STRUCTURAL AND FUNCTIONAL CHANGES IN LYSOSOMES OF REGIONAL LYMPH NODES AND LIVER OF DOGS WITH TOXIC HEPATITIS

Yu. I. Borodin,* T. A. Korolenko, A. E. Malygin, A. B. Pupyshev, E. P. Sharaikina, and G. S. Yakobson

UDC 616.36-002-099-0.7:[616.428+616.36]-091.8

Structural and functional changes in lysosomes of cells in the regional lymph nodes and liver were investigated in the course of toxic hepatitis caused by single or repeated administration of CCl_4 to dogs. Total activity of the lymph node lysosomal enzymes studied (β-galactosidase, acid RNase, cathepsin D) exceeded the corresponding activity in the liver of intact dogs, and this reflects the barrier or protective function of the organ. With the development of acute toxic hepatitis a sharp increase was found in acid RNase and cathepsin D activity. At the times of testing (8 and 30 days) the parameters studied had not returned to normal. Meanwhile the mass of the regional lymph nodes and the relative number of macrophages and neutrophils in their sinuses were increased. The increase in lysosomal enzyme activity in the regional lymph nodes following liver damage is connected with the increased functional load on the lymph nodes, which are involved in the hydrolysis of biopolymers brought to the regional lymph nodes with the lymph flow.

KEY WORDS: lymph node; lysosomes; toxic hepatitis.

Subcellular structures (lysosomes) are found in nearly all cells in man and animals [2]. High activity of lysosomal enzymes is found in the blood leukocytes [5], the Kupffer cells of the liver [7], and macrophages [8]. The protective function of the lympho-reticuloendothelial system as a whole is performed largely by the vacuolar system of the cells, the main structural unit of which is the lysosomes. After injury to the liver the metabolic products formed are discharged into the lymph and the barrier-filtration function of the regional lymph nodes is activated. The object of this investigation was to study structural and functional changes in the regional lymph nodes and the activity of lysosomal enzymes in the course of toxic injury to the liver.

EXPERIMENTAL METHOD

Experiments were carried out on 85 mongrel dogs of both sexes. The body weight of the animals of the control and experimental groups did not differ significantly. To discover general principles in the response of the regional lymph nodes to liver damage, two variants of administration of a hepatotoxin were used: single and repeated. Toxic hepatitis was produced by peroral administration of CCl_4 to fasting animals in the morning in a dose of 1 ml pure CCl_4/kg body weight. In the case of repeated (5 doses) administration the poison was given at intervals of 24 h. The animals were killed 1, 3, and 8, and 30 days after the final dose. For biochemical investigations a piece of liver weighing 2-3 g was removed and washed thoroughly with 0.25 M sucrose with 1 mM EDTA (pH 7.5) to remove blood. The lymph node was freed from the surrounding adipose tissue and cut into small pieces. To prepare homogenates of the liver and lymph nodes a mechanical homogenizer with Teflon pestle was used. To assess the state of the lysosomes the total activity (with 0.1% triton X-100 solution) [11] of β-galactosidase, acid RNase, and cathepsin D was determined. The substrates were p-nitrophenol-β-D-galactopyranoside (from Chemopol, Czechoslovakia), polymerized RNA, and hemoglobin (from Reanal, Hungary) respectively. When the activity of these enzymes was determined the recom-

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Department of Normal Anatomy and Central Research Laboratory, Novosibirsk Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 428-431, October, 1978. Original article submitted December 5, 1977.

0007-4888/78/8610-1317$07.50

TABLE 1. Activity of Acid RNase (in μg RNA/min/mg protein), Cathepsin D (in μg tyrosine/min/mg protein), and β-Galactosidase (in μmoles p-nitrophenyl/min/mg protein) in Homogenate of Liver and Lymph Nodes from Dogs during Development of Toxic Hepatitis (M ± m)

Enzyme	Organ	Intact animals	After CCl_4 poisoning						
			single			5 doses			
			1 day	3 days	8 days	1 day	3 days	8 days	30 days
Acid RNase	Liver	5,4±1,08	3,90±0,73	5,64±1,50	5,15±0,98	6,57±0,72	6,24±0,80	7,03±0,32	5,50±0,66
	Lymph node	18,6±2,43	38,95±4,58*	—	33,57±3,68*	55,02±8,50*	24,55±2,43	43,57±4,13*	58,46±13,78
Cathepsin D	Liver	1,50±0,17	3,21±0,29*	2,23±0,34	3,57±0,19*	6,05±0,68*	4,42±0,26*	2,68±0,23*	2,0±0,14
	Lymph node	1,33±0,12	7,33±1,05*	—	6,38±0,40*	9,91±0,30*	4,87±0,59*	2,85±0,28*	7,12±0,53*
β-Galactosidase	Liver	1,36±0,1	0,64±0,082*	1,20±0,21	1,34±0,12	1,01±0,14	2,06±0,29	1,55±0,28	1,15±0,13
	Lymph node	4,72±0,51	2,96±0,24*	4,07±0,34	3,14±0,51	3,23±0,09*	2,90±0,31	1,20±0,002*	2,18±0,23*

*P < 0.05 compared with corresponding control.

mendations of Barrett [6] were followed. The results were subjected to statistical analysis by means of Student's t-test.

A parallel histological study was made of the liver tissue and regional hepatic lymph nodes, on material fixed in Bouin's and Carnoy's fluid. Sections were stained with hematoxylin-eosin, azure II-eosin, and picrofuchsin by Van Gieson's method. Total cell counts on films of the regional lymph nodes were performed by Duplevskaya's method [3]. The cell composition of the sinuses of the lymph nodes was determined in standardized sections [1].

EXPERIMENTAL RESULTS

Circulatory and trophic disturbances and necrobiotic changes were found in the liver of the experimental animals after a single dose of CCl_4. The necrobiotic changes were less marked after three days, and the circulatory and trophic disturbances diminished after eight days. However, the structure of the liver was not completely restored. After repeated administration of CCl_4 only circulatory and trophic disturbances were observed in the animal's liver, and these gradually diminished until the 30th day after the end of poisoning. The development of toxic hepatitis was accompanied by a significant increase in the weight of the regional lymph nodes (Fig. 1) and in the number of small lymphocytes in their total cell counts [4], and by changes in the cell composition in the sinuses (Fig. 2). A sharp increase in the relative number of macrophages and neutrophils was observed 1 and 3 days after poisoning by a single dose and at all times of investigation after repeated administration of CCl_4.

Activity of β-galactosidase and acid RNase in the homogenate of regional lymph nodes of intact dogs was more than three times higher than in the liver homogenate (Table 1). Cathepsin D activity in these organs was practically identical. In toxic hepatitis, whether after single or repeated administration of CCl_4, similar changes were observed in the activity of the lysosomal enzymes studied, and the normal pattern was not restored during the period of investigation. In both variants of the experiments acid RNase activity in homogenates of lymph nodes was twice to three times higher than the corresponding activity in intact animals. Cathepsin D activity was significantly increased: by 5-7 times in the lymph nodes and by 1.5-2 times in the liver (Table 1).

What is the connection between the state of the lymph nodes and activation of the lysosomal apparatus of the cells? The higher activity of acid RNase and β-galactosidase in the lymph nodes of the intact dogs than in the liver was due primarily to the fact that the liver participates in active defense against various foreign substances. During the development of toxic hepatitis and during spontaneous recovery after injury the sharp increase in acid RNase and cathepsin D activity in the regional lymph nodes reflects an increase in the functional load of the lymph nodes performing their barrier-filtration and drainage function. Consequently, in response to the increased arrival of breakdown and metabolic products formed in the liver, an adaptive increase in the activity of the lysosomal enzyme evidently took place.

To what extent are changes in the lysosomes connected with or determined by the cell composition of the lymph nodes? An increase in cathepsin D and acid RNase activity in the regional lymph nodes, incidentally, was observed simultaneously with an increase in the number of macrophages and neutrophils — cells rich in lysosomal enzyme and actively ingesting foreign substances — in the medullary sinuses. Under these circumstances, corresponding to the increase in the relative number of macrophages there was an approximately equal increase in the activity of lysosomal enzymes in the homogenates. Activation of the function of the lymph node is probably brought about to a large extent not by induction of lysosomal enzymes in the cells, but through a change in the cell composition of the organ — an increase in the number of macrophages and neutrophils rich in lysosomal enzymes. In rats, two populations of lysosomes are found both in the spleen and in the lymph nodes: those from macrophages and those from lymphocytes [9]. These populations differ in their enzyme composition. In particular, the latter is richer in cathepsin D. Total acid phosphatase and β-glycuronidase activity in the rat spleen is higher than in the liver [10]. Unfortunately, because of the small size and frequent absence of hepatic regional lymph nodes in rats, their biochemical investigation is difficult and it is practicable only on large laboratory animals (dogs).

By analogy with results obtained on rats [9, 10] it can be tentatively suggested that the increase in total lysosomal enzyme activity in the regional lymph nodes is due primarily to an increase in the number of macrophages, although an increase in the number of small lymphocytes, rich in cathepsin D, in the cell counts must also be borne in mind.

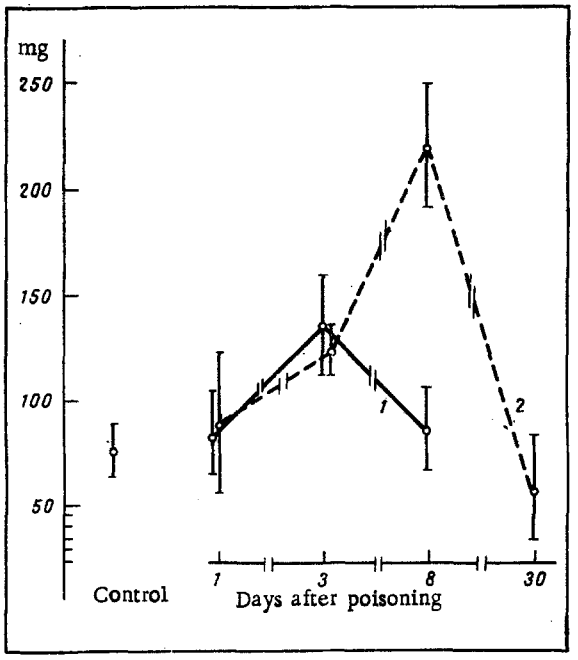

Fig. 1

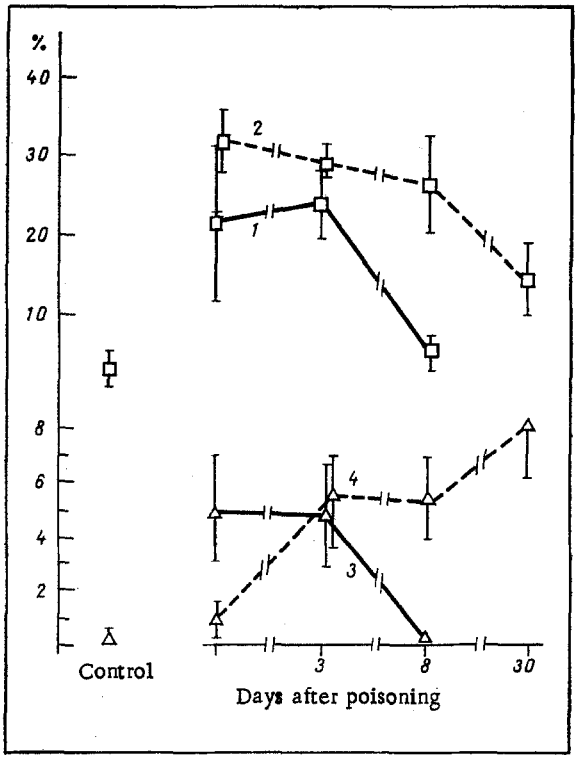

Fig. 2

Fig. 1. Weight of regional hepatic lymph nodes (in mg) during course of toxic hepatitis. 1) Single dose of CCl_4; 2) repeated administration of CCl_4. Number of animals in each group 10.

Fig. 2. Changes in cell composition of regional lymph nodes (in %) in course of toxic hepatitis. 1 and 2) percentage of macrophages after single and repeated administration of CCl_4 respectively; 3 and 4) percentage of neutrophils after single and repeated administration of CCl_4 respectively.

LITERATURE CITED

1. L. I. Bulgakova, Arkh. Anat., No. 11, 56 (1975).
2. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
3. A. P. Duplevskaya, "Lymph node cytomorphology and the clinical picture of certain diseases associated with lymph node lesions," Author's Abstract of Candidate's Dissertation, Dnepropetrovsk (1961).
4. E. P. Sharaikina, in: Lymphatics and Blood Vessels (the Microcirculation from Experimental and Clinical Aspects) [in Russian], Novosibirsk (1976), p. 195.
5. J. L. Avila and J. Convit, Biochim. Biophys. Acta, 293, 397 (1973).
6. A. J. Barrett, in: Lysosomes. A Laboratory Handbook (edited by J. T. Dingle), Amsterdam (1972), p. 110.
7. T. J. C. Van Berkel, J. K. Kruij, and J. F. Koster, Eur. J. Biochem., 58, 145 (1975).
8. G. H. Bourne, J. F. Danielli, and K. W. Jeon, Int. Rev. Cytol., 36, 201 (1973).
9. W. E. Bowers, J. Exp. Med., 136, 1394 (1972).
10. W. E. Bowers, J. T. Finkenstaedt and C. de Duve, J. Cell. Biol., 32, 325 (1967).
11. R. Wattiaux and C. de Duve, Biochem. J., 63, 606 (1956).