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The pig as an experimental model for mid-dermal burns research

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ABSTRACT

This was a novel, prospective and interventional animal study designed to develop and evaluate a new infliction device for the experimental burn model.

Four paired sets of contact burns measuring 36 mm diameter were inflicted on the dorsum of an anesthetized pig using a stainless steel round bar heated up to 80–110 °C. The bar was applied using a push–pull force gauge designed to control 1 kgf mechanical force applied to the skin for a period of 20 s. The left dorsum was used for macroscopic observation and the right dorsum was used for histopathological evaluation. A total of eight burns were covered with moist saline dressings and given daily treatments of xylocaine (lidocaine HCl) gel. This procedure was followed for a period of 24 days. Full-thickness biopsies were obtained for histologic analysis to determine the extent of injury.

Statistical analysis showed a high correlation between the exposure temperature and histopathological assessment. The results found the depth of injury to the collagen (Seg_1) correlated with the temperature (T_i) at which the burns was inflicted, $Seg_1 = 0.038T_i - 2.57$ ($r = 0.973$, $P < 0.05$). Also, the histological studies show a high correlation between the depth of collagen denaturation in wounds and the exposure temperature, $Seg_1 = 0.0268T_i - 0.165$ ($r = 0.991$, $P < 0.05$). This model is useful to assess more closely the therapeutic agents used for wound healing in experimental burn wounds.

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1. Introduction

Thermal injuries are among the most common medical problem throughout the world. The clinical evaluation of burns is based on the following grading scale: I° (superficial burns with injury localized to the epidermis), II°a (superficial dermal burns with injury to the epidermis and superficial dermis), II°b (deep dermal burns with injury through the epidermis and deep into the dermis), III° (full skin thickness burns with injury through the epidermis and dermis into subcutaneous fat), and IV° (burns causing injury to underlying tissue structure such as muscle, tendons, and bone) [1,2]. Cutaneous wound healing is an intricate process involving inflammation, reepithelialization, matrix deposition and contraction [3]. Methods to accelerate wound healing remain an active area of research which includes herbal remedies, animal/insect products used as wound healing agents, and organisms used as wound debridement through biosurgery [4]. Clinical practice is mainly limited to macroscopic observation of wound healing parameters. Standardized histopathological evaluation is beneficial for a better understanding of the pathophysiology of burns and wound healing processes. As a part of a larger study to investigate the therapeutic agents used for wound healing in experimental burn wounds, we aimed to create a novel reproducible burn model of inducing superficial to mid-dermal burns.

Previously, the pig has proved to be a reliable animal model for evaluating wound healing [5–9]. They are used due to their great anatomical and physiological similarity to human beings. Their skin closely resembles human skin, and thus responds to therapeutic treatments much as our does [10,11]. Among the site selection studies for ensuring uniform burns, a porcine model of epidermal wound healing noted that variation between the anatomical regions was found to be negligible [12]. Singer and McClain had found the paravertebral region especially suitable in pigs rather than ventral abdominal undersurface, which were the results of recurrent trauma to burns during movement or lying [13]. Two papers by Davis et al. had reported a regional healing difference with the hind quarters healing faster; they had therefore limited the second-degree burn studies on the paravertebral zone anterior to the pig's coxal tuberosity [14,15]. Wang et al. had recommended that caudal part healed significantly better than those near the cranial part of the thoracic rib in a porcine burn model [16].

In the experiments described below, topical lidocaine in the treatment of superficial burns was application after burn creation and during dressing changes. Brofeldt et al. have shown that topical 5% lidocaine cream applied to the burn wound at a concentration of 1 mg/cm^2 offers significant decrease in pain of long duration of up to 4–6 h without associated systemic side-effects [17]. Moreover, lidocaine is the currently available local analgesic agent which has potent anti-inflammatory effects [18]. Reports of toxicity associated with topical use a 3% lidocaine emulsion or a 4% aqueous solution for dressing changes of partial thickness skin graft donor sites, lidocaine plasma concentrations have been corroborated significantly less than recommended toxic plasma concentration of >5 mg/l [19].

In this preliminary experimental design, choosing the appropriate depth of injury is important so as to create uniform burn wounds and to assess the effects on burn healing of various therapeutic agents. Here, we anticipate that using the minimum number of wounds on the dorsum of the same pig and allow attempts to evaluate the macroscopic and histological changings resulting from the infliction of standard burns. On the basis of our knowledge of the prior research experiences, we report that burns located on the thoracic paravertebral zone of the pig in the uniform healing response. The thoracic paravertebral region here begins at iliac crest and extends to scapula crest along the dorsal midline, while bilateral distance to the dorsal axis within a range of 6 cm. The surface area of the dorsum makes it possible to inflict burns of sufficient size and number. In the present study, we designed a new infliction device using mechanical force applied to the skin by a push–pull force gauge. The more human-like skin of the domestic pig was used. The second-degree burn wound model meets the clinical uses of wound dressing for conservative treatment needs.

2. Materials and methods

2.1. Animal

This study was conducted in the University Animal Research Laboratory at our institution. A castrated male pig (Landrace, 2 months old) weighing approximately 20 kg was used in this study. The pig had access to a standard chow diet and water *ad libitum*, and its daily gain in body weight was up to 1 kg. It was allowed one week to adapt to the environment before the investigation began and did not have access to food the night before any procedures. All experiments were performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University (IACUC Approval No.: NTU-101-EL-11), and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [20]. Animal was kept for 24 days for observation of the healing process. At the end of the experiment, the pig was sacrificed with an overdose of thiamylal sodium.

2.2. Burn model and experimental protocol

The test subject/animal/pig was subjected to burns while under anesthesia and analgesia. It was given intramuscular injection of a combination of atropine (0.06 mg/kg, Astar Pharmaceutical Co., HsinChu, Taiwan), Zoletil® 50 (4.4 mg/kg, Virbac Laboratories, Carros, France), and xylazine (2.2 mg/kg, Lloyd, Iowa, USA). The pig was then intubated endotracheally (Mallinckrodt™ 4.5 mm i.d., Covidien, MA, USA). Throughout this process it was maintained under anesthesia with 0.5–2.5% isoflurane (Abbott Laboratories, Berkshire, UK) inhalant anesthetic as needed, which was delivered through a volume regulated respirator (VIP 3000 veterinary vaporizer, Midmark Corp., OH, USA). An intravenous catheter was placed in an ear vein, and sterile 5% glucose injection was administered intravenously at a rate of 3–4 ml/kg/h. The flank and dorsum