CHANGES IN SOME ENZYME SYSTEMS AND METABOLITES OF LIPOGENESIS IN EXPERIMENTAL TUBERCULOSIS

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The general principles governing quantitative changes in phospholipids (PL) in the blood in tuberculosis are known [1, 2, 4, 5]. It is interesting to study differences in PL metabolism in the lung tissue of animals with experimental tuberculosis by the discovery of qualitative and quantitative changes in these compounds and the intermediate products of their biosynthesis, as well as deviations in the activity of various enzymes catalyzing the initial steps of phosphate production. This applies in particular to glycerophosphate (GP), the principle product predetermining the subsequent stages of glycerolipid biosynthesis. In order to study the biochemical mechanism of GP formation, a series of investigations was carried out to determine glycerokinase (GK) and glycophosphate dehydrogenase (GPD) activity, for these enzymes catalyze two processes in opposite directions simultaneously. Differences in their relative activity, due to the character of the quantitative relations between NAD and NADH, make the study of differences in the intensity of oxidation reactions of GP into dihydroxyacetone phosphate (DHAP), as well as processes of its reduction into GP, particularly important. The discovery of an imbalance between these two reactions, reflected ultimately in the pool of GP and, consequently, of PL-glycerides, may be useful when assessing the specific nature of changes in lipid—lipid relations in this particular biological system and in this particular disease. It may also be decisive in determining the pool of their active regulation in a direction advantageous for the sick organism.

The aim of this investigation was to study phospholipid metabolism of lung tissue in experimental tuberculosis.

EXPERIMENTAL METHOD

Experiments were carried out on 38 guinea pigs. A model of pulmonary tuberculosis was formed by subcutaneous injection of a virulent strain of *Mycobacterium tuberculosis* ($H_{37}Rw$) in a dose of 0.0001 mg. The animals were killed on the 30th day by intramuscular injection of hexobarbital; the lungs were isolated in the cold and the visible bronchi and blood vessels were removed simultaneously; the tissue was homogenized in a glass homogenizer with a mixture of 0.25 M sucrose and 0.1 mM EDTA in the ratio of 1:5, respectively. At autopsy on the animals, widespread tuberculosis was found in the form of miliary foci, which in some places were confluent, with the formation of infiltrating areas with caseous liquefaction. The index of involvement was maximal (22 points). GK and GPD activity and the GP concentration in the tissues were determined as in [9], the glycerol concentration by the method in [7], and the DHAP concentration by a microspectrophotometric method [8]. Total lipids were extracted from acetone powders of lung tissue. Individual PL were fractionated by thin-layer chromatography [6] followed by determination of their content of lipid phosphorus [3].

EXPERIMENTAL RESULTS

Our observations showed that GPD activity in intact lung tissue in the reaction of reduction of DHAP to GP (direct reaction) was about 7.5 times greater than GK activity. Comparison of the data relating to the study of the intensity of reactions

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TABLE 1. Level of Some Lipid Metabolites and Enzyme Activity (in μmoles NADH/g tissue) in Normal and Tuberculous Lung Tissue

Parameter	Control (n=10)	Experiment (n = 28)		
Glycerophosphate	0,19±0,01	0,04±0,02	p	0,05
Dihydroxyacetone phosphate	1,45±0,02	1,98±0,01	p	0,05
Glycerol, µg/g	6,8±0,15	8,2±0,3	p	0,05
Glycerokinase	0,2±0,01	0,04±0,01	p	0,05
Glycerophosphate dehydrogenase	1,54±0,06	0,9±0,03	p	0,001
Glycerophosphate dehydrogenase (reverse reaction)	1,98±0,12	1,21±0,09	p	0,05

for GP formation indicates the importance of the glycolytic pathway for the supply of GP in the lungs. High GPD activity in the reaction of oxidation to DHAP (reverse reaction) also was found in the lung tissue of intact animals (Table 1).

Analysis of the results demonstrated inhibition of GPD activity in the direct reaction throughout the period of development of tuberculosis by about 41.5%, and this was accompanied by a sharp decline in GK activity to about 80%. Inhibition of the glycerokinase and glycolytic pathways of formation of GP, as a key metabolite in the process of lipogenesis, was characterized by a sharp fall in its concentration in the test tissues by about 78.9%. Changes observed also were accompanied by increases in concentrations of DHPA and free glycerol by about 36.0% and 16.1%, respectively. We did not undertake a special study of glycerophosphate acetyltransferease (GPAT) activity. The regulatory role of this enzyme in reactions of lipid metabolism is the conversion of GP into phosphatidic acids (PA), the principal intermediate products of phosphatide formation. However, an opinion of the activity of this enzyme can be obtained indirectly from the yield of PA. The results indicate a sharp degree of inhibition by about 57%. The reaction of tissue PA biosynthesis is known to be effected also by direct acylation of DHAP with the formation of acylhydroxyacetone phosphate, which is subsequently converted into acyl-GP, i.e., into PA. It can accordingly be postulated that DHAP accumulation in the lungs in tuberculosis points to inhibition of the pathway of PL biosynthesis described above. Thus the results of these investigations are evidence of the inhibitory action of tuberculosis disease on GPD activity in both the direct and the reverse reaction.

According to the experimental evidence, reflected in Table 2, the presence of a characteristic set of individual PL fractions, distributed on the chromatogram in the following order, is found in lung tissue: lysophosphatidylcholines (LPC), monophosphoinositides (MPI), sphingomyelins (SPM), phosphatidylcholines (PCh), phosphatidylserines (PS), phosphatidylethanolamines (PEA), and cardiolipins (CL). The development of tuberculosis was accompanied by a statistically significant fall of the PS concentration by about 20.9%, PEA by 28.3%, and PCh by 59.1%, with a parallel relative rise of the MPI and SPM concentrations by 51.1% and 31.0%, respectively. The changes observed took place against the background of a considerable reduction of the total PL concentration by about 35.1%. LPC was found in the lung tissue of intact animals only in trace amounts, whereas in tuberculosis the level of these lipids was significantly higher, further evidence of activation of phospholipase A_2, as one of the most important pathogenetic factors in this disease.

In the modern view PCh are ascribed the role of principal surface-active compounds. The sharp decrease in their concentration which we found in this disease is therefore confirmation of the disturbance of the surfactant function of lung tissue taking place in it. Under these circumstances, definite importance must be attached to changes in concentrations of the remaining lung tissue lipid fractions. In the normal functioning of the biological systems of the body, including cell membrane formations and subcellular organelles, great importance is attached to the phylogenetically established constancy of relations between quantities of individual PL constituting the spectrum of these compounds characteristic of the biological system of the lungs. In this connection the results of a study of the specific character of deviations in the value of the ratio (K) between total NPL and APl are quite informative. According to our findings, K for the ratio NPL/APL in pathologically changed lung tissue is appreciably reduced due to a decrease in the content of individual representatives of the NPL (PCh and PEA). This introduces substantial but unwanted corrections into the constancy of the qualitative and quantitative set of PL mentioned above, and this in turn leads to a combination of disturbances in the physiological activity of the cell.

Disturbances of lipid metabolism in the lung tissue in tuberculosis are evidence of the depth of the disorders taking place in its structural and functional status, with the risk of grave complications also in the pattern of PL-protein relations. These are mainly concerned with changes taking place in the regulatory systems of lipid environment of the membrane proteins,

TABLE 2. Changes in Neutral and Acid Phospholipids (in μg lipid phosphorus/g dry weight) in Lung Tissue in Tuberculosis

Porction	Control (n=10)	Experiment (n=28)
Lysophosphatidylcholines	—	41,1±2,1
Phosphoinositides	100,0±3,1	139,0±2,0
Sphingomyelins	98,0±4,1	131,1±1,1
Phosphatidylcholines	225,1±2,3	320,0±3,4
Phosphatidylethanolamines	74,1±3,1	53,0±2,3
Phosphatidylserines	153,0±9,4	121,1±1,9
Cardiolipins	26,1±1,1	27,1±1,6
Total PL	676,3±16,3	603,4±14,1
NPL/APL	1,5±0,01	1,1±0,01

Legend. *p < 0.05. NPL) Neutral phospholipids, APL) acid phospholipids

whose functional activity is largely dictated by the fluidity of the biological membrane, which in turn is determined by the stability of its lipid component. Disturbances of the latter taking place during the development of pathological changes create a qualitatively new profile of their effect on the catalytic activity of membrane proteinases — enzymes and receptors. Both are highly important for the onset, development, and generalization of the tuberculosis focus. They lead to the formation of a "pathological" type of tissue metabolism, with all the complications arising therefrom.

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