



## Effects of enoxaparin and rivaroxaban on tissue survival in skin degloving injury: an experimental study

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**Objective:** The aim of this study was to evaluate the effects of the antithrombotic agents enoxaparin and rivaroxaban on tissue survival following skin degloving injury in an experimental rat tail model.

**Methods:** The study included 24 rats divided into three equal groups of 8; the enoxaparin group (Group 1), the rivaroxaban group (Group 2) and the saline control group (Group 3). A degloving injury was created by making a circular incision 5 cm distal to the base of the tail; manual traction was applied to the tail skin distal to the incision. After 15 minutes, the ends of the incision were sutured back in place. Antithrombotic agents were administered immediately after suturing and repeated once a day for 15 days. At the end of Day 15, the experiment was terminated. Gross morphological tissue survival and histopathology were evaluated.

**Results:** Histopathological examination of the enoxaparin and rivaroxaban groups revealed that the skin was mostly normal or intact with minimal inflammation. The mean length of necrotic area was significantly higher in the saline group compared to the enoxaparin and rivaroxaban groups ( $p < 0.05$ ). No statistically significant differences were noted between the rivaroxaban and enoxaparin groups ( $p = 0.451$ ). The mean extent of skin necrosis was significantly higher in the control group than the study groups ( $p < 0.05$ ), while there was no significant difference in the length of necrotic area between Group 1 and 2 ( $p = 0.722$ ).

**Conclusion:** Rivaroxaban and enoxaparin improved tissue survival in skin degloving injuries in terms of gross morphological and histopathological findings in a rat tail model.

**Key words:** Degloving injury; enoxaparin; rat; rivaroxaban; tissue survival.

Ring avulsion injury, which usually involves the ring finger, occurs due to traumatic avulsion of the skin and subcutaneous tissue from the underlying deep fascia.<sup>[1,2]</sup>

Urbaniak et al. classified ring avulsion injuries into three types: Type 1, circulation adequate; Type 2, cir-

ulation inadequate; and Type 3, complete degloving or complete amputation.<sup>[3]</sup> In Type 2 and 3 ring injuries, excellent microsurgery is needed for good outcomes.<sup>[1-4]</sup> In Type 1 ring injuries, it may sometimes be possible to use the degloved skin as a full-thickness graft when re-

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plantation or revascularization is not possible. Survival of the avulsed flap requires blood circulation between the flap and flap bed, which is in turn dependent on formation of anastomoses between the vessels.<sup>[5]</sup> Such formation takes approximately 72 hours.<sup>[6]</sup> At this point, pharmacological agents might aid in revascularization, improve microcirculation and prevent thrombosis of newly formed vessels. The inhibition of coagulation factors Xa and IIa is augmented by enoxaparin. Factor Xa catalyzes the conversion of prothrombin to thrombin and ultimately prevents fibrin clot formation.<sup>[7]</sup> Enoxaparin has been reported to improve capillary density by 33% without any bleeding complications,<sup>[8]</sup> and to aid in the survival of congested flaps in animal studies.<sup>[9]</sup> Rivaroxaban, a novel oral antithrombotic agent, binds specifically and directly to the active site of factor Xa and has a longer half-life than enoxaparin.<sup>[7,10]</sup> Rivaroxaban has been shown to prevent arterial and venous thrombosis in experimental animal studies.<sup>[11]</sup>

We hypothesized that enoxaparin and the oral agent rivaroxaban, which has a similar mechanism of action as enoxaparin, might improve the survival of avulsed skin in rat tails by facilitating microcirculation and preventing microthrombus formation.

The aim of this study was to investigate the effects of enoxaparin and rivaroxaban on tissue survival in terms of gross morphological and histopathological findings following skin degloving injury in an experimental rat tail model.

## Materials and methods

All procedures were performed in the Practice and Research Laboratory of our University after approval was obtained from the Local Committee on Animal Research Ethics. The study included 24 male Sprague-Dawley rats (weight: 230 to 280 g). Rats were randomized into 3 groups (n=8): the enoxaparin group (Group 1), the rivaroxaban group (Group 2) and the saline control group (Group 3).

The rat was anesthetized subcutaneously with a combination of 50 mg/kg ketamine hydrochloride (Ketalar®; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun®; Bayer, Istanbul, Turkey). The surgical method of choice was the degloving injury model reported by Oz-tuna et al.<sup>[12]</sup> The skin and subcutaneous tissues were incised circumferentially 5 cm distal to the base of the tail. Next, using the thumbs and index finger, a moderate manual force was applied to the tail at the distal end of the incision. When a 3-cm-long avulsion of the skin and subcutaneous tissue from the underlying tendon and vascular tissue was achieved, traction was ceased (Fig. 1).

Fifteen minutes after injury, the ends of the incision were re-approximated and sutured using 4/0 vicryl. A single dose of buprenorphine (0.04 mg/kg) was applied subcutaneously as an analgesic immediately after the incision was closed, and continued daily for 15 days.

Immediately after the degloved skin was sutured, rats in Group 1 rats received a subcutaneous dose of enoxaparin (100 anti-Xa IU/kg) (Clexane®; Sanofi-Aventis, Istanbul, Turkey).<sup>[9]</sup> Group 2 rats received 3 mg/kg of rivaroxaban (Xarelto®; Bayer, Istanbul, Turkey) in 2 mL of saline solution by gastric lavage.<sup>[13]</sup> In Group 3 rats, 1 mL of saline solution (I.E. Ulagay, Istanbul, Turkey) was applied subcutaneously. Drug administration continued for 15 days. During the study period, no rat was excluded because of death or self-mutilation.

Rats were sacrificed on Day 15 and the lengths of the necrotic tail regions were measured in millimeters using a ruler. Circumferential lengths of necrosis at the proximal and distal boundaries were measured. In the inter-group comparisons, a reduction in the length of necrotic regions was considered 'positive' and an increase in the length of necrosis was considered 'negative'.

Tissues samples from all groups were prepared for embedding in paraffin and sectioned in longitudinal orientation, fixed in 10% neutral buffered formalin and stored in 5% formic acid. Sections were stained with hematoxylin-eosin (HE) and examined by light microscopy by an experienced pathologist. Tissue lesion sections were evaluated using the scale developed by the National Pressure Ulcer Advisory Panel<sup>[14]</sup> (NPUAP; 1989) (Table 1). Stage 1 and 2 ulcers are generally considered to be the 'least severe' and Stage 4 ulcers 'the most severe'.

All data were recorded and analyzed using SPSS for Windows v.15.0 (SPSS Inc., Chicago, IL, USA). The Kruskal-Wallis test was used for statistical analysis of gross morphological and histopathological differences among groups. The Tukey/Bonferroni correction was applied to correct for comparative differences. Compari-



**Fig. 1.** The creation of a degloving injury site in the tail of the rats. [Color figure can be viewed in the online issue, which is available at [www.aott.org.tr](http://www.aott.org.tr)]

**Table 1.** The National Pressure Ulcer Advisory Panel (NPUAP) scale.

Stage	
0	Normal skin
1	Intact skin and intact epidermis with inflammation
2	Partial thickness loss of skin layers involving epidermis
3	Full-thickness skin loss, or necrosis of subcutaneous tissue extending down to the fascia
4	Full-thickness skin loss with extensive destruction, tissue necrosis or damage to muscle, bone or supporting structures such as tendon and joint capsule

sons at the 95% confidence interval and that were associated with  $p$  values of less than 0.05 were considered significant.

## Results

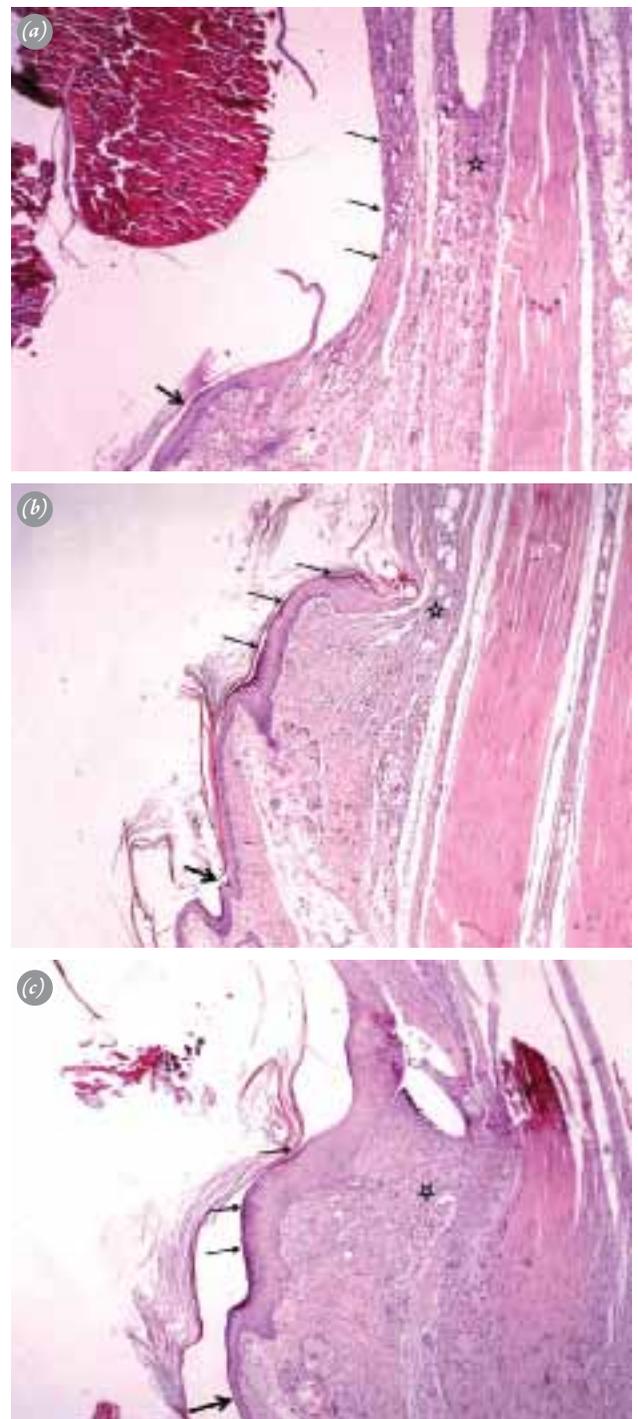
Ischemic areas had developed at the avulsed tail regions at the end of Day 15. Demarcation lines were evident and the length of each region was measurable. In the enoxaparin and rivaroxaban groups, 6 of 8 tails had healed completely or showed little necrosis (Fig. 2a), whereas 5 of 8 rats in the control group had prominent necrotic areas (Fig. 2b). The mean length of the necrotic area was significantly higher in the control group than in the other groups ( $p < 0.05$ ). There were no statistically significant differences in the length of the necrotic area between the enoxaparin and rivaroxaban groups ( $p = 0.451$ ) (Table 2).

The mean histopathological stage was significantly higher in the control group (Fig. 3a) compared to the enoxaparin (Fig. 3b) and rivaroxaban (Fig. 3c) groups ( $p < 0.05$  for both comparisons). There was no statistically significant differences in histopathological stage be-



**Fig. 2.** (a) Intact epidermis in the rivaroxaban and enoxaparin group and (b) necrotic part of skin of the tail in the control group. [Color figure can be viewed in the online issue, which is available at [www.aott.org.tr](http://www.aott.org.tr)]

tween the enoxaparin and rivaroxaban groups ( $p = 0.722$ ) (Table 2).



**Fig. 3.** Histological examination showed, (a) loss of epithelium (thin arrows), dense infiltration of inflammatory cells reaching to muscular tissue, and wide tissue destruction (star) in a rat from the control group, (b, c) full-thickness superficial re-epithelization (thin arrows) with inflammation (star) in the rats from the rivaroxaban and enoxaparin groups, respectively, just near to the normal epithelium (large arrows) (HE,  $\times 40$ ). [Color figure can be viewed in the online issue, which is available at [www.aott.org.tr](http://www.aott.org.tr)]

**Table 2.** The length of necrosis (mean±SD) in the tails of rats and histopathological stages according to groups.

	Group 1 (Enoxaparin) (Mean±SD)	Group 2 (Rivaroxaban) (Mean±SD)	Group 3 (Saline) (Mean±SD)
Length of necrosis (mm)	4±2	5±2	14±5
Histopathological stage	0.92±0.30	0.96±0.38	2.46±0.82

## Discussion

In the present study, enoxaparin and rivaroxaban was beneficial for the healing of avulsed skin in a rat tail model. We hypothesize that enoxaparin and rivaroxaban increased microvascular blood circulation via prevention of microthrombus formation, thus enabling delivery of more growth factors to the injured area, enhancing revascularization.

Healing problems encountered in degloving injuries have stimulated studies on factors likely to enhance microcirculation and the development of new therapeutic strategies.<sup>[8,12,15,16]</sup> Enoxaparin has been reported to improve microcirculation,<sup>[7]</sup> increase capillary blood flow,<sup>[6,17]</sup> and reduce interstitial inflammation and capillary compression.<sup>[18]</sup> Rivaroxaban, a novel antithrombotic agent, binds specifically and directly to the active site of factor Xa and has a longer half-life than does enoxaparin.<sup>[10-11]</sup> Rivaroxaban can be administered orally.<sup>[19]</sup>

Oztuna et al.<sup>[12]</sup> developed the rat tail degloving injury model. After simple reattachment of an avulsed flap, pentoxifylline was administered for 10 days. The authors measured the length of necrotic skin and showed that pentoxifylline improved tissue preservation and was of great utility in skin degloving injuries. Milcheski et al.<sup>[16]</sup> developed a hind limb degloving injury model in rats in which flaps were repositioned and sutured. The control group received only a saline solution, a second group received pentoxifylline and a third group allopurinol and were observed over 7 days. Allopurinol and pentoxifylline reduced the extent of necrosis. The necrotic borders were irregular in the injury model of Milcheski et al.<sup>[16]</sup> and it was necessary to measure the necrotic areas for comparison of groups. However, Oztuna et al.<sup>[12]</sup> measured the necrotic length in the rat tail and reported statistically significant results. In the present study, all rats were of similar weight, and the necrotic borders were near-circumferential, both proximally and distally. Additionally, the circumferential lengths of necrosis (both proximally and distally) were similar. We did not measure necrotic areas because we found a statistically significant difference in terms of length of necrosis between the enoxaparin and rivaroxaban group and the control group. Therefore, we agree with Oztuna et al.<sup>[12]</sup>

that simple measurement of the length of necrosis is adequate when Type 1 ring degloving injuries are studied using the rat tail model. Our duration of therapy (15 days) was longer than previous studies.<sup>[12,16]</sup> As our results are similar to those of both cited studies, we suggest that long-term therapy may be unnecessary.

We reattached avulsed skin 15 minutes after the creation of a degloving injury. Enoxaparin and rivaroxaban therapy commenced immediately. Our treatment protocol can be considered fast when taking into account the timing of suturing and initiation of therapy. We are aware that it is difficult to create these ideal conditions in current practices. Nevertheless, we know that the dermal vascular plexus is interrupted due to the injury. However, the formation of these capillary anastomoses requires approximately 72 hours.<sup>[6]</sup> Therefore, vascular structures would not be reinstated simply because the wound is sutured early. In addition, Urbaniak et al.<sup>[3]</sup> suggested that skin repair must be delayed for a few days in order not to cause tension around circumferential laceration in Type 1 degloving injuries. Ultimately, we believe that minor changes in the timing of suturing of avulsed skin and minor deviations of the lag period between surgery and the first implementation of treatment would not have a significant effect on tissue survival.

Our study had several limitations. First, we used the Type 1 ring injury model, which is a simple injury with adequate circulation of the skin flap. Therefore, our results are not relevant for degloving injuries with inadequate circulation, infections, crush injury or amputation. Second, we repaired the avulsed skin rapidly; this may not always be possible in clinical practice. Third, we administered drugs for 15 days. Shorter therapy protocol may yield similar results. Fourth, the use of moderate manual force to create a skin degloving injury may be difficult to reproduce and we did not evaluate the degree of neurovascular damage of the degloving injury. Fifth, it is important to consider that the healing capacity of rats is greater than that of humans. Finally, we did not use different doses of rivaroxaban and enoxaparin. Future studies should be designed using different injury models and therapy protocols.

In conclusion, enoxaparin and rivaroxaban signifi-

cantly improved survival of skin degloving injuries in a rat tail model. As rivaroxaban is an oral drug, its use is easier than that of the subcutaneous administration of enoxaparin. Further studies are necessary to assess whether these results are applicable in humans.

**Conflicts of Interest:** No conflicts declared.

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