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Changes in plasma RANKL-osteoprotegerin in a Prospective, Randomized Clinical trial of Initial Antiviral Therapy: A5260s

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Abstract

Background—The contributions of the Receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) axis to cardiovascular and bone disease in treated HIV-1 infection is not well-defined.

Setting—Prospective, observational, longitudinal study.

Methods—In a subset analysis of a prospective randomized clinical trial, 234 HIV-1-infected antiretroviral therapy (ART)-naïve participants received tenofovir-emtricitabine plus either atazanavir/ritonavir, darunavir/ritonavir, or raltegravir and achieved plasma HIV-1 RNA <50 copies/ml by week 24 and thereafter. Associations between plasma RANKL, OPG or RANKL/OPG ratio levels with total, hip, and spine bone mineral density loss (BMD) or progression of carotid artery intima-media thickness (CIMT) were assessed longitudinally over 96 weeks.

Results—Over 96 weeks all treatment groups had similar and sustained declines in plasma RANKL, increases in plasma OPG, and subsequently, decreases in the RANKL/OPG ratio. There

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Authors contributions:

J.C, J.S., G.M., T.B, T.K. were responsible for the study concept and design. C.M. and L.J. carried out the statistical analyses. T.K. and C.M drafted the manuscript. T.K., O.Y, M.D, J.S., J.C., G.M. T.B. collected the data. All co-authors participated in discussions about the design of the study, interpretation of the findings, and critically reviewed the manuscript.

were no associations between plasma RANKL or RANKL/OPG ratio levels with total, hip, and spine bone mineral density loss or progression of carotid artery intima-media thickness; however, plasma OPG in successfully treated HIV-infected patients (week 48 and 96) was associated with spine BMD loss.

Conclusions—In virologically suppressed HIV-infected patients, the evolution of bone disease could be linked to plasma OPG levels; however the role of plasma levels of RANKL and RANKL/OPG ratio in the prediction of morbidity in treated HIV-1 infection may be limited.

Keywords

HIV; Receptor activator of nuclear factor kappa-B ligand (RANKL); osteoprotegerin (OPG); cardiovascular disease (CVD); bone disease

INTRODUCTION

Comorbidities such as osteoporosis and cardiovascular disease (CVD) contribute to increased morbidity and mortality in HIV- infected persons, on potent antiretroviral therapy (ART). The cross-talk between the immune and the skeletal systems has been implicated in the pathogenesis of cardiovascular and bone disease in the setting of HIV infection despite suppressive ART [1–3]. The receptor activator of the nuclear factor kappa B (NF-kB) ligand (RANKL)/osteoprotegerin (OPG) system plays a critical role in regulating bone metabolism, the immune system, and CVD. In the setting of inflammation, RANKL expression is induced in osteoblasts and synovial fibroblasts and promotes osteoclast activity [3]. OPG binds RANKL, counteracts the effects of RANKL and is induced in multiple tissues including bone [3]. Both RANKL and OPG are also produced by myeloid cells, lymphocytes which are involved in inflammation, osteoclastogenesis and atherogenesis [3]. A higher ratio of circulating RANKL/OPG in the setting of inflammation promotes osteoclastogenesis and atherogenesis [3].

We have previously reviewed the conflicting data on the role of the RANKL/OPG axis in HIV infection [3]. *In vitro* and *in vivo* studies have suggested that both HIV-1-RNA and ART can directly and independently affect the RANKL/OPG levels. Plasma levels represent only a small fraction of the total tissue levels/activity of RANKL/OPG [3]. However, there is limited data in the setting of ART initiation as to whether increased plasma levels of the RANKL/OPG axis are associated with surrogate measures of CVD and bone disease in chronic HIV infection. Elucidating the role of RANKL/OPG axis in systemic inflammation and immune dysfunction in the setting of suppressed viremia may help us understand mechanisms of HIV-related CVD and bone disease.

ACTG A5260s was a prospective substudy nested into an ART treatment study (A5257), in which participants were randomized to tenofovir disoproxil fumarate-emtricitabine (TDF/FTC) plus either raltegravir (RAL) or atazanavir/ritonavir (ATV/r) or darunavir/ritonavir (DRV/r) [4–6]. In this study we found that ATV/r was associated with slower progression of carotid intima-media thickness (ΔCIMT) compared to DRV/r or RAL [4] and that markers of inflammation and immune activation inconsistently and partially decreased after these

regimens [5]. We also found that bone mineral density (BMD) losses were lowest among those receiving 96 weeks of RAL compared to protease inhibitors (PIs) [6].

Herein, we describe for the first time in the setting of a large randomized ART initiation trial, the effects of different successful ART regimens on plasma levels of RANKL, OPG, and the ratio (RANKL/OPG [3]) on surrogate markers of CVD and bone disease. In view of limited data that suggest favorable effects of integrase inhibitors compared to PIs on markers of inflammation [7–9], we hypothesized that RAL would increase plasma OPG and reduce the plasma RANKL and RANKL/OPG ratio compared to both PIs.

METHODS

Study Design and Participants

A5260s included 328 HIV-infected, ART-naïve adults with no CVD or diabetes mellitus. The design of this substudy (clinicalTrials.gov identifier: NCT00851799) has been previously reported [4, 6] and was approved by the Institutional Review Boards at all participating institutions; all participants provided written informed consent. To avoid the confounding effects of viremia on plasma levels of RANKL and OPG, the analysis population was restricted to a subset of 234 (71%) virologically suppressed participants (no ART interruptions > 7 days, HIV-1 RNA <50 copies/ml by study week 24 and thereafter).

Measurement of biomarkers, CIMT and BMD (total, lumbar spine, hip)

Plasma RANKL and OPG were determined at study entry prior to ART initiation and after 48 and 96 weeks on treatment, as previously described [6]. CIMT and BMD were determined before ART initiation and after 48 (CIMT), 96 (CIMT, BMD), and 144 weeks (CIMT). Primary results and methodologies are reported elsewhere [4, 6].

Statistical Analyses

Pairwise treatment group comparisons of changes from baseline were assessed by Wilcoxon rank sum tests. Associations between on-treatment levels of the biomarkers and BMD change (% change from baseline to week 96) were evaluated with linear regression models, adjusted for randomized treatment, stratification factors (screening viral load and 10-year Framingham risk score) and baseline biomarkers levels. Consistent with the study primary analysis, associations between biomarkers and CIMT were examined using mixed effects linear regression models with random intercept and slope and unstructured covariance matrix on the random effect, adjusted for baseline CIMT, time (years), and baseline Framingham Risk Score and HIV-RNA stratification factors [4, 6]. All treatment group comparisons were assessed with a 2.5% alpha level; all other comparisons used a 5% level, without adjustment for multiple comparisons. All analyses were performed with SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Baseline characteristics

Baseline demographic characteristics of the 328 participants from the A5260s study were previously described [10, 11]. The 234 (220 in the current substudy) participants included in the successfully treated population had similar baseline demographic characteristics across treatment groups [5]. Briefly, 90% were men, 48% white, with a median age of 36 years, CD4+ cell count 338 cells/mm^3 and HIV-1 RNA 4.6 log10 copies/ml. Levels of RANKL, OPG and RANKL/OPG per timepoint are presented in Table 1. No notable differences in distributions are seen across treatment groups (Supplemental Tables 1, 2, Supplemental Figure 1).

Changes in plasma RANKL, OPG

Consistent with the declines in markers of inflammation [5], decreases in plasma RANKL from baseline were observed at week 48 that were sustained to week 96 among all participants (Table 1) and among all treatment groups (Supplement Table 1). Specifically, levels of RANKL at 96 weeks were on average 50% lower than baseline measures. Increases (approximately at least 10% higher than baseline measures) in plasma OPG from baseline were noted only at week 96 among all participants (Table 1) and among all treatment groups (Supplement Table 1).

Associations between RANKL, OPG, RANKL/OPG ratio, BMD and CIMT

In baseline-OPG adjusted models, there was a trend to higher OPG levels at 48 and 96 weeks being associated with spine (but not hip) BMD loss (p=0.07). Examining OPG levels on-study, without baseline adjustments, higher level of OPG at week 48 and 96 were associated with a larger decrease in spine BMD [1.04% (p=0.039) and 1.27% (p=0.034)]. Associations were not observed between levels of RANKL and RANKL/OPG and lumbar spine and total hip BMD (p≥0.36) (Table 2). No associations between CIMT and levels of RANKL, OPG, RANKL/OPG at 48 weeks (were apparent (p≥0.35) (Table 2).

DISCUSSION

In this prospective study of ART-naïve participants who achieved virologic suppression after initiation of TDF/FTC along with either RAL, ATV/r or DRV/r, plasma RANKL and RANKL/OPG ratio were not associated with BMD or CIMT change. Higher OPG level on ART was associated with greater spine BMD loss in models that did not adjust for baseline OPG level and marginally in baseline-adjusted models. RAL did not appear to have a more favorable effect on increasing OPG and decreasing RANKL and RANKL/OPG ratio compared to the PIs. To our knowledge, this is the largest prospective study that explored associations of plasma RANKL and OPG with markers of CVD and bone disease. Overall, given prior *in vivo* and *in vitro* data that support a major role of the RANKL-OPG axis in pathogenesis of CVD, bone disease and HIV infection and the fact that plasma levels represent only a small fraction of the total tissue levels/activity of RANKL/OPG [3], these results suggest that plasma levels of the RANKL-OPG axis may not be the best surrogate markers [3, 12] to predict bone disease and CVD in chronic treated HIV infection.

The differential effects of integrase inhibitors compared to PIs on RANKL/OPG remain unclear[3]. We did not find any benefit of RAL over PIs on reducing RANKL or RANKL/OPG ratio during the first 96 weeks of successful treatment. A strength of our study is that, in contrast to prior RAL switch or intensification-related studies, prior ART does not confound our data since we enrolled only ART-naïve participants and followed them for 96 weeks, a longer duration than prior studies. However, changes in levels of RANKL and OPG were also measured in the setting of initiation of different ART regimens during a period where there may be major changes in inflammation and immune activation that may also affect the RANKL/OPG axis [3]. Thus, the specific effect of ART on the RANKL/OPG axis may best be examined in HIV-uninfected persons receiving ART for HIV prevention or in HIV-infected persons on longer term potent ART who switch ARTs.

Consistent with the hypothesis that the RANKL/OPG axis contributes to HIV-related bone disease, we found that participants with higher plasma OPG after 48 weeks of ART had more spine BMD loss. Increased levels of OPG in HIV-infected individuals may reflect a compensatory mechanism to downregulate increased bone resorption [3]. However, like others, we did not find an association between plasma RANKL/OPG ratio with measures of bone disease [13].

Reports of associations between circulating levels of OPG or RANKL with CVD in HIV-uninfected subjects have been conflicting [3]. Despite evidence from retrospective studies that serum RANKL was negatively associated with the coronary artery plaque burden and calcium score in HIV-infected individuals on ART compared to controls [12], similar to our data, in a smaller prospective study of HIV-positive participants on ART there were no significant associations of serum RANKL, OPG, and RANKL/OPG with CIMT [14]. In the largest study to date that has investigated the associations of plasma levels of RANKL and OPG with subclinical atherosclerosis in chronic HIV infection, only higher OPG (but not lower RANKL) concentrations were associated with the presence of coronary artery calcium, mixed plaque, and coronary stenosis > 50% in HIV infected men [15]. Thus, plasma OPG rather than plasma RANKL levels may be a better surrogate marker for HIV-related morbidity.

Our study had several limitations that have previously been described [5]. Briefly these include limited power to detect effect sizes with adjustment for multiple biomarker comparisons, selection bias of A5260s participants when restricting to the cohort of virologically suppressed individuals on potent ART, and inclusion of mostly men, which may limit generalizability of our findings. Finally, the relatively younger age of our study population without clinical CVD, may have limited our ability to study the cross talk between RANKL/OPG, CVD, and bone disease.

In conclusion, in this prospective study plasma levels of the RANKL and RANKL/OPG were not associated with CIMT and BMD change after 96 weeks among treatment-naïve individuals initiating and remaining on successful ART regimens of TDF/FTC with RAL, ATV/r or DRV/r. Our data suggest that higher levels of plasma OPG may predict BMD loss in ART treated HIV infected individuals. Given that plasma levels of RANKL and OPG may not reflect local tissue expression and in view of the role of RANKL/OPG axis in vascular

calcification and bone disease [3], these results highlight the need to further understand the role of the RANKL/OPG axis at the tissue (arteries, bone, and lymphocytes) level. Larger prospective studies that will also explore tissue levels of RANKL, OPG and CVD endpoints such as plaque may more definitely address the role of RANKL/OPG axis in HIV-related CVD and bone disease. Elucidating the role of the RANKL/OPG axis in HIV infection may lay the foundation for novel strategies to prevent future bone or CVD in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive Summary Statistics [Median (IQR)]. RANKL: OPG ratio was calculated as RANKL pmol/L over OPG pmol/L to create unit-less ratio. RANKL and OPG are presented on the log 10 scale. Changes in RANKL and OPG were summarized as fold-change from baseline, and were estimated by back-transforming the mean difference between levels on-treatment and at baseline on the log10 scale.

	N	Week 0	N	Week 48	N	Week 96	N	Δ (0-48)	N	Δ (0-96)
RANKL:OPG	228	0.36 (0.15, 0.71)	223	0.28 (0.13, 0.45)	220	0.16 (0.05, 0.34)	(−)	(−)	(−)	(−)
RANKL	231	1.49 (1.13, 1.74)	223	1.31 (1.03, 1.52)	220	1.14 (0.68, 1.43)	220	0.74 (0.43, 1.24)	218	0.54 (0.26, 0.98)
OPG	228	0.61 (0.51, 0.69)	223	0.61 (0.53, 0.68)	220	0.66 (0.57, 0.75)	217	0.98 (0.82, 1.22)	215	1.11 (0.87, 1.43)

Δ (0-48): Week 48-fold change from baseline

Δ (0-96): Week 96-fold change from baseline

Table 2

Regression Estimates (CIs) for Hip, Spine, Bone mineral density (BMD) loss, longitudinal CIMT over 3 years and plasma levels of RANKL, OPG and RANKL/OPG ratio in successfully treated population

	Study Week 48		Study Week 96 (ΔBMD)/Week 144 (ΔCIMT)	
	Estimate (CI)	**p-value**	**Estimate (CI)**	**p-value**
Hip ΔBMD				
Adjusted RANKL[1]	0.17 (−0.20, 0.55)	0.36	−0.08 (−0.35, 0.19)	0.57
Adjusted OPG[1]	0.11 (−0.99, 1.21)	0.84	−0.24 (−1.15, 0.67)	0.60
RANKL:OPG Ratio[2]	−0.004 (−1.22, 1.21)	0.99	0.10 (−1.13, 1.34)	0.87
Spine ΔBMD				
Adjusted RANKL[1]	0.01 (−0.40, 0.42)	0.96	−0.13 (−0.43, 0.17)	0.40
Adjusted OPG[1]	−1.10 (−2.29, −0.08)	0.07	−0.93 (−1.93, −0.08)	0.07
RANKL:OPG Ratio[2]	0.49 (−0.82, 1.81)	0.46	0.12 (−1.24, 1.48)	0.87
ΔCIMT[3]				
RANKL	1. 87 (−2.95, 6.70)	0.44	(−)	(−)
OPG	−0.16 (−13.51, 13.18)	0.98	(−)	(−)
RANKL:OPG Ratio	−1.62 (−4.99, 1.76)	0.35	(−)	(−)

Notes:

[1] Parameter estimates for RANKL and OPG are presented as per 0.3 log10 units, which is equivalent to a 2-fold difference

[2] Models adjusted for randomized treatment, stratification factors, and baseline biomarker levels. Extreme RANKL:OPG ratios greater than 2 (range: 2.1 to 8.99, n=17) are set equal to 2

[3] All models adjusted for baseline CIMT, time (years), and baseline Framingham Risk Score and HIV-RNA stratification factors. Annual rate of change modeled as the interaction between the level of the biomarker at week 48 and time (years).