

Higenamine Decreased Oxidative Kidney Damage Induced By Ischemia Reperfusion in Rats

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Abstract

The aim of this research is to determine protective effects of higenamine on kidney tissue injury caused by ischemia reperfusion. In this study, 24 Sprague Dawley female rats were divided into 3 groups. The groups were designed as follows; control, ischemia reperfusion, and ischemia reperfusion + higenamine. Some oxidant, antioxidant and inflammatory parameters were evaluated in kidney tissues at the end of the experimental procedure. It was confirmed that the oxidant and inflammatory parameters of kidney tissue increased and antioxidant parameters decreased in ischemia reperfusion group compared to control group. Antioxidant parameters increased while oxidant and inflammatory parameters decreased in the ischemia reperfusion + higenamine group compared to ischemia reperfusion group. These results have demonstrated that higenamine administration as single dose is effective against oxidative kidney damage originating from ischemia reperfusion.

Keywords: Ischemia reperfusion, Higenamine, Kidney, Oxidative stress, Inflammation, Rat

Higenamin Ratlarda İskemi Reperfüzyonunun Neden Olduğu Oksidatif Böbrek Hasarını Azaltır

Öz

Bu araştırmanın amacı, higenaminin, iskemi reperfüzyonunun neden olduğu böbrek dokusu hasarı üzerine koruyucu etkilerini belirlemektir. Bu çalışmada 24 adet Sprague Dawley dişi sıçan üç gruba ayrıldı. Bu çalışmanın grupları aşağıdaki şekilde tasarlanmıştır; kontrol, iskemi reperfüzyonu ve iskemi reperfüzyonu + higenamin grupları. Deney sonunda elde edilen böbrek dokularındaki bazı oksidan, antioksidan ve inflamatuvar parametreler değerlendirildi. İskemi reperfüzyon grubu kontrol grubu ile kıyaslandığında, böbrek dokudaki oksidan ve inflamatuvar parametrelerin arttığı fakat antioksidan parametrelerin azaldığı belirlendi. Tedavi grubu (iskemi reperfüzyonu + higenamin) yalnızca iskemi reperfüzyon grubu ile kıyaslandığında antioksidan parametreler artarken, oksidan ve inflamatuvar parametreler azaldı. Bu sonuçlar, tek doz higenamin uygulamasının, iskemi reperfüzyon kaynaklı oksidatif böbrek hasarına karşı etkili olduğunu göstermiştir.

Anahtar sözcükler: İskemi reperfüzyon, Higenamine, Böbrek, Oksidatif stres, İnflamasyon, Sıçan

INTRODUCTION

Decreasing the blood flow, reperfusion and systemic inflammatory response may lead to kidney ischemia reperfusion (IR) injury ^[1]. Acute kidney injury (AKI) is related to a severe mortality, great economic, and social burdens, particularly in critically ill cases ^[2-4]. In the long term, AKI

may cause chronic kidney disease and end-stage kidney disease ^[5]. Reactive oxygen species (ROS), are related to the early phase of inflammation, necrosis and apoptosis in kidney IR injury ^[6]. It has been suggested that increased ROS production during kidney IR is one of the most important reasons of kidney damage with extensive interstitial edema, tubular flattening with brush border



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microvilli loss, tubular dilation, brush border shedding, casts and obstruction [7,8]. Elevated malondialdehyde (MDA) levels were shown due to IR injury and activities of antioxidants (ROS scavenger) such as catalase (CAT) and superoxide dismutase (SOD) were determined to be essential to prevent the toxic effects of MDA [9]. MDA is a lipid peroxidation product and it is used to evaluate the oxidative stress levels in *in vitro* and *in vivo* conditions [10]. Linas et al. [11] indicated that kidney IR injury was aggravated by activated neutrophils. Neutrophil activation is related with myeloperoxidase (MPO). Furthermore, proinflammatory cytokine and ROS production is associated with active neutrophils [12]. IR injury aggregates inflammatory cells, releases inflammatory factors (TNF- α , IL-8, and IL-6, etc.) and increases adhesion molecules [13,14]. Up to day, there has been no efficient therapy against kidney IR injury [15,16].

Higenamine (Hig) (1-[4-hydroxyphenyl)methyl]-1,2,3,4-tetrahydroisoquinoline-6,7-diol) is an alkaloid and was first derived from *Aconitum* [17]. As a source for higenamine, *Aconitum japonicum* Thunb has been used for collapse, tumor, bronchial asthma, rheumatic fever, edema, painful joint, and syncope treatment for centuries in China and Japan [18]. In 1976, aconite root was used for the first isolation of higenamine [19]. Higenamine has pharmacological features including immunomodulatory, antiapoptotic, anti-inflammatory and antithrombotic effects [17]. This research was planned to detect the protective effect of higenamine against kidney oxidative damage induced by IR.

MATERIAL and METHODS

This study was initiated with approval (2019-69) of Atatürk University Experimental Animals Local Ethics Committee. Experimental phase of the present research was performed at Atatürk University Experimental Animal Research and Application Center and the animals were supplied from the same place. Animals were kept in standard cages in laboratory environment provided with humidity, 20-22°C temperature and 12 h light/dark cycle control. They were fed with standard pellet feed and water. They were fasted 12 h before the experiment to prevent anesthesia complications.

Groups and Ischemia Reperfusion Model

All procedures were performed under anesthesia of 10 mg/kg *i.p.* xylazine hydrochloride (Rompun[®], Bayer, Istanbul) and 60 mg/kg *i.p.* ketamine (Ketalar[®], Pfizer, Istanbul). The number of animals and there should be at least 8 animals in each group if the animals were divided into 3 groups were determined by 5% deviation, type 1 error (α) 0.05 and type 2 error (β) (Power = 0.80) power analysis. Three groups each containing 8 Sprague Dawley female rats (240 \pm 10 g) were set. Control group; the back region was shaved, cleaned and opened with an incision under anesthesia and then closed without IR model or a medication. I/R group; the incision area was cleaned with povidone iodine, opened

with an incision under anesthesia and then, bilateral kidney arteria and veins were blocked with an atraumatic microvascular clamp for 1 h. In reperfusion period, blood circulation was allowed for 24 h by opening the clamps. Incision closed with silk 3/0 suture. IR + Hig group; higenamine was administered to rats intraperitoneally at a dose of 10 mg/kg 30 min before reperfusion. Later as described in I/R group, the IR model was created. At the end of the experiment, the right kidneys of all decapitated rats were collected with capsulespa. Finally, when the experiment ended, the kidney tissues were washed and kept frozen until the biochemical analysis. Higenamine was purchased from Sigma Aldrich (Missouri, USA).

Analysis of Biochemical Parameters

The kidney samples (right kidney samples with capsules, each sample 100 mg) were homogenized with phosphate buffer (2 mL). The homogenized kidney tissues were centrifuged at 5000 rpm at +4°C for 20 min; and the supernatants obtained in this way were transferred to microcentrifuge tubes. Kidney tissue samples were processed for MDA assay to determine lipid peroxidation status as described by Ohkawa et al. [20]. The results were given in μ mol/g protein. SOD activity was measured as defined by Sun et al. [21]. The results were presented in U/mg protein. We also quantified kidney injuries by measuring tissue MPO activity, using a protocol developed by Bradley et al. [22]. The results of MPO activity in tissue samples were presented in U/g protein. The total antioxidant status (TAS) value was evaluated with a commercially available kit (Rel Assay Diagnostics). Total oxidant status (TOS) measurement was done with a commercially available kit (Rel Assay Diagnostics). TAS and TOS results were presented as nmol/L. TOS to TAS ratio was accepted as the oxidative stress index (OSI). OSI level was detected as follows: $OSI = [(TOS, \mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / (TAS, \text{mmol Trolox equivalent/L}) \times 10]$. OSI has been proposed to be better in demonstrating the oxidative state more precisely compared to TOS value [23].

Statistical Analysis

Statistical analysis was performed using IBM SPSS Package Program (version 22.0). Experimental results were reported as mean \pm standard deviation. The normality assumption was confirmed by the Kolmogorov Smirnov test. One-way ANOVA was used to compare the experimental groups with the control. Multiple comparisons were made using the Post hoc Tukey test.

RESULTS

While TAS value decreased significantly, TOS and OSI levels increased in I/R group compared to control group. TOS and OSI values decreased but TAS level increased in I/R + Hig group compared to group I/R (Table 1, $P < 0.05$).

Table 1. TAS (mmol/L), TOS ($\mu\text{mol/L}$) and OSI values of control, I/R and I/R+Hig groups			
Groups/Parameters	Control	I/R	I/R+Hig
TAS (mmol/L)	2.60 \pm 0.15	1.39 \pm 0.15 ^a	2.53 \pm 0.15 ^b
TOS ($\mu\text{mol/L}$)	7.09 \pm 0.53	10.05 \pm 0.93 ^a	7.50 \pm 0.68 ^b
OSI	0.27 \pm 0.02	0.72 \pm 0.10 ^a	0.29 \pm 0.03 ^b

The results are presented as mean \pm SD (n=8). ^aP<0.05 versus to control group, ^bP<0.05 versus to I/R group

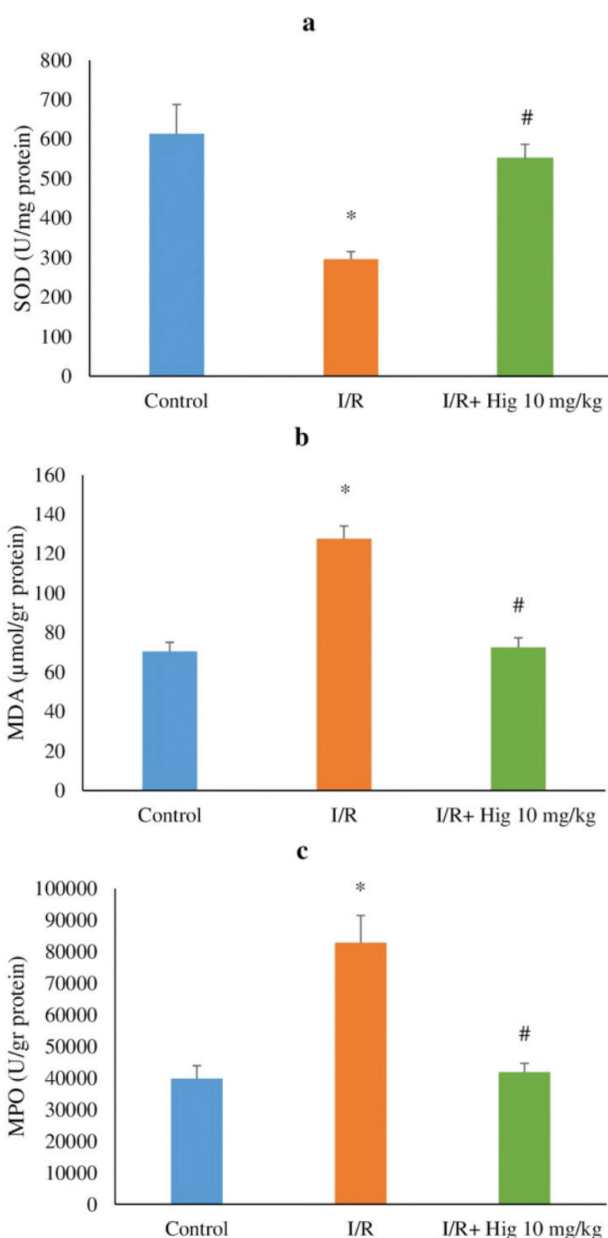


Fig 1. Biochemical parameters in kidney tissue. a- SOD levels, b- MDA levels and c- MPO activity. Data are expressed as mean \pm SD; * P<0.05 versus to control group, #P<0.05 versus to I/R groups

Superoxide dismutase enzyme activity increased in I/R + Hig group compared to I/R group. The antioxidant and anti-inflammatory properties and protective effects of higenamine against IR-induced kidney damage have been demonstrated by biochemical results (as shown in Fig.

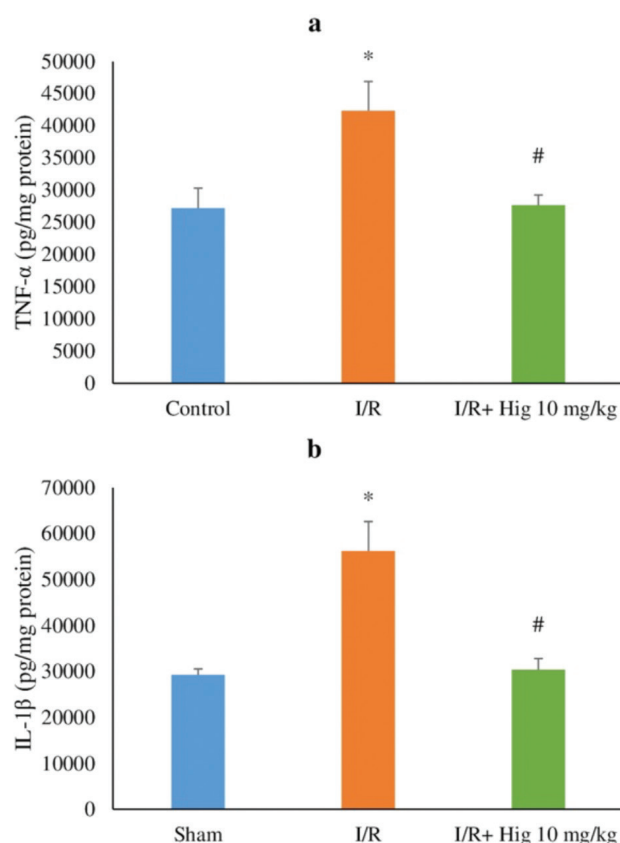


Fig 2. Inflammatory markers in kidney. a- TNF- α levels and b- IL-1 β levels. Data are expressed as mean \pm SD (n=8). * P<0.05 versus to control group, #P<0.05 versus to I/R groups

1a). However, MPO activity (Fig. 1b), MDA (Fig. 1c), TNF- α (Fig. 2a) and IL-1 β (Fig. 2b) levels increased in I/R group compared to control group and decreased in I/R + Hig group compared to IR group (P<0.05).

DISCUSSION

Acute kidney injury, especially in developing countries, is related to mortality and morbidity [24]. AKI usually occurs due to IR injury [25]. Kidney IR injury is a major reason for AKI with various origins such as kidney transplantation, shock and low cardiac output [26]. Kidney injury following kidney transplantation may also lead to kidney IR injury [27]. In the reperfusion phase, oxygen derived free radicals occur [28]. Tissue injury induced by IR injury is based on oxidative stress and this condition is supported with a strong body of evidence [29]. To remove toxic ROS, cells have several

natural defense systems, including SOD enzyme. Increased ROS that is generated during IR may cause endogenous antioxidant depletion^[30]. The protecting enzymes (SOD, CAT) perform against the devastating actions of ROS and these molecules comprise TAS. TAS measurement provides the evaluating of all antioxidant levels in a biological sample^[31]. TOS to TAS ratio is confirmed as OSI, which is an indication of oxidative stress^[32]. Reaction between ROS and lipids causes lipid peroxidation in biological membranes during kidney IRI^[33] and ultimately, enzymatic actions like ion pump activity (plays role on inhibition of DNA transcription and repair) is affected. If an uncontrolled lipid peroxidation continues, it may end with cell death^[34,35]. MDA, bioproducts of lipid peroxidation, indicates oxidative stress. CAT and SOD indirectly show free radical generation ability. These are positive and negative markers for oxidative stress level^[36]. MDA was clearly increased in a kidney IR model in rats^[37].

The inflammatory response is another important part of the pathophysiology implicated in kidney IRI^[38]. Some proinflammatory cytokines such as IL-2, IL-6, TNF- α and IL-1 β are released during kidney IRI^[39,40]. TNF- α takes an important part in the beginning and continuation of the inflammatory response. Further, TNF- α could lead to endothelial damage, apoptosis and even kidney failure^[41]. IL-1 is a proinflammatory cytokine involved in several inflammatory processes^[42]. When the inflammatory response is regulated at the early stage effectively, this presents a vital step for prevention and treatment of kidney injury^[43,44].

The present study is the first report about the positive effect of higenamine on the kidney IR. There is no study available in the literature showing the same effect and study models. However, there are several studies showing the antioxidant and antiinflammatory properties of higenamine that support the results of this study. In the present study, reduction of IL-1 β and TNF- α levels in kidney IR model in rats by higenamine, suggesting that higenamine decreased IR-induced kidney injury. In a rat model of cerebral IR, higenamine improved functional state of nerves and significantly stopped the increase in TNF- α , IL-1, and IL-6 levels while decreasing the axonal nerve degeneration^[45]. Induction of ROS and MDA production, and SOD activity inhibition arising from neuronal cell injury triggered by oxygen-glucose deprivation/reperfusion were attenuated by higenamine^[46]. Higenamine increased antioxidant level and reduced MDA, TNF- α and IL-1 β levels in a collagen-induced arthritis study^[47]. According to published reports, higenamine possesses a variety of pharmacological properties, including dilation of blood vessels and bronchi, immuno-modulation, antiinflammation, antiapoptosis and anti-oxidation^[18]. In parallel with these studies, in the present study antioxidant and antiinflammatory properties of higenamine have been shown in kidney IR model in rats. In IR group, TAS and

SOD values decreased while TOS, OSI, MDA, MPO, TNF- α , and IL-1 β levels increased compared to control group and higenamine treatment reversed these levels.

Oxidative stress in kidney tissue was assessed to determine the possible mechanisms of the protective effect of higenamine against IR-induced kidney injury and it was observed that oxidative stress decreased with higenamine. To make effective changes in the clinical management of IR, the pathogenesis of IR-induced organ damage should be better understood for the development of therapeutic strategies. Clearly observed in IR studies is that suppression of inflammation and oxidative stress can provide significant contributions to IR treatment. In the present study, inflammation and oxidative stress pathways are suppressed by higenamine and this promises hope in the treatment of IR.

Higenamine provides a protection against IR-induced kidney injury with its antioxidant and antiinflammatory properties. We have indicated that treatment with higenamine reduces kidney injury in experimental animals exposed to IR model. Moreover, further researches are necessary to explain the other protective mechanisms in IR-induced kidney tissue injury.

CONFLICT OF INTEREST STATEMENT

None.

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