Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/burns

Non-severe burn injury leads to depletion of bone volume that can be ameliorated by inhibiting TNF-α

Emily O'Halloran [a,1], *Jasreen Kular* [b,1], *Jiake Xu* [b], *Fiona Wood* [a,c,d], *Mark Fear* [a,c,e,*]

[a] *Burn Injury Research Unit, School of Surgery, University of Western Australia, Perth, Australia*
[b] *School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Australia*
[c] *The Fiona Wood Foundation, Royal Perth Hospital, Perth, Australia*
[d] *Burns Service of Western Australia, Department of Health, Perth, Australia*
[e] *School of Medicine, University of Notre Dame Australia, Fremantle, Australia*

ARTICLE INFO

Article history:
Accepted 2 September 2014

Keywords:
Burn injury
Bone
TNF-α

ABSTRACT

Bone loss after severe burn injury is well established, and is thought to be a consequence of the severe hyper-metabolic response as well as changes in cytokine and glucocorticoid levels that decrease bone synthesis and increase rate of loss. However, 90% of presentations are for non-severe burns which do not elicit this response. Little is known about whether these non-severe injuries may also affect bone tissue, and whether other mechanisms may be involved.

To investigate whether bone loss occurs after a non-severe burn injury we used a mouse model of an approximately 8% total body surface area (TBSA) full-thickness burn and micro-CT. We also assessed whether blocking TNF-α after a burn injury by administration of an antibody could modulate the impacts of the burn on bone tissue.

There was a significant loss of trabecular bone volume of (3.27% compared to 5.27%, $p = 0.0051$) after non-severe burn injury. Trabecular number was significantly decreased (0.57/mm after injury compared to 1.02/mm controls, $p = 0.0051$) and spacing increased after burn injury (0.40 compared to 0.28, $p = 0.0083$). Anti-TNF-α antibodies significantly improved trabecular bone volume (8.53%, $p = 0.0034$) and number after burn injury (1.28/mm, $p = 0.0034$). There was no significant change observed in cortical bone after burn injury or administration of anti-TNF-α antibodies.

These findings show that non-severe burn injury can lead to changes in bone metabolism. Monitoring bone density in patients with non-severe injuries and interventions to limit the impacts of the inflammatory storm may benefit patient recovery and outcomes.

* *Corresponding author at*: Burn Injury Research Unit, M318, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia. Tel.: +61 8 6488 8133.
E-mail address: mark@fionawoodfoundation.com (M. Fear).
[1] These authors contributed equally to this work.
http://dx.doi.org/10.1016/j.burns.2014.09.004
0305-4179/

1. Introduction

Bone loss after severe burn injury is well established and is thought to be in part a consequence of the severe hyper-metabolic response which results in sarcopenia and immobilisation, together with elevated cytokine and glucocorticoid levels which directly alter the balance of bone synthesis and degradation [1–5]. Catecholamines and cortisol area elevated in response to burn injury and stress for up to two years post severe burn and are primary mediators of the hyper-metabolic response and bone loss [2,3]. Bone loss has been demonstrated within two months following a severe burn [4], is sustained, and increases the risk of post-burn fracture [5].

Severely burned patients are lacking in bone formation and synthesis, resulting in slow bone remodelling and turnover rates which increase fracture risk [4–8]. Others have shown significant decreases in serum osteocalcin and parathyroid hormone levels for 9–36 months post-burn, associated with profound decreases in bone mineral content and bone mineral density [6,9]. A recent review by Klein states that immobilisation could lead to urinary calcium wasting and reduced bone formation, leading to reduced bone calcification [9]. In summary, bone loss after severe burn injury is well established, with important contributions of inflammatory and stress mediators likely to be critical. However, little is known about the impact of non-severe burn injury on bone.

Until recently non-severe burns (<15% TBSA) were thought to cause a localised injury response with negligible systemic impact. However, more recent data suggests that a minor burn injury can elicit long-term systemic changes [2], although the mechanisms are not yet well understood.

Non-severe burns are common injuries. In the Australian state of Victoria (population ~5 million), approximately 3800 people per year who do not require admission are known to present to hospital emergency departments with a burn injury; and many more present directly to general practitioners for definitive management [3]. In contrast to patients requiring hospital admission for burn injury, 90% of non-admitted burn injured patients are injured by contact with hot substances, rather than by exposure to flames [3]. The majority of admissions in Western Australia are for burns of <10% TBSA, which mimics other developed countries [14]. Age groups most at risk are <4 and 15–29 years [14]. Therefore it is clear that many burn patients will have long life-expectancy after injury and the consequences of any systemic effect, even if relatively minor, may ultimately lead to significant problems.

Here we have investigated, for the first time, the systemic effect of non-severe burn injury on bone morphology. We have also investigated whether limiting the inflammatory response, through the use of anti-TNF-α antibodies, can modulate the effect of the burn injury on bone.

2. Methods

2.1. Ethics approval

All experiments were approved by The Animal Ethics Committee (AEC) of The University of Western Australia (UWA) and performed in accordance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (AEC ethics number: RA/3/100/1032 and RA/3/100/899).

Adolescent female C57BL/6 (Animal Resource Centre, Western Australia) were maintained in standard housing with food and water provided *ad libitum*. Mice were divided into groups as follow:

Activity analysis groups

1. Wild type C57BL/6 with 8%TBSA thermal injury ($n = 5$).
2. Wild type C57BL/6 with no thermal injury ($n = 5$).

Bone analysis groups

3. Wild type C57BL/6 with 8%TBSA thermal injury ($n = 6$).
4. Wild type C57BL/6 with no thermal injury ($n = 6$).
5. Wild type C57BL/6 with 8%TBSA thermal injury + anti-TNF antibody ($n = 7$).

2.2. Monitoring activity levels

These experiments were conducted independently of the bone analysis. Mice were allocated to burn injury and control groups ($n = 5$ per group). Mice were caged individually with access to a voluntary exercise wheel for a period of 28 days from the date of injury (or sham treatment). Electronic pedometers were attached to the voluntary exercise wheels and distance travelled was recorded.

2.3. Murine burn wound model

Full-thickness burn wounds were generated following a validated protocol as previously described [17–19]. Briefly, 9-week old wild type C57BL/6 female mice were anaesthetised in a closed chamber with a continuous flow of 4% isoflurane and high flow oxygen. Mice were shaved on the dorsum and swabbed with Betadine antiseptic solution. The mid lower dorsum received a full-thickness contact burn *via* a 10-s application of a 20 mm diameter brass rod (8% TBSA) heated to 95 °C or sham treatment (no heated rod). Mice were administered analgesic (buprenorphine, 0.1 mg/kg) subcutaneously in the right shoulder at the time of injury and 12 h post burn. After injury, mice were returned to cages and given feed and water *ad libitum*. Continuous analgesia was maintained by administering oral paracetamol (0.01 mg/ml) in drinking water for five days following the burn procedure.

2.4. Treatment group

Post-injury, one group of animals ($n = 7$) received 20 mg/kg IP injection of anti-TNF-α monoclonal antibody (CNTO 5048, provided by Janssen Pharmaceuticals) on day 0, day 1 and day 3 post-injury. These administration time-points were chosen to have maximum impact on blocking the acute inflammatory cascade initiated by the injury. In previous studies TNF-α levels in burn injured mice have been shown to be maximal at 24 h post-injury and to steadily decline after this point in a severe burn injury [20]. In this more moderate injury