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A comparison of rat degloving injury models

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ABSTRACT

Objective: Two different rat models for degloving injury were described in the literature. Our aim in this study is to compare these rat models to determine which one is more reliable and reproducible.
Methods: We surgically induced degloving injury on tails and left hindlimbs of Wistar albino rats (n = 8), and sutured the avulsed tissues back in their original positions after a waiting period. We observed the changes in the avulsed flaps every other day for 10 days. At the end of follow-up period we evaluated the lesions in avulsed flaps by macroscopic measurement of necrosis and histological ulcer scoring using the National Pressure Ulcer Advisory Panel (NPUAP) Scale.
Results: The average length of necrosis in avulsed tail flaps was 28.42 ± 3.04 mm, whereas there was no necrosis in avulsed hindlimb flaps ($p < 0.05$). The average ulcer score of the lesions in tail and left hindlimb were 3.42 ± 0.78, and 1.28 ± 0.48, respectively ($p < 0.05$). Despite the lack of visible necrosis TUNEL staining revealed an increased amount of apoptotic cells in avulsed hindlimb flaps. Literature review revealed a significant variability in previous studies in terms of the amount of necrosis observed in tail degloving injury model.
Conclusion: Tail degloving injury model proved to be a more reliable animal model for degloving injuries. However, standardization of the magnitude of degloving force is required to decrease the variability of necrosis observed in the literature.

Introduction

Degloving (avulsion) injury is defined as the separation of the skin and subcutaneous tissues from the underlying deep fascia.[1,2] The most common emergency treatment of degloving injury is suturing the avulsed tissue back in its original anatomical position. Despite the treatment, the outcome is usually partial or total necrosis of the avulsed tissue since the subcutaneous vascular plexus nourishing the overlying tissue is injured.[1,2] Experimental studies focusing on the treatment of degloving injuries used two different rat models for degloving injury: the tail degloving injury model described by Oztuna et al,[3] and the hindlimb degloving injury model described by Milcheski et al.[4] For an objective evaluation of the effects of different treatment methods, experimental degloving injury models should be reliable and easily reproducible. More specifically the extent of skin necrosis should be similar in all animals that are subjected to the same method of degloving injury.

Our aim in this study is to compare the previously described rat models for degloving injury in terms of reliability and reproducibility. We induced degloving injuries in rat tails and hindlimbs and measured the amount of tissue necrosis in each model. We also examined and compared the degloved tissues histologically.

Materials and methods

All the animal experiments were approved by Institutional Animal Care and Use Committee (protocol # 2015-43). We created degloving injury in both tails and left hindlimbs of Wistar albino rats (n = 8) under anesthesia induced by intraperitoneal (ip.) injection of a mixture of 50 mg/kg Ketamine Hydrochloride (Ketalar®, Pfizer, İstanbul), and 10 mg/kg Xylazine (Rompun®, Bayer, İstanbul).

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Surgical technique

Tail degloving injury model

We performed a circular incision of skin and subcutaneous tissue 5 cm distal to the root of tail protecting underlying tendon and vascular structures as described previously.[3] Then, we applied a traction force to the tail distal to the incision using thumb and index fingers, avulsing the skin and subcutaneous tissue 3 cm distally (Fig. 1A). We preserved the distal subcutaneous attachments of the tail skin. After 15 min, we sutured the avulsed tissue back in its original position using 5/0 polypropylene suture (Prolene®, Ethicon Inc., Somerville, NJ).

Hindlimb degloving injury model

We first performed a circular incision of skin and subcutaneous tissue in proximal left hindlimb following inguinal and gluteal creases. Afterwards, we avulsed the skin down to the ankle joint using a surgical towel clamp as described previously (Fig. 1B).[4] We sutured the resultant distally based avulsed flap in its original position 5 min later using 5/0 polypropylene suture (Ethicon Inc.).

Data collection

Postoperatively, we applied povidone/iodine solution (Batticon, ADEKA, İstanbul) to the surgical wounds to prevent infection. We survived the animals until postoperative day 10. We observed the changes in the avulsed flaps every other day, and took pictures of the avulsed flaps under general anesthesia. On postoperative day 10, we measured the longest distance between incision and tip of necrotic skin using a ruler in tails and left hindlimbs of the animals. We considered warm, pink-white and pliable tissue as viable skin; and brown-black, cold and hardened tissue as necrotic skin.

Following measurements, we removed the avulsed flaps as a single circular block for further histologic examination. We fixed the tissues in 10% neutral buffered formalin and kept them in 5% formic acid until further processing. Following routine histopathological preparation, we cut the embedded tissues into 5 μm thick sections and stained the sections with Hematoxylin-Eosin (HE) stain. The stained sections were evaluated under light microscope by an experienced pathologist blinded to the study. The tissue lesions were scored using National Pressure Ulcer Advisory Panel (NPUAP) Scale (Table 1).[5]

Additionally, we performed TUNEL Assay in tissue sections to evaluate the extent of apoptosis. We cut the embedded tissues into 5–6 μm thick sections and transferred the sections to polylysine coated histologic slides. We stained the apoptotic cells using ApopTag Plus Peroxidase InSitu Apoptosis Detection Kit (S7101, Chemicon, MA) following the manufacturer's instructions. Briefly, we deparaffinized the sections using decreasing concentrations of xylene and alcohol. We incubated the slides with 0.05% proteinase K solution for 10 min followed by %3 hydrogen peroxide solution for 5 min to prevent endogenous peroxidase activity. Following phosphate buffered saline (PBS) wash, we incubated the slides first with Equilibration Buffer for 6 min, and then with a working solution (%70 μl Reaction Buffer + %30 TdT Enzyme) at 37 °C for 60 min in a humid chamber. We incubated the slides with Stop/WashBuffer for 10 min, and anti-digoxigenin-peroxidase for 30 min. We used diaminobenzidine (DAB) enzyme substrate to stain the apoptotic cells and counterstained the nuclei with Harris Hematoxylin prior to coverslipping the slides. The stained sections were evaluated by a Novel N-800M microscope by an experienced pathologist blinded to the study.

Table 1
Pressure ulcer definition and stages.

Stage	Histological findings
0	Normal skin
1	Visible inflammation but intact skin with non-blanchable redness of a localized area.
2	Partial thickness loss of dermis presenting as a shallow open ulcer
3	Full thickness tissue loss. Subcutaneous fat may be visible but bone, tendon or muscle are not exposed
4	Full thickness tissue loss with exposed bone, tendon or muscle

Statistical analysis

Statistical analysis was performed by Student's t test using SPSS statistical software 18.0. Statistical significance was set at $p < 0.05$.

Results

All animals survived until the study endpoint. Avulsed tail flaps were cyanotic, and distal tail segments were edematous in all animals on postoperative day 2. The cyanosis in the avulsed tail flaps progressed and we observed a full thickness necrosis by postoperative day 10 (Fig. 2). On the other hand, there was a mild cyanosis along the incision lines in avulsed hindlimb flaps on postoperative day 2 which disappeared totally on postoperative day 4. We did not observe any surgical wound infection but there was a partial dehiscence in one hindlimb wound which healed by secondary intention. We also observed a total wound dehiscence measuring 4 mm in the tails of two rats secondary to full thickness necrosis of the skin. The average length of necrosis in avulsed tail flaps was 28.42 ± 3.04 mm, whereas there was no necrosis in avulsed hindlimb flaps ($p < 0.05$) (Fig. 3).

Histologically, we observed full thickness epidermal loss and ulceration accompanied by a heavy inflammatory infiltrate in the

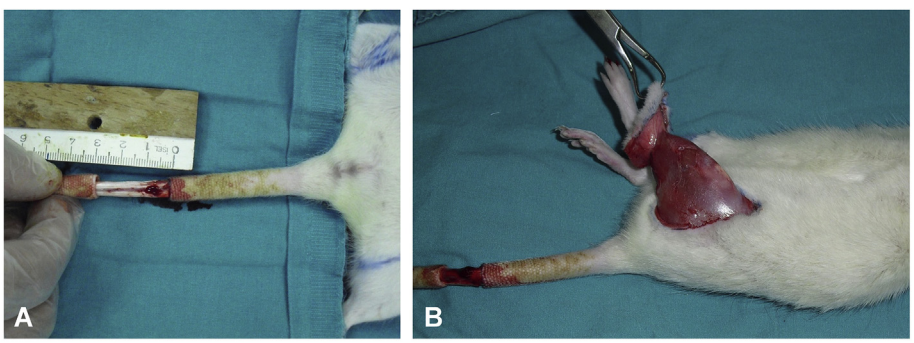

Fig. 1. A) A 3 cm long avulsed tail flap and the underlying tendon and dorsal vascular structures. **B)** Degloved hindlimb flap following a proximal circumferential incision.

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