PROTECTION OF ERYTHROCYTES BY α-TOCOPHEROL AGAINST HEMOLYSIS INDUCED BY THERMAL TRAUMA

G. I. Bekyarova, M. P. Markova, and V. G. Kagan

UDC 616.155.18-02:616-001.17]084:[615.356:577.161.3]-092.9

KEY WORDS: burns; hemolysis of erythrocytes; lipid peroxidation; α-tocopherol.

Early and steadily increasing anemia is a characteristic feature of burns [5]. Immediately after burning destructive changes develop in the cells as a result both of the direct thermal effect and the action of plasma hemolytic factors [5, 7, 13]. However, the concrete molecular mechanisms lying at the basis of cell damage in burns and, in particular, of hemolysis of the erythrocytes, have not yet been explained [5].

It has been suggested that a basic role in the destabilization of erythrocyte membranes is played by products arising as a result of activation of lipid peroxidation (LPO) and also of endogenous phospholipases A_2 (free fatty acids – FFA – and lysophospholipids) [4, 6, 16]. However, the contribution of each of the factors to the thermally induced erythrocyte damage is not known.

In the investigation described below the role of activation of LPO and of phospholipases A_2 in erythrocyte damage in the early period of burns was evaluated by using the ability of α-tocopherol (TP) to prevent the accumulation of LPO products, by interacting with lipid radicals [8, 9], and also to form stable complexes with products of phospholipid hydrolysis by phospholipases A_2 [1, 3].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170-200 g. A standard IIa-IIIb burn covering 15-20% of the body surface was inflicted by means of a radiant heat apparatus for 7 sec. The rats were divided into five groups: 1) control intact, 2) control intact + TP), 3) burns, 4) burns + TP (immediately after burning), 5) TP (3 h beforehand) + burns. D,L-α-tocopherol (Serva, West Germany) was injected intraperitoneally in a dose of 20 mg/kg body weight. Blood for analysis was taken from the jugular vein under superficial ether anesthesia, 3 h after burning. The LPO level was judged from the concentration of malonic dialdehyde (MDA) and of fluorescent end products (of the Schiff bases type). MDA was estimated spectrophotometrically by the reaction with 2-thiobarbituric acid [12], and concentrations of Schiff bases were determined by measuring the intensity of fluorescence of lipid extracts in chloroform [15]. Florescence spectra were recorded on a Hitachi-Perkin-Elmer MPF-4 spectrofluorometer. The criterion of the degree of damage to the erythrocyte membrane was spontaneous hemolysis in an iso-osmotic medium, and hemolysis induced by oleic acid (20 μM) in iso-osmotic medium. The degree of hemolysis was expressed as a percentage of total hemolysis, obtained by replacing the phosphate buffer by distilled water. The concentration of extraerythrocytic hemoglobin in the plasma was determined by the standard cyanohemoglobin [5, 16].

EXPERIMENTAL RESULTS

After thermal trauma the concentrations of LPO products were found to be increased by more than 50% compared with the control values (Table 1). This is in agreement with data in the literature on activation of LPO in the acute period of burns [12, 14]. TP lowered the level of endogenous LPO products in animals of the control group and caused a sharp fall in

---

Higher Medical Institute, Varna. Institute of Physiology, Bulgarian Academy of Sciences, Sofia 1113. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 413-415, April, 1989. Original article submitted May 30, 1988.

 © 1989 Plenum Publishing Corporation

TABLE 1. Effect of TP on Blood LPO Activity (M ± m)

Experimental conditions	Schiff bases, relative units/ml	MDA concentration, nmoles/ml	Burns + TP
1. Control (4)	7,2±0,4	4,9±0,7	37,0±4,6
2. Control + TP (4)	7,0±1,2	1,2±0,4	9,06±3,1
3. Burns (4)	13,7±0,6	8,9±1,5	55,6±12,7
4. Burns + TP (5)	11,2±0,7	7,8±0,9	43,0±4,7
5. TP + burns (4)	8,2±0,7	2,8±0,3	20,8±4,2
p_{1-3}	<0,01	<0,05	<0,05
p_{3-5}	<0,05	<0,05	<0,05
p_{4-5}	<0,05	<0,05	<0,05
p_{2-4}	<0,05	<0,05	<0,05
p_{2-3}	<0,01	<0,05	<0,05
p_{1-2}		<0,05	<0,05

Legend. Here and in Table 2, number of experiments shown in parentheses.

TABLE 2. Effect of TP on Spontaneous Erythrocyte Hemolysis, Concentration of Extraerythrocytic Hemoglobin, and Hemolysis of Erythrocytes Induced by Oleic Acid (M ± m)

Experimental conditions	Hemolysis, %	Concentration of extraerythrocytic hemoglobin in plasma, mg/ml	Hemolysis induced by oleic acid, %
1. Control (5)	4,5±0,3	0,35±0,03	22,4±1,1
2. Control + TP (5)	3,4±0,2	0,34±0,03	19,0±1,4
3. Burns (5)	9,8±0,8	1,13±0,04	51,6±2,5
4. Burns (5) + TP	7,4±0,3	0,78±0,05	42,5±4,0
5. TP + burns (5)	5,4±0,4	0,56±0,03	36,5±2,2
p_{1-3}	<0,001	<0,001	<0,001
p_{1-4}	<0,001	<0,001	<0,05
p_{1-5}	<0,001	<0,01	<0,001
p_{2-3}	<0,001	<0,01	<0,001
p_{2-4}	<0,001	<0,001	<0,001
p_{2-5}	<0,001	<0,01	<0,001
p_{3-4}	<0,001	<0,01	<0,05
p_{4-5}	<0,001	<0,01	
p_{3-5}	<0,001	<0,001	<0,01

their concentration when injected before burning. The action of TP given after burning was much weaker.

The model of thermal trauma used significantly reduced the stability of the erythrocyte membrane, as shown by a raised level of spontaneous hemolysis, an increased concentration of extraerythrocytic hemoglobin, and increased sensitivity of the erythrocytes to the action of oleic acid (Table 2). TP had only a very weak stabilizing effect in the control group of animals, but had a powerful protective action, when used both before and after burning. Two circumstances must be mentioned: 1) injection of TP into the animals after thermal trauma caused a very small decrease in the level of accumulated LPO products, and 2) a strong stabilizing action of TP on erythrocytes was observed when given both before and after thermal trauma, although in the first case the effect was rather more marked. Comparison of these facts leads to the conclusion that the stabilizing action of TP in burns cannot be reduced simply to its ability to prevent (or abolish) the accumulation of LPO products [2, 3, 10], but it is also due to the nonantioxidant effects of vitamin E. This nonantioxidant stabilizing action of vitamin E may be based on its ability of exert an organizing action on the molecular mobility of polyene lipids in the membrane bilayer, and also its ability to form complexes with free fatty acids and lysophospholipids [1, 3]. Accordingly, the stabilizing effect of TP when given to animals both before and after burning, on erythrocytes incubated in the presence of oleic acid, merits attention (Table 2).

Lowering of the level of LPO products by TP when administered after burning can probably be explained by its action on "secondary" activation of LPO, arising as a result of hemolysis of erythrocytes, release of hemoglobin, and breakdown of hydroperoxides, branching the oxidation chains of lipids in the erythrocyte membranes, catalyzed by hemoglobin [11, 15].

It can be concluded from these results as a whole that the protective effect of TP in burns is not simply due to its antioxidative action, but is largely realized also by its "nonradical" stabilizing properties and, above all, its ability to form complexes with FFA and with lysophospholipids.

LITERATURE CITED

1. A. N. Erin, V. I. Skrypin, and V. E. Kagan, Dokl. Akad. Nauk SSSR, 273, No. 2, 489 (1983).
2. I. M. Ivanov and V. I. Tarusov, Free-Radical Lipid Oxidation under Normal and Pathological Conditions [in Russian], Moscow (1976), pp. 105-108.
3. V. E. Kagan, E. P. Serbinova, and Ts. Stoichev, Priroda, No. 5, 32 (1986).
4. G. S. Levin and Ts. L. Kuznetsova, Lipid Metabolism in Hemorrhage and Shock [in Russian], Tashkent (1982).
5. G. Ya. Levin and Ya. A. Sheremet'ev, Gematol. Transfuziol., No. 2, 52 (1983).
6. V. G. Mkhitaryan, M. I. Agadzhanov, and P. A. Kazaryan, Vopr. Med. Khimii, No. 6, 698 (1979).
7. S. Baar, J. Exp. Pathol., 55, 187 (1974).
8. J. G. Bieri, Vitam. Horm., 34, 31 (1976).
9. J. H. Brown and S. H. Pollock, J. Nutr., 102, 1413 (1972).
10. A. T. Diplock and J. A. Lucy, FEBS Lett., 29, 205 (1973).
11. G. W. Grams and S. Escin, Biochemistry (Washington), 11, 606 (1972).
12. A. Larcan, Vet. Med., 57, 3005 (1976).
13. R. L. Harris, J. Trauma, 22, 194 (1982).
14. A. L. Tappel, Ann. N.Y. Acad. Sci., 355, 18 (1980).
15. H. Tomai, M. Miki, and M. Mino, J. Free Radical Biol. Med., 2, 49 (1986).
16. K. Yagi, Lipid Peroxides in Biology and Medicine, New York (1982).