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The Effect of Burn Injury on Serum Concentrations of Sclerostin and FGF23

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Abstract

Severe burn injury results in acute bone resorption followed by an adynamic state, most likely due to changes brought about by the inflammatory and glucocorticoid responses to the injury. There is a consequent increase in annual extrapolated fracture incidence in children. While osteoblasts have been reported to disappear from the bone surface and stem cell differentiation into osteoblasts is impaired, the effect of burns on osteocyte function is unknown. We measured serum concentrations of two osteocyte proteins, sclerostin and fibroblast growth factor (FGF)-23 between 6–60 days post-burn in pediatric patients, ages 5–18 years who had participated in a randomized controlled double-blind study of acute administration of pamidronate to prevent the resorptive bone loss. While FGF-23 was undetectable in all samples, the plot of sclerostin concentration versus time post-burn yielded a statistically significant difference between slopes, –2.5 in the placebo control group and +3.5 in the group receiving pamidronate, $p=0.016$ by ANCOVA. The FGF23 data suggest that osteocytes may be apoptotic, although the sclerostin data may indicate partial preservation of osteocyte function in subjects receiving pamidronate or an ectopic source of sclerostin.

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Disclosures

Gordon L Klein discloses that he participated in a meeting of the Bone Toxicity Advisory Board for Novartis Pharmaceuticals in August 2012.

David N Herndon has no conflicts of interest to disclose.

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Statement of Conflict of Interest

GLK has served on the Bone Toxicity Advisory Board of Novartis Pharmaceuticals in August 2012. No other co-author has a conflict of interest

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Keywords

burns; osteocytes; FGF23; sclerostin

Introduction

Burn injury of 40% total body surface area (TBSA) in children is associated with loss of up to 7% of lumbar spine bone mass in as little as 4–6 weeks and nearly 3% of total body bone mineral content by 6 months post-burn[1]. These changes can persist for at least 18–24 months [2]. The consequence of this bone loss is an annual extrapolated fracture incidence that is doubled in boys and 50% higher in girls compared to normal age-matched peers [3]. The mechanisms of bone loss include at a minimum the systemic inflammatory response as indicated by elevation of cytokines, such as interleukins (IL-) 1 β and IL-6 [4] and the stress response that increases urinary free cortisol production from 3–8 times the upper limit of normal [4,5]. Both responses are potentially exacerbated by sepsis and repeated surgical interventions.

The initiation of these adaptive responses by the burn injury results in osteoblast apoptosis and impaired stem cell differentiation into osteoblasts [4], early increase in bone resorption [6] followed by an adynamic or hypodynamic state within two weeks of the burn[4].

How burn injury affects osteocyte function, however, is unclear. Yet the question is important since the osteocyte is the body's mechanosensor [7]. If osteocytes are apoptotic, the increased annual extrapolated fracture incidence could be explained on that basis.

A previously completed randomized double-blind, controlled trial (RCT) of the bisphosphonate pamidronate, when given to children within the first ten days following burn injury, completely prevented their resorptive bone loss [1] and the effect lasted for at least two years [2]. We reasoned that analysis of the serum for osteocyte-specific proteins from patients enrolled in this RCT should provide useful data on osteocyte viability following burn injury.

Methods

We identified frozen serum specimens from patients ages 5–18 years who completed the RCT of pamidronate between 2000 and 2004. Samples used in the study had been taken between 6 and 60 days post-burn of 40% TBSA. Serum was analyzed for two osteocyte specific proteins, fibroblast growth factor (FGF)- 23 and sclerostin. All samples were stored at –80°C without previous thawing. Under these conditions no losses of aged specimens have been encountered in the lab of PTL and CJR.

Serum sclerostin (SOST) concentration was measured using human SOST ELISA (R&D Systems, Minneapolis MN). The assay sensitivity was 1.7pg/ml. The intra- and inter-assay variations were 2.0% and 9.5% respectively. Serum FGF-23 concentration was measured using human FGF-23 ELISA (EMD Millipore Corporation, Billerica MA). The assay

sensitivity was 3.5pg/ml. The intra- and inter-assay variations were 9.4% and 6.6% respectively. All assays were performed in duplicate.

Serum concentrations of osteocyte factors were plotted against time post-burn. An ANCOVA modeled the effect on sclerostin due to an interaction between treatment (placebo versus pamidronate) and time following injury. Statistical analysis was performed using R statistical software (R Core Team 2012)[8]. A 95% level of confidence was assumed.

Results

A total of 21 samples were obtained for FGF23 and sclerostin concentration determinations. Two-thirds of them had been drawn between midnight and 4AM. Three others were drawn outside this time frame and four samples were not specifically marked as to the time drawn. Utilizing the values of sclerostin obtained in the assays there were no significant differences between the sclerostin concentrations in the group of specimens obtained between midnight and 4 AM and the group obtained outside that time frame, $p=0.48$ on unpaired t test, so the results of all samples were pooled. Data were obtained from serum samples from 9 subjects who received pamidronate and from 8 subjects who received placebo. Ages and sex distribution were not different between the two groups. FGF23 was undetectable in nearly all specimens regardless of whether the subjects received pamidronate (see Table 1). Serum was also located from 11 subjects who received pamidronate and from 21 who received placebo for measurement of phosphorus (P) concentration. P was significantly different between those receiving pamidronate and placebo as the mean concentration from days 6 to 60 was 3.39 ± 0.46 mg/dL ($n=11$) in the pamidronate group compared to 3.89 ± 0.28 mg/dL ($n=21$) in the controls, $p=0.0006$. With serum sclerostin concentrations, the ANCOVA showed that the slope of sclerostin concentration with time differed significantly by treatment group, $p=0.016$, with a slope of +3.5 in subjects receiving pamidronate as opposed to -2.5 in subjects receiving placebo, as shown in Figure 1.

Discussion

The results of this investigation demonstrated that FGF23 concentration in the blood was undetectable regardless of pamidronate administration. This would suggest that osteocytes are apoptotic in the face of large quantities of endogenous glucocorticoids [4,5]. The glucocorticoid data are published elsewhere [9] and also did not differ between pamidronate and placebo control groups. In addition to low FGF 23 concentrations, the other main phosphaturic agent in the body, parathyroid hormone, is also low secondary to cytokine-mediated up-regulation of the parathyroid calcium-sensing receptor [10]. Therefore, in burns phosphate is preserved, possibly to provide ATP to supply the increased energy requirements of these patients. However, the dynamics of FGF 23 in a burn setting are not clear and thus the interpretation of the data should be made with some caution. Thus, for example, FGF23 may be structurally altered by the burn injury making the assay more difficult, and serum concentrations of 1.25-dihydroxyvitamin D will be low due to the hypoparathyroidism and the lack of constitutive binding proteins, such as albumin, for at least six months post-burn. These data have been documented in previous publications [10,11]. It should be noted that while the difference between serum P concentrations in the

pamidronate and placebo groups was statistically significant, both mean values were within the normal range. Furthermore, since low FGF23 and low PTH concentrations suggest that the body is conserving phosphorus the difference in serum P concentrations between the two groups could possibly reflect a difference in tissue utilization of phosphate between groups.

The interpretation of the sclerostin data is more complex. There is an apparent difference in the slopes of the circulating sclerostin concentrations between subjects receiving pamidronate and those receiving placebo. The positive slope in the subjects receiving the bisphosphonate may indicate preservation of some osteocyte function, an interpretation supported by the work of Bellido and Plotkin [12]. However, it may also reflect sclerostin from an as yet unidentified ectopic source such as muscle or may reflect local activity in bone not attributable to osteocytes.

Were there to be a retention of some osteocyte function following bisphosphonate administration, this could serve as a partial explanation for the reduction in muscle protein breakdown seen in subjects who received pamidronate [9]. That is, that some osteocyte function may be needed to send paracrine signals to muscle.

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Highlights

- We studied serum of burned children on pamidronate or placebo for FGF23 and sclerostin.
- Serum FGF23 levels are not detectable in children 6 - 60 days post- burns 40% TBSA.
- The slope of sclerostin v time: positive with pamidronate and negative with placebo

Figure 1.

Plot of sclerostin concentration in serum versus time post-burn in subjects who received a single dose of pamidronate (open circles) and those who received placebo (solid circles) with ANCOVA estimates overlaid with 95% confidence intervals. The normal range of the assay is from 67–300 pg/ml.

TABLE 1

SERUM CONCENTRATIONS OF FGF-23 AND SCLEROSTIN FOLLOWING BURN.

Controls	Days Post Burn	Serum FGF-23 (pg/ml)	Serum Sclerostin (pg/ml)
	30	ND	144.67
	8	ND	79.69
	11	2.4	103.75
	9	ND	163.62
	25	ND	64.53
	12	ND	220.13
	42	ND	53.92
	37	ND	47.69
Pamidronate			
	22	ND	71.53
	30	ND	121.34
	6	ND	184.70
	40	ND	196.45
	20	3.69	36.76
	56	ND	312.36
	14	ND	105.30
	46	ND	197.84
	29	ND	199.04

ND= non-detectable; pg= picograms.