Scripta Scientifica Medica, vol. 28 (1993), pp 62-66

ALPHA-TOCOPHEROL STABILIZES ERYTHROCYTE MEMBRANE DURING THE EARLY STAGE AFTER THERMAL TRAUMA

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Standard thermal trauma of 3rd a - 3rd b degree, of 15-20 per cent of body surface was induced on white male Wistar rats narcotized with thiopental. The changes of the concentration of thiobarbituric acid (TBA)- reactive products, the activity of some antioxidant enzymes such as superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G-6-PD), and catalase as well as the percentage of haemolysis were investigated during the early post-burn period (24, 48, and 72 hours after burning). It was demonstrated that erythrocyte haemolysis increased along with activation of lipid peroxidation (LPO) after thermic injury. Alpha-tocopherol treatment in a dose of 20 mg/kg body mass reduced the elevated levels of TBA-reactive products and enhanced erythrocytic antioxidant defence and resistance. It could be clarified that LPO activation played an important role in the haemolysis and that alpha-tocopherol stabilized erythrocyte membrane after burns.

Key-words: Thermic injury, lipid peroxidation, alpha-tocopherol, erythrocytes, antioxidant enzymes, rats

INTRODUCTION

Changes in structural and functional properties of erythrocytes play an important role for microcirculatory disturbances after thermal trauma (4, 13). Red blood cells are transformed into exynocytes which are inclined to aggregation and haemolysis (3). These changes characterize the prelytic state of erythrocytes (5).

Lipid peroxidation (LPO) is an important factor for destabilization of erythrocyte membrane (2). Disorders in functions and integrity of red blood cells are associated with high plasma concentration of lipoperoxides and low erythrocytic levels of alpha-tocopherol (12). Data about alterations of the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glucose-6-phosphate dehydrogenase (G-6-PD) after burn injury are scarce and contradictory

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(10). Alpha-tocopherol has protective effect on erythrocytes in haemolytic anaemia (7).

The purpose of the present study is to investigate the effect of alpha-tocopherol on the resistance of erythrocyte membrane during the early stage after experimental thermal trauma.

MATERIAL AND METHODS

Male Wistar rats weighing 222 ± 54 g (mean ± SD) were divided into three groups: 1. Controls (n = 51); 2. Burned (n = 62), and 3. Burned but treated with alpha-tocopherol (n= 54). They were fed a laboratory chew ad libitum. All burns were made under thiopental narcosis (in a dose of 30 mg.kg^{-1} body mass) on the back of animals by using an apparatus for heat convection on 15-20 per cent of the total body surface at 3rda until 3rdb degree. Alpha-tocopherol (Serva, Germany) was intraperitoneally applied in a dose of 20 mg. kg^{-1} body mass immediately after thermal trauma as well as at the 24th and 48th hour after burns. Blood samples for analysis were taken from the jugular vein at the 24th, 48th, and 72nd hour after thermal injury.

LPO in erythrocytes was estimated by the concentration of thiobarbituric acid (TBA) reactive products. They were spectrophotometrically determined at wave length of 532 nm with extinction coefficient of 1,56.10^{-5}.M^{-1}.cm^{-1} (11). Erythrocytic SOD activity was measured by the method of Misra and Fridovich (1972), G-6-PD one was assessed by Boehringer's (Germany) test reactives, and catalase one was estimated after Asatiani's method (1969). Jager's method (1968) was used to determine erythrocyte haemolysis. Results were presented as the means ± SEM. Statistical significance was estimated by Student-Fisher's *t*-test.

RESULTS

The concentrations of TBA-reactive products increase during the interval studied by 38, 74, and 80 per cent ($p < 0,001$), respectively, in comparison with

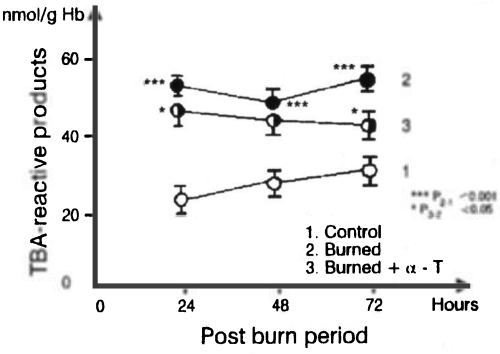

Fig. 1. Changes of TBA-reactive product level after burns and alpha-tocopherol treatment

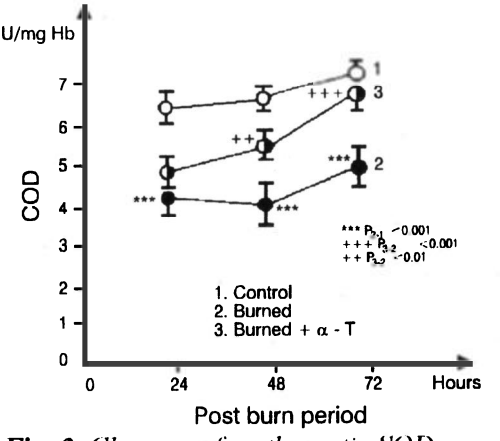

Fig. 2. Changes of erythrocytic SOD activity after burns and alpha-tocopherol treatment

their initial values (Fig. 1). The content of these products of LPO decreases significantly by 22 per cent at the 24th hour and by 38 per cent at the 72nd hour, respectively.

SOD activity reduces most significantly by 45 per cent at the 48th hour after thermic trauma (Fig. 2). It enhances statistically significantly at the 24th and 48th hour and normalizes at the 72nd hour after burning and alpha-tocopherol treatment. There is no significant reduction of the catalase index during the early stage after burn injury as well as after alpha-tocopherol treatment (Fig. 3). G-6-PD activity enhances by 68 and 78 per cent at the 48th and 72nd hour after burns, respectively, when compared with that in control animals. The activity of this enzyme decreases 48 hours after alpha-tocopherol treatment and remains significantly elevated as compared with that of controls (Fig. 4).

Haemolysis of erythrocytes increases by 400 per cent at the 24th hour after burns. This percentage decreases, however, significantly after alpha-tocopherol treatment by 26 per cent at the 24th hour and by 38 per cent at the 72nd hour after thermic trauma (Fig. 5).

DISCUSSION

Our results demonstrate that there is a LPO activation manifested by elevated blood concentration of TBA-reactive products. Other authors also report high plasma lipid peroxide levels in the early stage after thermal trauma (10). Activity of antioxidant enzymes protecting erythrocytes against free-radical damage changes to a different extent and dynamics.

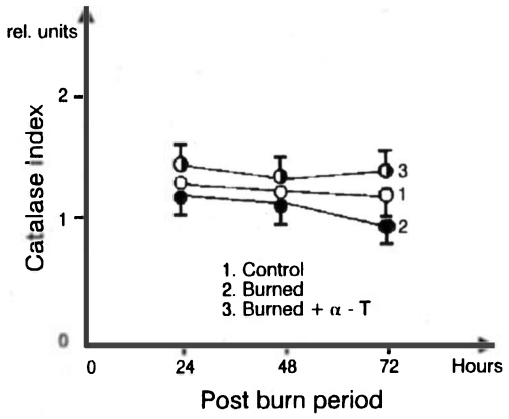

Fig. 3. Changes of catalase index after burns and alpha-tocopherol treatment

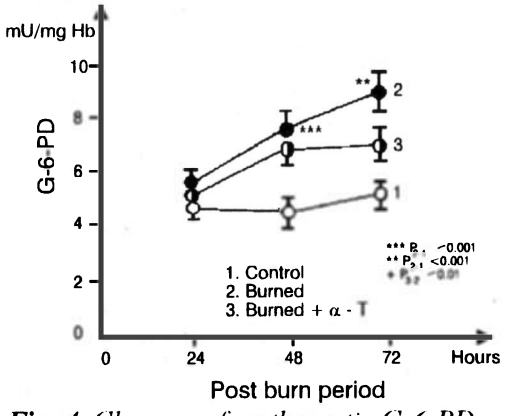

Fig. 4. Changes of erythrocytic G-6-PD activity after burns and alpha-tocopherol treatment

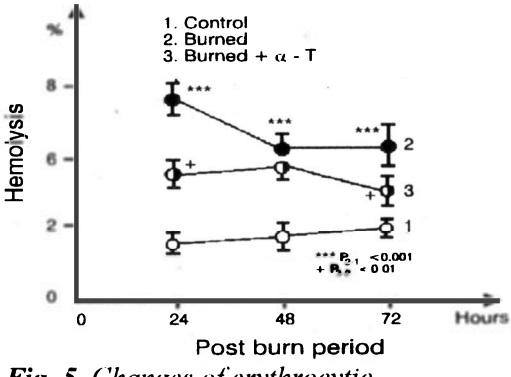

Fig. 5. Changes of erythrocytic haemolysis after burns and alpha-tocopherol treatment

SOD activity reduces after burns. It could be due to oxidation of SH-groups in the active centre of the enzyme by oxygen species. It has been reported that hydrogen peroxide inhibits SOD activity (6). However, we are not able to indicate the concrete reasons for the absent essential changes in the activity of this enzyme. Erythrocytic G-6-PD activity enhances after burn injury. Our data suggest the assumption that this enzyme of the pentose-phosphate cycle supports a high level of reduced glutathione to protect against free-radical damage probably by intensified production of NADH in erythrocytes.

Alpha-tocopherol restricts the extent of haemolysis during the acute phase after burns. This fact could be due to its ability to react with free radicals as well as to improve the antioxidant defence of erythrocytes and protect them against oxidative injuries (8).

CONCLUSION

Based on these data the following coclusions can be drawn:

1. LPO activation plays a substantial role in erythrocytic haemolysis after burn trauma.

2. Alpha-tocopherol treatment stabilizes erythrocyte membrane during the early stage after thermal trauma.

REFERENCES

1. Асатиани, В. С. В: Ферментные методы анализа. Москва, 1969.- 2. Владимиров, Ю. А. *Патол. физиол. эксперим. тер.*, 1980, № 4, 17-19.- 3. Заец, Т. Л., В. А. Лавров, А. И. Марчук, И. М. Носова. *Бюлл. эксперим. биол. мед.*, 1990, № 1, 27-30.- 4. Лавров, В. А., Т. Л. Заец. *Бюлл. эксперим. биол. мед.*, 1990, № 2, 43-50.- 5. Черницкий, Е. Л., А. В. Воробей. Структура и функция эритроцитарных мембран. Минск, 1981.- 6. Bartosz, G., T. Tannert, R. Fried, W. Leyko. *Experientia*, **34**, 1978, 1464.- 7. Bieri, J., R. Corach, V. Hulberd. *New Engl. J. Med.*, **308**, 1983, 1063-1074.- 8. Diplock, A., J. Lucy. *FEBS Lett.*, **29**, 1973, 205-220.- 9. Misra, P., J. Fridovich. *J. Biol. Chem.*, **274**, 1972, 1370.- 10. Nishigaki, I., M. Hagihara, M. Hiramatsu. *Biochem. Med.*, **24**, 1980, 185-189.- 11. Porter, N. N., Y. Norton, I. Ramdas. *Biochim. Biophys. Acta*, **441**, 1976, 596-599.- 12. Sasaki, J., G. Gottam, C. Baxter. *J. Burn Care Rehabil.*, **4**, 1983, 251-253.- 13. Yuan, Y., F. Zhiyand, Z. Zhiholg. *Burns*, **14**, 1988, 365-368.

Das Alpha-Tocopherol stabilisiert die Erythrozytenmembran während des frühen Stadiums nach einem thermischen Trauma

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Zusammenfassung: Ein standartisiertes thermisches Trauma (des Grades IIIa -

IIIb, auf 15 - 20 % der Körperfläche) wurde bei weißen männlichen Wistar Ratten, die mit Thiopental narkotisiert worden waren, verursacht. Während des frühen Stadiums nach der Verbrennung (an der 24., 48. und der 72. Stunde) wurden die Veränderungen in der Konzentration der reaktiven Produkte der Thiobarbitursäure (TRP) und die Aktivität einiger antioxidativen Enzyme wie der Superoxiddismutase (SOD), Glukose-6-Phosphat-Dehydrogenase (G-6-PD) und Katalse untersucht. Dabei studierte man auch die prozentige Änderung der Hämolyse. Es wurde gezeigt, daß nach dem thermischen Trauma die Hämolyse der Erythrozyten parallel zu der Aktivierung der Lipidperoxidation (LPO) steigte. Die Behandlung mit Alpha-Tocopherol in einer Dosierung von 20 mg/kg Körpermasse reduzierte den erhöhten Spiegel der TRR und vergrößerte den antioxidativen Schutz und die Resistenz der Erythrozyten. Es wurde deutlich, daß die Aktivierung der LPO eine wesentliche Rolle bei der Hämolyse der Erythrozyten spielte, und daß das Alpha-Tocopherol die Erythrozytenmembran nach der Verbrennung stabilisierte.

Alpha-tocophérol stabilise la membrane d'érythrocytes dans un premier stade d'un trauma thermique

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Résumé: Un trauma thermique standartisé (IIIa - IIIb degré, sur 15-20 % de la superficie corporelle) a été provoqué sur des rats blancs mâles Wistar, qui étaient assoupis sous narcose par l'action de thiopental. On a suivi les changements que le contenu des acides thiobarbiturs (TBA) a subi - les produits réactifs dans le sang, l'activité de quelques enzymes antioxydantes dans les érythrocytes comme superoxyde dismutase (SOD), glucose-6-phosphate déhydrogénase (G-6-PD), catalase et hémolyse des érythrocytes pendant la periode aiguë, après le trauma thermique (24, 48 et 72 heures). Les données de l'analyse montrent que après un trauma thermique l'hémolyse des érythrocytes s'augmente quand s'active la péroxydation lipide. Après un traitement avec alpha-tocophérol (20 mg/kg de masse corporelle) le contenu de TBA-produits réactifs diminue, tandis que la protection antioxydante et la résistance des érythrocytes s'activent. On arrive à la conclusion que l'activation de la péroxydation lipide joue un rôle important dans l'hémolyse des érythrocytes, tandis que l'alpha-tocophérol stabilise la membrane d'érythrocytes lors d'un trauma thermique.