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EXPERIMENTAL STUDY

Analysis of the effects of heparin and enoxaparin on degloving injuries

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Abstract: *Background:* Heparin and low molecular weight heparin are the most frequently used antithromboembolic drugs in fractures.
Objectives: We aimed to compare the effects of heparin and enoxaparin, which are used as standard treatment, on viability in degloving injuries.
Methods: Thirty rats were used in the study. Three groups were composed including 10 rats in each group. Degloving injuries were formed in the tails of the rats. Enoxaparin was injected subcutaneously to the rats in group 1. Standard heparin was injected subcutaneously to the rats in group 2. Serum physiologic solution was injected subcutaneously to the rats in group 3. The experiment was ended on day 15. The tails of the rats were evaluated clinically and histopathologically.
Results: There was a statistically significant difference in the clinical results ($p < 0.05$). There was a statistically significant difference in the histopathological results ($p < 0.05$).
Conclusions: We encountered positive effects of both heparin and enoxaparin on the treatment of degloving injuries in this experimental study. However, the findings of this study should be supported and improved by new experimental and especially clinical studies *(Fig. 3, Ref. 18)*. Text in PDF *www.elis.sk.*
Key words: degloving injury, heparin, enoxaparin.

"Degloving" is a soft tissue injury defined as traumatic avulsion of the skin and subcutaneous tissue, together with the underlying deep fascia. Skin includes a dermal vascular plexus. A break in the dermal vascular plexus is formed due to the separation of vessels from the skin in degloving type of injury. Skin necrosis can be seen as a result.

De-gloving occurs frequently due to shear forces as a result of contact of the extremity with circling objects forming a high speed of friction, such as car wheels or motorcycle accidents. Degloving injuries are divided into two groups as open and closed injuries (1). Skin is generally open in degloving injury due to shear forces.

Open de-gloving injury was first defined by Slack (2) in 1952. Slack evaluated the association of these injuries with wheel injuries and defined these injuries as friction injuries.

Slack, in addition, proved in his study that the skin necrosis was due to loss of muscle support of subcutaneous tissue in secondary sepsis, and delayed skin gangrene. Closed degloving injury may occur when the underlying deep fascia raises the subcutaneous tissue without superficial tears (1). Most frequently seen degloving injuries are reported to be in the leg in 31.6 %, in femur in 21.1 %, in pelvis in 21.1 %, and in the feet in 13.2 % of cases (3).

Data related to the results of treatment of degloving injuries are scarce. Two points have to be taken into account for the results: these are cosmetic results and extremity function.

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Standard heparin acts by binding to an inhibitor enzyme, antithrombin III. Lower molecular heparin compounds are obtained by chemical and enzymatic depolimerization of heparin (4). LMWH lowers the risk of bleeding by minimally effecting on aPTT; while, heparin increases the bleeding risk by lengthening the coagulation time (aPTT, TT). We evaluated whether the antithrombotic substances such as heparin and enoxaparin have an effect on degloving injuries in this study. We thought that they might have beneficial effects on wound healing by preventing microthrombi during neovascularization.

Methods

This study was performed in the animal laboratory in Gaziantep University School of Medicine, Department of Physiology. The study was conducted with the contributions of Physiology, Pathology, Biostatistics, Histology, and Pharmacology Departments. Test animals Ethics Committee approval was obtained from the Ethics Committee of Gaziantep University School of Medicine prior to the study.

Thirty Sprague-Dawley adult male rats were used in this study. Randomly selected rats were maintained in 3 different cages including 10 rats each. The first cage was selected as Enoxaparin group, second as Standard Heparin group, and the third was selected as serum Physiologic solution group and required information was written on the cages.

Mean weight of the rats was 230 g (220–240). Rats were maintained in the optimal rat shelter in the physiology laboratory

under 22 degrees centigrade constant heat and in 12-hour light, 12-hour dark cycle.

Rats were fed by single type palette bait and same fountain water. None of the rats was lost in any group during the study period.

Study Groups

Three main groups were identified and separated to be analyzed at the end of 15 days.

GROUP 1 (n = 10): Enoxaparin group
GROUP 2 (n = 10): Standard heparin group
GROUP 3 (n = 10): Serum physiologic group

Production of degloving injury

Anesthesia

Atropine sulphate (Atropin® amp. Biosel, Istanbul), 0.18 mg/kg was given to the rats used in the study intramuscularly for anesthetic premedication to prevent respiratory system obstruction. Prior to the operation, 50 mg/kg cefazolin sodium (Sefazol flk. Mustafa Nevzat, Istanbul) was administered intraperitoneally for prophylactic antibiotherapy. Ketamine hydrochloride (Ketalar flk. Parke Davis, Istanbul) 50 mg/kg was administered intraperitoneally to provide operative anesthesia. The status of anesthesia was checked according to the reaction of the rats to skin pressurizing every 5 minutes.

Postoperative analgesia was provided with morphine HCL (Morfin HCL amp. Biosel, Istanbul) subcutaneously in a dose of 10 mg/kg.

Degloving wound production and fixation method

After the anesthesia was performed with the described method, rats were covered with sterile green sheets after local field cleansing with betadine solution. A circular incision on the skin and subcutaneous tissues using a no 11 blade was performed on the tails of the rats 5 cm distally to the beginning of the tail. The proximal part of the incision was fixed and a 3 cm avulsion of the skin and subcutaneous tissues was obtained with controlled traction applied to the tail from the distal part of the incision, and then, the traction was ended. The two ends of the incision of the degloving injury field were approximated manually and primarily sutured with 4/0 traumatic vicryl (Vicryl, ETHICON. Somerville, NJ), after waiting for 10 minutes.

Drug treatment

After standard application of the same operations to all the rats in the experiment groups, daily injections of enoxaparin 100 U/kg, standard heparin 200 μ/kg, and serum physiologic solution 0.5 cc were administered s.c. in the 1st, 2nd, and the third groups, respectively after cleansing the local region with betadine solution (4, 5).

Termination of experiments

Experiments were terminated with killing of all the rats in all the groups at the end of day 15 by cervical dislocation.

Evaluation of the results

Following the termination of the experiments at day 15, first the necrotic areas in the tails were measured; then the clinical examinations were performed. Finally, samples were taken from the region of degloving injury for histopathological evaluation. The samples of tail tissue were fixed in 20 % neutral formaldehyde solution and were kept in 5 % formic acid. The materials taken into paraffin blocks after routine histopathological preparation were cross sectioned in 5μm by Leica Rotary microtome. Cross sections were stained with Hematoxylin-Eosin and Hematoxylin Van Giesson dyes and examined afterwards. Tissue micrographs were evaluated by a pathologist through a binocular research microscope attached to a digital camera. Tissue lesions in all the specimens were evaluated according to a scale prepared according to NPUAP (National Pressure Ulcer Advisory Panel) (6) and were staged as follows:

Stage 0; normal skin
Stage 1; inflammation dominant healthy skin and healthy epidermis
Stage 2; skin loss partially affecting the epidermis
Stage 3; full thickness skin loss, subcutaneous tissue necrosis, full thickness skin necrosis advancing into the fascia
Stage 4; full thickness skin loss and large tissue destruction and necrosis or necrosis advancing into the muscles, and full thickness lesion advancing even into the tendon, and joint capsule.

Results

Clinical findings

Skin necrosis was at a measurable level at the end of day 15. The dimension of the necrotic region in the enoxaparin group was

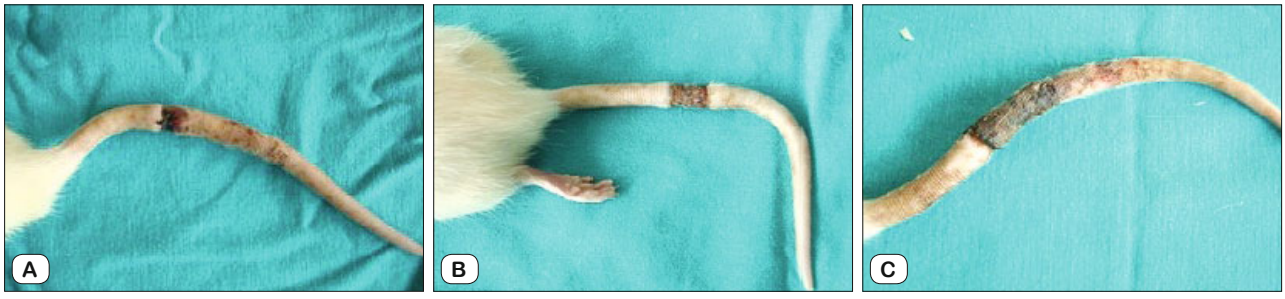

Fig. 1. A) Clinical viewing of Necrosis in Heparin Group (15th day). B) Clinical viewing of Necrosis in Enoxaparin Group (15th day). C) Clinical viewing of Necrosisin Serum Physiologic Group (15th day).

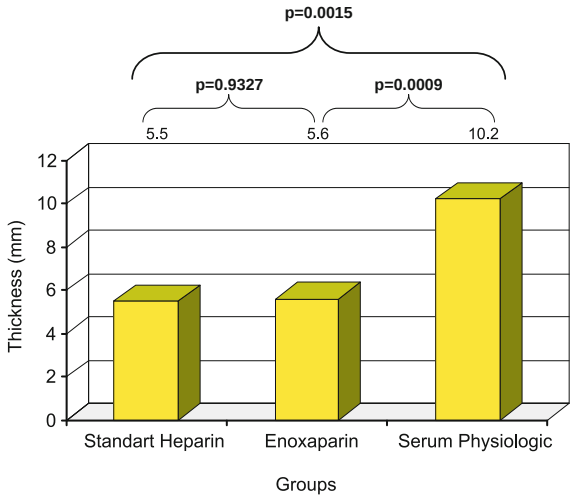

Fig. 2. Mean Necrosis Thickness in the Groups (15th day).

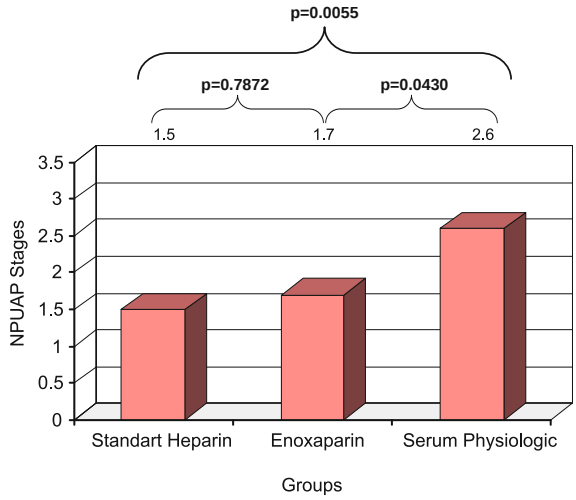

Fig. 3.Histopathological staging results in the Groups (15th day).

close to the one in the heparin group; however, the measured skin necrosis in the serum physiologic group was greater than that of the other two groups (Figs 1A, 1B, 1C).

The associations between the study groups and the control group were evaluated with unpaired Student T test for statistical analysis at the end of day 15. A p-value lower than 0.05 was accepted as significant (Fig. 2).

Histopathological findings

Staging values obtained with the evaluation of tissue samples taken from standard heparin, enoxaparin, and serum physiologic groups at the end of the 15th day are shown in Figure 3. Mann–Whitney U test was used for statistical analysis to compare the histological evaluation among the groups and p values less than 0.05 were accepted as significant.

Clinical findings, histopathological findings, and results of statistical analysis demonstrated that the differences between the heparin and enoxaparin groups were not significant. There was a significant difference between the results of the serum physiologic group and both the enoxaparin and standard heparin groups.

Discussion

"Degloving" is a soft tissue injury due to a traumatic avulsion of the skin and subcutaneous tissue from underlying deep fascia. Entin et al (7) evaluated the factors causing degloving injury in three groups. First is the presence of a cylinder circling with the compression forces on the extremity; second is the circling movement resulting in friction and an important torch; third is the presence of stripping forces that peel the soft tissues off the bones. These factors affect the degree and size of the trauma. In addition, Entin reported that the incidence of degloving injury is lower in young patients since they have a more elastic tissue. Clinical studies support the observations of Entin on age. Degloving injuries are seen more frequently in elderly patients. This is due to the changing resistance against shear forces and secondary changes in the quality of subcutaneous tissue. On the other hand, degloving injuries are seen more often in patients with skin and subcutaneous tissue pathology (such as rheumatoid arthritis) (3, 7).

In degloving injuries with viability problems, to give the opportunity for healing elf with postponing the debridement may result in irreversible infections. Serious extremity infections may even cause amputations (8).

The differentiating characteristic of a de-gloving injury from other injuries is the injury in neurovascular structures caused by the traumatic mechanisms. Vascular separation in the avulsed skin results in necrosis due to the separation of blood support. Pure degloving injury is an avulsion with intact underlying anatomic structures such as tendons, bones, and joints. Skin and subcutaneous tissues are damaged seriously and separate from underlying fascia and muscles. The most frequently used technique in this type of injury in which a variety of methods are used for treatment is debridement of the avulsed part and full thickness skin graft placement obtained from the degloved flap to cover the defect (9,10).

We developed circular shaped skin avulsions in rat tails in this study. This model resembles type I ring injury and could be used as a standard method. For good results in type II and type III ring injuries, precise microsurgical interventions are needed for treatment. However, no specific therapy is needed to maintain the viability of the avulsed skin in type I injuries. We thought that enoxaparin and standard heparin could be used in this clinical scenario to provide the viability of avulsed part of the skin. Other potential agents might be used in the treatment of degloving injuries in extremities.

Standard heparin and enoxaparin are anticoagulant agents that are intensively used in deep venous thrombosis prophylaxis in orthopedics clinics. The maintenance of viability of the skin grafts is thought to be due to the state of vascular anastomosis between the donor and recipient fields. We thought that these two agents could prevent the formation of microthrombi that might develop in the phase of vascular anastomosis formation, and thus effect tissue perfusion and viability when starting this study.

There are studies related to the use of low molecular weight heparins and standard heparins in the prevention of thrombosis and for wound healing in replantation, flap surgery and vascular

surgery (11). There is a healing mechanism common to all wounds. Standard heparin and LMWH's inhibit thrombin formation that is effective in the coagulation phase of the phases of wound healing.

It has been reported that heparin and derivatives fasten wound healing by decreasing the destruction in tissues and cells, and increasing neoangiogenesis, thus, supporting revascularization, granulation, and epithelisation (12, 13). In addition, heparin and derivatives positively affect wound healing by increasing the cellular aggregation and new vessel formation, and by decreasing cellular destruction (14–16). Heparin is demonstrated to augment wound healing by increasing the vascularization and arranging cellular renovation (12, 16). These studies in the literature support the results of our study.

Abbruzzese et al (17) reported that enoxaparin had no effect on local tissue thrombosis, inflammatory markers, and muscle necrosis. In spite of its potent in vivo activity, no effect to decrease thrombus formation after muscle injury, thrombosis or ischemia reperfusion of enoxaparin was detected. Hadlock et al (18) evaluated the effects of subcutaneous low molecular weight heparin in the treatment of microarterial thrombosis in rats, and reported that enoxaparin had no effect on microarterial thrombus formation. Although these studies suggest that low molecular weight heparins have no effect on microarterial thrombosis, we saw that they had clinical positive effects.

We took all the samples from the rat tails distal to the field of degloving trauma for histopathological examinations. In this way, we evaluated the wound healing area more efficiently. The appearance of inflammatory cells and a healthy epidermis was predominant on histopathological examination at the end of day 15 in standard heparin and enoxaparin groups. The microscopic appearances of the specimens of the two groups were similar as well. Full thickness skin loss and skin loss partially affecting the epidermis were preponderant in the group that received physiologic serum. Thus, standard heparin and enoxaparin positively affected the healing of degloving injuries during this time period. Statistical analysis supported us. No differences were found between the enoxaparin group and heparin group in statistical analysis ($p > 0.005$). This result demonstrated us that when compared no advantage of either one was identified among the positive effects of standard heparin and enoxaparin on healing of degloving injuries. We thought that histopathological examinations might demonstrate personal differences no matter what; therefore, obtaining an optimum result might have been impossible. We planned to overcome this disadvantage by performing a clinical evaluation. Clinical evaluation results were similar to the results of the histopathological evaluations.

In conclusion, with clinical and histopathological results, when taken separately or together, it was demonstrated that standard heparin and enoxaparin had positive effects on the viability and healing of de-gloving injuries, which most certainly supported our hypothesis. Although there is no similar study in humans in accordance with the findings of our study, we came to the decision that standard heparin and enoxaparin have positive effects on the viability and healing of degloving injuries.

Conclusions

The treatment of degloving injuries, pure or with fractures, poses serious problems in orthopedic clinics.

Standard heparin and enoxaparin have positive effects on wound healing.

Standard heparin and enoxaparin are antithrombotic drugs and their positive effects on the viability of degloving injuries were demonstrated as a result of our experimental study.

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