EXPERIMENTAL STUDY

Effect of pheniramine maleate on rat skeletal muscle ischemia-reperfusion injury

Kubilay Erol, M.D.,[1] Eser Y. Sozmen, M.D.,[2] Ülkü Küçük, M.D.,[3] Levent Küçük, M.D.[4]

[1]Department of Orthopedics and Traumatology, Emot Hospital, İzmir-*Türkiye*
[2]Department of Medical Biochemistry, Ege University Faculty of Medicine, İzmir-*Türkiye*
[3]Department of Pathology, Tepecik Training and Research Hospital, İzmir-*Türkiye*
[4]Department of Orthopedics and Traumatology, Medicana International Hospital, İzmir-*Türkiye*

ABSTRACT

BACKGROUND: Skeletal muscle ischemia-reperfusion injury (IRI) is a common clinical problem encountered after tourniquet application or replantation. This study investigated the effect of pheniramine maleate (Ph), which is frequently used in clinical practice to reduce IRI, and compared its efficacy in IRI with N-acetylcysteine (NAC), a molecule that has been shown to be effective in IRI.

METHODS: Twenty-eight male Sprague–Dawley rats were randomly divided into four groups (sham, ischemia-reperfusion [IR], IR+Ph, IR+NAC; n=7 rats per group). Ischemia was induced in the lower right extremities of rats for 3 h using a femoral artery clamp and an elastic tourniquet. Ph and NAC were administered intraperitoneally 15 min before ischemia was terminated. At 24 h after reperfusion, levels of thiobarbituric acid reactive substance (TBARS), catalase (CAT), myeloperoxidase (MPO), superoxide dismutase (SOD), polyadenosine diphosphate ribose polymerase (PARP), and neutrophil infiltration were evaluated. Inducible nitric oxide synthase (iNOS) density in muscle tissue was evaluated by immunohistochemical methods after 1 week.

RESULTS: SOD, MPO, PARP, CAT, and TBARS levels in muscle tissue were significantly lower in the sham group compared with the other groups ($p<0.001$). All parameters except TBARS were lower in the NAC and Ph groups than in the IR group ($p<0.001$). Neutrophil infiltration in the muscle tissue samples from the IR group was significantly increased compared with the NAC and Ph groups ($p<0.05$). iNOS staining was not observed in the sham and NAC groups.

CONCLUSION: Ph is effective at reducing experimental rat skeletal muscle IRI.

Keywords: Inducible nitric oxide synthase; ischemia-reperfusion; N-acetylcysteine; pheniramine maleate; skeletal muscle.

INTRODUCTION

Skeletal muscle ischemia-reperfusion injury (IRI) is commonly seen in peripheral vascular injury, compartment syndrome, revascularization crush syndrome, and tourniquet application in orthopedic surgery.[1,2] Skeletal muscle ischemia causes energy depletion and accumulation of metabolic products. It is accepted that an excessive inflammatory response which is consisting of free oxygen radicals (FOR), cellular infiltration, and proinflammatory cytokines is involved in the formation of IRI.[3] Tissue damage and apoptosis occur due to these metabolic and cellular changes during the reperfusion period.[3,4] The tissue damage can cause loss of contractility, disability, limb amputation, multisystem organ dysfunction, and even death.[3–5]

It has been shown in animal studies that many agents such as glutamine, l-arginine, and lipoxin reduce IRI in skeletal muscle. However, their clinical utility appears to be limited.[6–8] Therefore, we need new studies for evaluating the effect of new agents which can be easily applied in clinical practice.

N-acetylcysteine (NAC) is used as a mucolytic agent in clinical practice. It exerts antioxidant properties as a glutathione

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Address for correspondence: Kubilay Erol, M.D.
Emot Hastanesi, Ortopedi ve Travmatoloji Kliniği, 35230 Konak, İzmir, Türkiye
Tel: +90 232 - 441 01 21 E-mail: kubilayerol89@gmail.com

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precursor and inhibits the synthesis of inflammatory transcription factors. NAC has been shown to reduce the severity of IRI in the lung, kidney, heart, and skeletal muscle in animal studies.[9–12]

Pheniramine maleate (Ph) is a first-generation antihistamine that blocks H1 histamine receptors. Its favorable adverse effect profile and low cost make it a frequently used drug in clinical practice. It decreases the severity of IRI by reducing capillary permeability and preventing the release of inflammatory mediators through inhibiting the inflammatory nuclear transcription factors.[13–15] Due to these pharmacological effects, Ph attenuates IRI by preventing inflammation and leukocyte infiltration. Furthermore, Ph prevents the formation of FORs that cause IRI.[14] Although animal studies have shown that Ph decreases IRI in the kidney, brain, lung, and small intestine, there are no studies about the effect of Ph on skeletal muscle IRI.[15–18]

In this study, we hypothesized that Ph reduces IRI in skeletal muscle by anti-inflammatory effect. Thus, we aimed to investigate the effect of Ph in an animal model of skeletal muscle IRI and to compare its effectiveness with NAC.

MATERIALS AND METHODS

Animals

The Institutional Committee on Ethics in Animal Use approval was obtained from our institution (No. 2017-017). Animal care and experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC). Twenty-eight male Sprague–Dawley rats weighing 250–350 g were used in the experiment. All animals were kept under 12-h day-night cycles and were fed ad libitum. The ambient temperature of the cages was kept at 20°C–24°C, and the humidity was held between 50% and 60%.

Study Design and Model of Ischemia-Reperfusion (IR)

Twenty-eight Sprague–Dawley rats were randomly divided into four groups of seven rats in each.[5,17] Groups were determined as control (sham; n=7), IR (IR; n=7), IR+Ph (n=7), and IR+NAC (n=7) (Fig. 1)[16,17] Intraperitoneal ketamine hydrochloride (80 mg/kg Ketalar; Parke-Davis, Detroit, MI, USA) and xylazine hydrochloride (8 mg/kg Rompun; Bayer, Leverkusen, Germany) were used for anesthesia. 500 mg paracetamol was added to 500 ml of drinking water for post-operative analgesia. Before the surgical procedure, the right inguinal area of the rats was shaved while the animal was in the supine position, and an antiseptic was applied. An oblique 2-cm incision was made on the inguinal region, and the femoral artery and vein were dissected. An atraumatic vascular mini clamp (Vascu-Statt® Plus Aproximator-Mini; USA) was applied to the femoral artery. In addition, an elastic tourniquet was applied on the trochanteric region of the femur to prevent collateral circulation.

Under anesthesia, the femoral artery and vein of the right lower extremity of the animals in the control group were

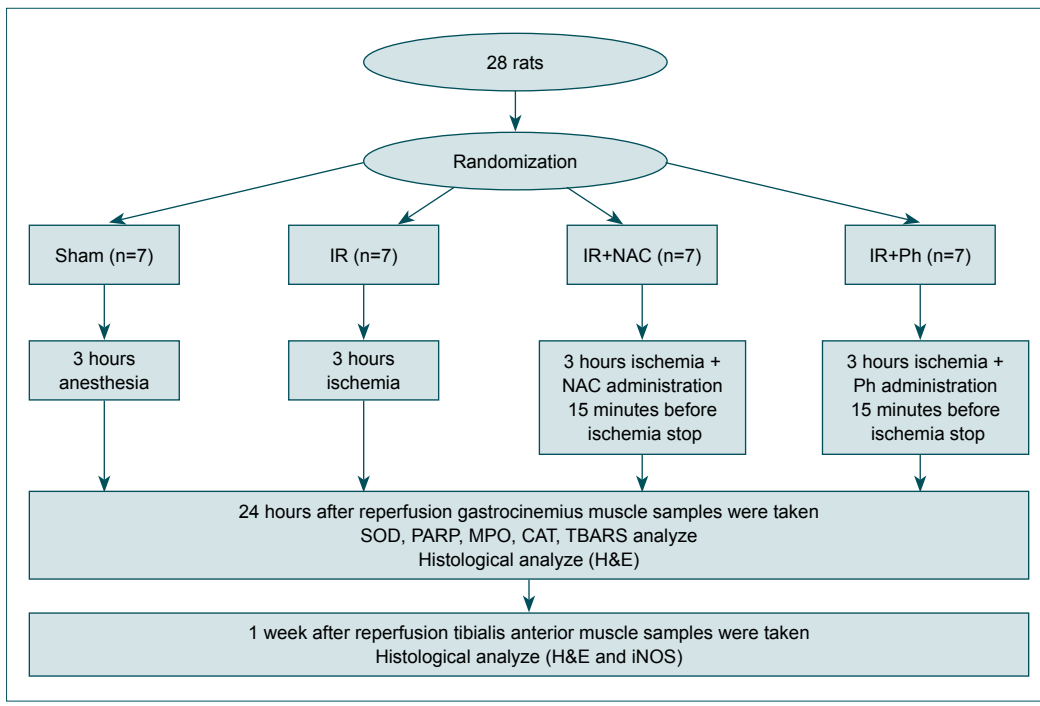

Figure 1. Schematic representation of study design IR: Ischemia reperfusion, NAC: N-acetylcysteine, Ph: Pheniramine maleate, SOD: Superoxide dismutase, PARP: Polyadenosine diphosphate ribose polymerase, MPO: Myeloperoxidase, CAT: Catalase, TBARS: Thiobarbituric acid reactive substance, iNOS: Inducible nitric oxide synthase.

dissected, and the skin was closed 3 h later without applying ischemia. Ischemia was applied to the right lower extremity of the animals in the IR+Ph group for 3 h; and 15 min before termination of ischemia, 10 mg/kg of Ph (Avil; Sandoz, Holzkirchen, Germany) was administered by intraperitoneal injection.[16,17] In the IR+NAC group, ischemia was applied to the right lower extremity of the animals for 3 h; and 15 min before cessation of ischemia, 20 mg/kg of NAC (Asist; Bilim Ilac, Istanbul, Turkey) was administered intraperitoneally.[9]

Tissue Sampling

Tissue samples were dissected from the right lower extremity gastrocnemius muscles of all rats under anesthesia at 24 h after reperfusion. Tissue samples were washed with saline, and half of the samples were fixed with 10% formaldehyde to prepare for hematoxylin-eosin staining. The remaining tissue samples were frozen at −80°C for biochemical examinations and stored until the day of analysis. The same procedure was applied to the tibialis anterior muscle 1 week after reperfusion. Tissue samples were washed with saline, placed in a 10% formaldehyde container for histopathological and immunohistochemical examination. After the tissue samples were harvested, the animals were sacrificed with high-dose anesthesia (ketamine hydrochloride, 150 mg/kg).

Preparation of Tissue Homogenates

Tissue samples were homogenized in phosphate buffer (0.5M, pH=7.0; 1/10 w/v). The homogenate was centrifuged for 5 min at 700 × g at 4°C to sediment unbroken cells and cellular debris.[19] The determination of superoxide dismutase (SOD) and catalase (CAT) activities and thiobarbituric acid reactive substance (TBARS) content in the supernatants was carried out immediately.

Quantification of TBARS

After dilution in the required amount of tissue homogenates, TBARS measurements were performed by incubation with TBARS solution (0.12 M thiobarbituric Acid [TBA] in 15% trichloroacetic acid and 1% HCl) for 30 min at 95°C.[20] TBARS levels were calculated using the 1,1,3,3-tetramethoxypropane standard curve.

Measurement of SOD Activity

SOD activities were measured according to Sözmen et al.[21] on the basis of the inhibition of autoxidation of epinephrine by SOD at 480 nm, with a Thermo Scientific Varioskan Flash-3001 spectrofluorometer (Thermo Fisher Scientific, USA). The assay was calibrated using purified SOD, and one unit of enzyme was defined as the amount of enzyme that inhibits 50% of the autoxidation of epinephrine.

Measurement of CAT Activity

CAT activities were determined as described by Sözmen et al.,[21] in which the degradation of hydrogen peroxide (H_2O_2) was recorded spectrophotometrically at 240 nm in a UV plaque with a Thermo Scientific Varioskan Flash-3001 spectrofluorometer (Thermo Fisher Scientific, USA). One unit of CAT was defined as the amount of enzyme that decomposes 1 µmol of H_2O_2/min under specific conditions.

Measurement of Myeloperoxidase (MPO) and Polyadenosine Diphosphate Ribose Polymerase (PARP) Activity

MPO measurements were carried out with a commercially available ELISA kit (MyBiosource®, catalog no. MBS046496; USA). PARP measurements likewise were carried out with a commercially available ELISA kit (SunRed®, catalog no. 201-11-0224; China).

Hematoxylin-eosin Staining

Muscle tissue samples were fixed in 10% formaldehyde solution for 1 day. Routine paraffin embedding procedure was performed, and 5-µm-thick sections were taken from blocked tissues for hematoxylin-eosin staining. Hematoxylin-eosin-stained slides were evaluated by a pathologist who was blinded to the study. The number of neutrophils at ×400 magnification in samples taken at 24 h and the number of mononuclear cells at ×400 magnification in samples taken at the end of week 1 were scored as mean by evaluating five different areas as follows: 0=≤3 inflammatory cells; 1=3–10 inflammatory cells; 2=11–20 inflammatory cells; 3=21–30 inflammatory cells; 4=31–40 inflammatory cells; and 5=≥41 inflammatory cells.[22]

Immunohistochemical Inducible Nitric Oxide Synthase (iNOS) Staining

Five-µm-thick sections were taken from the paraffin blocks onto the polylysine-coated slides. After the deparaffinization of these sections, iNOS monoclonal antibody (Clone SP126; Springbio, USA) was applied with an automatic staining system (Dako autostainer 48 link device, Denmark) using a universal kit. iNOS density in tissue samples was rated as 0=negative, 1=mild, 2=moderate, and 3=severe.[23]

Statistical Analysis

SPSS 25.0 (IBM Corporation, Armonk, NY, USA) was used to analyze variables. To compare quantitative variables with each other, the one-way analysis of variance was used for parametric tests, and the Games-Howell test was used for post hoc analysis. The Kruskal–Wallis test was used for non-parametric analysis, and Dunn's Test was used for post hoc analysis. Comparisons of treatment groups for the variables of inflammation and iNOS were made with the Fisher-Freeman-Holton test. $P<0.05$ was considered statistically significant.

RESULTS

TBARS levels, which are indicators of lipid peroxidation, were significantly increased in all groups compared with the sham group ($p<0.001$). TBARS levels were decreased in the treat-

Table 1. Tissue oxidant and antioxidant levels

Treatmant	Sham	IR	IR+Ph	IR+NAC
TBARS (nmol/mg prt)[a] Median	0.11	1.02*	0.46*	0.57*
MPO (U/mg prt)[a] Median	4.50	20.26*	12.64*†	14.84*†
PARP (ng/mg prt)[b] Mean± SD	0.70±0.14	20.88±1.83*	11.82±1.43*†	11±1.14*†
CAT (U/mg prt)[b] Mean±SD	2.36±0.36	13.19±1.61*	11.18±1.66*†	6.70±1.09*†‡
SOD (U/mg prt)[b] Mean± SD	1.18±0.31	13.10±1.36*	9.62±0.95*†	8.34±0.62*†

[a]Kruskal-Wallis test (Monte Carlo); post-hoc test: Dunn's test. [b]One-way analysis of variance (robust statistic: Brown-Forsythe); post-hoc test: Games-Howell.
CAT: Catalase; IR: Ischemia-reperfusion; Min: Minimum; max: Maximum; MPO: Myeloperoxidase; NAC: N-acetylcysteine; PARP: Polyadenosin diphosphate ribose polymerase; Ph: Pheniramine maleate; SD: Sandard deviation; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substance.
*p<.05 between the sham and the other groups. †p<.05 between the IR and the treatment groups. ‡p<.05 between the IR + Ph and the IR + NAC groups.

Table 2. Histological and histopathological findings

Treatmant	Sham	IR	IR+Ph	IR+NAC
Severity of neutrophil infiltrationa Median	0	4.33*	2*†	1†
Severity of monocyte infiltrationa	0	1.33*	1.60*	0.50
iNOS positivitya, n (%)				
Positive	0 (0.0)	7(100.0)	4 (57.1)	3 (42.9)
Negative	7 (100.0)	4 (57.1)	6 (85.7)	7 (100.0)
iNOS staining intensityb	0	0.60 (0/3)	0.14	0

[a]Kruskal-Wallis test (Monte Carlo); post-hoc test: Dunn's test. [b]Fisher-Freeman-Halton (Monte Carlo).
iNOS: Inducible nitric oxide synthase; IR: Ischemia-reperfusion; max: Maximum; min: Minimum; NAC: N-acetylcysteine; Ph: Pheniramine maleate. *p<.05 between the sham and the other groups. ‡p<.05 between the IR and the treatment groups.

ment groups compared with the IR group, but there was no statistically significant difference between treatment groups (p=0.065 and 0.222 for Ph group and NAC group, respectively) (Table 1).

MPO activity, which is used as an indicator of neutrophil infiltration in tissue, was increased in all groups compared with the sham group (p<0.001). MPO levels were significantly lower in the NAC and Ph groups compared with the IR group (p<0.001). There was no significant difference between the NAC and Ph groups (p=0.306) (Table 1).

PARP, which has increased activity in DNA chain fractures and apoptosis, was increased in all groups compared with the sham group (p<0.001), whereas PARP levels were significantly lower in the groups given NAC and Ph compared with the IR group (p<0.001). No significant difference was found between the NAC and Ph groups (P=0.836) (Table 1).

SOD and CAT activity levels were increased in all groups compared with the sham group (p<0.001). SOD activity was significantly increased in the IR group compared with the IR+NAC and IR+Ph groups (p<0.001). Increase of SOD and CAT activities was significantly less in the NAC group compared with the IR group (p<0.001) (Table 1).

In the tissue samples of gastrocnemius muscle taken 24 h after reperfusion, inflammation was not observed in the sham group. The intensity of inflammation in the muscle tissue was

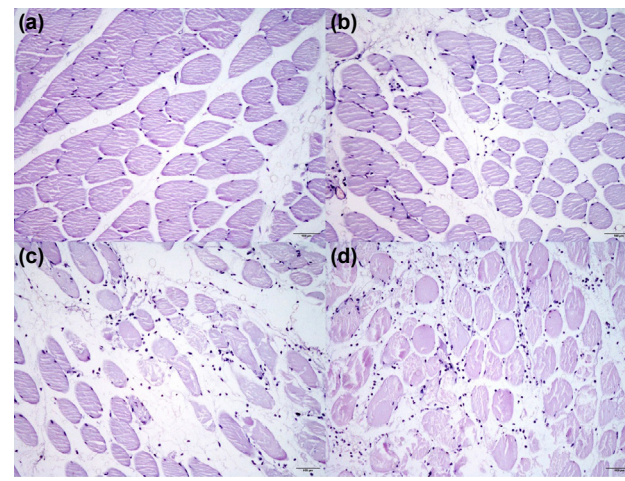

Figure 2. Histological analysis 24 h after reperfusion in gastrocnemius muscle tissue samples with hematoxylin-eosin staining. **(a)** Absence of neutrophil infiltration in the sham group (×200); (b) neutrophil infiltration of moderate intensity in the ischemia-reperfusion (IR) + N-acetylcysteine (NAC) group (×200); (c) mild neutrophil infiltration in the IR + pheniramine maleate (Ph) group (×200); and (d) severe neutrophil infiltration in the IR group (×200).

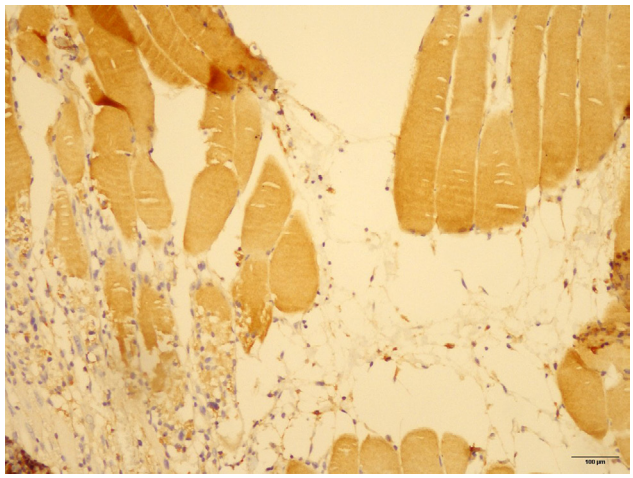

Figure 3. Histological and histopathological analysis 1 week after reperfusion in tbialis anterior muscle tissue samples. (a) Stained with inducible nitric oxide synthase (iNOS) in the ischemia-reperfusion (IR) group(×200).

significantly increased in the IR group compared with the NAC and Ph groups ($p<0.05$). No significant difference was found between the NAC and Ph groups (Table 2 and Fig. 2).

In the tibialis anterior muscle tissue samples taken 1 week after reperfusion, mononuclear cell infiltration, which is an indicator of chronic inflammation, decreased in the NAC and Ph groups compared with the IR group, but without any statistically significant difference. The muscle tissue samples taken in the sham group and in the IR+NAC group did not stain with iNOS, whereas the muscle tissue samples excised from one rat in the IR+Ph group and three rats in the IR group stained with iNOS. There was no significant difference between the groups in terms of iNOS staining density (Table 2 and Fig. 3).

DISCUSSION

Tourniquet applications and amputations cause prolonged ischemia in skeletal muscle. Ischemic damage can lead to many local and systemic postoperative complications such as muscle dysfunction, necrosis, severe acidosis, and myoglobinuria.[2–4,24] After reperfusion, activated neutrophils, FORs, and endothelial dysfunction cause increased local and systemic damage.[2,3] Many studies have shown that tissue damage substantially occurs in reperfusion period.[2,3,25] Therefore, reducing IRI will increase the success of the surgery by preventing local and systemic side effects. Our study showed that; Ph application in the pre-reperfusion period effectively reduced IRI by alleviating histological damage and decreasing SOD, CAT, MPO, and PARP levels in the muscle tissue.

FORs, which are formed as a result of IRI, break the DNA chain by disrupting the structure of nucleic acids. Oxidative damage as a result of IRI increases tissue PARP activity which is an enzyme involved in repairing DNA chain fractures. It has been shown that inhibition of PARP in the heart muscle[26] and skeletal muscle[27] reduces IRI in previous animal studies. Neutrophils and cytokines also play a major role in the development of IRI. Keskin et al.[28] indicated that ischemic preconditioning reduced IRI by reducing neutrophil infiltration in rat muscle tissue, while Sotoudeh et al.[12] reported similar beneficial effects of NAC on ischemic injury of rat lung tissue. Similarly, in our study, NAC and Ph significantly decreased IRI with inhibition of PARP and reducing neutrophil infiltration. Ph may exert this anti-IRI effect by preventing migration, diapedesis of neutrophils and by decreasing the release of inflammatory cytokines, and interleukins.

The measurement of MPO, an enzyme found in the cytoplasm of neutrophils, provides information about the ratio of activated neutrophils in the tissue. Wang et al.[29] showed that the modeled SOD coordination compound reduced MPO activity in rat skeletal muscle by decreasing IRI. Similarly, Cuzzocrea et al.[11] demonstrated that NAC decreased IRI by reducing the MPO activity in rat brain tissue and Nosálová et al.[18] showed that Ph decreased intestinal IRI by decreasing the MPO level. In our study, the MPO levels decreased significantly in the Ph group ($p=0.02$). However, the same effect was not observed in the NAC group ($p=0.191$). As a reason for that, we suggest that Ph may have reduced extravasation from the vein due to its anti-inflammatory and vasoconstrictory effects. Thus, it may have decreased the number of activated neutrophils more than NAC does.

The iNOS enzyme which is found in macrophages is induced after IRI, causing the formation of peroxynitrite radicals. The previous studies have shown that inhibition of the iNOS enzyme reduces IRI.[30] In our study, staining with iNOS antibody was not observed in tissue samples taken 1 week after reperfusion in the sham and in IR+NAC groups. Only one rat in the IR+Ph group and three rats in the IR group demonstrated iNOS staining. In previous animal studies, it was noted that samples were taken 2–24 h after reperfusion to evaluate iNOS level.[23,31,32] However, in our study, the staining of iNOS was evaluated in the chronic period, and it was noted that no iNOS activity was detected, especially in the NAC group. NAC directly inhibits the iNOS expression gene at the molecular level, a finding supported by Bergamini et al.[10] As a result, NAC and Ph were considered effective for preventing iNOS enzyme activity; however, the difference was not detected in statistics due to the limited number of cases.

SOD and CAT which are released as a result of IRI are antioxidant enzymes that enable the degredation of FORs into harmless forms. Sahin et al.[33] demonstrated that SOD and CAT activities were increased in oxidative damage in rats. Sirmali et al.[34] showed beneficial effects of erdosteine, Vitamins C and E in rat lung model of IRI by reducing SOD and CAT activities. In our study, a significant increase in activities of SOD and CAT was observed in the group with IRI compared with the groups given NAC and Ph. This result suggested that NAC and Ph had a protective effect against IRI by reducing the activities of SOD and CAT.

FORs formed as a result of IRI disrupt the structure and functions of lipids, which are the basic building blocks of the cell, by causing peroxidation of lipids. There is no test that directly measures the level of malondialdehyde (MDA), the end product of lipid peroxidation. Measurement of TBARS is used in many systems to reflect the MDA level. However, TBA reacts with sialic acid, aldehyde compounds, and oxidized lipids. Koksal et al.[9] reported that, as a result of IRI in rat muscle tissue, the TBARS level was significantly higher in the IR group than in the sham and IR+NAC groups. Yürekli et al.[16] studied with a rat brain model of IRI. They found that MDA levels were significantly higher in the IR group than the sham and Ph-treated groups. However, they indicated that methylprednisolone did not significantly decrease the MDA level in rat brains. Da Silveira et al.[35] reported that NAC had no significant effect on MDA levels in rat skeletal muscle model of IRI. In our study, TBARS levels were significantly higher in the IR group compared with the sham group. Although no significant difference was observed between the NAC, Ph, and IR groups.

Extremity ischemia usually occurs as a result of unexpected and sudden accidents. Due to the unpredictable nature of vascular injury events, ischemia is an unavoidable situation. However, it is possible to prevent or reduce IRI by treatment that administered before reperfusion. Previous animal studies have shown that Ph attenuates IRI in rat brain, lung, kidney, and mesenteric tissue.[15–18] Likewise in our study, it was shown that Ph administered before the reperfusion significantly reduced skeletal muscle IRI. Ph is a safe, low cost, and well-known pharmacological agent which is used commonly for treatment of various medical conditions in daily clinical practice over many years. Ph may be an effective and low-cost candidate for prevention and reduction of IRI in clinical practice. As a result, IRI which is frequently occured situation after amputations, tourniquet applications, acute arterial occlusions, cerebrovascular events, cardiovascular surgeries, and causes tissue damage, will be significantly attenuated by Ph administration. However, the use of Ph as a treatment option must be investigated by new animal and clinical studies to determine timing and dosing of Ph administration. Our study will lead to these new studies.

The limitations of our study are the limited number of animals in the groups (for ethical reasons) and we do not know the optimal administration time and dose of pheniramine in IRI. Future studies will focus on these subjects.

Conclusion

Ph, thanks to its low adverse effect profile and low treatment cost, is used in the treatment of hypersensitivity reactions. This study found that Ph significantly reverses the biochemical and pathological reactions caused by IRI in rat skeletal muscle and can be as effective as NAC in reducing IRI. However, there is a need for additional clinical and experimental studies on this subject to determine the routine use of Ph for this application.

Ethics Committee Approval: This study was approved by the Ege University Animal Experiment Ethics Committee (Date: 29.03.2017, Decision No: 2017-017).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: K.E., L.K.; Design: K.E., L.K., E.Y.S.; Supervision: L.K., Ü.K., E.Y.S.; Resource: K.E., E.Y.S., Ü.K.; Materials: Ü.K., E.Y.S.; Data: K.E., E.Y.S., Ü.K.; Analysis: L.K., Ü.K., E.Y.S.; Literature search: K.E., Ü.K.; Writing: K.E., Ü.K., L.K.; Critical revision: K.E., Ü.K., L.K., E.Y.S.

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DENEYSEL ÇALIŞMA - *ÖZ*

Feniramin maleatın sıçan iskelet kasındaki iskemi reperfüzyon hasarına etkisi

Dr. Kubilay Erol,[1] Dr. Eser Y. Sozmen,[2] Dr. Ülkü Küçük,[3] Dr. Levent Küçük[4]

[1]Emot Hastanesi, Ortopedi ve Travmatoloji Bölümü, İzmir
[2]Ege Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, İzmir
[3]Tepecik Eğitim ve Araştırma Hastanesi, Patoloji Bölümü, İzmir
[4]Medicana International Hastanesi, Ortopedi ve Travmatoloji Bölümü, İzmir

AMAÇ: İskelet kası iskemi reperfüzyon hasarı (İRH) turnike uygulamaları ve replantasyon sonrası görülen klinik bir problemdir. Bu çalışmada, klinik pratikte sık kullanılan bir molekül olan feniramin maleatın (Ph) İRH'nı azaltmadaki etkisinin belirlenmesi ve İRH'nı azaltmada etkinliği kanıtlanmış olan N- asetil sisteinle (NAC) karşılaştırılması amaçlanmıştır.
GEREÇ VE YÖNTEM: 28 adet Sprague-Dawley sıçan rastgele yedişerli dört gruba ayrıldı. Gruplar sham, iskemi-reperfüzyon (İR), İR + Ph, İR + NAC olarak belirlendi. Sıçanların sağ alt ekstremitesinde femoral arter klempi ve elastik turnike ile üç saat süreyle iskemi oluşturuldu. Ph ve NAC, iskemi bitiminden 15 dakika önce intraperitoneal uygulandı. Reperfüzyondan 24 saat sonra kas dokusunda, tiobarbiturik asitle reaksiyon veren maddeler (TBARS), katalaz (CAT), süperoksit dismutaz (SOD), miyeloperoksidaz (MPO), poli adenozin di fosfat riboz polimeraz (PARP), nötrofil infiltrasyonu ve bir hafta sonra iNOS monoklonal antikor pozitifliği değerlendirildi.
BULGULAR: Sham grubunda kas dokusundaki MPO, TBARS, PARP, SOD, CAT düzeyleri diğer gruplara oranla belirgin olarak düşük bulundu ($p<.001$). MPO, PARP, SOD, CAT düzeyleri İR + Ph grubunda ve İR + NAC grubunda İR grubundan belirgin olarak düşük bulundu ($p<.001$). Nötrofil infiltrasyonu İR grubunda İR + NAC ve İR + Ph grubundan belirgin olarak yüksek bulundu ($p<0.005$). İRH azaltılmasında Ph ve NAC'ın etklili olduğu, ikisi arasında anlamlı fark olmadığı bulundu.
TARTIŞMA: Feniramin maleat sıçan iskelet kasında oluşan İRH'nın azaltılmasında etkin bir moleküldür.
Anahtar sözcükler: CAT; feniramin maleat; iNOS; iskemi reperfüzyon; SOD.

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