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Evaluation and comparison of the effect of hypothermia and ozone on ischemia–reperfusion injury of skeletal muscle in rats

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ABSTRACT

Background: Tourniquet-induced ischemia–reperfusion, which affects local and distant organs, is very common in orthopedic surgery. Hypothermia is used in traumatic tissue during ischemic period commonly. Ozone (O_3) has been recommended as a novel therapeutic agent in various medical conditions. The objective of the study was to evaluate and compare the effect of hypothermia (H) and O_3 on ischemia–reperfusion injury of skeletal muscle in rats by measuring oxidative parameters and inducible nitric oxide synthase (iNOS) levels.

Materials and methods: Eighteen rats (Wistar albino) were separated into five groups randomly (sham, IR, IR + H, IR + O_3, IR + H + O_3; $n = 6$). The lower right extremity of all rats was subjected to 2 h of ischemia and 22 h of reperfusion clamping the common iliac artery and using the rubber-band technique at the level of the lesser trochanter under general anesthesia. Two hours of hypothermia were applied during the first 2 h of reperfusion in two groups. O_3 was applied in two groups. All rats were sacrificed after the IR period with high dose of anesthesia. The tibialis anterior muscle and blood were saved. Levels of superoxide dismutase, glutathione peroxidase, MDA, NOx, and interleukin-1β were measured in the muscle. Creatinine kinase, lactate dehydrogenase, aspartate aminotransferase, urea, creatinine, and electrolytes were measured in serum. Immunohistochemical iNOS staining was performed on muscle samples.

Results: The levels of MDA, NOx, and interleukin-1β in muscle were raised in the IR group compared with those in the sham group. The same parameters were lower in the groups of IR + H, IR + O_3, and IR + H + O_3 compared with those in the IR group. Superoxide dismutase and glutathione peroxidase activities in muscle were lower in the IR group compared with those in the sham group; however, same parameters were higher in the groups of IR + H, IR + O_3, and IR + H + O_3 compared with those in the IR group. Score and intensity of iNOS staining in skeletal muscle in the IR group was increased compared with that in the sham

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group and decreased in the groups of IR + H, IR + O_3, and IR + H + O_3 compared with that in the IR group. Levels of creatinine kinase, aspartate aminotransferase, and K in the three treatment groups decreased compared with those in the IR group.

Conclusions: These findings showed that hypothermia, which has more affect, and O_3 decreased the tourniquet-induced IR injury in the rat's muscle-skeletal system by reducing the levels of oxidative and nitrosative stress parameters and enhancing antioxidant enzymes. Hypothermia and O_3 had no synergistic effect. Hypothermic reperfusion and O_3 preconditioning might be beneficial in skeletal muscle IR injury-associated tourniquet.

1. Introduction

Ischemia is a restriction in blood supply to tissues, typically associated to vascular trauma, vascular disease, fracture, compartment syndrome, and tourniquet usage during extremity surgeries. Reestablishment of blood flow results in tissue injury more than during ischemic period. Reoxygenation causes release of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This phenomenon is known as ischemia–reperfusion (IR) injury [1].

ROS increase levels of malondialdehyte (MDA) indicating oxidative stress, which involves lipid peroxidation of the cellular membrane [2]. RNS cause formation of nitrite-nitrate (NOx) indicating nitrosative stress, which is measured by serum nitrit–nitrat. Antioxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), which together preserve cells from ROS and RNS. However, the antioxidant enzyme system is insufficient to deal with a rapid-onset condition like ischemic-reperfusion period [3].

Ozone (O_3) was first used in World War I in the treatment of infection. It has been shown that O_3 has a successful effect on ischemia, inflammation, and infection because of its powerful oxidant capacity. Though O_3 has strong potent oxidant impact, some studies reported that ozone preconditioning (O_3-PC) application has preserved the tissue against IR injury, which is similar to the effect of ischemic preconditioning [4,5].

Hypothermic exposure to tissue during ischemia is well established and clinically commonly used method, as hypothermia increases the critical ischemia time and replantation success, reducing inflammation and edema [6]. In addition, some studies showed that hypothermia during reperfusion period had protective effect against the IR injury, decreasing edema, acidosis, oxygen consuming, and muscle damage due to its vasoconstrictive effect [7,8].

Although some studies reported that hypothermic reperfusion decreased edema and muscle damage, it is relatively unexplored whether hypothermia during reperfusion can suppress the generation of oxygen free radicals after the IR period. Therefore, we evaluated and compared the effect of hypothermia during reperfusion and O_3 on oxidative and nitrosative stress-associated tourniquet IR injury in skeletal muscle of rats.

2. Materials and methods

The animal model of the IR study was started after approval by the Animal Ethics Committee (GATA). Thirty male rats (Wistar albino, weight range 225–330 g) were separated into five groups. These groups were sham, IR, IR + H, IR + O_3, and IR + H + O_3 ($n = 6$). Rats were kept in standard condition and fed with standard diet and water in the research laboratory at the same temperature (24°C) and 12 h of dark–light period.

2.1. Model of IR

Rodent anesthetic mixture (a dose of 85 mg/kg and 12.5 mg/kg of ketamine and xylazine 150:30 mg/mL) was administered intramuscularly for anesthetizing all rats. Anesthesia was maintained with injection of extra doses of the mixture during the surgery. The inguinal area was shaved and skin cleaned with a 10% solution of povidone-iodine (Betadine; Purdue Products, Stamford, CT). Body temperature was measured with a rectal probe continuously and kept at approximately 31.2°C–34.5°C with a surgical lamp.

We dissected and clamped the common iliac artery by introducing a 2-cm incision over the medial site of the right hind extremity. Then, a rubber tourniquet was located at the level of the trochanter lesser to prevent collateral blood flow. At the end of the ischemic period, the clamp and tourniquet were removed and skin was closed with sticks. All rats had 22 h of the reperfusion period. After a total of 24 h, the IR period was finished, and animals were sacrificed by cervical dislocation. The sham group was exposed to the same procedure, but without the IR period. The IR group was exposed to 2 h of ischemia and 22 h of reperfusion. The IR + H group had 4 h of local hypothermia added to 2 h of ischemia and 22 h of reperfusion. The IR + O_3 group had six doses of O_3 in addition to 2 h of ischemia and 22 h of reperfusion. The IR + H + O_3 had 4 h of local hypothermia and six doses of O_3 in addition to 2 h of ischemia and 22 h of reperfusion. Flow of the study detail is given in Table 1.

2.2. Model of hypothermia

We designed a water tank made of wood for our hypothermia model (Fig. 1). The upper surface of the water tank was sealed by holed glass. We put water in the tank until there was 3-mm space under the glass to prevent body temperature decrease. The right extremities of six rats in groups IR + H and IR + H + O_3 were put in a latex glove. Then animals were placed on the upper surface of the glass of the tank. The right lower extremities were hanged down from holes into the water. The water temperature of the tank was kept at 12 ± 2°C by putting ice. Room temperature was kept at 20°C during the period of hypothermia. At the end of the 4 h of local