CHANGES IN PERMEABILITY AND COMPOSITION OF LYSOSOMAL MEMBRANES OF LIVER CELLS AFTER THERMAL BURNS

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An important role in destructive changes in tissues, which are well marked after burns, is played by intensification of autolysis, due to lysosomal enzymes [1, 2]. Since the increase in activity of lysosomal enzymes in the cell in different physiological and pathological states is often accompanied by damage to lysosomal membranes, the question of the stability of these membranes in burns is of great interest. Data in the literature on this question are scanty [4] and were obtained mainly by morphological investigations [3], which, despite their advantages, do not enable quantitative evaluation of the degree of damage to the membrane, do not provide information on changes in its composition, and so on.

The object of this investigation was to study the permeability and chemical composition of lipids of lysosomal membranes of liver cells in thermal burns.

EXPERIMENTAL METHODS

Experiments were carried out on albino rats weighing 150-200 g, on which a flame burn covering 15-20% of the body surface and of the IIIB degree was inflicted. The investigations were carried out 1 h and 7 days after burning. The rats were decapitated and the liver was homogenized at 0-4°C in a glass Potter's homogenizer in 0.25 M sucrose solution (pH 7.4) containing 0.001 M EDTA for 90 sec at 1200 rpm. The final dilution of the homogenate was 1:19 (w/v). An enriched lysosomal fraction was isolated by De Duve's method [6]. As an indicator of the stability of the membranes the total and free activity of cathepsin D, a marker enzyme of the lysosomal matrix, was determined by Anson's method [5]. Free activity was determined in the supernatant after centrifugation at 17,000 g.

Total activity was determined in the homogenate after centrifugation at 1000g and after destruction of the lysosome with Triton X-100 in a final concentration of 0.2%. Cathepsin D activity was expressed in micrograms tyrosine per milligram protein or per gram wet weight of tissue. The stability of the lysosomal membrane was judged from the ratio of free to total activity of cathepsin D.

TABLE 1. Total and Free Cathepsin D Activity in Liver of Burned Rats (M ± m)

Experimental conditions	Free activity, % of total	Total activity, μg tyrosine/mg protein
Control	10,8±2,0	20,9±1,6
1 h after burning	33,1±4,5 (+200)	20,5±1,6
P	<0,01	<0,1
7 days after burning	25,2±3,2 (+150)	23,3±1,3
P	<0,05	<0,1

Legend. Change in % given in parentheses.

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TABLE 2. Composition of Phospholipids and Neutral Lipids in Lysosomal Fractions of Liver in Control and 1 h and 7 Days after Burning (M ± m)

Name of lipid	Control	1 h after burning	7 days after burning
	In % of total phospholipids		
Phosphatidylcholine	5,1±2,5	39,5±1,8	53,2±1,5
Lysolecithin	2,1±0,2	1,1±0,1	0,40±0,06
Sphingomyelin	3,9±0,3	7,3±0,7	8,0±0,5
Phosphatidylinositol	9,0±0,8	12,7±1,3	4,7±0,3
Phosphatidylserine	4,1±0,4	7,5±0,7	1,5±0,1
Phosphatidylethanolamine	24,6±1,3	23,8±1,3	24,3±1,2
Cardiolipin	3,6±0,3	5,5±0,5	4,3±0,2
	In % of total neutral lipids		
Cholesterol esters	13,1±2,1	15,1±1,2	7,2±3,1
Triglycerides	45,4±2,4	37,3±3,1	62,5±7,1
Free fatty acids	13,2±2,3	11,4±3,2	5,7±3,1
Cholesterol	21,4±2,7	25,0±1,2	23,5±0,8
Diglycerides	1,9±0,6	5,1±0,5	1,9±0,4
Neutral lipids: phospholipids	1,0±0,1	0,5±0,1	0,75±0,2

The lipid composition of the membranes of the enriched lysosomal fraction was determined by thin-layer chromatography on silica-gel. To determine the content of individual phospholipids, the method of two-dimensional thin-layer chromatography [9] was used. Neutral lipids were separated by unidimensional thin-layer chromatography [7] and estimated quantitatively by a densitometric method.

EXPERIMENTAL RESULTS

The experiments showed that 1 h after burning free cathepsin D activity in the liver of the burned rats was considerably increased but there was no change in total cathepsin D activity. Free activity expressed as a percentage of the total was correspondingly increased threefold, indicating a significant decrease in the stability of the lysosomal membranes of the liver cells and an increase in their permeability. Both free and total cathepsin D activity 7 days after burning showed changes in the same direction (Table 1). Meanwhile changes in the chemical composition of the lipids of the lysosomal membranes were found (Table 2).

As the experiments to study lipids of liver lysosomes showed, significant quantitative and qualitative changes in their composition were observed both 1 h and 7 days after burning. Particularly marked quantitative changes were observed in the ratio between neutral lipids and phospholipids. Whereas under normal conditions this ratio was close to 1, 1 h after burning it fell to 0.5, and later it increased somewhat to 0.75. The concentration of triglycerides fell at first and then rose. The initial changes in neutral lipids must evidently be regarded as associated with stress reaction and activation of energy metabolism in the burned animals. A statistically significant increase in the fraction of diglycerides 1 h after burning must be particularly noted.

Among phospholipids the most labile components after burns were the minor fractions: sphingomyelin, phosphatidylinositol, and phosphatidylserine. Whereas the relative content of sphingomyelin increased both 1 h and 7 days after burning, the content of phosphatidylinositol and phosphatidylserine in the liver lysosomes was reduced 7 days after burning. The fraction of phosphatidic acid, it will be noted, disappeared after burns.

The order of the changes observed may vary, but the following scheme corresponds reasonably to the true state of affairs: 1 h after burning and during the subsequent week, under the influence of certain as yet unidentified factors, the quantitative and even the qualitative composition of neutral lipids and phospholipids of the lysosomal membranes in the liver of the burned rats changed. As a result of this change the permeability of the lysosomal membranes was disturbed and the lysosomal enzymes were able to enter the cytoplasm. This could be responsible for the intensification of autolysis and the development of the destructive changes in the liver.

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