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Annals of Biological Research, 2012, 3 (8):4099-4102
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Effects of N-acetylcysteine upon the rat serum enzyme changes in skeletal muscle ischemia reperfusion

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ABSTRACT

Skeletal muscle ischemia reperfusion injuries can occur with diseases, trauma and during surgical procedures. Ischemia reperfusion injury is characterized by the production of oxygen free radicals leading to disturbances in vasomotility and microvascular permeability. The objective was to evaluate effects of N-acetylcysteine as a scavenger of radical oxygen species on the serum enzyme changes secondary to muscle ischemia reperfusion. Twenty wistar male rats were divided randomly into two experimental groups: group ischemia-reperfusion (group I) and group ischemia-reperfusion + N-acetylcysteine (group II). After ketamine (50 mgkg^{-1}) and xylazine (10 mgkg^{-1}) anesthesia, femoral artery was exposed and undergone 2h of ischemia, 24h of reperfusion. Rats that were treated with N-acetylcysteine given intravenously at a dose of 150 mgkg-^{1}, immediately before reperfusion. After 24h of reperfusion, the blood samples were collected and submitted for evaluation of serum aspirate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), lactate dehrdrogenase (LDH) values to identify the ischemic damage occurring in the skeletal muscle tissue. Enzymatic parameters (CPK, AST, ALT and LDH) measured for demonstrating ischemia induced muscle injuries were lower in the group receiving N-acetylcysteine. In this study, N-acetylcysteine as an antioxidant substance was demonstrated to have protective effects on acute ischemia and reperfusion injury of the skeletal muscle in lower extremities.

Key words N-acetylcysteine. Skeletal Muscle. Ischemia reperfusion. Serum enzyme changes. Rat.

INTRODUCTION

Limb ischemia reperfusion injury is one of the most common types of injuries that occur in a variety of conditions such as trauma, disease and surgery. The local and remote consequences of limb ischemia and reperfusion injury continue to be a serious clinical problem for general vascular surgeons. A variety of animal models (canine, rabbit, rat) have been used to mimic the clinical situation of acute limb ischemia followed by reperfusion and test potential therapeutic interventions [1- 9]. These animal models have provided strong evidence that an inflammatory response mediated by neutrophils, cytokines and reactive oxygen species play a significant role in the pathogenesis of acute ischemia reperfusion injury.

Several antioxidants like superoxide dismutase, catalase, mannitol, dimethyl sulfoxide, iloprost and zinc aspartate have proven to be efficient in attenuating the changes in skeletal muscle ischemia reperfusion injury [10, 11, 12].

N-acetylcysteine is particularly suitable for the attenuation of oxidant-mediated injury as it not only has a direct antioxidant effect by means of free radical scavenging properties but also replenishes depleted cellular glutathione stores restoring intrinsic cellular antioxidant defenses [13, 14, 15, 16]. The widely accepted use of N-acetylcysteine in current clinical practice confers an additional advantage for its potential use in the context of compartment syndrome, ischemia reperfusion injury or trauma, as the toxicity profile of the drug is well established and clinicians already are experienced in its use.

This experimental study was performed in rats subjected to temporary clamping of the femoral artery, with the purpose of evaluating effects of N-acetylcysteine on the serum enzyme changes secondary to muscle ischemia reperfusion.

MATERIALS AND METHODS

All rats of the present research were cared according to the norms of the Islamic Azad University Faculty of Specialized Veterinary Sciences Tehran Iran laboratory of animal experimentations; this investigation was approved by the Committee of Ethics in Research with animals in Islamic Azad University.

Twenty Wistar male rats weighing 250–300 g were used in this study. All rats were kept at a constant room temperature under standard conditions with food and water ad libitum in individual plastic cages with soft bedding. Animals were divided randomly into two experimental groups of ten rats each: group ischemia-reperfusion (group I) and group ischemia-reperfusion + N-acetylcysteine (group II).

Anesthesia was induced using intramuscular ketamine (50 $mgkg^{-1}$) plus xylazine (10 $mgkg^{-1}$). After induction of anesthesia, the left hind limb was completely clipped. After clipping, disinfecting and dropping (using a sterile technique), a skin incision was made on medial surface of the left hind limb. After isolated the femoral artery and vein from the surrounding structures, femoral artery was exposed and clamped with a mini bulldog forceps. Before clamped the femoral artery, 250 IU heparin was administered via the jugular vein to prevent clotting. All animals were undergone 2h of ischemia by occlusion femoral artery with a vascular clamp and 24h of reperfusion. Rats were maintained in a dorsal recumbency and kept anesthetized throughout the duration of the ischemic period. Additional doses were given as necessary to maintain anesthesia during the experiment. Body temperature was maintained with a heating pad under anesthesia. In group II N-acetylcysteine (150 $mgkg^{-1}$) was injected intravenous immediately before reperfusion. Following the ischemic period, the vascular forceps was removed and then surgical site was routinely closed. After surgery, fluid losses were replaced by intraperitoneal administration of 5ml of warm (37˚C) isotonic saline, and rats were returned to their cages with food and water ad libitum during the reperfusion period. The analgesic nalbuphine hydrochloride (2 $mgkg^{-1}$) was used via subcutaneous during observation time.

After 24h of reperfusion, the blood samples were collected from jugular vein and submitted for evaluation of serum aspirate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), lactate dehrdrogenase (LDH) values to identify the ischemic damage occurring in the skeletal muscle tissue.

Statistical analyses were carried out using SPSS statistical software (version 12.0). Results were expressed as the mean +/- standard deviation. The Mann–Whitney *U* Test. and Student's *t* test were employed to analyze two groups consecutively. *P* value < 0.05 was accepted as being statistically significant.

RESULTS

All of rats tolerated operation and survived until the final study period. Data belonging to AST, ALT, CPK and LDH measurements from blood samples after reperfusion are shown in Table 1.

AST measurements of Group II were significantly lower than the measurements in Group I. ALT levels were significantly higher in the group I when compared with the group II. CPK levels of Group II were significantly lower than those of Group I. LDH levels obtained from Group II were significantly lower than levels in Group I.

TABLE 1- Serum aspirate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), lactate dehrdrogenase (LDH) concentrations (units/Liter).

Group	N	AST Mean ± SD*	ALT Mean ± SD*	CPK Mean ± SD*	LDH Mean ± SD*
I	10	354.9±11.9	98±3.3	1415±425	1539±230
II	10	189.5±5.8	52±2.55	811±248	412±80

* *There is significant difference (P<0.05) between two groups.*

DISCUSSION

It is well recognized that ischemia followed by reperfusion in skeletal muscle represents an important clinical problem in many vascular diseases, musculoskeletal trauma and orthopedic surgery. The use of a tourniquet to achieve ischemia is a common practice in orthopedic surgery and bleeding of upper and lower limbs. When orthopedic surgeons administer a tourniquet allowing for surgery under bloodless conditions, the respective muscles suffer from ischemia and following reperfusion [17]. This harmful situation has been studied with regard to functional [18] and metabolic [19, 20] alterations in muscle or focusing on the vascular bed [21, 22]. These studies demonstrated that ischemia reperfusion injury is characterized by the production of oxygen free radicals leading to disturbances in vasomotility and microvascular permeability.

Various antioxidant therapies reportedly prevent distant organ and local skeletal muscle injury after ischemia reperfusion injury [23, 24].

N-acetylcysteine is one such antioxidant [25, 26]. In clinical practice, N-acetylcysteine is used primarily to reduce hepatocyte injury after acetaminophen overdose [27]. N-acetylcysteine also has been used as nephroprotective prophylaxis before administration of radiographic intravenous contrast in patients with renal impairment [28]. It protects lung epithelial cells against oxidant injury mediated by activated neutrophils in vitro and against pulmonary oxygen toxicity in vivo [29]. N-acetylcysteine also reduces oxidative burst activity but enhances phagocytosis in rodent and human neutrophils [30]. N-acetylcysteine is not simply an antioxidant drug. It acts as a glutathione precursor, as a chemical reductant of oxidized thiols, as a scavenger of radical oxygen species, as a vasodilator and also improves microcirculation by restoring the decreased activity of endothelium-derived relaxing factor and may have antiaggregan features [31].

We evaluated the effects of N-acetylcysteine on skeletal muscle injury caused by ischemia reperfusion injury to the lower extremities. Ischemia caused by nontraumatic vascular clamping was maintained for 2h, followed by 24h of reperfusion. At the end of the reperfusion period, skeletal muscle injury was assessed by enzymatic parameters.

It was also found that N-acetylcysteine administrations were associated with lower plasma LDH and CPK levels compared to the ischemia reperfusion group. This difference demonstrates a massive increase in muscle tissue injury occurred in the ischemia reperfusion group.

Both AST and ALT, which have typically been used as markers of liver pathology [32], are also found in muscle and thus increases in their levels partly attributed to levels in muscle. The higher increases in these enzymes largely may be reflecting liver injury as a remote organ injury. Sagara *et al* showed that liver function deteriorated due to limb ischemia reperfusion and that the extent of deterioration corresponded to the ischemic period [33].

This study was designed to determine the potential of N-acetylcysteine to attenuate the reperfusion injury to skeletal muscle after ischemia reperfusion by assessed enzymatic parameters.

The biochemical parameters used were the serum dosing of aspirate aminotransferase, alanine aminotransferase, creatine phosphokinase, lactate dehrdrogenase, where it is possible to observe the level of injury. Enzymes dictating ischemia induced muscle injury were lower in rats receiving N-acetylcysteine, which can be explained by the protective features of the antioxidant effects of N-acetylcysteine molecule.

CONCLUSION

This study confirmed that temporary occlusion of the femoral artery in rats leaded to serum enzyme changes in group I. N-acetylcysteine as an antioxidant substance was demonstrated to have protective effects on acute ischemia and reperfusion injury of the skeletal muscle in lower extremities.

REFERENCES

[1] F Blaisdell, *Cardiovasc Surg*, **2002**, 10, 620.
[2] J Blebea, RA Cambria, D DeFouw, RN Feinberg, RW Hobson, WN Duran, *J Vasc Surg*, **1990**, 12(6), 657.
[3] M Belkin, JG Wright, RW Hobson, *J Vasc Surg*, **1990**, 11(1), 77.
[4] M Belkin, CR Valeri, RW Hobson, *J Vasc Surg*, **1989**, 9(1), 161.
[5] J Freischlag, D Hanna, *J Surg Res*, **1991**, 50, 648.
[6] JM Klausner, H Anner, I Paterson, L Kobzik, CR Valeri, D Shepro, H Hechtman, *Ann Surg*, **1988**, 208, 761.
[7] WC Sternbergh, B Adelman, J Vasc Surg, **1992**, 16, 30.
[8] WC Sternbergh, TM Tuttle, RG Makhoul, HD Bear, M Sobel, AA Fowler, *J Vasc Surg*, **1994**, 20(3), 474.
[9] A Sotoudeh, M Ashrafzadeh Takhtfooladi, A Jahanshahi, AH Khiabanian Asl, H Ashrafzadeh Takhtfooladi, M Khansari, *Acta Cir Bras*. **2012**, 27(2), 168-171.
[10] RJ Karthuis, DN Granger, MI Townsley, AE Taylor, *Circ Res*, **1985**, 57, 599.
[11] J Blebea, RA Cambria, D Defouw, RN Feinberg, RW Hobson, WN Duran, *J Vasc Surg*, **1990**, 12, 657.
[12] E Atahan, Y Ergun, E Belge Kurutas, E Cetinus, U Guney Ergun, *J Surg Res*, **2007**, 137, 109- 116.
[13] GR Bernard, Am J Med, **1991**, 91, 54S–59S.
[14] CJ Davreux, I Soric, AB Nathens, RW Watson, ID McGilvray, ZE Suntres, PN Shek, OD Rotstein, *Shock*, **1997**, 8, 432–438.
[15] MJ McKenna, I Medved, CA Goodman, MJ Brown, AR Bjorksten, KT Murphy, AC Petersen, S Sostaric, X Gong, *J Physiol*, **2006**, 576, 279–288.
[16] K Sjo¨din, E Nilsson, A Hallberg, A Tunek, *Biochem Pharmacol*, **1989**, 38, 3981–3985.
[17] M Hayashi, H Hirose, E Sasaki, S Senga, S Murakawa, Y Mori, Y Furusawa, M Banodo, *J Surg Res*, **1998**, 80, 266-271.
[18] IB Racz, G Illyes, L Sarkadi, J Hamar, *Eur Surg Res*, **1997**, 29, 254-263.
[19] CA da Cruz, CA Massuda, J Cherri, CE Piccinato, *J Cardiovasc Surg*, **1997**, 38, 473-477.
[20] BB Rubin, G Chang, S Liauw, A Young, A Romaschin, PM Walker, *Am J Physiol*, **1992**, 263, H1695-H1702.
[21] L Birk-Sorensen, J Fuglsang, HB Sorensen, CL Kerrigan, LC Petersen, HB Ravn, VE Hjortdal, *Blood Coagul Fibrinolysis*, **1999**, 10, 157-165.
[22] WN Duran, H Takenaka , RW Hobson, *Semin Vasc Surg*, **1998**, 11, 203- 214.
[23] R McLaughlin, D Bowler, CJ Kelly, E Kay, D Bouchier-Hayes, *Eur J Surg*, **2000**, 166, 375–379.
[24] BA Perler, AG Tohmeh, GB Bulkley, *Surgery*, **1990**, 108, 40–47.
[25] C Koksal, *J Surg Res*, **2003**, 111, 236- 239.
[26] F Saricaoglu, D Dal, AE Salman, OA Atay, MN Doral, MA Salman, K Kilinc, U Aypar, *Acta Anaesthesiol Scand*, **2005**, 49, 847- 851.
[27] RJ Flanagan, TJ Meredith, *Am J Med*, **1991**, 91, 131S- 139S.
[28] M Tepel, M van der Giet, C Schwarzfeld, U Laufer, D Liermann, W Zidek. *N Engl J Med*, **2000**, 343, 180- 184.
[29] O Ortolani, A Conti, AR De Gaudio, E Moraldi, Q Cantini, G Novelli, *Am J Respir Crit Care Med*, **2000**, 161, 1907- 1911.
[30] AR Heller, G Groth, SC Heller, R Breitkreutz, T Nebe, M Quintel, T Koch, *Crit Care Med*, **2001**, 29, 272- 276.
[31] JM Tredger, *Transplantation*, **2000**, 69, 703- 704.
[32] J Holoman, J Glasa, S Galbavy, D Danis, A Molnarova, J Kazar, *Bratisl Lek Listy*, **2002**, 103, 70- 75.
[33]. G Sagar, O Ogikubo, H Takeyama, T Tada, N Suzuki, H Okamoto, T Otsuka, *Nagoya Med J*, **2009**, 50, 83-91.