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PROTECTIVE ACTION OF SUPEROXIDE DISMUTASE IN EXPERIMENTAL MYOCARDITIS

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The method of hyperbaric oxygenation (HBO) is being applied on an ever-increasing scale for the treatment of cardiovascular diseases. However, the problem of protection of the patient against overdosage of HBO is still unsolved. The more intensive formation of the superoxide anion (O_2^-), which appears in such cases, may lead to oxidative destruction of the cell and, in particular, to stimulation of lipid peroxidation (LPO) [6, 9]. Administration of superoxide dismutase (SOD), which renders O_2^- harmless, has a protective action against certain pathological processes with an inflammatory component [2, 3, 12].

The investigation described below showed that injection of exogenous SOD can reduce the severity of the functional and morphological manifestations of adrenalin myocarditis during periodic exposure to increased doses of HBO.

EXPERIMENTAL METHOD

Adrenalin myocarditis was induced in chinchilla rabbits by slow (in the course of 2-3 min) intravenous injection of 1% caffeine solution (20 mg/kg) and 0.1% adrenalin solution (0.2 ml) with an interval of 2 min. Control animals received physiological saline. Sessions of HBO were given under a pressure of 2.5 atm for 1 h daily for 3 days. The contractile function of the left ventricle was assessed from the pressure inside the ventricle recorded by a Mingograph-82 electromagnometer under conditions of relative rest, and also at the 5th second after complete occlusion of the ascending aorta. The rate of contraction and relaxation of the myocardium of the left ventricle also was determined. The potential working capacity of the left ventricle was calculated by the formula in [10] and the intensity of functioning of structures by the method in [5].

SOD activity was determined by a method based on the ability of the enzyme to inhibit O_2^--mediated autooxidation of adrenalin [1]. To study the effect of exogenous SOD on cardiac activity a purified preparation of SOD from bovine blood was injected intravenously into rabbits in a dose of 1 mg 3 times a day for 3 days. The first injection of SOD was given 30 min before injection of adrenalin. The enzyme, isolated from bovine blood by a modified method of McCord and Fridovich [11], had activity of 2000 Units/mg protein. Isoelectric focusing in polyacrylamide gel revealed two bands with isoelectric points of 4.6 and 4.8, possessing SOD activity.

To determine endogenous SOD activity the tissues were ground in a mortar with liquid nitrogen, homogenized on ice in a Potter homogenizer (glass–glass) for 5 min at 5000 rpm in K-phosphate buffer with 0.9%

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TABLE 1. Effect of SOD on Contractile Function of Left Ventricle in Rabbits (M ± m)

Parameter	Experimental conditions	Stage of experiment	
		rest	loading
Pressure developed, mm Hg	Control (10)	116 ± 8	220 ± 10
	Myocarditis (6)	103 ± 6	190 ± 8*
	Myocarditis + HBO (6)	106 ± 8	184 ± 8
	Myocarditis + HBO + SOD (6)	109 ± 8	224 ± 11†
Intensity of functioning of structure, mm Hg/min·g	Control	10.5 ± 1.8	17.8 ± 2.1
	Myocarditis	8.0 ± 1.4	12.2 ± 1.6*
	Myocarditis + HBO	7.2 ± 1.2	10.0 ± 1.8
	Myocarditis + HBO + SOD	10.2 ± 1.4	16.3 ± 1.3†
Rate of contraction, mm Hg/sec	Control	2540 ± 201	4474 ± 216
	Myocarditis	2216 ± 198	3620 ± 180*
	Myocarditis + HBO	2285 ± 276	3288 ± 192
	Myocarditis + HBO + SOD	2409 ± 166	4209 ± 171†
Rate of relaxation, mm Hg/sec	Control	1722 ± 124	2086 ± 220
	Myocarditis	1587 ± 116	1601 ± 107*
	Myocarditis + HBO	1516 ± 145	1598 ± 118
	Myocarditis + HBO + SOD	1698 ± 171	1916 ± 103†
Coefficient of potential working capacity, %	Control		100 ± 7
	Myocarditis		75 ± 5*
	Myocarditis + HBO		69 ± 9
	Myocarditis + HBO + SOD		104 ± 8†

*$P < 0.05$ compared with control.
†$P < 0.05$ compared with myocarditis.

NaCl, pH 7.4 (9 : 1), and centrifuged at 700g for 10 min. The supernatant was used for measurement of SOD activity. Areas of the heart where the conducting system was most probably localized were identified by the method in [15].

EXPERIMENTAL RESULTS

On the 3rd day of development of adrenalin myocarditis a tendency was observed for the intensity of functioning of structures (IFS), and the rate of contraction and relaxation of the left ventricle at rest to fall. With maximal loading due to occlusion of the aorta, a decrease was observed in the rate of contraction and relaxation of the left ventricle, accompanied by a fall in IFS and the potential working capacity of the myocardium (Table 1). HBO is known to induce a therapeutic effect in some types of cardiac lesion [4]. However, in adrenalin myocarditis, HBO under the conditions used not only did not improve, but actually worsened the myocardial contractility a little (Table 1); this was particularly clearly reflected in IFS and the rate of contraction and relaxation under maximal loading conditions. One of the mechanisms of development of adrenalin-induced myocarditis and one of the causes of the unfavorable effect of superoxygenation of the myocardium under these circumstances could be intensification of formation of products of enzymic and nonenzymic oxidation of adrenalin in the tissues, including H_2O_2, $O_2^{\cdot-}$, and adrenochrome. For instance, it has recently been shown that adrenochrome can cause necrosis of cardiomyocytes and reduce myocardial contractility [13].

The importance of activated forms of oxygen in stimulation of LPO in the heart during stress has been suggested [6, 7]. Adrenalin myocarditis is a convenient model with which to study the pathochemical role of $O_2^{\cdot-}$ in cardiology, not only because adrenalin loading ought to facilitate increased formation of active forms of oxygen, but also because of a decline in the level of SOD activity in different parts of the heart after adrenalin loading (Fig. 1). It is a particularly interesting fact that in areas of heart tissue adjacent to the conducting system, SOD activity falls very considerably in myocarditis. Such a change must be regarded as predisposing to the onset of cardiac arrhythmias when $O_2^{\cdot-}$ production in the tissue is increased.

A rather unexpected finding was that the level of SOD activity was quite high in the heart valves and wall of the aorta – structures rich in connective-tissue cells. It was shown previously that SOD activity is low in connective-tissue formations of the locomotor system [2]. It can be tentatively suggested that differences in connective-tissue structures of the body with respect to their SOD activity levels are dependent on differences in the intensity of oxygenation in the tissues.

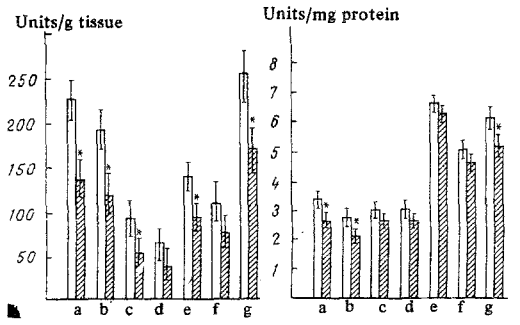

Fig. 1. Changes in SOD activity in different parts of the rabbit heart during adrenalin-induced myocarditis. Unshaded columns – control, shaded columns – myocarditis. a) Left ventricle, b) right ventricle, c) left atrium, d) right atrium, e) aorta, f) valvular apparatus, g) areas of location of conducting system. Ordinate, SOD activity. Results of 10 experiments given. *$P < 0.05$ compared with control.

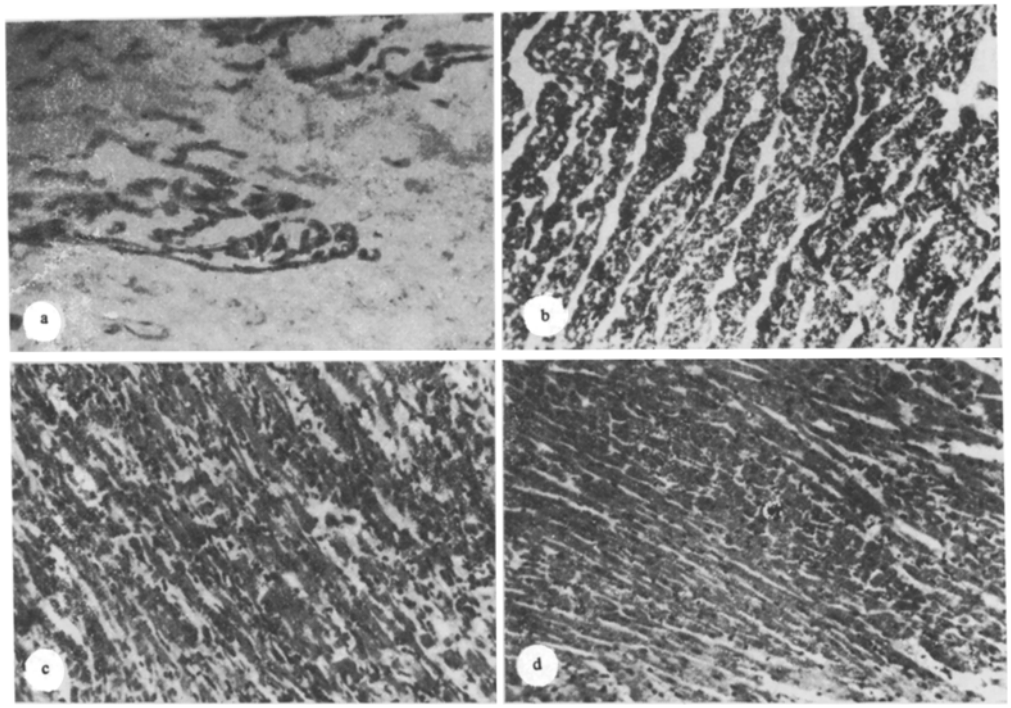

Fig. 2. Severity of necrotic changes (reflected in succinate dehydrogenase activity, a, b) and leukocytic infiltration (b, d) in adrenalin-induced myocarditis, after HBO therapy without (a, c) and with (b, d) injection of SOD. a, b) Nachlas' reaction, 140×; c, d) hematoxylin–eosin, 100×.

The only experimental approach available at present to prove the participation of O_2^- in a physiological or pathological process in vivo is to inject SOD or its analogs into the animal. Injections of SOD into rabbits have been found to reduce the severity of morphological and functional manifestations of myocarditis treated by HBO; the number of necrotic zones and the intensity of leukocytic infiltration were reduced (Fig. 2) and the contractile function of the heart was restored (Table 1).

The protective effect of SOD in adrenalin myocarditis may be due to several causes: inhibition of adreno-

chrome formation (see above), detoxication of the O_2^- formed by nonenzymic oxidation of adrenalin, reduction of the harmful action of "oxidative phagocytosis" [8] of leukocytes infiltrating the heart tissues in myocarditis. Exogenous SOD evidently does not penetrate into cardiomyocytes and, as a result of this, these mechanisms must be attributed primarily to extracellular (intercellular) events [3]. At the same time, if it is recalled that preliminary injection of SOD before exposure to hyperoxia increases the resistance of myocardial homogenates to ascorbate-induced LPO [14], it can be postulated that extracellular SOD has an indirect action on intracellular antioxidative systems.

The level of SOD activity also remained low in the myocardium of animals with adrenalin-induced myocarditis treated with HBO, and receiving or not receiving injections of SOD.

Irrespective of the concrete mechanism of action of exogenous SOD, the data given above point to the desirability of using this enzyme preparation in the prevention and treatment of myocardiopathies associated with a disturbance of catecholamine metabolism, for example, in stress situations, and also for preventing side effects of HBO in myocarditis.

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