CLINICAL STUDY

Distribution of strontium and mineralization in iliac bone biopsies from osteoporotic women treated long-term with strontium ranelate

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Abstract

Objective: To investigate interactions between strontium (Sr) and bone mineral and its effects on mineralization in osteoporotic women treated long-term with Sr ranelate (SrRan).

Design: In this study, 34 iliac bone biopsies were analyzed after 2, 12, 24, 36, 48, and 60 months of treatment with SrRan.

Methods: Sr global distribution was analyzed by X-ray cartography and the percentage of bone area containing Sr was calculated in the bone samples. The focal distribution of Sr in all bone samples was investigated by X-ray microanalysis. The degree of mineralization was assessed by quantitative microradiography.

Results: Absent from old bone formed before the beginning of treatment, Sr was exclusively present in bone formed during this treatment with a much higher focal Sr content in new bone structural units than in old ones. A progressive increase in the extent of areas containing Sr was observed during treatment. The focal bone Sr content in recently formed bone was constant over treatment. Secondary mineralization was maintained at a normal level during treatment.

Conclusion: Thus, the quality of bone mineralization (density and heterogeneity at tissue level) was preserved after a long-term treatment with SrRan.

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Introduction

Strontium ranelate (SrRan) is an effective antiosteoporotic drug developed for the treatment of postmenopausal osteoporosis (PMOP). *In vitro* and *in vivo* experimental studies (1–9) demonstrate that SrRan has a unique dissociating effect on bone remodeling by maintaining bone formation and decreasing bone resorption, resulting in a prevention of bone loss and an increase in bone mass and strength (9–13). In clinical studies, Sr provides early and sustained vertebral and nonvertebral (including hip) antifracture efficacy and increases the bone mineral density at the spine and hip levels (14–18). Human studies also show an improvement of microarchitecture under SrRan treatment (19–21).

The rate of bone remodeling and the mean duration of secondary mineralization of bone influence the degree of mineralization of bone (DMB) (22–26). When bone remodeling rate increases, the birthrate of new bone structural units (BSUs) increases, resulting in less time to complete secondary mineralization and,

ultimately, DMB decreases. This is observed with anabolic agents used to treat osteoporosis such as parathyroid hormone (27, 28). Conversely, when bone remodeling rate is decreased (treatment with anti-resorptive agents such as bisphosphonates, selective estrogen receptor modulators, or estrogens), the birthrate of BSUs is greatly decreased, their lifespan increases, the duration of secondary mineralization is greatly increased, and the DMB increases (23, 25, 29–31).

In a recent study on PMOP women treated for 36 months with SrRan, we have shown that Sr was exclusively present in bone formed during treatment and was heterogeneously distributed with higher focal concentrations in recent bone formed during treatment than in old bone formed before the beginning of treatment (32). The X-ray cartography, illustrating the extent of bone areas containing Sr and thus the formation activity of Sr during treatment, showed an increase in bone areas containing Sr until 36 months, more in cancellous than in cortical bone (32). Secondary mineralization was maintained at a normal level during the treatment (32). These observations

confirmed the previous ones in monkeys receiving SrRan for 13 weeks (33) and 52 weeks (34). However, the incorporation of Sr within the apatite crystals represented a maximum of one calcium (Ca) ion over ten substituted by one Sr ion in monkeys (33, 34) and a maximum of 0.5 Sr ion in women (35, 36).

We hypothesize that even after a long-term treatment (up to 60 months) and whatever the distribution of Sr in bone, quality of bone mineralization (DMB and the heterogeneity index (HI)) is maintained at tissue level. Thus, in iliac bone biopsies taken from PMOP women after a prolonged treatment with SrRan, the main objectives of this study were to assess i) the global distribution of Sr, Ca, and phosphorus elements in both cortical and cancellous bone tissue on all the area of the biopsy; ii) the focal bone contents of Sr, Ca, and phosphorus in bone formed before and during treatment in cortical and cancellous tissues; and iii) the level of the secondary mineralization of bone.

Materials and methods

Patients and iliac bone samples

Transiliac bone biopsies were obtained from PMOP women (72 ± 5 years) enrolled in three randomized, double-blinded, placebo-controlled clinical trials, STRATOS (16), SOTI (14), and TROPOS (17). The main biopsies analyzed were obtained after 36 ($n=17$), 48 ($n=7$), and 60 ($n=7$) months of treatment with 2 g/day of SrRan (Protelos – Protos – Protaxos – Osseor – Bivalos, Servier, Suresnes, France). All the patients also received daily Ca (up to 1000 mg) and vitamin D (up to 800 UI) according to their needs. Before biopsy, patients received 1 g/day tetracycline for 2 days, followed by a 10-day tetracycline-free interval, and then received 1 g/day tetracycline for two other days. Biopsies were performed within 5–7 days of the last dose of tetracycline (19).

Bone samples were fixed in 70% alcohol, dehydrated in absolute alcohol, and then embedded in methyl methacrylate without prior decalcification (32). The global cartography (a very time-consuming method) was performed on ten biopsies taken after 2 ($n=1$), 12 ($n=1$), 24 ($n=1$), 36 ($n=4$), 48 ($n=2$), and 60 months ($n=1$) of treatment. All bone samples were used for focal X-ray microanalysis and quantitative microradiography. The biopsies taken at 36 months of treatment, previously partly published (32), were included in this study to show a global evolution until 60 months of treatment.

The samples presented in this study were blind-selected by Servier among samples allowing a global study of the entire bone core. These bone samples have been analyzed by histomorphometry and also showed increased mineral apposition rate (MAR) and osteoblastic surfaces (19).

Cartography by global X-ray microanalysis

For the X-ray cartography, the ten embedded bone samples were surfaced with an alumina suspension (1 μm), carbon-coated, and then fixed in the specimen holder (32).

Global X-ray microanalysis was performed using a Camebax electron microprobe (Cameca, Gennevilliers, France) equipped with an energy-dispersive X-ray spectrometer Kevex Super Quantum Mark 5 (Kevex Instruments, Valencia, CA, USA), coupled with the analysis system Noran Voyager III (Noran Instruments, Middletown, WI, USA). Sr $L\alpha$, P $K\alpha$, and Ca $K\alpha$ were the main elements analyzed on the same whole area of bone samples, including osteons and packets formed before and during treatment, on a thickness of 1 μm . Secondary electron images (morphology of the area measured) and X-ray images (topographical distribution of the main elements analyzed) were performed for each biopsy at a voltage of 15 kV, which was the lowest allowing the analysis of the main elements constituting bone mineral without destroying the sample. The resolution chosen for cartography was 256×256 pixels and the counting time for each pixel was 0.07 s, allowing duration of acquisition of 90 min for each region of interest (1 mm^2). This was the largest area analyzable at the lowest magnification of $\times 100$ and preserving an overlapping band allowing the mapping of the complete image of the bone sample. To perform one cartography, a large number of acquisitions and a precise reconstruction were necessary for each sample.

The quantitative image analysis of the cartography required i) the manual elimination of pixels due to background noise, ii) the binarization and dilatation allowing the obtaining of continuous areas, and iii) the calculation of the percentage of bone area, where Sr is present, relative to the total analyzed bone area delimited by the presence of Ca. The percentage of the analyzed bone area containing Sr (number of pixels of Sr over number of pixels of Ca) was calculated for cortical, cancellous, and total (cortical + cancellous) bone tissues.

Focal X-ray microanalysis

The embedded bone samples were surfaced with an alumina suspension (1 μm), carbon-coated, and then fixed into the specimen holder (32). The focal measurements were performed on 1 μm^2 areas. These analyzed areas included old bone formed before treatment and recent bone formed during treatment from both cortical and cancellous bone. Bone was considered as recent if osteons or endosteal borders were not covered by other BSUs, when there is a large haversian canal, or in the border of trabeculae. Old bone corresponded to interstitial cortical bone and to the center of trabeculae. In each category of tissue, 15 different measurements were performed (15 in

Figure 1 Global (a) and focal (b) distribution (or cartography) of phosphorus (P $K\alpha$), calcium (Ca $K\alpha$), and strontium (Sr $L\alpha$) from a PMOP woman treated with 2 g/day SrRan for 60 months. The secondary electron image (SEI) gave the global topography of the area analyzed and allowed the discrimination between recent (R) and old (O) bone. The distribution of elements reflected the formation activity of the treatment. Strontium was only present in recent bone (R) formed under treatment, and the BSUs containing Sr also contained less phosphorus and calcium elements.

recently formed and 15 in old cortical bone, 15 in recently formed, and 15 in old cancellous bone), i.e. a total of 60 measurements in each bone sample. Recent bone formed during treatment was separated from old bone based on morphological criteria.

Focal X-ray microanalysis was performed using the same equipment previously mentioned for X-ray cartography. Sr $L\alpha$, P $K\alpha$, and Ca $K\alpha$ were the main elements analyzed at the same place at a voltage of 15 kV. Each measurement represented a count of 100 s at the same place. For each element, contents were expressed as weight and atomic percentages (100% corresponding to the total elements analyzed, i.e. Ca, phosphorus, Sr, magnesium, sodium, and oxygen). Finally, the weight and atomic ratios Ca/P, Ca+Sr/P, Sr/Ca, Sr/P, and Sr/Ca+Sr were also calculated. Secondary electron images and X-ray images were performed to illustrate the focal Sr distribution in bone tissue.

Quantitative microradiography

Thick sections (about 150 μm) were cut from the bone samples used for the focal X-ray microanalysis using a precision diamond wire saw (Well, Escil, Chassieu, France) and were progressively ground to a thickness of 100 μm and polished with an alumina suspension (1 μm). The thickness of the section was measured with an accuracy of 1 μm using a precision micrometer (Compac, Geneva, Switzerland). Bone sections were then cleaned with ultrasounds and microradiographed (23, 26).

Microradiography was performed using a PW 1830/40 X-ray diffraction unit equipped with a PW 2273/20 diffraction tube (Philips, Limeil-Brevannes, France). The nickel-filtered copper $K\alpha$ radiation was used under 25 kV and 25 mA. A Geola high-resolution film (Slavich International Wholesale Office, Vilnius, Lithuania) was exposed for 20 min simultaneously with the entire 100 μm -thick section and the aluminum standard. For quantitative evaluation of X-ray

absorption by the bone section, a calibration reference system composed of aluminum step-wedge was exposed on each microradiograph (23, 26). Automatic programs (MorphoExpert and Mineralization) were used to analyze gray levels of the aluminum standard (Explora Nova, La Rochelle, France). A digital camera (actual resolution: 1600 $\times$ 1200 pixels or 800 $\times$ 600 after binning) captured the microscopic image of the microradiograph. After calibration using the aluminum reference system, the measured region of bone tissue was automatically selected and then the gray levels were segmented after bone threshold. Subsequently, the values of the gray levels obtained at the pixel level (size 2.82 μm at a magnification of $\times 2.5$) were converted into values of mineralization after plotting a calibration curve based on the values obtained from the aluminum standard. Finally, the variables reflecting the secondary mineralization were expressed in grams of mineral per cubic centimeter of bone tissue. Results were measured separately in cortical, cancellous, and total (cortical+cancellous) bone tissues. The main variable measured

Figure 2 Evolution of the global distribution of strontium in total (cortical+cancellous) bone (%) according to the duration of treatment. There was a progressive extension of bone areas containing strontium.

for each sample was the mean DMB, and the heterogeneity of the distribution of the DMB, calculated as the width at half-maximum of the curve (23, 26). However, a limitation of this technique was the underestimation of DMB in a few measurements of trabecular bone, related to the partial filled volume effect. However, this error in measurement slightly influences the global results because it is comparable in all groups.

Statistical analysis

Results were expressed as mean $\pm$ s.d. Owing to the small number of patients in each group, nonparametric tests were used: the Kruskal–Wallis test was performed to analyze the effect of time. The Mann–Whitney *U* test was used for the comparison of means between groups. Correlations between two parameters were tested with the Spearman test. Significant difference was accepted for $P \leq 0.05$.

Results

Global distribution of Sr in bone tissue

In SrRan-treated PMOP patients, Sr was only detected at the mineral substance level. Indeed, Sr was detected neither in the uncalcified organic matrix (osteoid tissue) nor in the marrow and/or bone cells.

The X-ray cartography allowed the visualization of the BSUs containing Sr, Ca, and phosphorus (Fig. 1a). In cortical and cancellous bone, Sr was always heterogeneously distributed and almost exclusively present in the recent bone formed during treatment compared with old bone formed before the beginning of treatment (Fig. 1b). The osteons and packets containing Sr corresponded to BSUs involved in formation activity during a part of the period of treatment but not necessarily during all the duration of the treatment. Old interstitial bone did not contain Sr. BSUs containing Sr (formed under treatment) also contained less Ca and phosphorus than old interstitial bone (Fig. 1b).

Table 1 Focal X-ray microanalysis of the major elements contained in recent (formed during treatment) and old (formed before treatment) bone tissues from cortical and cancellous bone, as weight %, from patients treated with 2 g/day of SrRan. Sr was almost always absent in old bone and was present in recent bone. The focal bone Sr content in recent bone was constant from 2 to 60 months of treatment.

Treatment (months)	n	Bone	P (weight %)	Ca (weight %)	Sr (weight %)	Ca/P	Ca+Sr/P	Sr/Ca+Sr
2	1	Cortical						
		Recent	13.79 (0.70)	33.78 (1.91)	1.59	2.45 (0.08)	2.46 (0.07)	0.003 (0.013)
		Old	13.66 (0.31)	34.22 (0.97)	0.00 (0.00)	2.51 (0.06)	2.51 (0.06)	0.000 (0.000)
		Cancellous						
12	1	Recent	13.65 (0.49)	32.09 (1.22)	1.81 (0.27)	2.35 (0.10)	2.40 (0.07)	0.018 (0.027)
		Old	13.84 (0.51)	34.54 (1.66)	0.00 (0.00)	2.49 (0.07)	2.49 (0.07)	0.000 (0.000)
		Cortical						
		Recent	13.39 (0.71)	32.37 (1.71)	1.42 (0.27)	2.42 (0.08)	2.52 (0.07)	0.042 (0.008)
24	1	Old	13.39 (0.73)	34.40 (1.97)	0.00 (0.00)	2.57 (0.04)	2.57 (0.04)	0.000 (0.000)
		Cancellous						
		Recent	13.88 (0.97)	32.95 (1.99)	1.25 (0.36)	2.38 (0.08)	2.47 (0.08)	0.036 (0.010)
		Old	13.66 (0.77)	34.75 (2.14)	0.00 (0.00)	2.54 (0.06)	2.54 (0.06)	0.000 (0.000)
36	17	Cortical						
		Recent	14.72 (0.96)	37.22 (3.65)	2.18 (0.29)	2.52 (0.12)	2.67 (0.12)	0.056 (0.007)
		Old	13.99 (0.85)	35.91 (2.84)	0.01 (0.04)	2.57 (0.10)	2.57 (0.10)	0.000 (0.001)
		Cancellous						
48	7	Recent	13.75 (1.13)	34.32 (3.03)	2.23 (0.39)	2.50 (0.07)	2.66 (0.07)	0.061 (0.009)
		Old	14.20 (1.14)	37.35 (3.37)	0.03 (0.07)	2.63 (0.08)	2.63 (0.08)	0.001 (0.002)
		Cortical						
		Recent	15.04 (0.85)	36.84 (3.08)	1.74 (0.95)	2.45 (0.14)	2.57 (0.14)	0.045 (0.024)
60	7	Old	15.03 (0.79)	39.04 (3.21)	0.00 (0.04)	2.60 (0.13)	2.60 (0.13)	0.000 (0.001)
		Cancellous						
		Recent	14.97 (0.95)	36.09 (3.12)	1.73 (0.89)	2.41 (0.13)	2.53 (0.13)	0.046 (0.023)
		Old	15.14 (0.86)	38.52 (3.22)	0.00 (0.03)	2.54 (0.11)	2.52 (0.26)	0.000 (0.001)
60	7	Cortical						
		Recent	13.99 (0.98)	32.28 (2.83)	1.57 (0.61)	2.31 (0.08)	2.42 (0.09)	0.046 (0.018)
		Old	13.88 (0.74)	34.29 (2.25)	0.00 (0.02)	2.47 (0.07)	2.47 (0.07)	0.000 (0.001)
		Cancellous						
60	7	Recent	13.90 (0.77)	31.24 (1.95)	1.55 (0.65)	2.25 (0.09)	2.36 (0.09)	0.047 (0.020)
		Old	14.04 (0.70)	34.01 (2.04)	0.03 (0.18)	2.42 (0.07)	2.42 (0.07)	0.001 (0.005)
		Cortical						
		Recent	14.43 (1.39)	33.42 (3.13)	1.44 (0.97)	2.32 (0.11)	2.42 (0.09)	0.041 (0.024)
60	7	Old	14.17 (1.24)	34.76 (3.26)	0.04 (0.16)	2.45 (0.08)	2.46 (0.08)	0.001 (0.004)
		Cancellous						
		Recent	14.95 (1.68)	34.14 (4.19)	1.37 (0.79)	2.28 (0.11)	2.37 (0.10)	0.038 (0.019)
		Old	14.85 (1.69)	35.91 (4.18)	0.06 (0.23)	2.42 (0.09)	2.42 (0.08)	0.002 (0.007)

The X-ray cartography revealed that Sr was distributed and was present up to 48% of the total bone area ($27.53 \pm 16.02\%$, mean $\pm$ s.d. from all data). Whatever the duration of treatment, the percentage of bone area with Sr was higher in cancellous bone ($36.25 \pm 28.52\%$) than in cortical bone ($24.70 \pm 15.34\%$). The global distribution of Sr showed, in a small number of samples, a regular and progressive extension of the areas containing Sr from 2 months (2%) until 36 months (35%), to finally reach a value of 48% at 60 months (Fig. 2).

Focal contents of elements measured in bone tissue

Sr was absent from bone formed before the beginning of treatment and was almost exclusively present in recent bone formed during treatment (Table 1, Fig. 3) as evidenced by tetracycline double labeling. The focal Sr content measured in total bone was constant whatever the duration of treatment (Fig. 3) and showed similar evolutions in cortical and cancellous bone (Table 1). In total bone, the focal content of Sr in bone formed during treatment reached early a content close to the one measured after 48–60 months of treatment (Table 1, Fig. 3). Indeed, the BSUs containing Sr revealed mean Sr contents not significantly ($P=0.81$) different between 2, 12, 24, 36, 48, and 60 months. After 36 months of treatment, Ca, Ca/P, and Ca+Sr/P were significantly lower ($P \leq 0.01$) in recent bone formed during the treatment than in old bone formed before treatment initiation. In recent bone, the Sr/Ca+Sr ratio showed a replacement of Ca ions by Sr ions from 0 to 6% (Table 1). In old bone, the Sr/Ca+Sr ratio was always zero.

Figure 3 Evolution of the focal bone strontium content in total (cortical + cancellous) bone (weight %) according to the duration of treatment. Strontium was almost always absent in old interstitial bone formed before the initiation of treatment. In recent bone formed during treatment, the focal strontium content was constant all along the duration of treatment.

Figure 4 Microradiograph (a) illustrating the heterogeneity of the secondary mineralization from a PMOP woman treated with 2 g/day of SrRan for 48 months and (b) evolution of the degree of mineralization of bone (DMB, black bars) and of the heterogeneity index (HI, white bars) of total (cortical + cancellous) bone according to the duration of SrRan treatment compared with DMB and HI measured in patients treated with placebo (PLA, dark and light gray bars respectively) during 36 months (31). DMB and HI remained constant whatever the duration of treatment.

Secondary mineralization of bone

Up to 60 months of treatment, DMB, reflecting the secondary mineralization of bone, remained constant in cortical (1.12 ± 0.08), cancellous (1.13 ± 0.07), or total bone (1.13 ± 0.07). The HI (of the distribution of DMB) was constantly heterogeneous with a mean of 0.25 ± 0.08 in total bone (Fig. 4, Table 2).

Discussion

To assess the impact of Sr on bone mineralization even after a long-term treatment, we found in this study that i) Sr was heterogeneously distributed in bone tissue and was exclusively present in bone formed after the beginning of treatment; ii) bone area containing Sr increased until 60 months of treatment; iii) the extent of

Table 2 Variables reflecting the secondary mineralization (DMB) and (HI) measured in cortical and cancellous bone of patients treated daily with 2 g of SrRan. These variables were constant whatever the duration of treatment. Range corresponds to the lowest and the highest values.

Treatment (months)	n	Bone	Secondary mineralization mean (s.d.)/range	
			DMB (g/cm ³)	HI (g/cm ³)
36	17	Cortical	1.13 (0.07)/ 1.00–1.24	0.21 (0.07)/ 0.11–0.32
		Cancellous	1.14 (0.06)/ 1.04–1.25	0.21 (0.06)/ 0.13–0.32
48	7	Cortical	1.11 (0.09)/ 1.01–1.23	0.32 (0.14)/ 0.14–0.58
		Cancellous	1.12 (0.05)/ 1.05–1.20	0.24 (0.07)/ 0.16–0.40
60	7	Cortical	1.12 (0.11)/ 0.96–1.31	0.27 (0.08)/ 0.15–0.36
		Cancellous	1.11 (0.09)/ 0.99–1.29	0.28 (0.07)/ 0.17–0.41

areas containing Sr was often higher in cancellous than in cortical bone; iv) the focal Sr content was constant in bone formed during treatment; and v) whatever the duration of treatment and the content of Sr in bone, the secondary mineralization was maintained at a normal level.

Distribution of Sr in bone

Sr was taken up by bone mineral and heterogeneously distributed. Owing to tetracycline double-labeling, we were able to identify bone formed under treatment and we could observe that Sr was almost exclusively present in the bone formed under treatment (32). BSUs containing Sr were more numerous in cancellous than in cortical bone. This was probably explained by the fact that cancellous bone is richer in new bone than cortical bone, due to a higher remodeling rate or to greater surface-to-volume ratio in cancellous tissue (25). The global distributions of Sr observed in this study were in good agreement with those already reported in monkeys (33, 34) and in women (32). Nevertheless, focal Sr contents in areas containing Sr were similar after 2–60 months of treatment, i.e. that for a given patient, and independent of the duration of treatment, bone Sr uptake was constant without progressive enrichment at the same site. The evolution of the global bone Sr content measured chemically (32) was thus due to extended bone area containing Sr but not to its focal accumulation. The observation of a limited number of cartographies of Sr showed an increment in the number of BSUs having had a formation activity during a part of the treatment. During the 60 months of treatment, a large part of the old interstitial bone did not contain Sr. Finally, in the same patients, the relative change in lumbar spine bone mineral density from baseline

followed the same evolution than that of total iliac bone areas containing Sr (data not shown).

Quantification of Sr in bone

Focal bone Sr content in old bone formed before treatment was very low, if not zero, and similar to the amount of Sr reported in the iliac bone from placebo-treated PMOP patients, in old bone of patients treated for 36 months with SrRan (32), and from healthy subjects or untreated osteoporotic patients (37). After 2–60 months of treatment with SrRan in PMOP women, about the same amount of Sr was taken up by bone tissue (1.59 ± 0.70 weight %, mean $\pm$ s.d. of all time points) and was almost exclusively present in recent bone formed during treatment. This strongly suggested that Sr was taken up early by bone mineral and/or with the same ratio (Sr vs Ca) whatever the duration of treatment. Moreover, the small variations of focal Sr content in recent bone (Fig. 3) can be explained by the variations in blood Sr levels because there was a significant positive correlation ($P=0.0017$) between these two parameters. The Sr/Ca + Sr ratio is the most relevant to define how many Ca atoms were replaced by Sr atoms. Our data showed that the replacement of Ca by Sr was between 0 and 6%. These results are in the same order of magnitude than data recently published (35, 36) and confirmed the small rate of substitutions between Ca and Sr. The heterogeneity of focal Sr distribution between bone formed before or during treatment observed in this study has also been reported in monkeys receiving SrRan (33, 34). Only recent bone formed during treatment took up Sr by ionic substitution within the apatite crystal lattice and by ionic exchange of Ca ions by Sr ions in the hydrated layer of the crystals (33, 35), but we were not able to measure in which proportions for each site of the crystal. Recently, it has been reported that, in goats receiving 40 mg/kg per day of Sr for 16 weeks, there was no change in the bone crystal morphology and structure, and a limited amount of Sr replaced Ca in apatite crystals (38). Another study on a very small number of patients receiving daily 2 g SrRan for 36 months (35) also confirmed that Sr preserved the thickness and the length of bone mineral crystals.

Sr and mineralization of bone

DMB and HI were constantly at a normal value in treated PMOP patients whatever the duration of the SrRan treatment. Moreover, DMB and HI values, from 36 to 60 months, were comparable to data from patients treated with placebo (Ca and vitamin D) for 36 months (32) (Fig. 4). Furthermore, the mean DMB from patients receiving a prolonged treatment was close to the mean DMB from controls, premenopausal women (23), and PMOP patients treated for 36 months with SrRan (32, 36). Moreover, these values are also

comparable to untreated osteoporotic patients, with a mean DMB of 1.042 ± 0.095 and 1.084 ± 0.092 in cortical and cancellous bone respectively (29). These data have been recently confirmed in untreated postmenopausal patients (39). Thus, the secondary mineralization was not modified by Sr as previously reported in animals (6, 11, 33–35) and human (19, 32) studies. However, we know that the presence of Sr ions, larger than Ca ions, leads to a greater attenuation of X-rays. Thus, it remained possible that a decrease in DMB in recent bone was masked by the presence of Sr in bone, resulting in an unchanged DMB. SrRan increased bone formation and decreased bone resorption (1–5, 9, 24, 32). Indeed, in SrRan-treated PMOP women (19), the maintenance of the primary mineralization of bone (mineral apposition rate) has been shown in addition to a decrease in osteoid thickness and an increase in osteoblastic surfaces (+38% in cancellous and endocortical bone). Finally, even in women receiving a prolonged SrRan treatment, a preservation of the normal bone secondary mineralization process was observed. This was also the case in monkeys treated with high doses of SrRan (33, 34) and in PMOP women treated for 36 months with SrRan (32, 35, 36).

Conclusions

In conclusion, during 60 months of treatment with SrRan, i) Sr was almost exclusively distributed in recent bone formed during the treatment; ii) the areas containing Sr increased during 60 months of treatment; iii) the Sr focal content in recent bone was constant at each time point; iv) whatever the duration of treatment and the content of Sr in bone, the secondary mineralization (density and heterogeneity at tissue level) was maintained within the normal range. Finally, after a prolonged treatment with SrRan, the quality of bone mineralization in PMOP women was unchanged.

Declaration of interest

P J Meunier and G Boivin serve as consultants for the Servier Company. All other authors have no conflicts of interest.

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