Effects of Tourniquet Induced Ischaemia on Malondialdehyde and Muscle Enzymes Under Regional Anaesthesia

REJYONEL ANESTEZİ ALTINDA TURNİKE İSKEMİSİNİN MALONDİALDEHİT VE KAS ENZİMLERİNE ETKİLERİ

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Özet

Objectives: Ischaemia-reperfusion injury due to tourniquet application is a good in vivo model to examine the resultant ischaemic damage from a biochemical perspective. The aim of the study was to investigate the effects of tourniquet induced injury by determining creatinine phosphokinase (CPK), glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH) activities and malondialdehyde (MDA) levels under regional anaesthesia.

Material and Methods: Blood samples were collected from 11 patients undergoing orthopaedic surgery under regional anaesthesia (either spinal or combined-spinal epidural anaesthesia) to determine CPK, SGOT, LDH activities and MDA levels preoperatively as control (baseline), 1 minute (min) before tourniquet release (BTR), 1, 5 and 30 min after tourniquet release (ATR).

Results: There were no significant differences in CPK, SGOT, LDH activities and MDA levels with respect to control but, MDA levels determined at 30 min ATR significantly increased with respect to 5 min ATR ($p<0.05$).

Conclusion: Patients undergoing orthopaedic surgery under regional anaesthesia do not require any antioxidant prophylaxis against lipid peroxidation induced ischaemia-reperfusion injury via tourniquet application lasting 109.6±34.8 min.

Key Words: Lipid peroxidation products, CPK (creatinine phospho kinase) SGOT (glutamic oxaloacetic transaminase), LDH (lactic dehydrogenase), MDA (malonyl dialdehyde), Tourniquet application, Ischaemia-reperfusion injury

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Summary

Amaç: Turnike uygulamasına bağlı iskemi-reperfüzyon hasarı oluşan iskemik hasarın biyokimyasal açıdan araştırılması için uygun bir in vivo modeldir. Bu çalışmanın amacı rejyonal anestezi altında kreatinin fosfokinaz (CPK), glutamik oksaloasetik asit transferaz (SGOT), laktik dehdirogenaz (LDH) aktiviteleri ve malondialdehit (MDA) düzeylerini tespit ederek turnikeye bağlı hasarın etkilerini araştırmaktır.

Materyal ve Metod: Spinal ya da kombine spinal epidural anestezi ile ortopedik cerrahi geçirecek 11 olgu çalışmaya alındı. CPK, SGOT ve LDH aktiviteleri ile MDA düzeylerini belirlemek için preoperatif olarak (kontrol), turnike açılmadan 1 dk önce, turnike açıldıktan 1, 5 ve 30 dk sonra kan örnekleri alındı.

Bulgular: CPK, SGOT ve LDH aktiviteleri ve MDA düzeylerinde kontrole göre anlamlı bir fark gözlenmedi fakat turnike açıldıktan 30 dk sonra belirlenen MDA düzeyleri turnike açıldıktan 5 dk sonraki değerlerden istatistiksel olarak anlamlı şekilde yüksek bulundu ($P<0.05$).

Sonuç: Rejyonal anestezi altında ortopedik cerrahi girişim geçiren hastalarda ortalama 109.6±34.8 dakikalık turnike uygulamasına bağlı iskemi-reperfüzyon hasarının neden olduğu lipid peroksidasyonuna karşı antioksidan proflaksi gerekmediği sonucuna varıldı.

Anahtar Kelimeler: Lipid peroksidasyon ürünleri, CPK, SGOT, LDH, MDA (malondialdehit), Turnike uygulaması, İskemi-reperfüzyon hasarı

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Haemostasis by pneumatic tourniquet is a popular technique for orthopaedic surgery of the extremities since it provides a bloodless operation field. However, consequent ischaemia leads to tissue anoxia, and if it prolongs, it will lead to cellular damage in addition to accentuated injury by reperfusion of ischaemic tissues (1,**2).**

Free radicals and lipid peroxidation products known to contribute to tissue injury associated with a wide range of acute and chronic conditions

include ischaemia-reperfusion injury due to tourniquet release which is an anticipated phenomenon during orthopaedic surgery (3). Malondialdehyde (MDA), which is one of the cytotoxic aldehydes, is a minor end product of peroxidation and it disrupts the structure of biological membranes (4). It has been reported that there was an elevation in MDA levels during limb surgery requiring tourniquet use under general anaesthesia in humans (5). Additionally, injury to muscle is manifested by abnormal permeability of the cell membrane to cytoplasmic enzymes including creatinin phosphokinase (CPK), glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) (6). However, the effect of ischaemia-reperfusion injury induced by limb tourniquet on MDA levels and muscle enzymes during ischaemic insult and reperfusion under regional anaesthesia have not been thoroughly investigated yet.

The aim of the study was to investigate the effects of ischaemia-reperfusion injury leading to oxidative stress and muscle injury by determining MDA levels and CPK, SGOT and LDH activities, respectively during tourniquet application under regional anaesthesia.

Methods

Patients

This study was approved by Hospital Ethics Committee on Clinical Investigation and written informed consent was obtained from each of the participants. Eleven unpremedicated ASA (American Society of Anesthesiologists) physical status I or II patients (6 female, 5 male patients), undergoing lower extremity surgery requiring tourniquet, without any metabolic, renal or hepatic disturbances and not receiving any antioxidant agents were enrolled in the study. The limb to be operated was elevated for ten minutes and wrapped with an elastic bandage to minimize venous content. A pneumatic double cuffed limb tourniquet was placed around the thigh. After achieving adequate anaesthesia by either spinal or combined spinal-epidural anaesthesia using bupivacaine 12.5 mg (Marcaine® heavy 0.5%, AstraZeneca, Sweden), the tourniquet was inflated between 350 to 400 mm Hg until the rapid cessation of arterial inflow to provide a bloodless operation field. Occlusion was released at the end of the operation, thereby allowing recirculation of the extremity.

Sample Preparation and Storage

The blood samples were collected from an indwelling radial artery catheter inserted under local anaesthesia to avoid repeated venous punctures. CPK, SGOT and LDH activities and MDA levels were determined before tourniquet application as control (baseline), 1 min. before tourniquet release (BTR), 1, 5 and 30 minutes after tourniquet release (ATR). Blood samples were centrifuged within 10 minutes and the supernatant was stored at -70°C until analysis.

Biochemical Analysis

All of the blood samples were analyzed for CPK (RAXT, Biocon kit, at 340 nm wavelenght), SGOT and LDH (DAX-Technicon 48, Biocon kit at 340 nm wavelenght) activity by spectrophotometry. Results were expressed as U L^{-1}.

Free Radical Assay

The lipid peroxides formed by peroxidation of the free radicals converted into MDA react with thiobarbituric acid (TBA) to form a colored complex. The degree of lipid peroxidation was determined as TBA reacting substances. Plasma (0.5 mL) and trichloracetic acid (200 g L^{-1}) were mixed. Then, TBA (6.7 g L^{-1}) was added to this mixture and boiled for 30 minutes. Following addition of N-butanol (4 mL), the mixture was centrifuged for 10 minutes at 3.000 r.p.m. Results were obtained as micromol L^{-1} at 535 nm wavelength by Milton Ray Spectronic 3000 Array (U.S.A).

Statistical Analysis

Results were expressed as mean ± SD. One way ANOVA was used to interpret the results among the sampling times followed by post hoc test in case of detecting significant differences. A p value less than 0.05 was considered as statistically significant.

Results and Discussion

The mean age, weight, height of the patients and duration of tourniquet application were 59 ± 16 years, 69 ± 8 kg, 162 ± 6 cm and 109.6 ± 34.8 min, respectively.

The CPK, SGOT and LDL activities did not show any statistically significant differences among the sampling times and remained within the clinical limits as well (Figure 1).

MDA levels determined at different time points did not show a statistically significant difference with respect to control, but MDA levels of 30 min ATR significantly increased when compared to the MDA levels of 5 min ATR (Table 1).

The MDA levels in the late reperfusion phase demonstrated a significant increase versus those in the mid-reperfusion phase which was not control but not control associated without any apparent alterations in enzymes such as; CPK, SGOT and LDH. This result was considered to be due to muscle injury caused by tourniquet ischaemia-reperfusion under regional anaesthesia.

There are numerous complications due to tourniquet induced ischaemia. Although very rare, one of the most severe is rhabdomyolysis which is defined as an injury of the skeletal muscle allowing its contents to escape (6). Injury is manifested by abnormal permeability of the cell membrane to cytoplasmic enzymes including CPK, SGOT and LDH which are identifiable in plasma or urine as indicators of muscle damage (7). In contrast to this study, Day and Zale (6) found an increase in the postoperative CPK and LDH activities only in 2 patients out of 40 whereas, a decrease in the postoperative SGOT activities were found only in 1 patient out of 40 with respect to preoperative values. In our study,

Table 1. Malondialdehyde (MDA) levels (u mol L^{-1}) (Mean ± SD)

Baseline (Control)	4.56 ± 1.49
1 min BTR	4.31 ±1.54
1 min. ATR	4.31 ± 1.54
5 min. ATR	3.98 ± 1.31
30 min. ATR	7.21 ±4.82*

BTR: Before tourniquet release
ATR: After tourniquet release (reperfusion)
*P<0.05 versus 5 min ATR.

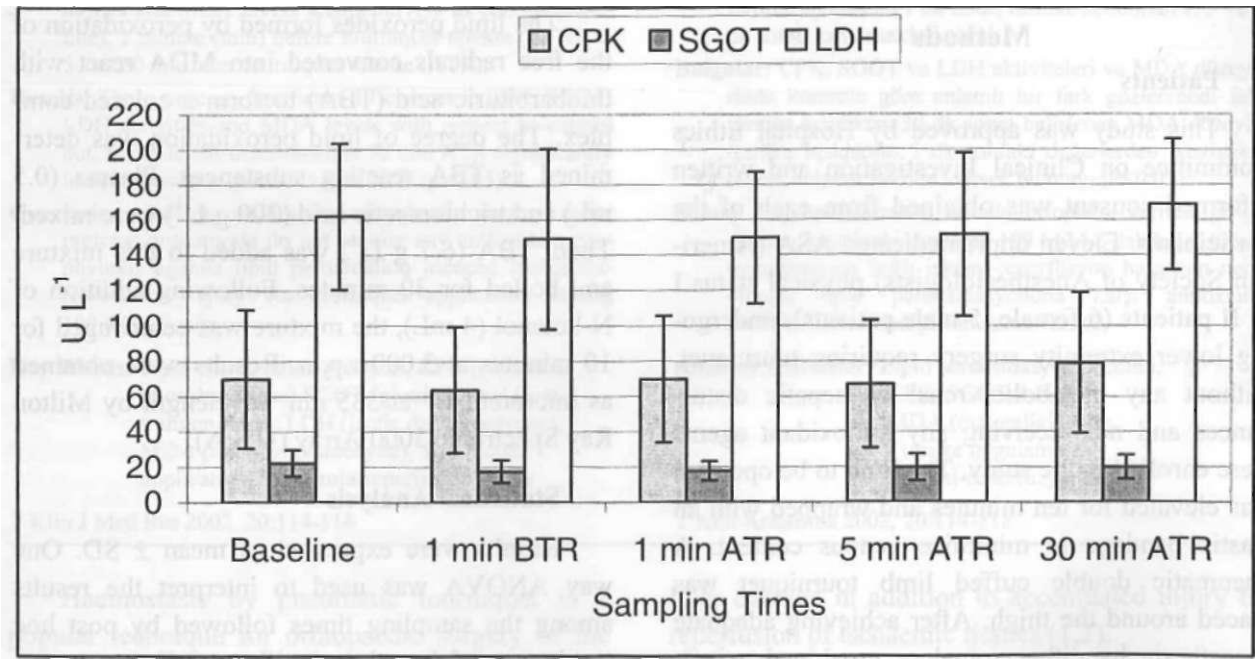

Figure 1. CPK (U L^{-1}) levels of baseline (control), 1 min BTR, 1 min ATR, 5 min ATR (n=l 1) and 30 min ATR (n=10); SGOT and LDH (U L^{-1}) levels of baseline (control), 1, 5 and 30 min ATR (n=l 1). The normal limits were between 25 -1! U L for CPK, 5 - 45 U L^{-1} for SGOT and 100 - 450 U L^{-1} for LDH.

there were no statistically significant differences in the mean CPK, SGOT and LDH activities among the sampling times. On the other hand, Chiu et al (1) demonstrated that there were no changes in CPK activities with 1 and 2 hours of ischaemic periods until after 1 hour of tourniquet release in dogs, since the tolerance of skeletal muscle of the dog to ischemia was assumed to be similar to that of the humans. In parallel to this study, we did not find a significant difference in CPK activity probably because of the relative early determination of the last CPK activity (30 minutes ATR) and relative short duration of tourniquet duration. Thus, skeletal muscle injury evaluated by CPK, SGOT and LDH elevations were found to be inapparent with a 109.6 ± 34.8 min tourniquet duration which was reliable in the present study.

Injury can occur, even though skeletal muscle is relatively insensitive to the deleterious effects of ischaemia and subsequent reperfusion. Numerous studies are found in the literature concerning the local, systemic, metabolic and morphological effects of pneumatic tourniquets on skeletal muscles (8,9). The effects of continuous tourniquet application resulted in a significant muscle necrosis after a two hour tourniquet application at 350 mm Hg in rabbits (10), but it is recommended not to exceed approximately 2 hours in humans (6).

Damage from oxygen free radicals has been documented in many different tissues after reperfusion, including skeletal muscle (11-13). Limb ischaemia from prolonged use of tourniquet in orthopaedic surgery may have profound effects on muscle function following restoration of blood supply (14) because of possible production of free radicals during reperfusion of ischaemic skeletal muscle which might be mediators of the resulting damage (12,13). There are numbers of potential intracellular sites for the production of free radicals within muscle such as mitochondrial electron transport systems, membrane-bound oxidases, infiltrating phagocytic cells and xanthine-oxidase within endothelial tissue closely associated with muscle (15). Oxygen radicals formed in a variety of reactions are prevented by several antioxidants. Consequently, when endogenous antioxidant defence capabilities are exceeded by oxidant flux, tissue injury occurs (16). Elevation in the lipid peroxidation causes an increase in membrane penneability, decrease in intracellular ATP levels leading to activation of membrane-bound phospholipases which further potentiate membrane damage (17-19). The present study demonstrated that ischaemia reperfusion induced lipid peroxidation has been assumed to be compansated by endogen protective mechanisms because MDA levels during reperfusion phase (1, 5 and 30 min. ATR) did not show any significant differences with respect to control. We performed regional anaesthesia with bupivacaine and we did not use any anaesthetic drugs known to have antioxidant properties to sedate patients during regional anaesthesia such as; propofol.

In the current study, the TBA assay which has been the most popular and simple method is used as an indicator of lipid peroxidation in biological samples as described in the previous studies (20,21). Interassay variation known as coefficient of variation estimated with the standard solution of MDA was less than 2% and results for duplicate samples varied within 2%.

Although ischaemia reperfusion injury was associated with an increase in CPK activities which were measured by chemiluminescence in rabbits (22), neither MDA (lipid peroxidation end products) nor enzymes used as indicators of resultant muscle damage showed any significant differences. The discrepancy between the studies might be due to the different determination methods of CPK.

It has been reported that inosine, a stable metabolite of adenosine, attenuated the local and the systemic proinflammatory responses associated with skeletal muscle reperfusion injury in mice (23). The present study demonstrated that patients undergoing orthopaedic surgery under regional anaesthesia do not require any antioxidant prophylaxis against ischaemia-reperfusion injury induced lipid peroxidation via tourniquet application lasting approximately 109 min. since increase in MDA levels could be balanced by endogen protective compansatory mechanisms. However, further studies are needed to elucidate the accurate underlying mechanism.

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