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The effects of S-nitrosoglutathione on intestinal ischemia reperfusion injury and acute lung injury in rats: Roles of oxidative stress and NF-κB

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ABSTRACT

Background: Intestinal ischemia and reperfusion (I/R) induces oxidative stress, inflammatory response, and acute lung injury. S-nitrosoglutathione (GSNO), a nitric oxide donor, has been documented to have protective effects on experimental ischemia models.

Aim: The aim of this study was to examine the effect of GSNO on I/R-induced intestine and lung damage and detect the potential mechanisms emphasizing the protective role of GSNO.

Methods: Intestinal I/R was induced by occluding the superior mesenteric artery for 30 min followed by reperfusion for 180 min. GSNO was administered intravenously before reperfusion period (0.25 mg/kg). The levels of lipid peroxidation, reduced glutathione, and myeloperoxidase (MPO), histopathological evaluation and immunohistochemical expressions of both nuclear factor KappaB (NF-κB) and inducible nitric oxide (iNOS) in intestine and lung tissues were assessed.

Results: Histolopathologic evaluation demonstrated that intestinal I/R induced severe damages in the intestine and the lung tissues. Histopathological scores decreased with GSNO treatment. GSNO treatment reduced lipid peroxidation and MPO levels and inhibited expression of NF-κB and iNOS in the intestine.

Conclusion: Our results suggest that GSNO treatment may ameliorate the intestinal and lung injury in rats, at least in part, by inhibiting inflammatory response and oxidative stress.

1. Introduction

Intestinal ischemia and reperfusion (I/R) injury leads to apoptosis, release of proinflammatory cytokines, oxidative stress, production of reactive nitrogen species, intestinal barrier breakdown and bacterial translocation (Li et al., 2017; Mészáros et al., 2017). Moreover, oxidative stress, which produces inflammatory cytokines and reactive oxygen species (ROS), could further exacerbate the intestinal damage (Zu et al., 2016). Intestinal I/R injury is observed in a variety of clinical conditions such as mesenteric artery occlusion, intestinal transplantation, vascular surgery procedures, and trauma (Li et al., 2017; Mallick et al., 2004; Chen et al., 2003).

Intestinal I/R injury has been reported not only to induce local injury of the small intestine but also the production of proinflammatory mediators in the blood stream. These mediators can provoke remote organ dysfunction including acute lung injury (ALI) (Zu et al., 2016; Kim et al., 2012). ALI is characterized by increased pulmonary endothelial leakage and accumulation of inflammatory cells in the lung tissue (Mo et al., 2014; Zhang et al., 2015). Physiopathological mechanisms of ALI include the excessive production of ROS and nitric oxide (NO), sequestration of the polymorphonuclear neutrophils, the release of cytokines and other inflammatory mediators, and the promotion of apoptosis (Zhang et al., 2015; Zhao et al., 2014; Bayomy et al., 2014). ROS have important roles in the occurence of ALI (Kim et al., 2012; Liu et al., 2007). ROS can cause epithelial and endothelial injuries in the lung tissue by provoking several proinflammatory cytokines (Zu et al., 2016). Injury of intestinal barrier function during ischemia causes the translocation of bacteria and endotoxins to the systemic circulation that is associated with the systemic inflammatory response (Sukhotnik et al., 2016). Several studies suggest that excessive production of NO derived by inducible nitric oxide synthase (iNOS) aggrevates the microvascular injury in the lung by ROS (Zhou et al., 2003). S-nitrosoglutathione (GSNO), an endogenous metabolite of glutathione, has been reported to reduce the production of peroxynitrite and to display neuroprotection against ischemic injury (Khan et al., 2009; Shunmugavel et al., 2012). For example, several studies have demonsrated that GSNO reduces neuronal injury after experimental cerebral ischemia and traumatic brain injury models (Khan et al., 2009;

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Khan et al., 2005; Khan et al., 2012; Yin et al., 2013). It is suggested that GSNO has antiinflammatory effect by the inhibiting the NF-κB, cell adhesion molecules, cytokines, and iNOS (Khan et al., 2009; Khan et al., 2012).

The mechanisms of intestinal I/R injury are very complicated and different treatment agents have been tried to reduce intestinal and lung injury in experimental models. The purpose of the present study is to investigate the protective role of GSNO on late period of I/R in intestine and lung injury and to reveal the protective mechanism of GSNO against intestinal I/R injury.

2. Materials and methods

2.1. Animals

Adult male Wistar Albino rats weighing 250–325 were employed in the present study. They were kept under controlled conditions (at temperature of 22 ± 2 °C and 12 h light-dark cycles) and were fed with rat chow diet and tap water. Rats were fasted prior to operation and were allowed just free access to water. The experimental protocols were confirmed by the Bulent Ecevit University of Ethical committee of experimental animals. All chemical purchased from Sigma (Sigma-Aldrich Chemical Company; MO, USA).

2.2. Experimental groups

Twenty four rats were designed into three groups each containing 8 animals: control group, I/R group, and GSNO treated group. Control group: laparatomy was performed without superior mesenteric mesenteric artery (SMA) ligation to be used as the sham-operated group; I/R group: SMA were clamped for 30 min followed by 3 h reperfusion; GSNO-treated group: animals underwent occlusion of SMA. GSNO (0.25 mg/kg) was injected via the tail vein just before the beginning of reperfusion period. Animals in control and I/R groups were received equal volume of saline. The GSNO dose was reported to be neuroprotective in cerebral ischemia model previously (Khan et al., 2015).

2.3. Induction of intestinal I/R injury

Intestinal I/R injury was induced as previously explained (Ozacmak et al., 2014). Briefly, rats were anesthetized with sodium thiopenthal (90 mg/kg) by intraperitoneal injection. Midline abdominal incision was performed and the SMA was isolated and ligated with a microvascular clamp for 30 min. Intestinal ischemia was monitored by disappearance of pulse in the SMA. After ischemia period, the clamp was released and the bowel perfusion was controlled by the presence of pulse in mesenteric circulation. At the end of the 3-h reperfusion period, intestinal and lung tissues were collected for histopathologic and biochemical analyses.

2.4. Biochemical analyses

2.4.1. Myeloperoxidase activity measurement

Myeloperoxidase (MPO) activity were measured in intestine and lung tissues as marker of neutrophil infiltration in to the tissues. MPO activity was measured according to the Bradford method (Bradley et al., 1982). Briefly, tissues were homogenized with potassium phosphate buffer including hexadecyltrimethyl ammonium bromide. After centrifugation, the supernatants were used for the determination of MPO activity in the presence of *o*-dianisidine dihydrochloride. Absorbances were measured at 460 nm (UV- 1280 UV–vis spectrophotometer, Shimadzu, Japan).

2.4.2. Malondialdehyde determination

Malondialdehyde (MDA) reflects lipid peroxidation in tissues and measured by spectrophotometric method (Casini et al., 1986). Briefly, homogenized tissues (with %10 Tricholoroacetic acid) heated with thiobarbituric acid and the absorbances were determined at 535 nm.

2.4.3. Glutathione determination

Reduced glutathione (GSH) levels were measured spectrofotometric method described previously (Aykac et al., 1985). Briefly, the supernatant acquired by the same procedure mentioned above was used for the GSH determination. After adding DTNB solution, the absorbance was measured at 412 nm.

2.5. Histopathological examination

Histolopathologic samples were taken from the terminal ileum and isolated lobe of the lung. The histopathological assesments were performed by two different pathologist in a blinded manner. Tissue samples were fixed formalin solution and embeded paraffin and then sectioned at 4–5 μm thickness. Tissue slices were stained with hemotoxilen and eosine (H&E). The samples were evaluated with light microscopy (Leica DM4000, Wetzlar, Germany). Histopathological evaluation of injury in intestine were performed scoring method defined by Chiu et al (Chiu et al., 1970). Briefly, a normal villus appearance was assigned as 0; Occurrence of subepithelial Gruenenhagen gap and capillary congestion were scored as 1; Increased subepithelial space and separation of epithelial layer from lamina propria observed in score 2; Massive epithelial separation and denudation of some villi tips as considered grade 3; there are denudation on villi and dilated capillary structure scored as grade 4; digestion and decomposation of lamina propria with ulseration and hemorrhage were sored as grade 5.

The score of lung injury was evaluated according to Pirat et al. (2006). Semi-quantitative analyses of injury was performed and scored as absent (0), minimal changes (1), mild (2), moderate (3), or severe injury (4). The lung injury scores were evaluated by neutrophil infiltration, interstitial edema, airway epithelial-cell damage, hyaline membrane formation, hemorrhage, total lung injury score.

2.6. Immunohistochemistry analyses

Immunohistochemical analyses of iNOS and NF-κB were evaluated as previously established. Briefly, following the deparaffinization procedures, tissue section slides were incubated with %10 H_2O_2 to inhibit endogenous peroxidase activity. All immunohistochemical procedures were performed on the automated immunohistochemistry instrument (Roche Ventana, Benchmark ULTRA, USA). The sections were incubated with primary antibodies to NF-κB/p65 (RB-9242-R7, Thermo Scientific/LabVision) and iNOS (rabbit polyclonal antibody, RB-9034-R7, Lab Vision, Fremont, CA, USA). The biotinylated goat antipolyvalen (Lab Vision) was added for secondary antibody. 3,3′-diaminobenzidine tetrahydrochloride was used for color occurence and H&E was performed for counter staining. Pictures were assesed at 40× magnification with a microscope (Nikon E800 Eclipse, Japan). Nuclear and cytoplasmic immunoreactivity was evaluated as staining intensity in semi-quantitative manner and then sections were scored as: 0 (no staining); 1 + (weak staining); 2 + (moderate staining); 3 + (intense staining). NF-κB immunoreactivity was assessed in the cytoplasm of small bowel and lung tissues of I/R and GSNO-treated I/R groups because no nuclear staining was observed. However, both cytoplasmic and nuclear staining were evaluated in the I/R group both tissues.

2.7. Statistical analyses

Statistical analyses were evaluated with an SPSS 22.0 statistical software program. Data were presented as means ± standard error (SEM). Statistical significance was determined using Kruskal-Wallis test for differences between groups followed by a post-hoc Bonferroni test to evaluate the differences with in the groups. $P < 0.05$ was considered statistically significant.