Potential for Correcting Syndromal Metabolic Disorders by Means of Enzyme Therapy

F. N. Gil'miyarova, V. Yu. Golenishchev, and V. M. Radomskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 7, pp. 23-25, July, 1995
Original article submitted July 1, 1994

Measurement of metabolites in the cardiac muscle of rabbits at different stages of myocardial infarction showed elevated levels of intermediate metabolic products with marked acidic properties in the infarcted area as well as in the adjacent and more distant (apparently intact) areas. Introduction of exogenous lactate dehydrogenase into the circulation led to enhanced metabolic processes in the cytoplasm and mitochondria of cardiac and skeletal muscle cells. This suggests that lactate dehydrogenase may be recommended for use as an agent helping to normalize oxidative processes, compensate for energy deficiency, and correct metabolism in tissues.

Key Words: *myocardial infarction; carbohydrate/lipid metabolism; skeletal muscle; cardiac muscle; enzyme therapy*

Some of the pathogenic factors for a wide range of diseases are energy deficit in tissues, disrupted balance between oxidized and reduced metabolites, and generally elevated levels of polar oxo- and hydroxy acids with marked acidic properties [3,9]. Impaired utilization of unified metabolites provides a basis for energy deficiency and alters the supply of structural tissue elements with water and the orientation and reactivity of functional groups in macromolecules [2].

The purpose of the present study was to explore the possibility of utilizing a preparation of the enzyme lactate dehydrogenase as a biogenic agent for correcting the energy debt and metabolic acidosis.

MATERIALS AND METHODS

A total of 21 random-bred rabbits weighing 2.5-3.5 kg were used. In 11 of them myocardial infarction was produced by occlusion of the left coronary artery. The remaining 10 rabbits, kept in the vivarium under the same conditions as the test animals, served as intact controls.

At different times after myocardial infarction production, metabolites were measured in the infarcted area (left ventricle wall), in the zone around the infarcted area (anterolateral wall of the left ventricle), and in putatively intact areas (posterior wall of the left ventricle and the right ventricle).

The cardiac tissues of test and control rabbits were assayed for malate, oxaloacetate, lactate, pyruvate, α-glycerophosphate, and dihydroxyacetone phosphate using a standardized procedure [4], for protein by a biuret method, and for glycerol [7], free fatty acids [8], and glucose and glycogen [1].

In order to find out whether the metabolic shifts that occurred could be corrected with the enzyme lactate dehydrogenase, the metabolites listed above were measured in the cardiac tissues of control and test rabbits after instilling a lactate dehydrogenase preparation (M_4 isoenzyme) [5] into one auricular concha of each rabbit in a dose of

Department of Biological and Clinical Chemistry, State Medical Institute, Samara (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0007-0668$12.50

5000 U/kg body weight. The results were treated statistically using Student's t test.

RESULTS

The concentrations of α-glycerophosphate, dihydroxyacetone phosphate, glycerol, higher fatty acids, oxaloacetate, lactate, and pyruvate in the infarcted, periinfarct, and intact areas were found to be highest on day 3 after coronary artery occlusion (Table 1), and, as shown in this table, most of these metabolites were still present at significantly elevated levels on days 10 and 30 postocclusion, i.e., during the period of scarring. These findings point to their abnormal utilization not only in the infarcted and adjacent areas, but also in the areas considered to be intact. The only exceptions were glycogen and glucose (data not shown), suggesting that the compounds predominantly utilized in the cardiac muscle following the onset of myocardial infarction were carbohydrates. Because oxidative processes proceeded at reduced rates and anaerobic catabolism prevailed, incompletely oxidized metabolic products were accumulating in the myocardium, posing a real risk of metabolic acidosis and indicating that the osmotic regimen of cardiac muscle cell work was impaired and that the cardiac tissue was overloaded with metabolic material and suffering energy deficiency. Since there is a quantitative predominance of enzymes over substrates in tissues [6], the accumulation of metabolites, such as oxaloacetate and dihydroxyacetone phosphate, produced via many metabolic pathways reflects a functional weakness of enzymatic processes.

TABLE 1. Metabolite Levels in Different Myocardial Areas in Rabbits at Different Times after Myocardial Infarction ($M \pm m$)

Metabolite	Control	Time post-occlusion, days	Myocardial area		
			intact	infarcted	periinfarct
Malate, μmol/g	0.79±0.02				
		3	0.68±0.04*	0.64±0.03*	0.66±0.02*
		10	0.74±0.09	0.70±0.05*	0.71±0.03*
		30	0.79±0.08	0.78±0.09	0.77±0.08
Oxaloacetate, μmol/g	0.06±0.01				
		3	0.92±0.07*	0.11±0.01*	0.98±0.08*
		10	0.82±0.05*	0.55±0.02*	0.79±0.06*
		30	0.73±0.05*	0.38±0.02*	0.64±0.04*
Lactate, μmol/g	5.32±0.33				
		3	8.63±0.67*	10.52±0.76*	9.91±0.64*
		10	6.89±0.43*	6.08±0.38*	7.96±0.52*
		30	6.51±0.46*	5.94±0.29*	6.18±0.36*
Pyruvate, μmol/g	0.16±0.01				
		3	0.21±0.01*	0.21±0.02*	0.27±0.01
		10	0.19±0.02*	0.20±0.01*	0.18±0.02
		30	0.17±0.02	0.13±0.01*	0.18±0.02
α−Glycerophosphate, μmol/g	1.05±0.03				
		3	1.38±0.08*	1.62±0.12*	1.51±0.08*
		10	1.39±0.07*	1.14±0.09	1.37±0.05*
		30	1.25±0.03	1.06±0.03	1.23±0.04*
Dihydroxyacetone phosphate, μmol/g	0.243±0.02				
		3	0.31±0.01*	0.31±0.02*	0.34±0.03*
		10	0.24±0.02	0.26±0.01*	0.21±0.02
		30	0.25±0.01	0.28±0.01*	0.22±0.02
Glycerol, mg/g					
		3	7.15±0.48*	7.95±0.44*	8.19±0.53
		10	6.18±0.24*	6.12±0.33*	5.88±0.35*
		30	5.39±0.31	5.81±0.28	4.75±0.22
Free fatty acids, μmol/g	16.72±0.41				
		3	7.11±4.77*	35.71±3.92*	40.82±4.06*
		10	0.52±3.83*	30.64±2.52*	30.17±3.62*
		30	1.65±2.31*	23.72±2.39*	22.87±2.38*

Note. *$p<0.01$.

TABLE 2. Effect of Exogenous Lactate Dehydrogenase on Metabolite Levels in Rabbit Skeletal Muscle ($M \pm m$)

Metabolite	Control rabbits	Test rabbits
Alanine aminotransferase, μmol/ml×h	1.96±0.21	5.25±0.18
Aspartate aminotransferase, μmol/ml×h	3.29±0.29	6.04±0.32
Aldolase, μmol NADH/min/g	0.19±0.01	0.45±0.01
Glyceraldehyde−3−phosphate dehydrogenase, μmol NADH/min/mg	0.26±0.02	0.39±0.01
Lactate dehydrogenase, μmol NADH/min/mg	0.81±0.16	3.25±0.25
Malate dehydrogenase, μmol NADH/min/mg	4.63±0.04	3.91±0.21
Glutamate dehydrogenase, μmol NADH/min/mg	0.04±0.003	0.02±0.002
Glutamate, μmol/g	0.25±0.02	0.57±0.06
Pyruvate, μmol/g	0.05±0.004	0.08±0.006
2−Oxoglutarate, μmol/g	0.12±0.02	0.11±0.01
Oxaloacetate, μmol/g	0.15±0.006	0.08±0.005
Glucose, mmol/liter	2.12±0.04	1.82±0.009

Note. Here and in Table 3: the results are statistically significant at $p<0.01-0.001$.

TABLE 3. Effect of Exogenous Lactate Dehydrogenase on Metabolite Levels in Rabbit Cardiac Muscle ($M \pm m$)

Metabolite	Control rabbits	Test rabbits
Alanine aminotransferase, μmol/ml×h		
supernatant	7.25±0.32	10.07±0.18
mitochondria	12.68±0.61	14.11±0.43
Aspartate aminotransferase, μmol/ml×h		
supernatant	6.11±0.18	5.68±0.21
mitochondria	11.11±0.32	11.71±0.32
Aldolase, μmol/min/mg		
supernatant	0.12±0.01	0.15±0.01
mitochondria	0.01±0.001	0.02±0.001
Glutamate dehydrogenase, μmol NADH/min/mg	0.08±0.004	0.06±0.003
Glutamate, μmol/g	0.53±0.003	0.75±0.05
2−Oxoglutarate, μmol/g	0.12±0.005	0.10±0.005

The presence of exogenous lactate dehydrogenase (LDH) in the bloodstream of rabbits enhanced metabolic processes in the skeletal and cardiac muscles (Tables 2 and 3). The effect was more pronounced in skeletal muscles, suggesting that the LDH isoenzyme used shows a high affinity for skeletal tissue. Intensified amino acid metabolism via transamination and activation of glycolysis at both the central and terminal stages of amino acid conversions were recorded. Of special note is the intensive utilization of 2-oxoglutarate, oxaloacetate, pyruvate, and lactate in the metabolic processes.

The results of this study warrant submitting this lactate dehydrogenase preparation to clinical trials with a view to eliminating the metabolic debt and preventing metabolic acidosis in cases where incompletely oxidized metabolic products accumulate in large amounts.

REFERENCES

1. N. P. Meshkova and S. E. Severin (eds.), *Biochemistry* [in Russian], Moscow (1979), pp. 35-43.
2. A. S. Gavrish and V. S. Paukov, *Vestn. Akad. Med. Nauk SSSR*, № 10, 31 (1988).
3. F. N. Gil'miyarova, V. N. Fatenkov, V. P. Mishurova, *et al.*, *Sov. Med.*, № 1, 7 (1980).
4. F. N. Gil'miyarova, V. M. Radomskaya, and L. N. Vinogradova, *Instructions on the Use of Standardized Clinical Laboratory Methods of Investigation* [in Russian], Moscow (1986), pp. 31-36.
5. F. N. Gil'miyarova, V. M. Radomskaya, *et al.*, *Byull. Eksp. Biol. Med.*, **116**, № 10, 376-378 (1993).
6. B. I. Kurganov and E. A. Lyubarev, *Biokhimiya*, **54**, № 5, 716 (1989).
7. M. I. Prokhorova, in: *Methods of Biochemical Investigations* [in Russian], Leningrad (1982), pp. 146-148.
8. E. A. Stroev and V. G. Makarova, in: *Biochemistry* [in Russian], Moscow (1986), pp. 124-128.
9. V. N. Fatenkov, G. V. Gracheva, V. P. Mishurova, *et al.*, *Kazan. Med. Zh.*, **38**, № 1, 37 (1977).