Cardioprotective Effect of Ischemic Postconditioning on the Model of Isolated Heart

L. N. Maslov*,, A. S. Gorbunov*,**, and Yu. B. Lishmanov*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 3, pp. 290-291, March, 2012
Original article submitted November 29, 2010

Irreversible cardiomyocyte damage was induced by 45-min global ischemia followed by 30-min reperfusion in Langendorff-perfused isolated rat heart. Cell damage was assessed by the release of creatine phosphokinase into the perfusate. The hearts were subjected to the following postconditioning protocols: 1) three cycles of 10-sec reperfusion and 10-sec ischemia, total cycle time 20 sec; 2) six cycles of 10-sec reperfusion and 10-sec ischemia, total cycle time 20 sec; 3) three sessions of 20-sec reperfusion and 20-sec ischemia, total cycle time 40 sec; 4) 6 cycles of 20-sec reperfusion and 20-sec ischemia, total cycle time 40 sec; 5) 3 cycles of 30-sec reperfusion and 30-sec ischemia, total cycle time 60 sec. It was found that only postconditioning with a total cycle time of 40 sec or 60 sec prevents myocardial reperfusion injury.

Key Words: *heart; ischemia; reperfusion; postconditioning*

Increased resistance of organs and tissues to the damaging effect of postischemic reoxygenation achieved by several sessions of short-term ischemia under the conditions of reperfusion is known as ischemic postconditioning (IP). The phenomenon of IP was found in 2003 by a group of psychologists from Atlanta (USA) [5] in the *in vivo* experiments on dogs. Later it was found that IP can be reproduced in experiments on the isolated perfused heart [1,3,4] in which some researchers used three cycles of alternating 30-sec reperfusion intervals followed by 30-sec ischemia [3], the others performed three cycles of reperfusion ischemia/reperfusion (I/R; 10 sec/10 sec each) [1]. The hearts are usually subjected to six cycles [4]. At the same time, some researchers in isolated heart experiments have failed to reproduce the phenomenon of postconditioning by any protocol [2]. Hence, the standard model of IP currently does not exist.

The purpose of the study was to compare several models of IP and evaluate their cardioprotective effect.

MATERIALS AND METHODS

Experiments were carried out on isolated hearts from Wistar male rats weighing 250-300 g anesthetized with ethyl ether. The hearts were removed from the chest cavity and rapidly placed in a bath with cooled to 4°C Krebs–Henseleit solution until the spontaneous contractions ceased. Then the cannula was introduced into the ascending aortic arch and retrograde Langendorff perfusion with Krebs–Henseleit solution was performed with oxygenated solution (37°C, pH 7.4) containing (in mM): 120 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20 $NaHCO_3$, and 10 D-glucose (MP Biomedicals, Irvine).

In a control series of experiments, the heart was adapted to normoxic perfusion for 20 min and then subjected to 45-min total ischemia and 30-min reperfusion. The degree of necrotic damage to cardiomyocytes was assessed by creatine phosphokinase (CPK) level in the perfusate throughout the entire reperfusion period. CPK activity was assessed using enzymatic CK-NAc kits (Analyticon Biotechnologies AG – Lichtenfeis) and calculated per gram of heart tissue. We used the following postconditioning protocols: 1) three cycles of 10-sec reperfusion and 10-sec ischemia, total

* Institute of Cardiology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk; ** Tomsk State Pedagogical University, Russia. ***Address for correspondence:*** Maslov@cardio.tsu.ru. L. N. Maslov

0007-4888/12/1533-0313

cycle time 20 sec; 2) six cycles of 10-sec reperfusion and 10-sec ischemia, total cycle time 20 sec; 3) three sessions of 20-sec reperfusion and 20-sec ischemia, total cycle time 40 sec; 4) 6 cycles of 20-sec reperfusion and 20-sec ischemia, total cycle time 40 sec; 5) 3 cycles of 30-sec reperfusion and 30-sec ischemia, total cycle time 60 sec. Each group comprised 14 hearts. Since the experiments were conducted in autumn and winter, control (I/R) and postconditioning experiments were performed daily to eliminate possible influence of seasonal variations in the heart tolerance to I/R on the results of experiments.

The results were analyzed by Mann–Whitney test.

RESULTS

Three cycles of 10-sec reperfusion and 10-sec ischemia had no significant effect on CPK level in the perfusate in comparison with the controls (I/R without postconditioning, Fig. 1). Six 20-sec I/R cycles also failed to achieve greater tolerance of the heart to the pathogenic effects of reperfusion. Postconditioning with 3 40-sec I/R cycles reduced CPK concentration in the perfusate by 19% in comparison with controls. Six 40-sec I/R sessions decreased CPK level by 29% in comparison with the control group (Fig. 1). The maximum cardioprotective effect of IP was recorded when 3 60-sec I/R cycles were used. In this case, CPK level in the perfusate was by 32% lower than in control group (Fig. 1).

Thus, according to our data, increasing tolerance of the heart to reperfusion injury can be achieved only using several I/R cycles of 40-60 sec. We can assume that there was a threshold for the duration of I/R that induced signaling pathways involved in postconditioning. This threshold is a I/R cycle with a duration of <40 sec, but >20 sec.

The theoretical and practical significance of the work is that new data on the cardioprotective effect of IP were obtained. The results can be useful for cardiac surgery departments conducting operations under cardiopulmonary bypass; departments and institutions studying problems of heart ischemia and reperfusion injury; pharmaceutical companies developing drugs to treat cardiovascular diseases.

This work was supported by the Federal Agency for Science and Innovation (government contracts No. 02.740.11.0714, No. 11.519.11.2016, No. 11.519.11.2028) and Russian Foundation for Basic Research (grants No. 10-04-00288, No. 11-04-00467, No. 11-04-98001, No. 11-04-98000, No. 11-04-98004).

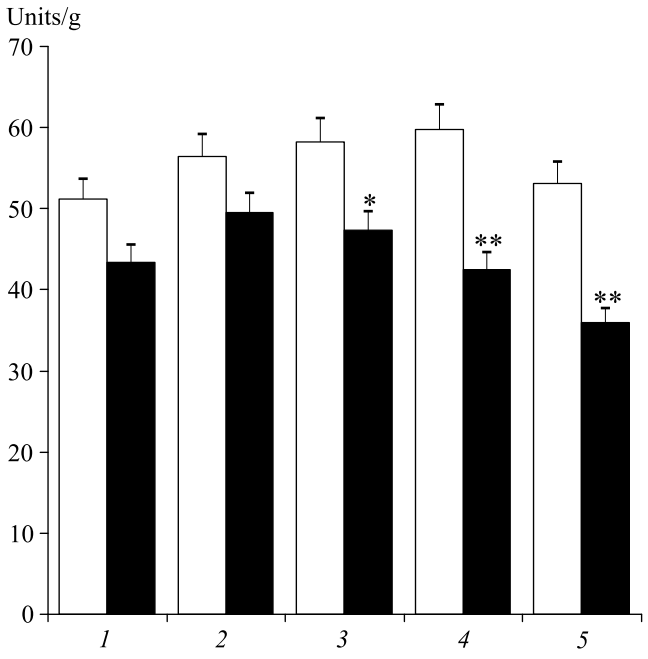

Fig. 1. CPK activity in the perfusion solution after IP. Light bars, control; dark bars, IP. *1*, three cycles of 10-sec reperfusion and 10-sec ischemia, cycle time 20 sec; *2*, six cycles of 10-sec reperfusion and 10-sec ischemia, cycle time 20 sec; *3*, three sessions of 20-sec reperfusion and 20-sec ischemia, cycle time 40 sec; *4*, 6 cycles of 20-sec reperfusion and 20-sec ischemia, cycle time 40 sec; *5*, 3 cycles of 30-sec reperfusion and 30-sec ischemia, cycle time 60 sec.*$p<0.05$, **$p<0.01$ compared with the control.

REFERENCES

1. M. D. Goodman, S. E. Koch, G. A. Fuller-Bicer, and K. L. Butler, *Am. J. Physiol. Heart Circ. Physiol*., **295**, No. 4, H1649-H1656 (2008).
2. M. L. Kaljusto, T. Mori, S. Mohammad Husain Rizvi, *et al*., *Scand. Cardiovasc. J.*, **40**, No. 6, 334-341 (2006).
3. R. Schreckenberg, T. Maier, and K. D. Schlüter, *Br. J. Pharmacol*., **156**, No. 6, 901-908 (2009).
4. D. van Vuuren, A. Genis, S. Genade, and A. Lochner, *Cardiovasc. Drugs Ther*., **22**, No. 5, 391-397 (2008).
5. Z. Q. Zhao, J. S. Corvera, M. E. Halkos, *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, **285**, No. 2, P. H579-H588 (2003).