JOURNAL OF SURGICAL RESEARCH 186 (2014) 240–245

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

# Dexmedetomidine protects against ischemia–reperfusion injury in rat skeletal muscle

*Xu Dong, MM,*[a] *Qunzhi Xing, MM,*[a,*] *Yu Li, MM,*[a] *Xuechang Han, MM,*[a] *and Lixia Sun, MM*[b]

[a] *Department of Anesthesiology, The First Affiliated Hospital, Henan Science and Technology University, Luoyang, Henan, China*
[b] *Department of Clinical Laboratory, The First Affiliated Hospital, Henan Science and Technology University, Luoyang, Henan, China*

ARTICLE INFO

*Article history:*
Received 24 May 2013
Received in revised form
14 July 2013
Accepted 26 July 2013
Available online 19 August 2013

*Keywords:*
Ischemia–reperfusion injury
Skeletal muscle
Dexmedetomidine
Reactive oxygen species

ABSTRACT

*Background:* Dexmedetomidine (DEX) has been shown to decrease ischemia–reperfusion (I/R) injury in kidney and brain tissues. In this study, the effects of DEX were evaluated in skeletal muscle during I/R injury.

*Materials and methods:* Animals were divided into four groups: sham-operated (sham group), saline + I/R, DEX + I/R, and α-tocopherol + I/R groups. Hind limb ischemia was induced by clamping the common femoral artery and vein. After 4 h of ischemia, the clamp was removed and the animals underwent 2 h of reperfusion. Animals in the drug treatment group received DEX or α-tocopherol by intraperitoneal injection 1 h before reperfusion. We measured plasma concentrations of interleukin 1β and tumor necrosis factor α levels using an enzyme-linked immunosorbent assay. The right gastrocnemius muscle was harvested and immediately stored at −80°C for the assessment of superoxide dismutase (SOD) and catalase (CAT) activities as well as glutathione (GSH), malondialdehyde (MDA), and protein oxidation (PO) levels. DEX (25 μg/kg) and normal saline (10 mL/kg) were administered by intraperitoneal injection 1 h before reperfusion.

*Results:* Plasma tumor necrosis factor α or interleukin 1β levels increased significantly in the I/R group ($P < 0.01$ compared with sham group) and decreased significantly in the DEX group ($P < 0.01$ compared with I/R group). Muscle tissues of the I/R group had significantly decreased SOD, GSH, and CAT activities and increased levels of MDA and PO content compared with the sham group. The activity of antioxidant enzymes in the DEX + I/R group was greatly elevated compared with that in the I/R group (SOD, 1.068 ± 0.120 *versus* 0.576 ± 0.072 U/mg protein; GSH, 2.436 ± 0.144 *versus* 1.128 ± 0.132 μmol/g; and CAT, 69.240 ± 6.456 *versus* 31.884 ± 6.312 U/mg protein; $P < 0.01$), whereas the levels of MDA and PO content were clearly reduced (23.268 ± 3.708 *versus* 53.604 ± 5.972 nmol/g protein and 1.908 ± 0.192 *versus* 5.208 ± 0.612 nmol/mg protein, respectively; $P < 0.01$). Moreover, DEX exhibited more potent antioxidant activity than vitamin E in the skeletal muscle I/R.

*Conclusions:* We found that DEX exhibits protective effects against skeletal muscle I/R injury. These results underscore the necessity of human studies with DEX to determine if it is beneficial for preventing skeletal muscle I/R injury.



* *Corresponding author*. Department of Clinical Laboratory, The First Affiliated Hospital, Henan Science and Technology University, No. 24 Jinghua Road, Luoyang, Henan 471003, China. Tel./fax: +86 379 64830680.
E-mail address: qunzhixing666@outlook.com (Q. Xing).
0022-4804/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.07.052

## 1. Introduction

Ischemia–reperfusion (I/R) injury in skeletal muscle is inevitable in many vascular and musculoskeletal traumas, diseases, and free tissue transfers and during time-consuming reconstructive and transplantation surgeries [1]. Ischemia often leads to energy depletion, accumulation of toxic metabolic products, increase in tissue acidity, activation of phospholipase and lysozymes, and cell damage [2]. Reperfusion can then worsen the injury by inducing cellular infiltration and generating reactive oxygen species (ROS), which leads to further cell damage [3–6]. Skeletal muscle I/R injury often results in the loss of contractile function and in severe cases can lead to disability, limb amputation, and even death.

Dexmedetomidine (DEX) is a highly selective and potent α2-adrenoreceptor agonist, which was approved by the Food and Drug Administration in 1999 for patients during the first 24 h of mechanical ventilation in the intensive care unit [7]. A number of studies have demonstrated that DEX has a protective effect against I/R injury in several organs, including heart [8], brain [9], and kidney [10], which is thought to be because of the antioxidant and anti-inflammatory properties of the compound [11]. However, whether DEX influences I/R injury of skeletal muscle remains unclear. Therefore, based on the characteristics of DEX, we hypothesized that it may have antioxidant and anti-inflammatory effects against I/R injury in rat skeletal muscle.

In this study, we evaluated the antioxidant effects of DEX against skeletal muscle I/R injury in a rat model. We analyzed several antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and protein oxidation (PO) levels in muscle. We also assessed plasma levels of various inflammatory mediators including tumor necrosis factor α (TNF-α) and interleukin β (IL-1β).

## 2. Materials and methods

### 2.1. Animals

The experimental protocol used in this study was approved by the Animal Ethics Review Committee of the Faculty of the First Affiliated Hospital, Henan Science and Technology University, and adhered to National Institutes of Health guidelines for the use of experimental animals. Forty adult Wistar rats weighing between 200 and 250 g were included in this experimental study. Animals were housed in individual cages in a temperature-controlled room with alternating 12-h light–dark cycles and acclimatized for a week before the study. Food was removed 8 h before the study, but all animals were allowed free access to water and rat chow diet at all other times.

### 2.2. Experimental design

The animals were randomly divided into four groups of 10 animals each [12]. Group 1 (sham group) rats were sham operated by exposing the femoral artery and then closing the incision without inducing I/R. Group 2 (saline + I/R group) rats were subjected to 4 h of femoral pedicles occlusion followed by 2 h of reperfusion. Animals with I/R injury received saline (0.2 mL) with intraperitoneal (i.p.) injection 1 h before the reperfusion. Group 3 (DEX + I/R group) rats were subjected to 4 h of femoral pedicles occlusion followed by 2 h of reperfusion. Animals with I/R injury received DEX (25 μg/kg) with i.p. injection 1 h before the reperfusion. Group 4 (α-tocopherol + I/R group) rats were subjected to 4 h of femoral pedicles occlusion followed by 2 h of reperfusion. Animals with I/R injury received α-tocopherol (10 mg/kg; Sigma, St. Louis, MO) with i.p. injection 1 h before the reperfusion. The dose of DEX used was based on previous studies [13,14].

### 2.3. Induction of I/R

All surgical procedures were performed after the rats had been sufficiently anaesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg; Sigma), which was determined by assessing the eye touch response. The lower abdomen and groin were shaved and sterilized with povidone iodine solution and then the animals were placed in a supine position. A heat lamp was used to maintain the body temperature at 37 ± 0.5°C during the experiment. The right groin vessels were exposed through a transverse groin incision, and the right femoral artery was isolated by clamping with an atraumatic microvascular clamp. After 4 h of ischemia, the microvascular clamp was removed and the lower extremity was inspected for restoration of blood flow.

### 2.4. Collection of blood and tissue samples

At the end of the reperfusion period, blood was drawn from the inferior vena cava using heparinized syringes. Blood samples were collected into polyethylene tubes and centrifuged (2500*g* for 25 min at 4°C) to separate the supernatant for determination of cytokine concentrations. The right gastrocnemius muscle was also harvested and immediately stored at −80°C for biochemical analysis.

### 2.5. Biochemical assays

At the end of each experimental procedure, the extracted gastrocnemius muscle was washed three times in cold isotonic saline. The tissue was then homogenized with three volumes of ice-cold Tris–HCl buffer (pH 7.4). GSH measurements were taken using a modification of the Boyne and Ellman procedure [15]. Briefly, after centrifugation at 2000*g* for 10 min, 0.5 mL of supernatant was added to 2 mL of 0.3 mol/$H_2PO_4$–$H_2O_2$ solution. A 0.2 mL solution of dithiobisnitrobenzoate (0.4 mg/mL in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of $1.36 \times 10^5$/M/cm. The results were expressed in nanomoles per 100 milligram of protein in tissue samples.

The total SOD activity was measured according to the method previously described by Fridovich [16]. The principle of the method is based on the inhibition of nitroblue tetrazolium reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the