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Beneficial Effect of α-Tocopherol in Renal Ischemia-Reperfusion in Rats

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ABSTRACT—We evaluated the effects of α-tocopherol (vitamin E) on the products of lipid peroxidation and serum creatinine levels in a rat model of renal ischemia-reperfusion. The animals were submitted to sham operation or renal ischemia-reperfusion, and they were pretreated with α-tocopherol or the vehicle saline. In four groups, we analyzed the lipid peroxidation products by measuring malondialdehyde and chemiluminescence levels. In the other three groups, we studied the serum creatinine levels after the procedures. In our study, the pretreatment with α-tocopherol reduced significantly the lipid peroxidation of renal cells and renal dysfunction induced by renal ischemia-reperfusion in rats.

Keywords: α-Tocopherol, Renal ischemia-reperfusion, Lipid peroxidation

Renal ischemia-reperfusion is a necessary procedure in circumstances such as surgical revascularization of renal arteries, surgical treatment of suprarenal aortic aneurysms, partial nephrectomies and renal transplantation. Oxygen free radicals (OFR) were shown to contribute to the cellular damage induced by ischemia-reperfusion, probably due to their lipidic oxidative characteristics, and several agents have been used to minimize the OFR action in renal ischemia-reperfusion (1).

α-Tocopherol (vitamin E) is localized in the cell membranes and contributes to their stability and seems to protect the membrane lipids against oxidative damage (2).

The purpose of these studies was to investigate the efficacy of α-tocopherol in the reduction of injury induced by OFR in a rat model of renal ischemia-reperfusion.

Male Wistar rats weighing 250 to 330 g were randomly assigned to the experiments for analysis of the renal cell lipid peroxidation and measurement of serum creatinine levels. All experiments were approved by the local Committee for Animal Use and Care. The animals received general anesthesia with 50 mg/kg, i.p. of thiopental solution (Cristália, São Paulo, Brazil; 50 mg/ml). Renal ischemia-reperfusion was performed through a left flank incision (2 to 2.5 cm) followed by the dissection of the left renal pedicle so as to expose the renal vessels. Non-traumatic vascular clamps were used to stop blood flow. Reperfusion was established by removing the clamps. The abdominal wall (muscular layer and skin) was closed with 3.0 polypropylene and 4.0 mononylon sutures. The animals were randomly distributed into four groups: I) control group (n = 10): rats pretreated with 0.9% NaCl solution, 1 ml/kg, i.p., given 1 h before the sham-operation; II) ischemia group (n = 9): rats undergoing renal ischemia for 50 min; III) ischemia-reperfusion group (n = 10): rats undergoing renal ischemia for 50 min and reperfusion for 50 min; and IV) α-tocopherol group (n = 10): rats pretreated with α-tocopherol solution (Aldrich, Milwaukee, WI, USA; 30 mg/ml), 30 mg/kg, i.p., 72, 48, 24, 6 and 1 h before ischemia for 50 min followed by reperfusion for 50 min.

Immediately after the procedures, the renal tissue was excised to measure OFR's-induced lipid peroxidation of the renal cell membranes by the thiobarbituric acid reactive substances method (TBARS) (3), which measures the malondialdehyde level (MDA), expressing values in nmol /mg of protein, and chemiluminescence (CL) initiated by *tert*-butyl hydroperoxide (4), with the results expressed as counts per second/mg of protein of luminous energy emitted due to the return of excited carbonyls and single oxygen to the fundamental state during lipid peroxidation.

The other three groups of rats were used to evaluate renal

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function: V) control group (n = 10): rats pretreated with 0.9% NaCl solution (1 ml/kg, i.p.), 1 h before the sham-operation; VI) ischemia group (n = 10): rats undergoing renal ischemia for 50 min; and VII) α-tocopherol group (n = 10): rats pretreated with α-tocopherol solution as described in group IV and underwent ischemia for 50 min. Serum creatinine levels were measured 24, 96 and 192 h after the proceedings.

The one-way analysis of variance (ANOVA) method and Bonferroni's *t* test were used to analyze CL and MDA values and serum creatinine levels. A *P* value below 0.05 was considered significant. All data are presented as mean ± S.E.M.

The levels of MDA are shown in Table 1. Differences on the levels of MDA were observed among all the groups (P<0.05). The pretreatment with α-tocopherol reduced the MDA concentration in renal ischemia-reperfusion (0.36 ± 0.03 vs 1.68 ± 0.21 nmol/mg of protein, P<0.05).

Table 1. Effect of α-tocopherol on renal level of malondialdehyde (MDA) in rats submitted to renal ischemia-reperfusion

Group	MDA (nmol/mg protein)
I (control)	0.74 ± 0.08
II (ischemia)	1.06 ± 0.23[a]
III (ischemia-reperfusion)	1.68 ± 0.21[a,b]
IV (α-tocopherol+ischemia-reperfusion)	0.36 ± 0.03[a,b,c]

Each value represents a mean ± S.E.M.; n = 10 (each group). ANOVA followed the Bonferroni *t*-test. [a] vs group I, P<0.05; [b] vs group II, P<0.05; [c] vs group III, P<0.05.

Table 2. Effect of α-tocopherol on chemiluminescence levels in rats submitted to renal ischemia-reperfusion

Group	Maximum Chemiluminescence (cps/mg protein)
I (control)	4334 ± 655
II (ischemia)	7062 ± 1461[a]
III (ischemia-reperfusion)	9215 ± 1294[a,b]
IV (α-tocopherol+ischemia-reperfusion)	4660 ± 218[a,b,c]

Each value represents a mean ± S.E.M.; n = 10 (each group). ANOVA followed the Bonferroni *t*-test. [a] vs group I, P<0.05; [b] vs group II, P<0.05; [c] vs group III, P<0.05.

The differences in lipid peroxidation, measured by the chemiluminescence method, were also statistically significant among all the groups (Table 2). Furthermore, the kidney-levels of chemiluminescence were significantly reduced in the ischemia-reperfusion group of rats pretreated with α-tocopherol (4660 ± 218 vs 9215 ± 1294 cps/mg of protein, P<0.05).

The values of serum creatinine levels are shown in Table 3. The data revealed that the renal ischemia (Group VI) had significantly increased serum creatinine levels at 24 and 96 h after the surgical procedure, when compared with the control group (P<0.05). Furthermore, the pretreatment with α-tocopherol significantly protected renal function in the rats subjected to renal ischemia (P<0.05).

OFR, such as superoxide ($\cdot O_2^-$), hydroxyl radical ($\cdot OH^-$) and hydrogen peroxide (H_2O_2), have been considered the principal mediators of injury induced by ischemia-reperfusion (3). The OFR can induce cell injury through lipid peroxidation reactions of mitochondrial, lysosomal and plasma membranes, thus altering membrane structure and function (1, 5, 6).

Vitamin E (α-tocopherol) reacts with hydroxyl radicals and hydrogen peroxide and thus avoids the perpetuation of lipid peroxidation (2). It has been demonstrated that α-tocopherol administration reduced the ischemia-reperfusion injury (7). Furthermore, pretreatment with α-tocopherol can protect pancreatic function against ischemia-reperfusion injury (8). In the kidney, Demirbas et al. (9) demonstrated that the addition of α-tocopherol to the commercial Euro-Collins solution improved renal function and decreased lipid peroxidation rate caused by OFR in a canine kidney autotransplantation model. On the other hand, the injection of α-tocopherol before renal ischemia had a mild effect on the MDA content in rats (10). Moreover, dietary enrichment of rats with α-tocopherol was effective in suppressing the renal epithelial lipid peroxidation in a rat model of bilateral renal ischemia-reperfusion (11). Dietary deficiency of vitamin E seems to lead to greater structural and functional renal impairment and increased lipid peroxidation following renal ischemia (12).

Our results showed that pretreatment with α-tocopherol

Table 3. Effect of α-tocopherol on serum creatinine in different times of reperfusion after the renal ischemia in rats

Group	24 h	96 h	192 h
V (nonischemic)	0.43 ± 0.08	0.31 ± 0.10	0.42 ± 0.01
VI (ischemia)	2.98 ± 0.89[d]	1.22 ± 0.66[d]	0.43 ± 0.07
VII (α-tocopherol+ischemia)	0.75 ± 0.15	0.55 ± 0.07	0.47 ± 0.05

Each value represents a mean ± S.E.M.; n = 10 (each group). ANOVA followed the Bonferroni *t*-test. [d] vs groups V and VII, P<0.05.

reduced lipid peroxidation of renal cellular membranes and ameliorated the renal function in a model of normotermic renal ischemia-reperfusion in rats, but further investigations are needed to substantiate these results mainly in the clinical setting.

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