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Effect of Preconditioned Hyperbaric Oxygen and Ozone on Ischemia-Reperfusion Induced Tourniquet in Skeletal Bone of Rats

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Background. **The aim of the study was to investigate effect of I/R injury on bone tissue and protective role of hyperbaric oxygen precondition (HBO-PC) and ozone precondition (O_3-PC) on I/R injury by using biochemical analysis.**

Materials and Methods. **Thirty-two rats were included in study. The animals were divided into four equal groups: sham operation, I/R, I/R+HBO and I/R+O_3. One session of 60 min, 3 ATA, 3–4 L/min, 100% oxygenation was defined as one dose of HBO. First dose of HBO was administrated 72 h before ischemia. Subsequent, one-dose of HBO administrated per 12 hours until ischemia time (total seven doses); 0.7 mg/kg ozone/oxygen mixture intraperitoneally was defined as one dose of ozone. First dose of O_3 was administered 72 h before ischemia (total four doses). I/R model was induced in anesthetized rats by unilateral (right) femoral artery clipping for 2 h followed by 22 h of reperfusion. The right tibia and were harvested. Tissue was assayed for levels of malondialdehyde (MDA) and protein carbonyl (PCO), activities of superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px).**

Results. **MDA and PCO levels were increased in I/R group. SOD activity was increased; GSH-Px activity was decreased in I/R group. MDA and PCO levels were decreased, SOD and GSH-Px activities were increased in both HBO+I/R and O_3+I/R groups.**

Conclusion. **It has been shown that levels of MDA and PCO in bone were increased followed by 2 h of ischemia and 22 h of reperfusion period. Ozone-PC and HBO-PC has protective effect against skeletal bone I/R injury by decreasing levels of MDA and PCO, increasing activities of SOD and GSH-Px in rats.**

Key Words: **HBO; ozone; preconditioning; IR Injury; bone; malondialdehyde (MDA); superoxide dismutase (SOD); glutathione peroxidase (GSH-Px).**

INTRODUCTION

During the reperfusion of ischemic tissue, oxygenated blood causes blood cell sequestration, and increases number and activities of oxidants. Reperfusion increases the hazardous effects of early ischemic injury by release of cytokines and reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), superoxide radical ($O_2^{\cdot}$), and hydrogen peroxide (H_2O_2) and by the activation of complement system. This phenomenon has been named "ischemia-reperfusion (I/R) injury" [1].

Skeletal muscle I/R may occur with vascular problems, including atherosclerotic occlusive disease, arterial thrombosis, or arterial embolism, organ transplantation, cardiovascular surgery, and vascular trauma [2]. In addition, limb ischemia-reperfusion occurs often in surgical procedures using a tourniquet to provide a bloodless surgery field [3].

Although hyperbaric oxygen (HBO) causes oxidative stress [4] similar to ischemic-preconditioning (IPC), HBO-PC generates a nonlethal level of ROS, which induces ischemic tolerance and has protective effect after severe ischemic attacks [5, 6]. Some studies have shown that HBO-PC reduces I/R damage by its antioxidant

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effect, hyperoxygenation, and vasoconstriction [7–9]. HBO is believed to exert its antioxidant effect by reducing leucocyte recruitment and activation, edema, cellular necrosis, and increasing the efficacy of antioxidant enzymes [10]. The effect of HBO-PC on IR injury was studied in brain, spinal cord, and myocardial muscle [11, 12, 13]. To the best of our knowledge, effect of HBO-PC on IR injury in musculoskeletal system has not been studied before in the English literature.

Studies on ozone (O_3) have determined its primary effect is to increase the resistance by stimulation of immune mediators [14]. It is mainly used in medicine for infected wounds, colitis, proctitis, circulatory disorders, and viral diseases. Although O_3 has potent oxidant effect, some studies has shown that it has antioxidant effect [15]. It has been shown that ozone-precondition (ozone-PC) has protective effect on I/R injury in liver and kidney [16, 17]. Chen *et al.* [16] reported that IPC and ozone-PC had a similar protective effect, however; they had no synergistic effect on IR injury in kidney.

There are many studies investigating I/R injury in different tissue, such as kidney, liver, lung, testis, brain, heart muscle, and skeletal muscle in the literature [18]. Although tourniquet is used often in orthopedic surgery, effect of tourniquet induced IR on normal bone is not clear in the literature. However, some studies showed that fracture resulted in oxidative stress by increasing MDA both animals [19] and humans [20]. In addition, one study [21] reported that IR increased bone formation, while another study [22] indicated IR did not influence fracture healing. We wondered whether IR affects the bone like other tissues or not. Therefore, we postulated that I/R produces oxidative stress in bone tissue like other organs, and HBO-PC and ozone-PC have protective effect on tourniquet-induced I/R injury measuring biochemical parameters, including MDA, PCO, SOD, GSH-Px.

MATERIAL AND METHODS

Animals

This experimental study was accepted by the Ethics Committee of our institution (GATA Ethics Committee). Thirty-two adult male Wistar Albino rats weighing between 280 and 340 g were included in study. They were kept under standard conditions. During the experiment, except under effect of anesthesia, animals were fed standard rat chow and water *ad libitum* and housed in cages with controlled temperature and 12-h light/dark cycle. The animals were divided into four equal groups: (1) sham operation, (2) I/R, (3) I/R+HBO-PC, and (4) I/R+ozone-PC.

HBO Preconditioning Model

HBO-PC was administered in a special animal hyperbaric chamber (Etimesgut Military Equipment Factory, Ankara, Turkey) was used for exposure. One dose of HBO consisted of 60 min, 3 ATA, 3–4 L/min, 100% oxygenation (one-dose HBO). HBO-PC was started 72 h before ischemia. Subsequent, one-dose of HBO administrated per 12 h until ischemia time. Total number of HBO-PC was seven (Table 1). Compression and decompression of the chamber was completed in aproximately10 min. All HBO-PC were administrated at the same hour on the morning (08.00 a.m.) and in the evening (08.00 p.m.) to adjust to biological body rhythm.

O_3 Preconditioning Model

One dose of O_3 consisted of in 0.7 mg/kg ozone/oxygen mixture intraperitoneally (i.p.). Gaseous mixture was administered to each animal approximately 2.3–3.0 mL of 60 μg/mL i.p. O_3-PC was started 72 h before ischemia. Total number doses of O_3 were four (Table 1). Ozone was produced from medical-grade oxygen (O_2) using electrical corona arc discharge, by the O_3 generator (model OZONOSAN Photonik 1014; Hansler GmbH, Iffezheim, Germany), which provides the gas flow and ozone concentration to be controlled in real time by photometric determination, as recommended by the Standardization Committee of the International Ozone Association. The flow-rate of O_3 was maintained constantly at 3 L/min during the experiments, and it represented only about 3% of the gas ($O_2 + O_3$) mixture. Tygon polymer tubing and single-use silicon-treated polypropylene syringes (ozone resistant) were utilized throughout to ensure constant O_3 concentrations.

The Ischemia-Reperfusion Model

After 2 h, the last dose of HBO and O_3 ischemia-reperfusion model was carried out. Intramuscular injection of a rodent anesthetic mixture (a dose of 85 mg/kg and 12.5 mg/kg) was used for anesthesia in all rats (ketamine and xylazine 150:30 mg/mL). Additional doses of the mixture were administrated until end of the procedure. Inguinal area was shaved and the skin was cleaned with a 10% solution of povidone-iodine (Betadine; Purdue Products, Stamford, CT). Body temperature was monitored with a rectal probe and maintained at approximately 36.5–37.5°C with a heating pad. After rat was anaesthetized, common iliac artery was found using inguinal incision. Common iliac artery was clamped, and collateral blood flow was blocked with a rubber arterial tourniquet at the proximal femur. At the end of the 2 h of ischemia, the clamp and rubber tourniquet were removed. Skin was closed by 2.0 vicryl. Mixture of ketamine and xylazine that we used had effect of anesthesia 2 h during the

TABLE 1

HBO and O3 Preconditioning

HBO O_3	HBO	HBO O_3	HBO	HBO O_3	HBO	HBO O_3				
							I	R	Scarification	
−72	−60	−48	−36	−24	−12	0	+2	+4	+22	(h)

HBO = hyperbaric oxygen; O_3 = ozone; I = ischemia; R = reperfusion.