Long-Term Treatment With Strontium Ranelate Increases Vertebral Bone Mass Without Deleterious Effect in Mice

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It was previously shown that strontium ranelate (SR; S12911-PROTOS®, Institut de Recherches Internationales Servier, Courbevoie, France) can modulate bone metabolism in rats and mice. To determine the long-term effects of SR on vertebral bone metabolism in adult mice, the compound or the vehicle was given in the diet to normal male and female mice for 104 weeks at the dose of 200, 600, or 1,800 mg/kg/d corresponding to 0.78, 2.34 or 7.01 mmol Sr^{2+}/kg/d. SR dose-dependently increased plasma strontium concentration, as well as exposure to the drug. Histomorphometric analyses of indices of bone volume, bone formation, and resorption were determined in the endosteal vertebral bone. SR significantly increased the trabecular bone volume by 25% and 59% in females treated with SR 600 and 1,800 mg/kg/d, respectively. This was associated with a 27% and 62% increase in mineralized bone volume. Bone volume was also significantly increased by 17% and 38% in male mice treated with SR 200 and 1,800 mg/kg/d, respectively. In parallel, SR increased the osteoblastic surface by 131% in males. In addition to this stimulatory effect on bone formation, a 52% decrease in osteoclastic surface, and a dose-dependent decrease in osteoclastic number (30% to 47%), was observed in female mice. Finally, SR even at the highest dose tested did not alter the osteoid thickness, indicating no deleterious effect on bone mineralization. Altogether, these findings show that SR simultaneously increases bone formation and decreases bone resorption in male or female mice, which results in increased vertebral bone mass in both genders without deleterious effect on bone mineralization.

THERE IS ACCUMULATING evidence that strontium ranelate (SR, S12911) affects bone cells and bone metabolism in vitro and in vivo.[1,2] SR administration at low doses was shown to inhibit bone resorption and/or to stimulate bone formation in rodents, monkeys, and humans.[3-7] In vitro, it was found that SR stimulates bone formation and inhibits bone resorption.[8,9] Moreover, SR increases rat calvaria osteoblastic cell replication and function[10] and inhibits osteoclast function in vitro.[11,12] Thus, SR appears to have a dual effect on bone formation and resorption in vitro and in vivo, suggesting that SR treatment may be useful for increasing bone mass in osteopenic animals.[2] Indeed, experimental studies in estrogen-deficient rats[13,14] and immobilized rats[15] showed that administration of SR improved trabecular bone mass by decreasing bone resorption while maintaining or increasing bone formation. Accordingly, recent evidence indicates that SR may be useful for improving bone mass in patients with osteopenic disorders.[16,17] Since improvement in bone mass may require long-term treatment, it is important to determine if long-term treatment with SR still has beneficial effects on bone metabolism and bone mass without inducing deleterious effects on bone growth or mineralization.

The aim of the present study was therefore to investigate the long-term effects of SR administration on vertebral bone metabolism in adult mice. The data show that SR decreases indices of bone resorption or increases bone formation in female and male mice. An increase in vertebral bone volume was also demonstrated in both genders without inducing deleterious effects on bone mineralization.

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MATERIALS AND METHODS

Animals

Twenty male and 20 female 6-week-old $B_6C_3F_1$ mice were randomized into 5 groups and weighed 1 week before the beginning of the treatment. The animals were treated daily with SR (5-[bis(carboxymethyl)amino]-2-carboxy-4-cyano-3-thiophenacetic acid, distrontium salt; S12911-PROTOS®, Institut de Recherches Internationales Servier, Courbevoie, France). This compound, which has a molecular weight of 513.5, contains 2 stable strontium ions per molecule (ie, 34.13% wt/wt) and was given in the diet at a dose of 200, 600, or 1,800 mg/kg/d (ie, 0.78, 2.34, or 7.01 mmol Sr^{2+}/kg/d) for 104 weeks. Control animals were given the vehicle only. The animals were fed a standard rodent chow containing 0.74% calcium. Body weight was recorded once during the acclimation phase, weekly for the first 13 weeks, every 2 weeks from weeks 13 to 53, and then once every 4 weeks until the end of the study (last weighing in week 101).

Plasma Biochemistry and Calculation of Exposure

Blood was collected at the jugular vein under ether anesthesia. Plasma was obtained by centrifugation and samples were stored at –20°C until analysis. Plasma strontium levels were determined with a method adapted from Mauras et al[18] using an inductively coupled plasma optical emission spectrometry technique.

One plasma sample was obtained on 4 occasions: after 26, 52, 78, and 104 weeks of treatment. The exposure of the animals to strontium could be estimated at each occasion from these plasmatic concentrations by the calculation of the area under the curve over 24 hours ($AUC_{0\text{-}24h}$). Since SR was given in the diet and the steady state was reached,[19] the $AUC_{0\text{-}24h}$ (mmol · h/L) at steady state was calculated using the following formula: $AUC_{0\text{-}24h} = Cp \times 24$, where Cp is the plasma strontium concentration (mmol/L). One $AUC_{0\text{-}24h}$ for each treatment group was obtained at the 4 occasions described above, and the mean of these 4 values was calculated to reflect the mean exposure over the whole treatment period.

Bone Histomorphometry

The mice were killed and the first 2 caudal vertebrae were removed, cleaned of skin and connective tissue, and dehydrated in

70% ethanol. Samples were randomized to allow measurement without knowledge of the gender and treatment group, before being embedded, undecalcified, in methylmetacrylate.[20] Serial longitudinal sections 7 μm thick were obtained using a Jung K microtome (Nußloch, Germany) equipped with a tungsten carbide blade, and sections were stained with toluidine blue. Except cartilage and vertebral lengths, histomorphometric indices of formation and resorption were determined on standardized areas in the entire endosteal bone, including the endocortical surface, and excluding the primary spongiosa, as previously described.[3,4] Structural parameters were determined using a Biocom software imaging system (Les Ulis, France).[13,15] Others parameters were measured using a Zeiss ocular integrator, mounted on an Olympus microscope (Rungis, France).[20] The following conventional static and dynamic histomorphometric indices were measured[21]: trabecular bone volume (%), mineralized bone volume (%), osteoid volume (%), osteoid surface (%), osteoid thickness (μm), osteoblast surface (%), osteoclast surface (%), osteoclast number (mm^{-2}), trabecular thickness (μm), trabecular separation (μm), and trabecular number (mm^{-1}). In addition, the dimensional parameters of length of vertebrae (mm) and cartilage thickness (μm) were evaluated on the bone sections.

Statistics

All histomorphometric parameters are expressed as the mean ± SEM. The effects of SR were evaluated separately in male and female mice. Differences between untreated and treated groups were analyzed using the statistical package super-ANOVA with a subsequent Scheffé F test in case of significance (Macintosh, Abacus Concepts, Berkeley, CA). Differences between mean values were evaluated with a minimal significance of $P < .05$.

RESULTS

There was no sign of toxicity in the animals even at the high dose level and there was no modification in body weight and food and water consumption. Actual achieved intakes of strontium ranelate were 199.5 ± 15.2 mg/kg/d, 601.9 ± 45.6 mg/kg/d, and 1,806.2 ± 138.9 mg/kg/d in males and 199.9 ± 13.9 mg/kg/d, 594.5 ± 38.5 mg/kg/d, and 1,816.4 ± 99.0 mg/kg/d in females treated with 200, 600, and 1,800 mg/kg/d of SR, respectively, corresponding to 0.78, 2.34 and 7.01 mmol Sr^{2+}/kg/d, respectively.

Mean body weight was similar in all groups during the first year of the study. Thereafter, the body weight of mice given 1,800 mg/kg/d was slightly but generally significantly higher than that of controls (maximum, +4% for males and +6% for females). At the last weighing time point of the study (week 101), the overall comparison was significant either in males or in females. At this time, the body weight was 36.2 ± 0.38 g, 37.0 ± 0.38 g, 36.9 ± 0.37 g, and 36.7 ± 0.32 g for males receiving 0, 200, 600, and 1,800 mg/kg/d, respectively, and 35.4 ± 0.50 g, 36.5 ± 0.54 g, 36.5 ± 0.50 g, and 37.0 ± 0.53 g for females, respectively.

Plasma Strontium Increases in Treated Mice

Treatment with SR dose-dependently increased plasma strontium concentrations (0.015 to 0.181 mmol/L, and 0.023 to 0.266 mmol/L for treated males and females, respectively) and the subsequent exposure of the animals to the dose, over the 104 weeks of treatment (Fig 1). $AUC_{0\text{-}24}$ was 0.37, 1.59, and 4.39 mmol · h/L and 0.55, 1.74, and 6.55 mmol · h/L for males and females, respectively (Fig 1B). Importantly, for a given dose group, females had a higher exposure than males, and $AUC_{0\text{-}24}$ were significantly higher in females (+50% and +47%) than in males in groups treated with 200 or 1,800 mg/kg/d, respectively ($P < .001$ in each group).

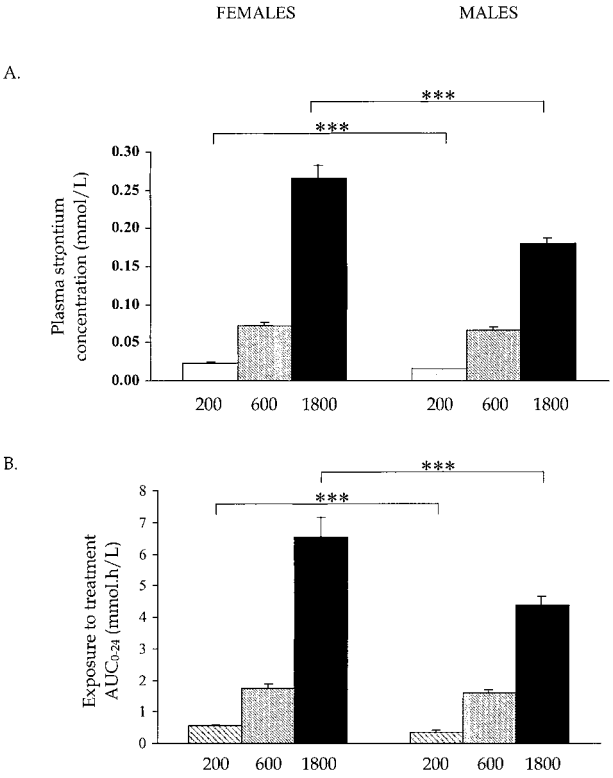

Fig 1. Treatment with SR dose-dependently increases plasma strontium levels (A) and strontium exposure (B) measured by the $AUC_{0\text{-}24h}$ in normal mice treated over 104 weeks. Data are expressed as the mean ± SEM (n = 20/group). ***$P < .001$ *v* males at the same dose.Doses are in mg/kg/d of SR and correspond, respectively, to 0.78, 2.34 and 7.01 mmol Sr^{2+}/kg/d.

SR Increases Bone Formation and Decreases Bone Resorption

Because SR dissociates and ranelic acid is not absorbed in the gut, the effects of SR on bone metabolism reflected the effects of strontium. The histomorphometric parameters of bone formation (osteoid surface, osteoblastic surface) and bone resorption (number of osteoclasts and osteoclast surface) differed between male and female mice in the control group ($P < .001$). As a consequence, significant differences in these parameters were also observed between males and females at a given treated group (data not shown). However, in both genders, a 104-week period of treatment with SR dose-dependently increased histomorphometric indices of bone formation. Both osteoid and osteoblastic surfaces were significantly increased in males treated with 600 and 1,800 mg/kg/d as compared with the control group, with a maximal effect of +81% and +131% for osteoid surface and osteo-

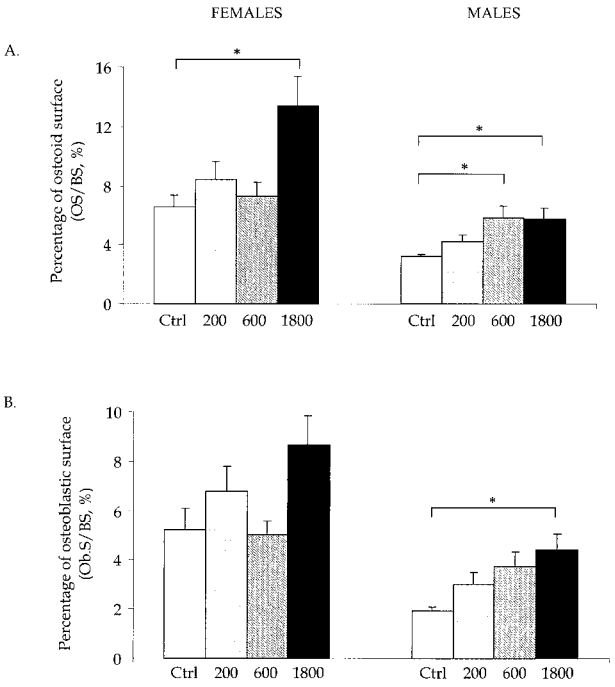

Fig 2. Effects of SR on histomorphometric indices of bone formation in normal mice treated over 104 weeks. Data are expressed as the mean ± SEM (n = 20/group). *$P < .05$ *v* the control (Ctrl) group. In the control group, males and females differed ($P < .001$). Doses are in mg/kg/d of SR and correspond, respectively, to 0.78, 2.34 and 7.01 mmol Sr^{2+}/kg/d.

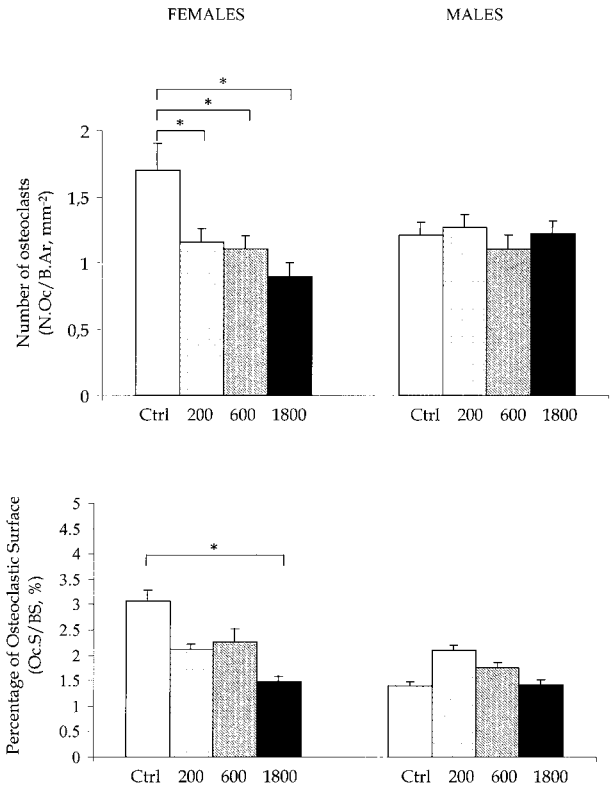

Fig 3. Effects of SR on histomorphometric indices of bone resorption in normal mice treated over 104 weeks. Data are expressed as the mean ± SEM (n = 20/group). *$P < .05$ *v* the control (Ctrl) group. In the control group, males and females were different ($P < .001$ for osteoclast surface and $P < .01$ for number of osteoclasts). Doses are in mg/kg/d of SR and correspond, respectively, to 0.78, 2.34 and 7.01 mmol Sr^{2+}/kg/d.

blastic surface, respectively, at the highest dose (Fig 2). In females, only the highest dose significantly increased osteoid surface (+103%) as compared with the control group (Fig 2). In contrast, SR effects on bone resorption differed in males and females. In female mice, SR decreased osteoclastic surface in a dose-related manner with a maximal effect (-52%, $P < .05$) at 1,800 mg/kg/d. The number of osteoclasts was also significantly dose-dependently decreased by 30% to 47% in female mice (Fig 3). In contrast, osteoclastic surface and osteoclast number (Fig 3) were not significantly changed in male mice after 104 weeks of treatment. These results indicate that SR had different effects on bone metabolism in mice according to gender, with a more acute effect on bone formation in male mice, and a significant inhibitory effect on bone resorption in female mice.

SR Increases Vertebral Metaphyseal Bone Volume

Administration of SR increased vertebral bone volume in both male and female mice. In females, the metaphyseal mineralized bone volume increased by 27% ($P < .05$) and 62% ($P < .05$) in animals treated with SR 600 and 1,800 mg/kg/d, respectively (Fig 4). Similarly, the total bone volume increased by 27% ($P < .05$) and 62% ($P < .05$) in these animals (Table 1). A significant increase in mineralized vertebral bone volume was also observed in males treated with 200 and 1,800 mg/kg/d (+17% and +38%, respectively, $P < .05$ in each case) (Fig 4). However, trabecular separation, thickness, and number did not change significantly (Table 1). These data indicate that the changes in bone formation and resorption induced by SR result in increased trabecular bone mass in both genders.

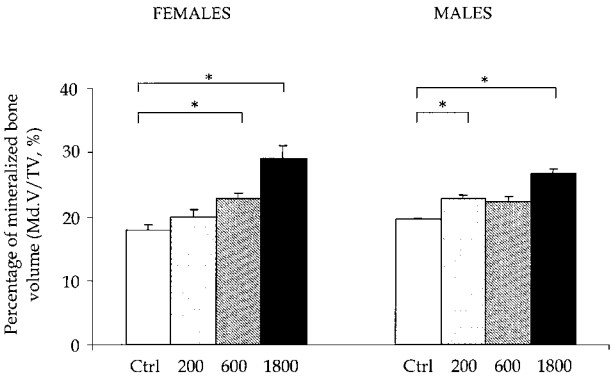

Fig 4. Effects of SR on the mineralized vertebral bone volume measured in the metaphysis in normal mice treated over 104 weeks. Data are expressed as the mean ± SEM (n = 20/group). *$P < .05$ *v* control (Ctrl) group. Doses are in mg/kg/d of SR and correspond, respectively, to 0.78, 2.34 and 7.01 mmol Sr^{2+}/kg/d.

Table 1. Effects of Strontium Ranelate on Bone Structural Parameters in Vertebral Bone in Normal Mice Treated Over 104 Weeks

	Females				Males			
SR (mg/kg/d)	Trabecular Bone Volume (%)	Trabecular Thickness (μm)	Trabecular Separation (μm)	Trabecular No. (mm^{-1})	Trabecular Bone Volume (%)	Trabecular Thickness (μm)	Trabecular Separation (μm)	Trabecular No. (mm^{-1})
0								
Mean	18.6	102	261	2.9	20.1	90	147	4.3
SEM	0.9	3.0	20	0.1	0.7	1.9	11	0.2
200								
Mean	20.3	109	266	2.8	23.5*	98	132	4.4
SEM	0.8	3.7	20	0.1	0.7	2.7	6	0.1
600								
Mean	23.3*	103	226	3.1	23.1	85	149	4.2
SEM	1.3	4.2	19	0.2	0.9	2.2	6	0.1
1,800								
Mean	29.6*	117	214	3.1	27.4*	96	142	4.3
SEM	1.2	4.5	16	0.1	0.7	4.1	6	0.1

NOTE. (n = 20 per group).
*$P < .05$ compared with control group.

SR Has No Deleterious Effect on Bone Metabolism

As shown in Table 2, long-term treatment with SR did not increase osteoid thickness in male or female mice, indicating that the treatment had no deleterious effect on the bone mineralization process. At the highest dose, the cartilage thickness was slightly but significantly increased (+13%, $P < .05$) (Table 2). However, this marginal effect did not affect vertebral growth as total length was not affected (Table 2).

DISCUSSION

The present study shows that long-term treatment with SR significantly increases bone formation and reduces bone resorption in normal male and female adult mice, leading to increased metaphyseal bone volume in vertebral bone. The administration of SR led to a dose-dependent increase in plasma strontium concentrations, confirming the previous results in rats, mice, and monkeys.[3,6] Bone strontium content is known to be directly related to strontium plasma levels and to be dependent on time of treatment and gender.[19] Indeed, plasma strontium levels and plasma $AUC_{0\text{-}24}$ (and presumably strontium levels in bone marrow as well) were higher in females compared with males for a given dose, presumably because of differences in pharmacokinetics between both genders.[19]

Interestingly, the distinct plasma strontium levels in male and female mice were associated with different effects on bone metabolism. Indeed, SR decreased bone resorption indices in females but not significantly in males. The observed inhibitory effect of SR on bone resorption in female mice is in agreement with previous findings in rodents.[3,4,13] This may be related to a direct and specific effect of strontium on osteoclast activity.[11,12] The effect of strontium on bone resorption may be highly dependent on its local concentration around osteoclasts, as demonstrated for calcium.[22] Because the incorporation of strontium into bone depends on the gender,[21] the distinct effect of

Table 2. Effects of Strontium Ranelate on Osteoid Thickness and Cartilage Parameters in Vertebral Bone in Normal Mice Treated for 104 Weeks

	Females			Males		
SR (mg/kg/d)	Vertebral Length (mm)	Cartilage Thickness (μm)	Osteoid Thickness (μm)	Vertebral Length (mm)	Cartilage Thickness (μm)	Osteoid Thickness (μm)
0						
Mean	3.1	92	2.5	3.6	93	2.0
SEM	0.1	2.5	0.3	0.1	2.1	0.1
200						
Mean	3.2	91	2.7	3.3*	90	2.2
SEM	0.1	1.7	0.3	0.1	2.7	0.3
600						
Mean	3.3	93	2.3	3.4	91	2.8
SEM	0.1	3.5	0.3	0.1	2.7	0.6
1,800						
Mean	3.3	104*	2.7	3.4	101	3.1
SEM	0.1	3.1	0.4	0.1	3.5	0.7

NOTE. (n = 20 per group).
*$P < .05$ compared with control group.

SR on bone resorption in female and male mice may thus be related to different strontium bone uptake. In contrast to the distinct effects of SR on bone resorption in male and female mice, SR increased bone formation in both genders. This is in accordance with previous studies in rodents, monkeys, and humans.[3-7] Although the mechanism of action of SR on bone formation is not known, in vitro studies suggest that SR stimulates the replication of osteoprogenitor cells.[10] Overall, the dual and independent effects on bone resorption and formation of SR resulted in increased bone volume in both genders. This was not related to an effect of SR on skeletal growth, since body weight was not changed during the first year of treatment and was similar to control animals at the end of treatment. Although no significant changes in trabecular bone thickness or separation could be detected under treatment, this may be related to the caudal vertebral site, since earlier studies have shown significant changes in trabecular architecture in long bones of rats treated with strontium[8] and SR.[15,23]

It was suggested that strontium may inhibit bone mineralization in growing animals fed a low-calcium diet.[24] High bone strontium content was also recently reported to be associated with osteomalacia in uremic rats.[25] An important aim of this study was therefore to investigate if long-term treatment with relatively high doses of SR may affect bone mineralization. We found that long-term administration of high doses of SR (up to 1,800 mg/kg/d, ie, 7.01 mmol Sr^{2+}/kg/d) did not induce any alteration of bone mineralization, as shown by the lack of increase in osteoid thickness, an indicator of defective mineralization.[26] Only the dose of 1,800 mg/kg/d very slightly increased cartilage thickness, suggesting a possible alteration in growth plate mineralization. This effect was, however, marginal and did not affect vertebral growth even after 104 weeks of treatment. Consistent with the present data, it was recently shown that SR (0.1 to 1 mmol/L Sr^{2+} in the culture medium) increases cartilage matrix synthesis in isolated human chondrocytes without stimulating the chondroresorption process.[27] Our finding that long-term treatment with SR at the doses administered had no deleterious effect on bone metabolism or mineralization in mice is in accordance with previous studies in normal or ovariectomized rats.[5,13,28]

The results of the present study therefore demonstrate that long-term administration (104 weeks) of SR at dose levels up to 1,800 mg/kg/d (ie, 7.01 mmol Sr^{2+}/kg/d) increases bone formation and reduces bone resorption in male or female mice, and increases bone volume in both genders without inducing deleterious effect on bone metabolism. This suggests that SR may be of potential interest in the treatment of osteopenic disorders.

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