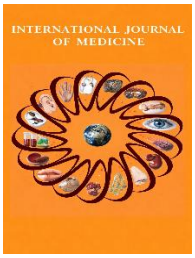

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Original Article

Renoprotective effects of ellagic acid on renal ischemia-reperfusion-induced

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Abstract

Background: This experimental study examines the potential protective features of ellagic acid (EA) on renal tissue injury caused by ischemia-reperfusion.

Methods: In the experimental process, animals were assigned to three groups including sham, ischemia-reperfusion (I/R), and I/R+EA 75 mg/kg (treatment group). Following the experiment, renal tissue samples were collected, several oxidative and antioxidant parameters were analyzed.

Results: When the I/R group is compared to the sham group, the oxidant and inflammatory parameters elevated while antioxidant parameters performed declining. On the other side, antioxidant values increased and oxidant, inflammatory parameters decreased in the treatment group when it is compared to the I/R group. Also, the HAVCR1 immunopositivity of the I/R group was severe level while it was diminishing in the treatment group.

Conclusions: These results demonstrated that EA administration is effective against oxidative renal damage induced by I/R.

Keywords: ellagic acid; inflammation; kidney; oxidative stress

1. Introduction

Renal ischemia-reperfusion (I/R) injury is an important reason for acute kidney injury (AKI) which can be observed in several clinical conditions including shock and renal transplantation [1,2]. AKI is characterized by renal function loss and high morbidity rate [3]. I/R injury

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occurs in case of rapid recirculation following ischemia [4]. Following the recirculation, reactive oxygen species (ROS) and inflammatory substances reach excessive levels and it exacerbates the injury [5].

ROS enhance oxidative stress level and inflammatory cytokine production [6]. I/R-induced ROS generation activates leukocyte infiltration which enhances more ROS and cytokine expression [7]. ROS are metabolic products and scavenged by antioxidant enzymes including superoxide dismutase (SOD) [8]. During the reperfusion stage, blood flow restarts and it enhances the ROS production. Excessive ROS levels overcome scavenging capacity and lead to injury. Malondialdehyde (MDA) occurs due to lipid peroxidation and reflects oxidative stress [9].

Different agents with anti-inflammatory, antioxidant, and radical scavenging properties have been examined against I/R injuries [10-13]. Natural plant-based studies were performed because of their low side effects and strong therapeutic features [14]. Ellagic acid (EA) is a natural agent and it is frequently in various nutrients including tea, berries, nuts, and grapes [15]. This research was performed to find out the possible protective effects of EA against renal oxidative damage induced by I/R.

2. Methods

2.1. Experimental animals and ethical approval

The current study was confirmed by Atatürk University Experimental Animal Ethics Committee (protocol no: 02.03.2018-52). Experimental steps of the study were carried out at Atatürk University Experimental Animals Research and Application Center and rats were acquired from the same center. They were housed in appropriate laboratory conditions including humidity, light/darkness cycle, and temperature. Rats had access to both water and food ad libitum. Twelve hours before the experiment, they were debarred from food but were allowed to drink water. This study was conducted in accordance with the Declaration of Helsinki.

2.2. Groups and experimental design

The animals that exposed to surgical procedure were fixed in face-down positioning (FDP), shaved, and disinfected. Povidone-iodine was preferred for disinfection. And also, anesthesia was applied to animals before the surgical intervention. 10 mg/kg xylazine hydrochloride (Rompun®, Bayer, Istanbul) and 60 mg/kg ketamine (Ketalar®, Pfizer, Istanbul) were preferred for the anesthesia (intraperitoneally, i.p.). EA was purchased from Sigma Aldrich, USA. Twenty-four male Wistar Albino rats at 220-260 g weights were grouped as;

1. Sham group: No intervention was carried out on animals. Only the back region was incised and sutured via 3/0 silk suture.
2. I/R Group: Same steps were followed by the sham group. After incision, renal arteries and veins were occluded by using microvascular clamps, and ischemia was made for 60 minutes. Then, the clamps were released and the renal blood flow was recovered for 24 hours.
3. I/R+EA 75 mg/kg Group (EA 75 mg/kg Group): All procedures were performed as described in the I/R group. In addition, a single dose of 75 mg/kg EA was applied i.p. to the animals prior to reperfusion.

Following the experiment, the animals were sacrificed by anesthesia and renal tissues were removed.

2.3. Biochemical analysis

Each renal tissue sample was weighed as 100 mg and 2 mL of phosphate buffer solution (PBS) was used for the homogenization. After the homogenization, samples were centrifuged and kept at -80 ^{0}C. MDA measurement depends on the determination of the compound which occurs due to the reaction between thiobarbituric acid (TBA) and MDA [16]. Total antioxidant status (TAS) and total oxidant status (TOS) were gauged with appropriate kits (Rel Assay Diagnostics). Oxidative stress index (OSI) means the rate of TOS to TAS. Tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) values were gauged via appropriate kits (Elabscience, Wuhan, China). Myeloperoxidase (MPO) was determined with the measurement of the complex which results from the reaction between o-dianisidine and MPO [17]. The activity of SOD was gauged with the inhibition degree of formazan dye formation [18].

2.4. Immunohistochemical (IHC) examination

Kidney tissue samples were placed in 10% neutral formalin for 2-4 days and embedded into paraffin blocks. 5-µm thick sections were stained as immunohistochemical and investigated under a microscope. Following the deparaffinization by xylene and rehydrated in graded alcohols, sections were incubated for 10 minutes in 0.3% H_2O_2 to quench the activity of endogenous peroxidase. Sections were heated to reveal antigens in the tissue in an antigen retrieval solution for ten minutes. They were subjected to anti-hepatitis A virus cellular receptor 1 (HAVCR1) (Cat. no.bs-2713R, dilution 1/200; Bioss, USA). After washing in PBS, incubation was done with exposing mouse and rabbit specific HRP/DAB detection IHC kit (Abcam: ab80436), the secondary antibody. As a chromogen, 3,3′diaminobenzidine (DAB) was applied and sections were counterstained with hematoxylin. Positive cells in immunostaining sections were examined under a light microscope. Immunoreactivity was evaluated as 0 (none), 1 (mild), 2 (moderate), and 3 (severe).

2.5. Statistical analysis

SPSS statistical software (SPSS for Windows, version 20.0) was used for analysis. The data of biochemical parameters were demonstrated as mean ± standard deviation (SD) by applying the One-Way ANOVA test. Tukey test was preferred for intergroup comparisons. All immunohistochemical data were shown in mean (±) standard error (S.E.). Differences between the groups were determined via Kruskal-Wallis. Dual comparisons among groups were determined via Mann-Whitney U-test. The differences were admitted significant when $p<0.05$.

3. Results

3.1. Biochemical results

When the I/R and sham groups were compared, TAS and SOD values decreased while TOS, MDA, MPO concentrations, and OSI values elevated in the I/R group. All these parameters were reversed significantly in EA applied group when it is compared to the I/R group [Table 1].

Table 1. Comparisons of TAS, TOS, OSI, SOD, MPO, and MDA parameters among the experimental groups

Experimental groups (n=8)	Sham (1)	I/R (2)	EA 75 mg/kg (3)
TAS (mmol/L)	2.38 ± 0.34	1.92 ± 0.17[a]	2.44 ± 0.29[b]
TOS (µmol/L)	8.35 ± 1.4	11.07 ± 1.89[a]	8.30 ± 0.89[b]
OSI (arbitrary unit)	0.35 ± 0.08	0.57 ± 0.11[a]	0.34 ± 0.05[b]
SOD (U/mg protein)	295.37 ± 13.34	203.33 ± 11.62[a]	298.93 ± 20.24[b]
MPO (U/g protein)	34820.78 ± 7390.79	87362.41 ± 10092.62[a]	35342.77 ± 5955.54[b]
MDA (µmol/g protein)	48.62 ± 5.59	73.73 ± 9.44[a]	46.72 ± 6.55[b]

Results were expressed as Mean ± S.E.
Data were analyzed by one-way ANOVA followed by Tukey as post-test.
[a]$p<0.001$ compared to the sham group. [b]$p<0.005$ compared to the I/R group.

3.2. Effect of EA on inflammatory markers

Renal I/R promotes a marked increase in TNF-α and IL-1β levels [Figure 1a and 1b] in renal tissue. On the other hand, the i.p. administration of EA increased the cytokine levels.

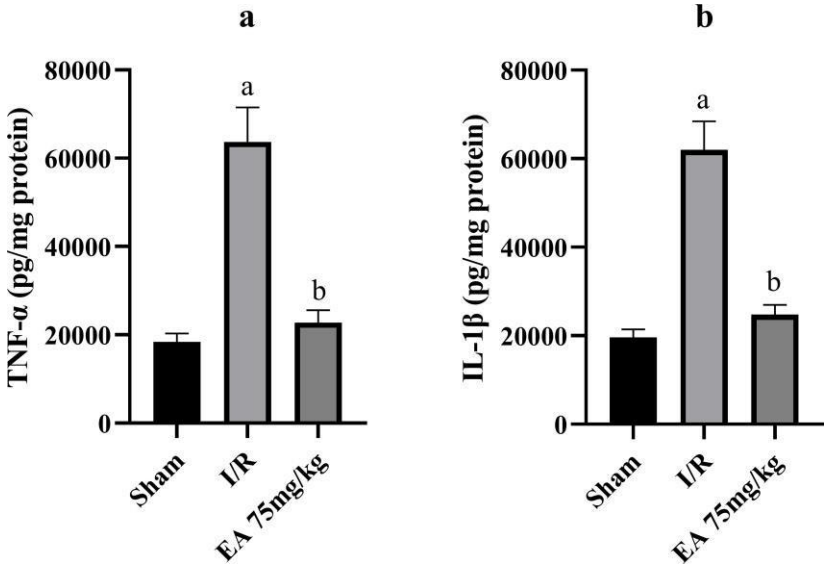

Figure 1. Effect of EA on RIR-induced changes in proinflammatory cytokines: (a) TNF-α and (b) IL-1β levels. Results were expressed as Mean±S.E. n=8. Data were analyzed by one-way ANOVA followed by Tukey as post-test. [a]$p<0.001$ compared to the sham group, [b]$p<0.001$ compared to the I/R group.

3.3. Immunohistochemical examination

A statistically significant difference was detected in immunohistochemical staining with HAVCR1 ($p<0.05$). While HAVCR1 immunopositivity was not observed in the sham group, it was intensive in tubular epithelial cells of I/R and EA treatment groups. But the immunopositivity was less in the EA treatment group when it is compared to the I/R group [Figure 2].

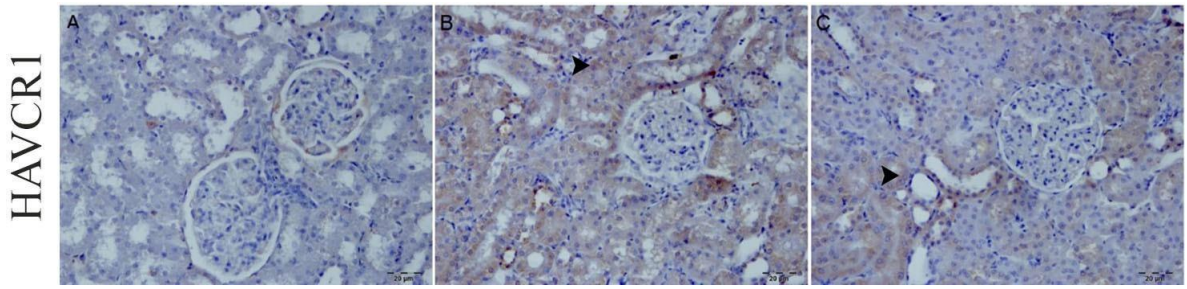

Figure 2. A) Sham group, B) I/R group, intensive HAVCR1 immunopositivity in tubular epithelial cells C) EA 75 mg/kg group, mild HAVCR1 immunopositivity in tubular epithelial cells (arrowhead). IHC (20X magnification).

4. Discussion

Ischemia causes permanent damage in tissues through decreased blood flow [19]. Reperfusion also leads to severe tissue injury [20]. I/R-induced AKI is a serious clinical condition that influences about %10 of hospitalized patients [21,22]. I/R-induced renal injury is one of the most common reasons for acute renal insufficiency and results from several conditions including renal transplantation and shock [23,24]. ROS related cellular damage ends up with I/R injury [25]. Lipid peroxidation is closely related to I/R-induced tissue injury and MDA is used to point out lipid peroxidation and to reflect the oxidative stress [26]. MDA occurs due to lipid peroxidation and frequently preferred to show ROS activity indirectly [27,28]. Here, MDA showed an increase in the I/R group while there was a significant decrease in the EA treatment group. MDA is a part of various studies about renal I/R [29,30]. Antioxidant systems including SOD take a role in the protection of tissues against ROS and oxidative damage. In the current study, the SOD level increased with EA treatment. If the oxidant system overcomes the antioxidant activity, this leads to oxidative stress. OSI reflects the oxidative stress degree [31,32]. TAS measurement demonstrates the entire antioxidant activity in a biological sample [33]. The oxidative balance improved in favor of antioxidants in the current study.

The immune system plays a role in renal I/R injury pathophysiology besides hypoxic injury [34]. Enhanced inflammatory reactions, generation of TNF-α, IL-1β, and other proinflammatory cytokines contribute to I/R injury [35,36]. In the current study, the level of TNF-α, IL-1β elevated in the I/R group and decreased with EA treatment. MPO is a pro-inflammatory enzyme and stored in neutrophils with high levels [37]. ROS generation is associated with the injury of lipid membrane structure which enhances MPO production [38]. MPO takes a role in the pathogenesis of renal I/R injury and is mostly used as an indicator of an inflammatory response [39].

Kidney injury molecule-1 (KIM-1) is a transmembrane glycoprotein in proximal tubule cells [40]. KIM-1 is also called T cell immunoglobulin mucin-1 (TIM-1) or hepatitis A virus cellular receptor 1 (HAVCR1) [41]. KIM-1 production on tubular renal epithelial cells elevates during renal injuries [42,43]. KIM-1 is strongly related to renal tubular injury [44]. Therefore, KIM-1 may be an important parameter for kidney diseases. The current study demonstrated HAVCR1 levels increased in the I/R group and it was reversed with EA treatment.

EA demonstrates several biological features such as antioxidant activity [45]. EA both acted on ROS and lipid peroxidation by diminishing them in a previous study [46]. EA therapy prevented cisplatin-induced nephrotoxicity and declined plasma creatinine and urea levels [47]. Similar to these data, here, EA demonstrated antioxidant and anti-inflammatory activities in the renal I/R model in rats. In the I/R group, antioxidant activity (TAS and SOD) declined while the oxidant and inflammatory system (MDA, MPO, TNF-α, IL-1β, TOS, OSI) parameters elevated. EA treatment reversed these parameters.

4.1. Conclusion

Treatment with EA significantly attenuated renal injury and showed protection against I/R-induced renal injury. Further researches may be useful to investigate the different protective mechanisms on I/R-induced renal tissue damage.

Conflict of interest

The authors declare that they have no conflict of interest.

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