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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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Background/aim: To compare the protective efficacy of erdosteine and vitamins C and E against renal injury caused by hind limb ischemia-reperfusion (I/R).

Materials and methods: Rats were split into 4 groups: group I as the control, group II as I/R, group III as I/R + erdosteine, and group IV as I/R + vitamins C and E. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) tissue levels were determined.

Results: MDA levels were found comparable with the control group in groups II and III. However, they were considerably decreased in group IV when compared to group II ($P < 0.01$). Additionally, SOD, CAT, and GSH-Px activities were considerably ($P < 0.05$) decreased in group II. While CAT and GSH-Px activities were restored ($P < 0.01$) by vitamin E and C treatment, SOD activity was not significantly affected. While GSH-Px activities were higher ($P < 0.05$) with erdosteine administration, SOD and CAT activities were unchanged.

Conclusion: The protective effect of vitamins C and E is higher than that of erdosteine treatment in reducing the oxidative stress after renal ischemia in this animal model.

Key words: Erdosteine, vitamin E, vitamin C, ischemia-reperfusion, antioxidant enzymes, oxidative stress, kidney

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

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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

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.

	SOD (U/g protein)	CAT (k/g protein)	GSH-Px (U/g protein)	MDA (nmol/mg protein)
I: Control (n = 10)	590.7 ± 178.6	6.19 ± 2.03	81.3 ± 18.7	1.40 ± 0.31
II: I/R (n = 10)	458.4 ± 105.2	2.42 ± 1.51	62.3 ± 9.9	1.63 ± 0.27
III: I/R + erdosteine (n = 10)	517.1 ± 100.6	3.94 ± 1.62	83.1 ± 19.5	1.44 ± 0.29
IV: I/R + vitamins C and E (n = 10)	544.9 ± 110.1	5.51 ± 2.17	85.5 ± 26.0	1.05 ± 0.31
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

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_2O_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_2O_2 (30). The decreased SOD activity could be another sign of increased oxidative stress. Vitamin C, vitamin E, and erdosteine

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

1. Frink M, Kaudel CP, Hildebrand F, Pape HC, Klempnauer J, Winkler M, Krettek C, van Griensven M. FTY720 improves survival after transient ischemia and reperfusion of the hind limbs. J Trauma 2007; 63: 263–267.
2. Burne-Taney MJ, Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H. Acute renal failure after whole body ischemia is characterized by inflammation and T-cell-mediated injury. Am J Physiol Renal Physiol 2003; 285: F87–F94.
3. Hamar J, Racz I, Ciz M, Lojek A, Pallinger E, Furesz J. Time course of leukocyte response and free radical release in an early reperfusion injury of the superior mesenteric artery. Physiol Res 2003; 52: 417–423.
4. Harkin DW, Barros D'sa AA, McCallion K, Hoper M, Halliday MI, Campbell FC. Circulating neutrophil priming and systemic inflammation in limb ischaemia-reperfusion injury. Int Angiol 2001; 20: 78–89.
5. Cocks RA, Chan TY. Alteration in leukocyte adhesion molecule expression following minor, moderate and major trauma. Eur J Emerg Med 1997; 4: 193–195.
6. Faist E, Schinkel C, Zimmer S. Update on the mechanisms of immune suppression of injury and immune modulation. World J Surg 1996; 20: 454–459.
7. Moore FA, Moore EE. Evolving concepts in the pathogenesis of postinjury multiple organ failure. Surg Clin North Am 1995; 75: 257–277.
8. Angele MK, Faist E. Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. Crit Care 2002; 6: 298–305.
9. Eppihimer MJ, Granger DN. Ischemia/reperfusion-induced leukocyte-endothelial interactions in postcapillary venules. Shock 1997; 8: 16–25.
10. McIntyre TM, Modur V, Prescott SM, Zimmerman GA. Molecular mechanisms of early inflammation. Thromb Haemost 1997; 78: 302–305.
11. Green DR, Faist E. Trauma and the immune response. Immunol Today 1988; 9: 253–255.
12. Fardoun RZ. The use of vitamin E in type 2 diabetes mellitus. Clin Exp Hypertens 2007; 29: 135–148.
13. Kutlu M, Naziroglu M, Simsek H, Yilmaz T, Sahap Kükner A. Moderate exercise combined with dietary vitamins C and E counteracts oxidative stress in the kidney and lens of streptozotocin-induced diabetic-rat. Int J Vitam Nutr Res 2005; 75: 71–80.
14. Simsek M, Naziroglu M, Erdinc A. Moderate exercise with a dietary vitamin C and E combination protects against streptozotocin-induced oxidative damage to the kidney and lens in pregnant rats. Exp Clin Endocrinol Diabetes 2005; 113: 53–59.
15. Tuttle KR, Anderberg RJ, Cooney SK, Meek RL. Oxidative stress mediates protein kinase C activation and advanced glycation end product formation in a mesangial cell model of diabetes and high protein diet. Am J Nephrol 2009; 29: 171–180.
16. Haidara MA, Mikhailidis DP, Rateb MA, Ahmed ZA, Yassin HZ, Ibrahim IM, Rashed LA. Evaluation of the effect of oxidative stress and vitamin E supplementation on renal function in rats with streptozotocin-induced Type 1 diabetes. J Diabetes Complications 2009; 23: 130–136.
17. Kuvandik G, Duru M, Nacar A, Yonden Z, Helvaci R, Koc A, Kozlu T, Kaya H, Sogut S. Effects of erdosteine on acetaminophen-induced hepatotoxicity in rats. Toxicol Pathol 2008; 36: 714–719.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–275.
19. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 86: 421–431.
20. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497–500.
21. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121–126.
22. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70: 158–169.
23. Calikoglu M, Tamer L, Sucu N, Coskun B, Ercan B, Gul A, Calikoglu I, Kanik A. The effects of caffeic acid phenethyl ester on tissue damage in lung after hindlimb ischemia-reperfusion. Pharmacol Res 2003; 48: 397–403.
24. Özkaya D, Naziroğlu M, Armağan A, Demirel A, Köroglu BK, Çolakoğlu N, Kükner A, Sönmez TT. Dietary vitamin C and E modulates oxidative stress induced-kidney and lens injury in diabetic aged male rats through modulating glucose homeostasis and antioxidant systems. Cell Biochem Funct 2011; 29: 287–293.

25. Fulop T, Larbi A, Douziech N. Insulin receptor and ageing. Pathol Biol 2003; 51: 574–580.

26. Fatehi-Hassanabad Z, Chan CB, Furman BL. Reactive oxygen species and endothelial function in diabetes. Eur J Pharmacol 2010; 636: 8–17.

27. Kim SS, Gallaher DD, Csallany AS. Vitamin E and probucol reduce lipophilic aldehydes and renal enlargement in streptozotocin-induced diabetic rats. Lipids 2000; 35: 1225–1237.

28. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. P Natl Acad Sci USA 1989; 86: 6377–6381.

29. Nazıroğlu M. Role of selenium on calcium signaling and oxidative stress- induced molecular pathways in epilepsy. Neurochem Res 2009; 34: 2181–2191.

30. Lee JY, Kim HS, Park CS, Kim MC. Erdosteine in renal ischemia-reperfusion injury: an experimental study in pigs. J Vet Med Sci 2010; 72: 127–130.

31. Erdogan H, Fadillioglu E, Yagmurca M, Uçar M, Irmak MK. Protein oxidation and lipid peroxidation after renal ischemia-reperfusion injury: protective effects of erdosteine and N-acetylcysteine. Urol Res 2006; 34: 41–46.

32. Gurel A, Armutcu F, Cihan A, Numanoğlu KV, Unalacak M. Erdosteine improves oxidative damage in a rat model of renal ischemia-reperfusion injury. Eur Surg Res 2004; 36: 206–209.

33. Ozkaya YG, Agar A, Yargicoglu P, Hacioglu G, Bilmen-Sarikçioglu S, Ozen I, Alicigüzel Y. The effect of exercise on brain antioxidant status of diabetic rats. Diabetes Metab 2002; 28: 377–384.

34. Yurdakul T, Kulaksizoglu H, Pişkin MM, Avunduk MC, Ertemli E, Gokçe G, Barişkaner H, Büyükbaş S, Kocabas V. Combination antioxidant effect of α-tocoferol and erdosteine in ischemia-reperfusion injury in rat model. Int Urol Nephrol 2010; 42: 647–655.