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Low Doses Hypoxia Effectively Reduces the Size of Necrosis in Rats with Experimental Myocardial Infarction

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Abstract: This paper presents results on the effect of a single session of hyperbaric oxygenation (HBO) therapy on prevalence zone of ischemia and myocardial necrosis in experimental animals. Compared with "high-doses" of HBO therapy, application of the so-called "small" doses (oxygen pressure at 0.02 MPa) was more favorable. The results of this study suggest the effectiveness of HBO in irreversible obstruction of coronary arteries, as well as more efficient use of HBO in the "low-dose".

Key words: Rats · High doses hypoxia · Small doses hypoxia · Necrosis · Myocardial Infarction

INTRODUCTION

Treatment strategy for acute myocardial infarction (MI) is to as quickly as possible restore adequate oxygen supply of the heart muscle [1-6]. A logically sound idea is to reinforce oxygen delivery to blood by increasing the concentration of dissolved oxygen in the plasma (pO2), which will allow to reach it by inhalation under increased pressure (hyperbaric oxygen therapy, HBOT) [2, 3].

In an experimental model (IM) in a condition of "ischemia-reperfusion", it was shown that the inhalation of hyperbaric oxygen at an excess pressure of 0.05-0.2 MPa can significantly reduce the area of irreversible myocardial changes, improve survivability and reduce the severity of pathological remodeling of myocardium [7, 8]. However, clinical data are not as clear as reflected in the meta-analysis of the Cochrane Library [9]. One reason for the discrepancy between the experimental and clinical data can be toxic effects of hyperbaric oxygen, especially in a condition of "reperfusion injury" [10-12]. In addition, in clinical practice, it is not always possible to achieve full restoration of blood flow [13], a phenomenon referred to as "no-reflow" or "low-reflow".

In this regard, it is essential to understand whether HBOT have a positive impact in terms of complete occlusion of the coronary vessel, on one hand or there are differences in the effects of hyperbaric oxygen, used under different pressures on the other hand.

Classical model of experimental MI is a surgical ligation of coronary artery in aseptic conditions. The model reproduced well, but requires a well worked out surgical technique [14].

Therefore, the aim of the present work was to study the effect of a single session of HBOT on prevalence zone of ischemia and myocardial necrosis:

- In a condition of complete irreversible coronary artery occlusion;
- "Low-" and "high-doses" modes of HBOT (0.02 MPa and 0.2 MPa, respectively).

MATERIALS AND METHODS

Laboratory Animals: The object of this experimental study was 60 rats weighing 200-250 g of both sexes between the ages of 9 months to 1 year and were in standard vivarium conditions. All procedures were performed according to protocols approved by our University Committee for Use and Care of Laboratory Animals (in accordance with the ethical requirements of the Helsinki Declaration, revised 2000).

Model of Myocardial Infarction: Experimental MI in the rats was reproduced by ligation of the left coronary artery (CA) as described by Ye *et al*. [14], with some modifications. The total duration of the operation took

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15-20 minutes, the chest was left open 45-60 seconds, making it possible to use non- inhalation anesthesia. Animals were awake in 15-20 minutes after surgery; the fatality rate did not exceed 10%. In some of the rats, CA ligation was not conducted; after opening the chest, it was sutured again (sham-operated animals).

In each rat, electrocardiogram in lead II before and after 15-30 minutes of operation (speed of 50 mm / s, 1 mV = 10 mm) was recorded. The criterion for inclusion of the operated animals for further study was the appearance of typical postoperative ischemic changes [15]. Of the 60 operated animals, 46 were included.

Planimetric Study: All morphological studies were conducted 24 hours after the onset of MI as described [16]. The animals were injected with 2 mL of Evans Blue into the jugular vein and sacrificed by decapitation and the heart were removed, the left ventricle was isolated and cut crosswise into 7 equal parts to the thickness of the rings-slices that were weighed. Ring-sections were scanned at 1200 dpi on both sides. Then the sections were stained with 1% solution of TTC (2,3,5-Triphenyltetrazolium chloride) and scanned again. Scanned images were downloaded into a computer. The results were processed using Photoshop CS2. The following were calculated: 1) the area of risk, expressed as a percentage of the mass of undyed myocardial tissue from the total left ventricular mass (staining with Evans Blue); 2) the zone of ischemia, expressed as a percentage of the mass of stained tissue (staining with TTS) by weight of the risk zone and 3) zone of necrosis, expressed as a percentage of the mass of unstained tissue (staining technique TTS) by weight of the risk zone.

HBO Session: HBO was conducted in a hyperbaric pressure single chamber BLKS-303 MK (Khrunichev state research and production space center, Russia), intended for treatment session using hyperbaric oxygenation in compliance with safety regulations. The HBOT session was conducted within 3 hours from the time of occlusion of blood vessels. The duration of a session was 60 minutes and the pressure - 0.02 MPa and 0.1 MPa.

In accordance with the protocols of the experiment, animals were divided into the following groups:

Group 1, n = 7. The natural occurrence of MI. HBOT session after CA ligation was not performed, morphological studies were performed 24 hours after the onset of MI.

Group 2, n = 7. HBOT procedure performed at an excess oxygen pressure of 0.02 MPa.

Group 3, n = 6. HBOT procedure performed at an excess oxygen pressure of 0.1 MPa.

Statistical Analysis: Data were processing with the STATISTICA 6.0 programme package using methods of nonparametric statistics: the Mann-Whitney test for independent groups and the Wilcoxon test for dependent groups. Results are presented as median (Me) and 25-75 percentile.

RESULTS AND DISCUSSION

The weight of the animals included in this experiment was similar in all groups. The mass of the left ventricle in all groups was also similar.

Twenty-four hours after CA ligation in the control group, median (Me) of the risk area was 31.72% from the mass of the left ventricle (Figure 1). In other words, about one third of the left ventricular blood flow was disrupted, which was considered as an area of risk. In animals exposed to HBO, the zone of risk was lower.

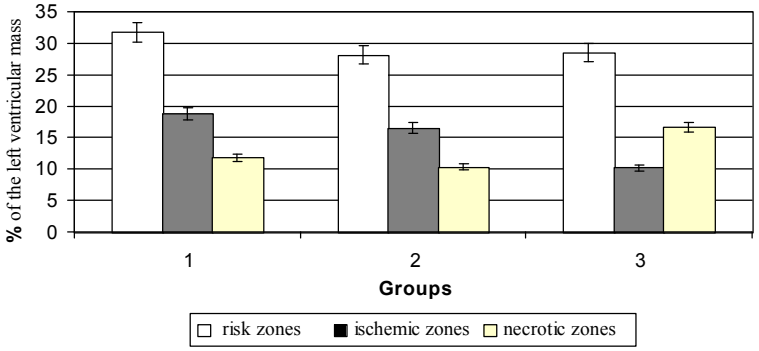

Fig. 1: Percent (%) of risk zones, ischemia and necrosis of the left ventricular mass.

Table 1: Ischemic zones and necrosis (%) of the risk areas (Me, 25-75% Quartile)

Group	Zones of ischemia (%)	Zones of necrosis (%)	Ratio of zones of ischemia (%) to necrosis (%)
1 (without HBOT, n=7)	62.22 [3] (35.26 - 62.91)	37.78 [2] (37.09 - 64.74)	1.7:1
2 (HBOT after 3 hrs 0.02 MPa, n=7)	66,65 [3] (58.73 - 72.78)	33,35 [3] (27.22 - 41.27)	2:1
3 (HBOT after 3 hrs 0.1 MPa, n=6)	37.17 [1,2] (30.32 - 42.73)	62,83 [1,2] (57.27 - 69.68)	0.6:1

[1]- $p < 0.05$ in comparison with the group 1;

[2]- $p < 0.05$ in comparison with the group 2;

[3]- $p < 0.05$ in comparison with the group 3.

The risk zone is the area of the myocardium with impaired blood supply [8] and a tendency towards the reduction may be due to the fact that a slight increase in the partial pressure of oxygen promotes the opening of collaterals [17] and when the partial pressure exceeds 0.05 Mpa, vasoconstrictor effects of hyperoxia begin to show.

When using the TTC dye, area of risk was clearly divided into ischemic zones (areas of cardiac tissue that retained dehydrogenase, which serves as a substrate for the TTC) and the necrotic zones (areas of cardiac tissue in which dehydrogenase was sharply reduced or absent).

In assessing the zones of necrosis and ischemia (Table 1), it is noteworthy that in the natural course of MI (group 1), the ratio of zones of ischemia and necrosis was 1.7:1, which was slightly lower than that given in the literature and may be due to methodological issues [18-20]. In the application of hyperoxia pressure at 0.1 MPa ischemic zone decreased in favour of the necrotic zone, for example, in the application of HBOT in the 0.1 MPa mode, size of necrotic tissue was significantly increased.

Application of the so-called "small" doses of HBOT (oxygen pressure at 0.02 MPa) was more favorable. It is interesting to note that in the group 3 animals (that were exposed to HBO in the 0.1 MPa mode), zones of necrosis were substantially increased in the risk areas, whereas zones of ischemia were reduced. In the group 2 animals (effect of HBO in the 0.02 MPa mode), zones of necrosis were almost twice less; however, areas of ischemia increased. This means that there was a rise in the volume of myocardial tissue affected by ischemia, although still retains the ability to recover. In our view, these results support the emerging in recent years, more effective representation of low-dose HBO. Itwas shown that 40-minute exposure of oxygen at a pressure of 0.03 MPa in healthy volunteers significantly reduces oxidative stress indicators [20]. Kim *et al*. [21] believed that the infarct-limiting effects of HBO associated with activation of antioxidant mechanisms, including the activation of catalase, since the positive effects of HBOT are nullified in the presence of catalase inhibitor.

It seems a logical assumption that the increase in the partial pressure of oxygen in the blood may limit the zone of necrosis. In IM experimental models, it was shown that in different layers of myocardium, cell death does not occur simultaneously (the process begins with the endocardium) and lasts for at least 4-6 hours, covering all sectors of the myocardium [7, 13]. It is known, for example, that with the dye TTC areas of necrotic tissue is clearly defined within 6 hours after acute occlusion (CA) in rats and then within 48 hours, the distribution of "necrotic wave" of the final (on histological data) formation of the zone of necrosis [22]. It may be associated with a mosaic dying of cells and the phenomenon of hibernation. Perhaps this phenomenon is associated with the development of collateral circulation. Jia and Sato [17], studied the distribution of the microparticles in the heart tissue of rats after ligation of the standard (CA), the collateral circulation develops within 15-30 minutes after occlusion: the dye was found in the outer layer of the ischemic zone. After 3 hours, the dye was detected in the whole area of risk. The authors believe that, in addition to the development of the capillary circulation, there is a retrograde reflux of blood from a large vein into the venules and capillaries.

It should be noted that the effect of HBOT remains, at least for 24 hours after the session. The results of dos Santos *et al*. [16] are close to those obtained in this study. The authors observed a decrease in the zone of myocardial necrosis during the HBOT procedure immediately after the creation of ischemia in the rats and subsequent transfer of the animals into atmospheric conditions for 24 hours. On the other hand, Sterling *et al.* [8] found positive effects of HBOT (at 0.15 MPa) in experimental myocardial infarction in rabbits, when HBO was conducted directly at the time of modeling of myocardial ischemia and in its subsequent reperfusion. We believed that this issue of HBOT in acute (MI) is still in a stage of accumulation of experimental and clinical data. Heterogeneity of experimental and clinical models, various applications of HBOT does not allow to formulating precise conclusions. It is believed that the use

of HBOT in acute coronary syndrome is a clinical prospect; however, the existing evidence base is currently insufficient to recommend a method in general clinical practice [9].

Overall, our results indicated the effectiveness of HBO in irreversible obstruction of coronary arteries, as well as more efficient use of HBO in the "low-dose".

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