FREE RADICAL OXIDATION AND ERYTHROCYTE AGGREGATION AFTER THERMIC INJURY EFFECT OF ALPHATOCOPHEROL

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Key-words: thermic injury — free radical oxidation — erythrocyte aggregation — alphatocopherol — microcirculation

Disseminated intravascular coagulation and «sludge» phenomenon development are a characteristic manifestation of thermic shock. Erythrocyte aggregation presents an important link in the mechanism of this pathological process (8, 10, 13). Erythrocyte involvement in this phenomenon is related to alteration of their electrical loading as well as to loss of their deformability (3, 5). The concept that conformational reconstruction of erythrocyte membranes due to essential structural changes is of vital importance for aggregation becomes more and more popular, indeed (2). In this sense, the clarifying of the role of free radical oxidation (FRO) as one of the main factors changing membrane structural-functional activity, in the mechanism of erythrocyte aggregation after burning as well as in the changing of the erythrocyte sedimentation rate (ESR) and hematocrit (Ht) presents a definite interest, indeed.

Material and methods

Experimental thermic injury of III[rd] a — III[rd] b — degree and of 15 — 20 per cent of body surface was moulded in white male rats with body mass of 200 — 250 g. Animals were divided into three groups: a) healthy b) burned untreated c) burned and treated with alphatocopherol. DL-alphatocopherol («Serva») was intraperitoneally injected in a dose of 20 mg/kg b. w. immediately after burning, on the 24[th] and 48[th] hour after thermic injury. Certain parameters were estimated in the following time intervals: on the 24[th], 48[th] and 72[nd] hour.

The activity of FRO processes was estimated according to thiobarbituric products (TBP) contents. TBP were spectrophotometrically determined (11) at wave lenth of 532 nm with extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$. Intravascular erythrocyte aggregation was determined after the method of Lopotnikov and Harash (1982). ESR changes were followed-up after Panchenko and Ht level was estimated by using of a microhematocrit centrifuge.

Our results were statistically processed after the method of variation analysis and compared according to Student-Fisher's t-test.

Results and discussion

Our data demonstrate a significant increase of blood TBP level after thermic injury in comparison with controls (healthy animals). Levels increase most

considerably on the 24th and 48th hour (approximately two times). Then they diminish (insignificantly) without normalization on the third day. Alphatocopherol administration results in TBP content reduction in all the intervals studied. This decrease is most considerable and reliable on the 24th hour ($p < 0.01$) (fig. 1). Hematocrit values are maximal on the 5th hour ($p < 0.01$). Then they decrease and tend to fall below the normal limits on the 72nd hour. However, when alphatocopherol is injected Ht values do not change essentially in comparison with burned untreated animals' ones (fig. 2).

During the acute period after thermic injury intravascular erythrocyte aggregation increases reliably in the aforementioned intervals by 51, 76, 60 and 34 per cent, respectively, as compared with that in control animals. Alphatocopherol induces erythrocyte aggregation reduction which is most statistically significant on the 24 hour ($p<0.01$) when compared with that in burned untreated rats (fig. 3).

ESR increases considerably still on the 5th hour after thermic injury and then this rate decreases but remains reliably higher than

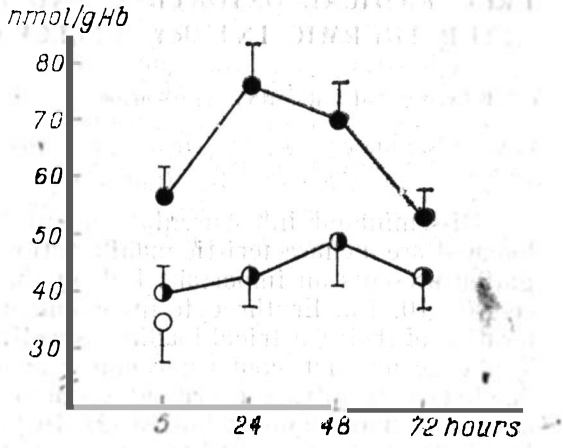

Fig. 1. Change of TB-product concentration in the blood after thermic injury in rats (III[a] — III[a] degree, 15—20 per cent of body surface).
Legend:
O — controls — intact
● — burned untreated
◗ — burned treated with alphatocopherol.
* $P_{2-1} < 0.05$; ** $P_{2-1} < 0.01$; *** $+ P_{2-1} > < 0.001 + p < 0.05 < p + 0.01$.

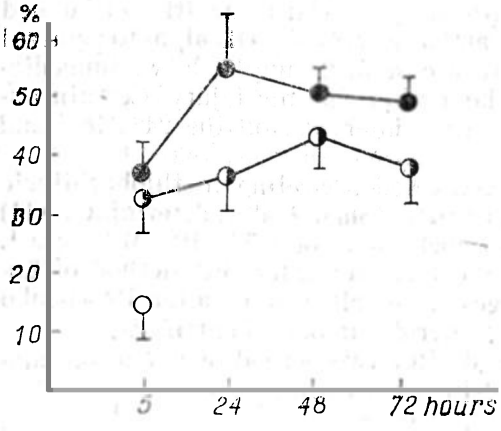

Fig. 2. Hematocrit changes after thermic injury in rats (III[a] — III[b] degree, 15—20 per cent of body surface). Legend — see fig. 1.

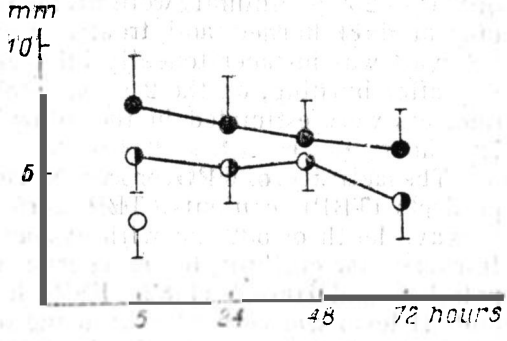

Fig. 3. Change of erythrocyte aggregation after thermic injury in rats (III[a] — III[b] degree, 15 — 20 per cent of body surface). Y-axis — changes in per centage in comparison with control values. X-axis — interval of observation. Legend — see fig. 1.

the normal level. After alphatocopherol application, except for the 48th hour, ESP decreases but does not normalize till the 72nd hour (fig. 4).

Data presented show that FRO activity increases still on the 5th hour and remains high during the first days after thermic trauma. Paralelly, erythrocyte aggregation, ESR and Ht elevates, too.

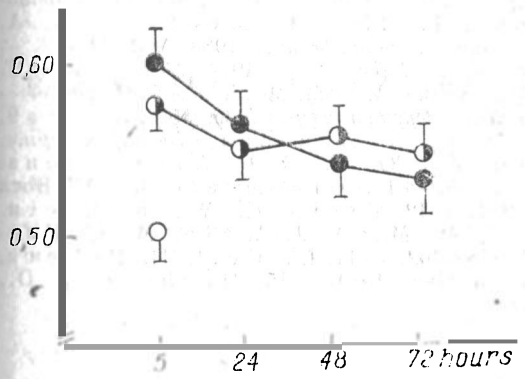

Fig. 4. ESR changes after thermic injury in rast (III[a] — III[a] degree, 15 — 20 per cent of boyd surface).
Legend — see fig. 1.

We suppose that the increased aggregation capacity is due to serious structural alterations of erythrocyte membrane influenced by the accumulated endogenous FRO derivatives. Probably, peroxide modification results in redistribution of ionized groups on erythrocyte membrane surface and in electrokinetic potential reduction (2,9). The close relationship between activation of FRO processes and erythrocyte aggregation is in concordance with literature data available (1, 9, 14).

It can be assumed that aggregation capacity increase is due to a change of elastic properties and to transformation of discocytes into spherocytes because of conformational alterations in lipid and polypeptide components during the direct action of FRO derivatives on erythrocytes (15). FRO activation induce the formation of high-molecular compounds of the type of lysolecithine and of the free fatty acids which stimulate disc-spherocytic transformation probably by means of PL-ase activity increase (7, 14).

It can be presumed that FRO is an important link in the mechanism of erythrocyte aggregation and, of course, not the only one link. Probably, to that process changes of plasma blood composition (i. e. decreased albumin level, increased concentration of high-molecular components, fibrinogen, gammaglobulin /2/) which disturb erythrocyte suspension stability in hemoconcentration as well as the more insufficient effect of alphatocopherol on these parameters contribute to a certain extent, too.

Alphatocopherol due to its antioxidative properties diminishes FRO activation and this results in electrokinetic potential stabilization (12). By this way, it contributes to the creation of an electromagnetic balance between erythrocytes and vascular wall (4). Alphatocopherol prevents erythrocyte aggregation and thus contributes to blood viscosity reduction and microcirculation improvement after burning.

Our results obtained argue for the statement that FRO is one of the determinant factors stimulating erythrocyte aggregation. This conclusion is confirmed by our data demonstrating a close relationship between FRO, erythrocyte aggregation as well as alphatocopherol protective action.

REFERENCES

1. Арутюнян, Р. А., С. А. Баджинян, А. К. Абрамян, Н. Р. Овисян. Депон. рукопись в ВНИИМИ МЗ СССР. Москва, № 6130—83. — 2. Габриелян, З. С., С. З. Агопов. Клетки крови и кровообращение. Ереван, 1985, 399 с. — 3. Гусенова, Ф. М., В. А. Соколова, В. П. Матвеенко, и др. *Патол. физиол. эксперим. тер.*, 1986, № 2, 11—14. — 4. Казанов, Ю. М. *Кардиология*, 1981, 1981, № 8, 110—116. — 5. Левин, Г. Я., Р. И. Муразян, Ю. А. Шереметьев, П. Р. Панченко. *Гематол. и трансфузиол.*, 1983, № 2, 52—58. — 6. Лопотников, В. А., Л. М. Хараш. *Лабор. дело*, 1982, № 7, 5—7. — 7. Меерсон, Ф. З. Адаптация, стресс, профилактика. Москва, Медицина, 1981, 288 с. — 8. Мчеедлишвили, Г. И. *Патол. физиол. эксперим. тер.*, 1986, № 2, 3—11. — 9. Панасенко, О. М., Р. И. Шалина, О. А. Авизова. *Бюлл. эксперим. биол. и мед.*, 1985, № 4, 434—439. — 10. Штыхно, Ю. М., З. Р. Атаджанова. *Патол. физиол. эксперим. тер.*, 1985, № 2, 30—35. — 11. Greenwald, R. A. Boca Raton, Florida, CRC Press Inc., 1936, 197—204. — 12. Escin, C. W., K. Escin. *Biochemistry*, 11, 1972, 606—610. — 13. Guest, M. M., D. J. Wain, M. E. Frawier. *J. Burn Care Rehabil.*, 4, 1983, No 3, 158—162. — 14. La Cell, И. Р. Винц, Л. А. Сантяно. Мембраны и болезнь. Москва, 1980. — 15. Hochstein, D., S. K. Jain. *Fed. Proc.*, 40, 1981, 183—188.

СВОБОДНОЕ РАДИКАЛЬНОЕ ОКИСЛЕНИЕ И АГРЕГАЦИЯ ЭРИТРОЦИТОВ ПОСЛЕ ТЕРМИЧЕСКОЙ ТРАВМЫ. ЭФФЕКТ АЛЬФАТОКОФЕРОЛА

Г. Бекярова, И. Козарев, Т. Янкова

РЕЗЮМЕ

Повышеннаяа грегация клеток крови является одним из наиболее важных патогенных факторов нарушений микроциркуляции. Авторы поставили перед собой цель изучить влияние активирования продуктов свободного радикального окисления на агрегационную способность эритроцитов, реакции оседания эритроцитов и гематокрита в первые часы и дни после получения термической травмы IIIа и IIIб степени (15—20% поверхности тела) у белых крыс самцов.

Устанавливается, что параллельно с нарастанием содержания ТБК-продуктов крови, агрегация эритроцитов, скорост оседания эритроцитов и гематокрит повышаются. Применение альфатокоферола приводит к уменьшению количества ТБК-продуктов, понижению агрегации и скорости оседания эритроцитов и не оказывает влияния на гематокрит.

Полученные результаты дают основание предполагать, что повышенное количество продуктов свободного радикального окисления стимулирует агрегацию эритроцитов при термической травме, а альфатокоферол имеет защитную функцию.