DISTURBANCES OF CARDIAC CONTRACTILITY IN ISCHEMIC SHOCK: PROTECTIVE EFFECT OF ANTIOXIDANTS AND PHOSPHOLIPID LIPOSOMES

M. V. Bilenko, A. A. Morgunov,
T. D. Churakova, V. G. Bulgakov, and
P. G. Komarov

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Ischemic shock, complicating restoration of the blood flow in massive organs deprived of their blood supply for a long time (tourniquet syndrome, compressed or replanted limb syndrome, crush syndrome, etc.), is accompanied by disturbance of the central hemodynamics, impairment of the coronary blood flow, and weakening of myocardial contractility [13]. It has also been shown on a model comprising application of a tourniquet for 6 h followed by reperfusion of the hind limb in rats that ischemic shock is accompanied by an increase in the concentration of lipid peroxidation (LPO) products in the blood and vital organs [1, 14]. Intensification of LPO processes and their generalization in the body also have been found in shock of different etiology: traumatic, hemorrhagic, and burn shock [4, 6, 12, 14].

Meanwhile, the contribution of LPO processes to the mechanism of disturbances of the coronary blood flow and of myocardial contractility in shock remains unstudied, and the possibility of preventing and treating these disturbances with the aid of antioxidants has virtually not been investigated. The present study was devoted to an examination of these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 458 female Wistar rats weighing 200-260 g. Ischemic shock was produced by application of tourniquets to the two hind limbs at the level of the upper third of the thigh for 6 h, after which the tourniquets were removed. In the experiments of group 1 (404 rats) the severity of ischemic shock was estimated on the basis of the survival rate of the animals which were under observation for 3-7 days. In group 2 (54 rats) contractility of the heart was evaluated, for which purpose the hearts were removed from heparinized (3 mg/kg) rats under hexobarbital anesthesia (70 mg/kg) at the following times: before application of the tourniquets (initial data), 1, 6, and 12 h after removal of the tourniquets (experimental). Cardiac contractility was studied on a model of coronary perfusion of the heart with Krebs-Henseleit solution, oxygenated with carbogen, at pH 7.4 by Langendorff's method in the modification in [10].

The volume velocity of coronary perfusion (VCP) was determined by measuring the outflow from the pulmonary artery, and cardiac contractility by measuring the proximal pressure developed in the left ventricle (P_{max}), using an isovolumic baloon technique. The heart rate (HR) was counted on the ECG. All parameters were recorded on an SP-1400 monitor ("Statham") 15, 30, 45, and 60 min after the beginning of perfusion of the heart.

In the control series no preparations were administered, whereas in the experimental series antioxidants were injected: ionol (2,6-di-tert-butyl-4-methylphenol) and a sulfur-containing derivative of 2,2,6,6-tetramethylpiperidine (1-hydroxy-TMP), which, in aqueous solutions, forms nitroxyl radicals, possessing antioxidant activity [3].

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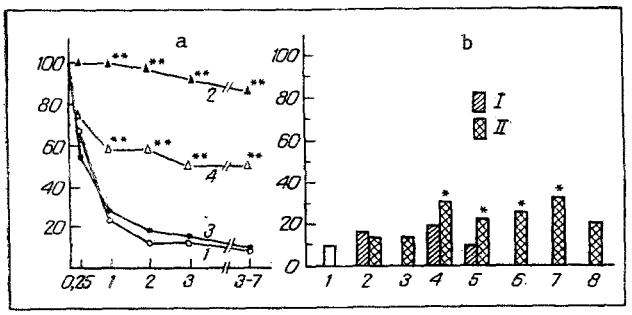

Fig. 1. Dynamics of survival rate of rats after ischemia and reperfusion of the limbs, in animals treated with antioxidants (a) or PL liposomes (b). Abscissa: a) time of observation (in days); ordinate, survival rate (in %). a: 1) Without preparations (control); 2) ionol 4 h before application of tourniquets; 3) the same 30 min before removal of tourniquets: 4) 1-hydroxy-TMP; b: 1) without PL (control); 2, 3) PCh and OPCh respectively, in the form of monolayer liposomes; 4-6) PCh, PCh + PEA, and OPCh respectively, in the form of multilayer liposomes; 7, 8) PCh and OPCh respectively in the form of multilayer liposomes + ionol; I) PL in a dose of 50 mg/kg; II) PL in a dose of 100 mg/kg. *$p < 0.05$, **$p < 0.01$ compared with control.

Ionol was injected into the rats in a dose of 120 mg/kg 4 h before application of tourniquets to the limbs or 30 min before removal of the tourniquets, in the form of an aqueous suspension in Tween-80; 1-hydroxy-TMP was given in a dose of 36 mg/kg 1 h before removal of the tourniquets.

In one series of experiments, egg phospholipids (PL) were injected 30 min before removal of the tourniquets: phosphatidylcholine (PCh), a mixture of PCh with phosphatidylethanolamine (PEA) in the ratio of 1:5 (by weight), and oxidized PCh (OPCh). The PL were used in the form of monolayer or multilayer liposomes in doses of 50 and 100 mg/kg in 3-5 ml of physiological saline. PCh and OPCh, in doses of 100 mg/kg, also were injected together with ionol (120 mg/kg) in the form of multilayer liposomes. All preparations were injected intraperitoneally, and in the control experiments an equal volume of physiological saline was used.

Preparation of the PL, isolation of PCh and PEA, preparation of the liposomes and evaluation of their monolayer state were described previously [2]. PCh were oxidized by addition of an alcoholic solution of PCh in Tris-buffer (10 mM) in a final concentration of 2 mg/ml in a Fe^{++}–ascorbate system (2.5 μM and 200 μM respectively), with constant aeration with oxygen, and with the periodic (every 2 h) addition of ascorbate, at room temperature and at pH 7.4. OPCh were isolated by the method in [9]. The high index of oxidation of OPCh (ratio of optical density of lipid solution at 232 nm to its optical density at 215 nm 0.90) was achieved by prolonging the incubation time to 6 h. In the experiments with PL + ionol, the preparation was added to a solution of PCh or OPCh in chloroform.

EXPERIMENTAL RESULTS

Determination of the survival rate of the rats with ischemic shock (on the 3rd day of reperfusion) showed that in the series without the preparations virtually all the rats died in the course of 24 h reperfusion of the limbs (Fig. 1a). Injection of ionol 4 h before application of tourniquets to the limbs and of 1-hydroxy-TMP 1 h before removal of the tourniquets led to a considerable increase in the number of surviving animals (90 and 50% of the initial number; $p < 0.01$ and $p < 0.05$ respectively). Ionol, injected 30 min before reperfusion of the limbs, had no protective effect.

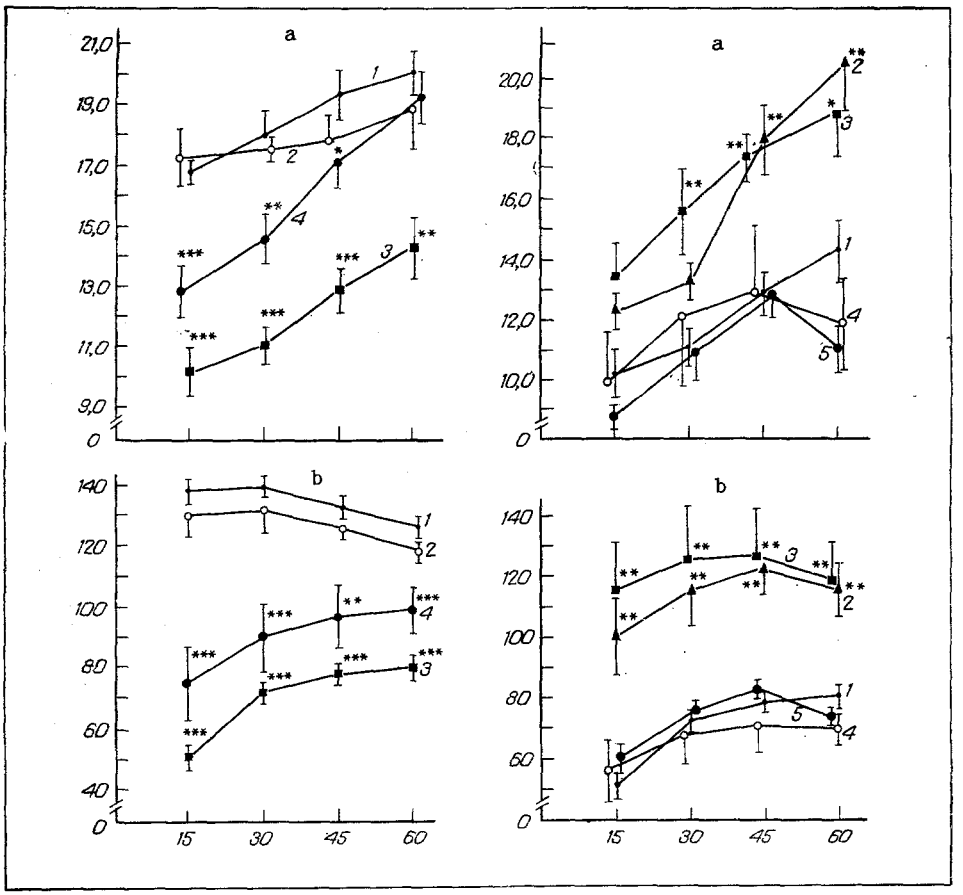

Fig. 2

Fig. 3

Fig. 2. Velocity of coronary perfusion (a) and contractility of the heart (b), taken from rats after different periods of perfusion of the ischemic limbs. 1) Intact rats, 2-4) after reperfusion of ischemic limbs for 1, 6, and 12 h respectively. Ordinate: a) VCP (in ml/min/g wet weight of tissue); b) P_{max} (in mm Hg). Abscissa, duration of perfusion (in min). $p^* < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with hearts of intact rats.

Fig. 3. Velocity of coronary perfusion (a) and contractility of heart (b) taken from rats after 6 h of perfusion of ischemic limbs of animals treated with antioxidants and liposomes from PCh and OPCh. 1) Without preparations (control); 2) ionol 4 h before application of tourniquets; 3) 1-hydroxy TMP; 4) PCh in the form of multilayer liposomes, in a dose of 100 mg/kg; 5) OPCh – same conditions as PCh. Ordinate: a) VCP (in ml/min/g wet weight of tissue); b) P_{max} (in mm Hg). Abscissa, duration of perfusion (in min). $p^* < 0.05$, $^{**}p < 0.01$ compared with control.

The effect of PL of different composition on the severity of ischemic shock, assessed by the criterion of survival of the rats, was weaker than the effect of the antioxidants and depended on the type of lipsomes and the dose of PL. Irrespective of their composition and of the dose of the PL contained in them, monolayer liposomes did not increase the percentage of surviving rats, whereas the effect of multilayer liposomes was dose-dependent: in a dose of 50 mg/kg PCh or PCh + PEA had no protective effect, but in a dose of 100 mg/kg all PL (PCh, PCh + PEA, OPCh) moderately but significantly increased the number of surviving rats (Fig. 1b). The type of PL used and their degree of oxidation had no effect on the action of the liposomes.

Combined administration of PCh and OPCh with ionol, in the form of multilayer liposomes, either did not change the survival rate of the rats or actually worsened it a little compared with that observed in animals treated with PL alone, without ionol; this corresponds to the absence of a protective effect of ionol given in the same dose 30 min before reperfusion of the limbs without PL (Fig. 1a).

Evaluation of contractility of the heart, taken from rats with ischemic shock at various times after reperfusion of the ischemic limbs, showed (Fig. 2) that the values of VCP and P_{max} for the heart taken from the rats 1 h after the beginning of perfusion of the ischemic limbs do not differ from values obtained in the heart removed from intact animals. In hearts taken after 6 and 12 h of limb reperfusion the values of VCP and P_{max} during the first 15 min of isolated perfusion were significantly depressed (62-73 and 39-48% of the value in the intact heart respectively); there was no significant change in HR. On continued perfusion the value of VCP had a strong, and the value of P_{max} a less strong, tendency toward recovery.

The results of experiments to study the protective action of antioxidants and PL of different composition on VCP and the contractile function of the heart, performed on the heart taken from rats after 6 h of limb perfusion, are shown in Fig. 3.

Injection of ionol and 1-hydroxy TMP, which effectively increase the survival rate of animals with ischemic chock, also led to an increase in VCP and P_{max} in the rat heart at nearly every stage of its isolated perfusion. Injection of PCh and OPCh into the animals in a dose of 100 mg/kg in the form of multilayer liposomes, while leading to a moderate increase in the percentage of surviving rats, did not affect the value of VCP or the contractility of the heart.

Correlation analysis between the percentage of survivng animals on the 3rd day after reperfusion of the ischemic limbs and the value of P_{max} in the heart taken 6 h after reperfusion of the limbs, and value estimated 15 min (r_1) and 45 min (r_2) after its isolated perfusion in vitro, revealed strong correlation ($r_1 = 0.65$, $r_2 = 0.68$; $p < 0.05$); this indicates that an essential role in the mechanism of death of the animals from ischemic shock is played by damage to the contractile function of the heart.

The results show that antioxidants and, to a lesser degree, egg phospholipid liposomes, reduce the severity of ischemic shock, as is shown by an increase in the number of surviving animals and improvement of the contractile function of the heart, when assessed under identical conditions of adequate perfusion in vitro.

The high protective effect of the antioxidant ionol, in agreement with earlier data [1], was observed when the compound was injected before application of the tourniquets to the limbs and was absent if it was injected after their application, regardless of the way in which it was given (in Tween-80, in the composition of PCh or OPCh liposomes), whereas the antioxidant 1-hydroxy-TMP exhibited its activity when given 1 h before removal of the tourniquets, i.e., when it was able to act only during the reperfusion period. These observations broaden the possibilities of the use of this new compound, and evaluation of its effect when given in combination with other antioxidants becomes promising.

The beneficial effect of egg PL liposomes observed when the severity of ischemic shock is assessed in terms of the survival rate of the animals was moderate in character and was exhibited only when multilayer, but not monolayer, liposomes were used. This result can evidently be attributed to the ability of multilayer liposomes to leave the blood plasma more rapidly and to accumulate in the liver [5], for assuming the possibility of adsorption of circulating LPO products on the liposomes [8], this may lead to their elimination from the blood and their transport into the liver, thereby promoting metabolism of the LPO products on account of the peroxidase function of the cytochrome P 450-dependent mono-oxygenase system [1, 11]. Such a mechanism may perhaps explain the presence of the weak protective effect of liposomes containing PL with different degrees of saturation, and even of OPCh, which does not rule out a toxic effect of endogenous LPO products formed during shock and circulating in the blood stream, not in liposomal form, but in the composition of high-density lipoproteins. The protective effect of liposomes with different composition also has been demonstrated in other pathological conditions accompanied by intensification of LPO (the long-term crush syndrome, CCl_4 poisoning) [7, 8].

The absence of a protective effect of egg PL on contractility of the heart is most probably due to the weakness of their effect, although it may also be connected with the ability of liposomes to accumulate selectively in the liver [5] as mentioned above, or with their unfavorable action on cardiac contractility, revealed when different kinds of PL are added to the perfusion medium of the heart [2].

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