EFFECT OF CONTRYCAL ON ACTIVITY OF DEHYDROGENASES AND THEIR ISOZYMES IN MUSCLES AND ORGANS OF ANIMALS WITH DEVELOPING GRANULATION TISSUE

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The effect of contrycal on the state of the enzyme systems of the muscles, liver, kidneys, and heart was investigated in rats with developing granulation tissue. This protease inhibitor was found to stimulate lactate and malate dehydrogenase activity and also the isozyme spectrum of these enzymes. The action of the inhibitor was manifested as a change in the state of the enzyme systems both at the site of injury (granulations and underlying tissue) and in certain internal organs (liver and kidneys).

KEY WORDS: lactate dehydrogenase; malate dehydrogenase; isozymes; protease inhibitor, contrycal; muscles; liver; kidneys; heart.

The therapeutic efficacy of protease inhibitors during the healing of infected wounds and in the treatment of burns and other pathological states [1, 4, 6] depends on the study of their effect on metabolic processes in the body and, in particular, on the biochemical processes taking place during wound healing. In previous investigations the writers showed [3] that contrycal has a marked action on the morphological structure and metabolism of granulation tissue. On the other hand, it produces changes in metabolism not only at the site of injury, but in the body as a whole [2], and for that reason it is essential to elucidate the nature of its effect on metabolic processes in the organs and tissues of animals with developing granulation tissue.

In this investigation the effect of contrycal was studied on the activity of enzyme of glycolysis and the Krebs' cycle, namely lactate (LD) and malate (MD) dehydrogenases, and their isozyme spectrum in the muscles and organs of animals.

EXPERIMENTAL METHOD

Male rats weighing 150-200 g were used. Growth of granulation tissue was induced by means of a nichrome coil, 35 mm and 4 mm in diameter, implanted subcutaneously in the dorsal region. On the day of the operation and the next two days the control animals received an intraperitoneal injection of 1 ml 0.14 M NaCl and the experimental animals an injection of contrycal in a dose of 5000 units (ATU) in 1 ml of 0.14 M NaCl. The rats of both groups were decapitated simultaneously on the seventh day after the operation. The liver, kidneys, heart, and skeletal muscles from two regions, adjacent to and remote from the zone of granulations, were taken for investigation. Total MD and LD activity was determined spectrophotometrically [5, 10] and expressed in activity units/mg protein. Protein was determined by Lowry's method. Fractionation into isozymes was carried out by electrophoresis on agar [7, 8].

EXPERIMENTAL RESULTS AND DISCUSSION

Determination of total LD activity and its isozyme spectrum in skeletal muscle (Table 1) showed that both the total LD activity and its isozyme composition in the region adjacent to granulation tissue were virtually indistinguishable from their values in the remote region. Injection of contrycal led to a significant increase in

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TABLE 1. Effect of Contrycal on Activity of LD, MD, and their Isozymes in Muscles and Organs of Rats with Developing Granulation Tissue (M ± m)

Tissue studied	Series	LD					
		total activity (in Wroblewski units/mg protein)	isozymes, %				
			LD_1	LD_2	LD_3	LD_4	LD_5
Muscles	Control						
	Adjacent region	3833,8±341,2	16,4±1,3	20,5±0,6	17,0±1,7	18,6±1,1	29,1±0,9
	Remote region	4223,0±230,7	14,8±1,6	18,5±1,0	15,0±0,9	22,6±0,8	31,5±0,5
	Experiment						
	Adjacent region	5524,0±250,0*	17,0±1,1	20,5±3,4	21,9±2,4	22,9±3,3	39,9±2,4*
	Remote region	4428,0±16,8	19,0±2,5	20,0±0,8	20,5±1,2	21,6±1,4	31,2±1,9
Liver	Control	4794,0±443,0	16,8±2,5	11,2±0,7	8,5±1,4	13,7±3,1	47,3±0,8
	Experiment	4576,0±263,0	10,5±0,6*	12,4±0,9	10,0±0,7	14,1±1,3	52,9±1,5*
Kidneys	Control	2404,0±171,4	29,3±1,3	23,3±1,7	10,5±0,0	15,4±2,5	21,4±0,8
	Experiment	3054,0±445,2	30,0±1,5	25,7±1,3	15,5±2,2*	11,0±1,7	17,3±2,5
Heart	Control	4020,0±101,2	28,3±0,9	27,8±0,6	21,0±1,2	11,8±0,9	11,6±0,9
	Experiment	4388,0±221,0	28,6±1,6	27,0±1,4	22,8±0,0	12,9±1,7	8,6±1,4

Tissue control	Series	MD				
		total activity (in Bucher units/mg protein)	isozymes, %			
			MD_1	MD_2	MD_3	MD_4
Muscles	Control					
	Adjacent region	182,7±22,5	7,5±1,9	22,4±0,7	35,2±0,6	34,8±1,3
	Remote region	262,0±9,3	6,2±1,7	19,6±2,8	37,0±1,2	37,1±2,6
	Experiment					
	Adjacent region	237,6±5,1*	3,75±0,9	16,9±3,2	41,5±2,7	40,1±2,3
	Remote region	336,0±15,5*	5,23±0,4	18,8±1,7	39,4±2,2	36,4±0,5
Liver	Control	277,0±34,1	4,2±0,8	7,8±1,7	44,3±4,2	43,9±2,4
	Experiment	301,7±30,7	3,3±0,1	5,9±1,2	46,0±2,6	45,0±1,7
Kidneys	Control	311,2±22,5		8,3±3,1	55,6±2,5	35,5±1,0
	Experiment	362,4±39,8		5,5±0,6	48,8±1,9	45,5±1,2*
Heart	Control	476,5±14,3	16,3±1,2	20,8±1,7	31,3±1,9	31,5±2,6
	Experiment	528,7±13,8	16,7±0,8	22,5±1,2	30,2±1,9	30,4±0,3

Legend. P calculated relative to control; values for which $P < 0.05$ marked by asterisk.

total LD activity in the adjacent area with a simultaneous change in the isozyme spectrum: An increase in the activity the LD_5 fraction was found compared with its value in the remote region.

The study of the effect of contrycal on the LD isozyme profile in the various internal organs showed that the greatest changes occurred in the liver: Activity of fraction LD_1 was reduced and that of fraction LD_5 was increased a little. This modification of the isozyme composition of LD did not affect the total activity, which remained unchanged. No significant changes in the indices studied could be observed in the heart or kidneys.

Determination of the total activity of MD and activity of its isozymes (Table 1) showed a significant decrease of 30.6% of total activity of the enzyme in the control group of animals in the muscles adjacent to the granulation tissue compared with the corresponding MD activity in the distant muscle ($P < 0.01$). No differences were found in the isozyme profile of the regions of muscle tissue compared.

Administration of contrycal caused a significant increase in the total activity of the enzyme in both test muscles by 30 and 28%, respectively. The ratio between the values of MD activity in the adjacent and distant muscles remained unchanged, i.e., the relative decrease in total MD activity was preserved in the region adjacent to the zone of granulations, and the percentage inhibition was the same as in the group of animals not receiving contrycal (30%). No significant changes were observed in the MD isozyme spectrum under the influence of contrycal.

During investigation of MD activity in the internal organs after administration of contrycal, definite changes were found in isozyme profile of the kidneys: MD_4 activity was increased by 28.1% whereas MD_3 activity was slightly reduced. No significant changes in the isozyme composition of the liver and heart were found after administration of contrycal under the conditions of these experiments. The marked tendency toward an increase in total MD activity under the influence of contrycal in the organs studied will be noted.

The results of these investigations thus agree with the existing view that muscle tissue adjacent to a zone of granulations is involved in the pathological process. Evidence of this is given the marked inhibition of total MD activity found in these experiments.

According to data in the literature administration of contrycal in the treatment of suppurative wounds and burns led to more rapid healing and to normalization of protein metabolism in the case of lesions produced by heat [1].

Metabolic changes in the same direction, namely activation of aerobic processes, under the influence of contrycal were observed previously in granulation tissue: the aerobic fractions LD_1 and LD_2 were inhibited and the anaerobic LD_5 fraction was activated, with a slight increase in total activity [3].

It can be concluded from these results that contrycal stimulates activity both of MD, an enzyme of anaerobic processes. This effect is not confined to a change purely in the total activity of the enzymes, but it also extends to their isozyme spectrum. The action of protease inhibitors is manifested as a disturbance of the state of the enzyme systems both at the site of injury (granulations and adjacent tissue) and in certain internal organs (liver and kidneys).

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