European Journal of Pharmacology 793 (2016) 28–34

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

The effect of memantine on trinitrobenzene sulfonic acid-induced ulcerative colitis in mice

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ARTICLE INFO

Keywords:
Memantine
NMDA receptors
Colitis
Antagonist
Inflammation
Mice

ABSTRACT

Previous reports suggest a significant role for N-Methyl-D-aspartate (NMDA) activation in inflammatory processes. So, this study was conducted to investigate the effect of memantine, a commonly used NMDA receptor antagonist, on inflammatory changes in mice model of colitis. Colitis was induced by intracolonic instillation of trinitrobenzene sulfonic acid (TNBS) (40 mg/kg). Animals received memantine (12.5, 25 and 50 mg/kg, i.p.), glutamate (2 g/kg, p.o.) or dexamethasone (1 mg/kg, i.p.) 24 h before TNBS instillation and daily thereafter for 4 days. The colonic injury was measured by clinical, macroscopic, microscopic and biochemical analysis. Memantine significantly attenuated the body weight loss, colon weight, the plasma levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and colon level of tumor necrosis factor-α (TNF-α) and myeloperoxidase (MPO); as well as macroscopic and microscopic signs of colitis. Oral administration of glutamate had no significant effect on investigated parameters. Memantine as a NMDA antagonist may provide a novel venue for the development of strategies for the treatment of ulcerative colitis.

1. Introduction

Ulcerative colitis is a chronic inflammatory disease involves primarily the mucosa and submucosa of the colon (Arihiro et al., 2002) with clinical characteristics such as diarrhea along with blood and/or mucus, nausea, abdominal pain, fatigue, fever, weight loss, inflammation, and colon ulcers (Minaiyan et al., 2015).

This disease is associated with elevation of inflammatory markers such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and myeloperoxidase (MPO) activity (Motavallian et al., 2012).

L-glutamate is well recognized as a major excitatory neurotransmitter in the brain but there are now evidences indicate that glutamate acts as a signaling transmitter in non-neuronal tissues (Pacheco et al., 2006; Storto et al., 2006; Zhou et al., 2006). Some studies also indicate that N-Methyl-D-Aspartate (NMDA) receptors, a group of ionotropic glutamate receptors, have also an important role in the peripheral tissues such as colon (Hrabovszky et al., 2006). Moreover, previous studies suggested the involvement of NMDA receptors in inflammatory processes. NMDA receptor suppression inhibits activation and proliferation of T lymphocytes (Kubera et al., 2006) while, the receptor activation can stimulate production of pro-inflammatory cytokines from lymphocytes. These findings suggest that NMDA receptor inhibition might be beneficial for the treatment of various inflammatory conditions. In this regard, dextromethorphan as an NMDA receptor antagonist attenuated heat-induced acute lung inflammation in rats (Yang et al., 2012). Moreover, ketamine, another NMDA antagonist, frequently has shown anti-inflammatory effects in both in vivo and in vitro experiments (Kock et al., 2013). Although, little attention has been paid to the involvement of NMDA receptors in pathogenesis of colitis, there is a possible application for memantine as a safe and well tolerated uncompetitive NMDA receptor antagonist in the treatment of colitis. Memantine is currently used in clinic for Alzheimer's disease and dementia with good safety profile (Levin et al., 2009).

The present study was undertaken to determine whether memantine has a beneficial effect on the TNBS induced ulcerative colitis in mice.

2. Material and method

2.1. Animals

Male Syrian mice weighting 25–30 g were used in the experiment. The animals were kept in condition with temperature of 20–23 °C and

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http://dx.doi.org/10.1016/j.ejphar.2016.10.032
Received 17 July 2016; Received in revised form 24 October 2016; Accepted 24 October 2016
Available online 27 October 2016
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12:12 h light/dark cycle. Except when fasting, food and water were freely available.

The experiments were made in accordance with guidelines of the Ethics Committee of the Isfahan University of Medical Sciences, Isfahan, Iran.

2.2. Chemicals

Memantine was purchased from Sobhan daroo Company (Tehran, Iran). Dexamethasone was also obtained from Raha Pharmaceutical Company (Isfahan, Iran). TNBS, hexadecyl trimethyl-ammonium bromide (HTAB) and O-dianisidine dihydrochloride were bought from Sigma- Aldrich (St. Louis, Mo, USA).

The ELISA kits for mouse TNF-α, IL-1β and IL-6 were purchased from Boster Co. (Pleasanton, CA, USA). Glutamate was bought from MP biomedical (Netherland). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.3. Animal weight measurement

Each mouse was weighted daily prior to induction of colitis and subsequently over the experimental period.

2.4. Animal groups

The mice were randomly divided into the following groups: Sham group (normal saline, 10 ml/kg, i.p.), colitis control group (normal saline, 10 ml/kg, i.p.), reference group (dexamethasone, i.p., 1 mg/kg), memantine (12.5, 25 and 50 mg/kg, i.p.) and glutamate (2 g/kg, p.o.) groups.

All drugs were administered once daily from 24 h prior to induction of colitis until 4 days thereafter. Colitis was induced in all animals except Sham group. Each group consisted of six mice.

2.5. Induction of colitis

The mice were fasted 16–18 h prior to the TNBS enema. Animals were anesthetized with ether and colitis was induced by instillation of TNBS (40 mg/kg) in 50% ethanol with a 3-cm long tube in the colon via the anus. Animals were hold in head down position for 90 s after instillation of TNBS (Minaiyan et al., 2014a).

2.6. Macroscopic measurement of colonic inflammation

Animals were euthanized using ether at the fourth day after receiving TNBS, (i.e. 24 h after the last treatment). The colon was dissected out, and its length and weight were assessed and scored respectively.

Macroscopic scoring of damage was accomplished according to Wallace et al. method (1989).

Then, colon was fixed on a white sheet and a photo was taken using a Canon camera (Powershot G9, 12 megapixel, Japan) to measure erosion area. The erosion area was measured using Fiji-win 32 software, an image processing and analysis software (NIH image for the Macintosh) (Minaiyan et al., 2014b).

Then, the colon tissue was cut in three segment and then was processed for H & E staining, tissue MPO activity and ELISA.

The segment for MPO activity was frozen in liquid nitrogen and kept at −70 °C. The part for H & E staining was fixed in formalin (10% solution in phosphate buffer saline (PBS)).

2.7. Assessment of MPO activity

Colonic MPO activity, a marker of neutrophil accumulation, was measured according to Kim et al. method (2012). Briefly, colon segments were weighted and homogenized in 1 ml of potassium phosphate (50 mM, pH=6) with 0.5% HTAB using polytron homogenizer for 4 min at 30 Hz. The homogenate was centrifuged (13400*g*, 4 °C) and supernatant was stored at −70 °C until to be used.

A 7 microliter of the supernatant was mixed with PBS solution (pH=6) containing 0.0005% hydrogen peroxide and 0.167 mg/ml O-dianisidine dihydrochloride. The change in absorbance values was measured using an ELISA reader at 450 nm (three readings at 30 s intervals).

One unit of MPO activity was defined as the amount that degraded 1 μmol of hydrogen peroxide per minute at 25 °C. MPO activity was expressed as units/100 mg of tissue.

2.8. Histopathological evaluation of colon damage

Colon specimens which were fixed in formalin solution (10% formalin in PBS), embedded in paraffin, sliced into 4 μm-thick sections; then, the sections were deparaffinized with xylene, and stained with hematoxylin and eosin (H & E) and scored according to the criteria previously described by Dieleman et al. (1998). Total colitis index was calculated by summing 3 sub-scores (inflammation severity, inflammation extent, crypt damage) on hematoxylin and eosin stained and coded slides.

2.9. Blood hematocrit and cytokines assessment

The mice were examined for blood hematocrit (Eckhardt et al., 2010). Thereafter, blood samples, obtained via cardiac puncture, were centrifuged at 1000*g* for 10 min at 4 °C. The supernatants were stored at −70 °C until assay. On the other hand, colon samples were homogenized and the homogenates were frozen until analysis. The levels of cytokines (IL-1β, IL-6, TNF-α) were determined by means of a commercially available ELISA kit according to the manufacturer's instruction. Briefly, the microplate provided in these kits has been coated with an antibody specific to each cytokine. Plasma or homogenate samples and standards were added to the appropriate plate wells with a biotinylated detection polyclonal antibody from goat specific for IL-1β, IL-6 and TNF-α, and incubated for 60 min at 37 °C.

After 3 times washing with PBS, Avidin-Biotin-Peroxidase Complex was added to each well and incubated for 30 min at 37 °C. After 5 times washing, 100 μl TMB (tetramethyl-benzidine) color developing agent was added and the reaction was stopped after incubation for 20 min in dark at 37 °C. Then, the plates were read at 450 nm.

2.10. Data analysis

Results are expressed as mean ± S.E.M. for parametric data and median (range) for non-parametric data. Statistical comparisons were made by one-way ANOVA, followed by a post hoc Tukey test, for parametric data, or by Kruskal-Wallis test followed by Dunn's test for non-parametric data. SPSS (version 18) were used in data analysis. A P value of < 0.05 was considered significant..

3. Results

3.1. Effect of memantine on body weight changes

Colitis caused weight loss during experimental period in colitis control group. Mice treated with dexamethasone (1 mg/kg, i.p.) or memantine (25 and 50 mg/kg, i.p.) showed a significant improvement of weight loss in experimental period compared with colitis control group. However, glutamate had no significant effect on body weight loss. Additionally, the weight of mice increased regularly in sham group compared to colitis control (Fig. 1).

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