STRUCTURAL AND ENZYMIC DISORGANIZATION OF BIOLOGICAL MEMBRANES IN LIVER CELLS OF RATS WITH THERMAL BURNS

T. L. Zaets

UDC 617-001.17-07:616.36-018.1:576.314/-092.9

KEY WORDS: cell membrane; burns; liver.

The state after extensive and deep thermal burns is characterized by the development of dystrophic and destructive processes, whose intensity varies in different organs outside the burned zone. These changes are based on disturbances of the normal properties of cell membranes [2, 3, 5, 6]. This is mainly the case with cell and lysosomal membranes. Meanwhile we know that in response to the harmful action of several toxic external environmental factors, universal labilization of membranes develops in the body [4]. Such a response may perhaps be characteristic also of the post-burned state, but no information on this matter can be found in the literature.

The aim of the present investigation was to study the structural integrity and enzymic properties of the cell membrane and of membranes of the mitochondria, lysosomes, and microsomes of liver cells of rats with thermal burns.

EXPERIMENTAL METHOD

Experiments were carried out on 183 albino rats weighing 170-200 g: Intact animals and rats in which a burn of the IIIB degree covering 15-20% of the body surface was inflicted under superficial ether anesthesia by application of a spirit flame. Structural integrity and permeability of the cell membranes were judged by the release of relatively or strictly hepatospecific enzymes into the blood stream: alanine aminotransferase (AlT) [14], histidase [1], ornithine carbamoyl-transferase (OCT) [13], and by activity of the enzyme 5'-nucleotidase, firmly bound with the membrane [11], in the fraction rich in plasma membranes [10].* Structural integrity of the membranes of the intracellular organelles was judged from nonsedimented activity after appropriate differential centrifugation [9] of the mitochondrial matrix enzyme malate dehydrogenase (MDH) and the membrane-bound mitochondrial enzyme succinate dehydrogenase (SDH) [12], the lysosomal matrix enzyme cathepsin D [7], and the membrane-bound microsomal enzyme glucose-6-phosphatase (G6P) [15], and also by the activity of these enzymes in the corresponding isolated organelles.

EXPERIMENTAL RESULTS

AlT activity in the blood was found to be increased 1 h after burning and histidase and OCT activity was very sharply increased (Fig. 1). Enzyme activity of AlT, histidase, and OCT in the blood remained high 24 h after burning, but during the following week histidase and OCT activity fell, although it still remained significantly above the initial level. Although all the enzymes studied are considered to be hepatospecific, AlT can also enter the blood stream from muscle, which undergoes dystrophic changes for a long time after burning, and histidase can enter the blood stream not only from the liver, but also from destroyed skin cells. OCT activity in the blood reflects damage to the hepatocyte cell membrane, which as the results show, took place during the first few hours after burning. In this period it was not yet possible to find any significant decrease in 5-nucleotidase activity in the fraction rich in plasma membranes. This decrease, by 20-25% of the initial level, occurred 1-7 days after burning. The cell membrane, which had now become permeable for cytoplasmic enzymes, evidently could still retain much of the activity of the enzyme firmly bound with it; permanent lose of activity of this enzyme indicates disorganization of the membrane.

*The author is grateful to Dr. Biol. Sci. A. V. Pogosova for help with the histidase determination.

All-Union Burn Center, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 43-45, October, 1983. Original article article submitted April 1, 1983.

 0007-4888/83/9610-1390$07.50

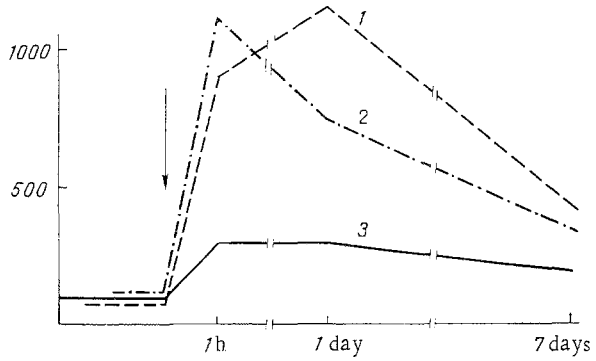

Fig. 1. Time course of changes in activity of hepatospecific enzymes in blood serum of burned rats. 1) Histidase; 2) OCT; 3) AlT. Arrow indicates time of burning. Abscissa, time after burning; ordinate, activity (in % of control).

TABLE 1. Nonsedimented Activity of Mitochondrial and Microsomal Enzymes and Activity in Liver Organelles of Rats with Thermal Burns (M ± m)

Experimental conditions	MDH		SDH		G6P	
	NA	mitochondria, units/mg protein	NA	mitochondria, ΔE/100 mg protein/min	NA	microsomes, μg P_i/mg protein/15 min
Control (16)	14,0±1,8	1,8±0,15	12,7±3,4	6,0±0,7	2,8±0,7	17,3±2,0
Burns:						
1 h (16)	19,2±3,5	1,5±0,14	8,3±1,5	8,0±0,3*	2,6±0,4	21,2±1,4
1 day (8)	28,0±1,3**	1,56±0,13*	24,0±3,5**	3,9±0,7**	14,5±4,5***	9,4±1,8**
7 days(8)	25,5±1,2**	1,6±0,5	17,2±2,0*	4,1±0,4*	6,3±1,3**	21,3±2,7

Legend. NA) Nonsedimented activity (in % of total activity). Number of experiments shown in parentheses. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

Equally early changes were observed in permeability of the lysosomal membrane, which started to rise 1 h after burning and reached a maximum for cathepsin D and acid phosphatase (a two-threefold increase) after 24 h.

The state of permeability and structural integrity of the mitochondrial and microsomal membranes was judged from the level of nonsedimented activity of marker enzymes and activity of these enzymes in the organelles.

No significant increase in the level of nonsedimented activity of mitochondrial and microsomal enzymes could be found 1 h after burning. Only a small decrease in SDH activity in the mitochondria pointed to disturbance of the enzyme function of the mitochondria. An increase in nonsedimented MDH, SDH, and G6P activity by 100, 90, and 418% respectively was found 24 h after burning, evidence of a marked increase in permeability of the mitochondrial and microsomal membranes and their disorganization, for not only enzymes of the matrix, but also membrane-bound enzymes were present in the cytosol. Activity of the latter in the organelles was appreciably reduced: SDH activity in the mitochondria by 35%, G6P activity in the microsomes by 50%, a further indication of disorganization of the membranes of these organelles. Marked disturbances of membrane permeability in the mitochondria and, to a lesser degree, in the microsomes were still present 7 days after burning.

It can be concluded from these results that thermal trauma has a universal membrane-damaging action in organs remote from the burned zone. Permeability of the cell membrane and of membranes of the mitochondria, lysosomes, and microsomes is increased, so that enzymes of the matrix of the corresponding organelles can be released into the cytosol. Evidence of profound disorganization of the biological membranes is given by a decrease in the activity of enzymes firmly bound with these membranes. Processes catalyzed by enzymes are disturbed. The cell membrane and lysosomal membrane are the most vulnerable to burn trauma and their permeability is disturbed in the early stages after burning. Damage to mitochondrial and microsomal membranes, as well as profound disorganization of all membranes, developed during the first day after burning.

With effect from the first few hours after injury and during the next 7 days of investigation, burn trauma is thus characterized by profound and universal pathological changes in the system of cell membranes of the liver with their disorganization and with changes in activity of membrane-bound enzymes.

LITERATURE CITED

1. V. A. Burobin, in: Methods of Investigation of Some Enzymes in Clinical Medicine [in Russian], No. 6, Moscow (1967), p. 22.
2. T. L. Zaets, E. B. Burlakova, V. K. Sologub, et al., Byull. Éksp. Biol. Med., No. 7, 60 (1980).
3. A. A. Kiyashko, S. A. Smorshok, and M. P. Gariyan, in: Pathology of Membrane Permeability [in Russian], Moscow (1975), p. 30.
4. R. V. Merkur'eva, Vopr. Med. Khim., No. 2, 35 (1982).
5. M. I. Remizova and N. I. Kochetygov, Patol. Fiziol., No. 3, 63 (1976).
6. S. A. Smorshok, K. K. Volkov, and I. L. Tyurina, in: Abstracts of Proceedings of the 9th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Minsk (1981), pp. 357-358.
7. M. Anson, J. Gen. Physiol., 22, 79 (1939).
8. H. Bergmeyer (ed.), Methoden der enzymatischen Analyse, Weinheim (1974).
9. C. De Duve, Exp. Cell Res., 7, Suppl., 169 (1959).
10. L. Heppel and R. Hilmor, Methods Enzymol., 2, 540 (1955).
11. D. Nevill, J. Biophys. Biochem. Cytol., 8, 413 (1980).
12. R. Pennington, Biochem. J., 80, 649 (1961).
13. H. Reichard, Acta Chir. Scand., 126, 45 (1963).
14. R. Silber, M. Conclyn, and G. Grusky, J. Clin. Invest., 56, 1324 (1975).
15. M. A. Swanson, Methods Enzymol., 2, 541 (1955).

SPATIAL ORGANIZATION OF LOW-DENSITY LIPOPROTEINS OF THE HUMAN AORTA (A FLUORESCENT PROBE STUDY)

G. E. Dobretsov, M. M. Spirin, A. S. Kuznetsov, and A. V. Popov

UDC 616.132-004.6-092: 616.132-02:577.122.856

KEY WORDS: aorta; low-density lipoproteins; structure; fluorescent probes.

Low-density lipoproteins (LDL) constitute the principal transport form of cholesterol in organs and tissues. Interaction between plasma LDL and the wall of the aorta is currently regarded as the key to the understanding of formation of the atherosclerotic focus [5]. Several properties of LDL of the aortic wall have been investigated [7-9, 11], but the spatial organization of these lipid—protein complexes has not yet been studied.

The aim of the investigation described below was to study the spatial structure of LDL isolated from the human aorta. The method used has only recently been developed and is based on recording the transfer of energy between fluorescent probes and from protein to probes [2, 3].

EXPERIMENTAL METHOD

LDL were isolated within the density range 1.006-1.063 g/cm^3 as described previously [6] from blood plasma obtained from healthy donors and from 14 men aged 39-52 years dying accidentally and free from atherosclerosis of the intima of the aorta. The LDL fraction of the aorta used in the study had the same elution volume on gel-filtration on Sepharose 4B as

Department of Biophysics, N. I. Pirogov Second Moscow Medical Institute. Department of Biochemistry, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 10, pp. 45-47, October, 1983. Original article submitted November 19, 1982.

0007-4888/83/9610-1392$07.50