Metabolic Correction Reduces the Area of Acute Ischemic Myocardial Infarction in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 3, pp. 267-269, March, 2006
Original article submitted April 6, 2005

The possibility of decreasing the degree of irreversible alterations in cardiomyocytes with original saline reperfusion solution enriched with L-aspartic acid, D-glucose, and D-mannitol was studied on experimental rats with regional ischemia and reperfusion. Infusion of the test solution into the left ventricle during the early reperfusion stage significantly reduced the area of myocardial infraction. This effect was accompanied by improvement of energy metabolism and decrease in damage to cell membranes in the risk zone. Our results indicate that metabolic protection during reperfusion increases myocardial resistance to ischemic and reperfusion stress.

Key words: *myocardial infraction; reperfusion; metabolic correction; energy metabolism; cardiomyocyte membranes*

Current efforts are focused on the development of new approaches to metabolic correction of myocardial dysfunction induced by ischemia and reperfusion. Much attention is given to the synthesis of new drugs for the treatment of coronary heart disease [7]. Introduction of L-aspartic acid and D-mannitol into the composition of cardioplegic solution improves functional recovery of the isolated rat heart after total ischemia [2].

This work was designed to test the hypothesis that treatment with the original reperfusion solution (RS) during the early reperfusion stage can improve myocardial resistance to acute coronary occlusion.

MATERIALS AND METHODS

Infraction of the left ventricle (LV) was induced by occlusion of the anterior descending coronary artery (ADA) followed by reperfusion of the risk zone. Experiments were performed on male Wistar rats (300-450 g) under conditions of jet ventilation with an oxygen-containing mixture. Preparation of animals was followed by a 30-min period for hemodynamic stabilization and blood sampling (basal state). Regional myocardial ischemia was induced by 40-min occlusion of ADA and 60-min reperfusion.

RS was enriched with D-glucose and L-aspartic acid that provide synthesis of ATP and guanosine triphosphate in the cytosol and mitochondria at low intracellular oxygen concentration. The solution also included D-mannitol, which scavenges reactive oxygen species and prevents cell edema and sarcolemmal ruptures. Trisamine entering the composition of RS serves as the regulator of tissue pH. RS consisted of 137.0 mM NaCl, 4.7 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 20.0 mM potassium aspartate, 20.0 mM glucose, 20.0 mM mannitol, and 10.0 mM trisamine. pH was 7.5±0.1 at 22°C. Osmolarity was 380±5 mOsm/liter. The protective effect of RS was estimated by its ability to reduce the area of myocardial infarction, improve energy metabolism, and decrease the degree of damage to the cell membrane. RS was infused into LV (per-

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0007-4888/06/1413-0296

fusion rate 20 μl/min) over the first 5 min of reperfusion using a CMA/100 microinjection pump (Carnegey Medicine). Control animals received physiological saline under similar conditions. The animals exhibiting LV fibrillation were excluded from further studies. Acid-base balance in the arterial blood was monitored on an ABL-30 gas analyzer (Radiometer) and maintained at a physiological level.

By the end of the study, the hearts from some animals were stained with 2% Evans solution. Tissues of the risk zone were rapidly cut out from the hearts of other animals. LV were separated from stained hearts. Sections (~1 mm) were incubated with 1% solution of 2,3,5-triphenyltetrazolium chloride in 0.1 M phosphate buffered saline (pH 7.4) at 37°C for 10 min. The samples were dried and weighted to determine the weight of LV. The area of myocardial infarction was estimated by means of computerized planimetry using Imagecal software. We calculated the percentage of the risk zone relative to the weight of LV, as well as the ratio between the areas of myocardial infarction and risk zone [1]. Tissue of the risk zone was rapidly frozen in liquid nitrogen to obtain protein-free extracts. The concentrations of ATP, phosphocreatine, and creatine were determined enzymatically [5]. Further studies were performed to determine basal metabolic indexes. Heart tissue of animals was taken by the end of 30-min stabilization after preparation. Heart tissue of control animals was sampled by the 10th minute of reperfusion (5-min administration of physiological saline into LV and 5-min reperfusion). Heart tissue of treated animals was cut off by the 10th minute of reperfusion (5-min administration of RS into LV and 5-min reperfusion).

The results were analyzed by Student's t test. The differences were significant at $p<0.05$.

RESULTS

Administration of RS into LV over the first 5 min of reperfusion had no effect on blood pressure and acid-base balance in the arterial blood. During the overall period of reperfusion these indexes did not differ from the basal level (blood pressure 116±6 mm Hg, pH 7.40±0.03, Pco_2 18.6±1.8 mm Hg, Po_2 235.0±14.0 mm Hg). Thus, the animals of both groups had stable hemodynamic parameters. Adequate aerobic metabolism was maintained at the system level.

Table 1 shows the effects of RS on the concentration of energy metabolites in the risk zone of rat LV by the 10th minute of reperfusion. RS significantly improved ATP reduction in the risk zone (up to 80.0±3.1% of the basal level *vs*. 69.4±3.2% in control animals, $p<0.05$). Treated rats were characterized by a more pronounced phosphocreatine overshoot and lower loss of intracellular creatine (up to 54.6±2.9% of the basal level *vs*. 47.4±1.4% in control animals, $p<0.05$). Total creatine level (phosphocreatine and creatine) was much higher after RS administration (72.1±2.7% of the basal level *vs*. 62.5±1.8% in the control, $p<0.05$).

No intergroup differences were revealed in the percentage of the risk zone relative to the weight of LV. However, treatment with RS decreased the area of myocardial infarction by 1.5 times compared to the control (Fig. 1).

Administration of RS for 5 min decreased the degree of myocardial injury induced by acute occlusion of ADA and further reperfusion. The protective effect of RS is associated with modulation of metabolism in ischemic cardiomyocytes. L-aspartic acid entering the composition of RS improves aerobic resynthesis of adenine nucleotides and phosphocreatine during the early reperfusion stage [4]. This hypothesis is supported by a higher level of ATP and phosphocreatine in the risk zone compared to the control. It can be hypothesized that high concentration of glucose in RS (20 mM) reduces energy deficit in myocardial cells [6]. ATP produced during anaerobic/aerobic glycolysis preserves the integrity of sarcolemma in ischemic myocytes [4]. Our results show that RS administration during

TABLE 1. Concentration of Metabolites in the Risk Zone of Rat LV Myocardium by the 10th Minute of Reperfusion with RS (μmol/g dry tissue weight, $M±m$, $n=8$)

Parameter	Basal state	Group	
		control	experimental (RS)
ATP	22.33±1.20	15.50±0.70*	17.84±0.71*+
Phosphocreatine	23.78±1.59	23.00±1.19	26.52±1.20
Creatine	53.62±0.90	25.41±0.77*	29.30±1.60*+
Total creatine	77.40±1.12	48.41±1.37*	55.84±2.08*+

Note. n, number of animals per group. $p<0.05$: *compared to the basal level; +compared to the control group.

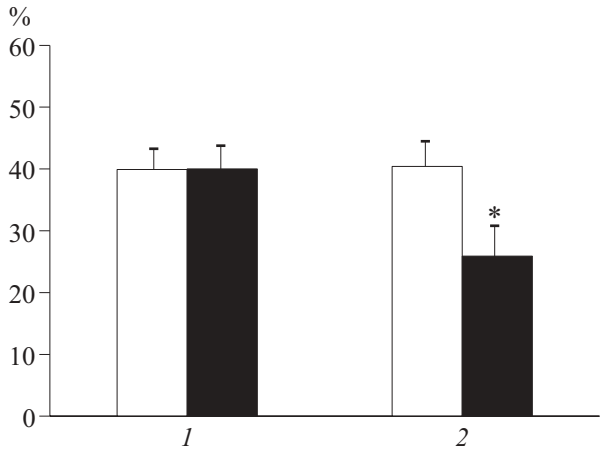

Fig. 1. Area of the risk zone and myocardial infarction after 60-minute reperfusion with reperfusion solution (RS). Light bars, control (n=14); dark bars, treatment (RS, n=14). Percentage of the risk zone relative to the weight of the left ventricle (%, *1*); ratio between the areas of myocardial infarction and risk zone (%, *2*). *p<0.05 compared to the control group.

the early reperfusion stage increases total creatine level in the risk zone (Table 1). Creatine loss is mainly due to sarcolemmal ruptures. These data illustrate a decrease in the release of intracellular creatine into the circulation, which alleviates damage to cell membranes. D-Mannitol entering the composition of RS not only provides appropriate osmolarity of this solution, but also protects the sarcolemma. This effect is related to antioxidant activity of D-mannitol [3]. We believe that well-balanced ionic composition of RS and the presence of components normalizing intracellular metabolism and alleviating damage to cell membranes determine a decrease in the area of myocardial infarction during reperfusion (Fig. 1).

Our results show that metabolic correction of postischemic myocardium during the early stage of reperfusion is an effective approach to preventing cardiomyocyte death.

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