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Original article

Early reduced bone formation following burn injury in rats is not inversely related to marrow adiposity

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ABSTRACT

Objectives: The objective of the study was to determine whether postburn reduction of bone formation occurred earlier than 2–3 weeks after burn injury and whether that reduction was inversely related to marrow adiposity.
Methods: Using a rat model of burn injury with sacrifice at 3 days postburn, we measured serum osteocalcin, a biomarker of bone formation, as well as a regulator of glucose metabolism, and counted tibial marrow adipocytes.
Results: Serum osteocalcin was reduced as early as 3 days postburn, coinciding with a trend toward decline in marrow adipocyte number rather than demonstrating an inverse relationship with adipocyte count.
Conclusions: Factors that may be responsible for the dissociation include lack of circulating sclerostin, previously reported, increased energy demands following burn injury, increased sympathetic tone and perhaps oxidative stress. The relationship between bone formation and marrow adiposity is complex and subject to a variety of influences.

1. Introduction

Burn injury in children initiates a cascade of catabolic responses, including a systemic inflammatory response and a stress response. As a consequence, these children suffer from growth retardation and resorptive bone loss as well as muscle wasting, all of which prolong and complicate rehabilitation.

We have previously shown that biochemical evidence of bone resorption begins on day 1 following burn in a sheep model of childhood burn injury [1] while the earliest evidence of reduced bone formation in children was seen 2 weeks following a severe burn as evidenced by a disappearance of osteoblasts from the bone surface and reduction of osteoblast differentiation [2,3]. These occurrences were thought to be at least in part due to excessive endogenous glucocorticoid production [3] but the onset of reduced bone formation had not been specifically studied. In an attempt to determine whether onset of reduced bone formation occurred earlier than 2 weeks postburn, necessitating a reassessment of the pathophysiology of reduced bone formation, we studied a rat model of burn injury for biomarkers of reduced bone formation. In addition, marrow adipocyte content and adipocyte area were evaluated to see whether it was increased following severe burns, as marrow adiposity may be inversely related to bone density [4].

2. Methods

Following approval from our Institutional Animal Care and Use Committee, protocol 0506032, we studied 27 8-week-old Sprague Dawley rats assigned randomly to burn or control groups. Those assigned to the burn group received a 60% scald burn under isoflurane anesthesia and were sacrificed on study day 4 (3 days postburn).

2.1. Bones

Eight tibias, 4 per group, were randomly selected and placed in 10% formalin and refrigerated overnight. They were then

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transferred to phosphate buffered saline. Specimens, n = 4 per group, were decalcified and marrow adipocytes quantified using BioQuant 2016 software version 16.1.6 M (Bioquant, Nashville, TN, USA). The area quantified included the proximal tibia 1.0 mm from the growth plate and 0.5 mm from the endocortical surface including an area of approximately 3.0 mm. Adipocyte counts were expressed per mm^2.

2.2. Blood

Blood samples obtained at sacrifice were centrifuged and serum was collected and stored at −80 °C. Osteocalcin concentration was measured by using the rat osteocalcin ELISA kit (Aviva Biosystems, San Diego, CA, USA). Aliquots of 5 μL of serum from 12 control and 15 burned rats were analyzed. Data were analyzed using GraphPad Prism Software version 5.0 (GraphPad Software Inc., La Jolla, CA, USA) and expressed as mean ± standard error of the mean.

2.3. Statistics

Means and standard deviations were compared using unpaired t-tests; $p < 0.05$ was taken as a significant difference.

3. Results

Fig. 1 shows the osteocalcin concentration in the rat serum at 3 days postburn. In the burned group (n = 15), serum osteocalcin concentration was significantly lower at 3 days postburn, $p < 0.001$. Fig. 2 shows the adipocyte number per mm^2 counting area. Of note is that the burned group (n = 4) showed a trend toward reduced adipocyte count compared to unburned controls ($p = 0.24$). The adipocyte area in each group showed no significant differences. These findings show that at 3 days postburn bone formation as measured by its biomarker osteocalcin was lower in burned rats than in unburned controls while marrow adipocyte count was not different between the 2 groups but showed a trend toward reduction, indicating that in burn injury there is an early onset of reduced bone formation in a time frame similar to that of increased resorption as well as the disruption of any existing reciprocal relationship between marrow adiposity and bone formation. .

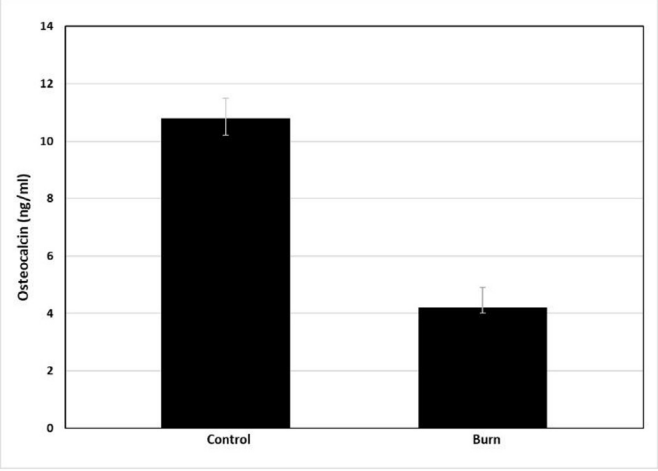

Fig. 1. Serum osteocalcin concentration at 3 days from the start of the experiment in control rats (n = 12) and in those receiving a 60% scald burn (n = 15). Serum osteocalcin was significantly reduced in burned rats, $p < 0.0001$.

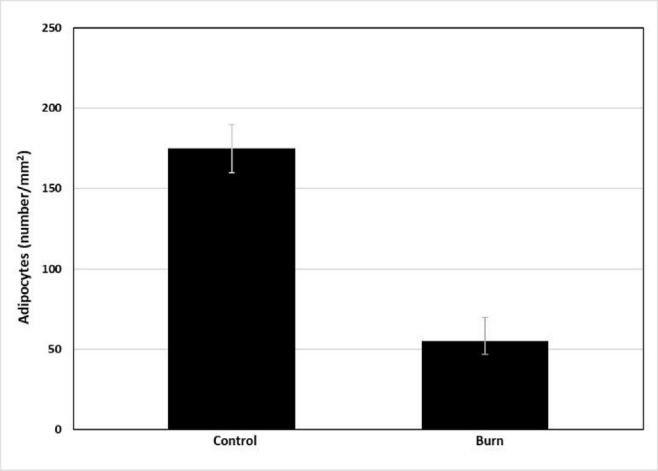

Fig. 2. Number of marrow adipocytes per mm^2 at 3 days from the start of the experiment in control rats (n = 4) and in those receiving a 60% scald burn (n = 4). Marrow adipocyte count trended lower in the burned rats, $p = 0.24$.

4. Discussion

Our data showed a significant reduction in serum osteocalcin concentration at 3 days postburn in rats while marrow adipocytes showed a trend toward reduction instead of the expected increase. This is the earliest that bone formation has been reported to be reduced following severe burn injury, the previous data indicating that bone formation was reduced when studied at 2 weeks postburn [2,3]. The data also show that the widely reported reciprocal relationship between marrow adipocytes and osteoblasts is not present acutely following burn injury. While the early reduction in new bone formation was not expected, the reduction trend in marrow adipocytes was not surprising since it follows a pattern of reduced white adipocytes in other tissues of the body following severe burn injury [5]. While this finding may be due to increased energy demands of the postburn body as well as to increased sympathetic drive [6]. On the other hand, other conditions of reduced marrow adiposity are associated with rapid bone loss in mice, including lactation and vertical sleeve gastrectomy [7,8]. The reduction of sclerostin, which is also found acutely following burn injury [9], may also account for fewer adipocytes as sclerostin is known to be an important contributor to the acquisition of adipocytes by marrow fat [10].

The reduction in bone formation by 3 days postburn is the earliest that this occurrence has been reported. One potential cause for the reduced bone formation is oxidative stress as the adaptive migration of forkhead box O (FOXO) transcription factors to the nucleus of osteoprogenitor cells is associated with a blockage of beta catenin binding to T-cell transcription factors in the nucleus that normally trigger the osteoblastogenesis pathway for marrow stem cells [9]. In addition, certain pro-inflammatory cytokines can also suppress osteoblastogenesis [10–12] and could likely explain both the increased resorption and the decreased formation. Both mechanisms could be operative in this case in addition to excessive endogenous glucocorticoid output. The relative contributions of each still need to be established. The data also confirm that the relationship between marrow adipogenesis and osteoblastogenesis is likely complex and various factors, such as increased energy requirements, may supercede the reciprocal relationship of marrow fat and osteoblasts, although a situation in which both are present in abundance has not yet been described.

4.1. Limitations of the study

The small number of bone specimens used for quantitation of marrow adipocytes and adipocyte area limits our ability to conclude definitively whether marrow adipocyte count changed following burn injury. However, there was clearly no increase in marrow adiposity in relation to the lower serum osteocalcin. In addition, our analysis of bone formation could have benefitted from examination of more than one biomarker. However, if osteocalcin were released during bone resorption, one would expect higher circulating osteocalcin concentrations in serum rather than the lower concentrations that we observed following the burn. Therefore, it is likely that the lower osteocalcin concentrations observed following burn injury do reflect reduced bone formation.

5. Conclusions

The relationship between bone formation and marrow adiposity is complex and requires further study of the relative influences of a variety of metabolic factors.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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