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PREVENTION OF POSTRESUSCITATION HEART FAILURE WITH IONOL

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UDC 616-036.882-08-036.8:616.12-008.46-085.272.4

KEY WORDS: heart in postresuscitation period; lipid peroxidation; antioxidant ionol.

Activation of lipid peroxidation (LPO) in heart muscle is observed in emotional-painful stress [9], hypoxia, and reoxygenation [8, 16]. Together with activation of phospholipases and the detergent action of an excess of fatty acids [13], this process plays an important role in the mechanism of injury to the lipid bilayer of the cardiomyocyte membranes and disturbances of cardiac function in these states. The mechanism of disturbances of the contractile function of the heart observed during resuscitation after clinical death [6, 10], is not clear. Since stress, hypoxia, and reoxygenation are observed in various combinations in the course of this process, it can be tentatively suggested that LPO activation and injury to the membrane apparatus of the cardiomyocytes both play a role in postresuscitation heart failure.

To test this hypothesis, in the investigation described below LPO activity and the contractile function of the heart were studied in animals during resuscitation after clinical death, after which an attempt was made to prevent the disturbances of contractile function thus revealed by means of the synthetic antioxidant ionol (2,6-di-tert-butyl-4-methylphenol).

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 190-220 g anesthetized with pentobarbital (25 mg/kg). There are four series of experiments: I) control animals; II) animals resuscitated from clinical death; III) animals receiving ionol; IV) animals receiving ionol before clinical death. Each group contained 10 or 11 animals. Clinical death, for a duration of 4 min, was induced by acute bleeding through the carotid artery, and the animals were resuscitated by centripetal injection of the lost blood and artificial ventilation of the lungs. Ionol was injected intraperitoneally in a dose of 100 mg/kg daily for 3 days. For biochemical tests and for the study of its contractile function the heart was removed 6 h after resuscitation. Lipids were extracted from the myocardium by the method in [15]. Accumu-

Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Central Research Laboratory, Omsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 13-16, April, 1983. Original article submitted September 7, 1982.

 0007-4888/83/9504-0418$07.50

TABLE 1. Effect of Ionol on LPO Activation in Myocardium of Resuscitated Rats (M ± m)

Series of experiments	Content of hydroperoxides in polyene lipids, relative units	Intensity of fluorescence of Schiff bases, relative units
I-Control	0,112±0,009	3,95±0,50
II-Clinical death	0,228±0,010	7,00±0,72
III-Ionol	0,107±0,008	3,14±0,36
IV-Ionol + clinical death	0,143±0,010	4,65±0,45
P_{I-II}	>0,001	>0,01
P_{II-IV}	<0,01	<0,05

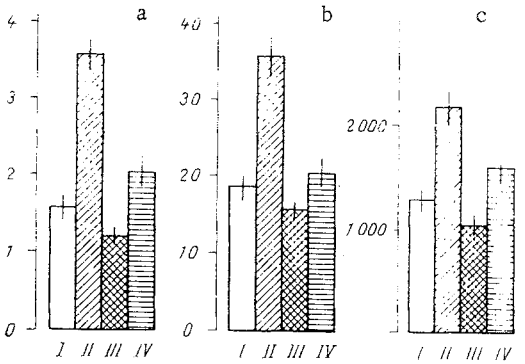

Fig. 1. Effect of ionol on enzyme activity in coronary perfusate from isolated rat hearts 6 h after resuscitation. I-IV) Series of experiments; a, b) activity of CPK and GOT, respectively (in μmoles/g pyruvate/h); c) RNase activity (in o.d.u.).

lation of hydroperoxides in polyene lipids was estimated from the UV absorption spectra of solutions of lipids in a methanol–hexane (5:1) mixture, determined on the SF-26 instrument [11] and expressed in optical density units (o.d.u.). The end products of LPO were determined as fluorescence of lipids in chloroform with a fluorescence excitation maximum at 365 nm and emission maxiumum at 436 nm [12] on a "Bian-130" fluorometer, calibrated with 1% solution of quinine sulfate in 0.1 N H_2SO_4. Myocardial contractility was studied on the isolated heart, contracting at different frequencies (120, 300, 400, and 500 min^{-1}) around a latex balloon filling the left ventricle [14]. The heart was perfused with Krebs–Henseleit solution saturated with carbogen. The pressure in the left ventricle was measured on an EM2-01 electromanometer and recorded together with its first derivative. Activity of creatine phosphokinase (CPK) [5], aspartase aminotransferase (GOT) and acid RNase [1] was determined in perfusate flowing through the coronary system and the loss of these enzymes per gram dry weight of myocardial tissue per hour was calculated.

EXPERIMENTAL RESULTS

Preliminary injection of ionol reduced mortality in the early post-resuscitation period from 48.0 to 24.5%, i.e., by half. Data on the effect of clinical death and ionol on the content of LPO products in heart muscle are given in Table 1.

It will be clear from Table 1 that 6 h after resuscitation the content of hydroperoxides in polyene lipids isolated from the myocardium was doubled, whereas that in the Schiff bases was increased by 1.8 times. Injection of ionol significantly reduced accumulation of LPO products in the myocardium after resuscitation. Ionol had no effect on the content of these products in the hearts of intact animals. Consequently, excessive activation of LPO, characteristic of the postresuscitation period, may be considerably reduced by means of ionol, which possesses high antiradical activity and inhibits free-radical oxidation of lipids [3].

Release of enzymes into the coronary blood flow 30 min after the beginning of perfusion is illustrated in Fig. 1. The hearts of rats resuscitated from clinical death were found to secrete twice as much CPK and GOT, and 1.8 times as much RNase as in the control. Release of

TABLE 2. Disturbance of Contractility of Heart Muscle after Clinical Death, with Different Contraction Frequencies, and Their Prevention by Ionol (M ± m)

Parameter	Series of experiments	Frequency of cardiac contractions, min^{-1}			
		120	300	400	500
Systolic pressure, mm Hg	I	84±2,9	98±3,0	122±5,1	118±5,0
	II	53±3,0	57±3,9	54±3,2	54±1,3
	III	92±4,0	104±4,0	129±4,7	122±5,1
	IV	74±3,1	88±5,0	98±4,9	86±4,7
P_{I-II}		<0,001	<0,001	<0,001	<0,001
P_{II-IV}		<0,001	<0,001	<0,001	<0,001
Diastolic pressure mm Hg	I	4,3±0,40	0,8±0,10	6,9±0,53	12,2±1,03
	II	4,1±0,25	7,4±1,02	23,5±2,19	35,3±2,17
	III	3,9±0,30	1,0±0,10	5,5±0,43	12,7±1,15
	IV	4,6±0,33	2,8±0,20	12,0±1,61	24,5±1,85
P_{I-II}		>0,05	<0,001	<0,001	<0,001
P_{II-IV}		>0,05	<0,001	<0,001	<0,001
Rate of contraction, mm Hg/sec	I	1341±87	—	—	—
	II	818±55	—	—	—
	III	1563±102	—	—	—
	IV	1283±117	—	—	—
P_{I-II}		<0,001			
P_{II-IV}		<0,01			
Rate of relaxation, mm Hg/sec	I	807±47	—	—	—
	II	409±39	—	—	—
	III	878±52	—	—	—
	IV	722±66	—	—	—
P_{I-IV}		<0,001			
P_{II-IV}		<0,001			
Diastolic defect, mm Hg/sec	I	—	—	26,5±2,3	53,2±3,7
	II	—	10,5±1,4	72,0±6,4	165,5±10,6
	III	—	—	21,5±1,8	47,3±4,1
	IV	—	—	51,7±3,8	93,1±5,8
P_{I-II}				<0,001	<0,001
P_{II-IV}				<0,01	<0,001

enzymes into the perfusate is known to be an objective criterion of injury to cardiomyocytes. Ionol, if administered before clinical death, reduced the outflow of enzymes almost to the control level and, consequently, prevented injury to the heart during clinical death and resuscitation.

Release of enzymes into the coronary perfusate, reflecting the degree of injury to the myocardium, correlates closely with postresuscitation disturbances of cardiac contractile function. It follows from Table 2 that with an initial frequency of 120 contractions/min depression of the contractile function characterized by lowering of the systolic pressure and of the rate of contraction by 1.6 times, and of the rate of relaxation by half, was observed. With binding of a higher contraction rate the postresuscitation disturbances of contractile function were manifested even more distinctly: The hearts of control rats responded to an increase in contraction frequency by an increase in developed pressure (positive inotropic effect), whereas the hearts of resuscitated rats responded to a rising contraction rate by a fall of developed pressure by 2.5 times (negative inotropic effect) and by disturbance of diastolic relaxation of the myocardium. This was expressed quantitatively as a 5-8-fold increase in diastolic pressure, compared with a 1.5-2.5-fold increase in the control; the diastolic defect in this case began to appear at a frequency of 300 min^{-1} and was more than three times higher than the control level at a frequency of 500 min^{-1}. Injection of ionol into intact rats did not cause significant changes in myocardial contractility, but at the same time injection of ionol before clinical death reduced the disturbances of cardiac contractility after resuscitation: The developed pressure and also the rate of contraction and relaxation, with an initial frequency of 120 min^{-1}, were significantly higher than for hearts of animals not protected by ionol. With the binding of a faster frequency of contraction, the protective effect of ionol was even more distinctly exhibited: The positive inotropic effect was preserved, but the diastolic effect which developed, although it exceeded the control level, was reduced by almost half compared with animals not receiving ionol before clinical death.

The increase in the diastolic defect by a factor of several times observed during binding of a high contraction rate in the postresuscitation period is evidence of a reduction in the effectiveness of the membrane calcium pump of the cardiomyocytes, which is responsible for relaxation of heart muscle. Stress and hypoxia, an excess of catecholamines, and LPO activation, arising during agony, clinical death, and subsequent resuscitation with reoxygenation, may perhaps injure membrane pumps of the sarcoplasmic reticulum and sarcolemma responsible

for the timely removal of calcium from the myofibrils and realization of diastolic relaxation, whereas ionol prevents these manifestations of injury.

Preliminary injection of ionol thus limits overactivation of LPO and abolishes postresuscitation injury to the heart, estimated on the basis of release of enzymes from cardiomyocytes and disturbance of contractility of the heart.

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ACTIVE ADJUSTMENT OF THE ARCHITECTONICS (STRUCTURE) OF MOTOR UNITS

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UDC 612.741.014.2.612.748

KEY WORDS: neuromuscular diseases; motor unit; muscle fibers; denervation-reinnervation process.

The principles governing the distribution of muscle fibers forming the motor units (MU) of normal muscles [4, 7], and also of muscles in different stages of recovery of function after denervation, have been studied in detail in investigations by electrophysiological, morphological, and histochemical methods. The principles and ability of preserved axons of motor nerves to undertake compensatory innervation by branching, to which the name sprouting has been given [2, 8], have also been studied. It has also been shown that the intensity of sprouting rises with a decrease in the number of preserved neurons, and this is reflected in the well-known phenomenon of grouping of muscle fibers of the same histochemical type [5].

Meanwhile the character of distribution of preserved nerve fibers has not yet been adequately analyzed. The data given below suggest that in the course of reinnervation the motoneuron concentrates the zone of distribution of its own axons in a narrower region than normally, and actively deprives muscle fibers located in the areas of MU remotest from the center, of its influences.

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 16-20, April, 1983. Original article submitted October 27, 1982.