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The protective effects of sildenafil in acute lung injury in a rat model of severe scald burn: A biochemical and histopathological study

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ABSTRACT

Severe burn induces biochemical mediators such as reactive oxygen species that leads to lipid peroxidation which may have a key role in formation of acute lung injury (ALI). Sildenafil is a selective and potent inhibitor of cyclic guanosine monophosphate specific phosphodiesterase-5. Sildenafil preserves alveolar growth, angiogenesis, reduces inflammation and airway reactivity. The purpose of the present study was to evaluate the effects of different dosages of sildenafil in ALI due to severe scald burn in rats. Twenty-four rats were subjected to 30% total body surface area severe scald injury and were randomly divided into three equal groups as follow: control, 10 and 20 mg/kg sildenafil groups. Levels of malondialdehyde (MDA), activities of glutathione peroxidase (Gpx), catalase (Cat), total oxidative stress (TOS), and total antioxidative capacity (TAC) were measured in both tissues and serums. Oxidative stress index (OSI) was calculated. A semi-quantitative scoring system was used for the evaluation of histopatological findings. Sildenafil increased Gpx, Cat, TAC and decreased MDA, TOS and OSI. Sildenafil decreased inflammation scores in lungs. Our results reveal that sildenafil is protective against scald burn related ALI by decreasing oxidative stress and inflammation and the dosage of 10 mg/kg could be apparently better than 20 mg/kg.

1. Introduction

Severe burn causes a posttraumatic inflammation that leads to systemic inflammatory response syndrome (SIRS), sepsis, and multiple organ dysfunction syndrome (MODS), especially acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) which are common causes of morbidity and mortality [1]. The mechanism behind the ALI/ARDS due to severe burn remains unclear. However, previous studies demonstrated a variety of biochemical mediators are involved in these pathophysiological mechanisms and the development of ALI related burn [1–4]. Transportation of elevated pro-inflammatory mediators released from both gut and burn site to lung by intestinal lymphatic system could play a key role in ALI/ARDS formation [5]. These mediators induce neutrophil sequestration as well as activation of endothelial cells in lungs. Systemic neutrophil sequestration causes cellular

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damage in lung tissue due to lipid peroxidation by promoting the production of reactive oxygen species (ROS) [1,6]. The proinflammatory effects of ROS include endothelial damage, neutrophil reinforcement, cytokine release, and mitochondrial injury [7]. Thus, ALI/ARDS following burn appears to be mediated by ROS via the formation of super oxygen radicals [1].

Effects of various agents on oxidative damage due to severe burn were evaluated in remote organs including lungs in previous studies [3,4,8–12]. Sildenafil is a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase-5 (PDE-5). PDE-5 catalyzes the hydrolysis of cGMP. Inhibition of PDE-5 causes increased concentration of c-GMP and cyclic adenosine monophosphate (cAMP). The cyclic nucleotides c-AMP and c-GMP are known as second messengers which have major roles in various cellular processes, like inflammation [13]. Sildenafil induces endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), which generate NO. Thus, sildenafil has a relaxant effect on pulmonary vascular smooth muscles as well as smooth muscle cells of the arterioles via nitric oxide (NO)-depended mechanism [14]. This agent also preserves alveolar growth and angiogenesis [15], reduces inflammation and airway reactivity in animal models [16]. Additionally, as a PDE-5 inhibitor, sildenafil is expected to potentiate platelet inhibition and improve the microcirculation. It was shown that sildenafil therapy significantly improved postoperative skin flap viability by vasodilatation and platelet inhibition [17] as well as promoting neovascularization on healing left colonic anastomoses [18]. Furthermore, positive effects of sildenafil have been demonstrated in oxidation–antioxidation balance by reducing oxidative stress in inflammatory events [16,18–23]. However, there is no study in the literature using sildenafil in order to prevent severe burn induced ALI. The purpose of present study was to evaluate the effects of different dosages of sildenafil in ALI/ARDS due to severe scald burn in a rat model.

2. Materials and methods

The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23, revised 1996) and approval has been received from Institutional Animal Ethics Committee at Cumhuriyet University.

2.1. *Animals*

A total of 32 adult female Wistar Albino rats weighing between 200 and 250 g were included in the study. Animals were provided by the Experimental Animals Center, Cumhuriyet University, Sivas, Turkey. The animals were fed *ad libitum* with standard diet and water throughout the experiment. All animals were housed separately and kept under standard conditions of room temperature (22–24 °C) and a 12 h light/12 h dark cycle.

2.2. *Burn procedure*

The dorsal surfaces of rats were shaved closely, and rats were secured in a constructed template device. The surface area of the skin on the dorsal surface exposed through the template device was immersed in 98 °C water for 12 s. All test animals were quickly dried after each exposure to avoid additional injury. With the use of this technique, full-thickness dermal burns comprising 30% of the total body surface area (TBSA) were obtained [3].

2.3. *Anesthesia and management of pain*

Animals were anesthetized with i.p. xylazine (5 mg/kg) and ketamine (30 mg/kg) during the scalding and burn procedure, and 1 mg/kg morphine was administered intra-muscular just before immersing each of them to the boiling water.

2.4. *Chemicals*

All the chemicals used in experiments were purchased from Sigma Chemical Co. (Munich, Germany). Sildenafil was obtained from Pfizer (Istanbul, Turkey).

2.5. *Experimental design*

Animals were randomly divided into equal four groups as follows: group S (no burn, no medication), group I (scald control) were administrated perorally (p.o.) 2 ml 0.09% NaCl, group II: 10 mg/kg p.o. sildenafil, and group III: 20 mg/kg p.o. sildenafil just after the scald burn (Fig. 1). All animals were administered 2 ml/100 g body weight of lactated Ringer's solution subcutaneously just after the burn for fluid resuscitation. Then, all animals were located in their own cages and let them free to reach food and water. The reason for the

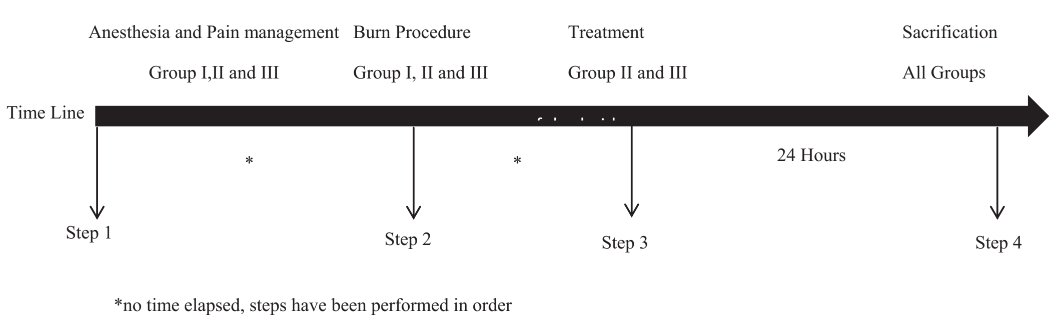

Fig. 1 – Schematic view of the experiment.