8. A. G. Stolyarova, Zh. Nevropatol. Psikhiatr., No. 2, 199 (1973).
9. N. N. Anichkow, in: Atherosclerosis (E. V. Cowdry, etc.), New York (1933), pp. 271-322.
10. A. Barbeau, Can. Med. Assoc. J., 101, 59 (1969).
11. S. Bertelsen, Acta Pathol. Microbiol. Scand., 53, 335 (1961).
12. J. H. Bragdon, in: Lipids and Steroid Hormones in Clinical Medicine, Philadelphia (1960), p. 6.
13. S. Margolis and D. Capuzzi, in: Blood Lipids and Lipoproteins, (G. J. Nelson, ed.), Wiley, New York (1972), pp. 825-880.
14. B. P. Nery and C. S. Frings, Clin. Chem., 19, 1201 (1973).

PATHOGENETIC ROLES OF ACTIVATION OF LIPID PEROXIDATION AND PROTECTIVE EFFECT OF SODIUM SELENITE DURING ISCHEMIA AND REPERFUSION OF THE MYOCARDIUM

P. F. Litvitskii, A. Kh. Kogan, A. N. Kudrin, and L. O. Luk'yanova

UDC 616.127-005.4-092:616.127-008.939.15-02:615.31:546.33'23

KEY WORDS: myocardial ischemia; reperfusion; lipid peroxidation; antioxidants.

A temporary reduction of the coronary blood flow is accompanied by a disturbance of the contractile function and rhythm of the heart not only in the period of myocardial ischemia (MI), but also when perfusion of the coronary arteries is resumed [4-7]. The development of arrhythmias and depression of the contractile process are the dominant factors of the disturbance of the systemic hemodynamics in the initial stage of postischemic restoration of the coronary blood flow and they essentially form the cardiac reperfusion syndrome [9]. The mechanism of development of this syndrome is evidently based on summation of metabolic-functional and structural changes in the heart arising during its ischemia and subsequent reperfusion (RP). Among the metabolic disorders in the RP stage, accompanied by myocardial hyperoxia, particular attention should be paid to the process of free-radical lipid peroxidation (FRLP). This is because of data showing, first, the intensification of FRLP during myocardial hyperoxia [13], second, the protective effect of an antioxidant – α-tocopherol – during reoxygenation of the previously ischemized myocardium *in vitro* [13], and third, as the writers' previous investigations revealed, biphasic activation of FRLP during reversible MI: in the period of ischemia and on resumption of the blood flow in the coronary arteries of the heart [9]. With the above facts in mind, the writers postulated the important pathogenetic role of activation of FRLP in the development of both the ischemic and the reperfusion syndromes during reversible disturbance of the coronary blood flow [9]. To obtain proof of this hypothesis, an attempt was made to compare the dynamics of FRLP and cardiac function during transient myocardial ischemia (TMI) for different durations and also to study the effect of sodium selenite, an active inhibitor of FRLP, on cardiac activity during TMI.

EXPERIMENTAL METHOD

Experiments were carried out on 89 noninbred male albino rats weighing 190-210 g. TMI was produced by the method described previously [4, 6] under urethane (1200 mg/kg) anesthesia with artificial ventilation of the lungs with atmospheric air. The duration of the period of MI before myocardial RP was 10, 20, 40, or 120 min. Lipids were extracted from the damaged area of the myocardium by the method in [11]. The intensity of FRLP was determined by a chemiluminescence method using a quantum measuring device for recording weak light fluxes [1]. The cardiac frequency was recorded on the ECG and the peak actual (P_{act}) and maximal (during isometric contraction of the heart, P_{max}) pressure within the left ventricle

Department of Pathophysiology and Pharmacology, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR F. I. Komarov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 3, pp. 271-274, March, 1981. Original article submitted June 20, 1980.

 0007-4888/81/9103-0270$07.50

(the latter indirectly reflects the strength of the cardiac contractions) also were recorded. The mean rate of rise and fall of pressure in the left ventricle, reflecting the rate of contraction and relaxation of the myocardium, respectively, and also Opie's index [14], an integral index of cardiac function, were calculated. An aqueous solution of sodium selenite was injected intraperitoneally in a dose of 30 mg/kg, 90 min before induction of TMI. All the animals were divided into two groups: those developing TMI after preliminary injection of sodium selenite (experiment) and those not receiving sodium selenite (control).

EXPERIMENTAL RESULTS

The development of TMI in animals receiving prophylactic sodium selenite was marked by a lesser degree (compared with the control) of activation of FRLP and depression of the contractile function of the heart both during MI and during subsequent RP. During the MI period, for instance, the intensity of chemiluminescence of lipids was lower than in the control: by 33% in the case of MI for 10 min, and 14.6 and 8.2% for MI lasting 40 and 120 min, respectively (Table 1). The greater antioxidant effect of sodium selenite in the initial (first 10 min) stage of MI is interesting. It is evidently attributable to the fact that selenium inhibits FRLP not only at the stage of breakdown of lipid peroxides, but also at the stage of formation of active free radicals.

A study of the dynamics of contractile function of the heart during MI and prophylactic administration of sodium selenite showed that it was depressed by a lesser degree than in the absence of the compound (Table 2). For instance, whereas in animals of the control series P_{max} fell after 10 min of MI by 4.4% compared with the background, by 9.2% after 40 min, and by 16.7% after 120 min, in animals receiving sodium selenite the fall in P_{max} was 2.5, 7.1, and 9.9%, respectively. The process of contraction and, in particular, of relaxation of the myocardium was less severely affected, as is shown by the lesser degree of depression of the rise and fall of pressure within the left ventricle. The higher values of Opie's index in the animals receiving sodium selenite are further evidence of the increased resistance of the heart as a whole to ischemic injury.

During the period of postischemic RP in rats receiving sodium selenite the decrease in the intensity of FRLP was less (by 11-44%) than in the control, and this was accompanied by a lesser degree of decrease in the indices of myocardial contractility. Two facts were discovered. The first was the higher intensity of FRLP and the high antioxidant activity of sodium selenite after a long (40-120 min) reduction of the coronary blood flow compared with those observed after a short reduction (10 min). This can evidently be attributed to the fact that resumption of the coronary blood flow after prolonged ischemia is accompanied, on the one hand, by massive uptake of calcium ions by the mitochondria [10], the uncoupling of oxidative phosphorylation, and an increase in the partial pressure of oxygen in the myocardium, and on the other hand, by calcium activation of phospholipase A [15], injury to the membranous structures of the cardiomyocytes under the influence of that enzyme, "denudation" of their lipid layers and the consequent creation by these two processes of the necessary conditions for intensification of FRLP, which was also observed in the control animals. Sodium selenite, an antioxidant with "antiradical" and "antiperoxide" action, effectively inhibits these processes. After short-term (10 min) MI, RP does not lead to any significant retention of calcium in the mitochondria or significant activation of phospholipases [10, 15], and it is thus accompanied by a lower intensity of formation of active free radicals. The second fact was the lesser degree of depression of contractile properties of the myocardium in animals receiving sodium selenite, as shown by the higher indices for the dynamics of the force (P_{max}) and rate (rate of rise and fall of pressure in the left ventricle) of the contractile process. This applies most to the rate of fall of pressure in the period of diastolic relaxation of the myocardium. These findings suggest a lesser degree of alteration of the subcellular membranous structures participating in processes of energy provision (mitochondria), contraction (myofibrils), and electromechanical coupling (sarcoplasmic reticulum) during postischemic RP of the myocardium under the influence of the antioxidant sodium selenite.

The experimental results thus support a significant pathogenetic role for activation of FRLP during MI and its subsequent RP. Evidence for this is given, in particular, by the coincidence of the times of intensification of FRLP and depression of the contractile function of the heart during the period both of reduction and of restoration of the coronary blood flow. Inhibition of FRLP by means of sodium selenite is accompanied by an increase in the resistance

TABLE 1. Effect of Sodium Selenite on Intensity of Chemiluminescence of Lipids in Myocardium (in CPS) during Ischemia and RP (M ± m)

Experimental conditions	MI for 10 min	RP, min		MI for 20 min	RP, min		MI for 40 min	RP, min		MI for 120 min	RP, min	
		10	40		10	40		10	40		10	40
Control ($n = 35$)	56,8±1,8	29,5±2,5	31,5±1,0	28,5±1,3	34,1±2,8	42,3±2,7	30,2±0,9	40,2±4,6	50,8±3,9	36,1±1.8	54,3±4,8	53.5±1,1
Sodium selenite ($n = 40$)	37,9±1,5*	24,6±2,1	27,9±4,9	26,7±2,0	30,0±2,8	23,4±1,3*	25,8±1,3	26,4±0,8*	28,9±1,9*	29,3±0,8*	40.0±0.9*	44,7±1,0*

*P < 0.05 compared with the control.
Legend: n) Number of tests.

TABLE 2. Dynamics of Indices of Contractile Function of the Heart during Myocardial Ischemia and RP (M ± m)

Index	Experimental conditions	Background	MI for 10 min	RP, min		Background	MI for 40 min	RP, min		Background	MI for 120 min	RP, min	
				10	40			10	40			10	40
P_{max}, mm Hg	Control (n = 15)	204±1,5	195±1,4	198±2,3	198±2,4	206±2,6	187±2,9	188±2,5	179±1,7*	204±2,1	170±3,3*	164±2,8*	171±2,6*
	Sodium selenite (n =10)	206±0,7	201±1,5	202±2,8	197±2,3	209±1,2	194±2,6	190±2,0	197±2,5	201±2,4	181±2,9	180±2,6	186±2,8
Mean rate of rise in pressure in left ventricle, mm Hg/sec	Control	1465±50	1424±67	1555±84	1650±78	1851±92	1474±57*	1431±65*	1373±82*	1860±51	1242±60*	1192±54*	1220±42*
	Sodium selenite	1859±62	1708±65	1766±66	1822±72	2190±45	1849±48*	1865±50*	1928±52	1843±54	1369±55*	1320±44*	1362±49*
Mean rate of fall in pressure in left ventricle, mm Hg/sec	Control	659±12	826±22*	640±14	639+20	677±24	659±29	596±19	558±18	787±22	608±29	602±24	594±26*
	Sodium selenite	618±34	580±22	583±13	626±11	614±23	658±27	683±38	674±25	772±24	698±26	686±25	689±22
Opie's index $\times 10^3$	Control	26,9±0,5	32,3±0,7*	27,9±0,8	29,0±0,8*	29,8±0,8	27,3±1,2*	25,3±0,7*	24,2±0,5*	23,6±0,1	19,2±0,1*	19,0±0,1*	18,7±0,1*
	Sodium selenite	31,6±0,4	28,0±0,9*	26,4±0,5*	27,3±0,6*	29,3±0,4	28,1±1,4	30,3±1,2	28,3±1,1	23,1±0,1	21,4±0,1*	21,1±0,2*	21,5±0,3*

*P = 0.05 compared with background.

of the heart to TMI, and this is expressed by a lesser degree of depression of the contractile properties of the myocardium in the experimental animals than in the controls. Taken as a whole these facts provide an experimental basis for a new principle of pathogenetic treatment of TMI by means of inhibitors of FRLP and, in particular, with sodium selenite. This is in harmony with similar conclusions regarding myocardial infarction of coronary and sympathomimetic genesis [2, 3].

The data described above may have definite relevance to clinical practice, for TMI produced in animals is an experimental model of forms of human coronary heart disease which are accompanied by a transient reduction of the coronary blood flow (angina pectoris, the "intermittent coronary syndrome," the ischemic period of myocardial infarction with surgical revascularization of the ischemic zone).

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Peroxidation of Lipids in Biological Membranes [in Russian], Moscow (1972).
2. A. Kh. Kogan, A. N. Kudrin, and S. N. Nikolaev, in: Free Radical Oxidation of Lipids under Normal and Pathological Conditions [in Russian], Moscow (1976), pp. 71-74.
3. A. N. Kudrin, A. Kh. Kogan, V. V. Korolev, et al., Kardiologiya, No. 2, 115 (1978).
4. P. F. Litvitskii, in: The Circulation under Conditions of High Mountains and Experimental Hypoxia [in Russian], Dushanbe, (1978), pp. 159-161.
5. P. F. Litvitskii and Yu. M. Medvedev, in: Current Problems in Experimental Simulation of Pathological Processes and Methods of Their Treatment [in Russian], Moscow (1979), pp. 85-87.
6. P. F. Litvitskii and Yu. M. Medvedev, in: Current Problems in Experimental Simulation of Pathological Processes and Methods of Their Treatment [in Russian], Moscow (1979), pp. 85-87.
7. P. F. Litvitskii, T. B. Zubkova, Yu. M. Medvedev, et al., Krovoobrashchenie, No. 6, 13 (1979).
8. P. F. Litvitskii, Byull. Éksp. Biol. Med., No. 5, 532 (1980).
9. P. F. Litvitskii, A. Kh. Kogan, and L. O. Luk'yanova, in: Problems in Experimental and Clinical Pathology [in Russian], L'vov (1980).
10. R. Jennings and A. C. Shen, in: Myocardiology, Baltimore (1972), pp. 639-656.
11. J. Folch, M. Lees, G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
12. C. Guarnieri, R. Ferrany, W. Nayler, et al., J. Mol. Cell. Cardiol., 9, No. 9, Suppl., 16 (1977).
13. C. Guarnieri, F. Flamigni, and C. Caldarera, J. Mol. Cell. Cardiol., 11, No. 9, Suppl. No. 2, 20 (1979).
14. L. Opie, J. Physiol. (London), 180, 529 (1965).
15. W. Weglicki, E. Owens, R. Ruth, et al., J. Mol. Cell. Cardiol., 11, 65 (1979).