Validation of a surgical wound healing model in Sprague-Dawley rats

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Abstract

BACKGROUND: Wound healing is a biological process that can be altered by drugs in specific therapeutic classes. The aim of the study was to validate a wound healing rat model with tensile strength measurements. MATERIALS AND METHODS: Two (2) full thickness skin incisions (50 mm in length) were created in the thoracolumbar area of adult male Sprague-Dawley rats and the wounds were sutured using a standardized suture pattern. Daily intramuscular saline and dexamethasone (4 mg/kg) injections were used as negative and positive controls, respectively. On Day 4 after surgery, rectangular skin samples were tested within 6 hrs of collection. Skin samples with standardized dimensions (15 X 40mm) were tested under tension until wound ruptured and the remaining ten (10) mm of wound was used for histology evaluation (H&E). The maximal tension was recorded for each skin sample and compared between groups. RESULTS: Mean tensile strength was 0.55N in saline-treated rats and 0.23N in dexamethasone-treated rats ($p<0.05$). A significant reduction in body weight on Day 4 when compared to pretreatment was also noted for the dexamethasone group ($p<0.0005$) but no statistically significant difference was noted for saline-treated rats. Histology was compatible with a delayed wound healing in dexamethasone-treated rats. CONCLUSION: The model identified a dexamethasone-induced reduction in tensile strength with characteristic histological changes. This rat model is considered adequate for preclinical evaluation of perioperative wound healing.

Materials and Methods

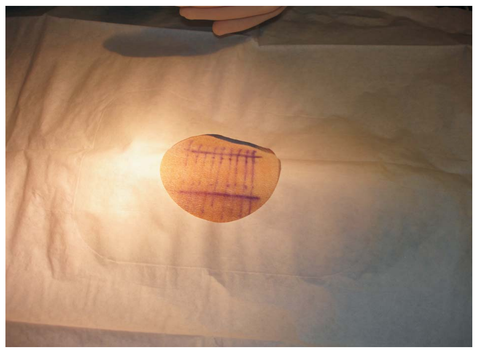
Animal Preparation and Analgesia
Animals were anesthetized with isoflurane and were placed on a heating pad during the surgery. A surgical skin marker was used to delineate 2 incision sites of 5 cm in length with marks for sutures every 0.5 cm. The skin was aseptically prepared using chlorexidine and isopropyl alcohol. The surgical site was covered with gauze soaked in chlorexidine pending surgery initiation. Buprenorphine (0.03ml/animal, 0.3 mg/ml) was given at least twice a day, starting after completion of wound suture for at least 2 days following surgery.

Surgery
Animals received prophylactic antibiotics (penicillin procaine, 300 000 IU/ml, 0.02 ml/animal)prior to the surgery and twice a day for two (2) days following surgery. Once deep anesthesia was confirmed, two full-thickness cutaneous incisions were made longitudinally, one on each side of mid-line. The subcutaneous tissue was also incised to ensure that it did not interfere with tensile strength measurement. The wound was sutured with simple interrupted sutures using non-absorbable sutures (polypropylene 4-0) and a reverse cutting needle. At surgery completion, animals were given 2.5 ml of Lactate Ringer's solution as fluid therapy. Half of the animals received dexamethasone (4 mg/kg, IM) for 4 days while the second half of the animals received saline at the same dose volume.

Biomechanical testing
Skin samples were collected from all animals on Day 4 and were disposed horizontally on DMEM and penicillin and/or streptomycin-soaked gauzes in hermetic containers pending analysis. Two (2) rectangular samples with standard dimensions were excised from each skin samples as shown in Figures 1 and 2. The samples were mounted on 2 bases which were attached to the loading cell in the vertical plane as shown in Figures 3 and 4. A Mach-1 A400.25 biomechanical tester was used to apply gradually increasing tensile load until rupture. Figure 3 represent the force measured by the load cell of the biomechanical tester during the tensile strength test. Tensile strengths were performed blinded to study groups.

Histology
Histopathological examination was performed on collected wound sites (at least 3 sections) on approximately 1 cm of each wound (caudal) from each animal. Tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosine.

Surgery



Tensile strength

Figure 1

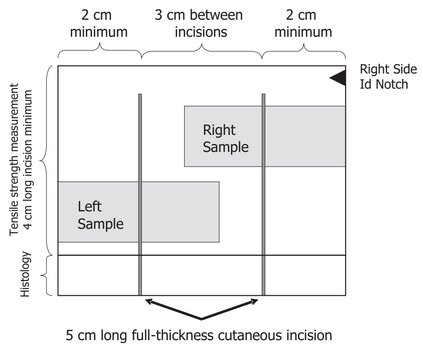


Figure 2

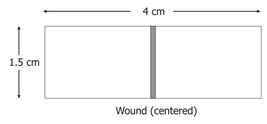


Figure 3

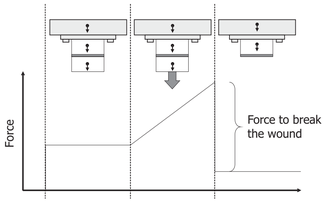
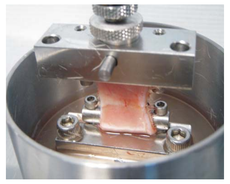
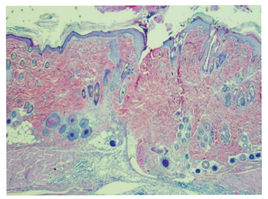


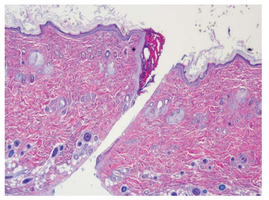
Figure 4



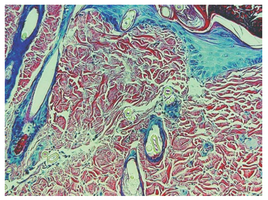
Histopathology



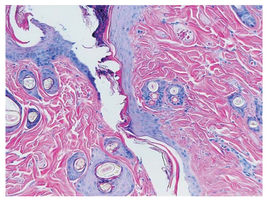
Saline treated animal Day 4 (250X)



Dexamethasone treated animal Day 4 (250X)



Saline treated animal Day 4 (1000X)



Dexamethasone treated animal Day 4 (1000X)

Results and Discussion

Development of new drugs including neovascularization inhibitors (e.g. VEGF) require non-clinical demonstration of the absence of adverse effects on wound healing[1]. Drug induced delayed wound healing has been reported in the rats [2] and TGF-β and IGF-I have been suggested as potential cellular mediators for the negative effects of steroids[3]. This study was undertaken to validate a non-clinical model allowing identification of delayed wound healing resulting from drug administration.

Tensile strength evaluations revealed that dexamethasone induced a statistically significant reduction of tensile strength when compared to treatment with saline. The average tensile strength for animals treated with dexamethasone was 23.24 grams compared to 55.08 grams for animals receiving saline ($P<0.05$).

Histopathological changes at wound edges were considered adequate and expected sequella corresponding to the well-known inflammatory/exudative phase of wound healing. Wound evaluations also revealed efficient wound healing by first intention in saline treated animals. As illustrated above, epithelial migration was adequate in the saline treated group while dexamethasone treated animals presented evidences of delayed wound healing.

Both evaluation modalities (tensile strength and histopathology) were useful for assessment of wound healing. Tensile strength provides a quantitative evaluation of the healing process which can be included with study results for regulatory submission.

Conclusion

This surgical rat model using tensile strength measurements and histopathological evaluations is considered valid to identify drug induced delayed wound healing.

References
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Special thanks to Isabelle Gilbert and Caroline Charette for their help during the study.