PREVENTION OF OXIDATIVE STRESS DUE TO TOURNIQUET APPLICATION ANALYSIS OF THE EFFECTS OF LOCAL HYPOTHERMIA AND SYSTEMIC ALLOPURINOL ADMINISTRATION

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In this study various methods for the prevention of oxidative stress due to tourniquet application were compared. An ischemia period of two hours followed by a reperfusion period of one hour was produced in the right posterior limb in 32 rats. In the first group no treatment was given. In the second group local cold application was performed before and after ischemia. In the third group allopurinol was given orally for 5 days before the procedure. In the fourth group both local hypothermia application and oral allopurinol treatment were given. Ultrastructural study of the gastrocnemius muscles in the various groups revealed that the least damage in the ultrastructure of the striated muscle was obtained using allopurinol alone and using a combination of allopurinol and local hypothermia. When tissue glutathione levels were measured, it was seen that a combination of allopurinol and local hypothermia was the best treatment for preventing oxidative stress.

Keywords : tourniquet ; oxidative stress ; hypothermia ; allopurinol.
Mots-clés : garrot ; stress oxydatif ; hypothermie ; allopurinol.

INTRODUCTION

A pneumatic tourniquet is frequently used during orthopedic surgery on limbs. It provides a bloodless operating field, lessens the amount of blood loss and makes the operation time shorter. As a counterpart it produces functional and microscopic alterations in the distal neuromuscular structures as a result of ischemia and reperfusion (10, 11, 16, 17).

Systemic allopurinol administration and local hypothermia are two commonly accepted treatments to prevent oxidative stress.

The aim of this study was to assess the effects of allopurinol and hypothermia on the prevention of oxidative stress during tourniquet application.

MATERIALS AND METHODS

The experiments were performed on 32 Wistar albino rats with body weights of 200-250 g. The rats were randomly divided into four groups, each containing eight rats. In the first group a rubber tourniquet was applied on the right posterior thighs of the rats for a period of 120 minutes. Then, 60 minutes of reperfusion was permitted (group T) (2). The same procedure was performed in the second group (group C) with a cold application that was started 10 minutes prior to the procedure and was maintained throughout with cold gel pockets (Cold Hot Pack, 3M Center, St.Paul, MN). Cold gel pockets contained 95% water and 5% nontoxic carboxymethylcellulose carbohydrate. The gels were frozen at -12°C (20). In the third (group A) and fourth groups (group A+C) 10 mg/kg allopurinol (Atabay-Istanbul) was given orally for five days before the procedure. The allopurinol dose protocol was the same as the one in a previous study about postischemic

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oxidative stress (I). The last doses were given two hours prior to the procedure. In the fourth group, in addition to allopurinol, local cold application as previously described was used. During cold application the temperature reached at the core of the muscle was not measured. The probable decrease in the temperature in the muscle during the procedure was considered negligible. After sacrifice, the gastrocnemius muscle was excised from both hind limbs. Contralateral extremities were used as controls. All these procedures were performed by the third and fourth authors.

The specimens were numbered, and no information about the group of the specimens was given to the first and second authors who made the ultrastructural analysis, to keep the analysis blind. The specimens were cut into pieces of 1 mm^3 and fixed in 1/5 M phosphate-buffered 2.5% glutaraldehyde at pH 7.4 and at +4°C. Following this, they were postfixed in 1/5 M phosphate-buffered 1% osmium tetroxide at +4°C. They were dehydrated in serial ethanol and embedded in a material that contained araldite CY212, dodesenil succinate (DDSA) and benzyl dimethylamine (BDMA). The thin sections were stained with lead citrate and uranyl acetate, and all fields were examined under a Zeiss EM-900 electron microscope.

Besides ultrastructural analysis, the total tissue glutathione level was measured by the technique described by Yeğen *et al.* (21). All measurements were made by the same team, who did not have any information about the group of the tissues. The total glutathione level was used as a quantitative indicator of oxidative stress. The decrease in total glutathione level was related to increased oxidative stress.

The Kruskal-Wallis one-way Anova test and paired t-test were used for statistical analysis of the data, and $p < 0.05$ was considered significant.

RESULTS

Ultrastructural analysis of the control group revealed that numerous nuclei lay just below the sarcolemma and on the edge of the myofibrils (fig. 1). When compared with the control group, the myofibrils in group T appeared atrophic. Degeneration in the mitochondria and disappearence of the cristae were seen (fig. 2). This appearance was considered "atrophy of the striated muscle". In group C similar structural alterations were seen (fig. 3). Nevertheless atrophy of the myofibrils and degeneration in the mitochondria were not as severe as in group T. The ultrastructural findings in group A were similar to those in the control group (fig. 4). In group A+C it was noted that the structure of the myofibrils and configuration of Z lines, H bands and M lines were normal. Slight degeneration of the mitochondria was occasionally observed (fig. 5). The ultrastructural findings in groups A and A+C showed that systemic allopurinol administration was superior to local cold application in prevention of oxidative stress due to tourniquet use.

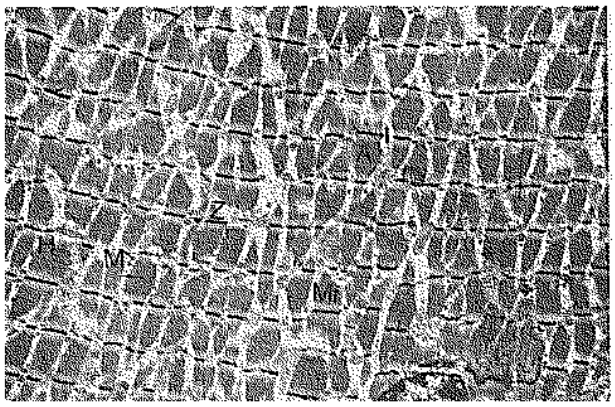

Fig. 1. — Normal ultrastructure of the striated muscle. Myofibrils (Mf) and normal configuration of A (A) and I (I) bands and their subgroups Z (Z) lines, H (H) bands and M lines (M) are seen. Mitochondria (⬟) with normal structure are also seen (lead citrate, × 6000).

Fig. 2. — Ultrastructural analysis of the T group. The nucleus (N) lies just under the sarcolemma and is flat. Some of the myofibrils (Mf) have dissolved, as in the case of atrophy. Interruption of the myofibrils gives the appearance of "teeth of a comb". Z lines (Z), H bands (H) and M lines (M) are irregular and occasionally disappear. Degeneration in the mitochondria (⬟) and disappearance of the cristae are noted (lead citrate, × 8800).

Fig. 3. — Ultrastructural analysis of the C group. The nucleus (N) lies under the sarcolemma. Destruction in some of the myofibrils (Mf) and occasional degeneration in Z lines (Z) and mitochondria are seen. The ultrastructure of the muscle seems to be better preserved than in the T group (lead citrate, × 6000).

Fig. 4. — Ultrastructural analysis of the A group. Structure and organization of the myofibrils correspond well with those in the control group. Localization and shape of the nucleus (N), arrangement of A (A) and (I) bands, Z lines (Z), H bands (H) and M lines (M) are normal. The structure of the mitochondria seems to be well preserved (lead citrate, × 8800).

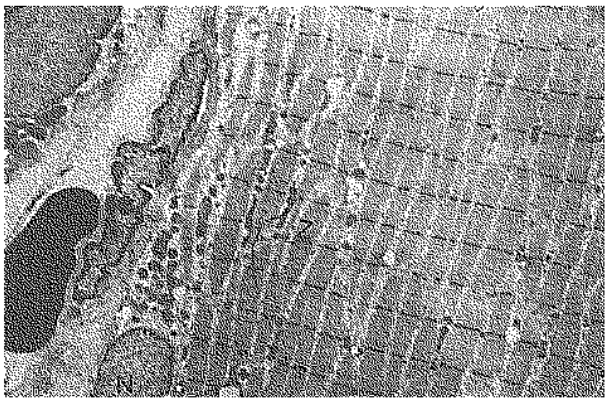

Fig. 5. — Ultrastructural analysis of the A+C group. The structure of the myofibrils is normal. Occasional slight degeneration in the mitochondria (⬇) and dense staining in the matrices are observed. The nucleus (N) lies just beneath the sarcolemma (lead citrate, × 6000).

Analysis of the average total glutathione levels of the ischemia-reperfusion groups revealed that there was a statistically significant difference between the average values (Kruskal-Wallis test, $p < 0.05$). On the other hand no significant difference was found between the average values of uninjured extremities (Kruskal-Wallis test, $p < 0.05$) (table I). In all ischemia-reperfusion groups, glutathione levels in the extremities treated were significantly different from the levels in uninjured extremities (paired t-test, T vs control ; $p < 0.001$), C vs control ; $p < 0.001$, A vs control $p < 0.001$ and A+C vs control $p < 0.05$) (table I).

DISCUSSION

Neuromuscular damage due to tourniquet application occurs in two phases : it begins with ischemia and increases during reperfusion. Cells use energy during ischemia to maintain ionic balance and homeostasis. Anaerobic metabolic pathways are used, and cell death due to damage to enzyme systems occurs. Production of free radicals, release of chemoreactant substances and increase in the adhesion of leukocytes are the three factors responsible for the tissue damage due to reperfusion. The hydroxyl radical is the most potent free radical, and it initiates the lipid peroxidation (3, 4, 7).

Several previous studies have revealed that tourniquet use leads to oxidative stress. Oxidative stress due to tourniquet use can be prevented by physiological (superoxide dismutase, catalase), pharmacological (allopurinol, antioxidants) or physical (hypothermia, hypoxic reperfusion) measures (6, 12, 13). None of the previous measures has definitely been found superior to the other for prevention of tissue damage.

Table I. — Analysis of total glutathione levels in the different groups. Values are means (standard deviation) (I/R = ischemia/reperfusion)

	Group T		Group C		Group A		Group A+C	
	Control	I/R	Control	I/R	Control	I/R	Control	I/R
Glutathione (μmol/gr)	2.25 (0.24)	1.25 (0.19)	2.29 (0.34)	1.67 (0.24)	2.27 (0.20)	1.72 (0.26)	2.32 (0.13)	2.12 (0.12)

Local hypothermia is known to be a reliable procedure for prolonging the tourniquet application time in operations longer than two hours. It decreases the metabolic rate of local striated muscle tissue ; thus tissue edema due to tourniquet use is reduced, and microvascular alterations due to ischemia are lessened (5, 8, 19, 20). We have observed some degeneration in the ultrastructure of the striated muscle in the local hypothermia group, but the alterations have been milder than in the tourniquet group without any treatment. It seems that cold application slightly lessens the harmful effects of tourniquet application on the ultrastructure of striated muscle, but does not definitely prevent postischemic oxidative stress. We have used local hypothermia at -12°C that has previously been suggested by Swanson *et al.* (19). We think that more physiological local hypothermia at -4°C as described by Ikemato *et al.* (8) may be equally effective to avoid some of the ultrastructural alterations. In this study, when the total tissue glutathione level of the ischemia-reperfusion group with and without cold application was analyzed, it was seen that local hypothermia had some beneficial effect on prevention of oxidative stress.

In the striated muscle, the source of the free radicals which are produced by ischemia-reperfusion is mostly xanthine oxidase. Allopurinol prevents the production of free radicals by inhibiting endothelial xanthine oxidase ; therefore pathological events during tissue reperfusion are avoided (1, 3, 9, 12, 18). Menger *et al.* (14) have stated that the flow paradox due to ischemia-reperfusion can be prevented successfully by superoxide dismutase and allopurinol applications. Their effects on the "no reflow phenomenon" which is lack of circulation after deflation of the tourniquet are reported to be limited (15). In this study, allopurinol has been found to be effective for the prevention of ultrastructural damage to striated muscle during tourniquet application. Dosage and administration time of allopurinol were the same as in a previous study (1) in which allopurinol and vitamin E were found to diminish the occurrence of oxidative stress and of postischemic edema in striated muscle. In this study, the effect of allopurinol on the total tissue glutathione level was nearly the same as that of cold application.

Ultrastructural analysis of all groups revealed that the least damage in the ultrastructure of the striated muscle was obtained by systemic allopurinol alone and by a combination of systemic allopurinol treatment and local cold application. When the tissue glutathione level was taken into consideration, the combination of systemic allopurinol administration and local hypothermia seemed to be the best one for reducing oxidative stress. Systemic allopurinol use and local hypothermia may have additive effects.

It can be concluded that tissue damage due to oxidative stress following tourniquet use can be reduced by a combination of allopurinol with local hypothermia and by systemic allopurinol alone prior to tourniquet use. Local cold application alone has limited beneficial effects and is not as useful as the previously mentioned measures in avoiding oxidative stress, because it slightly alters the ultrastructure of the striated muscle. We do not have any clinical experience in systemic allopurinol administration or local cold application in prevention of postischemic oxidative stress in skeletal muscle. We therefore need further investigations in higher animal models for prevention of postischemic oxidative stress before clinical applications.

Acknowledgment

The authors thank Lamia Pınar Yanıçoğlu, and Hale Sayan, from Gazi University, Faculty of Medicine, Dept. of Physiology, for their kind assistance in measuring glutathione levels. The authors also thank Hakan Ömeroğlu, from Osmangazi University, Faculty of Medicine, Dept. of Orthopedics and Traumatology, for his kind help in preparing and revising the manuscript.

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SAMENVATTING

D. ERDOĞAN, S. ÖMEROĞLU, S. SARBAN, O. Ş. ATIK. Voorkomen van oxidatiestress ten gevolge van tourniquets

In deze studie werden verschillende methoden onderzocht om de oxidatieve stress ten gevolge van tourniquet-gebruik te voorkomen. Een ischemieperiode van 2 uur gevolgd door reperfusieperiode van een uur werd verwezenlijkt in de rechterachterpoot van 32 ratten.
In de eerste groep werd geen behandeling gegeven, in de tweede groep werd lokale koude toegepast voor en na de ischemie en in een derde groep werd Alopurinol per os gegeven gedurende 5 dagen voor de procedure. In de vierde groep werd de lokale hypothermie en orale Alopurinol samen gegeven.
Ultrastructuurstudies van de gastrocnemiusspier in de verschillende groepen toonde aan dat de minste schade werd veroorzaakt bij deze groep met Alopurinol en de combinatie Alopurinol en locale hypothermie. Wanneer de weefselglutationenniveaus werden gemeten werd aangetoond dat de combinatie Alopurinol met locale hypothermie de beste preventie was van oxidatieve stress.

RÉSUMÉ

D. ERDOĞAN, S. ÖMEROĞLU, S. SARBAN, O. Ş. ATIK. Prévention du stress oxydatif dû au garrot pneumatique. Étude des effets de l'hypothermie locale et d'un traitement par allopurinol.

Ce travail a comparé différents modes de traitement utilisés pour éviter le stress oxydatif consécutif à l'emploi d'un garrot pneumatique. Les membres postérieurs droits de 32 rats répartis en 4 groupes, ont été soumis durant deux heures à une ischémie puis durant une heure à une reperfusion. Le 1er groupe n'a été soumis à aucun traitement. Dans le 2ème groupe, une hypothermie locale a été réalisée avant et après l'ischémie. Un traitement par allopurinol a été appliqué préalablement pendant 5 jours aux rats du 3ème groupe. Quant aux rats du 4ème groupe, ils ont été soumis à un traitement associant allopurinol et hypothermie locale. Les muscles gastrocnémiens ont été étudiés en microscopie électronique. Les muscles des extrémités controlatérales ont été utilisés comme contrôles. Cette étude a montré les altérations ultrastructurales les moins importantes dans les groupes qui avaient reçu le traitement par allopurinol associé ou non à une hypothermie locale et le traitement par allopurinol seul. En conclusion, cette étude nous a montré que la structure du muscle strié était mieux préservée dans les groupes traités par allopurinol ou a la fois par allopurinol et par hypothermie. Si l'on se base sur le taux de glutathion dans les tissus, il apparaît que l'association allopurinol et hypothermie locale a eu le meilleur effet protecteur vis-à-vis du stress oxydatif.