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Effects of burn location and investigator on burn depth in a porcine model

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ABSTRACT

Introduction: In order to be useful, animal models should be reproducible and consistent regardless of sampling bias, investigator creating burn, and burn location. We determined the variability in burn depth based on biopsy location, burn location and investigator in a porcine model of partial thickness burns.

Methods: 24 partial thickness burns (2.5 cm by 2.5 cm each) were created on the backs of 2 anesthetized pigs by 2 investigators (one experienced, one inexperienced) using a previously validated model. In one of the pigs, the necrotic epidermis covering each burn was removed. Five full thickness 4 mm punch biopsies were obtained 1 h after injury from the four corners and center of the burns and stained with Hematoxylin and Eosin and Masson's trichrome for determination of burn depth by a board certified dermatopathologist blinded to burn location and investigator. Comparisons of burn depth by biopsy location, burn location and investigator were performed with *t*-tests and ANOVA as appropriate.

Results: The mean (SD) depth of injury to blood vessels (the main determinant of burn progression) in debrided and non-debrided pigs pooled together was 1.8 (0.3) mm, which included 75% of the dermal depth. Non-debrided burns were 0.24 mm deeper than debrided burns ($P < 0.001$). Burn depth increased marginally from cephalic to caudal in non-debrided burns, but showed no statistical differences for these locations, in debrided burns. Additionally, there were also no statistical differences in burn depths from midline to lateral in either of these burn types. Burn depth was similar for both investigators and among biopsy locations.

Conclusions: Burn depth was greater for caudal locations in non-debrided burns and overall non-debrided burns were deeper than debrided burns. However, burn depth did not differ based on investigator, biopsy site, and medial–lateral location.

1. Introduction

Animal burn models are essential to the development of novel therapies and diagnostics. They also lead to a better understanding of the underlying mechanisms leading to burn progression and healing. Of all animal burn models, the porcine model is most commonly used due to the similarity of pig skin to that of humans [1–3].

We have previously developed and validated a porcine model of partial thickness burns in which a contact burn is created with a preheated aluminum bar that is applied to the

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skin on the back of the animal [4–6]. This model, as well as other similar models [7–13], relies on histological evaluation of skin punch biopsies taken at different time points from different locations on the same burn. With most standardized burn models, a basic assumption is that the burns are all uniform with very little variation in burn depth among and within burns and between different investigators who create the burns. A potential limitation of all animal burn models utilizing histological assessment of burn depth and healing is sampling bias where depth of injury and healing may differ based on the exact location of the biopsy within the wound. Thus differences in burn depth between and among the various burns may erroneously be attributed to differences in therapy. While a large number of porcine burn models have been described, other than a few reports on differences based on anatomical location [10,14], none have evaluated consistency of burn depth between various investigators and within the same burns.

In order to be useful, animal models should be reproducible and consistent regardless of sampling bias, investigator creating burn, and burn location. We determined the variability in burn depth based on biopsy location, burn location and investigator in a porcine model of partial thickness burns.

2. Methods

2.1. Study design

A prospective animal experiment was conducted to determine variation in burn depth based on investigator, anatomical location, and site of burn biopsy within the wound. The study was approved by the Institutional Animal Care and Use Committee. All animals were handled in accordance with the guidelines issued by the National Research Council [15].

2.2. Animals

Two female Yorkshire pigs weighing 20–25 kg were used for the study. The animals were acclimated for seven days prior to surgery. The animals were housed in separate pens and fed with a diet of Purina Mills Lab Diet food 5083 (Fisher and Sons) and given water ad lib. The animals were subjected to daily cycles of dark (12 h) and dark (12 h).

2.3. Sedation, anesthesia, and analgesia

The animals were fasted overnight and sedated with a combination of acepromazine 0.1 mg/kg, atropine 0.02 mg/kg, ketamine 20 mg/kg, and xylazine 2.2 mg/kg by intramuscular injection. The pigs were endotracheally intubated and maintained under a surgical plane of anesthesia with isoflurane 1.0–5.0% mixed with O_2 USP. The concentration of isoflurane was titrated to ensure adequate anesthesia while avoiding hypoventilation and apnea.

2.4. Burn creation and local treatment

The flank and back hair were clipped with electric clippers and the skin was scrubbed with soap and water. A rolled towel was placed under the lower abdomen of the animal to reduce a sway or lumbar lordosis during the wounding procedure.

A previously validated deep partial thickness porcine burn model was used in this study [4]. While under anesthesia, 24 deep partial thickness burns were created using a 2.5 by 2.5 by 7.5 mm, 150-g aluminum bar heated in a water bath to 80 °C (Fig. 1). Prior to creating the burns the location of the burns was traced with a pen using a template that ensured that the horizontal distance between the columns of burns was at least 2 cm and that the vertical distance between the rows of burns was at least 4 cm. Four columns of six burns each were created adjacent to the spinous processes of the vertebral bodies over the paravertebral muscles that served as a firm surface on which burns were created. The aluminum bar was blotted dry so as not to cause a burn from steam or scalding and then applied perpendicular to the skin surface, with a 2-kg pressure load for a period of 20 s [6]. This type of injury creates a deep partial thickness burn that involved approximately 50% of the thickness of the pig's dermis. After creating a burn, the surface of the aluminum bar in contact with the skin was wiped with an alcohol swab to remove any adherent debris. The bar was then placed back in the water bath for at least 2 min to allow proper temperature equilibration for the next burn.

Following the burning procedure the necrotic epidermis was gently removed from the surface of the burns in one of the animals by scraping the burn (debrided burn) with the blunt handle of a forceps. This is done to replicate rupture and subsequent removal of the burn blister in humans, since burns in pigs do not form blisters, possibly due to their dense skin [16]. In the second pig, the necrotic epidermis was left intact (non-debrided burn). Approximately one hour after injury, full thickness 4 mm punch biopsies were taken from all four corners (beginning at top left corner and continuing sequentially in a clockwise direction) and the middle of all burns. The animal was then euthanized with a lethal dose of pentobarbital.

2.5. Study outcomes

Burn depth was determined using histopathological assessment of the skin biopsies taken after the burn. Burn depth was determined by averaging 3 measurements of the vertical distance from the basement membrane to the deepest level of the burns at the center and at the two ends of the tissue slide. Depth of injury was measured separately for collagen, hair follicles and blood vessels.

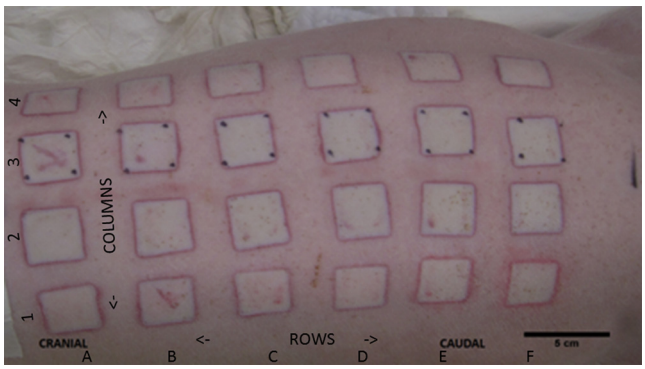

Fig. 1 – Creation of burns.