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ORIGINAL PAPER

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Tourniquet application and epinephrine injection to penile skin: is it safe?

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Abstract Although a tourniquet is frequently used in penile surgery there is still no consensus on safe application time. The aim of the present study is to investigate the effect of malondialdehyde (MDA) levels and histological changes in skin flaps after penile tourniquet application and epinephrine injection. A total of 36 male white New Zealand rabbits were randomly divided into six groups each containing six animals. A Mathieu-like flap was raised in all of the groups and a tourniquet was applied and the penis was subjected to ischemia for 10, 20 and 40 min in groups 1, 2 and 3, respectively. The flaps were then allowed to reperfuse for 5 min. Biopsies for MDA measurement were harvested in these groups. Subcutaneous 1/200,000 epinephrine was injected into penile skin in group 4 and 5 rabbits and biopsies for MDA measurement were harvested 10 and 40 min after injection. The control group was anesthetized without tourniquet usage or epinephrine injection. Specimens taken from the harvested flaps of all groups were submitted for histological evaluation. The mean MDA levels in all experimental groups were higher than in the control group and the difference was statistically significant. Edema, congestion and extravasation were observed in groups 1, 2 and 3. Minimal congestion and edema were observed in group 4 and severe edema and extravasation in group 5. Tourniquet usage for a duration of less than 10 min is clearly safer than prolonged usage. Epinephrine injection to penile skin may show a deleterious effect on wound healing.

Keywords Penile tourniquet · Epinephrine · Malondialdehyde · Ischemia-reperfusion · Hypospadias repair

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Introduction

The safe time for the application of a tourniquet in penile surgery has not been established and the times used vary according to the surgeon. Jean Louis Petit was the first surgeon to describe the screw tourniquet for limb surgery in 1718 and he was the first to use the term ''tourniquet'' [14]. Use of a rubber bandage for performing bloodless surgery was first introduced by Esmarch [14]. Several types of tourniquets have been used in different surgical specialties for the control of intraoperative bleeding. John Redman first described the application of a rubber band tourniquet in hypospadias surgery in 1983 [see 22]. This type of tourniquet is frequently used for several types of penile and clitoral surgeries.

When blood flow is restarted following such an ischemic period reperfusion injury may occur. This has been documented in many different tissues such as kidney, heart, intestines, liver, bone, skin and skeletal muscle. A similar cascade of events may also occur after the placement of a penile tourniquet.

Different doses of epinephrine are also used in penile surgery, either solely for vasoconstriction or, when in combination with local anaesthetic agents, for prolonging the efficacy of these agents in penile skin.

This experimental study was performed to investigate the time that a tourniquet can be safely used as well as to determine the effect of epinephrine on the skin in penile

surgery. Malondialdehyde (MDA: which is an end product of lipid peroxidation) levels were analyzed after varying durations of ischemia and histological evaluations were carried out.

Materials and methods

A total of 72 white New Zealand rabbits, weighing 1,000–1,500 g were used. The animals were housed under standardized conditions of light and temperature and were fed ad libitum. The experiments were performed in the animal research laboratory at Kirikkale University Medical Faculty with the approval of the Ethics Committee.

The first 36 animals were divided into six groups each containing six rabbits. A Mathieu-like flap was designed for all groups: group 1: 10 min tourniquet application + 5 min reperfusion, group 2: 20 min tourniquet application + 5 min reperfusion, group 3: 40 min tourniquet application + 5 min reperfusion, group 4: subcutaneous 1/200,000 epinephrine injection (10 min), group 5: subcutaneous 1/200,000 epinephrine injection (40 min), group 6: as a control (without tourniquet or epinephrine). The group information is summarized in Table 1. The same procedures were applied to the other 36 rabbits and specimens were obtained for histological evaluation.

The rabbits were anesthetized by intramuscular injection of ketamine hydrochloride (50 mg/kg) (Ketalar, Eczacibasi, Istanbul). After cleaning the inguinal region and penile area with 10% povidone-iodine solution, a standard rubber circular band was applied to the base of the penis. A Mathieu-like 0.8 cm long and 0.4 cm wide ventral flap was raised. After waiting 10, 20 and 40 min, as described above, the tourniquets were removed and the flaps were reperfused for 5 min. Reperfusion was confirmed with the restoration of penile skin color. For groups 4 and 5, 0.2 ml epinephrine (1/200,000) was injected subcutaneously into the penile skin and the skin flaps were harvested after 10 and 40 min. The control group was anesthetized without tourniquet or epinephrine injection. The flaps were removed for the determination of MDA levels.

All data were presented as mean ± SD. Kruskal-Wallis one-way ANOVA was used to investigate whether a differences was present between the groups . Differences between two groups were evaluated by Mann Whitney-U test. Differences were considered significant when the P value was less than 0.05.

Biochemical evaluation

The tissue samples were rinsed with 0.9% NaCl, and then homogenated in 1 ml of 0.9% NaCl. The homogenate was centrifuged at 1,500 *g* and 4°C for 10 min and the supernatant was used for analysis. A total of 50 µl of supernatant, 1 ml of DETBA (1,3 dietil 2-tiobarbituric acid) solution (DETBA 10 mM/l and K_2HPO_4 75 mM/l, pH 7.0), 100 µl EDTA (ethylenediamine tetra acetic acid) (18.75 mM/l) 100 µl H_3PO_4 (3 M/l) were mixed and heated for 45 min at 95°C in a water bath. After the mixture had cooled, 5 ml of n-butanol was added and mixed then centrifuged at 1,500 *g* for 10 min. The fluorescence of the supernatant butanol phase was measured with a Perkin-Elmer fluorometer at a wavelength of 515 nm excitation and 553 nm emission. TEP (1,1,3,3 tetraetoxy propane, Sigma, USA) was used for the calibration curve. Total protein level of the tissue homogenate was determined using the Lowry method and MDA levels were expressed as µmol/g protein [5, 18].

Table 1. Study groups

Groups	Ischemia time	Reperfusion time	Application
1	10 min	5 min	Tourniquet + flap
2	20 min	5 min	Tourniquet + flap
3	40 min	5 min	Tourniquet + flap
4	No ischemia	No reperfusion	1/200,000 adr. + flap (10 min)
5	No ischemia	No reperfusion	1/200,000 adr. + flap (40 min)
Control	No ischemia	No reperfusion	Only flap

Histological evaluation

Mathieu-like 0.8 cm long and 0.4 cm wide ventral skin flaps were harvested after the experimental procedures. Biopsy specimens were fixed in 10% buffered-formalin and embedded in paraffin blocks. Sections of 5 µm were obtained and stained with hematoxylin-eosin.

Results

The mean MDA level of group 1 (10 min tourniquet + 5 min reperfusion) was 2.49 ± 0.44 µmol/g protein, group 2 (20 min tourniquet + 5 min reperfusion) 2.72 ± 0.69 µmol/g, group 3 (40 min tourniquet + 5 min reperfusion) 3.09 ± 0.66 µmol/g, group 4 (epinephrine, 10 min) 3.90 ± 0.84 µmol/g, group 5 (epinephrine, 40 min) 4.48 ± 0.56 µmol/g and group 6 (control group) 1.64 ± 0.17 µmol/g.

The differences between the control group and all of the other groups were statistically significant ($P < 0.05$). In addition, the group 5 MDA level was significantly higher than the other study groups ($P < 0.05$) (Fig. 1).

Marked edema and congestion were observed in group 1, slight and moderate edema were noted in groups 2 and 3 respectively. Extravasation was noted in all of the flaps. Dilated lymphatic channels were observed in group 1 but not in the other groups. The morphology of the epithelium was normal in all the groups. Figs. 2, 3 and 4 show groups 1, 2 and 3, respectively.

Minimal congestion and edema were observed in group 4 (epinephrine 10 min). Extravasation was not encountered. Narrowing of the lumens of the vessels was obvious. Morphology of the epithelium was normal. Fig. 5 shows group 4.

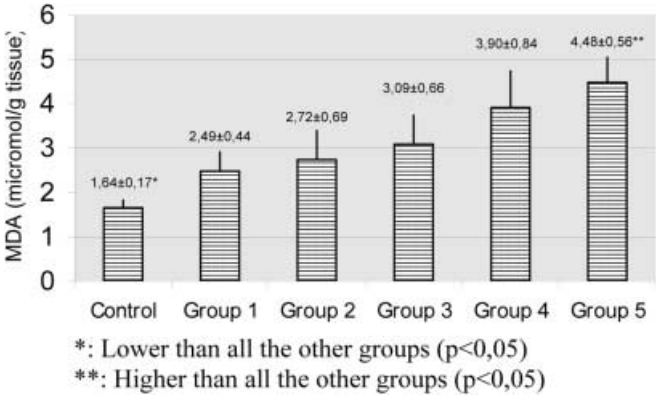

Fig. 1. Tissue MDA values of the groups. An *asterisk* indicates lower than all other groups and a *double asterisk* indicates higher than all other groups ($P < 0.05$)

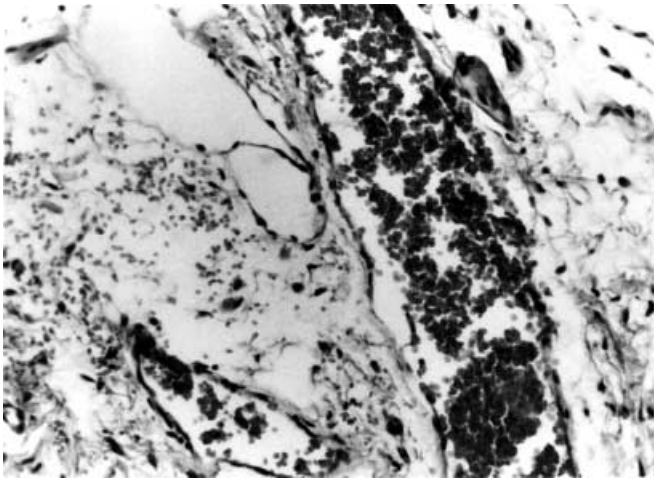

Fig. 2. Group 1.Vascular congestion, dilated lymphatic channels and erythrocyte extravasation. (H-E 200×)

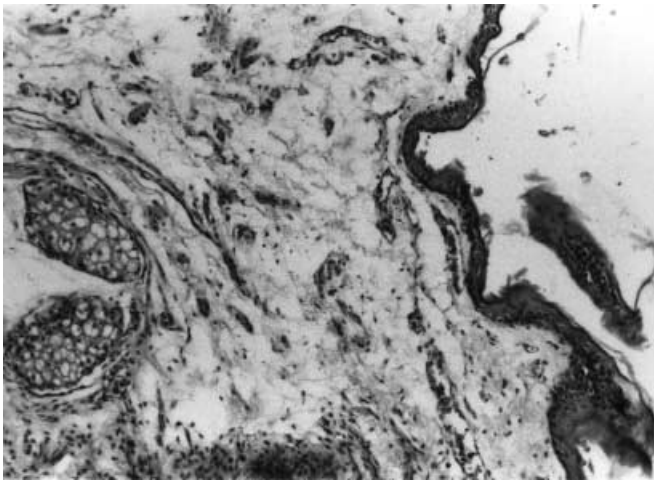

Fig. 3. Group 2. Slight edema and vascular congestion. (H-E 40×)

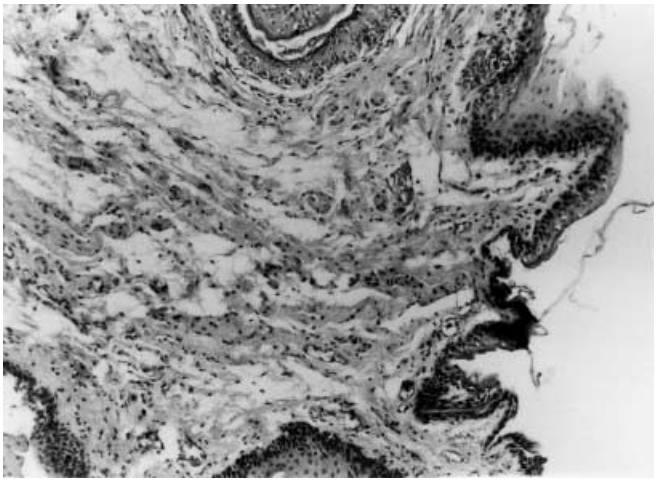

Fig. 4. Group 3. Moderate edema after 40 min of tourniquet application and 5 min of reperfusion. (H-E 40×)

Fig. 5. Group 4. Minimal edema in tissue and minimal congestion in vessels. (H-E 40×)

Congestion and edema were more severe in group 5 than in group 4. Extravasation was also seen in group 5. The epithelium was normal in this group as in the other groups (Fig. 6). Normal histological features were observed in the control group (Fig. 7).

Discussion

A bloodless field in hypospadias surgery can be maintained by using a vasoconstrictor or a tourniquet. The different tourniquet times and epinephrine dosages which have been used are summarized in Table 2. Redman was the first author to report the use of a rubber band tourniquet in hypospadias repairs in a series of 146 consecutive patients in 1981 [see 22]. Though various types of tourniquets have been used in hypospadias surgery over the years, a broad literature survey revealed that there is no consensus on optimal tourniquet

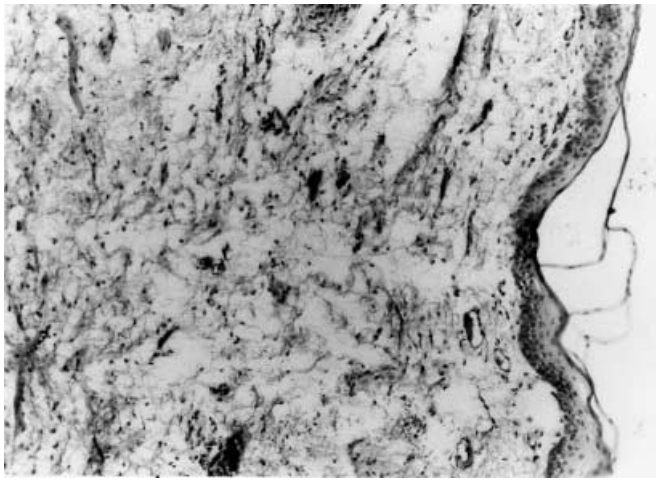

Fig. 6. Group 5. Marked edema following 40 min after epinephrine injection. (H-E 40×)

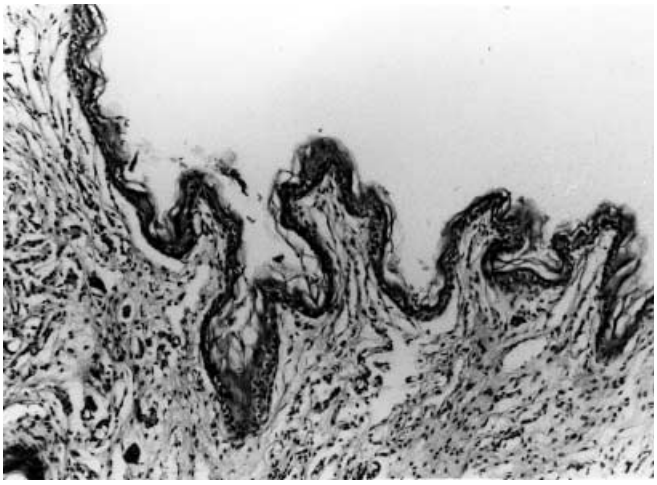

Fig. 7. Control group. Panoramic view of the penile skin. (H-E 40×)

time to be used or on the best epinephrine dosage. The duration of tourniquet application varied from 9 to 50 min during surgery. Some of the surgeons applied a tourniquet during the entire operation, while others advised intermittent release every 10 min [1, 2]. Harman and Scott applied a tourniquet only during the dissection period and Duckett and Keating used it intermittently with the infiltration of epinephrine into the penile skin [7, 12]. Redman reported an ischemia time of between 12 and 50 min with ischemia under 50 min was well tolerated. He also advocated the use of a tourniquet before the incision of the glans and release after closure [22]. Pathak reported the use of tourniquet application in 72 cases of minor penile surgery including circumcision, meatotomy, meatoplasty and chordee corrections with an average ischemia time of 9–14 min. He reported the occurrence of edema of the penis in 4.17% of cases after tourniquet release [21]. Davis and Harrold reported a graft failure after a two-stage, modified Cloutier operation because of tourniquet usage. He attributed the resultant hypoxia and oozing to the occurrence of reactive hyperemia following tourniquet release [4].

A similar controversy also exists on the optimal epinephrine dosage (such as 1/100,000, 1/200,000, 1/400,000 with or without lidocaine (such as 0.25%, 0.5%, 1%). The authors and their preferences for epinephrine dosage are summarized in Table 2.

Epinephrine has been used with lidocaine for prolonging anesthesia or alone for achieving local vasoconstriction. It has been reported that epinephrine containing solutions should not be injected into tissues supplied by end arteries such as fingers, toes, ears, nose and the penis and that 1/200,000 epinephrine provides optimal skin vasoconstriction [15]. The inadvertant use of sympathomimetic agents can lead to delayed wound healing, tissue edema or even necrosis because of the transient ischemic period which results from the vasoconstriction in the precapillary vessels and small venules which decrease the cutaneous blood flow.

Congestion and edema were observed in the penile skin infiltrated with epinephrine, and these findings were found to be more severe with increasing duration. Congestion and edema were noted in group 4 (10 min after epinephrine injection). Severe congestion and edema were observed as well as extravasation as an additional feature in group 5 (40 min after epinephrine injection). These findings are consistent with those previously reported after the use of vasoconstrictors. In addition, the MDA levels of group 5 were higher than in all other groups. The increase in MDA level was more pronounced in epinephrine-infiltrated skin samples. Moreover, MDA levels increased more with time in the epinephrine-infiltrated groups.

The damage caused by an ischemia-reperfusion (I/R) event has been documented in different tissues including skin, kidney, liver, bone and brain [3, 9]. The tolerance of tissues to ischemia and or reperfusion demonstrates a great variation in different organs [9, 16, 18, 24]. Tourniquet application and release is a frequently used model for studying the effects of ischemia and/or reperfusion [3]. In the present study, the application of a tourniquet caused an I/R injury in the penile skin flap, which is reflected in MDA levels. The tourniquet caused a significant increase in MDA levels in groups 1, 2 and 3. Thus, reperfusion injury starts in approximately 15 min (10 min tourniquet + 5 min reperfusion) and this continues in the 20 min and 40 min groups.

Table 2. Different preferences for tourniquet times and epinephrine dosages

	Epinephrine	Tourniquet
Duckett and Keating [7]	1/100,000 + 1% lidocaine/tissue	Intermittent
Devine and Horton [6]	1/200,000/tissue	
Rushton [23]	1/200,000 + 0.5% lidocain/tissue	Approximately 10 min
Belman and Belman [1]		Released about every 10 min.
Snodgrass [26]	1/100,000/tissue	
Smith [25]		Released every 10 min
Khanna [13]	1/400,000/tissue	
Rabinovitch HH [21]		Average 30 min, 40–50 min not infrequent
Ellsworth et al. [8]	1/100,000 + 0.25% marcaine/tissue	
Gilpin et al. [10]		45 min
Cloutier [2]		During surgery
Harman and Scott [12]		During dissection
Redman [22]		12–50 min, average 28 min
Pathak [20]		9–14 min

MDA levels of the penile skin of the rabbits increased in relation to the duration of reperfusion in our study. Initiation of the injury after 10 min may seem early but different tissues have different tolerances to I/R. Total blockage of the circulation of the penile skin was achieved after the placement of the tourniquet, and because the arteries of the penis are end arteries it has no collateral circulation. This may be one of the reasons for the early injury. Several studies show I/R injury of the skin. Concannon et al. reported a direct correlation between reperfusion injury and the duration of tourniquet ischemia in rabbit hindlimbs [3]. Their ischemia time varied from 1 h to 4.5 h with reperfusion intervals of 20 min. They also reported that allowing reperfusion intervals may increase the amount of damage and they recommended no reperfusion.

One of the reasons for the early injury may be the morphology of the penile skin which is thin, delicate and elastic. Regional variations exist in the thickness of the epidermis and in its microanatomy and function. Skin has several defense mechanisms against free radical damage such as anti-oxidant enzyme systems and the thickness of the horny layer. Menger et al. reported that capillary no-reflow in striated muscle ischemia reperfusion is a result of intravascular hemoconcentration, endothelial cell swelling and interstitial edema formation. These events result in capillary lumenal narrowing, which causes reduced tissue perfusion and delayed wound healing [19]. It is well known that edema has an inhibitory effect on wound healing and that this is more mechanical (pressure) than biochemical. Edema was noted in all experimental groups and this was more severe, especially 40 min after epinephrine infiltration and 40 min after tourniquet application. Extravasation, which is also an inhibitory factor for wound healing, was also observed in all of the tourniquet application groups.

A complication rate of about 10% has been reported for the Mathieu operation [11]. Besides technical errors, several mechanisms such as infection, edema and hemorrhage may lead to urethrocutaneous fistula formation following hypospadias surgery. Tourniquet application might be involved in the development of the adverse outcome after penile surgery. We hypothesize that severe edema and extravasation might have adverse effects on wound healing and skin flap vascularization in penile surgery, especially in hypospadias repair.

In conclusion, tourniquet usage even for the shortest period of 10 min had a negative effect on the penile skin. Operating under tourniquet control only during the flap dissection or glandular preparation phase without exceeding 10 min will preserve the viability of the tissues. Epinephrine injection to penile skin may also have a deleterious effect on wound healing. These two procedures, which are relatively routine in clinical practice, should be used with caution and further studies aiming to enhance the ischemic tolerance of the delicate penile skin against I/R are needed in order to decrease the rate of complications.

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