The authors are grateful to Corresponding Member of the Academy of Medical Sciences of the USSR Professor V. T. Ivanov for supplying the preparation of delta sleep inducing peptide used in the work.

LITERATURE CITED

1. I. P. Ashmarin and E. L. Dovedova, Dokl. Akad. Nauk SSSR, 255, No. 6, 1501 (1980).
2. M. M. Bogoslovskii, I. T. Karmanova, V. F. Maksimuk, et al., Zh. Evol. Biokhim. Fiziol., No. 7, 430 (1979).
3. V. Z. Gorkin, I. V. Verevkina, L. I. Gridneva, et al., in: Modern Methods in Biochemistry [in Russian], No. 3, Moscow (1968), p. 155.
4. N. N. Demin, I. G. Karmanova, V. F. Maksimuk, et al., Zh. Evol. Biokhim. Fiziol., No. 3, 257 (1980).
5. A. S. Sargesyan, L. V. Sumskaya, I. Yu. Aleksandrova, et al., Bioorg. Khim., 7, No. 3, 1125 (1981).
6. S. J. Hestrin, J. Biol. Chem., 180, 249 (1949).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 256 (1951).
8. I. I. Mikhaleva, A. V. Sargesyan, L. V. Sumskaya, et al., in: Peptides: Structure and Biological Function, Rockford, Illinois (1979), p. 901.
9. M. Monnier, L. Dubler, R. Gachter, et al., Experientia, 32, 548 (1976).
10. V. Popov, V. Prosler, C. Thiemann, et al., Acta Biol. Med. Germ., 26, 239 (1971).
11. G. A. Schoenenberger and M. Monnier, Proc. Natl. Acad. Sci. USA, 74, 1282 (1977).

ENZYMES DETOXICATING ACTIVE FORMS OF OXYGEN AND LIPID PEROXIDES IN EXPERIMENTAL MYOCARDIAL ISCHEMIA AND INFARCTION

V. Z. Lankin, A. Kh. Kogan,
A. L. Kovalevskaya, G. G. Konovalova,
D. R. Rakita, A. N. Kudrin, and
A. M. Vikhert

UDC 616.127-005.4+616.127-005.8]-092.9-07:616.127-008.931:577.152.2]-074

KEY WORDS: myocardial ischemia and infarction; superoxide dismutase; glutathione peroxidase; glutathione-S-transferase.

Recent experimental data have confirmed the important role of lipid peroxidation (LPO) in the pathogenesis of ischemic heart disease [5, 8]. Activation of LPO has been found during the formation of a myocardial infarct [4]; some natural and synthetic antioxidants have been found to inhibit the development of necrosis [4, 11]. Antioxidant enzymes — superoxide dismutase (SOD) and glutathione peroxidase (GP) — responsible for detoxication of the superoxide O_2 anion-radical and for decomposition of lipid peroxides [6], take part in the regulation of LPO *in vivo*. According to some workers [13, 15], a definite role in the utilization of lipid peroxides in the tissues may be played by certain glutathione-S-transferases (GTF), although this view has not been fully substantiated [9, 14]. The present writers showed previously that acute ischemia of the liver leads to a sharp fall in SOD and GP activity; a fall in the activity of antioxidant enzymes, moreover, may be one of the main causes of the intensification of LPO in ischemia [1].

The object of this investigation was to study changes in activity of SOD, GP, and GTF in the zone of ischemia and necrosis during the development of an infarct following coronary occlusion in rats.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. I. M. Sechenov First Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 58-60, May, 1982. Original article submitted November 27, 1981.

0007-4888/82/9305-0613$07.50

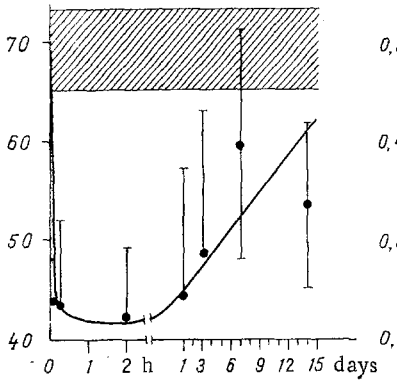

Fig. 1

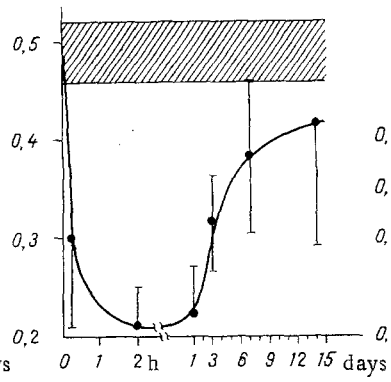

Fig. 2

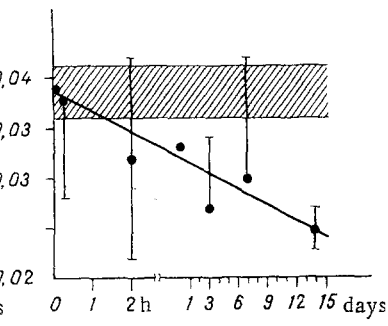

Fig. 3

Fig. 1. Changes in SOD activity in rats with myocardial ischemia and infarction. Here and in Figs. 2 and 3, shaded region represents level of activity of corresponding enzymes in intact animals and animals undergoing mock operation. Abscissa, time of experiment; ordinate, activity of corresponding enzymes (in units/mg protein).

Fig. 2. Changes in GP activity in rats with myocardial ischemia and infarction.

Fig. 3. Changes in GTF activity in rats with myocardial ischemia and infarction.

EXPERIMENTAL METHOD

Male Wistar rats weighing 190 ± 10 g were used. A myocardial infarct was produced by ligation of the left coronary artery 2 mm below the level where it crosses the left border of the conus arteriosus at its base, as described previously [2]. Operations were performed under endotracheal ether anesthesia. The presence of ischemia and infarction was determined from characteristic ECG changes (elevation of ST and deepening of the Q wave) in three standard leads. Ischemia was verified macroscopically by the presence of marked pallor, cyanosis, weakening of contraction, and dilation of the zone of occlusion; an infarct was verified by the presence of a zone of necrosis and the appearance of a postinfarct scar. Tissue for investigation was taken after decapitation of the rats on the zone of ischemia or infarction 2 and 15 min, 2 h, and 1, 3, 7, and 14 days after occlusion (five samples at each experimental point). These times correspond to stages of reversible ischemia (2-15 min), irreversible ischemia (2 h), necrosis (1 day), necrosis and granulation (3 days), and scar formation (7-14 days) [2, 3]. An equal number of intact rats and of rats killed at the same times after a mock operation (thoroacotomy and incision of the pericardium under endotracheal ether anesthesia) was used as the control. The tissues were kept until investigation in liquid nitrogen, then quickly thawed, homogenized in a glass hemogenizer with Teflon pestle in 0.05M Tris-HCl, pH 7.4, and centrifuged at 800g for 10 min. Blood from the patients with acute (1-3 days) myocardial infarction (men aged 53-55 years) was taken from a vein in the morning before breakfast and hemolyzed with 0.005 Tris-HCl solution, pH 7.4. Clinically healthy persons (61) of the same age group, with no hyperlipidemia or signs of ischemic heart disease, served as the control. SOD activity in the supernatant was determined by inhibition of reduction of nitro-BT in a xanthine-xanthine oxidase system [7], GP activity was determined by oxidation of NADPH in a coupled glutathione reductase system, using tert-butyl hydroperoxide as the substrate [7], and GTF activity was determined by the formation of glutathione conjugates with 1-chloro-2,4-dinitrobenzene [12]. Activity of the enzymes was measured at 25°C on an Aminco DW-2A spectophotometer (USA); the unit of SOD activity was taken to be the quantity of enzyme necessary to produce 50% inhibition of reduction of nitro-BT under the conditions of determination, the unit of GP activity the quantity of enzyme required to oxidize 1 μmole reduced glutathione, and the unit of GTF activity the quantity of enzyme required to conjugate 1 μmole glutathione. Protein in the enzyme preparations was determined by Lowry's method.

EXPERIMENTAL RESULTS

SOD activity in the myocardium was reduced by 50% 2 min after coronary occlusion and it remained low until the end of the experiments, although starting with the 7th day of the experiment there was a tendency for the level of its activity to return to normal (Fig. 1).

GP activity in the myocardium showed similar changes but these changes became significant later (after 15 min), when GP activity was reduced by 40% (Fig. 2). GTF activity in the myocardium was reduced by more than 30% with effect from the 3rd day after ligation of the coronary artery (Fig. 3). In patients with acute myocardial infarction the blood GP activity was higher (5.2 + 0.10 units/ml) than in the control (3.4 + 0.08 units/ml, $P < 0.001$). Acute myocardial infarction is thus accompanied by a significant fall in SOD activity in the zone of infarction, and by a corresponding fall in GP activity a little later. GTP activity in the zone of infarction, however, fell only in the stage of necrosis and granulation, and even then, not significantly. It can be tentatively suggested that the fall in activity of the antioxidant enzymes in the zone of myocardial damage is accompanied by their release into the blood stream, and this is confirmed by an increase in GP activity in the blood of patients with acute myocardial infarction. The sharp fall in SOD and GP activity in ischemia and myocardial infarction (Figs. 1 and 2) was evidently responsible for intensification of LPO in this pathology [4]. Similar changes in activity of antioxidant enzymes were observed previously by the present writers in acute ischemia of the liver [1]; characteristically in this case also SOD activity began to fall sooner than GP activity, correlating with a sharp increase in the content of lipid peroxides in the ischemized organ [1].

The results thus suggest that a fall in the activity of antioxidant enzymes and, in particular, of SOD in myocardial ischemia and acute myocardial infarction leads to the development of a syndrome of peroxidation and peroxide-induced injury of cell membranes in the ischemized zone of the myocardium [10].

The authors are grateful to A. N. Zakirova and A. K. Tikhaze for help with the work.

LITERATURE CITED

1. L. B. Dudnik, A. K. Tikhaze, A. V. Alesenko, et al., Byull. Eksp. Biol. Med., No. 4, 451 (1981).
2. A. Kh. Kogan, Patol. Fiziol., No. 3, 79 (1979).
3. A. Kh. Kogan, A. N. Kudrin, and S. M. Nikolaev, in: Free-Radical Oxidation of Lipids Under Normal and Pathological Conditions [in Russian], Moscow (1976), p. 68.
4. A. N. Kudrin, A. Kh. Kogan, V. V. Korolev, et al., Kardiologiya, No. 2, 115 (1978).
5. V. Z. Lankin, Kardiologiya, No. 8, 42 (1980).
6. V. Z. Lankin, in: Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981), p. 75.
7. V. Z. Lankin and S. M. Gurevich, Dokl. Akad. Nauk SSSR, 226, No. 3, 705 (1976).
8. V. Z. Lankin, A. N. Zakirova, B. Kh. Akhmedova, et al., Kardiologiya, No. 7, 93 (1980).
9. V. Z. Lankin, A. K. Tikhaze, A. L. Kovalevskaya, et al., Dokl. Akad. Nauk SSSR, 261, No. 6, 1467 (1981).
10. F. Z. Meerson, Kardiologiya, No. 6, 9 (1979).
11. F. Z. Meerson, L. M. Belkina, A. A. Ugolev, et al., Kardiologiya, No. 10, 81 (1980).
12. J. H. Keen and W. B. Jakoby, J. Biol. Chem., 253, 5654 (1978).
13. R. A. Lawrence and R. F. Burk, Biochem. Biophys. Res. Commun., 71, 952 (1976).
14. P. Morrissey and P. J. O'Brien, Can. J. Biochem., 58, 1012 (1980).
15. J. R. Prohaska and H. E. Ganther, Can. J. Biochem., 76, 737 (1977).