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Sclerostin: a candidate biomarker of SCI-induced osteoporosis

L. R. Morse,

Department of Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA, USA. Spaulding-Harvard SCI Model System, Spaulding Rehabilitation Hospital, Boston, MA, USA. The Forsyth Institute, Cambridge, MA, USA. Spinal Cord Injury Service, VA Boston Healthcare System, Boston, MA, USA

S. Sudhakar,

Spaulding-Harvard SCI Model System, Spaulding Rehabilitation Hospital, Boston, MA, USA

A. A. Lazzari,

Primary Care and Rehabilitation Sections, VA Boston Healthcare System and Boston University School of Medicine, Boston, MA, USA

C. Tun,

Rehabilitation Medicine Service, VA Boston Healthcare System, Boston, MA, USA

E. Garshick,

Pulmonary and Critical Care Medicine Section, Medical Service, VA Boston Healthcare System, Boston, MA, USA. Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

R. Zafonte, and

Department of Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA, USA. Spaulding-Harvard SCI Model System, Spaulding Rehabilitation Hospital, Boston, MA, USA

R. A. Battaglini

The Forsyth Institute, Cambridge, MA, USA. Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA, USA. Skeletal Biology Department, The Forsyth Institute, 245 First St., Cambridge, MA 02142, USA

R. A. Battaglini: rbattaglini@forsyth.org

Abstract

Summary—We assessed several circulating proteins as candidate biomarkers of bone status in men with chronic spinal cord injury. We report that sclerostin is significantly associated with bone mineral content and bone density at all skeletal sites tested. We found no association between bone and any other tested biomarker.

Introduction—Spinal cord injury results in severe osteoporosis. To date, no circulating biomarker of spinal cord injury (SCI)-induced osteoporosis has been identified. We recently reported that circulating sclerostin is associated with bone density in chronic SCI. In this study, we assessed several circulating proteins as candidate biomarkers of bone in men with chronic SCI.

Methods—We assessed the relationship between bone mineral content or bone density and the following circulating bone-related proteins: sclerostin, DKK-1, soluble receptor activator of

nuclear factor kappa B ligand, osteoprotegerin, osteocalcin, and c-telopeptide in 39 men with chronic SCI and 10 men with no SCI.

Results—After adjusting for age, lower sclerostin levels were significantly associated with lower bone mineral content and bone density at all skeletal sites tested ($p=0.0002$ – 0.03). No other circulating protein was associated with bone mineral content or bone mineral density ($p=0.18$ – 0.99).

Conclusion—These findings suggest that circulating sclerostin reflects the severity of bone loss and is a candidate biomarker of osteoporosis severity in chronic SCI.

Keywords

Bone biomarker; Osteoporosis; Rehabilitation medicine; Sclerostin; Spinal cord injury

Introduction

Spinal cord injury (SCI) causes rapid bone loss that increases the risk of low-impact fractures. More than 50 % of people with complete SCI will fracture at some point following their injury [1]. SCI-induced osteoporosis is unique in that the distal femur and proximal tibia are the most frequently fractured bones. Medical complications, including pressure ulcer formation, increased pain, spasticity, fracture non-union, and lower limb amputation, are common after fractures occur. Despite these serious complications, there are multiple barriers to diagnosis of osteoporosis, wide variations in treatment practices, and no standard of care for the detection, prevention, or treatment of SCI-induced osteoporosis [2–5]. Identification of a circulating biomarker of SCI-induced osteoporosis could advance clinical practice by providing a standardized approach for detecting significant bone loss, improving osteoporosis diagnosis, assessing fracture risk, and monitoring response to therapy.

Proteins produced primarily by cells within the bone microenvironment are attractive candidate biomarkers. Sclerostin and Dickkopf-related protein 1 (Dkk-1) are two secreted Wnt signaling antagonists produced almost exclusively by osteocytes. These two molecules can selectively inhibit Wnt/ β -catenin, suppressing the activity of osteoblasts as well as the viability of osteoblasts and osteocytes. Receptor activator of nuclear factor kappa B ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG), are also important regulators of bone homeostasis. RANKL is produced by osteocytes, osteoblasts, bone stromal cells, and activated T cells. The binding of RANKL to its receptor, RANK, is necessary and sufficient to induce osteoclast activation and resorption. OPG is an osteoprotective molecule produced by osteoblasts and stromal cells that blocks RANKL-stimulated osteoclast activation by competitively binding to RANK [6]. Osteocalcin is exclusively synthesized by osteoblasts, odontoblasts, and hypertrophic chondrocytes and regulates bone mineralization [7]. However, there is good evidence of several functions for osteocalcin outside the skeleton, including an important role in energy metabolism [8]. C-telopeptide is a product of osteoclastic hydrolysis of collagen by cathepsin K and is widely used as a biomarker of bone turnover due to its specificity and sensitivity [9].

We recently reported decreased sclerostin levels in chronic SCI in subjects with extremely low bone density [10]. These findings suggest that sclerostin is a potential biomarker reflecting bone mineral content in this population. In this study, we sought to compare sclerostin to other bone biomarkers and markers of bone turnover in subjects with chronic SCI (more than 2 years post-injury). Because sclerostin is produced by osteocytes which comprise over 90 % of all bone cells in the adult skeleton, we hypothesized that of all bone proteins tested, the association between bone and sclerostin would be the greatest.

Methods

Subjects

We studied a convenience sample of 39 subjects with chronic SCI recruited from veterans and individuals in the community previously participating in a larger epidemiological study assessing health at our VA facility [11, 12]. Subjects with SCI were eligible if they were 22 years of age or older, 2 years or more after injury, and had no other neurological condition (multiple sclerosis, stroke, and past polio). We also studied 10 subjects without SCI who were recruited from VA primary care clinics. Subjects without SCI were eligible for recruitment if they did not require an ambulatory aid, had no neurological conditions preventing independent walking, and did not have a history of osteoporosis. The study was approved by our institutional review boards, and all study subjects gave informed consent.

Motor score

Motor level and completeness of injury were confirmed by physical exam by a trained rater. Injury completeness was reported according to the American Spinal Injury Association Impairment Scale (AIS) as previously described [13]. Participants were classified as AIS A or B (motor complete, no motor function below the neurological level of injury); AIS C (motor incomplete, motor function preserved below the neurological level of injury, but with more than half of the key muscles below that level not strong enough to overcome gravity); or AIS D (motor incomplete, with more than half the key muscles below the neurological level of injury strong enough to overcome gravity). Injury severity was then classified in two categories: motor complete SCI (AIS A/B) or motor incomplete SCI (AIS C or D).

Assessment of bone mineral content by dual X-ray absorptiometry scanning

Bone mineral density (BMD) and bone mineral content (BMC) were determined by dual X-ray absorptiometry (DXA) scan using a fifth-generation GE iDXA densitometer. Fractures are most common at the knee (distal femur or proximal tibia) after SCI. Therefore, scans were performed at both SCI-specific (proximal tibia, distal femur) and standard skeletal sites (hip, radius) as previously described [4]. Unless there was a previous fracture or instrumentation, the nondominant lower extremity and radius were scanned. For the distal femur, the proximal edge of the region of interest (ROI) was set at 20 % of the femur length (measured from the lateral femoral condyle), and the distal edge was set at the visible intersection between the patella and the femur, excluding the patella from the ROI. For the proximal tibia, the proximal edge was set at the most proximal point of contact between the tibia and fibular head sites, avoiding regions of overlap between the fibula and the tibia. Scans were obtained in triplicate at the proximal tibia and distal femur. Customized research software supplied by General Electric was used to determine BMD at the knee. Total body scans were performed to determine total body and regional BMC. Because instrumentation of the spine was common causing metal artifact during scanning, leg BMC and arm BMC were used instead of total body BMC. As a standard procedure, a quality assurance phantom supplied by the manufacturer was measured at least every 2 days to confirm accuracy of the densitometer.

Biochemical analyses

Plasma samples were drawn into an EDTA tube and immediately delivered to the core blood research laboratory at our facility. The samples were centrifuged for 15 min at 2,600 rpm ($1,459\times g$) at 4 °C and stored at -80 °C until batch analysis. Circulating sclerostin (Alpco Diagnostics, Salem, NH, USA), Dkk-1 (R&D Systems, Minneapolis, MN, USA), soluble sRANKL (sRANKL; Alpco), and osteoprotegerin (Alpco) were quantified by enzyme-linked immunosorbent assay with detection limits of 8.9 pmol/L (sclerostin), 4.23 pg/

mL (Dkk-1), 0.02 pmol/L (sRANKL), and 2.8 pg/ml (OPG). 25 OH vitamin D was quantified by enzyme immunoassay (Immunodiagnostic Systems Inc., Fountain Hills, AZ, USA) with a detection limit of 2.0 ng/ml. Osteocalcin and C-telopeptide were quantified by electrochemiluminescence immunoassay on a 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA) with detection limits of 0.50 ng/mL (osteocalcin) and 0.01 ng/mL (C-telopeptide).

Variable definition

We considered sociodemographic factors and various health behaviors reported at the time of DXA scan. Participants were weighed and supine length measured for the calculation of body mass index (BMI). In subjects with severe joint contractures, length was self-reported ($n=6$).

Statistical analysis

All analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). Since the distribution of each biomarker except for 25 OH vitamin D was skewed, natural log-transformation was used to normalize the distribution of the outcomes and stabilize the variance for the comparisons provided for Table 2. *T* tests for independent samples and chi-square tests were used to assess differences in subject characteristics based on injury severity (SCI versus no SCI). Mean bone mineral density values at the proximal tibia and distal femur were calculated. General linear models (PROC GLM) were applied to assess relationships between biomarkers and age, bone density, and bone mineral content. Bone mineral density values were converted from grams per square centimeter to milligrams per square centimeter to derive sufficient digits to estimate the regression coefficient.

Results

Subject characteristics

Subject characteristics are presented based on presence of SCI (Table 1). All participants were male and the majority were white (91.8 %). Ages ranged from 30 to 78, with a mean of 55.8 ± 12.1 years. Duration of injury ranged from 4.1 to 42.6 years, with a mean of 22.4 ± 11.2 years. Twenty-nine subjects had motor complete SCI and 10 had motor incomplete SCI with nine out of the 10 AIS D SCI. Subjects with no SCI were older than subjects with SCI (61.7 ± 8.2 vs 54.3 ± 12.6 $p=0.08$) and also had greater BMI (29.0 ± 2.5 vs 26.6 ± 4.8 , $p=0.04$). Lower extremity BMD and BMC were significantly higher in the no-SCI group ($p<0.0001-0.0007$) when compared to the SCI group. There were no significant differences in arm BMC or radius BMD between the two groups ($p=0.86-0.97$).

Association between age and circulating bone protein levels

Circulating bone protein levels are presented in Table 2. Nine subjects had sRANKL values below the detection limit and all of these were assigned a value equal to one half of the detection limit (0.01 pmol/L) and included in the analysis. One subject with SCI had a vitamin D value of 142.63 ng/mL. Another subject with no SCI had a sclerostin level of 250 pmol/L. These values were considered to be outliers and excluded from the respective analyses. We examined the association between each bone protein and age. Only sclerostin ($p=0.0001$) and OPG ($p<0.0001$) levels increased significantly with age. There was no relationship between age and any other bone protein tested ($p=0.28-0.82$). We therefore adjusted all subsequent sclerostin and OPG analyses for age.

Association between candidate biomarkers and bone

We examined the association between each circulating bone protein and bone density or bone mineral content. Age-adjusted sclerostin levels were positively associated with leg BMC (Fig. 1, $R^2=0.33$, $p=0.0002$) and to a lesser extent arm BMC ($R^2=0.18$, $p=0.004$). Similarly, greater sclerostin levels were associated with greater BMD at all traditional and SCI-specific skeletal sites tested (Table 3). There was a significant positive association between leg BMC and OPG (Fig. 1, $p=0.02$). However, after adjusting for age, this association was no longer significant ($p=0.22$). None of the other bone proteins were associated with BMC or BMD (Fig. 1, $p=0.18-0.99$).

Discussion

In this study, we assessed several circulating proteins as candidate biomarkers of bone status in men with chronic SCI. We found that after adjusting for age, sclerostin is significantly associated with bone mineral content and bone density at all skeletal sites tested. We found no association between bone and any other tested biomarker. Previous studies have examined markers of bone turnover after SCI, including c-telopeptide and osteocalcin [14, 15]. While these biomarkers give insight into the balance between bone formation and bone resorption, these studies have not demonstrated a correlation between markers of bone turnover and bone density or bone mass. To our knowledge, ours is the first study to identify a potential circulating biomarker of SCI-induced osteoporosis.

Currently, a DXA-based diagnosis of osteoporosis has little clinical utility in SCI because fracture risk prediction by bone density category is not well-defined after SCI. Furthermore, fractures are most common at the knee (distal femoral metaphysis and proximal tibial metaphysis), and these skeletal sites are not included in standard clinical DXA scans. DXA scans are sometimes difficult to obtain for people with SCI because most facilities are not fully accessible for people with disabilities [5]. For a minority of patients, scan quality may be compromised by factors limiting correct scan positioning (contractures, spasms, and amputation) or the presence of scan artifact due to heterotopic ossification, artificial joints, or metal rods. In the current study, for every unit increase in sclerostin (picomoles per liter), leg bone mineral content increased by 4.3 ± 1.1 g and bone density at the distal femur increased by 2.9 ± 1.0 mg/cm². Although our sample size is small, we report a significant association between bone and age-adjusted sclerostin across a wide range of bone density and bone mineral content values. Our data suggests that in the absence of DXA testing, circulating sclerostin levels might be used to estimate bone density or lower extremity bone mineral content. There are limitations to using a serum biomarker alone to estimate bone density. Despite this, assessment of a circulating biomarker of SCI-induced osteoporosis might complement, or in some instances serve as a surrogate for bone density testing to assess fracture risk and monitor the response to antiresorptive therapies.

Sclerostin is a protein produced exclusively within the bone microenvironment and has been found to be associated with bone density in men and women 60 years and older [16] and in patients undergoing hemodialysis [17]. Marrow plasma (rather than circulating) levels of sclerostin more closely resemble conditions within the bone micro environment. To address this concern, a study of postmenopausal women [18] demonstrated that circulating and marrow plasma levels of sclerostin are strongly correlated, further validating the use of circulating sclerostin use as a biomarker. DKK1 is also produced exclusively by osteocytes within the bone microenvironment. No information exists regarding plasma and marrow levels of DKK1; however, our findings suggest that this correlation is not as strong as for sclerostin. In this study, we found no association between circulating DKK1 levels and bone density or bone mineral content. One possible explanation is that circulating DKK-1 levels are not reflective of DKK-1 activity within the bone microenvironment. It is possible that

DKK-1 is not released into the circulation, is degraded systemically, or is produced by other cell types outside of bone thereby making it a poor bone biomarker. It is also possible that DKK-1 is not regulated by mechanical loading. In one report [19], the ability of an anti-DKK-1 antibody to promote bone formation was tested in a rodent model of mechanical unloading. After anti-DKK-1 antibody administration, bone formation was greater in bones undergoing mechanical unloading compared to the control bones (normal mechanical loading), suggesting that DKK-1 may be upregulated by unloading. However, these findings were not conclusive since there was no direct assessment of DKK-1 in bone (i.e., histology and/or gene expression studies) in response to mechanical unloading.

Sclerostin is a Wnt signaling antagonist. The Wnt signaling pathway has recently been identified as central to the development of disuse osteoporosis [20–24]. Wnt binds to a co-receptor complex involving Frizzled receptor and low-density lipoprotein receptor-related protein (LRP)-5 or LRP-6, both present on osteoblasts. This binding stabilizes cytoplasmic β -catenin and causes it to translocate to the nucleus. Translocation of β -catenin, in turn, activates the transcription of genes that promote osteoblast proliferation, differentiation, and function, ultimately resulting in new bone formation. Sclerostin inhibits the Wnt pathway by preventing the formation of the Wnt-Frizzled-LRP5 complex by competitive binding to LRP5 [25, 26]. Mechanical unloading causes upregulation of sclerostin [27] and therefore inhibited bone formation via suppressed osteoblast activity and survival.

Animal models clearly demonstrate elevated sclerostin levels in response to mechanical unloading that is reversed with reloading [23]. Based on these reports, sclerostin levels are expected to be initially elevated with the withdrawal of mechanical loading after SCI. We previously reported a conceptual model of sclerostin-mediated bone loss after SCI [10]. Briefly, mechanical unloading (paralysis) in acute SCI subjects causes *greater* sclerostin levels, reduced bone formation, and rapid bone loss. In chronic SCI, extreme bone loss results in *lower* sclerostin levels due to a reduction in the number of sclerostin-producing osteocytes in osteoporotic bone. Therefore, the correlation between bone and sclerostin must be considered in the context of weight-bearing status. Significant periods of functional decline that alter ambulatory status might result in acute elevations in sclerostin. Under these conditions, sclerostin would be a mediator of disuse osteoporosis not a biomarker of bone density, and no correlation between bone and sclerostin would be expected. In the current study, all subjects were 4 years or longer post-injury and were considered to have chronic SCI.

There are limitations to the current study that must be considered. This is a small study limited to men. Larger, longitudinal studies that include women are needed to confirm these findings and to establish the utility of sclerostin in fracture risk determination and in monitoring the response to therapy. Also, limited information exists on the impact of medications on the relationship between sclerostin and bone. A larger study is needed to address this question. Despite these limitations, we propose that sclerostin may have utility in the development of SCI-specific clinical guidelines for the detection of bone loss.

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Fig. 1.

Association between candidate bone biomarker levels and arm or leg bone mineral content. Age-adjusted sclerostin levels were positively associated with leg BMC ($R^2=0.33$, $p=0.0002$) and to a lesser extent arm BMC ($R^2=0.18$, $p=0.004$). No association was seen between BMC and Dkk-1 ($R^2=0.00050$, $p=0.88$ for arms and $R^2=0.000025$, $p=0.97$ for legs), C-telopeptide ($R^2=0.01$, $p=0.48$ for arms and $R^2=0.0007$, $p=0.86$ for legs),

Osteocalcin ($R^2=0.01$, $p=0.50$ for arms and $R^2=0.04$, $p=0.18$ for legs), sRANKL ($R^2=0.002$, $p=0.78$ for arms and $R^2=0.007$, $p=0.57$ for legs), or age-adjusted OPG ($R^2=0.03$, $p=0.46$ for arms and $R^2=0.13$, $p=0.22$ for legs)

Table 1

Subject characteristics

Variable	SCI (N=39)	No SCI (N=10)	Total (N=49)
Demographics			
Age (mean±SD) years	54.3±12.6	61.7±8.2	55.8±12.1
White (%)	37 (94.9 %)	8 (80.0 %)	45 (91.8 %)
Duration of SCI (mean±SD) years	22.4±11.2	–	22.4±11.2
Injury severity			
Motor complete (AIS A/B)	29 (74.4 %)	–	29 (59.2 %)
Motor incomplete (AIS C/D)	10 (25.6 %)	–	10 (20.4 %)
Wheelchair use	30 (76.9 %)	–	30 (61.2 %)
BMI (mean±SD)kg/m ²	26.6±4.8	29.0±2.5	27.1±4.5
Underweight/normal(<25)	15 (38.5 %)	1 (10.0 %)	16 (32.7 %)
Overweight/Obese (≥ 25)	24 (61.5 %)	9 (90.0 %)	33 (67.4 %)
25 OH vitamin D (ng/mL) (range)	17.8–62.6	14.7–57.3 ^a	14.7–62.6
Low (<20 ng/mL)	4 (10.5 %)	3 (30.0 %)	7 (14.6 %)
Normal (≥ 20 ng/mL)	34 (89.5 %)	7 (70.0 %)	41 (85.4 %)
Bone mineral content (g)			
Leg BMC	830.6±266.6	1163.9±230.0	898.7±290.8
Arm BMC	486.7±98.3	485.3±88.9	486.4±95.6
Bone mineral density (g/cm ²)			
SCI-specific skeletal sites			
Distal femur	0.641±0.218 ^a	0.970±0.190	0.710±0.250
Proximal tibia	0.631±0.263 ^a	1.084±0.203	0.726±0.311
Traditional sites			
Total hip	0.729±0.209 ^b	1.045±0.154	0.796±0.237
Femoral neck	0.732±0.189 ^b	0.971±0.166	0.782±0.207
Radius	0.998±0.077 ^c	0.992±0.117	0.996±0.087
Osteoporosis diagnosis by hip bone density			
Hip BMD not available	2 (5.1 %)	–	2 (4.1 %)
Normal	8 (20.5 %)	5 (50.0 %)	13 (26.5 %)
Osteopenia	7 (17.9 %)	4 (40.0 %)	11 (22.4 %)
Osteoporosis/BMD lower than expected for age/gender	22 (56.4 %)	1 (10.0 %)	23 (46.9 %)

Percentages may not sum to 100 % due to rounding

^a₃₈

^b₃₇

^c₃₀

Table 2

Circulating levels of bone proteins and markers of bone turnover

Markers of bone turnover	SCI	No SCI
	Median (25–75 percentile)	Median (25–75 percentile)
C-telopeptide (ng/mL) ^a	0.3 (0.2–0.4)	0.2 (0.1–0.3)
Osteocalcin (ng/mL)	16.3 (12.3–21.0)	16.6 (11.7–20.4)
SrankL (pmol/L)	0.05 (0.02–0.10)	0.04 (0.02–0.07)
OPG (pg/mL)	81.6 (71.58–103.92)	94.2 (89.12–121.48)
Sclerostin (pmol/L) ^b	60.7 (38.6–80.0)	82.6 (70.3–143.9)
Dkk-1 (ng/mL)	254.9 (194.5–347.1)	324.7 (153.0–430.8)
vitamin D (ng/mL) ^b	33.2 (26.4–40.9)	24.0 (17.8–45.1)

^a₄₇^b₄₈

Table 3

Association between bone and sclerostin (adjusted for age) at SCI specific and traditional skeletal sites

Skeletal site	R^2	$\beta \pm SE$	p
SCI-specific sites			
Distal femur ($n=47$)	0.23	2.924 ± 1.006^a	0.006
Proximal tibia ($n=47$)	0.28	4.356 ± 1.211^a	0.001
Leg BMC ($n=48$)	0.33	4.3 ± 1.1^b	0.0002
Arm BMC ($n=48$)	0.18	1.2 ± 0.4^b	0.004
Traditional sites			
Total hip ($n=46$)	0.30	2.441 ± 0.898^a	0.009
Femoral neck ($n=46$)	0.26	2.290 ± 0.810^a	0.007
Radius ($n=39$)	0.13	0.988 ± 0.426^a	0.03

^a Milligrams per square centimeters per picomoles per liter^b Grams per picomoles per liter