EFFECT OF LIPID PEROXIDE OXIDATION ON ERYTHROCYTE FLEXIBILITY IN BURNS AND ALPHA-TOCOFEROL TREATMENT

G. Bekyarova, I. Kozarev, T. Yankova

Key-words: thermic trauma – lipid peroxide oxidation – erythrocytes – treatment – rats

Experimental investigations and clinical observations in cases of thermic shock reveal that microcirulation disorders rather often determined by disturbances of rheological properties of erythrocytes and partially of their flexibility present a characteristic peculiarity of this shock (3, 4). However, both pathogenetic mechanism and therapeutic influence concerning this important rheological index are not completely clarified yet and thus an object of experimental studies.

A lot of authors relate the reduced deformability after thermic trauma with changed structural-functional activity of the erythrocyte membrane influenced by several factors such as catecholamines, kinins, prostaglandins, free fatty acids, etc. (2, 10).

Recently, the concept is established that free-radical peroxide oxidation is one of the universal factors modifying membranes and reducing their functional activity (4). We could not find any data about the role of lipid peroxide oxidation (LPO) in the pathogenesis of disturbed erythrocyte flexibility in burns and after alpha-tocoferol (alpha-TF) treatment in the literature available.

In the present work we have the purpose to study the effect of activated LPO on erythrocyte flexibility during the acute period after thermic trauma and after alpha-TF treatment considering alpha-TF a possible protector.

Material and Methods

Standard thermic trauma of IIIrd-a – IIIrd-b degree up to a body surface of 10–15 per cent was induced by a heat radiation apparatus. Experimental white rats were divided into three groups: 1st – controls; 2nd – burned, and 3rd – burned and alpha-TF-treated. Alpha-TF (Fluka) was intraperitoneally injected at dosis of 20 mg/kg b.w. immediately after the thermic trauma as well as on the 24th and 48th h after it. Blood samples were taken under narcosis from the jugular vein after 5, 24, 48, and 72 hours. Blood LPO activity was evaluated according to diene conjugate (DC) and malondialdehyde (MDA) concentrations. DCs were determined after the method of Stal'naya and Garashvili (7) in μmol/ml and MDA – by means of thiobarbiturate test (11) in nmol/g Hb. Erythrocyte flexibility was estimated after the method of Tannert and Lux (20). The method of variation analysis was applied to evaluate statistically the results of the trial (D. Sepetliev, 1972).

Results and Discussion

Our results show that the concentration of primary LPO products, i.e. DCs increases maximally and significantly on the 5th hour. It reduces progressively after the 24th hour down to initial levels on the third day after thermic trauma. Alpha-TF reduces DC level in all the terms

of experiment down to insignificant values as compared with the normal ones exepting these on the 5th and 24th hour ($p < 0.05$). MDA level, i.e. secondary LPO product one, increases more considerably after burning. There is a significant MDA concentration increase even on the 5th hours after thermic trauma. The maximal level is reached on the 24th hour (table 1). Then it

Table 1

Changes of LPO product concentrations – DC /μmol/ml/; MDA /nmol/g Hb/ in the blood and erythrocyte flexibility after burns of III – a and III – b degree on a surface of 10–15%, in rats

Indexes	Groups	n	5 h	24 h	48 h	72 h
DC μmol/ml	1. H	6	20.67 ± 4.63	20.67 ± 4.63	20.67 ± 4.63	20.67 ± 4.63
	2. BU	6	58.34 ± 11.53	34.65 ± 2.24	24.46 ± 3.09	27.63 ± 1.68
	$p_2:p_1$		< 0.01	< 0.01	> 0.05	> 0.05
	3. B + ℒ-TF	6	43.8 ± 7.59	32.90 ± 7.17	23.34 ± 3.58	20.39 ± 1.75
	$p_3:p_2$		< 0.05	< 0.05	> 0.05	> 0.05
MDA nmol/g Hb	1. H	6	35.51 ± 5.19	35.51 ± 5.19	35.51 ± 5.19	35.51 ± 5.19
	2. BU	6	69.35 ± 3.58	70.33 ± 3.77	54.64 ± 6.60	55.97 ± 5.09
	$p_2:p_1$		H 0.001	< 0.001	< 0.05	< 0.01
	3. B + α-TF	6	53.65 ± 7.30	40.13 ± 7.95	47.68 ± 8.77	46.68 ± 7.75
	$p_3:p_1$		< 0.05	$p_3:p_2$ < 0.01	< 0.05	< 0.05
Flexibility tc/ts	1. H	6	0.88 ± 0.04	0.88 ± 0.04	0.88 ± 0.04	0.88 ± 0.04
	2. BU	6	0.67 ± 0.07	0.67 ± 0.06	0.66 ± 0.05	0.66 ± 0.04
	$p_2:p_1$		< 0.05	< 0.05	< 0.05	< 0.05
	3. B + ℒ-TF		0.71 ± 0.05	0.70 ± 0.03	0.64 ± 0.03	0.82 ± 0.05
	$p_3:p_1$		< 0.05	< 0.05	< 0.05	< 0.05

Legend:
H – healthy
BU – burned and untreated
B + ℒTF – burned and treated with ℒ-Tocoferol

decreases without reaching initial levels. Alpha-TF reduces the increased values (statistically reliably on the 24th h during the terms studied) but it does not induce their normalization. Erythrocyte flexibility decreases statistically significantly after burning in all the terms studied. However, it increases (excepting on the 48th hour) maximally and reliably on the 3rd day, practically to normal levels after alpha-TF treatment when compared with that of the untreated animals.

Our data indicate that in cases of thermic trauma LPO activation sets in resulting in a rapid transitory elevation of the concentration of the primary product, i.e. DCs, and more considrably expressed – of the end product, i.e. MDA. This is completely logical, i.e. primary product transforms into secondary one the latter being indicative of LPO activation.

Our comparative analysis demonstrates a definite correlation between the increased LPO product concentration and flexibility. Our results obtained coincide with data from experimetal and clinical investigations (9, 12, 17). As previously reported (17), MDA addition to erythrocyte suspension induces considerably the number of rigid erythrocytes and worsens their flexibility. R. R. Shilyaev (9) relates the disorders of cerebral circulation with changes of erythrocyte flexibility. These changes correlate with the increased concentration of LPO products.

Most probably, flexibility worsening established by us is due to profound structural transformation of erythrocyte membrane cytoskeleton. It is known that LPO products induce con-

formation changes as result from polymerization of erythrocyte membrane components accompanied by an increase of the concentration of cholesterol (8), saturated fatty acids with formation of spectrin macromolecular aggregates (14, 16) resulting in an increase of membrane microviscosity and rigidity (19). Because of activated peroxidation the activity of transport ATPases decreases. The electrolyte balance is disturbed, the erythrocyte volume increases and its shape is altered (1, 5, 6, 15). Thus changes in cytoskeleton and metabolism because of activated peroxidation reduce elastic properties and stimulate disc-spherocytic transformation. Spherocyte deformability requires much more efforts in comparison with discocyte membrane (16). Probably, this mechanism is of essential importance for worsened flexibility as in cases of burns structural heterogeneity as well as presence of large amounts of transformed forms of erythrocytes can be established (1).

The analysis of the results demonstrates that alpha-TF improves erythrocyte flexibility parallelly to limiting of LPO activation. This favourable influence is due to its membrane-stabilizing action and to its ability to build in itself into lipid bilayer structure and to ensure its space stability (13). Alpha-TF protective effect is determined by its capacity to interact with free radicals and active oxygen (4) and to inhibit their damaging action as well.

The discussion of the results obtained allows us to draw the conclusion that LPO activation is one of starting and even also decisive factors in the pathogenesis of worsened erythrocyte flexibility after thermic trauma. Thanks to its antioxidative and membrane-stabilizing action, alpha-TF protects erythrocyte membrane structure against damaging effect of LPO products and improves erythrocyte flexibility.

On the basis of data obtained and conclusions made we recommend alpha-TF including into the pathogenetic therapy of burns.

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ВЛИЯНИЕ ПРОДУКТОВ ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ НА ФЛЕКСИБИЛИТЕТ ЭРИТРОЦИТОВ ПРИ ОЖОГЕ И ЛЕЧЕНИИ Ł-ТОКОФЕРОЛОМ

Г. Бекярова, И. Козарев, Т. Янкова

РЕЗЮМЕ

Исследовано влияние усиленной липидной пероксидации в остром периоде ожоговой травмы (5, 24, 48, 72 часа после ожога) на флексибилитет эритроцитов. Ожог был IIIа – IIIб степени. Он охватывал 10 – 15 % поверхности тела.

С целью исследования этого влияния параллельно прослеживалось изменение количества диеновых коньюгат, представляющих собой промежуточный продукт малондиальдегида и конечный продукт перекисного окисления липидов в крови. Изменение количества диеновых коньюгат прослеживалось также по отношению к флексибилитету до и после ожого в указанные выше сроки.

Результаты исследования показывают, что с увеличением содержания продуктов перекисного окисления липидов в крови ухудшается флексибилитет эритроцитов в остром периоде после ожога.

В работе обсуждаются механизмы влияния продуктов перекисного окисления липидов на флексибилитет, как и результаты лечения Ł-токоферолом. Препарат применялся интраперитонеально по 20 мг/кг телесного веса по определенной схеме.