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The optimal time for early burn wound excision to reduce pro-inflammatory cytokine production in a murine burn injury model☆

*Ko-Chang Chang, Hsu Ma *, Wen-Chieh Liao, Chih-Kang Lee, Chia-Yi Lin, Chen-chien Chen*

Division of Plastic and Reconstructive Surgery, Department of Surgery, Taipei Veteran's General Hospital, Taiwan

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ABSTRACT

Background: A potential solution to prevent post-burn deleterious inflammatory responses is early burn wound excision. However, the most beneficial time point remains controversial. This animal study investigated the optimal time point for burn wound excision to reduce pro-inflammatory cytokines production after burn.

Methods: Forty-eight male Sprague-Dawley rats received scald burns with third-degree burns of 30% body surface area, and were then divided into eight groups by day of operation for excision. Group 1 ($n = 6$) received burn eschar excision on post-burn day (PBD) 1. Group 2 received excision on PBD2 ($n = 6$) and so on, while group 8 was the control group ($n = 6$) that did not undergo excision. The skin defect after excision was covered with a bovine-derived collagen dressing. Interleukin-1 (IL-1), IL-6, IL-10 and tumour necrosis factor-α were serially analysed by enzyme-linked immunosorbent assay (ELISA).

Results: We found that levels of all pro-inflammatory cytokines appeared to be lower after excision of full-thickness burns, but as the excision time was delayed from group 1 to group 7, the differences showed progressive decline.

Conclusions: We believe that the earlier the excision is performed, the more the level of pro-inflammatory cytokines can be lowered, and the better the post-burn inflammatory process can be modulated.

1. Introduction

Major burns are associated with an acute and persistent post-burn response, including changes in vascular permeability, alteration in the coagulation system, impairment of gut function, hypermetabolic response and immune depression [1]. These acute inflammatory responses are believed to be mediated by pro-inflammatory cytokines with a concomitant depression of the anti-inflammatory component. Interleukin-1 (IL-1) is an endogenous pyrogen that induces a variety of acute-phase reactions [2]. Tumour necrosis factor-α (TNF-α), or cachectin, is thought to be a primary mediator of the host response to inflammation [3]. Interleukin-6 (IL-6), originally identified as a B-cell differentiation factor, is thought to be an indicator of severity in the infectious phase, while interleukin-10 (IL-10) is regarded as the anti-inflammatory cytokine [4]. Thus, previous studies have suggested IL-1, IL-6, IL-10 and TNF-α can reflect the severity of the morbid condition after burn [5].

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* *Corresponding author at*: No. 201, 2nd Section, Shih-Pai Road, Taipei 112, Taiwan. Tel.: +886 2 2875 7542; fax: +886 2 2875 7041.
E-mail addresses: bubba4chuck@yahoo.com.tw (K.-C. Chang), ps@vghtpe.gov.tw, bubba4chuck@msn.com (H. Ma), liao5168@hotmail.com (W.-C. Liao), thelapradogato@yahoo.com.tw (C.-K. Lee), cylin8@gmail.com (C.-Y. Lin).

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The burn wound is considered to be a major source of inflammatory mediators that initiate and maintain the post-burn inflammatory response, and is characterised by hypermetabolism and immunosuppression. Hypermetabolic response with increased circulating levels of catabolic hormones and development of insulin resistance will result in raised energy requirement, muscle wasting and nitrogen imbalance [6,7]. A wide variety of immunological changes following thermal injury is exhibited, including impaired neutrophil function. In a previous animal study, neutrophils were found to be less sensitive to zymosan-activated serum infusion after 30% burns compared with sham burns, and rats with greater injury have fewer neutrophils in their wounds [8]. Susceptibility to wound infection and sepsis might be increased since prompt recruitment and delivery of neutrophils to a site of bacterial invasion is of prime importance in controlling infection. Decreased lymphocyte blastogenesis and impairment of interleukin-2 production were also found in previous studies [9]. It is generally accepted that burn trauma results in the suppression of both mechanical and immunological protection against sepsis.

To prevent these deleterious changes, a good solution appears to be to excise the burn wound before inflammatory response is maximised. Previous studies have suggested early burn wound excision and grafting, a surgical procedure performed to remove the burn wound eschar and cover the defect with skin graft or artificial skin substitutes [10–14]. When compared with conservative wound treatment with serial debridement and delayed grafting, early surgical intervention is associated with decreased blood loss [10], diminished wound infection [11,12] and shortened hospital stay [13,14]. Thus, early excision of full-thickness burn wound and immediate resurfacing have been considered the treatment of choice in the management of burns.

However, the most beneficial time point for early excision remains controversial. Herndon et al. suggested that early excision within 48 h is optimal for paediatric patients with massive burns [15], while other studies have described early excision in the range of 24 h to 7 days after a burn [10,14,16,17]. Due to ethical concerns, a prospective controlled clinical trial to investigate the optimal time point for burn wound excision is prohibited. Thus, we designed an animal study that compares post-operative changes of IL-1, IL-6, IL-10 and TNF-α, after burn in Sprague-Dawley rats, to determine the optimal time point at which burn wound excision can reduce pro-inflammatory cytokines by modulating inflammatory and metabolic responses.

2. Materials and methods

This animal study was approved by the Approval of Animal Use Protocol of Taipei Veterans General Hospital. Male Sprague-Dawley rats (300–350 g) were housed at 23 °C and 60% relative humidity with a 12-h light/dark cycle, with lights on at 07:00 h. Animals had free access to food (Laboratory Rodent Diet 5001®, Purina Mills, Richmond, IN, USA) and water, and were used after a minimum acclimatisation period of 7 days. The rats were anaesthetised intraperitoneally with ketamine 100 mg kg^{-1}. Hair was removed from the back by an electric shaver. The exposed skin was then scrubbed with Hibiscrub solution and 70% ethanol. The rat is then placed on its back in a mouldable metal wire cage, having their backs submerged in water at 90 °C for 20 s. This produced a full-thickness burn wound with an estimated 30% of body surface area (BSA), which was calculated by Meeh's formula [18]. All rats were scalded and then divided into eight groups: group 1: burn eschar excision on post-burn day (PBD) 1 ($n = 6$); group 2: excision on PBD2 ($n = 6$); group 3: excision on PBD3 ($n = 6$); group 4: excision on PBD4 ($n = 6$); group 5: excision on PBD5 ($n = 6$); group 6: excision on PBD6 ($n = 6$); group 7: excision on PBD7 ($n = 6$); while group 8 was the control group ($n = 6$) that did not undergo excision following burn.

All animals were resuscitated with an intra-peritoneal injection of lactated Ringer's solution 2 mL kg^{-1}% total body surface area (TBSA) immediately after the burn. In the excision groups (group 1 to group 7), incision was made with scalpel along the edges of burn eschar. After dissection with scissors, a plane can be easily identified between the cutaneous eschar and panniculus carnosus. The eschar was unroofed with minimal blood loss because the incision was mainly on the eschar. No apparent difference was found between different groups, in terms of blood loss, adhesion of burn wound and underlying wound base. After excision of full-thickness burn eschar, the wound was covered with Skintemp® (BioCore Medical Technologies, Topeka, KS, USA), a biological dressing that provides a porous bovine-derived collagen membrane, tailored to fit the skin defect. Running sutures with 4/0 nylon was used to attach the dressing to the wound firmly. Rats were housed in individual cages after excision. One-millilitre blood samples were obtained from the tail prior to the burn on PBD1, 3, 5 and 7 days and on post-excision days 1, 3, 5 and 7 and then collected in endotoxin-free ethylene-diamine tetra-acetic acid containing blood specimen tubes. The samples were kept on ice and then centrifuged at 3000 rpm for 60 s within 30 min of collection. The plasma was stored at −70 °C.

Cytokines were analysed by enzyme-linked immunosorbent assay (ELISA). The IL-1, IL-6, IL-10 and TNF-α kits were purchased from Bender Medsystem (Austria, Europe).

Statistical analysis was performed with the Mann–Whitney *U*-test for inter-group comparison. Comparisons before and after the excision were made by the Wilcoxon signed-rank test. Statistical significance was defined at $p < 0.05$ level for all comparisons.

3. Results

All wounds appeared dry and clean 1 week after excision. The biological dressing was adhered firmly to the wound without signs of dehiscence or infection. No apparent difference was discerned in the resected eschars that were sent for pathological studies, which showed that necrotic levels involved full-thickness skin and partial panniculus carnosus of subcutaneous muscle (Fig. 1).

When comparing post-operative cytokines levels with pre-operative levels, we found that levels of IL-1, IL-6 and TNF-α were lower after the excision. However, the difference