Activity of Lysosomal Apparatus in Rat Myocardium during Experimental Coronary and Noncoronary Myocardial Damage

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 6, pp. 626-628, June, 2000
Original article submitted February 8, 2000

Cardiac and plasma activities of marker lysosomal enzymes were studied in Wistar rats with metabolic (epinephrine) and occlusion (ligation of coronary arteries) myocardial infarction. Activity of all examined lysosomal enzymes significantly increased in the myocardium and blood plasma starting from the first day after ligation of the coronary arteries and was accompanied by leukocytic infiltration of the myocardium. Enzyme activity gradually decreased to postoperation day 14. In metabolic infarction leukocytic infiltration and specific activity of lysosomal enzymes rose gradually and attained maximum to postoperation day 14, while the signs of labilization of lysosomal membranes appeared from the first postoperation day. Plasma activity of lysosomal enzymes in metabolic infarction increased smoothly and peaked on day 14.

Key Words: *myocardial infarction; lysosomal enzymes; polynuclear leukocytes*

Despite considerable attention focused on atherogenesis, ischemia, and myocardial infarction, many molecular mechanisms of these diseases remain unclear. In particular, metabolic damage to the heart (diabetic cardiopathy and metabolic myocardial infarction) are poorly studied [2,5,12].

An essential role in the pathogenesis of noncoronary damages are played by disturbed hormonal regulation of energy metabolism and impaired structure and functional state of cell lysosomal apparatus [2], which can release active acid hydrolases into the cytoplasm, extracellular space, and blood thus inducing cell damage [1-3,6,9,10].

Our aim was to compare the structural and functional state of cardiac lysosomal apparatuses in rats with occlusion myocardial infarction (OMI) induced by ligation of the coronary arteries and epinephrine-induced (metabolic) myocardial infarction (MMI).

MATERIALS AND METHODS

OMI was induced in male Wistar rats (180-270 g) by ligation of the left coronary artery. Sham-operated rats served as the control.

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MMI was induced by subcutaneous injection of 0.2 ml norepinephrine (0.1%) for 1 week to intact or alloxan-diabetic rats (100-120 mg per rat).

Myocardial infarction was verified by ECG and morphological studies. On days 1, 3, 7, and 14 after surgical or pharmacological intervention, activity of marker lysosomal enzymes (cathepsin D, β-galactosidase, acid phosphatase, and acid DNase) in myocardium homogenate and plasma was measured as described elsewhere [4]. The results were analyzed statistically using STATGRAF and QUATTRO softwares.

RESULTS

The development of myocardial damage was accompanied by activation of plasma lysosomal enzymes on day 1 by 3-10 and 1.3-1.6 times in rats with OMI and MMI, respectively (Table 1). However, in rats with OMI plasma activity of lysosomal enzymes gradually decreased and on postoperation day 14 attained the normal (sham-operated rats), while in MMI-rats activity of lysosomal enzymes increased during the entire observation period with increasing the total dose of the hormone and on day 14 2.0-2.5-fold surpassed the normal (intact rats).

0007-4888/00/0006-530$25.00

TABLE 1. Plasma Activity of Lysosomal Enzymes (μmol/liter/min) (*M±m*)

Enzyme, experimental day		Control (*n*=10)	OMI (*n*=12-13)	MMI (*n*=18-39)
β-Galactosidase				
	1st	33.60±3.19	96.40±7.18*	39.6±3.0
	3rd	38.9±6.4	88.40±7.42**	66.40±4.08*
	14th	27.80±5.02	19.6±3.0	77.40±4.23*
DNAase				
	1st	4.40±0.52	27.9±1.5*	13.5±1.5*
	3rd	4.60±0.45	12.80±1.45*	18.2±1.2*
	14th	4.40±0.27	21.10±1.23*	25.40±1.34*
Acid phosphatase				
	1st	8.5±0.9	40.30±3.91*	25.9±3.7*
	3rd	8.10±1.01	25.8±3.1*	37.4±4.8*
	14th	8.00±0.52	9.60±0.48	37.2±2.3*
Cathepsin D				
	1st	0	10.90±1.22*	0
	3rd	Traces	3.30±0.17*	1.30±0.66*
	14th	0	2.40±0.33*	3.38±0.56*

Note. Here and in Tables 2 and 3: *$p<0.01$ compared to sham-operated rats.

TABLE 2. Activity of Lysosomal Enzymes (μmol/g protein/min) in Heart Myocardium Homogenate (*M±m*)

Enzyme, experimental day		Control (*n*=10)	OMI (*n*=12-13)	MMI (*n*=18-39)
β-Galactosidase				
	1st	8.60±0.89	6.00±0.66	6.30±0.43
	3rd	0.40±0.07	15.60±1.02*	10.4±0.9*
	14th	0.90±0.11	5.30±0.23	14.30±0.74*
DNAase				
	1st	3.00±0.02	11.90±0.09*	2.00±0.11
	3rd	5.50±0.43	12.60±1.01*	9.40±0.72*
	14th	2.30±0.03	3.90±0.18	11.40±0.93*
Acid phosphatase				
	1st	11.30±1.91	17.90±1.66*	8.1±0.2*
	3rd	2.80±0.05	10.4±1.0*	6.9±0.5*
	14th	3.60±0.07	4.60±0.42	19.7±1.3*
Cathepsin D				
	1st	0.50±0.03	0.80±0.04	1.40±0.14*
	3rd	9.70±1.37	1.80±0.06*	1.20±0.56*
	14th	3.00±0.32	0.8±0.1	1.80±0.26

The changes in activity of marker lysosomal enzymes in myocardium homogenate also differed between these groups. In OMI-rats specific activity of acid phosphatase, β-galactosidase, acid DNase, and cathepsin D markedly (1.5-3.0-fold) increased on days 1-3 (Tables 2 and 3), which may attest to polymorphonuclear leukocyte infiltration. Indeed, histological examination revealed a leukocytic infiltration zone around the necrotic focus as soon as on day 1 of OMI. Resorbtion of the necrotic area was accompanied by a gradual decrease in the number of polynuclear leukocytes.

Comparison of the dynamics of lysosomal enzyme activity in the myocardium and morphological changes showed that the highest acid hydrolase activity coincided with the most pronounced cell damage

TABLE 3. Ratio of Free and Specific Enzyme Activity in Control Rats and during MMI ($M \pm m$)

Enzyme	Control	MMI, experimental day		
		1st	3rd	14th
Acid phosphatase	0.38±0.02	0.53±0.03*	0.62±0.06	0.66±0.05*
Cathepsin D	0.25±0.02	0.31±0.05	0.25±0.03	0.38±0.01*

in the myocardium and considerably decreased during cicatrization of the necrotic foci.

Close positive correlation was revealed between activity of lysosomal enzymes in myocardial homogenate on day 1 with their plasma activity on postoperation days 1-3 (r=0.74-0.81). This may indicate that the release of lysosomal enzymes from damaged cardiomyocytes is partially responsible for elevated plasma activity.

By contrast, in MMI-rats leukocytic infiltration of the myocardium was minor during the first 3 days, but then gradually increased to day 14 of treatment. This probably explains peculiar dynamics of enzyme activity during MMI: minor but significant decrease in specific enzyme activity observed on day 1 was accompanied by a considerable enhancement of free hydrolase activity, which attests to labilization of lysosomal membranes known to be the first to react to hormonal disturbances [2].

Thus, an important role in cardiac damage is played by cell mechanisms, in which the activation of polymorphonuclear leukocytes and lysosomal apparatus (both in myocardial cells and polymorphonuclear leukocytes) is related to disturbed hormonal regulation characteristic of myocardial infarction of various genesis. This process may be considerably potentiated by LPO activation accompanying a drop in insulin synthesis [7], cytokine release (first of all, interleukin-6, which specifically activates B and D cathepsins [8]), and accumulation of oxidized LDL damaging lysosomes [11].

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