

Original Article

Circulating noggin levels following treatment with denosumab or teriparatide in postmenopausal women with low bone mass

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Abstract

Objectives: Noggin inactivates bone morphogenetic proteins (BMPs), possibly exerting negative effects on the skeleton. We aimed to compare the effect of agents with opposite impact on bone turnover on noggin circulating levels. **Methods:** In this observational, open label, non-randomized clinical study postmenopausal women with low bone mass were treated with either denosumab (n=30) or teriparatide (n=30). Serum samples were obtained at baseline, three and twelve months after treatment initiation. Prevalent fractures were recorded at baseline and lumbar spine bone mineral density (LS BMD) was measured at baseline and twelve months. Measured parameters included noggin, BMP-2, BMP-4, procollagen type 1 N-terminal propeptide (P1NP) and C-terminal cross-linking telopeptide of type 1 collagen (CTX). **Results:** Noggin levels remained unchanged after either denosumab or teriparatide treatment. Baseline noggin levels were not different between women with vs. without previous anti-osteoporotic treatment, or between those with vs. without vertebral or non-vertebral fractures and were not correlated with age or LS BMD. At twelve months, noggin levels were positively correlated with P1NP within the denosumab ($r_s = 0.47$; $p=0.014$), whereas negatively within the teriparatide group ($r_s = -0.43$; $p=0.019$). **Conclusions:** In postmenopausal women with low bone mass noggin levels were not correlated with bone parameters at any time point, except with P1NP at 12 months, and remained stable with both denosumab and teriparatide treatment.

Keywords: BMP, Denosumab, Noggin, Osteoporosis, Teriparatide

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Introduction

The secreted polypeptide noggin is a protein involved in the development of many body tissues, including nerve tissue, muscles, and bones. Noggin, encoded by the *NOG* gene, binds and inactivates members of the transforming growth factor-beta (TGF- β) superfamily, such as bone morphogenetic protein (BMP)-2 and -4, by preventing their binding to their receptors^{1,2}. BMPs are growth and differentiation factors considered to exert a positive effect on bone formation and thus bone mass³. Therefore, noggin is expected to have a negative impact on bone formation; indeed, in transgenic mice overexpressing noggin, decreased bone formation and severe osteoporosis were reported^{4,5}. On the other hand, the antagonism of BMP signaling through noggin is a prerequisite for normal development of the axial

skeleton, at least in mice^{6,7}, while noggin inactivation in mice has also been reported to cause osteopenia^{8,9}. In humans, reduced noggin activity due to mutations in the *NOG* gene or reduced sensitivity to noggin results in amplified BMP signaling and multiple synostoses syndromes^{10,11}. To the best of our knowledge, circulating noggin levels have not been evaluated in women with postmenopausal osteoporosis. Furthermore, there is no information regarding the effect of anti-osteoporotic therapy on noggin levels.

The primary aim of the study was to evaluate the effect of denosumab (Dmab), the most potent currently available antiresorptive¹², and teriparatide (TPTD), the only osteoanabolic currently available in the European Union, on noggin concentrations. Secondary aim was to evaluate potential associations of noggin levels with lumbar spine bone mineral density (LS BMD) and serum BMP-2 and -4, as well as with well-established bone turnover markers, namely procollagen type 1 N-terminal propeptide (P1NP) and C-terminal cross-linking telopeptide of type 1 collagen (CTX).

Patients and methods

Patients

This is an analysis of circulating noggin, BMP-2 and -4 levels in serum samples obtained from patients recruited and monitored regularly at the outpatient clinics for metabolic bone diseases of 424 General Military Hospital, Thessaloniki, Greece, in the context of a previously described observational, open label, non-randomized clinical study¹³. Postmenopausal women (mean age 65.8 ± 8.6 years, range 54 to 85 years) with low bone mass received either TPTD 20 µg/day (n=30; 13 treatment-naïve, 17 pretreated with bisphosphonates) or Dmab 60 mg/6 months (n=30; all treatment-naïve) for 12 months. Assigned treatment was decided by the patients' caring physician in line with everyday clinical practice. All patients were supplemented with calcium 1000 mg/d and vitamin D 800 IU/d throughout the study. Exclusion criteria were: i) history or presence of bone disease other than primary osteoporosis (e.g hyperparathyroidism, Paget's disease of bone, osteogenesis imperfecta), ii) medication known to affect bone metabolism (e.g glucocorticoids) within the last 3 years, with the exception of previous anti-osteoporotic treatment, iii) hip fracture or hip or knee replacement within 6 months before enrollment, iv) history of high energy fractures, v) any type of cancer, vi) renal and/or liver failure, vii) diabetes mellitus, viii) uncontrolled thyroid disease and ix) primary ovarian failure. The study was approved by the Ethics Committee of 424 General Military Hospital and was in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. Written informed consent was obtained from all the participants.

Methods

Baseline assessment was conducted as previously described¹³. Areal BMD was measured by dual energy X-ray

absorptiometry (DXA) at the LS of all patients at baseline and twelve months after Dmab or TPTD initiation, using a DPX-IQ densitometer (Lunar Corporation, Madison, WI, USA). Morning (8:00-9:00 pm) fasting blood samples were obtained from all patients at baseline, three and twelve months following treatment initiation. Samples were immediately centrifuged and stored at -80°C. Serum P1NP, CTx, parathyroid hormone (PTH) and 25-hydroxy-vitamin D (25OHD) were measured in one batch at the end of the previously described study¹³.

Additional unfrozen serum samples were used for the measurement of noggin, which was conducted blindly at the laboratory of FIANOSTICS GmbH, Wiener Neustadt, Austria. Noggin was measured using a recently developed high sensitivity fluorescent immunoassay based on plasmonic microtiter plates which increase the signal of fluorescent dyes several hundred-fold. Briefly the assay protocol includes: adsorptive coating of capture antibody in 50mM phosphate buffer (PBS)/150mM NaCl pH 7.4, over-night at 4°C followed by washing with PBS containing 0.1% Triton x100. Blocking of unspecific binding was achieved with a proprietary solution of FIANOSTICS containing synthetic polymers and mercapto-compounds. After another washing step, 20ul duplicates of standards/samples (serum) together with 25ul of anti-human NOG antibody labeled with AlexaFluor680 were incubated over night at RT temperature in the dark. Measurements were done using a standard fluorescence micro-plate reader. Samples reading above 100 pmol/l NOG were diluted with assay buffer and re-run to check for linearity of the signal.

Measurements of BMP-2 [ELISA, R&D Systems, Minneapolis, MN, US; minimum detectable dose (MDD) 4.3 pg/mL, intra-assay coefficient of variation (CV) 2.4-2.8%, inter-assay CV 5.3-7.3%] and BMP-4 (ELISA, R&D Systems, Minneapolis, MN, US; MDD 0.43 pg/mL, intra-assay CV 3.1-5.3%, inter-assay CV 5.3-5.8%) were conducted at the Bone Laboratory of the Technische Universität Dresden Medical Center, Dresden, Germany.

Statistical analysis

Data for continuous variables are presented as mean \pm standard error of the mean (SEM). Data for categorical variables are presented as absolute numbers. Kolmogorov-Smirnov test was used to test the normality of distribution of continuous variables. Spearman's coefficient was used to test for bivariate correlations. Chi-square test was used for between group comparisons of categorical variables. Independent T-test or Mann-Whitney test were used for between group comparisons of continuous variables. Within group comparisons of continuous variables were performed with the paired T-test or Wilcoxon Signed Ranks test, when the repeated measures were two, or repeated measures analysis of variance (ANOVA) or Friedman test, when the repeated measures were more than two. When needed, Bonferroni *post-hoc* adjustment was used for multiple pairwise comparisons. Repeated measures analysis of covariance (ANCOVA) was used to adjust within group comparisons for potential covariates. A two-sided p-value

Table 1. Comparative baseline, 3-month and 12-month data of the two groups.

	Group	Denosumab	Teriparatide	<i>p</i> -value (between groups)
Age (years)	Baseline	65.6 ± 1.5	65.2 ± 1.6	0.84
Age at menopause (years)	Baseline	47.4 ± 1.1	46.6 ± 1.0	0.58
Vertebral Fx (N)	Baseline	9	11	0.52
Non-vertebral Fx (N)	Baseline	4	8	0.20
25(OH)D (ng/ml)	Baseline	18.3 ± 1.6	25.9 ± 2.5	0.015
LS BMD (g/cm ²)	Baseline	0.871 ± 0.009	0.808 ± 0.010	<0.001
	Month 12	0.923 ± 0.011	0.865 ± 0.012	0.001
<i>p</i> -value (within groups)		<0.001	<0.001	
LS T-score	Baseline	-2.58 ± 0.07	-3.12 ± 0.08	<0.001
	Month 12	-2.13 ± 0.08	-2.66 ± 0.09	<0.001
<i>p</i> -value (within groups)		<0.001	<0.001	
BMI (kg/m ²)	Baseline	29.0 ± 0.8	27.1 ± 0.8	0.10
	Month 3	29.0 ± 0.8	27.2 ± 0.8	0.12
	Month 12	29.2 ± 0.8	27.6 ± 0.9	0.23
<i>p</i> -value (within groups)		0.46	0.19	
Noggin (pmol/l)	Baseline	14.0 ± 2.5	15.8 ± 2.4	0.46
	Month 3	13.6 ± 2.1	18.7 ± 3.4	0.29
	Month 12	12.3 ± 2.9	17.4 ± 3.2	0.10
<i>p</i> -value (within groups)		0.41	0.45	
P1NP (ng/ml)	Baseline	55.6 ± 3.7	50.6 ± 6.2	0.49
	Month 3	14.3 ± 0.7 ^a	104.5 ± 10.1 ^a	<0.001
	Month 12	21.8 ± 2.1 ^{a,b}	130.3 ± 12.0 ^{a,b}	<0.001
<i>p</i> -value (within groups)		<0.001	<0.001	
CTx (ng/l)	Baseline	439 ± 34	421 ± 52	0.77
	Month 3	38 ± 3 ^a	677 ± 85 ^a	<0.001
	Month 12	167 ± 24 ^{a,b}	788 ± 86 ^a	<0.001
<i>p</i> -value (within groups)		<0.001	<0.001	
PTH (pg/mL)	Baseline	46.9 ± 3.5	44.1 ± 4.4	0.62
	Month 3	73.0 ± 6.5 ^a	33.6 ± 3.9 ^a	<0.001
	Month 12	58.1 ± 5.6 ^b	40.0 ± 4.6	0.015
<i>p</i> -value (within groups)		0.001	0.027	

Data are presented as mean ± standard error of the mean (SEM) or absolute numbers

a: compared to baseline; b: compared to month 3

Abbreviations: BMI, body mass index; BMD, bone mineral density; Fx, fracture; LS, lumbar spine; P1NP, procollagen type 1 N-terminal propeptide; CTx, C-terminal telopeptide of type 1 collagen; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

<0.05 was considered statistically significant in all the tests. Statistical analysis was performed with SPSS for Macintosh, version 21.0 (IBM Corporation, New York, USA).

Results

Sixty postmenopausal women with low bone mass were assigned to either Dmab (n=30) or TPTD (n=30). Comparative baseline, 3-month and 12-month data are presented in Table 1. At baseline, age, age at menopause, BMI, previous vertebral and non-vertebral fractures, P1NP, CTx and PTH were similar

between groups. LS BMD and T-score were higher, whereas 25(OH)D levels were lower in the Dmab than the TPTD group.

Noggin levels were not different at baseline and they did not change after either Dmab or TPTD treatment (Table 1). After sequential adjustment for LS BMD; LS BMD and BMI; LS BMD, BMI and 25(OH)D; LS BMD, BMI, 25(OH)D and age; noggin levels remained essentially unchanged. As expected, both Dmab and TPTD improved LS BMD. P1NP and CTx levels decreased, whereas PTH levels increased after Dmab treatment, whereas the opposite trends were observed after TPTD treatment.

Baseline noggin levels were not different between women with vs. without previous anti-osteoporotic treatment, or between those with vs. without vertebral or non-vertebral fractures. Baseline noggin levels were not correlated with age, BMI, LS BMD, 25(OH)D, P1NP, CTx or PTH in the sum of patients. When analyzed per group, no correlations were observed between noggin levels and other parameters at month 3. At month 12, noggin was positively correlated with BMI ($r_s = 0.55$; $p=0.003$) and P1NP ($r_s = 0.47$; $p=0.014$) within the Dmab group, whereas negatively with P1NP within the TPTD group ($r_s = -0.43$; $p=0.019$).

Data on BMP-2 and BMP-4 are not presented, because many values were out of range or out of the y-axis, therefore, no secure data analysis could be performed.

Discussion

Our results suggest that neither antiresorptive (Dmab) nor osteoanabolic (TPTD) therapy causes specific alterations in circulating noggin levels in postmenopausal women with low bone mass. Several hypotheses can be formulated to explain these data. First, circulating noggin may not reflect the levels and activity of the protein in the bone microenvironment, as a small amount of locally acting cytokines leak to systemic circulation¹⁴. Second, part of serum noggin may originate from non-skeletal sources¹⁴. Notably, at 12 months, noggin levels were positively correlated with P1NP in the Dmab, whereas negatively in the TPTD group. No secure explanation exists for this inverse trend and no speculation could be made for a cause-effect relationship or an epiphenomenon. Further mechanistic studies are required to explore whether noggin partly mediates the effect of Dmab and/or TPTD on bone formation and its clinical implication.

In mice, BMP signaling has been reported to mediate bone anabolic action of PTH¹⁵. However, in our patients, TPTD treatment did not change the circulating levels of the BMP antagonist, noggin. In patients with beta-thalassemia major and osteoporosis treated with Dmab versus placebo for one year, noggin levels increased less with Dmab and were strongly correlated to distal radius BMD but not with LS BMD¹⁶. In the present study, we failed to verify an effect of Dmab treatment on noggin levels. Although we did not measure distal radius BMD in this study, we confirm the lack of correlation between noggin and LS BMD¹⁶.

Although other researchers have reported outcomes using the same BMP kits with us¹⁷, we encountered methodological difficulties in BMP-2 and BMP-4 measurements, which provided a considerable proportion of out of range values.

Our study has certain limitations. First, this study was not specifically designed to evaluate serum noggin levels, so an a priori power analysis had not specifically performed. Second, the number of participants is small, however, this is the first study evaluating circulating noggin in postmenopausal osteoporosis and the patients were followed-up for 12 months. Third, a fraction of patients were not treatment naïve, because of strict restrictions of Greek legislation in

TPTD administration. Finally, BMD was measured only in the LS; however, given the short duration of the study and the predominantly trabecular composition of the spine, LS was the measurement site expected to reveal the more prominent BMD changes at 12 months.

In conclusion, noggin levels were not affected by treatment with either Dmab or TPTD and were not correlated with prevalent fractures or LS BMD. Noggin levels were positively correlated with P1NP in the Dmab, whereas negatively in the TPTD group, a finding warranting further mechanistic studies.

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