Thus the induced very weak luminescence of the test tissues had a definite time course, reflecting LPO in different stages of development of the acetate gastric ulcer in the different groups of rats. The test substances modified LPO processes and accelerated healing of the gastric ulcer. The kinetics of LPO has been studied in adequate detail [1]. In accordance with these views the stage of the slow flash of ChL is accompanied by a chain reaction of free radicals of the RO'_2 type, modifying the structure of various biological substrates. The phenomenon of LPO activation in the very early stages (before 2 h) of development of gastric ulcer may lie at the basis of further changes in the relations between destructive and proliferative processes in the stomach at the later stages of development of the ulcer focus. This suggests that the ulcerostatic action of the comparison preparations and silatranes is due to their ability to inhibit LPO in the early stages of ulcer development, i.e., it can be tentatively suggested that one step in the mechanism of the ulcerostatic action of the test substances is their antioxidative effect.

LITERATURE CITED

1. Yu. A. Vladimirov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 4, 489 (1972).
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
3. M. G. Voronkov and V. M. D'yakov, Silatranes [in Russian], Novosibirsk (1978).
4. A. I. Zhuravlev, The Physicochemical Bases of Autoregulation in Cells [in Russian], Moscow (1968), pp. 7-14.
5. I. G. Kuznetsov, L. I. Slutskii, T. K. Gar, and M. G. Voronkov, Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Biol., No. 1, 116 (1986).

EMOXYPINE DURING REPERFUSION OF THE ISCHEMIC DOG MYOCARDIUM: EFFECT ON INFARCT SIZE AND PLASMA CREATINE KINASE ACTIVITY

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Determination of activity of the enzyme creatine kinase (CK), penetrating from necrotic tissue into the systemic blood flow, is one of the principal methods of quantitative assessment of heart damage in myocardial infarction. Meanwhile, restoration of the coronary blood flow in the ischemic myocardium, which is increasingly used in the treatment of acute myocardial infarction, leads to a sharp rise in the plasma CK level and may be the cause of a change in the ratio between plasma CK activity and the size of the infarct [15]. Release of intramyocardial proteins, linked with reperfusion, is due not so much to the flushing out of CK that has accumulated in the ischemic tissue as to damage to cardiomyocytes occurring during reperfusion [11]. It will be evident that this damage is determined by the supply of oxygen to the ischemic tissue, since reoxygenation after anoxic perfusion of the heart also causes CK release, but anoxic reperfusion of ischemic tissue, on the contrary, is not accompanied by any such release of CK into the perfusion fluid [10, 11]. Recent studies have shown that reperfusion damage can be diminished by means of antioxidants [12].

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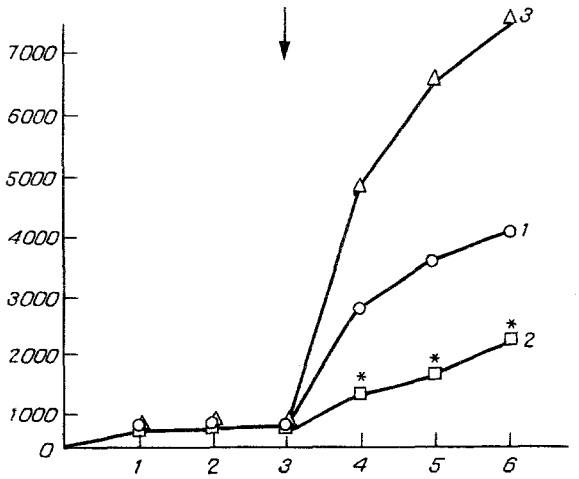

Fig. 1. Effect of emoxypine on plasma CK activity during reperfusion of the ischemic myocardium. Abscissa, time (in h); ordinate, plasma CK activity (in % of initial level). 1) Control, 2) emoxypine 10 mg/kg, 3) emoxypine 40 mg/kg. Arrow indicates restoration of coronary blood flow. $*p < 0.05$ compared with control.

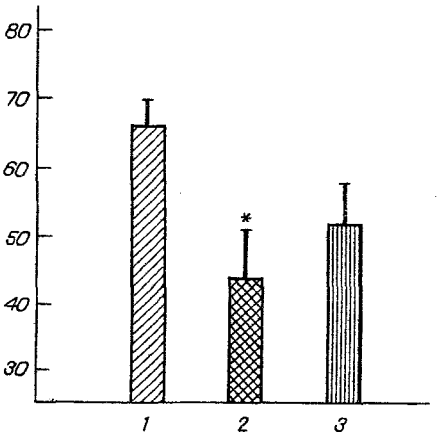

Fig. 2. Effect of emoxypine on infarct size during reperfusion of ischemic myocardium ($M \pm m$). Ordinate, infarct size (in % of risk zone). 1) Control, 2) emoxypine 10 mg/kg, 3) emoxypine 40 mg/kg. $*p < 0.05$ compared with control.

We have studied the action of the antioxidant emoxypine (2-ethyl-6-methyl-3-oxypyridine hydrochloride) on infarct size and plasma CK activity during reperfusion of the ischemic myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on dogs anesthetized with pentobarbital sodium (40 mg/kg). Myocardial ischemia was induced by applying a hemostat to the isolated anterior descending branch of the left coronary artery, and reperfusion was carried out 180 min later by removal of the hemostat. The size of the infarct was determined morphometrically as a percentage of the risk zone, as described previously [3]. CK activity was measured by the kinetic method using standard kits (Boehringer, West Germany) and expressed as a percentage of the initial level [2]. Emoxypine (10 and 40 mg/kg) as injected intravenously,

starting with the 120th minute of coronary occlusion, and continuing until the end of the experiment (0.1 of the dose in bolus form, the rest by drip).

EXPERIMENTAL RESULTS

The study of plasma CK activity showed that coronary occlusion was accompanied by a slow rise of the leakage of CK from the ischemic myocardium, but restoration of the blood flow after ischemia for 180 min led to a sharp increase in release of the enzyme into the bloodstream. For instance, at the 60th minute of reperfusion CK activity was 28 times higher than before ischemia, rising to 41 times higher at the 180th minute (Fig. 1). Similar results also have been obtained by other workers during reperfusion of the ischemic myocardium in dogs [2, 3, 9, 15]. The size of the infarct in the control experiments with reperfusion was 66 ± 4% of the risk zone. Administration of emoxypine, in a dose of 10 mg/kg led to significant limitation of infarct size, accompanied by a significant decrease in CK release into the blood plasma (Figs. 1 and 2). It was reported previously [3, 12] that various antioxidants can reduce infarct size during reperfusion of the ischemic myocardium, evidently on account of inhibition of generation of active forms of oxygen, arising as a result of restoration of the coronary blood flow, and making a significant contribution to damage of the cell components.

If the dose of emoxypine was increased to 40 mg/kg, although the size of the myocardial infarct was less than in the control, this difference did not reach the level of statistical significance (Fig. 2). Consequently, with an increase in the dose, reversal of the cardioprotective action of emoxypine was observed, as was also found in a previous investigation [1], in which the optimal concentration of the compound, giving the maximal reduction of hypoxic contracture and restoration of contractility of the papillary muscle during reoxygenation, was $5 \cdot 10^{-7}$ g/ml, whereas an increase in the concentration of the compound led to a significant increase of the diastolic pressure during hypoxia. In a concentration of $5 \cdot 10^{-4}$ g/ml, moreover, emoxypine not only increased the degree of hypoxic contracture, but also increased the tone of the intact papillary muscle. These features indicate the onset of Ca^{2+}-overloading with an increase in the dose of the compound, and this may probably be an additional damaging factor in our experiments with reperfusion of the ischemic myocardium. Overloading the cells with Ca^{2+} ions may be due to the fact emoxypine inhibits phosphodiesterase and leads to *c*AMP accumulation in the myocardial cells [7], facilitating an increase in Ca^{2+} inflow through the Ca^{2+}-channels of the sarcolemma. One cause of reversal of the protective action under the influence of high doses of emoxypine may be excessive agitation of this synthetic membrane protector in the phospholipid bilayer and a change in its conformation that leads to a direct increase in Ca^{2+}-permeability. For instance, low concentrations of ionol inhibit platelet aggregation, and this is known to be triggered by an increase in the Ca^{2+}-permeability of the platelet membranes, whereas an increase in the ionol concentration, on the other hand, intensifies platelet aggregation [6].

An increase in the dose of emoxypine to 40 mg/kg was accompanied by considerable release of CK into the bloodstream, the plasma CK activity in this group being actual higher than in the control (Fig. 1). These results indicate that during perfusion of the ischemic myocardium the size of the infarct does not always correspond to the amount of CK released into the blood plasma. In particular, the size of the infarct in the control group was rather larger than after administration of emoxypine in a dose of 40 mg/kg, whereas CK activity was reduced almost by half. Devries and co-workers [9] suggested that, besides the total quantity of CK released from the myocardium, the rate of rise of CK activity during the first hours of reperfusion should be used as a parameter reflecting the size of the irreversibly damaged myocardium, and in their experiments they found high correlation ($r = 0.92$) between the rate of rise of plasma CK activity in the first 30-60 min of reperfusion and infarct size. When emoxypine was given in a dose of 40 mg/kg this ratio also was disturbed, for in the control experiments a large infarct size was accompanied by a slower rate of rise of the plasma CK activity compared with that observed after administration of emoxypine.

In our view, increased release of CK during reperfusion of the ischemic myocardium, observed in the experiments with emoxypine (40 mg/kg), may be associated with improvement of drainage of the irreversibly damaged zone, leading to an increased supply of intramyocardial proteins to the systemic blood flow. When reperfusion is carried out after a long period of ischemia (≥90 min), so-called no-reflow zones are observed in the irreversibly damaged zone of the myocardium, and their appearance can be attributed in part to activation of leukocytes and generation of active forms of oxygen. Leakage of CK from the no-reflow zone into the systemic blood flow is evidently greatly retarded, and the CK is subjected to local proteolysis. Administration of the antioxidative enzymes superoxide dismutase (SOD) and catalase prevents microcirculatory damage and increases the subendocardial blood flow in the reperfused myocardium [14]. Improvement of drainage of the irreversibly damaged myocardium evidently lies at the basis of the considerable increase in plasma CK activity, compared with the control, during reperfusion of the ischemic myocardium when carried out against the background of administration of SOD and catalase [13]. Emoxypine also possesses antioxidative activity, which may lead to inhibition of the production of active forms of oxygen by the

leukocytes [8]. This preparation also dilates the coronary vessels [5] and inhibits platelet aggregation [6] and the conversion of fibrinogen into fibrin [4]. These facts suggest that emoxypine may improve the microcirculation in the reperfused myocardium and, consequently, may promote the more rapid release of CK into the systemic blood flow.

Thus when the protective action of substances is assessed during restoration of the coronary blood flow care must be exercised with the interpretation of data on plasma CK activity, for an increase in CK activity may be evidence not only of the further extension of the zone of cardiac damage compared with the control, but also of preservation of the integrity of the capillaries in the reperfused zone and the more rapid flushing out of intracellular enzymes into the systemic blood flow.

The water-soluble antioxidant emoxypine in a dose of 10 mg/kg is a promising therapeutic agent which limits the size of an infarct during reperfusion of the ischemic myocardium.

LITERATURE CITED

1. L. A. Vasilets, V. P. Mokh, G. N. Bogdanov and T. I. Guseva, Kardiologiya, No. 5, 83 (1987).
2. A. P. Golikov, O. A. Avilova, V. Yu. Polumiskov, et al., Byull. Éksp. Biol. Med., No. 10, 413 (1987).
3. A. P. Golikov, V. V. Pichugin, E. A. Konorev, et al., Kardiologiya, No. 7, 66 (1987).
4. A. N. Kleimenov, M. A. Rozenfel'd, E. B. Burlakova, et al., Vopr. Med. Khim., No. 1, 33 (1983).
5. E. A. Konorev, V. Yu. Polumiskov, L. A. Konorev, et al., Pharmacology and Scientific and Technical Progress [in Russian], Tashkent (1988), p. 187.
6. K. O. Muranov, S. B. Gashev, L. D. Smirnov, et al., Byull. Éksp. Biol. Med., No. 3, 337 (1986).
7. N. B. Polyanskii, L. L. Smirnov, A. A. Shvedova, et al., Vopr. Med. Khim., No. 1, 123 (1983).
8. C. J. Butteric, R. L. Baehner, L. A. Boxer, and R. A. Jersild, Am. J. Path., **112**, 287 (1983).
9. S. R. Devries, A. S. Jaffe, E. M. Geltman, et al., Am. Heart J., **117**, 31 (1989).
10. C. E. Ganote, J. Worstell, and J. P. Kaltenbahc, Am. J. Path., **84**, 327 (1976).
11. D. J. Hearse, S. M. Humphrey, and G. R. Bullock, J. Molec. Cell. Cardiol., **10**, 641 (1978).
12. S. R. Jolly, W. J. Kane, M. B. Bailie, et al., Circulat. Res., **54**, 277 (1984).
13. J. Nejima, D. R. Knight, J. T. Fallon, et al., Circulation, **79**, 143 (1989).
14. K. Przyklenk and R. A. Kloner, Circulat. Res., **64**, 86 (1989).
15. R. Roberts and Y. Ishikawa, Circulation, **68**, Suppl. 1, 83 (1983).