LIPID PEROXIDATION AND HIGH BLOOD ENZYME LEVELS IN BACTERIAL DISEASES OF THE CNS

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In different clinical forms of meningococcal infection, the severity of the patient's condition may vary [4]. Worsening of the clinical state is accompanied by marked biochemical changes in the blood, with the appearance of various enzymes in it.

Experimental and clinical evidence has now been obtained to show that one of the chief causes of cell damage and the first event in the chain of metabolic disturbances is injury to membranes, caused by the action of a particular bacterial toxin on the body as a whole, on an area of tissue, or on single cells, and that it is damage to the membranes which leads to the development of the pathological process [1, 2]. One of the factors causing damage to membranes is lipid peroxidation (LPO).

In the present investigation changes in the level of LPO products in the blood and in activity of certain cytoplasmic enzymes, namely aspartate transaminase (AST) and creatine phosphokinase (CPK), which appear in the blood when tissue cells are damaged, were studied in patients with meningitis of meningococcal etiology.

EXPERIMENTAL METHOD

Blood tests were done on 60 patients aged from 20 to 60 years, divided into two groups depending on the severity of course of the disease. Group 1 contained 20 patients with a mixed form of meningococcal infection, in whom the course of the disease was complicated by toxicoinfectious shock or edema and swelling of the brain, with disturbance of the vital functions. Group 2 contained 40 patients (15 with meningitis and 25 with a mixed form of meningococcal infection), in whom the course of the disease was uncomplicated. The control group consisted of 20 healthy blood donors aged from 20 to 40 years.

The biochemical parameters were determined on an OLLI-3000 biochemical analyzer (KONE, Finland). β-Lipoproteins (β-LP) were revealed by precipitation with $CaCl_2$ in the presence of heparin and measurement of turbidity at 620 nm; AST and CPK activity was determined by a kinetic method based on changes in extinction at 340 nm, using kits from KONE and Lachema (Czechoslovakia). The β-LP concentration was expressed in grams per liter, and enzyme activity in units per liter.

Serum concentrations of LPO products were judged by the malonic dialdehyde (MDA) level. To 0.5 ml of serum 3 ml of 1% phosphoric acid and 1 ml of 0.5% thiobarbituric acid were added. The samples were kept for 45 min at 100°C in a waterbath in test tubes with ground-glass stoppers. After cooling, 4 ml of N-butanol was added, and the mixture was shaken and centrifuged at 15,000 rpm for 15 min. The supernatant was decanted into a spectrophotometric cuvette with an optical path length of 1 cm, the absorption spectrum was recorded in the 500-580 nm band, and extinction at 532 nm was determined by the peak separation method. The concentration of LPO products was expressed in nanomoles per liter, the molar coefficient of extinction being taken to be $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ [1]. The concentration of diene conjugates was determined by the known method in a heptane–isopropanol system [3]. The concentration of hydroperoxides was considered to be proportional to extinction at a wavelength of 232 nm.

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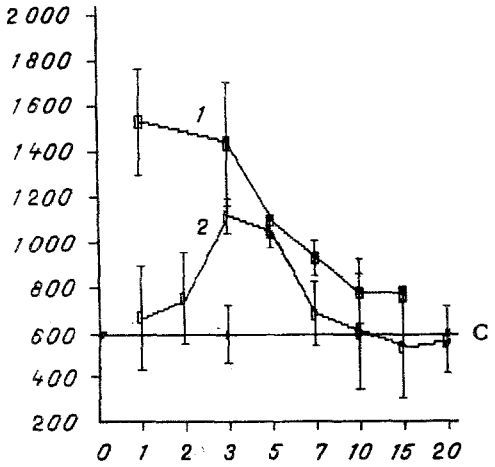

Fig. 1. Time course of MDA concentration (in nmoles/liter) in blood serum of patients with meningococcal infection, running a severe (1) or moderately severe (2) course. C) Control. Here and in Figs. 2-4: abscissa, duration of disease (in days).

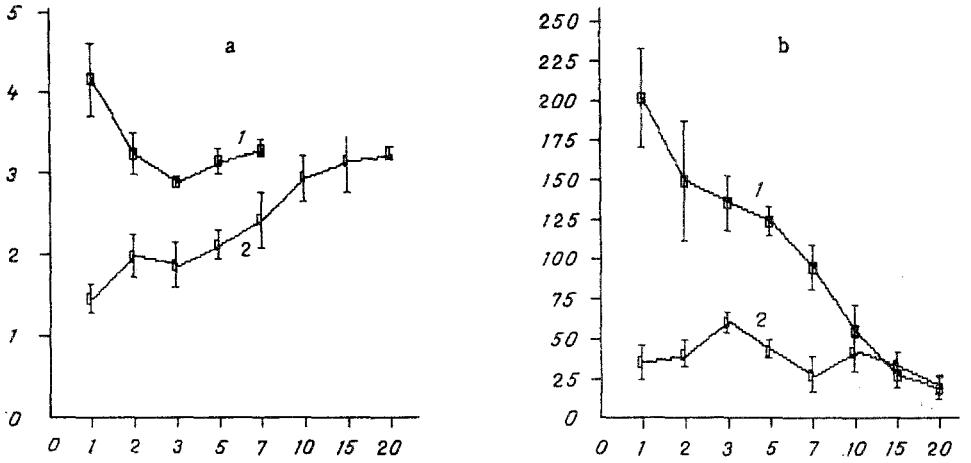

Fig. 2. Time course of concentration of diene conjugates (a) and AST activity (b) in blood serum of patients with meningococcal infection. Ordinate: a) optical density at 233 nm, b) AST activity (in o.d.u./liter). Remainder of legend as to Fig. 1.

The measurements were made on a "Response" spectrophotometer (Gilford, USA).

EXPERIMENTAL RESULTS

Measurement of the serum levels of secondary LPO products in patients in whom the disease ran a severe or moderately severe course revealed differences in the trend of the changes in LPO in these two groups. In the patients who were severely ill (Group 1) the MDA level on the 1st day of the disease reached a maximum, when it was 2.5 times higher than in the control (Fig. 1), but starting with the 5th day, it declined ($p < 0.001$). In the patients of group 2 (moderately severe illness) this parameter on the 1st day did not differ from the control (Fig. 1), on the 3rd day it was almost doubled, and on the 20th day it fell virtually to its original level ($p < 0.001$).

At the beginning of the disease, besides a raised MDA level, the patients also were found to have a high level of primary LPO products (diene conjugates), which also was significantly higher in the seriously ill group. In the patients of group 1 the level of LPO products gradually fell by 20-30% until the 5th day (Fig. 2a), whereas in the patients of group 2 it rose somewhat (by 30%). After the 5th day there was a parallel rise in the levels of diene conjugates in patients of both groups, which was more marked in the group with a moderately severe disease (group 2; Fig. 2). The level of primary LPO products in the control group was an order

TABLE 1. Maximal AST Activity (in U/liter) and β-LP Concentration (in g/liter) during Course of Disease in Patients with Severe Meningococcal Infection (M ± m)

Parameter	Days of disease				
	1st	2nd	3rd	5th	7th
AST	194,5±29,8	143,4±36,6	129,5±16,0	118,2±8,5	90,2±12,8
β-LP	8,38±0,44	7,03±0,26	6,64±0,33	5,96±0,22	5,39±0,20

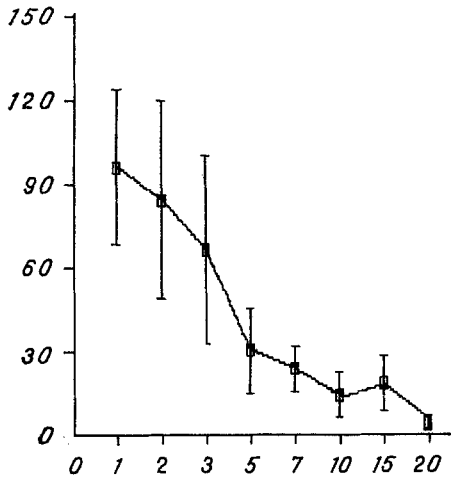

Fig. 3. Time course of serum CPK activity (in units/liter) in patients with severe meningococcal infection.

of magnitude lower, namely 0.234 ± 0.132 optical density unit (o.d.u.). This raised level of LPO products in the blood serum of patients with meningitis is probable evidence of activation of LPO processes, which may lead to a disturbance of function of the cell membranes and to an increase in their permeability, to cytolysis, and to the discharge of LPO products from the cells together with cytoplasmic enzymes.

To test this hypothesis the time course of AST activity in the course of the disease was studied. AST activity was found to increase in different patients at different times, and the increase was connected with a change in the concentration of LPO products. Data characterizing the trend of AST release in the patients studied are summarized in Fig. 2b. Release of the enzyme reached a maximum on the 1st, 2nd, 3rd, 5th, and 7th days of the disease. In the patients of group 2 AST activity was unchanged and was within normal limits (21.7 ± 10.5 U/liter). The coefficient of correlation between the change in AST activity and the change in concentration of LPO products was 0.91 and 0.79 in groups 1 and 2, respectively.

Changes in the β-LP level in these same patients were similar in character: it reached a maximum at the same times of the disease (Table 1). The coefficient of correlation between changes in AST activity and changes in the serum β-LP level was 0.92. In the patients of group 2 the β-LP level gradually rose from the 1st through the 15th days of the disease from 3.24 ± 0.67 to 6.95 ± 0.96 g/liter. The coefficient of correlation between the concentration of LPO products and the β-LP level was 0.95 and 0.95 in groups 1 and 2, respectively.

Thus when the level of LPO products in the group of severely ill patients was high, release of AST from the cells was observed. The level of LPO products could be raised for two reasons: first, due to the formation of LPO products in an infected cell and their subsequent discharge into the blood stream (as shown by high correlation between activity of the cytoplasmic enzyme and the concentration of LPO products), and second, due to an increase in the β-LP concentration in the blood, where they are the principal carriers of lipids.

The results show that the increase in the β-LP concentration observed during the first 5 days of the disease may be one cause of the raised level of LPO products. It can be tentatively suggested that this, in turn, leads to a disturbance of the barrier functions of the

membranes and to cytolysis of the cell. It is an interesting fact that the increase in AST activity in the blood serum of the severely ill patients could be observed only when the level of LPO products exceeded 1560 ± 237 nmoles/liter, and this could be a feature of diagnostic importance. The level of LPO products in the patients of group 2 was roughly one-third to one-half of that in group 1, and no increase was observed in their AST activity.

AST is known to be the cytoplasmic enzyme most widely distributed in man. CPK is a specific enzyme for heart and skeletal muscle. It was interesting to study the time course of activity of this enzyme during meningococcal infection also. Determination of CPK activity in the serum of patients of group 1 showed that it reached a maximum after the 1st day of the disease (94.9 ± 27.3 U/liter compared with a normal value of under 20 U/liter) and it returned to normal by the 10th day (3.84 ± 2.35 U/liter; Fig. 3). Patients of groups 1 and 2 with super-added herpetic infection, mainly on the 5th day, were the exception (CPK activity 109.9 ± 37.0 U/liter). CPK activity in the patients of group 2 was a little raised or normal, and no significant changes in its value were observed under these circumstances.

The marked specificity of CPK for the heart during considerable functional loading (tachycardia, vascular spasm, increased viscosity of the blood, and so on) explains the high level of the enzyme in the majority of seriously ill patients after the 1st day of the disease and its rapid return to normal immediately after recovery of the parameters indicated above. The coefficient of correlation between the change in CPK activity and the concentration of LPO products in the patients of groups 1 and 2 was 0.97 and 0.63, respectively. In addition, high CPK activity in the group of seriously ill patients after the 1st day correlated positively with the level of LPO products and hydroperoxides.

The main difference between the severely ill and the moderately severely ill patients is thus that immediately after the rise of the level of LPO products on the 1st day of the disease there was a sharp increase in AST and CPK activity, whereas in the patients of group 2 the level of LPO products was about two to three times lower and no sharp increase in enzyme activity was observed.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. Yu. A. Vladimirov, "The role of disturbances of the barrier and matrix functions of the lipid layer of biological membranes in pathology," Spoken Dissertation, Moscow (1985), p. 4.
3. V. P. Gavrilov and M. I. Mishkorudnaya, Lab. Delo, No. 3, 33 (1983).
4. V. I. Pokrovskii, L. I. Favorova, and N. N. Kostyukova, Meningococcal Infection [in Russian], Moscow (1976).