is still unknown. However, the results of the present investigation suggest that LPS activates B-cell division through activation of guanylate cyclase in the cell membrane. The action of LPS, under these circumstances, evidently does not involve the ion-transporting system of the membrane. Conversely, the mitogenic polyanion PAA activates B-cell division by increasing ionic permeability of the plasma membrane, without changing cyclase activity.

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USE OF LIPOSOMES AS A NONSPECIFIC DETOXICATOR IN THE CRUSH SYNDROME

A. V. Stefanov, V. M. Kreines, A. V. Dmitrieva, and V. K. Lishko*

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Phospholipids of the plasma membrane of cells play an important role in recognition and binding of various biologically active substances and toxins [8, 9]. It has been shown, for instance, that cholera toxin interacts with the ganglioside G_{JM}, isolated from the epithelium of small intestine, with a binding constant of 10^{-9} M. Addition of exogenous gangliosides sharply reduced the pathogenic action of cholera toxin, evidently by preventing it from binding with the cell membranes [10]. Artificial phospholipid vesicles (liposomes) are analogs of cell membranes and, because of their small size, they have a large total surface area. The high adsorptive activity of liposomes relative to proteins has often been described [7].

Considering that toxic metabolites appearing in the blood stream in ischemia are substances of protein nature and have high affinity for cell membranes [4], the writers postulated that injection of liposomes into animals with a model of endogenous poisoning would enable an additional target to be created for toxins with affinity for the membrane and would prevent or alleviate the course of toxic shock. To test this hypothesis a model of the crush

*Academician of the Academy of Sciences of the Ukrainian SSR.

Laboratory of Biochemistry of the Nerve Cell and Department of Physiology of the Circulation, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev.

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TABLE 1. Changes in Toxicity of Venous Blood Plasma (LD_{100}) in Postcompression Period of CS (M ± m)

Time of recording	Control animals with CS (sec)	Injection of liposomes, 25 mg/kg (sec)	Injection of liposomes, 50 mg/kg (sec)
End of compression period	593±23	635±52	635±52
Postcompression period			
5 min	309±44	326±21	405±27
30 min	330±42	653±50	975±84
1 h	298±28	637±47	485±39
2 h	187±37	587±49	715±52
3 h	283±26		712±53
4 h	312±22		710±51
5 h	201±22		615±55

syndrome (CS) was chosen. Toxic injury in the postcrushing period of CS develops rapidly, and the first phase of the pathological process is the phase of hemodynamic disorders, which is easily tested.

EXPERIMENTAL METHOD

Liposomes were prepared from ovolecithin, obtained by the method in [6] in the writers' modification. The phospholipid was dried in a round-bottomed flask in a vacuum evaporator until all solvent had been removed. A solution containing 17 mM NaCl was added to the resulting phospholipid film, and the flask was shaken until emulsification was complete. The suspension thus formed was treated with ultrasound. The model of CS was produced by applying a graduated spring press to a dog's hind limb with relative force of 3 kg/cm^2 for 3 h, which corresponds to moderately severe injury. Experiments were carried out on 10 mongrel dogs of both sexes weighing 7-15 kg, under chloralose-urethane anesthesia (50 and 500 mg/kg, intravenously). In the course of dissection the following vessels were mobilized: the femoral artery, through which a double-barreled catheter was introduced into the arch of the aorta to record the arterial pressure (BP) and cardiac output (CO); the external jugular vein for injection of cold physiological saline, and the common carotid artery, through which retrograde catheterization of the left ventricle was carried out. After the end of the preparatory operations heparin was injected (500 U/kg) into the animals. The state of the cardio- and hemodynamics was assessed by studying the following parameters: BP, measured electromanometrically, CO, determined by the thermodilution method with calculations of the total peripheral resistance (TPR) [1]. Pressure in the left ventricle (P_{lv}) and its first derivative (dP/dt) were recorded by means of an electromanometer, and Veragut's index (VI) [11] and the relaxation index (RI) [3] were calculated. The heart rate (HR) was determined from the ECG. The various parameters were recorded synchronously on an N338 multichannel automatic writer. Toxicity of the venous blood plasma (LD_{100}) also was determined by the paramecium test [5]. After the end of the experiment the animals were autopsied.

EXPERIMENTAL RESULTS

Liposomes were injected in doses of 25 and 50 mg phospholipid/kg body weight. The infusion began simultaneously with decompression of the limb. Control experiments showed that the doses of phospholipid used have no appreciable effect on the state of the animals' cardiovascular system.

In animals with a model of CS, a progressive increase in the toxic properties of the venous blood plasma took place in the early postcompression period (Table 1). By contrast, injection of liposomes prevented the increase in toxicity, and by the end of the period of observation it did not differ significantly from the control values.

Parallel with the increase in toxicity of the blood, 30 min after decompression myocardial contractility in animals with the untreated form of CS was reduced. VI fell by 27%, and P_{lv} and RI by 17 and 32%, respectively. Changes in contractility in animals receiving liposomes were transient (Fig. 1). The fall in VI toward the 30th minute and 2 h by 20 and 29% could be due both to inadequate adsorption of toxins and to the negative action of other metabolites,

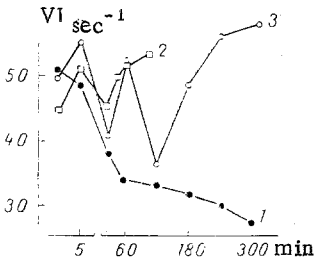

Fig. 1

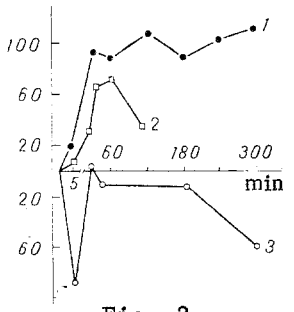

Fig. 2

Fig. 1. Changes in myocardial contractility in postcompression period of CS. Here in Figs. 2 and 3: 1) control animals with CS, 2) detoxification with liposomes in dose of 25 mg/kg, 3) detoxification with liposomes in dose of 50 mg/kg.

Fig. 2. Changes in TPR in postcompression period of CS.

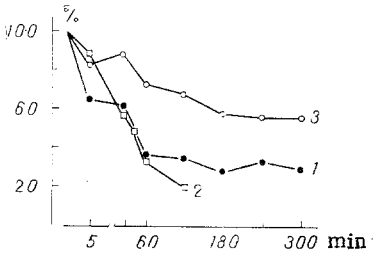

Fig. 3. Changes in BP in postcompression period of CS.

such as lactate, or ionic disturbances characteristic of CS, which were not corrected. Toward the end of the period of observation myocardial contractility was restored to its initial values, evidence that the functional state of the heart muscle was quite good.

The writers showed previously that spasm of peripheral vessels is toxic in genesis and develops parallel with the increase in toxicity of venous blood plasma [2]. After removal of the press from animals with the model of CS, TPR rose from 0.028 ± 0.003 to 0.051 ± 0.007 $N \cdot sec \cdot cm^{-5}$ ($P < 0.01$), and it remained at high values until the end of observation (Fig. 2). Injection of phospholipid in a dose of 25 mg/kg body weight reduced the severity of peripheral spasm, and by the end of the second hour after decompression TPR was 124% of its resting value. Injection of liposomes in a dose of 50 mg/kg prevented the development of generalized peripheral spasm. The value of TPR in the animals of this group 5 h after removal of the press was actually a little lower than at rest, possibly due to the uncorrected ionic disturbances.

Despite high values of TPR and centralization of the circulation in animals of the control group, depression of myocardial contractility and the decrease in CO led to a progressive fall of BP from 158 ± 14 to 110 ± 11.3 hPa. After injection of liposomes into the animal in a dose of 25 mg phospholipid/kg body weight BP also fell, but this decrease took place accompanied by virtually normal peripheral vascular tone and a very small reduction in myocardial contractility and CO. An increase in the dose of liposomes to 50 mg/kg enabled BP to be maintained within physiologically normal limits throughout the period of observation (Fig. 3).

Disturbances of the cardiohemodynamics thus revealed were caused by considerable morphological changes in the organs and tissues. At autopsy on animals with the model of CS, large and frequently transmural myocardial infarcts were found. At the same time, small focal lesions were observed along the coronary vessels with multiple subendocardial hemorrhages.

Multiple hemorrhagic foci also were found in the lungs, liver, kidneys, and mesentery. In animals undergoing detoxication by means of liposomes, no hemorrhagic foci were found in the lungs, liver, kidneys, or mesentery. Predominantly subendocardial focal hemorrhages were recorded in the myocardium.

The writers showed previously that products of modified tissue metabolism and proteolysis of cells, on entering the systemic blood flow from the injured limb after decompression, has a negative inotropic action on the myocardium and a direct constrictor action on the resistive vessels. Cardiac weakness developing as a result of this, despite the high vascular resistance, leads to a fall of BP which, in turn, leads to tissue hypoxia and reaccumulation of toxic metabolites.

Injection of liposomes completely prevented generalized peripheral spasm and substantially reduced the disturbances of myocardial contractility and cardiac output, evidence of neutralization of most of the toxic products. In the present experiments, for several hours after decompression of the injured segment, the functional state of the animals' cardiovascular system remained good.

Injection of a nonspecific parenteral sorbent (lecithin liposomes) simultaneously with decompression thus reduces the toxicity of the blood in animals in the postcompression period of CS, and thereby prevents the onset of hemodynamic disorders. As a result the characteristic vicious circle of CS, the principal pathological stage in the formation of the endogenous intoxication syndrome, does not arise.

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