Switching from tenofovir to abacavir in HIV-1-infected patients with low bone mineral density: changes in bone turnover markers and circulating sclerostin levels

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Background: Tenofovir is involved in accelerated bone mineral density (BMD) loss.

Methods: We recently published a hip BMD improvement at week 48 [+2.1% (95% CI: −0.6, 4.7) (P = 0.043)] in HIV-infected patients with osteopenia/osteoporosis randomized to switch from tenofovir to abacavir (n = 26), although without reaching statistical significance compared with those who maintained tenofovir (n = 28). Here, we present changes at week 48 in bone markers [C-terminal telopeptide of collagen type 1 (CTX), osteocalcin and procollagen type 1 N propeptide (P1NP)] as well as in circulating levels of three proteins involved in bone regulation [osteoprotegerin, receptor activator for NF-κB ligand (RANKL) and sclerostin, a selective regulator of bone formation through the Wnt pathway] in 44 of these patients. x2 or Fisher and Student t-tests were performed according to the distribution of the variables.

Results: Bone markers decreased only in the abacavir group [mean (SD) CTX changed from 0.543 (0.495) to 0.301 (0.306) ng/mL; mean (SD) osteocalcin changed from 23.72 (22.20) to 13.95 (12.40) ng/mL; and mean (SD) P1NP changed from 54.68 (54.52) to 28.65 (27.48) ng/mL (P < 0.001 in all cases), reaching statistical significance between the groups at week 48. Osteoprotegerin did not vary, but sclerostin significantly increased in the abacavir group [from 29.53 (27.91) to 35.56 (34.59) pmol/L, P = 0.002]. No significant differences in osteoprotegerin and sclerostin were detected between the groups at week 48. RANKL values were below the limit of detection in all samples.

Conclusions: The switch from tenofovir to abacavir seems to induce a positive effect on bone tissue, since bone turnover markers decreased. In addition, circulating sclerostin levels increased, a change associated with improved bone properties.

Keywords: osteoporosis, bone markers, osteoprotegerin, RANKL, HIV-infected patients

Introduction

Tenofovir is one of the antiretroviral agents involved in accelerated bone mineral density (BMD) loss.1,2 However, scarce data are available on the potential benefit of interrupting tenofovir in the case of decreased BMD.

We recently published data from a randomized pilot study (ClinicalTrials.gov number NCT 01153217) in HIV-infected patients with low BMD, conducted to assess changes in BMD after withdrawing tenofovir from a regimen.3 Briefly, 54 virologically suppressed patients under a tenofovir-containing regimen, with osteopenia/osteoporosis criteria (without receiving a specific treatment), were randomized to switch from tenofovir to abacavir (n = 26) or to continue with tenofovir (n = 28). Although differences between groups at week 48 did not reach statistical significance, hip BMD improved by 2.1% (95% CI: −0.6, 4.7) (P = 0.043) in the abacavir group while in the tenofovir group it did not change significantly [0.7% (95% CI: −0.9, 2.4) (P = 0.372)].
Since the mechanisms underlying these changes in bone density are not fully elucidated, we assessed changes in bone markers and three proteins involved in bone regulation in the same group of patients.

**Methods**

Forty-four patients had stored serum at baseline and week 48 (20 in the abacavir group and 24 in the tenofovir group). All samples were collected under fasting conditions and stored below –80 °C.

Changes from baseline to week 48 in bone markers [C-terminal telopeptide of collagen type 1 (CTX), a resorption marker, and osteocalcin and procollagen type 1 N propeptide (P1NP), markers of bone formation] were assessed. In addition, three proteins involved in bone regulation were also measured: osteoprotegerin, sclerostin and receptor activator for NF-κB ligand (RANKL). Sclerostin is a novel inhibitor factor of the Wnt signalling pathway that inhibits osteoblastogenesis and bone formation. It is recently considered of interest as a potential target for a new strategic therapy for osteoporosis.

CTX (Elexys 2010, Roche Diagnostics; normal range: men aged 30–50 years old, <0.584 ng/mL; men aged >50–70 years old, <0.707 ng/mL; pre-menopausal women, <0.573 ng/mL; and post-menopausal women, <1.008 ng/mL), osteocalcin (Elexys 2010, Roche Diagnostics; normal range: <22.0 ng/mL) and P1NP (Elexys 2010, Roche Diagnostics; normal range: men, 21–78 ng/mL; pre-menopausal women, 15.1–58.9 ng/mL; and post-menopausal women, 20.3–76.3 ng/mL) were measured by electrochemiluminescence immunoassay. Osteoprotegerin (Biomedica, Vienna; range: men, 0.46 pmol/L; and women, 0.37 pmol/L) were measured by ELISA.

**Statistical analyses**

Continuous variables are described as means and standard deviations (SDs). Proportions are given for categorical variables. To compare variables between study groups, χ² or Fisher and Student t-tests were performed.

Pearson’s correlation coefficients were calculated to evaluate linear relationships between bone markers and proteins involved in bone regulation (at baseline and week 48 as well as the difference between them) and BMD.

All analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA). A univariate bilateral P value <0.05 was considered significant.

**Results**

Table 1 summarizes the epidemiological and clinical characteristics for 44 patients. Both groups were well balanced in all parameters except P1NP, for which baseline levels were higher in the abacavir group than in the tenofovir group (P=0.013).

In the tenofovir group, CTX, osteocalcin and P1NP did not significantly vary at week 48 from baseline, while in the abacavir group all markers significantly decreased. Differences between groups at week 48 were significant for all three markers (Figure 1a).

Osteoprotegerin did not significantly vary in either group. In contrast, sclerostin significantly increased in the abacavir group. No significant differences in osteoprotegerin and sclerostin were detected between the groups at week 48 (Figure 1b). RANKL values were below the limit of detection (<0.02 pmol/L) in all samples assessed, at baseline and week 48.

Only in the abacavir group, a positive correlation was seen (r value of 0.68) in the difference between baseline and week 48 in CTX values and the difference between baseline and week 48 in osteocalcin values. No linear relationship was found between sclerostin and BMD (data not shown).

**Discussion**

Our results in HIV-infected patients show that switching from tenofovir to abacavir is followed by a reduction in the bone turnover markers, mean (SD)

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\begin{align*}
\text{CTX (ng/mL)} & \quad 0.543 (0.495) & \quad 0.424 (0.421) \\
\text{osteocalcin (ng/mL)} & \quad 23.72 (22.20) & \quad 21.91 (18.00) \\
\text{P1PN (ng/mL)} & \quad 54.68 (54.52) & \quad 38.81 (40.92) \\
\text{Bone markers, mean (SD)} & \quad & \\
\end{align*}
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HCV, hepatitis C virus; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone.

**Table 1. Epidemiological, HIV-related and biochemical baseline characteristics for 44 patients**
turnover rate and an increase in circulating sclerostin levels. To the
best of our knowledge, in this population these are the first data
exploring sclerostin, a selective regulator of bone formation
through the Wnt pathway.

The DXA scan is the gold standard test to assess BMD, but bone
biomarkers are also used to evaluate bone health.5 The serum CTX
marker is well correlated with the bone turnover rate.6 Collagen
type 1 is the substance produced by osteoblasts that makes up
the majority of bone’s non-mineral tissue. CTX is a specific peptide
sequence, corresponding to the portion that is cleaved during
d bone resorption by osteoclasts. Therefore, CTX serum levels are
proportional to osteoclastic activity. On the other hand, osteocal-
cin is a non-collagenous protein found in bones and also is pro-
duced by osteoblasts. Osteocalcin levels in serum are used as a
marker of the bone formation process.7 Finally, P1NP is derived
from collagen type 1. The precursor of collagen, procollagen,
contains a short signal sequence peptide, P1NP, and a terminal
extension peptide, the carboxy-terminal propeptide. The serum
concentrations of both peptides (P1NP and the carboxy-terminal
propeptide) indicate the synthesis rate of collagen type 1 and are
also used as markers of bone formation.

Among our patients, those who switched from tenofovir to
abacavir showed a significant reduction of all three bone markers
at week 48, indicating a reduction in bone turnover. In contrast,
markers remained completely stable in those patients who were
maintained on tenofovir. In the case of P1NP, levels at baseline
were higher in the abacavir group; this finding may be a potential
limitation for our results. However, patients receiving abacavir
achieved lower levels at week 48, while the tenofovir group remained
stable. Our findings totally agree with previously published data,8,9
confirming a deceleration of bone turnover after the cessation of
tenofovir.

Osteoprotegerin and RANKL are both involved in cytokine sig-
nalling in bone. Osteoprotegerin is a glycoprotein belonging to
the TNF receptor family, which acts as a decoy receptor by inhib-
iting the binding of RANK to RANKL receptors located on the
membrane of the osteoclasts, an essential step for the recruit-
ment, proliferation and activation of osteoclasts. Dysregulation
of the RANKL/RANK/osteoprotegerin system leads to distur-
bances of bone remodelling that underlie the loss of bone
mass. No significant changes were seen among our patients in
osteoprotegerin levels, similarly to the results from other
authors.4 We were not able to determine values for RANKL in
any sample, because circulating levels were below the detection
threshold of the technique.

Sclerostin is produced by osteocytes and has antianabolic
effects on bone formation. Recently, it has been identified as an
inhibitor factor of the Wnt signalling pathway (a network of pro-
teins that control cell-to-cell communication by sending signals
from receptors on the surface of the cell to DNA expression in
the nucleus). This inhibition led to down-regulation of osteoelastic
bone formation.10 In the patients who replaced tenofovir with
abacavir, sclerostin levels significantly increased. No previous
data on sclerostin in an HIV population are available to support
our results, but initial data have been published for non-HIV-
infected subjects.

Some controversial data have emerged from studies in animal
models.11,12 In humans, recently published studies show a posi-
tive correlation between serum sclerostin and improved bone
microarchitectural parameters13 and a lower risk of fracture.14,15

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Figure 1. (a) Changes from baseline to week 48 in mean bone markers (expressed as ng/mL) and (b) in mean osteoprotegerin and sclerostin (expressed as pmol/L). *In the tenofovir group, mean (SD) CTX changed from 0.42 (0.42) to 0.45 (0.43) ng/mL (P = 0.209), mean (SD) osteocalcin changed from 21.91 (18.00) to 20.45 (20.15) ng/mL (P = 0.430), mean (SD) P1NP changed from 38.81 (40.92) to 39.25 (39.42) ng/mL (P = 0.919), mean (SD) osteoprotegerin changed from 3.36 (2.84) to 3.17 (3.00) pmol/L (P = 0.450) and mean (SD) sclerostin changed from 29.99 (27.18) to 33.71 (31.44) pmol/L (P = 0.062). *In the abacavir group, mean (SD) CTX changed from 0.54 (3.00) to 0.31 (0.30) ng/mL (P < 0.001), mean (SD) osteocalcin changed from 23.72 (22.20) to 13.95 (12.60) ng/mL (P < 0.001), mean (SD) P1NP changed from 54.68 (54.52) to 28.65 (27.48) ng/mL (P < 0.001), mean (SD) osteoprotegerin changed from 2.47 (2.19) to 2.68 (2.50) pmol/L (P = 0.139) and mean (SD) sclerostin changed from 29.53 (27.91) to 35.56 (34.59) pmol/L (P = 0.002). **Differences between groups at week 48.
In contrast, results from other studies determined sclerostin as an independent predictor of bone loss or as a risk factor for fractures. Finally, no association between sclerostin levels and fracture risk was detected among 572 post-menopausal women followed prospectively for a median of 6 years (the OFELY study).

The role and importance of circulating sclerostin is still poorly understood, although a better knowledge of this protein’s function will surely contribute to understanding the pathogenesis of bone loss, since it can reflect local bone production. A possible explanation for these discordant results is the fact that serum sclerostin levels do not accurately reflect changes in the local production of sclerostin in bones. No linear relationships were found between BMD markers and, including circulating sclerostin levels, probably due to the small sample size of our study.

In conclusion, switching from tenofovir to abacavir seems to induce a positive effect on bone tissue, since it resulted in a reduction in markers of bone turnover while hip BMD increased 48 weeks after the switch. The switch to abacavir also resulted in increased levels of circulating sclerostin, which has been associated with improvements in bone properties such as density, microarchitecture and strength, and a decreased risk of fracture. However, a larger study is necessary to understand the possible association between sclerostin levels and BMD in this population.

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**References**


