Use of α-Tocopherol Emulsion for Antioxidant Protection of Ischemic or Stored Kidneys

V. I. Kirpatovskii, N. V. Nikiforova, Yu. V. Kudryavtsev, and O. N. Nadtochii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 499-502, May, 1996
Original article submitted April 6, 1995

The ability of α-tocopherol in the form of an emulsion to augment the antioxidant reserve of kidneys during their ischemia or storage is explored. Over 10 min after an intravenous injection of the emulsion into rats or rabbits at 10 mg/kg body weight, the mean α-tocopherol concentration in the renal cortical layer rose from 6.7±0.2 to 7.4±0.2 μg/g ($p<0.05$); the injection also slowed the accumulation of malonic dialdehyde in cortical layer homogenates of intact and ischemic kidneys during ascorbate-induced lipid peroxidation. In kidneys stored at 4°C in a preservative solution to which the α-tocopherol emulsion had been added (10 mg/liter), lipid peroxidation was found to be inhibited after 24 and 48 h of storage.

Key Words: *α-tocopherol; ischemia; storage; kidney*

Activation of free-radical processes is a key factor in the pathogenesis of ischemic damage to organs [2,9, 17]. Treatment with pharmaceutical preparations that inhibit the peroxidation of membrane lipids mitigates postischemic metabolic and functional disorders and slows the accumulation of lipid peroxidation (LPO) products [2,12,13,15].

An effective antioxidant with anti-ischemic properties is α-tocopherol (α-TP), whose beneficial effects have been demonstrated in ischemias of the kidney, heart, and liver [1,2,7,11,14,16]. However, because it is fat soluble, α-TP can be administered only by the intramuscular, intraperitoneal, or enteral route, so that its antioxidant and membrane-stabilizing effects are not manifested until 3-4 h postadministration and reach their maxima in 18-24 h [3,5,11].

We have developed a procedure for preparing α-TP in the form of an emulsion suitable for intravenous injection and for adding to preservative solutions. Essentially, the procedure involves extracting α-TP from its oil solution with dimethyl sulfoxide (DMSO) and mixing it with physiological saline or some other solution [6].

In the present study we evaluate the results obtained with the α-TP emulsion injected intravenously to augment the antioxidant reserve of ischemic or stored kidneys.

MATERIALS AND METHODS

In the first series of tests, the α-TP emulsion was assayed for its potential adverse effects on the kidney following intravenous administration. Freshly prepared emulsion was injected into 10 intact random-bred rats and 4 rabbits in an amount corresponding to 10 mg of α-TP per kilogram body weight (the DMSO dose being 200 mg/kg), and the animals were then observed for 30 days, during which period the clearance of endogenous creatinine was measured and samples of renal tissue were subjected to histological examination at various times.

In the second test series, the antioxidant effects of the α-TP emulsion and of a standard α-TP solution in oil were compared on 45 male Wistar rats divided into three groups. In group 1 ($n=15$), the left kidney was made ischemic for 90 min by compress-

Research Institute of Urology, Ministry of Health and Medical Industry of the Russian Federation, Moscow

0007-4888/96/0005-0452$15.00

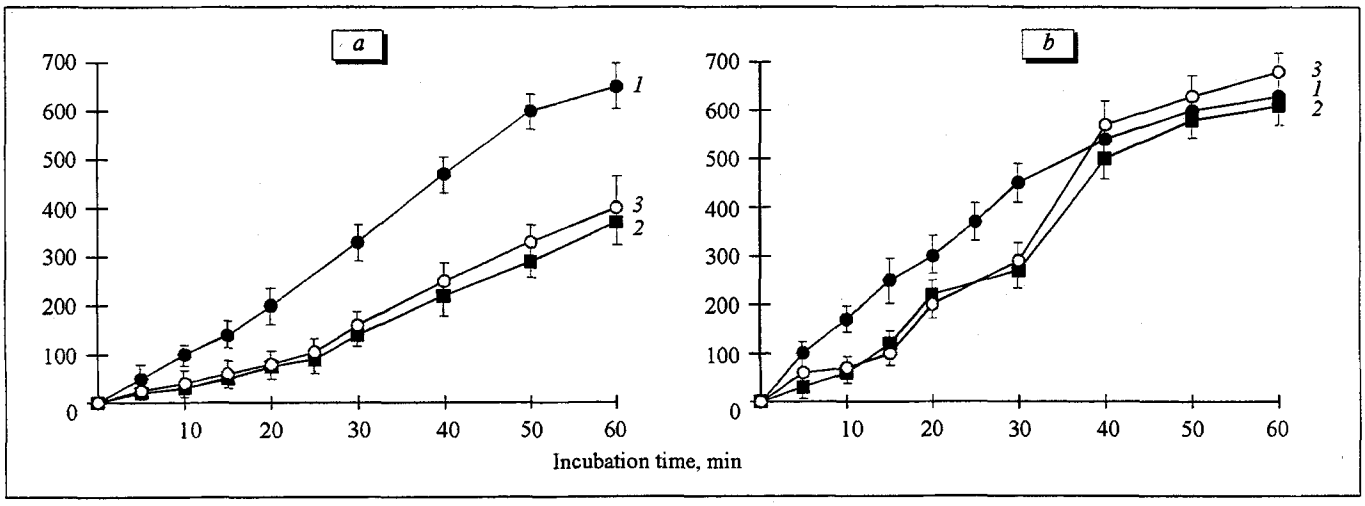

Fig. 1. Effect of intravenously injected α−tocopherol (α−TP) acetate emulsion on the accumulation of malonic dialdehyde (MDA) in renal cortical homogenates after LPO induction by ascorbate and Fe^{2+} *in vitro*. *a*) intact kidneys; *b*) kidneys after 90 min of thermal ischemia and 20 min of reperfusion. *1*) control tests; *2*) tests with intraperitoneal injection of α−TP acetate oil solution at 200 mg/kg 24 h before ischemia; *3*) tests with intravenous injection of α−TP acetate emulsion at 10 mg/kg 10 min before reperfusion. Ordinate: MDA concentration, nmol/g.

ing the renal vessels and neither the α-TP emulsion nor the standard α-TP oil solution was administered. In group 2 (n=14), a 30% oil solution of α-TP acetate was injected intraperitoneally at 200 mg/kg body weight 24 h before a 90-minute renal ischemia was produced as described above. Group 3 (n=16) received the emulsion intravenously at 10 mg α-TP/kg body weight after 80 min of the 90-min renal ischemia, i.e., 10 min before reperfusion of the kidney was started. Tissues of the intact kidneys and of the kidneys subjected to the 90-min ischemia followed by 20-min reperfusion were assayed for α-TP as described by Taylor *et al.* [18] and for malonic dialdehyde (MDA); MDA concentrations were measured in renal cortical homogenates at various times of their *in vitro* incubation after LPO induction with ascorbate and Fe^{2+} [8].

The third test series, carried out on five rabbits subjected to bilateral nephrectomy, was designed to assess the antioxidant activity of the α-TP emulsion added to a preservative (Eurocollins) solution used to store kidneys. One of the removed kidneys was washed, via the renal artery, with the standard Eurocollins solution cooled to 4°C, while the contralateral kidney was washed with the same solution, to which was added the α-TP extracted from its oil solution into DMSO (final concentrations: α-TP=10 mg/liter, DMSO=100 mg/liter). The kidneys were then stored in the same solution for 24 or 48 h, and the antioxidant effect of the added α-TP was estimated by measuring increases in the MDA concentration in renal cortical homogenates under normothermic aerobic conditions *in vitro* after LPO was induced with ascorbate and Fe^{2+} [8].

The test results were statistically analyzed by Student's t and Wilcoxon's U tests to estimate the significance of intergroup differences.

RESULTS

The first test series did not show any appreciable adverse effects of the intravenously injected α-TP emulsion on intact kidneys. The behavior of all animals remained normal and none of them died during the 30-day observation period. The urine of some animals was brown during the first 24 h postinjection, which was due to the excretion of colored DMSO metabolites [4]. The clearance of endogenous creatinine did not go beyond normal limits. Nor were any abnormalities detected on histological examination of renal tissue samples at various times after injection of the emulsion.

It follows from the above that no untoward effects actually materialized from the two potentially injurious factors associated with intravenous administration of the α-TP emulsion, namely, the toxicity

TABLE 1. Malonic Dialdehyde (MDA) Concentrations in the Cortical Tissue of Intact or Ischemic and Reperfused Kidneys after Injection of α-Tocopherol (α-TP) Oil Solution or Emulsion ($M\pm m$)

Test series	MDA concentration, nmol/g	
	intact kidneys	ischemic kidneys
Control (baseline)	21.0±2.0	25.1±1.9
α-TP oil solution	17.5±1.8	23.5±4.7
α-TP emulsion	21.0±2.0	23.0±3.1

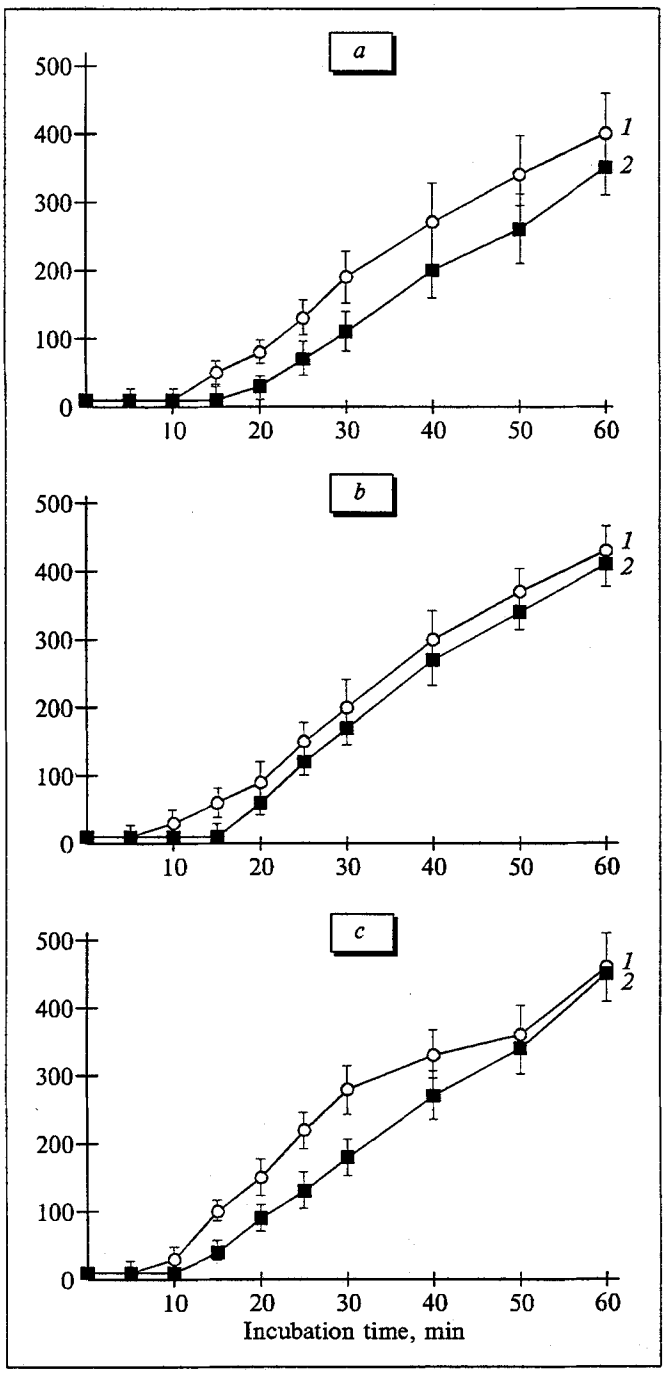

Fig. 2. Effect of α-tocopherol (α-TP) acetate emulsion added to preservative (Eurocollins) solution on the ascorbate- and Fe^{2+}-induced malonic dialdehyde (MDA) accumulation in cortical tissue homogenates prepared from kidneys stored at 4°C. *a*) unstored kidneys immediately after their washing with Eurocollins solution; *b*) after 24 h of storage; *c*) after 48 h of storage. *1*) standard Eurocollins solution (control tests); *b*) Eurocollins solution with added α-TP acetate emulsion. Ordinate: MDA concentration, nmol/g.

of the DMSO contained in the emulsion and the large α-TP micelles present in it that are capable of inducing capillary embolism. In fact, the DMSO dose used was well outside the toxic range [4].

Over the 24-h period after the intraperitoneal injection of the α-TP oil solution, the α-TP concentration in renal cortical tissue rose from 6.7±0.2 to 9.0±0.3 μg/g ($p<0.01$). In the tests where the emulsion was injected intravenously 10 min before reperfusion was started, the α-TP concentration in renal cortical tissue rose to 7.4±0.2 μg/g over the 10-min period ($p<0.05$ relative to the normal value), and this was accompanied by an increase in antioxidant activity of the renal tissue. Whereas the MDA concentrations in renal cortical tissue after the injection of α-TP in either form (as emulsion or oil solution) before or after ischemia declined insignificantly (Table 1), α-TP in both forms led to a marked inhibition of MDA accumulation in intact as well as ischemic and reperfused kidneys after LPO was induced with ascorbate and Fe^{2+} *in vitro* (Fig. 1, curves *2* and *3*). The oil solution and emulsion were almost equally effective in inhibiting LPO in these tests, and the period of the initial, relatively slow rise in MDA, reflecting the supply of tissues with endogenous antioxidants [8], was longer than in the control tests (25 min *vs.* 15 min; Fig. 1, *a*). After the ischemia, such a period was absent in the control tests, but it was well defined and lasted for about 15 min in the tests using α-TP in either form (Fig. 1, *b*).

Thus, although the renal concentration of α-TP was lower after the injection of its emulsion than after the injection of its oil solution, the two dosage forms produced similar antioxidant effects. In both test groups, the induced LPO was markedly inhibited as a result of an increase in the amount of available endogenous antioxidants and, unlike in the control group, an inhibitory effect was also observed during the postischemic period.

In the third test series using rabbit kidneys stored under hypothermal conditions, the MDA concentration in renal cortical homogenates rose insignificantly from the baseline level of 8.2±1.4 nmol/g to 8.6±1.0 nmol/g after 24 h of storage in the standard Eurocollins solution, followed by a significant rise to 20.1±2.4 nmol/g over the subsequent 24 h of storage in this solution ($p<0.05$). Lower MDA values were recorded for kidneys stored in Eurocollins solution containing DMSO-extracted α-TP: 6.2±1.1 nmol/g after 24 h of storage and 14.4±0.7 nmol/g after 48 h ($p<0.05$) *vs.* 8.3±0.9 nmol/g immediately after washing of the kidneys before storage.

In the tests where α-TP was added to the preservative solution, MDA accumulation during the ascorbate-induced LPO occurred at a significantly slowed rate in the cortical tissue homogenates prepared from unstored kidneys as well as those stored for 48 h (Fig. 2).

The results of this study indicate that the α-TP emulsion we have developed can be used both as an intravenous injection and as an additive to preservative solutions. These two uses are completely ruled out for the standard α-TP solution in oil.

The observed significant elevation of the α-TP concentration in renal tissue as early as 10 min after intravenous injection of α-TP emulsion indicates that this antioxidant is fairly rapidly delivered to parenchymal cells from the vascular bed and can thus inhibit LPO activation by effectively replenishing the store of endogenous antioxidants that are intensively expended during the postischemic period.

When added to a solution used to store kidneys, α-TP also increases the antioxidant reserve of kidney cells and inhibits LPO both before and after prolonged storage of the organ. Thus, even at a lowered temperature (4°C), α-TP can be transported to the renal parenchyma from emulsion vesicles present in the vascular bed.

Although the mechanism of this transport is not clear, it has been shown that when an aqueous solution of liposomes is mixed with α-TP dissolved in alcohol, the α-TP is rapidly incorporated into liposomal membranes, and that α-TP is also rapidly transferred from donor to acceptor vesicles after α-TP-loaded liposomes are mixed with liposomes not containing this antioxidant.

This study clearly demonstrates good prospects for the use of α-TP in the form of an emulsion to protect organs from ischemic damage.

REFERENCES

1. S. D. Artamonov, "The dynamics of heat production by a totally ischemic heart and the relation of the heat production changes produced by vitamin E to the degree of myocardial function recovery upon reoxygenation "(Synopsis of Dissertation) [in Russian], Moscow (1986).
2. M. V. Bilenko, *Damage to Organs Caused by Ischemia and Reperfusion* [in Russian], Moscow (1989).
3. E. B. Burlakova, S. A. Aristarkhova, and N. G. Khrapova, in: *Vitaminy* [in Russian], No. 8, Kiev (1975), pp. 30-36.
4. M. V. Danilenko and I. M. Turkevich, *Clinical Applications of Dimexide* [in Russian], Kiev (1976).
5. G. V. Donchenko, in: *Vitaminy* [in Russian], No. 8, Kiev (1975), pp. 43-60.
6. V. I. Kirpatovskii, Yu. V. Kudryavtsev, O. N. Nadtochii, and S. G. Emashov, *Byull. Izobret.*, No. 8 (1993).
7. V. I. Kirpatovskii, N. A. Onishchenko, T. A. Kozyreva, and V. N. Blyumkin, *Vestn. Akad. Med. Nauk SSSR*, No. 10, 48-52 (1981).
8. E. A. Neifakh, *Vopr. Med. Khimii*, No. 5, 451-456 (1969).
9. Yu. A. Petrovich and D. V. Gutkin, *Pat. Fiziol.*, No. 5, 85-92 (1986).
10. D. S. Rangelova, Zh. Zhelev, R. A. Bakalova, *et al.*, *Byull. Eksp. Biol. Med.*, **109**, No. 1, 37-39 (1990).
11. V. T. Chkheidze, "Increased protection afforded to the isolated heart through combined use of creatine phosphate and vitamin E" (Synopsis of Dissertation) [in Russian], Moscow (1990).
12. V. I. Shumakov, N. A. Onishchenko, and V. I. Kirpatovskii, *Pharmacological Protection of Transplants* [in Russian], Moscow (1983).
13. W. I. Bry, G. M. Collins, N. A. Halasz, *et al.*, *Transplantation*, **38**, 579-582 (1984).
14. C. Guarnieri and R. Ferrari, *J. Mol. Cell. Cardiol.*, **10**, 983-986 (1987).
15. N. A. Halasz, J. F. Bennett, W. I. Bry, *et al.*, *Cryobiology*, **22**, No. 6, 614 (1985).
16. S. Marubayashi, K. Dohi, K. Ochi, and T. Kawasaki, *Surgery*, **90**, 184 (1986).
17. M. S. Paller, J. R. Hoidal, and T. F. Ferris, *J. Clin. Invest.*, **74**, 1156-1164 (1984).
18. S. L. Taylor, M. P. Landen, and A. S. Tappel, *Lipids*, **11**, 530-538 (1976).