Protective effects of erdosteine, vitamin E, and vitamin C on renal injury induced by the ischemia-reperfusion of the hind limbs in rats

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1. Introduction
Ischemia and reperfusion (I/R) injury is a complicated process that involves various pathophysiological mechanisms (1–4). Organ or total-body injury caused by I/R injury is important in many surgical areas, such as trauma and cardiac surgery or transplantation medicine (2,5–7). I/R is the main cause of posttraumatic organ deficiency in these disciplines (7). Holding of white blood cells to the surrounding capillary endothelium is a critical pathophysiologic stage, regardless of the type of the I/R injury (8,9). As a result of adherence to endothelial cells, the neutrophils release proteases and oxygen radicals (10), which damages the endothelial layer. Furthermore, this causes higher permeability of capillaries and interstitial edema (5,6,8,11). Therefore, blocking the leukocyte infiltration, especially where the inflammation occurs, may be beneficial in decreasing damage after I/R.

In normal conditions, reactive oxygen species (ROS) levels are held under control by nonenzymatic species such as vitamins C and E, as well as enzymes, catalase (CAT), and glutathione peroxidase (GSH-Px) (12). However, in situations such as oxidative stress, where ROS production is out of control, damage to biomolecules such as protein, DNA, RNA, and lipids and cell structures is very likely (9–11).

There is some information in the literature about lipid peroxidation (LP) and evaluation of antioxidant levels of the I/R-injured kidney. However, the debate about the protective properties of vitamins C and E and erdosteine against the development of renal I/R injury in aging males is not settled. Dietary vitamins C and E have been
shown to improve the metabolic abnormalities caused by diabetic nephropathy (13–16). Hence, the experimental data support that vitamins C and E could be useful agents in diabetic nephropathy in younger animals (13,14). On the other hand, erdosteine is a mucolytic agent used to treat chronic pulmonary diseases. The protective efficacy of erdosteine in I/R injury was displayed in liver models (12–14,16,17). However, the therapeutic potentials of both vitamins C and E and erdosteine on antioxidant levels, lipid profiles, and renal function parameters have yet to be clearly elucidated.

Therefore, this experimental research investigates the possible protective effects of vitamins C and E and erdosteine against oxidative stress during I/R injury of the kidney as distant organ injury in rats.

2. Materials and methods

2.1. Experimental protocol
Forty adult male Sprague Dawley rats, weighing 253–360 g, were randomized. Animals were studied in 4 groups: control (group I, n = 10), I/R (group II, n = 10), I/R + erdosteine (group III, n = 10), and I/R + vitamins C and E (group IV, n = 10). Ketamine/xylazine injection at 100/10 mg/kg intramuscularly (im) was used to anesthetize the animals. In all groups, tourniquets were occluded proximally to both trochanter majors of the animals (hind limb model). The tourniquets were removed after 60 min of ischemia, followed by 120 min of reperfusion. In group III, animals were given oral erdosteine at 150 mg/kg daily, whereas in group IV, vitamins C and E were administered at 50 mg/kg im and 20 mg/kg intraperitoneally starting in both groups 3 days prior to the procedure. Finally, the control and I/R group animals were given equal amounts of saline at the same time, for the same period, and in the exact same way.

2.2. Biochemical procedure
The protein content of the kidney tissue samples was determined using the Lowry method (18).

2.3. Malondialdehyde determination
We estimated the malondialdehyde (MDA) level by using Draper and Hadley’s double heating method and expressed results in nanomoles per gram of protein (19).

2.4. Superoxide dismutase activity determination
Total (Cu-Zn and Mn) superoxide dismutase (SOD, EC 1.15.1.1) activity was expressed as units per milligram protein and calculated using the method of Sun et al. (20).

2.5. Catalase activity determination
CAT (EC 1.11.1.6) activity was expressed as k (rate constant) per gram protein and determined based on the method of Aebi (21).

2.6. Glutathione peroxidase activity determination
GSH-Px (EC 1.6.4.2) activity was expressed in units per gram protein and determined based on the method of Paglia and Valentine (22).

2.7. Statistical analysis
All variables were analyzed using SPSS 9.0. The values are listed as mean ± standard deviation (SD) of the mean. One-sample Kolmogorov–Smirnov test was used to analyze distribution of the groups. Biochemical results showed normal distribution. One-way ANOVA test was performed and least significant difference (LSD) was used for post hoc multiple comparisons.

3. Results
The mean LP and antioxidants in the kidneys of all groups are presented in the Table. Although the concentrations of MDA in the I/R group increased when compared with the control, this elevation was not significant statistically. The mean tissue concentrations of MDA were decreased in both the vitamins C and E and erdosteine groups, but statistical significance was only achieved with vitamins C and E (P < 0.01). The activities of SOD, CAT, and GSH-Px were significantly (P < 0.05) decreased in the I/R group. While activities of CAT and GSH-Px were restored (P < 0.01) by vitamin treatment, the SOD activities were not significantly affected. While the activities of GSH-Px were higher (P < 0.05) with erdosteine administration, the activities of SOD and CAT stayed stable.

4. Discussion
We have determined that kidney LP levels in I/R-injured rats were decreased by vitamins C and E and erdosteine treatments. In groups II and III, the levels of MDA were comparable with the control group. However, the levels of MDA were considerably reduced in group IV when compared to group II. SOD, CAT, and GSH-Px activities were decreased in the I/R group. While activities of GSH-Px were increased by both vitamins C and E and erdosteine, CAT activity remained stable. Furthermore, SOD activity was another parameter that remained the same regardless of the treatment. Hence, prophylactic administration of vitamins C and E to the animals resulted in reduced LP (MDA) levels and higher antioxidant enzyme levels. There is a lack of evidence about the protective effect of vitamins C and E and erdosteine treatments in rat kidneys with I/R injury (23,24). To the best of our knowledge, this study is the first to compare the effects of vitamins C and E and erdosteine treatments with the evidence of oxidative stress markers. Protein kinase C (PKC) activation generates superoxide anion radicals through phosphorylation of NADH oxidase. This increases oxidative stress, which in turn activates even more PKC (25,26). Higher aldose reductase activity may cause a defect in the defense system against free radicals (reduced GSH, β-carotene, or vitamin E or
GSH-Px inactivation) (13–16). We found kidney GSH-Px activities in I/R-injured rats to be less than the kidney GSH-Px activities in the control. On the other hand, LP levels between the I/R group and the control were similar.

Because of their roles in reactions that release hydroxyl radical and superoxide anion, vitamins and erdosteine cause decrease of GSH-Px activity in diabetic kidneys (12,25,26). Likewise, previous reports have demonstrated the protective effects of vitamins C and E on diabetic nephropathy (13,16,27). GSH’s cooperation with antioxidant vitamins such as vitamins C and E contributes to antioxidant defense against ROS. Vitamin C (ascorbic acid) is a free radical scavenger and it also acts as a reducing agent, transforming vitamin E to its active form (28). On the other hand, GSH causes oxidized vitamin C (dehydroascorbate) to return to its active form. Vitamin E can break the radical chain reaction and therefore prevent polyunsaturated fatty acid peroxidation in cellular and subcellular membranes by transferring its phenolic hydrogen to a peroxyl radical of a peroxidized polyunsaturated fatty acids (12). Additionally, vitamins C and E directly scavenge ROS and upregulate the enzymatic antioxidant activities (29). In accordance to the literature, we have found that treatment with vitamins C and E caused higher antioxidant levels and GSH and GSH-Px antioxidant values in I/R kidneys.

Erdosteine treatment also prevented the reduction of GSH-Px activities within the kidney. Increase of antioxidant enzyme activity in the erdosteine group could be a result of the free radical scavenging effect of this drug. The mechanism of effect of erdosteine on GSH-Px activity is not exactly known, but we suggest that erdosteine may act as a stimulating factor in GSH-Px activity during the reperfusion phase. This effect of erdosteine could be important in preventing I/R injury in the kidney (30). Some experimental studies supported erdosteine’s protective effect of the kidney after I/R in rats (31,32). In accordance with the previous studies, we have also found that the GSH-Px activities were considerably higher in the erdosteine group when compared with the I/R-injured group.

CAT and GSH-Px often metabolize hydrogen peroxide. When Fe^{2+} or other transition metals are present and CAT activity is decreased (as in this study), H\textsubscript{2}O\textsubscript{2} is reduced to a very highly oxidizing OH radical. The OH radical cannot be enzymatically detached from cells, but a free radical scavenger may detoxify it (7–10). Additionally in our study, CAT activity was significantly increased with administration of vitamins C and E.

In the present study, SOD activities were not significantly changed by vitamins C and E and erdosteine, although they decreased with I/R. This may be due to the duration of this experiment or the inherent organization of the kidney tissue. In the literature, there is no agreement on antioxidant enzyme levels in various organs during the I/R state. However, some studies that measured activities of SOD showed reduced levels of these enzymes (31,33,34). SOD protects cells against the toxic effects of superoxide radicals by catalyzing their conversion to H\textsubscript{2}O\textsubscript{2} (30). The decreased SOD activity could be another sign of increased oxidative stress. Vitamin C, vitamin E, and erdosteine

<table>
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<tr>
<th></th>
<th>SOD (U/g protein)</th>
<th>CAT (k/g protein)</th>
<th>GSH-Px (U/g protein)</th>
<th>MDA (nmol/mg protein)</th>
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<tr>
<td>I: Control (n = 10)</td>
<td>590.7 ± 178.6</td>
<td>6.19 ± 2.03</td>
<td>81.3 ± 18.7</td>
<td>1.40 ± 0.31</td>
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<tr>
<td>II: I/R (n = 10)</td>
<td>458.4 ± 105.2</td>
<td>2.42 ± 1.51</td>
<td>62.3 ± 9.9</td>
<td>1.63 ± 0.27</td>
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<tr>
<td>III: I/R + erdosteine (n = 10)</td>
<td>517.1 ± 100.6</td>
<td>3.94 ± 1.62</td>
<td>83.1 ± 19.5</td>
<td>1.44 ± 0.29</td>
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<tr>
<td>IV: I/R + vitamins C and E (n = 10)</td>
<td>544.9 ± 110.1</td>
<td>5.51 ± 2.17</td>
<td>85.5 ± 26.0</td>
<td>1.05 ± 0.31</td>
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P-values
I–II 0.026 0.0001 0.035 NS
I–III NS 0.010 NS NS
I–IV NS NS NS 0.011
II–III NS NS 0.022 NS
II–IV NS 0.001 0.011 0.0001

Table. The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in control, ischemia-reperfusion (I/R), I/R + erdosteine, and I/R + vitamins C and E. Mean ± SD. NS: Nonsignificant. One-way ANOVA, LSD.
could be scavengers for the free oxygen radicals. Thus, it is expected that they could prevent higher SOD activities in the I/R-injured kidney.

We found in an experimental I/R-induced animal study that, although vitamins C and E were superior, both the vitamin C and E combination and erdosteine effectively reduced renal injury in the kidneys of the rats. It can also be concluded that vitamins C and E and erdosteine improved the antioxidant levels in rat kidneys because of their inherent antioxidant features. Hence, we think that vitamins C and E and erdosteine may have potential as antiischemic agents for I/R-induced kidneys. Further studies in human kidneys are required to better confirm our findings.

References


