Journal of Surgical Research **164,** e325–e332 (2010)
doi:10.1016/j.jss.2010.08.013

β-Glucan Protects against Lung Injury Induced by Abdominal Aortic Ischemia-Reperfusion in Rats

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Submitted for publication April 28, 2010

Background. **Aortic ischemia-reperfusion (IR) is an important factor in the development of postoperative acute lung injury following abdominal aortic surgery. The aim of our study was to examine the effect of β-glucan on lung injury induced by abdominal aortic IR in rats.**

Material and Methods. **Thirty-two Wistar-albino rats were randomized into four groups (eight per group) as follows: the control group (sham laparotomy), aortic IR (120 min ischemia and 120 min reperfusion), aortic IR + β-glucan (β-glucan 50 mg/kg/d for 10 d was administered orally before IR), and control + β-glucan. Lung tissue samples were obtained for biochemical analysis. Protein concentrations in bronchoalveolar lavage fluid and lung wet/dry weight ratios were measured. Histologic evaluation of the rat lung tissues was also performed.**

Results. **Aortic IR significantly increased the levels of MDA, superoxide dismutase, catalase, and myeloperoxidase ($P < 0.05$ *versus* control).Whereas, β-glucan significantly decreased the lung tissue levels of MDA, superoxide dismutase, catalase, myeloperoxidase, ($P < 0.05$ *versus* aortic IR), and protein concentration in bronchoalveolar lavage fluid as well as wet/dry lung weight ratio. Histologic evaluation showed that β-glucan attenuated the morphological changes associated with lung injury.**

Conclusions. **The results of this study indicate that β-glucan attenuates lung injury induced by aortic IR in rats. We propose that this protective effect of β-glucan is due to (1) reduced systemic inflammatory response, (2) reduced oxidative stress and lipid peroxidation in the lung tissue, (3) reduced pulmonary microvascular leakage, and (4) inhibition of leukocyte infiltration into the lung tissue.**

Key Words: **β-glucan; lung injury; abdominal aorta; ischemia-reperfusion; reactive oxygen species.**

INTRODUCTION

During abdominal aortic surgery, cross-clamping and declamping of infrarenal abdominal aorta cause aortic ischemia and reperfusion (IR) periods, respectively. Aortic ischemia results in an ischemic insult to the lower extremities, and successive reperfusion results in injury to remote organs, including lungs [1]. Lung injury induced by aortic IR predisposes to development of acute lung dysfunction, which is still a frequent complication after elective infrarenal abdominal aortic surgery [2]. Lung injury induced by aortic IR is characterized by infiltration of activated leukocytes, increased oxidative stress, microvascular permeability, and systemic inflammatory response [3, 4]. Reactive oxygen species (ROS), polymorphonuclear leukocytes, complement system, and endothelial cells are the major cellular and humoral factors that are involved in the mechanisms of IR damage [5]. ROS-mediated cellular damage can be anticipated to occur when the oxygen is supplied to the tissue by reperfusion and ROS formation exceeds the high cellular detoxification capacity of lungs. Endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase protect cells from detrimental effects of ROS. Measurement of these enzymes indicates the magnitude of oxidative stress that occurs during IR injury [6]. ROS and ROS-mediated lipid peroxidation have been highly implicated in the pathogenesis of IR and its

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0022-4804/$36.00

complications, and malondialdehyde (MDA) is used as an indicator of the rate of lipid peroxidation [7].

β-Glucans are the glucose polymers derived from the cell wall of baker's yeast (*Saccharomyces cerevisiae*), fungi, and cereal plants [8–10]. Several mechanisms have been proposed for the protective effect of β-glucan molecule. These mechanisms are antibacterial, antifungal [11, 12], antitumoral [13], antilipid [14], antitoxic and radioprotective effects, stimulation of hematopoiesis, and improvement in wound healing process [15, 16]. In addition to these important functions, β-glucan is an effective antioxidant and a free radical scavenger. Investigators focused on β-glucan due to its beneficial effects, and the effects of β-glucan on lung injury have been investigated in experimental sepsis models. It is also reported that β-glucan decreased postoperative infection and mortality rates in major abdominal surgeries [17]. However, the role of β-glucan in infrarenal aortic IR-induced lung injury has not been determined yet. Thus, in the present study, we aimed to investigate possible potential protective effects of β-glucan on lung injury induced by aortic IR in rats. For this purpose, the effect of β-glucan on lung injury was assessed by biochemical and histologic analyses.

MATERIAL AND METHODS

Animal Models

Thirty-two Wistar-Albino rats of both sexes, weighing 200 to 250 g, were used for the experiment. Approval from the Animal Ethics Committee of Suleyman Demirel University Medical School (Isparta, Turkey) was obtained for the experiment (24.02.2009, no. 01/08). The rats were acquired from the university vivarium sources, and each was housed in an individual cage in a temperature and light-dark cycle controlled environment with free access to food and water. All rats received humane care, in compliance with the "Principles Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" documented by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication no. 85-23, revised 1985).

Experimental Design

Rats were randomly allocated to one of four experimental groups (n = 8/group); control group, control + β-glucan group, aortic IR group, and aortic IR + β-glucan group. The control group underwent midline laparotomy and dissection of the infrarenal abdominal aorta (IAA) without occlusion; the aortic IR group underwent laparotomy and clamping of the IAA for 120 min, followed by 120 min of reperfusion; the control + β-glucan group underwent laparotomy and dissection of the IAA without occlusion and received β-glucan; the aortic IR + β-glucan underwent 120 min of ischemia and 120 min of reperfusion and received β-glucan. Before the study, for 10 d, β-glucan capsule (Mustafa Nevzat Company, Istanbul, Turkey) 50 mg/kg was suspended in 2 mL of saline and given by intragastric gavage to the control + β-glucan and aortic IR + β-glucan groups. During the same period, the rats in the control and aortic IR groups also received 2 mL of saline per day. The dose of β-glucan was decided by taking the studies of Sener and Toklu into consideration [18, 19].

Aortic Ischemia-Reperfusion

Rats were anesthetized with ketamine hydrochloride (Ketalar, 50 mg/kg intramuscular Eczacıbaşı, Istanbul, Turkey) and anesthesia was maintained with supplementary intramuscular injections of ketamine hydrochloride [1]. Rats were placed supine under a heating lamp. The skin was aseptically prepared and a midline laparotomy was performed. Warm normal saline (10 mL) was instilled into the peritoneal cavity to help maintain fluid balance. The abdominal aorta was exposed by gently deflecting the loops of the intestine to the left with moist gauze swabs. An atraumatic microvascular clamp (Vascu-statts II, midi straight 1001-532; Scanlan Int., St. Paul, MN) was placed across the IAA. The abdomen was closed and the wound covered with plastic wrap to minimize heat and fluid losses. After 120 min, the microvascular clamp on the IAA was removed and the lower limbs were reperfused for 120 min. Aortic occlusion and reperfusion were confirmed by the loss and reappearance of satisfactory pulsation on the distal aorta. Thus, no-reflow phenomenon was excluded. At the end of reperfusion, a median sternotomy was done and blood samples were drawn from the right ventricles of all rats for biochemical analyses. A tracheostomy was performed using a 16-gauge intravenous cannula for the purpose of bronchoalveolar lavage (BAL). The left main bronchus and right lower lobe were clamped. The right upper and middle lobes were lavaged three times with 2 mL of saline containing 0.07 mmol/L ethylenediamine tetraacetic acid. This lavage fluid was cold-centrifuged at 1,500 × g for 20 min and frozen at –20°C. BAL fluid was subsequently used for measurement of protein concentration (BALprot) by the method of Lowry *et al.* [20]. The left upper lobe was removed for biochemical analyses and left lower lobe was removed for histological analyses. The wet/dry ratio of the right lower lobe was calculated after weighing the freshly harvested organ and heating it at 90°C in a gravity connection oven over a 72 h period until such time as the weight of the remaining residue was found to be constant. All rats were killed under anesthesia, and the specimens were harvested and allocated for further biochemical and histologic analyses.

Biochemical Analyses

Harvesting of the Tissue

Frozen tissue samples of the rat lung were weighed and homogenized (Ultra Turrax T25; Janke and Kunkel GmbH and Co., KG, Staufen, Germany) (1:10, wt/vol) in 100 mmol/L phosphate buffer (pH 7.4) containing 0.05% sodium azide in an ice bath. The homogenate was sonicated (Bandelin Sonoplus UW 2070; Berlin, Germany) for 30 s and centrifuged at 5000 g for 10 min. The supernatant was frozen at –78°C in aliquots until used for biochemical assays. The protein content of the supernatant was determined by the Lowry method [20]. In tissue samples, the levels of malondialdehyde (MDA), superoxide dismutase, catalase, and myeloperoxidase were measured.

Malondialdehyde

MDA levels, an indicator of free radical generation, were measured by the double heating method of Draper and Hadley [21]. The principle of the method is the spectrophotometric measurement of the colour produced by the reaction of thiobarbituric acid with malondialdehyde. For this purpose, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL supernatant in each centrifuge tube. The tubes were placed in a boiling water bath for 15 min and then cooled in tap water. The tubes were centrifuged at 1000 g for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L thiobarbituric acid solution, and was placed in a boiling water bath for 15 min. The solution was then cooled in tap water, and its absorbance was measured with a spectrophotometer (Shimadzu UV-1601; Kyoto, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the malondialdehyde-thiobarbituric acid