5. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, et al., Kardiologiya, No. 10, 28 (1985).
6. F. Z. Meerson, E. V. Shabunina, M. G. Pshennikova, and L. M. Belkina, Kardiologiya, No. 4, 87 (1987).
7. F. Z. Meerson and E. E. Ustinova, Metabolism, Structure, and Function of the Heart Cell [in Russian], Baku (1986).
8. F. Z. Meerson, E. V. Shabunina, and Yu. I. Malyshev, Kardiologiya (1987).
9. W. Loscher and H. Esenwein, Arzneim.-Forsch, 28, No. 5, 782 (1978).
10. W. Loscher and D. Schmidt, Epilepsia, 21, No. 6, 611 (1980).
11. W. Loscher and M. Votter, Neurosci. Lett., 47, No. 3, 325 (1984).
12. H. Selye, E. Bagusz, S. Grasso, and P. Mendell, Angiology, 11, 398 (1969).
13. J. E. Skinner, in: Stress and Heart Diseases, ed. by R. E. Beanish et al., Boston (1985), pp. 44-59.
14. I. F. Tulloch, D. S. Walter, G. M. Howe, and S. J. Howe, Neuropharmacology, 21, No. 6, 555 (1982).
15. S. Wong, A. Basset, J. Cameron, et al., Circulat. Res., 51, 486 (1982).

EFFECT OF HIGHLY DISPERSED COPPER POWDER ON SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE ACTIVITY IN EXPERIMENTAL MYOCARDIAL INFARCTION

G. G. Konovalova, V. Z. Lankin, O. A. Bogoslovskaya, N. N. Glushchenko, Yu. I. Fedorov, and A. M. Vikhert

UDC 616.127-005.8-092.9-092:[616.127-008.931:577.152.1[-02:615.31:546.56

KEY WORDS: myocardial infarction; copper; superoxide dismutase; glutathione peroxidase

An important role of active forms of oxygen in ischemic tissue damage has been postulated [7]. The discovery of proteolytic conversion of xanthine dehydrogenase into the oxidase form, accompanied by accumulation of hypoxanthine, the substrate of xanthine oxidase, in the course of ATP catabolism [7], which must inevitably be accompanied by increased generation of the superoxide anion-radical – $O_2^{\cdot-}$ – is experimental confirmation of this hypothesis. Our own [3] and other data [2] testify to the importance of reduction of enzymic utilization of $O_2^{\cdot-}$ on account of inhibition of superoxide dismutase (SOD) activity in the ischemic cardiomyocytes. Meanwhile an increase in the concentration of hydroxyl radicals ($OH^{\cdot}$ [5]) in perfusion fluid of the ischemic myocardium has been found with the aid of spin traps, and addition of SOD to a cardioplegic solution leads to effective protection of the myocardium in coronary occlusion [8].

The active center of cytosol SOD contains copper, which is responsible for the catalytic activity of the enzyme; it has been shown, moreover, that in animals with copper deficiency in the tissues, SOD activity is sharply depressed [13]. Copper deficiency in the body also leads to myocardial damage [6, 14] and to disturbance of lipid metabolism during the development of pathology of the cardiovascular system [12]. It is logical to suggest on the basis of the facts described above that administration of copper preparations in myocardial ischemia and infarction may have a beneficial action on the course of the pathological process.

The writers showed previously that administration of highly dispersed powders (HDP) of metals has great advantages over the corresponding salts because of their much lower toxicity and their more prolonged action [4]. Accordingly, in the investigation described below the effect of HDP of copper was investigated on the activity of antioxidative enzymes (SOD) and glutathione peroxidase (GP) in the ischemic myocardium and on survival of animals after coronary occlusion.

A. L. Myasnikov Institute of Clinical Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Institute of Chemical Physics, Academy of Sciencs of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. K. Shkhvatsabaya.) Translated from Byulleten' Éksperimental'noi Biologi i Meditsiny, Vol. 107, No. 2, pp. 154-157, February, 1989. Original article submitted December 14, 1987.

 0007-4888/89/0002-0160$12.50

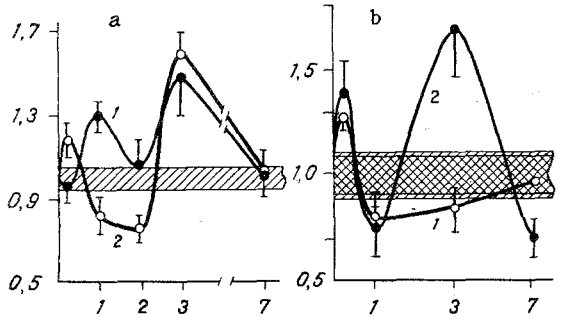

Fig. 1

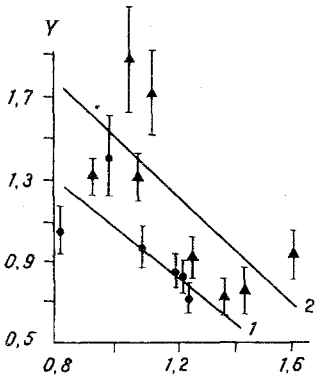

Fig. 2

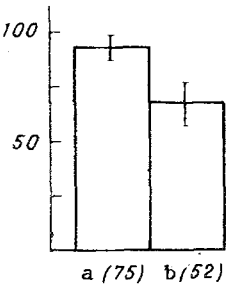

Fig. 3

Fig. 1. Changes in SOD (1) and GP (2) activity in rat heart after injection of HDP of copper. a) Intact animals, b) myocardial infarction. Ordinate, enzyme activity (in relative units). Abscissa, time of investigation (in days).

Fig. 2. Relationship between SOD (1) and GP (2) activity in zone of infarct and in zone of heart outside infarct. Ordinate and abscissa, activity of enzymes (in relative units/mg protein) in region of and outside infarct, respectively.

Fig. 3. Survival rate of rats on 3rd day after myocardial infarction caused by coronary occlusion and treated by injection of HDP of copper. Ordinate, number of surviving animals (in %). Abscissa: a) HDP of copper; b) control. Initial number animals given in parentheses.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170 ± 10 g. Myocardial infarction was induced by application of a ligature to the left coronary artery [3]. HDP of copper (particle size 50-100 nm) were obtained by the method in [1]. To prepare a suspension, weighed samples of metallic powder were dispersed in distilled water on a UZDN-2T ultrasonic disintegrator [4]. The suspension of the HDP of copper was injected subcutaneously 3 days before creation of an experimental model of myocardial infarction, in a dose of 0.2 mg/kg.

Samples of myocardium were homogenized in 50 mM K, Na-phosphate buffer, pH 7.4, and centrifuged at 4°C for 10 min at 800g. Hemoglobin in the hemolysates was precipitated by the method in [10]. Activity of the antioxidative enzymes was determined by means of Hitachi-220A spectrophotometer (Japan) and an FP-901 chemical analyzer (Finland) by the method in [3]. The protein concentration in the samples was determined by Lowry's method. The hemoglobin concentration was determined by the hemoglobin cyanide method, using "Medics" test kits (Finland). The concentrations of of copper and zinc in the heart were determined by atomic-absorption spectrophotometry on an AAC-5000 instrument (Perkin-Elmer, Sweden) [11] and expressed in μg/ml hemoglobin or μg/g tissue. The results were subjected to statistical and correlation analysis by the usual methods, using a Canon CX-1 computer (Japan).

Altogether 56 intact rats and 75 rats with myocardial infarction due to coronary occlusion were used in the experiments.

EXPERIMENTAL RESULTS

According to existing data an increase in the zinc/copper ratio can be regarded as a risk factor for the onset of pathology of the cardiovascular system [9]. The results of the present experiments indicate that, irrespective of the form (HDP or copper sulfate) and the dose (0.2 and 2.0 mg/kg) of copper injected into intact rats, direct dependence was observed between changes in zinc and copper in the myocardium (for a week after injection of the preparation), described by a linear regression equation:

$$[\Delta Zn] = 0.9; [\Delta Cu] - 0.64; r = 0.44 (p < 0.005).$$

The value of the regression coefficient (0.9) indicates that injection of copper had virtually no effect on the change in the zinc/copper ratio in the myocardium. Meanwhile injection of copper caused significant changes in activity of the antioxidative enzymes SOD and GP in the blood and heart of intact rats, respectively. The results of the present experiments points to the existence of positive correlation ($r = 0.65$; $p < 0.05$) in these animals between activity of copper–zinc SOD and the copper concentration in the blood, which can be described

by the equation: SOD = 60.7[Cu] + 1.28. The results confirm that SOD activity in the tissues can be increased deliberately by injecting the HDP of copper. SOD activity in the myocardium of the intact rats reached its peak level 3 days after injection of HDP of copper, when it was increased by more than 1.5 times (Fig. 1a). It must be pointed out that activity of GP, the enzyme responsible for lipoperoxide utilization, also was significantly increased in the myocardium of the intact rats (the increase was by almost 1.6 times; see Fig. 1a). The increase in GP activity in the myocardium of intact rats in response to injection of HDP of copper can be explained by correlation between the concentrations of copper and selenium (an element which is present in the active center of GP) in the tissues [15]. Thus injection of an HDP of copper, bringing about a marked increase in the antioxidative status of the myocardium, ought to increase enzymic protection of the myocardium in infarction, for the accumulation of active forms of oxygen during ischemia may lead to induction of lipid peroxidation [1], in whose regulation an active role is played by GP, which can utilize lipoperoxides [3]. Consequently, the results now obtained suggest that depressed activity of antioxidative enzymes in the ischemic myocardium can be corrected by injection of an HDP of copper.

The results of these experiments show that after injection of an HDP of copper into rats in a dose of 0.2 mg/kg, 3 days before coronary occlusion and 3 h after the operation a short-term increase is observed in the activity of antioxidative enzymes (SOD and GP) compared with the control (operation without preliminary injection of the HDP; Fig. 1b); GP activity in the zone of myocardial infarction, moreover, increased sharply on the 3rd day after ligation of the coronary artery (Fig. 1b). It is important to note that in the myocardium outside the zone of the infarct, activity of the antioxidative enzymes increased compared with that in the zone of ischemia and infarction when the operation was performed 3 days after injection of the HDP of copper, as shown by the existence of strong negative correlation between SOD activity ($r = 0.76$) and GP activity ($r = 0.74$) in the zone of infarction and in the myocardium outside that zone (Fig. 2). Thus when coronary occlusion was carried out in the presence of a raised level of antioxidative enzyme activity, after preliminary injection of an HDP of copper a transient increase in activity of the antioxidative enzymes is possible in the ischemic cardiomyocytes, accompanied by a persistent increase in activity of these enzymes in the myocardium outside the zone of infarction (Fig. 1b, 2). On the basis of these data significant limitation of the zone of infarction might be expected on account of increased activity of antioxidative enzymes in parts of the myocardium at a distance from the zone of infarction, and this undoubtedly ought to be reflected in the survival rate of the animals. In fact, according to the results of the four series of independent experiments, preliminary injection of an HDP of copper before coronary occlusion considerably increased the survival rate of the rats after the operation (Fig. 3).

The results of this investigation thus confirm the important role of enzymic utilization of active forms of oxygen and of lipoperoxides in the pathogenesis of myocardial ischemia and infarction, and also ways of therapeutic correction of myocardial damage with the aid of copper-containing preparations, able to increase activity of the antioxidative enzymes in the myocardium.

LITERATURE CITED

1. M. Ya. Gen, M. S. Ziskin, and Yu. I. Petrov, Dokl. Akad. Nauk SSSR, 127, No. 2, 366 (1959).
2. D. V. Gutkin and Yu. A. Petrovich, Byull. Éksp. Biol. Med., No. 1, 33 (1982).
3. V. Z. Lankin, A. Kh. Kogan, A. N. Kudrin, et al., Byull. Éksp. Biol. Med., No. 5, 58 (1982).
4. Yu. I. Fedorov, E. B. Burlakova, and I. P. Ol'khovskaya, Dokl. Akad. Nauk SSSR, 248, No. 5, 1277 (1979).
5. U. Blasig, B. Ebert, and X. Leve, Abstracts of Proceedings of the 3rd Symposium on Metabolism, Structure and Function on the Heart Cell [in Russian], Baku (1986), p. 128.
6. J. R. Goodman, J. B. Warshaw, and P. R. Dallman, Pediat. Res., 4, 244 (1970).
7. D. J. Hearse, A. S. Manning, J. M. Downey, and D. M. Jellon, Acta Physiol. Scand., Suppl. 548, 65 (1986).
8. S. R. Jolly, W. J. Kane, M. B. Bailie, G. D. Abrams, et al., Circulat. Res., 54, No. 3, 277 (1984).
9. Z. M. Klevay, Am. J. Clin. Nutr., 28, 764 (1975).
10. A. M. Michelson, K. Puget, P. Durosay, J. C. Bonnean, et al., Biochemical and Medical Aspects of Active Oxygen, Baltimore (1977), pp. 247-260.
11. L. Murthy, E. E. Menden, P. M. Eller, et al., Anal. Biochem., 53, 365 (1973).

12. H. G. Petering, L. Murthy, and E. O'Flaherty, J. Org. Food Chem., 25, 1105 (1977).
13. J. R. Prohaska and D. F. Gutson, Biol. Trace Elem. Res., 5, 35 (1983).
14. K. L. Stemmer, H. G. Petering, L. Murthy, et al., Ann. Nutr. Metab., 29, 332 (1985).
15. C. L. White, W. G. Hoekstra, and A. L. Pope, Trace Element Metabolism in Man and Animal, Berlin (1982), pp. 561-563.

PULMONARY CIRCULATION IN EMBOLIC PULMONARY EDEMA

N. V. Sanotskaya, V. V. Polikarpov, and D. D. Matsievskii

UDC 616.24-005.98-02:616.131-005.7]-07:616.24-005]-092.9

KEY WORDS: pulmonary circulation; pulmonary embolism; pulmonary edema; ultrasound.

One of the most threatening complications of pulmonary embolism is edema of the lungs [14]. Publications devoted to the study of the effect of pulmonary embolism on the pulmonary circulation mainly provide discrete data on changes in individual parameters of the pulmonary hemodynamics [10, 12, 14].

The aim of this investigation was to undertake a synchronized study of the blood flow in different parts of the vascular bed of the lungs and also of the pressure in the pulmonary arterial system and left atrium in fat embolism and mechanical embolism of the lungs before the moment of development of pulmonary edema.

EXPERIMENTAL METHOD

The linear and volume blood flow in the left lower lobar artery and vein were studied [8] by an ultrasonic method [5] in acute experiments on 19 cats weighing 3-5 kg, with an open chest and artificial ventilation of the lungs, under pentobarbital anesthesia (30-40 mg/kg, intraperitoneally). The blood pressure in the pulmonary artery was recorded by means of an electromanometer [6]. A cather was introduced through the upper lobar pulmonary artery into the lumen of the left pulmonary artery. The hydraulic resistance of the vascular bed of the lobe of the lung was calculated by means of an analog computer of our own design as the quotient obtained by dividing the mean values of pressure by the mean value of the blood flow in the pulmonary lobar artery. The balance between mean values of the blood flow along the artery and vein was estimated by the same method [9]. The pressure in the pulmonary artery and, in some experiments, in the left atrium also was measured. Three experiments were carried out with a closed chest and natural breathing.

A model of pulmonary embolism was created by intravenous injection of olive oil (1 ml/kg) in the course of 2 min, and a model of mechanical embolism by intravenous injection of a 2.5% suspension of lycopodium (1 ml/kg). All the parameters were recorded for 60-100 min. Repeated injections of oil or lycopodium were given in some of the experiments. The intensity of the developing pulmonary edema was estimated by calculating the pulmonary coefficient (PC) and the dry residue (DR), expressed as percentages [4].

EXPERIMENTAL RESULTS

Edema of the lungs developed regularly after 60 min in both fat and mechanical embolism. This was shown by a significant increase in PC (by 2.1 times for fat embolism, by twice for mechanical) and by reduction of DR (by 20 and 14.8%, respectively). In most animals the intensity of edema of the lungs was greater after injection of olive oil than after injection of lycopodium. Most animals died 60-100 min after induction of edema, four cats died after a shorter interval – varying from 10 to 36 min (after repeated injection of oil or lycopodium).

Laborary of Pathophysiology of Respiration and Bioengineering Laboratory, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Department of Pathological Physiology, Yaroslavl' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR B. I. Tkachenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 157-161, February, 1989. Original article submitted April 5, 1988.

