Pengaruh N-Acetylcysteine terhadap Kadar Malondialdehyde Jaringan dan Edema Jaringan pada Perlukaan Iskemi-Reperfusi Otot Rangka Tikus Putih (Rattus norvegicus)

Didyn Nuzul Ariefin, Erwin Ramawan

Department of Orthopaedic and Traumatology Faculty of Medicine Airlangga University / Dr. Soetomo General Hospital

ABSTRAK

Pendahuluan. Pelepasan radikal bebas merupakan salah satu mekanisme sentral perlukaan iskemi reperfusi. Radikal bebas dapat merusak membran melalui reaksi peroksidasi lipid. N-acetylcysteine adalah antioksidan, yang juga terbukti memperbaiki mikrosirkulasi dan menurunkan aktivasi neutrofil. Penelitian ini bertujuan untuk mengetahui efek protektif dan titrasi dosis N-acetylcysteine pada perlukaan iskemi reperfusi otot rangka dengan memeriksa malondialdehyde dan edema jaringan.

Bahan dan cara kerja. Desain penelitian ini adalah uji eksperimental dengan menggunakan 30 ekor tikus wistar. Iskemia kaki belakang pada tikus diinduksi selama 4 jam dengan ligasi vaskular dan pemasangan torniket kemudian diikuti reperfusi selama 1 jam. Sampel otot gastroknemius diambil pada akhir reperfusi. Kerusakan otot dievaluasi pada 5 kelompok: salin sebagai kelompok kontrol, pentoxifyyline 20mg/kgBB, N-acetylcysteine 200 mg/kgBB, N-acetylcysteine 400 mg/kgBB, dan N-acetylcysteine 800 mg/kgBB.

Hasil. Kelompok N-acetylcysteine menunjukkan kadar malondialdehyde jaringan otot yang lebih rendah secara bermakna dibandingkan kontrol. Tidak ada perbedaan bermakna kadar malondialdehyde jaringan otot antara kelompok N-acetylcysteine 200, 400 dan 800 mg/kgBB, serta antara kelompok N-acetylcysteine dengan pentoxifylline. Tidak ada perbedaan bermakna kandungan air jaringan antar kelompok eksperimen

Simpulan. N-acetylcysteine terbukti dapat menurunkan kerusakan membran yang terkait peroksidasi lipid pada model perlukaan iskemi reperfusi dari iskemi ekstremitas. N-acetylcysteine 200 mg/kgBB merupakan dosis yang direkomendasikan. Diperlukan penelitian lebih lanjut dengan waktu observasi yang lebih panjang serta parameter lain perlukaan iskemi reperfusi untuk mengetahui efek protektif dari N-acetylcysteine.

Kata kunci: perlukaan iskemi reperfusi, N-acetylcysteine, peroksidasi lipid, malondialdehyde jaringan, edema jaringan

Corresponding author:
Didyn Nuzul Ariefin, MD
Jl. Kenari 23 Rewwin Waru Sidoarjo 61256
Phone: 08121750700
Email: indrapura@gmail.com

The Effect of N-Acetylcysteine on Tissue Malondialdehyde Level and Tissue Edema in Ischemia Reperfusion Injury of Rat (Rattus norvegicus) Skeletal Muscle

ABSTRACT

Introductions. Generation of free radical is one of central mechanisms in ischemia reperfusion injury. It exerts deleterious effect on cell membrane through lipid peroxidation. N-acetylcysteine, an antioxidant, may improve microcirculation and attenuate neutrophil activation. The aim of this study was to scrutinize any possible protective effect of N-acetylcysteine in ischemia reperfusion injury of skeletal muscle by measuring tissue malondialdehyde and tissue edema, and to explore whether the effect was dose dependent or not.

Materials and methods. Thirty Wistar rats were employed in this study. Acute hind limb ischemia was induced for 4 h through vascular ligation and tourniquet application and followed by 1 hour reperfusion. Gastrocnemius muscle samples were obtained at the end of reperfusion. The results of N-acetylcysteine's protective effect toward malondialdehyde was also compared to pentoxifylline. Muscle injury was evaluated in 5 experimental groups: control (saline), pentoxifylline 20 mg/kgBW, N-acetylcysteine 200 mg/kgBW, N-acetylcysteine 400 mg/kgBW, and N-acetylcysteine 800 mg/kgBW.

Results. N-acetylcysteine groups had significantly lower muscle tissue malondialdehyde level as compared to the control group. There were no significant differences of muscle tissue malondialdehyde level among N-acetylcysteine 200, 400, 800 groups, and also between N-acetylcysteine and pentoxifylline group. There were no significant differences of tissue water content among experimental groups.

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

Key words : ischemia reperfusion injury, N-acetylcysteine, lipid peroxidation, malondialdehyde, 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.[1-3]

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 trans-membrane ionic gradients. ROS stimulate leukocyte activation and chemotaxis. These events may induce microvascular barrier dysfunction, that along with membrane injury may result tissue edema.[1,4,5] One of therapeutic strategies in IRI is antioxidant administration.[4] N-acetylcysteine (NAC) is an antioxidant that also serves as glutathione precursor. Glutathione, an important antioxidant defense, may scavenge hydroxyl radical generated during reperfusion. N-acetylcysteine is also proved to improve microcirculation and attenuate neutrophil activation.[6,7] Its beneficial effect in IRI has been demonstrated in brain, liver, kidney and cardiac

muscle.[8-12]

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 edema, and to explore whether the effect was dose dependent or not. Pentoxifylline was also tested for comparison, since it is widely used in revascularization procedures in Dr Soetomo hospital.

Materials and methods

Thirty adult male Wistar rats (weight 200-250 g) were divided into 5 experimental groups. Hind limb ischemia for 4 hour, followed by 1 hour reperfusion, was induced in all experimental groups. The rats were anesthetized by ketamine 5 mg/kg intramuscularly. Ischemia in left hind limb was attained by ligation of femoral vessel by nylon 6.0, continued by tourniquet application to prevent collateral circulation. The control group received saline solution (C group). Pentoxifylline group received pentoxifylline 20 mg/kg (PTX group). Three NAC groups received NAC 200 mg/kgBW, NAC 400 mg/kgBW and NAC 800 mg/kgBW respectively (P1, P2, P3 group). The drugs were given intravenously through the tail vein 15 minutes before reperfusion. After 4 hours ischemia, the ligation and tourniquet was released and reperfusion was ensured by patency test. Gastrocnemius muscle samples were obtained after 1 hour reperfusion period. Muscle tissue MDA was assayed in the form of thiobarbituric acid reacting substances (TBARS). Tissue edema was assessed by measurement of tissue water content using freeze drying method. This is a simple and reliable method to determine the increase in tissue fluid.[13] Experiments was conducted in biochemistry laboratory of FK UNAIR and Tissue & Biomaterial unit of Soetomo Hospital.

Results

The tissue MDA level in the control group (C) was 9.87±2.00 µmol/L. The tissue MDA level was 5.84±2.77 µmol/L in P1, 5.40±1.89 µmol/L in P2, and 4.76±2.54 µmol/L in P3. The tissue MDA level in pentoxifylline group was 7.50±2.50 µmol/L.

The results of analysis of variance showed that there were significant differences in tissue MDA levels between experimental groups p=0,007. The results of multiple comparisons through Least significant difference (LSD) test showed the influence of NAC on MDA levels, as indicated by the significant differences between control group with P1 (p=0,007), P2 (p=0,003), and P3 (p=0,001) groups. In this study, the decrease in tissue MDA levels was stratified according to dose increment.

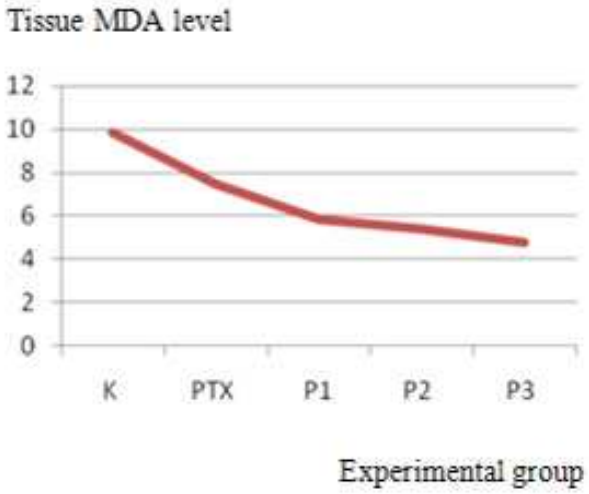

Figure 1. Average of tissue MDA level of each group

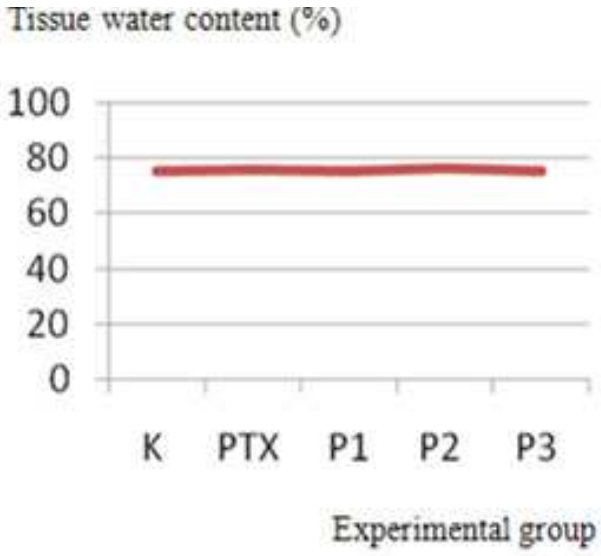

Figure 2. Average of tissue water content of each group

However, there were no significant differences among the three doses. This study suggests that a dose of 200 mg/kgBW had already significantly reduced tissue MDA, hence this is the recommended dose.

There were no significant difference between NAC groups and pentoxifylline group in tissue MDA level (Figure 1). The tissue water content was 75,24±1,46 % in the control group, 74.96± 1,72% in P1, 76.14± 1.23% in P2, 75.23±1.12% in P3, and 75.90±1.83 % in PTX. ANOVA test showed that there were no significant differences of tissue water content among experimental groups (p>0.05).(Figure 2)

Discussions

Ischemia reperfusion injury causes formation of ROS, characterized by an increase in lipid peroxidation during reperfusion.[14] Free radicals formed during reperfusion

resulted in lipid peroxidation reactions in the cell membrane. Saez and his colleagues[15] reported elevated levels of blood MDA, which is a product of lipid peroxidation, after bilateral ischemia in the rat hind leg.

This study demonstrated that NAC may reduce lipid peroxidation, which was characterized by decreased tissue MDA levels of muscle in IRI. This means that the NAC has a protective effect on the cell membrane against free radical attack. This effect may be due to its effect as an antioxidant which works directly or indirectly.[16] Reduced thiol groups in NAC can directly scavenge ROS which increases greatly in tissue with IRI. Moreover, these effects may also produce by the i creased availability of cysteine through the de-acetylation of NAC, thereby increasing the synthesis of GSH.[17,18] NAC may improve antioxidant systems such as superoxide dismutase and glutathione peroxidase.[19] In ischemic condition antioxidant defenses in the body become eroded, which is characterized by reduced glutathione.[1]

Protective effects of NAC on the cell membrane are very important. Lipid peroxidation on cell membrane causes serious damage including increased permeability of the membrane and disturbances of secretory function as well as ion exchange through trans-membrane ion gradients. Lipid peroxidation reactions which lead to the disintegration of the membrane can lead to cell death.[20] Inhibitors of lipid peroxidation was found to maintain the viability of skeletal muscle in IRI model.[21]

The results of multiple comparisons through the post hoc test showed no significant differences in tissue MDA levels between the control and PTX group, which was given pentoxifylline 20 mg/kgBW intravenously. The dose was determined according to a study by Kishi, et al.,[22] which examined protective effect of pentoxifylline on reperfusion injury in rat skeletal muscle after partial ischemia.[22] On ischemia reperfusion injury model of rabbit kidney, pentoxifylline was shown to reduce tissue MDA levels.[23] Study by Clark, et al.,[24] in pigs showed that pentoxyfilline can lower levels of MDA in reperfusion injury associated with lung transplantation. In contrast to those studies, in this study pentoxyfilline was not shown to decrease skeletal muscle tissue MDA levels. This may be due to differences of animal model, the organ explored, duration of IRI and time of drug administration.

ANOVA test showed no significant differences among control group and groups given NAC in tissue edema. This result was in contrast to study of Schaser, et al.,[6] who showed the effect of edema reduction in the model of closed severe soft tissue injury by intravenous administration of NAC 400 mg / kg of rats.[6] Our study was in parallel with the study by Inci, et al.,[11] on the protective effects of NAC on ischemia reperfusion injury after lung transplantation. Protective effects of NAC on pulmonary oxygenation was reflected from, decreased lipid peroxidation, increased glutathione levels and improvement of peak airway pressure. However, there was no significant difference in wet to dry weight ratio between the control and groups given NAC. Inci, et al.,[11] suggested that the decline in pulmonary edema which was not significant may be due to a short period of observation.

The water content may reflect water retention that occurs in the tissues, interstitial space, or intracellular space.[22] Ischemia reperfusion injury may induce edema, which possibly leads to the no reflow phenomenon.[25] The effect of NAC in reducing membrane damage in this study, which was reflected by the decrease in lipid peroxidation products, may potentially prevent water retention that causes intracellular edema.

Interstitial edema on IRI is induced by vascular barrier damage caused by the attachment of activated leukocytes on the vascular wall. Kearns, et al.,[7] who examined the protective effects of NAC on striated muscle in a model of compartment syndrome, reported that NAC did not reduce neutrophil infiltration acutely, but lower it after 24 hours. Longer period of observation was therefore needed to confirm NAC's effect on tissue edema in IRI.

There was no significant difference in tissue edema between the control group and pentoxifylline group given a dose of 20 mg / kg. This result was in contrast to the study by Kishi et al.,[22] who reported a decrease in edema by administration of pentoxifylline 20 mg/kg on IRI of skeletal muscle. Method for the induction of ischemia reperfusion on study by Kishi, et al.,[22] was slightly different. Ischemia time in Kishi's study was 90 minutes, while the time of ischemia in this study was 4 hours.[22] Blaisdell[3] suggested the degree of skeletal muscle damage to correlate directly with duration of ischemia. Thus, the damage produced by IRI in this study was probably greater than the study by Kishi, et al.[22]

Conclusions

It was concluded that NAC reduced tissue MDA level, an indicator of lipid peroxidation, in skeletal muscle ischemia reperfusion injury. However, NAC was not shown to reduce tissue edema in this IRI model. This study did not demonstrate any dose dependency of the effect. Therefore, the recommended dose was 200 mg/kg BW. 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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