Efficiency of Cardioplegic Solutions Containing L-Arginine and L-Aspartic Acid

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In experiments on rats we studied the effects of cardioplegic solutions with L-aspartic acid or L-arginine on functional recovery and metabolism of isolated working heart after 40-min normothermal global ischemia and 30-min reperfusion. After reperfusion of the hearts preventively protected with cardioplegic solution containing L-aspartic acid or L-arginine, coronary flow decreased in comparison with the initial values. As a component of cardioplegic solution, L-arginine was less efficient in recovery of contractility and cardiac output of the hearts in comparison with L-aspartic acid. In hearts protected with L-aspartic acid, the postischemic levels of ATP and phosphocreatine were significantly higher, and the level of lactate was significantly lower than in hearts protected with L-arginine. In comparison with L-arginine, L-aspartic acid is a more efficient component of cardioplegic solution in protection of the heart from metabolic and functional damages caused by global ischemia and reperfusion.

Key Words: *cardioplegia; L-aspartic acid; L-arginine; ischemia; reperfusion; cardiac function; metabolism*

Cardioplegic solutions (CS) containing L-aspartic acid (Asp) or L-arginine (Arg) are widely used to reduce ischemic and reperfusion damages to the heart during cardiosurgical interventions [9]. Under conditions of oxygen deficiency in cardiomyocytes, Asp stimulates anaerobic synthesis of ATP and guanosine triphosphate (GTP) in the mitochondria by the reactions related to production of succinate. In cytosol, Asp up-regulates transamination of glycolytic pyruvate into alanine, reduces accumulation of lactate, and moderates intracellular acidosis. Under conditions of normal oxygen supply to cardiomyocytes, Asp metabolism is coupled with production of tricarbonic acid cycle intermediates and with transfer of NADP reducing equivalents from cytosol to mitochondria [1]. Addition of Asp to CS improves recovery of contractile and pumping functions of the postischemic heart [8,9]. The protective effect of Arg on ischemized myocardium is related to production of nitric oxide ($NO^{\bullet}$) catalyzed by NO-synthase. The effects of $NO^{\bullet}$ results from dilation of coronary vessels, prevention of platelet aggregation and adhesion of neutrophils to coronary endothelium, and contribution into detoxification of superoxide radicals ($^{\bullet}O_2^-$) [9,11]. It was hypothesized that the cardioprotective effects of Arg on experimental blood-perfused hearts are caused by the direct influence of $NO^{\bullet}$ on blood cells [2]. Rationality of the addition of Arg into CS and its effects on metabolism in iscemized myocardium were little studied [3]. There are data on the damaging action of this amino acid on oxidative metabolism and mitochondrial functions in various cells [10]. Cardioplegic efficiency of Arg and Asp in the same experimental model was never compared. Our aim was to examine the effects of Arg and Asp as CS components on functional recovery and metabolism of isolated rat heart after global ischemia and reperfusion.

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MATERIALS AND METHODS

Experiments were carried out on the hearts from Wistar rats (body weight 392±9 g, heart weight 1.7±0.1 g) perfused by the method of Neely at a constant filling pressure of 15 mm Hg in the left atrium and the outflow left ventricular backpressure of 60 mm Hg [1]. In spontaneously beating heart, the pressure was recorded in the left ventricle and aorta; coronary flow and aortal volume were measured. The heart was perfused with oxygenated Krebs solution (95% O_2+5% CO_2, pH 7.4±0.1, 37°C) containing 11 mM glucose. CS with identical salt composition and equal pH contained either 21.5 mM Asp (perfusol) or 3 mM Arg (CS-Arg). The osmolarity of these CS was equalized with NaCl (Table 1).

After 20-min initial perfusion with Krebs solution (after Neely), the hearts were perfused for 5 min with one of the test CS (22°C, through the aorta under constant flow of 15±1 ml/min) and then subjected to 40-min normothermal global ischemia followed by 30-min Neely-reperfusion with Krebs solution at 37°C. At various stages of the experiment, the hearts were shock-frozen with a Wollenberger clamp cooled in liquid nitrogen. The frozen tissue was homogenized in cold 6% $HClO_4$, the proteins were precipitated by centrifugation, the supernatants were neutralized with 5M K_2CO_3 to pH 7.4, and $KClO_4$ precipitate was separated by centrifugation. The weight of dry specimens was determined by weighing of tissue samples after extraction of $HClO_4$ and overnight drying at 110°C [1]. In protein-free extracts, ATP, phosphocreatine, creatine, and lactate were essayed by enzymatic methods [7]. Total creatine was calculated as $\Sigma_{creatine}$=phosphocreatine+creatine. The data were processed statistically using Student's *t* test. Restoration of functional parameters of the heart during reperfusion was calculated as percentage of initial values in each experiment.

TABLE 1. Basic Parameters of Cardioplegic Solutions

Parameter	Perfusol	CS-Arg
NaCl, mM	105.0	114.0
KCl, mM	16.0	16.0
$MgCl_2$ $6H_2O$, mM	16.0	16.0
$CaCl_2$, mM	1.2	1.2
Asp, mM	21.5	—
Arg, mM	—	3.0
Mannit, mM	20.0	20.0
Trisamine, mM	5.0	5.0
Osmolality, mosmol/liter	340±5	340±5
pH at 22°C	7.6±0.1	7.6±0.1

Note. Dash (—) means the absence of the agent in CS

RESULTS

Table 2 shows the mean indices of contractile and pumping functions of the heart. There were no significant intergroup differences in changes of cardiac parameters during infusion of CS. Infusion of CS arrested the heart within 30-40 sec. To the end of reperfusion, coronary resistance and coro-

TABLE 2. Effect of Asp or Arg in Cardioplegic Solutions on Recovery of Functions of Isolated Rat Heart after Global Ischemia and 30-min Reperfusion

Parameter	Initial State (*n*=23)	Reperfusion (percent of initial, *M*±*m*)	
		perfusol (*n*=13)	CS-Arg (*n*=10)
Systolic pressure, mm Hg	114±1	77±2	67±4
Diastolic pressure, mm Hg	-5±1	339±21	286±19
Left ventricular developed pressure, mm Hg	118±1	75±3	68±7
HR, min^{-1}	237±3	103±4**	86±2
Contractile rate pressure product, mm Hg/min	27,274±557	79±4*	60±6
Coronary resistance, mm Hg/ml	4.10±0.05	100±3**	145±11
Coronary flow, ml/min	15±1	97±3***	68±5
Aortal volume, ml/min	26±1	39±4	36±9
Cardiac output, ml/min	44±1	62±3*	46±8
Stroke volume, μl/beat	199±4	64±3	60±8
Cardiac power output, ml×mm Hg	2656±47	60±2*	43±7

Note. Initial state was characterized by the parameters measured 20 min after the stabilization period. *p<0.05, **p<0.02, ***p<0.01 compared to CS-Arg group.

nary flow in the CS-Arg group were 145±11 and 68±5% of the initial values, respectively (Fig. 1, *c*; Table 2). By contrast, in the perfusol group both parameters returned to the initial values on reperfusion minute 1 and then remained unchanged. In hearts protected with CS-Arg, reperfusion restored contractile function to 60±6% of the initial value (Fig. 1, *a*). After 30-min reperfusion of the hearts in this group, stroke volume, cardiac power output, and cardiac flow output decreased to 60±3%, 43±7%, and 46±8% initial values, respectively (Fig. 1, *b*; Table 2). Perfusol was more efficient in restoration of contractile function, which attained 79±4% of the initial value to the end of reperfusion. This effect was primarily related to HR recovery. Virtually complete recovery of coronary resistance and coronary flow after perfusol cardioplegia followed by reperfusion resulted in better recovery of cardiac flow and power outputs (Fig. 1, *b*; Table 2).

To the end of global ischemia, the levels of ATP and phosphocreatine were significantly higher in perfusol-protected hearts than in the hearts treated with CS-Arg (Table 3). There were no intergroup differences between the levels of creatinine and total creatine ($\Sigma_{creatine}$). In CS-Arg group, the postischemic level of lactate was higher by 14.6 μmol/g dry tissue than in the perfusol group ($p<0.05$). To the end of reperfusion, ATP level restored to 60.0±3.2% and 46.9±2.4% in perfusol and CS-Arg groups, respectively ($p<0.05$). Replacement of Asp for Arg in CS had no effect on restoration of phosphocreatine and preservation of creatine in reperfused hearts. In the hearts arrested with perfusol, the content of lactate decreased to the initial value to the end of reperfusion, while in CS-Arg group it 2.4-fold surpassed the initial level.

In this study, we used saline perfusion to exclude neurohumoral effects and effects of amino acids on blood cells. This paradigm reveals the dif-

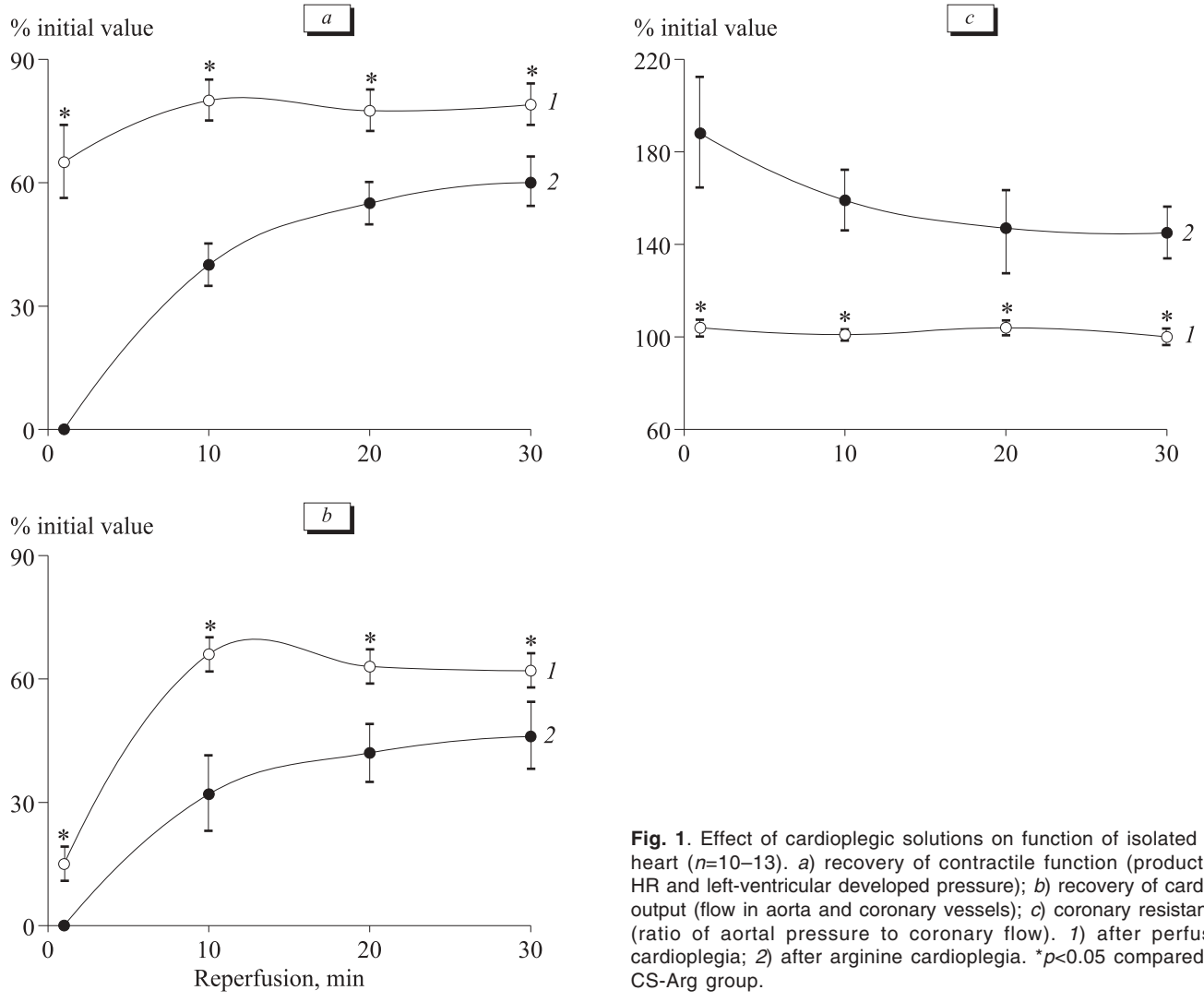

Fig. 1. Effect of cardioplegic solutions on function of isolated rat heart (n=10–13). *a*) recovery of contractile function (product of HR and left-ventricular developed pressure); *b*) recovery of cardiac output (flow in aorta and coronary vessels); *c*) coronary resistance (ratio of aortal pressure to coronary flow). *1*) after perfusol cardioplegia; *2*) after arginine cardioplegia. *$p<0.05$ compared to CS-Arg group.

TABLE 3. Effect of CS on Metabolic Status of the Heart after 40-min Ischemia and 30-min Reperfusion

Parameter, μmol/g dry tissue	Initial State (*n*=10)	Ischemia		Reperfusion	
		perfusol (*n*=5)	CS-Arg (*n*=5)	perfusol (*n*=13)	CS-Arg (*n*=10)
ATP	21.29±0.45	11.40±0.39*	8.54±0.76	12.73±0.69*	9.99±0.51
Phosphocreatine	23.40±0.54	5.78±0.38*	3.15±0.25	21.93±1.03	19.12±1.89
Creatine	53.44±0.28	68.18±6.23	70.85±6.24	37.72±3.24	36.11±2.29
$\Sigma_{creatine}$	76.84±0.45	73.96±5.54	74.00±4.33	56.63±0.96	55.79±2.07
Lactate	2.03±0.09	52.29±3.65*	66.93±4.39	1.31±0.14*	4.87±0.38

Note. For metabolite analysis, the hearts were frozen at the end of 20-min stabilization period (initial state), after 40-min global ischemia, and after the end of 30-min reperfusion. *p<0.05 compared to CS-Arg group.

ferences in the effects of Asp and Arg on metabolism and function of postischemic heart. Being added to the saline cocktail, Asp more effectively protected isolated rat heart from damage caused by global ischemia and reperfusion compared to Arg. The metabolic indices, function of the heart, and coronary flow recovered better, when perfusol was used for heart protection instead of CS-Arg.

Better cardiac recovery during reperfusion under the effect of Arg is usually explained by the formation of NO•, which improved coronary flow and inhibited platelet aggregation and/or neutrophil adhesion to coronary epithelium [2,3,11]. In our experiments, replacement of Asp with Arg in CS markedly increased coronary resistance and impeded recovery of coronary flow during reperfusion (Fig. 1; Table 2). This can be explained by insufficient synthesis of NO• from arginine or by binding of NO• to myoglobin heme or to Fe-S centers of other proteins (*e.g.* complexes I and II of the respiratory chain in mitochondria and/or aconitase in tricarbonic acid cycle) [5,10]. Probably, the latter mechanism explains the capacity of NO• to inhibit oxidative phosphorylation and to impede recovery of cardiac function during reperfusion. Another reason of poor efficiency of restoration of the contractile function of the heart treated with CS-Arg could be the negative inotropic effect of NO• [6]. It is caused by activation of cytoplasmic guanylate cyclase by NO•, which increases the level of intracellular cGMP and therefore inhibits Ca^{2+} current either directly or indirectly via stimulation of cGMP-dependent phosphodiesterase.

Production of NO• in the reaction catalyzed by NO-synthase needs NADPH as electron donor and molecular oxygen [11]. Probably, this explains the fact that the cardioprotective effects of Arg are more evident when it is applied during reperfusion, but greatly vary when this amino acid is added to CS [2,10]. Ambiguous effect of blood or saline arginine cardioplegia on the recovery of cardiac function and coronary blood flow, or on extrusion of ischemic markers into the myocardial outflow during reperfusion was observed in experimental and clinical studies [3,4,11], which agrees with the present findings. In contrast to Arg, Asp contributes to energy-producing aerobic and anaerobic reactions [9]. As a rule, inclusion of Asp into CS decreases the loss of macroergic phosphates and preserves the integrity of sarcoplasmic membrane in ischemized cardiomyocytes, which promotes ionic homeostasis, better recovery of aerobic metabolism and cardiac functions during following reperfusion [1,8]. Thus, our data experimentally substantiate rationality of the use of saline CS with Asp in cardiosurgery to minimize ischemic and reperfusion damages to the heart.

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REFERENCES

1. O. I. Pisarenko, V. S. Shul'zhenko, I. M. Studneva, and A. A. Timoshin, *Kardiologiya*, **44**, No. 4, 65-70 (2004).
2. M. R. Adams, W. Jessup, and D. Hailstones, *Circulation,* **95**, 662-668 (1997).
3. M. Carrier, M. Pellerin, P. L.Page *et al*., *Ann. Thorac. Surg.,* **66**, 108-112 (1998).
4. N. Hayashida, H. Tomoeda, and T. Oda, *Ann. Thorac. Cardiovasc. Surg.,* **6**, 27-33 (2000).
5. J. R. Lancaster, J. E. Hibbs, *Proc. Nat. Acad. Sci*., **87**, 1223-1227 (1990).
6. P. F. Mery, S. M. Lohmann, and U. Walter, *Ibid.*, **88**, 1197-1201 (1991).
7. H.U.Bergmeyer (Ed.), *Methods of Enzymatic Analysis,* New York (1974), pp. 1565-1569, 1772-1781, 2101-2110.
8. O. I. Pisarenko, F. L. Rosenfeldt, L. Langley *et al., Ann. Thorac. Surg.,* **59,** 1541-1548 (1995).
9. H. Taegtmeyer and T. Doenst, in: *Ischemia-Reperfusion Injury in Cardiac Surgery*, Georgetown (2001), pp. 79-89.
10. K. Takeuchi, F. X. McGowan, H. C. Danh, *et al*., *J. Mol. Cell. Cardiol.,* **27**, 1405-1414 (1995).
11. J. Vinten-Johansen and R. S. Ronson, in: *Ischemia-Reperfusion Injury in Cardiac Surgery*, Georgetown (2001), pp. 2-27.