Life Sciences 189 (2017) 23–28

Contents lists available at ScienceDirect

Life Sciences

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

Agmatine attenuates intestinal ischemia and reperfusion injury by reducing oxidative stress and inflammatory reaction in rats

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ARTICLE INFO

Keywords:
Agmatine
iNOS
Intestinal ischemia
Reperfusion injury
Oxidative stress

ABSTRACT

Aims: Oxidative stress and inflammatory response are major factors causing several tissue injuries in intestinal ischemia and reperfusion (I/R). Agmatine has been reported to attenuate I/R injury of various organs. The present study aims to analyze the possible protective effects of agmatine on intestinal I/R injury in rats.

Main methods: Four groups were designed: sham control, agmatine-treated control, I/R control, and agmatine-treated I/R groups. IR injury of small intestine was induced by the occlusion of the superior mesenteric artery for half an hour to be followed by a 3-hour-long reperfusion. Agmatine (10 mg/kg) was administered intraperitoneally before reperfusion period. After 180 min of reperfusion period, the contractile responses to both carbachol and potassium chloride (KCl) were subsequently examined in an isolated-organ bath. Malondialdehyde (MDA), reduced glutathione (GSH), and the activity of myeloperoxidase (MPO) were measured in intestinal tissue. Plasma cytokine levels were determined. The expression of the intestinal inducible nitric oxide synthase (iNOS) was also assessed by immunohistochemistry.

Key findings: The treatment with agmatine appeared to be significantly effective in reducing the MDA content and MPO activity besides restoring the content of GSH. The treatment also attenuated the histological injury. The increases in the I/R induced expressions of iNOS, IFN-γ, and IL-1α were brought back to the sham control levels by the treatment as well.

Significance: Our findings indicate that the agmatine pretreatment may ameliorate reperfusion induced injury in small intestine mainly due to reducing inflammatory response and oxidative stress.

1. Introduction

The intestinal ischemia reperfusion (I/R) injury is induced by intestinal transplantation, mechanical obstruction, mesenteric arterial occlusion, hemodynamic shock and several surgical procedures [1]. There are several mechanisms underlying I/R-induced intestinal damage; such as reactive oxygen species (ROS) [2,3], recruitment and activation of neutrophils [2,4], and altered nitric oxide (NO) metabolism [3]. Leukocyte adherence is suggested to have potential for excessive production of ROS and pro-inflammatory mediators such as cytokines [3,5,6]. Moreover, intestinal I/R damage results in increased levels of proinflammatory cytokine, exacerbating tissue injury through local and systemic responses [7]. I/R induces intense functional and structural changes in the intestine which may possibly cause impairment of nutrient absorption, disruption of permeability, bacterial translocation, and gastrointestinal dysmotility [8,9].

Intestinal I/R injury impairment in intestinal motility and the mucosal barrier function [4,10]. Production of ROS, alteration in NO metabolism, and proinflammatory cytokines lead to disturbance of intestinal motility during the process of intestinal I/R injury [2,3]. It is especially known that decreased intestinal motor function is related to lipid peroxidation induced cell membrane damage and an upregulation of the inducible nitric oxide synthase (iNOS) [11].

Agmatine is derived from the decarboxylation of L-arginine by the enzyme arginine decarboxylase [12]. Agmatine is considered as a neurotransmitter/neuromodulator in the body. Moreover, it is an agonist for the α-2-adrenergic and imidazoline receptors [12] as well as an antagonist for the NMDA receptor [13,14]. It also functions as an inhibitor of iNOS, reducing NO overproduction [15–17]. It has been shown that agmatine is quite protective against I/R injuries in many organs such as brain [16,18], spinal cord [19], retina [20], kidney [21], heart [22] and stomach [23]. However, at least to our knowledge, there is not any published study documenting its protective effect on intestinal I/R. Therefore, the present study aimed to evaluate the possible

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http://dx.doi.org/10.1016/j.lfs.2017.08.032
Received 24 July 2017; Received in revised form 25 August 2017; Accepted 29 August 2017
Available online 08 September 2017
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salutary effects of agmatine on I/R injury of the intestine and on the ileal muscle contractility. To reach that purpose, we measured malondialdehyde (MDA), reduced glutathione (GSH) and myeloperoxidase (MPO) activity in intestinal tissue. In addition, plasma concentrations of IFN-y and IL-1α, histopathological changes, expression of iNOS, and contractile responses were determined as well.

2. Materials and methods

2.1. Animals

Thirty-two adult male Wistar Albino rats (240–300 g) were used in the study. The animals were housed at 22 ± 2 °C and exposed to a 12 h/12 h light/dark cycle in a controlled environment provided by the Animal Care Unit of Bulent Ecevit University. The animals were fasted for 12 h with free access to tap water before surgical procedures. The experimental and surgical procedures in this study were authorized by the Animal Ethical Committee of the Bulent Ecevit University.

2.2. Experimental groups and surgical procedures

In all surgical procedures, sodium thiopental (60 mg/kg, ip) was used for inducing anesthesia and maximum care was taken for sterile conditions. The animals were subjected to midline laparotomy incision for induction intestinal I/R injury. The superior mesenteric artery was occluded with a clamp for 30 min to be followed by a 3-hour-long reperfusion period [24]. Agmatin (10 mg/kg) (Sigma-Aldrich Chemical Company; MO, USA) or saline as the vehicle was administered via intraperitoneal injection before the reperfusion period. Immediately after the end of this phase, the tissues of terminal ileum were carefully isolated and collected, which was followed by the evaluation of contractile responses to both carbachol and potassium chloride (KCl) in isolated-organ bath. For biochemical measurements, intestinal tissue samples were obtained and stored at − 80 °C. For histopathological and immunohistochemical analysis, intestinal tissue samples were immediately placed in 10% formalin and then embedded in paraffin. Plasma samples were also collected for the measurement of plasma cytokine levels.

The experimental animals were randomly assigned into four groups: 1) Sham control group: Laparotomy was performed without clamping the SMA and rats received saline only 2) I/R control group: Animals subjected to 30 min SMA occlusion and 3 h reperfusion period and rats received saline only; 3) Agmatine-treated sham control: Laparatomy was performed without clamping the SMA but treated with agmatine; and 4) Agmatine-treated I/R group: Rats exposed to I/R but treated with agmatine. Each group consists of eight animals.

2.3. Ileal longitudinal muscle contractility

The isolated ileal segments collected from the reperfused intestine (10 cm proximal to ileocecal valve) in all groups were removed, and suspended in 20 ml organ bath filled with Krebs solution that was warmed (37 °C) and continually aerated (5% CO_2 and 95% O_2 gas mixture). The solution had the following composition (in mM): NaCl 118.5; KCl 4.8; KH_2PO_4 1.2; $MgSO_4.7H_2O$ 1.2; $CaCl_2$ 1.9; $NaHCO_3$ 25; glucose 10.1. The initial resting tension of ileal segment was arranged to 2 g and lasted for 60 min for equilibration. After the equilibration period, responses to carbachol (10^{-7}–10^{-2} M) were obtained. Following the equilibration period, the contractile response to KCl (final concentration 30 mM) was also recorded by external force displacement transducer (FDA-10A, Commat Iletisim Co., Ankara, Turkey) and MP 30 software (Biopac Systems Inc., Santa Barbara, CA, USA).

2.4. MDA determination

The MDA level was determined in the ileal tissue samples as described previously [25].

2.5. GSH determination

The GSH level of the intestine was performed using Ellman's method, modified as described previously [26].

2.6. Measurement of tissue MPO activity

The MPO activity is generally used for to evaluate the amount of neutrophil accumulation in the tissues. MPO activity of tissue samples was measured with method described by Bradley et al. [27].

2.7. Histopathological examination

Segments of ileum were fixed in 10% paraformaldehyde solution. The tissues embedded in paraffin and stained with hematoxylin and eosin (H & E). Histolopathological scoring was made by one blinded pathologist to the experimental groups. Intestinal injury was evaluated on grading scale ranging from 0 to 4. The intestinal mucosal injury was evaluated according to Hierholzer method [28].

2.8. Immunohistochemical evaluation

After the deparaffinization, the intestinal sections were exposed to 10% hydrogen peroxidase to block endogenous peroxidase activity. The intestinal sections were incubated with specific primary antibody for iNOS (rabbit polyclonal antibody, Lab Vision, Fremont, CA). Sections were then incubated with biotinylated goat antipolyvalent (Lab Vision) for 20 min and streptavidin peroxidase complex (Lab Vision) for 20 min. Finally, 3,3′-diaminobenzidine (DAB) (Lab Vision) was employed as the chromogen. The sections were counterstained with hematoxylin. Evaluation of the sections were assessed by same investigator in blinded fashion. Immunoreactivity was graded using a semiquantitative scale for intensity of staining: 0 (absent); 1 + (few positive); 2 + (medium positive); and 3 + (high positive).

2.9. Plasma cytokine analyses

Plasma sample of animals were collected before sacrifice. They stored at − 80 °C until the determination of plasma cytokine levels. Measurements of rat IFN-γ and IL-1α in plasma were performed through a fluorescent bead immunoassay system, which is commercially available as a FlowCytomix Multiple Analyte Detection Rat Cytokine 6plex kit (eBioscience Bender MedSystems GmbH, Vienna, Austria). The cytokines analyses were made according to the manufacturer's protocol.

2.10. Statistical analyses

Statistical comparisons were assessed with an SPSS 22.0 statistical software program. Data were presented as means ± standard deviations (SD). Differences between groups were evaluated with Kruskal-Wallis followed by a post-hoc Bonferroni test to evaluate the differences with in the groups. A probability value of $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Contractile responses in isolated ileal segments

Carbachol (10^{-7} M–10^{-2} M) caused concentration-dependent contraction on the isolated rat ileal segments. Contractile response was significantly reduced in I/R group compared to that in sham operated control group. After I/R, the inhibition of carbachol-induced contraction was dramatically decreased by agmatine for the responses to

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