LIPID PEROXIDATION REACTIONS FOLLOWING ADMINISTRATION OF VARIOUS DOSES OF ZYMOSAN

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Recommendations for clinical use of bacterial polysaccharides are based on their broad spectrum of biological activity: they have low toxicity, they are not antigenic, and they cause virtually no tissue destruction. Administration of bacterial polysaccharides stimulates macrophages, which can not only ingest and digest microorganisms, but can also form active metabolites of oxygen with bactericidal action, but in some cases giving rise to undesirable side effects. The aim of the present investigation was to study the role of lipid peroxidation (LPO) products in the mechanism of these effects.

EXPERIMENTAL METHOD

Experiments were carried out on 32 male Wistar rats weighing 180-200 g. Zymosan (Reanal) was injected intravenously, taking account of the results of previous studies [3], in a dose of 100 mg/kg (experiments of series I, 20 mg per animal) and 25 mg/kg (experiments of series II, 5 mg per animal). The control rats received an intravenous injection of 0.85% of NaCl solution. Arterial blood in the experiments of series I was taken from the carotid artery and venous blood from the right ventricle. In the experiments of series II blood was taken from the right and left ventricles immediately after thoracotomy. The animals were deeply anesthetized with thiopental.

Blood oxygenation was measured by means of an electronic oxygen-saturation meter (Radiometer, Denmark) and the hematocrit index on a microhematocrit centrifuge (Radiometer). The reduced glutathione concentration was measured by the alloxan method [5], cholesterol as in [1], and lactate as in [2]. The pH of the blood was measured on an OP-215 microanalyzer (Radelkis, Hungary). The concentration of LPO products was determined by the known method based on determination of UV spectra after extraction of lipids by Folch's method.

EXPERIMENTAL RESULTS

In rats stimulated by zymosan the hematocrit index rose after 3 days (Fig. 1a). This increase was statistically significant, however, only in arterial blood in the experiments of series I. During the next 5 days the hematocrit index of the animals of this group fell compared with the control. In the experiments of series II there was only a tendency toward an increase on the 3rd day of observation and toward a decrease on the 5th day.

The time course of oxygenation of the arterial blood (Fig. 1b) on the whole was independent of the dose of zymosan given: a reduction of 20-30% of the blood oxygen saturation was observed on the 3rd day. In the experiments of series I, oxygenation was increased in the venous blood on the 3rd day, but on the 5th day it did not differ from the initial values. Oxygenation of arterial blood in the experiments of series II was below the normal phyisological level, due to the conditions of blood sampling. Hypoxemia, detected on the 3rd day, led to accumulation of initial LPO products in the venous blood ($p < 0.05$) in the experiments of series II, and also to an increase in both arterial ($p < 0.05$) and venous blood ($p < 0.05$) in series I. On the 5th day the level of LPO products was close to the control values (Fig. 1c).

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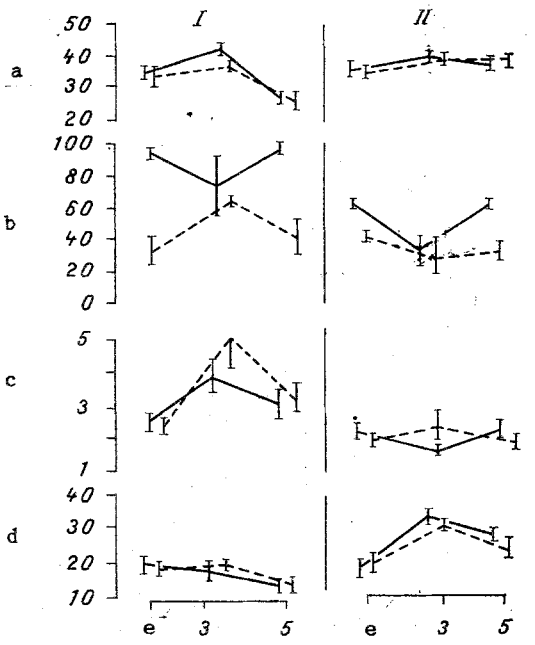

Fig. 1. Effect of various doses of zymosan on level of LPO products, of reduced glutathione, and on blood oxygenation. Abscissa, time of investigation (in days); ordinate, parameter studied. I) Zymosan (100 mg/kg); II) zymosan (25 mg/kg). a) Hematocrit index (in %); b) blood oxygen saturation (in %); c) level of diene conjugates (in D_{233}/ml); d) concentration of reduced glutathione (in mg%); e) control. Continuous line — arterial blood, broken line — venous blood.

Against this background the time course of the reduced glutathione concentration is interesting (Fig. 1d). In series I a gradual fall of the reduced glutathione level was observed on the 3rd day, and on the 5th day the decrease was statistically significant compared with the control. In the experiments of series II the glutathione concentration in the arterial and venous blood was increased on the 3rd day, but on the 5th day its level has returned to the initial value.

Measurement of the pH of the blood in the experiments of series II showed a shift toward the acid side ($p < 0.05$). A tendency was discovered for the lactate concentration to rise, also on the 3rd day, probably connected with activation of glycolysis, due to arterial hypoxemia and venous hyperoxia. The increase in the hematocrit index, mentioned above, which was more marked in the experiments of series I, must evidently be regarded as a compensatory mechanism in response to developing tissue hypoxia. The shift of the pH to the acid side was connected more with respiratory mechanisms and less with metabolic: on the 5th day the lactate concentration was below the control values in both venous and arterial blood (Table 1).

In the experiments of series II the concentration of total lipids was unchanged at all times of observation, whereas in series I their concentration was depressed on the 5th day. A redistribution of cholesterol was observed: on the 3rd day the cholesterol concentration in arterial blood reached the control level of venous blood, whereas that in venous blood reached the control level of arterial blood. The possibility cannot be ruled out that the rise of the cholesterol level in arterial blood was connected with a change in the metabolic function of the lungs and, in particular, with metabolism of the lung surfactant [4]. On the 5th day the arterio-venous cholesterol ratio returned to the control values.

The results of the experiments of series II (an increase in the reduced glutathione concentration and a fall in the level of diene conjugates in arterial blood, an increase in the cholesterol concentration in arterial blood accompanied by a decrease in venous blood) thus

TABLE 1. Changes in Cholesterol and Lactate Concentrations and pH of Blood after Administration of Zymosan (25 mg/kg) (M ± m)

Experimental conditions	Blood	Cholesterol, mg%	Lactate, mg%	pH
Control (5)	Arterial	127,2±25,0	3,04±0,27	7,30±0,03
	Venous	159,5±25,0	2,92±0,22	7,24±0,02
3rd day after injection of zymosan (5)	Arterial	160,0±86,0	3,60±0,40	7,31±0,01
	Venous	130,0±70,0	3,23±0,45	7,26±0,01
5th day after injection of zymosan (6)	Arterial	132,0±30,0	2,06±0,26	7,24±0,01
	Venous	172,0±40,0	2,11±0,10	7,18±0,01

Legend. Number of experiments given in parentheses.

indicate stimulation of the antioxidative system of the lungs following administration of small doses of zymosan. In the experiments of series I a toxic effect of zymosan was demonstrated (elevation of the level of diene conjugates, a decrease in the reduced glutathione concentration in arterial and venous blood).

Arterial hypoxemia with venous hyperoxia, developing after intravenous injection of 20 mg zymosan create favorable conditions for LPO activation. For instance, after injection of 5 mg zymosan a tendency toward an increase in the concentration of LPO products was observed in the venous blood, whereas after injection of 20 mg, the increase in diene conjugates was significant in both venous and arterial blood. In this connection, bearing in mind the physiological and pathophysiological effects of microbial polysaccharides, when zymosan is used in clinical practice strict attention must be paid to its dose.

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