

**THE EFFECT OF N-ACETYLCYSTEINE (NAC) ON TISSUE
MALONDIALDEHYDE (MDA) LEVEL AND TISSUE EDEMA IN ISCHEMIA
REPERFUSION INJURY OF RAT (*RATTUS NORVEGICUS*) SKELETAL
MUSCLE**

(A laboratory experimental study)

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ABSTRACT

Background: Generation of free radical is one of central mechanisms in ischemia reperfusion injury (IRI) . It exerts deleterious effect on cell membrane through lipid peroxidation. *N-acetylcysteine* (NAC), an antioxidant, that may improve microcirculation and attenuate neutrophil activation.

Objectives : The aim of this study was to scrutinize any possible protective effect of NAC in ischemia reperfusion injury of skeletal muscle by measuring tissue malondialdehyde (MDA) and tissue edema, and to explore whether the effect was dose dependant or not. Pentoxifylline was also tested for comparison

Methods : Acute hind limb ischemia in rats was induced for 4 h through vascular ligation and tourniquet application and followed by 1 h reperfusion. Gastrocnemius muscle samples were obtained at the end of reperfusion. Muscle injury was evaluated in 5 experimental groups: 1) control (saline), 2) Pentoxifylline 20 mg/kg, 3) NAC 200 mg/kg, 4) NAC 400 mg/kg, 5) 800 mg/kg.

Results : NAC groups had significantly lower muscle tissue MDA level as compared to the control group ($p < 0,05$). There were no significant differences of muscle tissue MDA level among NAC 200, 400, 800 groups, and also between NAC and Pentoxifylline group ($p > 0,05$). There were no significant differences of tissue water content among experimental groups ($p > 0,05$).

Conclusion : NAC was found to attenuate lipid peroxidation associated membrane injury in this IRI model of limb ischemia. NAC 200 mg/kg was the recommended dose. It was needed further investigation with longer follow up period and other parameters of IRI to study the protective effects of NAC.

Key words : Ischemia reperfusion injury, N-Acetylcysteine, lipid peroxidation, MDA, tissue edema

INTRODUCTION

The term ischemia-reperfusion injury (IRI) describes the experimentally and clinically prevalent finding that tissue ischemia with inadequate oxygen supply followed by successful reperfusion initiates a wide and complex array of inflammatory responses that may both aggravate local injury as well as induce impairment of remote organ function. IRI has been very important in orthopedic practice since it may compromise the clinical outcome of patients undergoing replantation, release of compartment syndrome, *free muscular flap*, *free myocutaneous flap*, or other revascularization procedures, even in technically successful operations. In revascularization after limb ischemia, skeletal muscle is particularly susceptible to the deleterious effects of IRI.

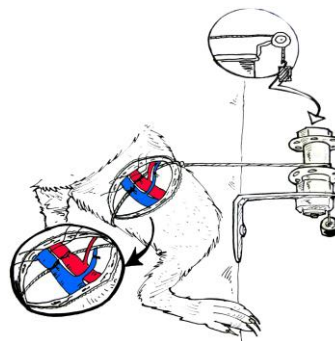
Reperfusion of ischemic tissue results in the formation of toxic *reactive oxygen species* (ROS). These compound directly damage cellular membrane by lipid peroxidation, which may produce loss of membrane fluidity, breakdown of membrane secretory functions and transmembrane ionic gradients. ROS stimulate leukocyte activation and chemotaxis. These events may induce microvascular barrier dysfunction, that along with membrane injury may result tissue edema. N-acetylcysteine (NAC), an antioxidant that is also proved to improve microcirculation and attenuate neutrophil activation.

MATERIALS AND METHODS

The objective of this study was to scrutinize any possible protective effect of NAC in ischemia reperfusion injury of skeletal muscle by measuring tissue malondialdehyde (MDA) and tissue oedema, and to explore whether the effect was dose

dependant or not. Pentoxifylline was also tested for comparison.

There were 5 experimental groups. Each group consists of 6 rats. Hind limb ischemia for 4 h, followed by 1 h reperfusion, was induced in all experimental groups.(Picture 1) The control group was rats received saline solution. Pentoxifylline group was rats received Pentoxifylline 20 mg/kg. Three NAC groups include rats received NAC 200 mg/kg, NAC 400 mg/kg and NAC 800 mg/kg. The drugs were given intravenously right before reperfusion. Gastrocnemius muscle samples were obtained at the end of reperfusion. Muscle tissue MDA was examined by thiobarbituric acid reagent. Tissue edema was assessed by measurement of tissue water content.



Picture 1. Femoral vessel ligation and tourniquet application

RESULT

The results showed that NAC groups had significantly lower muscle tissue MDA level as compared to the control group ($p < 0.05$). There were no significant differences of muscle tissue MDA level among NAC 200, 400, 800 groups, and also between NAC and Pentoxifylline group ($p > 0.05$). (Table 1) There were no significant differences of tissue water content among experimental groups ($p > 0.05$). (Table 2)

Table 1. Average of tissue MDA level of each group

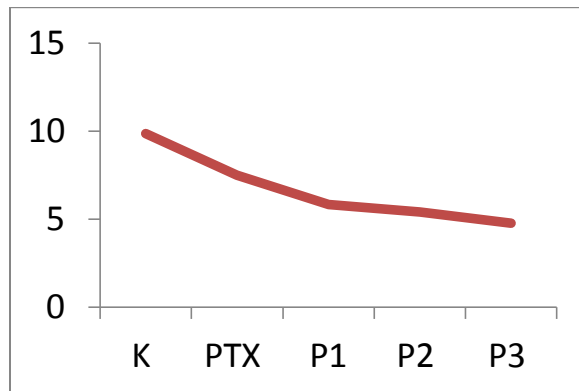
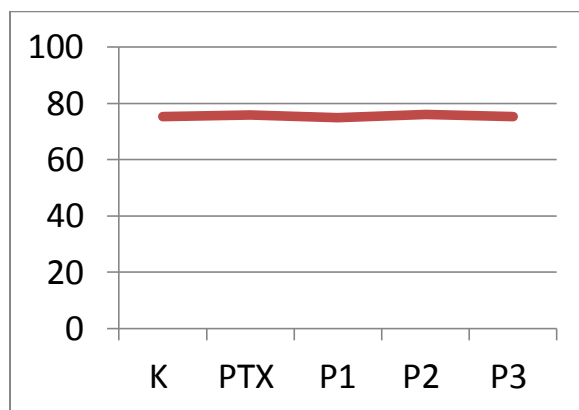


Table 2. Average of tissue water content of each group



DISCUSSION

There are some limitations in this study. Observation time to determine the effect of NAC was not long enough, when MDA and tissue edema examination was done only 1 hour after reperfusion. NAC administration was just single dose. It is required to be given several times or followed by slow infusion to determine further effect of NAC. Examination of tissue edema is done through the calculation of water content, by comparing the weight difference between wet and dry to wet weight. Drying tissue in this study carried out by the method of

freeze drying. Although this method has proven reliable, most researchers use method of heating for tissue drying. Examination of lipid peroxidation carried out using a reagent thiobarbituric acid (TBA). Although this method is accepted as marker of lipid peroxidation of many researchers, this method is considered non-specific. Examination should be done with other more stable markers such as F2 isoprostane and its metabolites which are peroxidation products of arachidonic acid.

It need a morphological evaluation by histology examination. to determine tissue damage and inflammatory cells in damaged muscle tissue due to direct ischemia reperfusion injury, to prove the protective effects of NAC. Further research are needed to examine the effect of NAC against systemic inflammation induced ischemia reperfusion injury of skeletal muscle that can lead to MODS.

CONCLUSION

It was concluded that NAC attenuated lipid peroxidation associated membrane injury in skeletal muscle ischemia reperfusion injury. This study did not demonstrate any dose dependency of the effect. Therefore, the recommended dose was 200 mg/kg bw. NAC and Pentoxifyllin, 1 hour after reperfusion, were not found to reduce tissue edema in this IRI model of skeletal muscle. This result did not exclude any possible effect of NAC in reducing IRI associated tissue edema since a short follow up period occupied in this study. Further investigations were needed to conclude the protective role of NAC in IRI with longer follow up period and other parameters measurement which indicate IRI.

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