РАЗДЕЛ I. ДИСФУНКЦИЯ ЭНДОТЕЛИЯ КРОВЕНОСНЫХ СОСУДОВ: ОБЩИЕ МЕДИКО-БИОЛОГИЧЕСКИЕ ВОПРОСЫ

CARDIO- AND VASOPROTECTIVE EFFECTS OF INTERMITTENT, NORMOBARIC HYPOXIC CONDITIONING IN A RAT MODEL OF MYOCARDIAL ISCHEMIA AND REPERFUSION

H. Fred Downey,[1] Ludmila M. Belkina[2], Elena A. Smirnova,[2] Robert T. Mallet,[1] Eugenia B. Manukhina[2]

[1]University of North Texas Health Science Center, Fort Worth, TX 76107, USA;
[2]Institue of General Pathology and Pathophysiology, Russian Federation

There is mounting clinical and experimental evidence that intermittent, hypoxic conditioning (IHC) is cardioprotective, especially in models of myocardial ischemia – reperfusion (IR) [1-8]. The current investigation tested the hypothesis that moderate, normobaric, intermittent hypoxia is both cardioprotective and vasoprotective in a rat model of myocardial IR.

Methods. Wistar rats weighing 250-280 g were subjected to normobaric IHC in a hypoxic chamber (5-8 cycles/d for 20 days, FIO_2 9.5 - 10% for 5 - 10 min/cycle, with intervening 4 min normoxia), a regimen that may have clinical applications. Control rats were sham-conditioned with 21% O_2.

One day after completing the IHC conditioning program (n=14) or the sham conditioning program (n=14), rats were anesthetized with urethane and placed on artificial respiration, ECG leads were attached, and the chest was opened. After a 30-min stabilization period, the main left coronary artery (LCA) was ligated for 30 min followed by 60-min reperfusion, while arrhythmias were monitored. Myocardial ischemia during LCA ligation was confirmed by regional cyanosis and progressive, marked ST segment elevation. Reperfusion was confirmed by an epicardial hyperemic response. When ventricular fibrillation occurred, the time to spontaneous defibrillation was measured.

Infarct size (IS) was evaluated with the triphenyl-tetrazolium chloride (TTC)–Evans blue technique. After 60 min of coronary reperfusion, the hearts were excised, perfused with 2% Evans blue dye to separate the left ventricular area at risk for infarction (AAR; tissue distal to the LCA ligature) from surrounding normal tissue (stained blue). The heart was then frozen at -20°C for 20 min, and cut into 5 2-mm-thick transverse slices. The slices were incubated for 20 min at 37°C in a 1% solution of TTC in phosphate buffer (pH=7.4) to differentiate the infarcted area (pale) from the non-infarcted AAR (red). Slices were fixed in a 10% formaldehyde solution, and photographed. The corresponding areas were measured by computerized planimetry, and infarct size was calculated as a percentage of the AAR.

In addition to parameters of cardioprotection, endothelial dysfunction was evaluated as an index of vasoprotective effects of IHC. Endothelium-dependent relaxation (EDR) was evaluated by the dilatory response to acetylcholine (10 μM) of norepinephrine (0.5 μM)-precontracted, isolated aortic rings from control and IHC rats with and without IR.

In all figures, * indicates a statistically significant difference ($p<0.05$) between IHC and Control.

Results. The 20-day normobaric hypoxic training did not induce any significant changes in the relative weight of right ventricle (Table 1), thus mitigating concerns about hypoxia-induced right ventricular hypertrophy.

Table 1 - Effect of IHC on the relative weight of right ventricle.

	BW	RVW/(RVW+LVW)	RVW/BW
Control	314 ± 31	0.23 ± 0.03	0.00061 ± 0.00008
IHC	342 ± 16	0.22 ± 0.03	0.00064 ± 0.00008

Values are means±SE from 14 animals per experimental group. BW = body weight, g; RVW = right ventricle weight, g; LVW = left ventricle weight, g

Total durations of ischemic extrasystole, ventricular tachycardia and ventricular fibrillation were significantly shorter in rats adapted to intermittent hypoxia than in untreated animals (Figure 1). However no protection against reperfusion arrhythmias was observed; the duration of reperfusion arrhythmias did not differ in hypoxia-adapted and non-adapted animals. The same was true for the occurrence of arrhythmias. All types of arrhythmias occurred less frequently in adapted than in control rats during ischemia (Figure 2). The proportion of rats with reperfusion arrhythmias was similar in control and IHC rats.

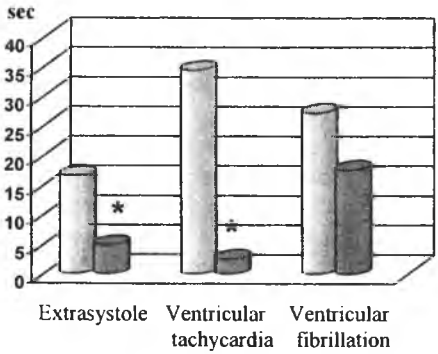


Fig. 1. Effect of IHC on duration of arrhythmias during ischemia. Light bars, control; dark bars, IHC. *Significantly different from control, $p<0.05$.

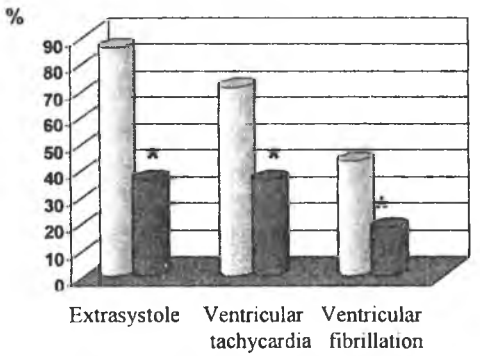
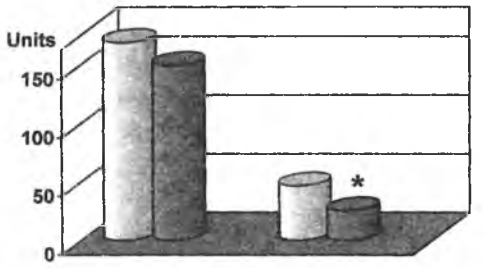


Fig. 2. Effect of IHC on occurrence (% of total experiments) of arrhythmias during ischemia. Light bars, control; dark bars, IHC. *Significantly different from control, $p < 0.05$.

A



B

Fig 3. Effect of IHC on infarct size (Panel A) and area at risk (Panel B). Panel A: Light bars, control; dark bars, IHC. *Significantly different from control, $p < 0.05$. Panel B: light segments, infarct zone; dark segments, non-infarcted area at risk.

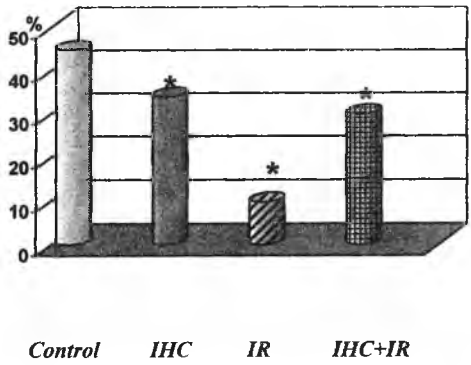


Fig. 4. Effect of prior IHC treatment on endothelium-dependent relaxation of isolated rat aorta following myocardial ischemia and reperfusion (IR). Values are percentage of pre-ACH treatment diameter.*Significantly different from control, $p < 0.05$. *Significantly different from IR, $p < 0.05$

Figure 3 illustrates the infarct size and area at risk (AAR) determined after 1 hr of reperfusion. In non-adapted rats, infarct size was almost twice as large as in control and constituted 30.6±4.6% of AAR, while in IHC rats, infarct size was only 17.7±3.5% of AAR ($p<0.05$). AARs did not significantly differ in IHC and control animals.

In aortic rings of Control animals, ACH caused a 46% increased in diameter of the pre-contracted rings. Figure 4 shows that IHC alone reduced pre-ischemic EDR to 33.9±3.3% ($p<0.05$ vs. control). Ischemia and reperfusion induced pronounced endothelial dysfunction but this disorder was completely prevented by prior IHC (29.9±2.9% after IR).

Discussion. Intermittent hypoxic conditioning (IHC) has been used for prevention and treatment of many diseases, including bronchial asthma, allergic neurodermatitis, hypertension, diabetes mellitus, Parkinson's disease, emotional disorders, dyslipidemia, paranoid form of schizophrenia, radiation toxicity, certain occupational diseases; and in sports [9]. The basic mechanisms underlying the beneficial effects of IHC, specifically IHC-induced cardioprotection are not completely understood but IHC is known to induce gene expression of protective proteins, stimulate antioxidant defenses, stabilize cell membranes, modulate NO synthesis, activate ATP-sensitive K^+ channels, prevent Ca^{2+} overload of cells, improve O_2 transport in tissues, and increase efficiency of oxygen utilization in ATP production [4-6, 8-10].

In clinical studies IHC showed a high anti-arrhythmic and anti-anginal efficacy in patients with ischemic heart disease, which exceeded the efficacy of drug therapy. An advantage of IHC over drug therapy is in the fact that IHC neither suppressed myocardial contractility nor impaired heart conductance and

provided more stable and long-term effect than anti-arrhythmic and anti-anginal medication [7, 11].

Experimental data obtained in the present study support the clinical application of the IHC method and demonstrate that IHC exerts not only anti-arrhythmic but also cell-protective and vasoprotective effects in the heart exposed to ischemia and reperfusion.

We recently proposed that IHC may suppress acute NO overproduction and oxidative stress during IR [8]. This mechanism may also protect both coronary and non-coronary blood vessels from the oxidative stress associated with IR [13-15]. Adaptive increases in antioxidant enzyme activities may also be protective [15].

Conclusions.

- Intermittent hypoxic preconditioning (IHC) protects rat heart against ischemic arrhythmias including extrasystole, ventricular tachycardia and ventricular fibrillation.
- IHC decreases the size of infarct zone formed after ischemia and reperfusion but does not influence the area at risk.
- IHC prevents aortic endothelial dysfunction after myocardial ischemia and reperfusion.
- Moderate, intermittent, normobaric hypoxia conditioning is both cardio- and vasoprotective in a rat model of myocardial IR.

Our positive findings may encourage clinical applications of this novel, non-invasive approach to protecting myocardium and vasculature threatened by ischemia and reperfusion.

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ДИСФУНКЦИЯ ЭНДОТЕЛИЯ И КИСЛОРОДСВЯЗЫВАЮЩИЕ СВОЙСТВА ГЕМОГЛОБИНА

Зинчук В.В.

УО «Гродненский государственный медицинский университет», Беларусь

Данные современной фундаментальной и клинической медицины указывают на то, что при заболеваниях сердечно-сосудистой системы одним из наиболее вероятных мест повреждения организма является эндотелий сосудистой стенки. В течение последних десятилетий кардинальным образом пересматриваются представления о его физиологической роли в организме. Эндотелий является не просто структурным обрамлением сосудов, а функционально активным органом с набором разнообразных функций. Его значимость иллюстрируется известной фразой J. Vane «Эндотелий – маэстро сердечно-сосудистой системы», а также названием актовой речи авторитетного исследователя по фармакотерапии эндотелия профессора Р. Григлевского «Эндотелий и моя жизнь» при присуждении ему звания Почетного Доктора Гродненского медицинского университета. В кли-