wu *et al.* showed that MEM decreased the release of pro-inflammatory factors in neuron-glia cultures exposed to lipopolysaccharide.[15] A reduction in the level of inflammatory cytokines by MEM has been reported in rats addicted to morphine.[14] Rajasekar *et al.*[16] reported that MEM inhibits neuroinflammation induced by STZ in astrocytes. In addition, recent studies have shown that MEM attenuates cardiac, pulmonary, and colonic inflammation in experimental models.[5,17-19]

The carrageenan-induced-rat paw edema is an appropriate model to assess the effects of anti-inflammatory drugs.[20] Inflammatory response due to carrageenan injection constitutes three phases that mediate by pro-inflammatory agents, including histamine, serotonin (primary phase), kinins (secondary phase), and prostaglandins, particularly E series (final phase). This response is quantified by an increase in hind paw thickness (edema).[21,22] The maximum level of edema in the hind paw appears about 3 h after injection of carrageenan.[23] Neutrophil's migration into the hind paw causes generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[21] Also, myeloperoxidase (MPO) secreted by neutrophils, initiates lipid peroxidation in the inflamed site.[24] Heretofore, no study was conducted on the effects of MEM against acute inflammation in the hind paw induced by carrageenan. In the present study, the effects of MEM on paw edema, histopathology, MPO activity, and malondialdehyde (MDA) levels were evaluated in carrageenan-induced inflammation in the hind paw of rats.

Materials and Methods

Drugs and chemicals

Carrageenan was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). MEM was purchased from Sobhan Darou Co. (Rasht, Iran). The other reagents were of a commercial analytical grade.

Animals

Thirty male Wistar rats with a weight range of 250 ± 30 and the age of 8–10 weeks were used in this study. Animals were maintained under standard conditions (12-h light/dark cycle, temperature 22 ± 1°C and 50 ± 10% humidity) with free access to water and standard laboratory animals' food. All experiments on animals were carried out following the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication, 8th edition, 2011) and approved by the Ethics Committee of the University (IR.UMSU.RES.1396.140; 2017-07-19).

Experimental design

The rats were randomly divided into five groups of six animals per group. Group 1 (carrageenan) received 0.1 mL of 1% carrageenan by subplantar injection into the right hind paw of the animals. Group 2 (DEXA) received 10 mg/kg of dexamethasone *ip* 20 min after injection of 0.1 mL of 1% carrageenan. Groups 3–5 served as MEM groups and received 5, 10, and 20 mg/kg of MEM *ip*, respectively, 20 min after injection of 0.1 mL of 1% carrageenan.

Assessing hind paw thickness

The inflammation was quantified by measuring the right hind paw thickness of animals using a digital caliper at 0, 1, 2, 3, 4, and 5 h after carrageenan injection.

Histopathological studies

The right hind paw of each rat was excised after euthanasia with pentobarbital and fixed in 10% formalin. Then, hematoxylin and Eosin (H&E) staining was used to evaluate the degree of inflammation and neutrophil infiltration. Histopathological changes such as edema and necrosis were scored by two pathologists in a single-blind fashion as 0, 1, 2, 3, and 4 for absent, mild (slight degree of inflammation), moderate (edema with degeneration of muscle fibers), moderate to severe (edema with myofibrillar degeneration and/or diffuse inflammation), and sever (edema with extensive myofibrillar degeneration and necrosis) pathological changes, respectively.[25,26]

Determination of MPO activity

MPO activity was considered as neutrophils activities according to the Bradley method.[27] In brief, Right animals' hind paws were homogenized in 50 mM potassium-phosphate-buffered solution (pH = 6.0) which contained hexadecyltrimethylammonium bromide (HTAB), then centrifuged at 4°C and 10,000 rpm and supernatant was collected. Thereafter, the absorbance was measured by a spectrophotometer (Cecil 9000, UK) at 460 nm and concentration was calculated by using the calibration curve ($y = 0.00616x - 0.0085$). The results were expressed as units of MPO in 100 mg weight of wet tissue (U/100 mg tissue).[27]

Measurement of tissue lipid peroxidation

Malondialdehyde (MDA) levels were measured in the right animals' hind paws by using the thiobarbituric acid reacting substance method.[28] Briefly, the hind paws of animals were homogenized in Tris–HCl buffer (0.1 M, pH = 7.4). Then samples were centrifuged at 4°C and 10,000 rpm and supernatant was collected. The tissue samples were reacted with thiobarbituric acid at acidic pH at high temperature in a boiling bath for 45 min. The absorbance of samples was determined at 532 nm by using a spectrophotometer (Cecil 9000, UK). *N*-Butyl alcohol was used as a blank. The results were presented as nanomoles per milligram protein.

Statistical analysis

All values are presented as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA). When the ANOVA analysis indicated significant differences, the Tukey *post hoc* test was performed to compare the mean values between the groups. Differences between means were considered significant at $P < 0.05$.

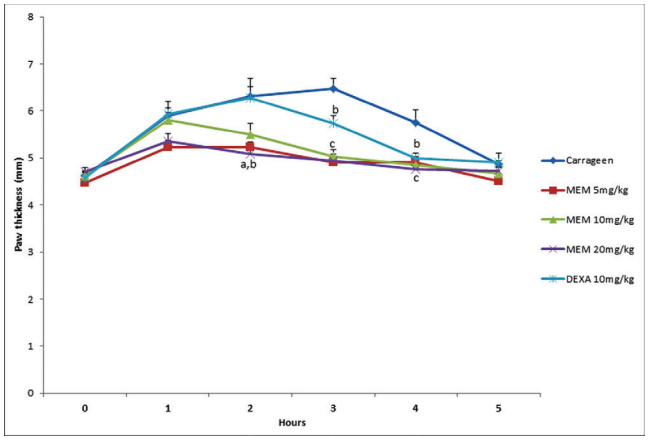

Figure 1: Effects of MEM on carrageenan-induced paw edema in rats at different times. Values are present as mean ± SEM (*n* = 6). Different letters (a–c) show significant differences (a: *P* < 0.05, b: *P* < 0.01 and c: *P* < 0.001) in comparison with the carrageenan group using one-way ANOVA with Tukey post hoc test

Results

Effects of MEM on paw thickness in carrageenan-induced hind paw edema

As shown in Figure 1, at the first hour after injection of carrageenan hind paw thickness of animals had no significant difference between the groups. At 2nd h, hind paw thickness in treated groups with MEM significantly ($P < 0.05$ and $P < 0.01$ at MEM 10 mg/kg and MEM 5 and 20 mg/kg, respectively) decreased in comparison to the carrageenan group. At 3rd h, MEM- and DEXA-treated groups showed a significant ($P < 0.001$ and $P < 0.01$, respectively) decrease in comparison to the carrageenan group. Also, at 4th h after injection of carrageenan, hind paw thickness significantly reduced in MEM ($P < 0.001$ at 5 mg/kg and $P < 0.01$ at MEM 10 and 20 mg/kg) and DEXA ($P < 0.01$) groups in comparison to the carrageenan group. After that, at 5th h, hind paw thickness of animals in all groups were similar to each other and no significant difference was observed.

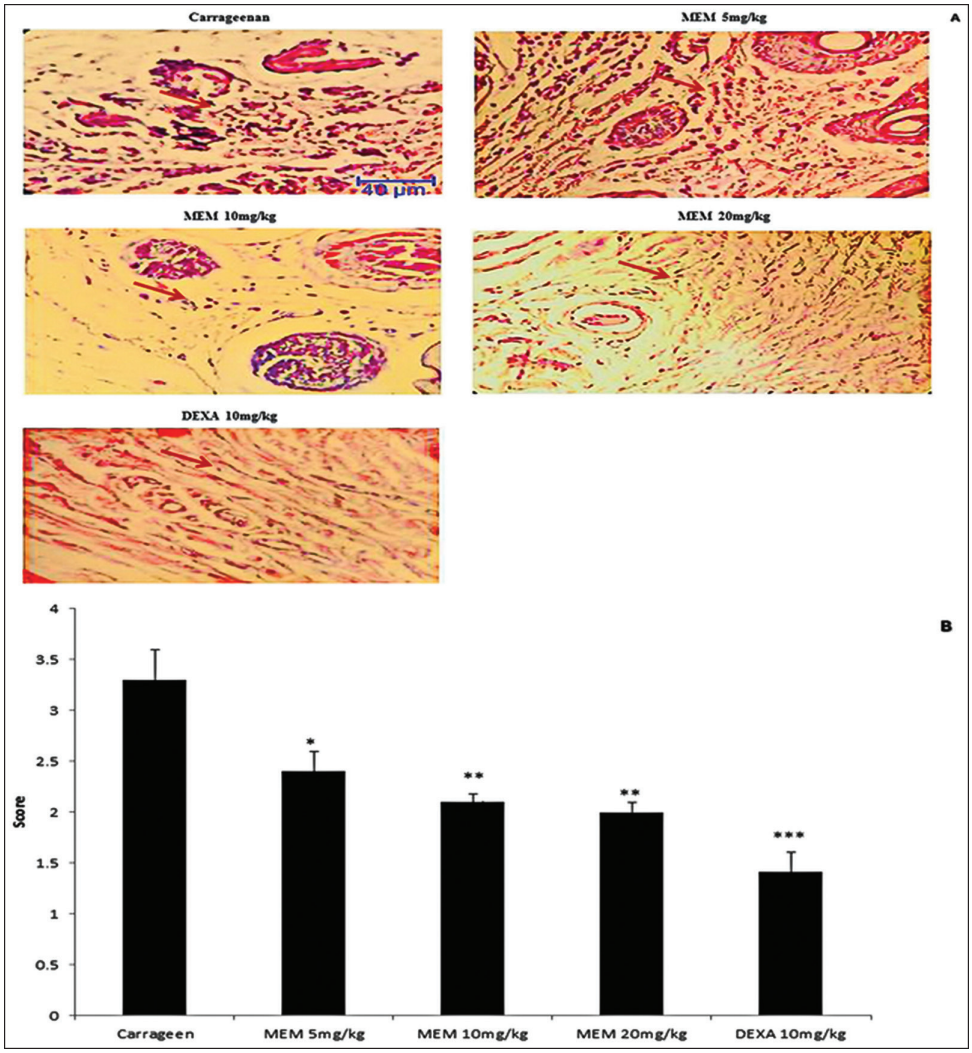

Figure 2: (A) Photomicrographs of sections of rat hind paws after H&E staining. (B) Grading of histopathological changes in the rats' hind paw tissues. Grades 1, 2, 3, and 4 represent low, moderate, high, and intensive pathological changes, respectively. Values are mean ± SEM. **P* < 0.05, ***P* < 0.01, and * *P* < 0.001 as compared to the carrageenan group using one-way ANOVA with Tukey post hoc test**

Effects of MEM on histopathology

The histological sections of the hind paw obtained from the carrageenan group showed a high number of leukocyte accumulation in the tissue with significant degeneration of muscle fibers. MEM treatment at all doses considerably decreased edema, leukocyte infiltration, and myofibril degeneration in comparison to the carrageenan group ($P < 0.05$ at 5 mg/kg and $P < 0.01$ at MEM 10 and 20 mg/kg). Also, administration of DEXA as a positive control markedly reduced leukocyte infiltration in the tissue ($P < 0.001$) [Figure 2].

Effects of MEM on MPO activity

Figure 3 indicates that administration of MEM at all doses significantly (MEM 5 mg/kg $P < 0.01$ and MEM 10, 20 mg/kg $P < 0.001$) decreased MPO activity in the hind paw of animals in comparison to the carrageenan group.

Effects of MEM on lipid peroxidation

Obviously, treating with MEM at all doses significantly ($P < 0.001$) reduced MDA levels in hind paw tissue [Figure 4].

Discussion

As mentioned earlier, NMDARs are participated in triggering inflammatory responses. In this study, MEM, an uncompetitive NMDAR antagonist, was administered to treat inflammation induced by carrageenan. Our results revealed that treatment with MEM significantly reduced hind paw thickness, leukocyte infiltration, MPO activity, and MDA levels.

As expected, injection of carrageenan induced hind paw inflammation that is characterized by increasing hind paw thickness and edema. The maximum amount of edema was observed at 3 h after carrageenan injection. The edema is one of the typical signs of inflammation following carrageenan injection which occurred due to action of pro-inflammatory agents generated by tissue or infiltrating cells in the inflamed site.[21,22,29]

Administration of MEM significantly reduced hind paw edema in animals. Similar to our findings, a study indicated that systemic administration of NMDAR antagonists decreased paw swelling induced by formalin.[30,31] As previously mentioned, histamine, serotonin, and prostaglandin mediate inflammatory response phases following carrageenan injection which induce hind paw edema.[21] MEM competitively inhibits human organic cation transporter 2 (hOCT2), a monoamine transporter that can translocate histamine and serotonin.[32] Therefore, it can be a reason for edema reduction by MEM. Moreover, another reason may relate to prostaglandin production. These bio-compounds sensitized by arachidonic acid and cyclooxygenase (COX) enzymes are catalysts of this reaction.[33] Evidence have indicated that MEM decreased the COX-2 level.[16] Wu *et al.*[15] showed that pre-treatment with MEM decreased PGE2. Thereby, a reduction in prostaglandin synthesis may be a reason for the anti-inflammatory effect of MEM and reduction of edema. On the other hand, MEM decreases the production of nitric oxide (NO)[13,15] that has a key role in the mediation of inflammation following carrageenan injection.[29]

MPO is a heme enzymatic protein that presents in neutrophil granulocytes. This enzyme is one of the local mediators of tissue injury and results from occurring inflammation in the damaged site.[24,34] Therefore, assessment of MPO activity is a dependable indicator of granulocytes infiltration and inflammation in different tissues.[35,36] MPO through formation of reactive oxygen and nitrogen intermediates which results in lipid peroxidation can cause host tissue damage.[24,34] Hence, a reduction in MPO activity is a good therapeutic strategy to treat inflammation.[34] Our study indicated that treatment with MEM decreased MPO activity in the carrageenan-injected hind paw of animals. These findings confirmed that MEM has anti-inflammatory effects. In line with our results, previous studies showed that MEM reduced MPO activity in various inflammatory conditions in different organs.[5,17,18]

Lipid peroxidation is one of the pathophysiologic consequences of acute inflammatory conditions.[24] Administration of MEM in the present study decreased lipid peroxidation which is

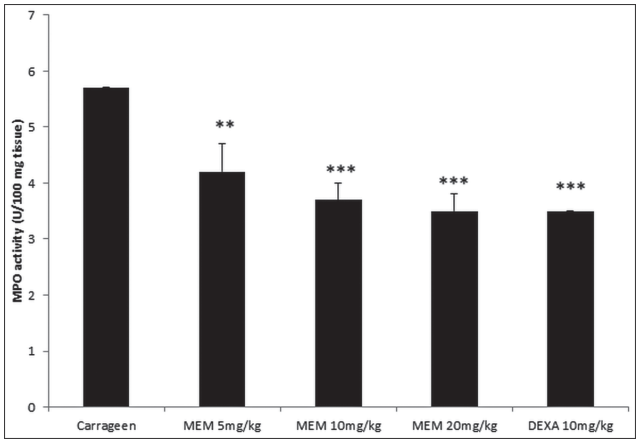

Figure 3: The effect of MEM on MPO activity in hind paws of rats injected with carrageenan. Values are present as mean ± SEM ($n = 6$). **$P < 0.01$ and ***$P < 0.001$ as compared to the carrageenan group using one-way ANOVA with Tukey post hoc test

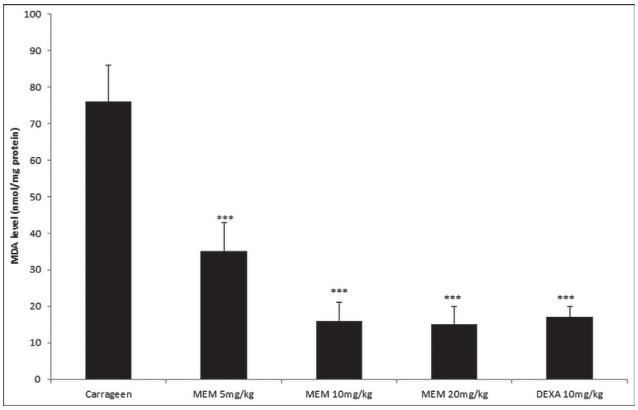

Figure 4: The effect of MEM on MDA levels in hind paws of rats injected with carrageenan. Values are present as mean ± SEM ($n = 6$). ***$P < 0.001$ as compared to the carrageenan group using one-way ANOVA with Tukey post hoc test

similar to other researchers' findings.[17,18] Reduction of lipid peroxidation may be related to a decrease in MPO activity, because MPO function is the main factor in the initiation of lipid peroxidation in the inflamed site.[24] Also, it may be associated with a reduction of ROS and RNS due to MEM treatment which can be inferred from previous studies.[13,15] To the best of our knowledge, this is the first study showing the anti-inflammatory effects of MEM in the carrageenan-induced hind paw edema, as an inflammation model in rats.

Conclusions

The present study demonstrated that MEM decreased paw edema, leukocyte infiltration, MPO activity, and MDA levels, and MEM can be considered as an effective anti-inflammatory agent.

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Conflict of interest

None declared.

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