DOI 10.1007/s10517-015-2849-1

Low Doses of Hyperbaric Oxygenation Effectively Decrease the Size of Necrotic Zone in Rats with Experimental Myocardial Infarction

E. A. Dotsenko, N. V. Nikulina, D. P. Salivonchik*, O. G. Lappo, A. I. Gritsuk*, and A. S. Bastron**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 158, No. 12, pp. 692-695, December, 2014
Original article submitted May 29, 2013

We studied the effect of a single session of hyperbaric oxygenation on the size of risk, ischemic, and necrotic zones in rat myocardium after irreversible occlusion of the coronary artery and excessive oxygen pressure of 0.02 and 0.1 MPa. Myocardium infarction was reproduced by ligation of the left coronary artery. The size of the risk, ischemic, and necrotic zones was planimetrically evaluated. Hyperbaric oxygenation (60-min session) was performed 3 h after artery occlusion at excessive oxygen pressure of 0.02 and 0.1 MPa. In rats not exposed to hyperbaric oxygenation, the risk zone median was 31.7% of the left ventricle weight, while after the session it did not exceed 25%. In spontaneous course of myocardium infarction, the ischemia to necrosis zone ratio was 1.7:1, while under conditions of hyperbaric oxygenation at oxygen pressure of 0.1 and 0.02 MPa, the these values were 0.6:1 and 2:1, respectively. Excessive oxygen pressure of 0.02 mPa is better than traditionally used 0.1 MPa, because it promotes redistribution of the ischemic and necrotic areas in the risk zone: the area of necrotic zone decreased at the expense of the ischemic zone. Hyperbaric oxygenation produces a positive effect on the myocardium under conditions of total occlusion of the coronary artery.

Key Words: *hyperbaric oxygenation; experimental myocardium infarction*

The therapy of acute myocardium infarction (MI) is aimed at the fastest recovery of adequate oxygenation of the myocardium [6]. It seems logical to increase blood oxygen release via increasing the concentration of plasma oxygen that can be reached by oxygen inhalation under hyperbaric oxygenation (HBO) conditions [3,6]. In cardiology, HBO is mostly used in CHD. However, there are only few publications on HBO application in acute CHD forms (acute coronary syndrome, MI) during the first day, when rapid recovery of oxygen balance in myocardium is essential [4].

According to clinical protocols, high-doses of HBO (excessive oxygen pressure 0.1 MPa) are recommended. In a classical manual on hyperbaric medicine [1], HBO application in doses <0.1 MPa is considered low effective. This is true for several clinical cases, for example, for CO intoxication or anaerobic infection. Experiments on the model of experimental MI under "ischemia/reperfusion" conditions have demonstrated [9] that hyperbaric oxygen inhalation at 0.1-0.2 MPa reduced the size of irreversible myocardium lesion and severity of pathological myocardium remodeling. However, addition of HBO to complex treatment of acute CHD does not significantly improve treatment efficiency, which is seen from the Cochrane Library systematic review [6]. One of the most likely reasons of diversity between experimental and clinical data is the toxic effect of oxygen. Patients with reperfusion myocardium impairments are extremely sensitive to it [1,5,8]. Obviously, the decrease of oxygen pressure excess will alleviate its toxic effects, but the direct

Belarussian State Medical University, Minsk, the Republic of Belarus; *Gomel State Medical University, the Republic of Belarus; **I. M. Sechenov Moscow State Medical University, Moscow, Russia. ***Address for correspondence:*** ed_dots@mail.ru. E. A. Dotsenko

anti-ischemic effect of hyperoxia under these conditions is under question. Moreover, complete blood flow recovery is not always achievable; this phenomenon is called "no-reflow" or "low-reflow". In light of this, it is important to understand, if HBO has a positive effect under conditions of total occlusion of the coronary artery and if there are any differences in the effects of hyperbaric oxygen delivered under different pressures.

Here we studied the influence of single HBO session on the size of ischemic and necrotic zones under conditions of irreversible and total coronary occlusion in low-dose and high-dose HBO modes (excessive pressure 0.02 and 0.1 MPa, respectively).

MATERIALS AND METHODS

The experiments were carried out on 9-12-month-old outbred male and female rats (n=30) weighing 200-250 g maintained under standard vivarium conditions. All manipulations were performed in strict adherence to the Declaration of Helsinki (2000).

MI was modeled by ligation of the left coronary artery at the level of the lower edge of the left auricle [10] with some modifications. The procedure took 15-20 min and the chest remained open for 45-60 sec, which made possible to apply non-inhalation narcosis. The animals awoke 15-20 min after the surgery. Mortality rate did not exceed 10%. Some rats (n=5) underwent sham operation (thoracotomy without ligation of the left coronary artery). In all rats, ECG was recorded in standard lead II before and 15-30 min after the surgery using a Malysh EK1T-04 electrocardiograph (type speed 50 mm/sec, 1 mV=10 mm). Inclusion criteria were typical ischemic changes appeared after the surgery [2]; 20 of 25 operated animals were included in the experiment.

All morphological studies were performed 24 h after MI modeling using the method described previously [9]. Evans blue solution (2 ml) was injected into the jugular vein and the animals were decapitated; the heart was removed, the left ventricle was isolated and cut transversally into 7 equal rings. The rings were weighed and then scanned at 1200 dpi definition from the both sides. Then the sections were stained with TTC (2,3,5-triphenyltetrazolium chloride) and repeatedly scanned. Scanned images were processed using Photoshop CS2 software. Risk zone was calculated as the percent of unstained tissue of the left ventricular myocardium from the whole left ventricle weight (vital Evans blue staining); ischemia zone was expressed as the percent of stained (TTC) tissue from the risk zone weight; necrosis zone was expressed as the percent of unstained (TTC) tissue from the risk zone weight.

HBO session was performed in an individual hyperbaric BLKS-303 MK pressure chamber (Khrunichev State Research and Production Space Center) developed for the treatment sessions according to safety instructions. The HBO session was performed within 3 h from coronary artery occlusion (HBO duration was 60 min, excessive pressure 0.02 and 0.1 MPa, compression and decompression time 15 min). According to the experimental protocols, the animals were divided into groups: in group 1 (n=7), MI run its natural course (HBO session after artery ligation was not performed, morphological studies were made 24 h after MI onset); in group 2 (n=7), HBO was performed with 0.02 MPa excessive oxygen pressure, in group 3 (n=6), HBO was applied with 0.1 MPa excessive oxygen pressure.

The data were processed by nonparametric Mann–Whitney U test for independent variables and Wilcoxon's test for dependent variables using Statistica 6.0 software and presented as the median and (25-75%) percentiles.

RESULTS

The weight of the experimental animals and the weight of the left ventricle (LV) were similar in all groups. No ischemic impairments of the myocardium were found in the sham-operated rats.

In the control group, the risk zone median 24 h after artery ligation was 31.72% of LV weight, *i.e.*, the blood supply was impaired in ~$^1/_3$LV that is considered as a risk zone. In animals subjected to HBO, the risk zone was less pronounced (Fig. 1).

The risk zone is myocardium areas with impaired blood supply and the tendency to its shrinkage can reflect openning of collaterals caused by partial oxygen pressure increase [1]. When the pressure ex-

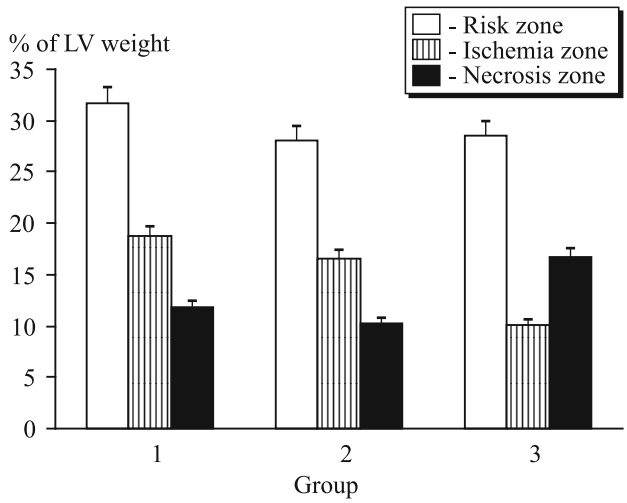

Fig. 1. Risk, ischemia, and necrosis zones.

TABLE 1. Ischemia and Necrosis Zones

Group	Ischemic zone, % of risk zone	Necrotic zone, % of risk zone	Ischemic/ necrotic zone, %
Group 1 (*n*=7)	62.22^{o} (35.26-62.91)	37.78^{o} (37.09-64.74)	1.7:1
Group 2 (*n*=7)	66.65^{o} (58.73-72.78)	33.35^{o} (27.22-41.27)	2:1
Group 3 (*n*=6)	37.17^{*+} (30.32-42.73)	62.83^{*+} (57.27-69.68)	0.6:1

Note. $p<0.05$ in comparison with *group 1, $^{+}$group 2, ^{o}group 3.

ceedes 0.05 MPa, vasoconstriction effects of hyperoxia appear.

On preparations stained with TTC, the risk zone was distinctively divided into ischemia zones (heart tissue with preserved dehydrogenases that use TTC as the substrate) and necrosis zone (heart tissue with sharply decreased concentration of dehydrogenases).

It is worthy of note, that in natural course of MI (group 1; Table 1), the ratio between the ischemic and necrotic zones was 1.7:1; this ratio is below the values reported previously, probably because of methodological differences. In HBO with 0.1 MPa excessive pressure (group 3), the size of the ischemic zone decreased and shifted towards necrosis, *i.e.* the volume of necrotic tissue significantly increased.

Application of low HBO doses (excessive oxygen pressure 0.02 MPa) showed better effect. In the risk zone of the myocardium, the volume of the ischemic tissue increased at the expense of a decrease of necrosis zone, that means the increase in the volume of ischemic, but reversibly damaged myocardium. These results confirm recently proposed concept on high effectiveness of low-dosed HBO. It was shown [8] that 40-min oxygen exposure at 0.03 MPa excessive pressure significantly decreases the parameters of oxidative stress in healthy volunteers. It is likely that infarction-limiting effects of HBO are associated with activation of antioxidant mechanisms, in particular, catalase activation, because the positive effects of HBO disappeared in presence of catalase inhibitor.

It seems logical that the increase of partial oxygen pressure in the blood flow can limit the necrosis zone. It was shown on the experimental models that cell death in different myocardium layers does not occur simultaneously: the process starts in the endocardium and lasts for at least 4-6 h involving all myocardium layers [5]. Thus, TTC staining showed that necrotic tissue areas can be distinctively visualized 6 h after acute coronary occlusion and then, the "necrotic wave" spreads over the next 48 h with final formation of the necrosis zone (according to histological data) [9].

It should be noted that the effect of HBO persists for at least 24 h after the session. Similar results were reported earlier [7]: the authors observed a decrease in the necrosis zone in the myocardium if HBO procedure was performed immediately after ischemia and then the rats were kept under standard conditions for 24 h. We suppose that the problem of HBO application in acute MI is at the stage of accumulation of experimental and clinical material. Heterogeneity of the experimental and clinical models and different variants of HBO application do not allow definite conclusions. It is believed that HBO application in acute coronary syndrome is a promising treatment option, but the reasons to recommend this method for routine clinical practice are insufficient [6].

These findings, on the one hand, attest to the effectiveness of HBO under conditions of irreversible occlusion of the coronary artery: in rats of the control group, the risk zone median was 31.7% from LV weight, while after HBO session it did not exceed 25%; but on the other hand, HBO application in low doses is more effective than in the high-dose mode. In natural MI course, the ratio between ischemia and necrosis zones was 1.7:1, after HBO at excessive oxyge pressure of 0.1 and 0.02 MPa, the corresponding values were 0.6:1 and 2:1, respectively.

Our findings suggest that low-dose HBO can be used in cardiological practice, because it promotes redistribution of ishemic and necrosis zones within the risk zone: increases the ischemic zone at the expense of a decrease in necrosis zone.

REFERENCES

1. *Handbook on Hyperbaric Medicine*, Ed. D. Mathieu, [Russian translation], Moscow (2009).
2. N. A. Zakharova, *Bull. Exp. Biol. Med.*, **46**, No. 6, 1454-1457 (1958).
3. A. N. Leonov, *Hyperoxia, Adaptation, Sanogenesis* [in Russian], Voronezh (2006).
4. S. A. Pataraya, *Vopr. Giperbarich. Med.*, **27**, No. 1, 28-30 (2006).
5. G. Asano, E. Takashi, T. Ishiwata, *et al., J. Nippon Med.*, **70**, No. 5, 384-392 (2003).
6. M. H. Bennett, J. P. Lehm, and N. Jepson, *Cochrane Database Syst. Rev.* No. 8, doi: 10.1002/14651858.CD004818 (2011)
7. L. dos Santos, A. J. Serra, E. L. Antonio, *et al*., *Clin. Exp. Pharmacol. Physiol.*, **36**, No. 5, 594-598 (2009).
8. S. Kim, T. Yukishita, K. Lee, *et al*., *Health*, **3**, No. 7, 432-436 (2011).
9. M. T. Vivaldi, R. A. Kloner, and F. J. Schoen, *Am. J. Pathol.*, **121**, No. 3, 522-530 (1985).
10. J. Ye, L. Yang, R. Sethi, *et al*., *Mol. Cell. Biochem.*, **176**, Nos. 1-2, 227-233 (1997).