Vitamin D supplementation and strontium ranelate absorption in postmenopausal women with low bone mass

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Abstract

Background: Strontium ranelate is used to treat osteoporosis. Calcium (Ca) and strontium (Sr) have common chemical features and are absorbed by the same pathways. Vitamin D has a main role in calcium intestinal absorption. The aim of this study was to investigate whether vitamin D status is a determinant of strontium ranelate absorption.

Methods: Twenty-five patients with vitamin D deficiency ($25(\text{OH})\text{D} < 50 \text{ nmol/l}$) and 25 with vitamin D sufficiency ($25(\text{OH})\text{D} > 75 \text{ nmol/l}$) underwent a 4-h oral Sr overload test. Sr absorption was evaluated as the fraction of absorbed dose and the area under the curve. After the baseline overload test, the deficient patients were treated until reaching sufficient vitamin D levels ($25(\text{OH})\text{D} > 75 \text{ nmol/l}$) and the test was repeated.

Results: Changing vitamin D status from deficient to sufficient resulted in a significant increase in $1,25(\text{OH})_2\text{D}$ ($24.97 \pm 4.64 \times 34.62 \pm 9.14 \text{ pg/ml}$, $P < 0.001$) and a reduction in parathyroid hormone ($73.87 \pm 37.50 \times 58.24 \pm 20.13 \text{ pg/ml}$, $P = 0.006$). Nevertheless, no differences were found in the parameters used to evaluate Sr absorption between the vitamin D deficient and sufficient groups. In addition, vitamin D3 replacement in the deficient group did not result in enhanced Sr absorption.

Conclusion: Vitamin D status did not interfere with strontium ranelate absorption. Taking into account the benefits of adequate vitamin D status in osteoporotic patients, we strongly recommend the treatment of vitamin D deficiency. However, the data demonstrate that such treatment does not enhance strontium ranelate absorption in patients with mild deficiency.

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Introduction

Osteoporosis is a chronic condition characterized by a reduction in bone mass, leading to diminished bone strength and an increased risk of fracture (1). Osteoporotic fractures are associated with a significant increase in morbidity and mortality rates and result in substantial healthcare expenditures. Thus, the aim of treatment is the prevention of fractures (1). Strontium ranelate is currently approved for osteoporosis treatment with significant anti-fracture efficacy (2, 3).

Strontium (Sr) is a bivalent ion found as a trace element in human fluids and takes no part in biological

cycles (4, 5). Like calcium (Ca), Sr is an alkaline earth metal (5). Previous studies have demonstrated homology between Ca and Sr absorption (4, 6, 7, 8). Animal studies using radioactive Ca and Sr reveal the same kinetic influx pattern (7, 8, 9). Moreover, evidence suggests that the transport through enteral cells is mediated by the same membrane carriers (7, 9).

Vitamin D is an important factor in Ca absorption (7, 10, 11, 12, 13, 14, 15). As very few foods naturally contain vitamin D and foods are not fortified worldwide,

the major source for most humans is exposure to sunlight (16, 17). Vitamin D deficiency is now recognized as a pandemic (17, 18, 19, 20) and can lead to secondary hyperparathyroidism, increased bone turnover and bone loss, mainly from cortical sites, such as the femoral neck (13, 16, 17, 21, 22, 23). Thus, all patients with osteoporosis should receive adequate amounts of vitamin D (24, 25).

A number of studies suggest that vitamin D status is a determinant of Ca absorption (10, 11, 12, 13). While the factors that regulate Sr have not yet been determined, they are assumed to be similar to those that regulate Ca metabolism (7, 10, 26). The aim of the present study was to determine whether vitamin D status is a determinant of strontium ranelate absorption in patients with low bone mass.

Subjects and methods

Fifty postmenopausal women evaluated on an outpatient basis at an osteometabolic disease clinic were divided into two groups based on vitamin D status: 25 with vitamin D deficiency – defined as serum levels of 25(OH)D below 50 nmol/l – and 25 with vitamin D sufficiency – defined as serum levels above 75 nmol/l. The deficient patients were treated with a weekly 50 000-IU cholecalciferol capsule for 8 weeks and the 25(OH)D levels were reassessed (24). Those that did not reach the desirable level (> 75 nmol/l) received a new course of treatment until the expected goal was reached. The patients presented either osteoporosis ($n=34$) or osteopenia ($n=16$) based on dual-energy X-ray absorptiometry, had normal values of total Ca, phosphorus (P) and creatinine (Cr) and no history of gastrointestinal disease.

This study received approval from the Human Research Ethics Committee of the *Universidade Federal de São Paulo* (Brazil). Signed informed consent was obtained from all participants.

Intestinal strontium absorption test

After an overnight fast, blood was drawn for baseline determinations of Cr, total Ca, P, albumin, intact parathyroid hormone (PTH), 1,25(OH)₂ vitamin D (1,25(OH)₂D) and Sr. A standard dose of strontium ranelate was then administered. One gram of strontium ranelate was dissolved in 200 ml of deionized water and administered orally. Blood was drawn for Sr determinations at 30, 60, 120 and 240 min. The patients were not permitted to eat during the 4-h Sr absorption test. The samples were collected in metal-free tubes and centrifuged

at 404 g for 10 min. The serum was separated and stored at –20 °C until analysis.

Analytical methods

Cr, P and Ca were measured using the automated colorimetric method (Olympus AU 640, automated analyzer; Olympus America, Inc., Diagnostic System Division, Melville, NY, USA). Chemiluminescence commercial assays (Elecsys 1010 immunoassay analyzer; Roche Diagnostics) were performed to quantify PTH. Mean intra-assay and inter-assay coefficients of variation were 1.01 and 6.45% respectively.

The 25(OH)D levels were determined by chemiluminescence immunoassay (LIAISON 25 OH Vitamin D Total-DiaSorin, Stillwater, MN, USA), with mean intra-assay and inter-assay coefficients of variations of 1.62 and 5.61% respectively.

1,25(OH)₂D was analyzed by RIA following the immunoextraction of the samples (1,25-dihydroxy vitamin D RIA, IDS, Boldon, UK). The coefficient of variation ranged from 7 to 11% and the recovery rate ranged from 97 to 108%.

Sr determinations were made using inductively coupled plasma mass spectrometry (Elan DRC-e mass spectrometry, Perkin Elmer Sciex ELAN DRC II, Toronto, Canada). All strontium samples were processed and analyzed in duplicate. Mean values were used. The intra-assay coefficient of variation was 4% and the recovery rates 91–105%. Sr absorption was analyzed as the fraction of absorbed dose (FAD) at each time (30, 60, 120 and 240 min) of the test and the total area under the curve (AUC).

Extracellular fluid was assumed to be 15% of body weight and FAD was calculated according to the following formula:

$$FAD\ t = \frac{(Sr\ t - Sr\ 0) \times 15\% \text{ body weight}}{Sr\ \text{administered dose}}$$

The AUC was calculated as the increment (above baseline) in the concentration–time curve over the 240 min of the test, determined by the trapezoid method and expressed as micromoles per litre per minute.

Statistical analyses

Statistical analyses were performed with the aid of the Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS, Inc.). Baseline features of the groups were expressed as mean and s.d. For all variables, the Kolmogorov–Smirnov test was used to determine the

normality of the data. The Student's *t*-test for unpaired data was employed to evaluate baseline features. The FAD of Sr at each time (30, 60, 120 and 240 min) and the AUC between deficient and sufficient patients were evaluated using the unpaired Student's *t*-test. The paired Student's *t*-test was used for the comparison of FAD and AUC in the deficient group before and after treatment. Pearson's correlation coefficients were calculated to assess interrelationships between the variables. All tests were two-tailed and the type 1 error level was set to 5%.

Results

Table 1 displays the characteristics of the participants in the vitamin D sufficient and deficient groups.

No differences were found in the parameters used to evaluate Sr absorption (FAD at any time and AUC) between the vitamin D deficient and sufficient groups (Figs 1A, B and 2A).

In the deficient group, the second oral Sr overload test was performed only when each individual achieved the desirable 25(OH)D level (> 75 nmol/l). The final mean 25(OH)D concentration was $99.92 (\pm 22.17)$ nmol/l, which was significantly higher than the baseline concentration. A significant increase was also found in 1,25(OH)₂D concentration after treatment with cholecalciferol in the deficient group. However, no significant differences were found in any Sr intestinal absorption parameters in the deficient group before or after treatment. Thus, vitamin D3 replacement in the deficient group did not result in enhanced Sr absorption (Fig. 2B).

Although all patients in both groups presented 1,25(OH)₂D within the normal range, a significant

difference in 1,25(OH)₂D levels was found between the sufficient and deficient groups ($P < 0.001$) at baseline (Fig. 3A). This difference disappeared following treatment for vitamin D deficiency ($P = 0.547$). A significant positive correlation was found between 25(OH)D and 1,25(OH)₂D (0.573 ; $P < 0.001$) at baseline. Levels of 1,25(OH)₂ also correlated with the total cholecalciferol doses used ($r = 0.335$; $P = 0.017$) and FAD at 120 min ($r = 0.305$; $P = 0.033$).

The cholecalciferol dose correlated positively with 25(OH)D levels ($r = 0.462$; $P = 0.001$) and negatively with PTH concentrations ($r = -0.357$; $P = 0.01$). An inverse association with borderline statistical significance ($r = -0.271$; $P = 0.05$) was found between PTH and 25(OH)D concentrations. PTH was similar in the deficient and sufficient groups ($P = 0.174$) at baseline, but the deficient group experienced a significant reduction following treatment ($P = 0.006$) (Fig. 3B).

Discussion

We report here the results of a study that evaluated the influence of vitamin D status on strontium ranelate absorption in postmenopausal women, the target population of this medication (2, 3). Postmenopausal women are also at risk for vitamin D deficiency because older individuals spend less time in the sun and older skin has significantly less capacity to synthesize vitamin D from sunlight than the skin of younger people (18, 23). In the present study, vitamin D status, defined according to 25(OH)D serum concentrations, seems not to be clinically relevant for the strontium ranelate absorption in patients with low bone mass. Only a mild significant positive correlation was found between 1,25(OH)₂D levels

Table 1 Characteristics of participants. Data are presented as mean $\pm$ s.d.

Variable	Sufficient <i>n</i> = 25	Deficient <i>n</i> = 25	<i>P</i>	Normal range
Age (years)	68.56 $\pm$ 7.78	68.40 $\pm$ 6.49	0.937	NA
Weight (kg)	59.98 $\pm$ 8.47	62.87 $\pm$ 8.41	0.231	NA
Height (m)	1.51 $\pm$ 0.05	1.52 $\pm$ 0.05	0.891	NA
BMI (kg/m ²)	26.03 $\pm$ 3.14	27.44 $\pm$ 3.46	0.154	20–25
25(OH)D (nmol/l)	98.4 $\pm$ 18.3	38.5 $\pm$ 13.5	$< 0.001^*$	20.0–150.0
Albumin (g/dl)	4.18 $\pm$ 0.27	4.26 $\pm$ 0.27	0.33	3.5–5.2
Total calcium (mg/dl)	9.26 $\pm$ 0.44	9.14 $\pm$ 0.44	0.34	8.6–10.2
Creatinine (mg/dl)	0.72 $\pm$ 0.12	0.71 $\pm$ 0.12	0.836	0.5–0.9
Phosphorus (mg/dl)	3.51 $\pm$ 0.51	3.47 $\pm$ 0.49	0.758	2.5–4.5
PTH (pg/ml)	62.28 $\pm$ 18.79	73.87 $\pm$ 37.5	0.174	15.0–65.0
D ₃ dose ^a (IU/day)	1712.4 $\pm$ 724.8	808.76 $\pm$ 689.5	$< 0.001^*$	NA
Basal Sr (μ mol/l)	0.54 $\pm$ 0.3	0.52 $\pm$ 0.31	0.823	NA
1,25(OH) ₂ D (pg/ml)	36.3 $\pm$ 10.2	24.97 $\pm$ 4.63	$< 0.001^*$	18.0–78.0

NA, not applicable; $^*P < 0.05$.

^aD₃ supplementation dose in use in previous 3 months.

Figure 1

(A) Profile of mean plasma strontium concentrations during the test in the three groups. (B) Profile of mean AUC in the three groups.

and later fractions of absorbed dose of Sr. As expected, a positive correlation was found between both vitamin D metabolites (25(OH)D and 1,25(OH)₂D) and between cholecalciferol dose in use and 25(OH)D levels. Changing vitamin D status from deficient to sufficient resulted in a significant increase in 1,25(OH)₂D and reduction in PTH levels (Fig. 2A and B).

The relation between Sr absorption and the active vitamin D metabolite (calcitriol) has been previously studied. Bolscher *et al.* applied a similar test to evaluate the influence of calcitriol on Sr absorption. Healthy volunteers were submitted to a basal strontium oral overload test using 2.5 mmol of Sr. After treatment with oral calcitriol, the test was repeated. A 35% enhance in Sr absorption was observed. A statistically significant difference was detected in a group of eight volunteers (26).

The effects of cholecalciferol supplementation had also been evaluated, albeit by a different route of administration. Although Khan *et al.* (27) used Sr as a surrogate for Ca absorption, they did not find any change in Sr absorption before or after an i.m. injection of vitamin D3 in a previously deficient adult population. A single vitamin D3 injection of 600 000 IU significantly increased mean 25(OH)D concentration and tended to lower PTH in volunteers with initially low 25(OH)D status, but no effect on Sr absorption was found (27). Although the study used strontium chloride at a lower Sr dose (4.8 mg/kg), the profile described was very similar to that found in the present study using strontium ranelate. The authors also used one of the same parameters to assess strontium absorption (AUC) and found no difference before or after intramuscular cholecalciferol treatment. This result suggests that vitamin D levels are not directly related to Sr absorption, which is in agreement with the present findings.

The evaluation of intestinal Sr absorption using an oral overload test may be influenced by diet. Animal studies have shown that absorption differs between solid and liquid sources (8). The majority of studies evaluating Sr absorption administered it only with water (6, 26, 27, 28, 29, 30). Some used a standard breakfast with a known Ca content based on the selection against Sr in favor of Ca in the intestinal tract (8, 28, 30, 31). A standard breakfast was shown to reduce the Sr FAD at each evaluation time as well as the variance within and between subjects, but the shape of the absorption curves remained unchanged (27, 30). The choice of the fasting state and administration of Sr dissolved in water could raise discussion on the modulating effect of a meal on the transit time through

Figure 2

FAD in deficient and sufficient groups (A) and before and after treatment (B).

Figure 3

Profile of 1,25(OH)₂D (A) and PTH (B) in the deficient group before (2) and after treatment (3). The mean values are emphasized.

the stomach and intestine. On the other hand, there is a lack of uniformity in the composition of the meals applied in the various tests described in the literature (27, 30, 31). Furthermore, the recommendation for the therapeutic use of strontium ranelate is that it should be taken at least 2 h before any meal. Therefore, the test in the present study was standardized with the fasting state.

The present study has a limitation that should be addressed. The presence of any gastrointestinal problem that could interfere with the absorption performance of the patients was not formally excluded, unless by the medical history. No specific evaluation was performed for that.

The two groups (sufficient and deficient) were similar with regard to baseline features, except for 25(OH)D (by design), 1,25(OH)₂D and cholecalciferol dose in use. Levels of 25(OH)D and 1,25(OH)₂D were correlated. At higher 25(OH)D concentrations and normal PTH, the correlation between the two vitamin D metabolites became stronger ($r=0.670$; $P<0.001$). Thus, it seems that 1,25(OH)₂D is a function of 25(OH)D, especially in individuals with adequate vitamin D status. At lower levels, secondary hyperparathyroidism is triggered to avoid the drop in 1,25(OH)₂D. Therefore, cholecalciferol supplementation is important to maintain adequate levels of 25(OH)D and 1,25(OH)₂D as well as to avoid the secondary elevation of PTH and its harmful effects.

The vitamin D thresholds chosen to define deficiency (<50 nmol/l) and sufficiency (>75 nmol/l) were determined in a manner to avoid debate. Despite the controversy over optimal levels of 25(OH)D, most experts agree levels less than 50 nmol/l (20 ng/ml) characterize vitamin D deficiency (24, 25, 32) and there is also

consensus regarding levels above 75 nmol/l (30 ng/ml) as sufficient (24, 25, 32). Thus, the different vitamin D status in the two groups evaluated herein was unquestionable.

Sr absorption has previously been studied mainly based on the homology with Ca. Although Dumond *et al.* (33) suggest that Sr transport is exclusively passive, other authors have found evidence regarding active intestinal Sr transport and indications for a common mechanism with Ca (4, 6, 7, 8). Bolscher *et al.* (26) found a stimulatory effect of 1,25(OH)₂D in Sr absorption. The authors evaluated Sr absorption in healthy volunteers before and after treatment with oral calcitriol and found enhanced Sr absorption as measured by FAD at 240 min and AUC. However, no correlation between serum levels of 1,25(OH)₂D and Sr absorption was demonstrated. In the present study, 1,25(OH)₂D correlated positively with FAD at 120 min. Although the association was found in only one of the variables analyzed, this result lends strength to the possibility of an effect of 1,25(OH)₂D on Sr absorption.

There is controversy over the association between the concentration of 25(OH)D and Ca absorption, which could be carefully extrapolated for Sr. A number of authors have found positive correlations (13, 16, 21, 22, 31, 34), whereas others have not (15, 22, 29, 35, 36). Graff *et al.* (35) found no significant difference in fractional Ca absorption between children with and without active rickets, despite the lower 25(OH)D concentrations in the former group. Need & Nordin (36) evaluated 461 postmenopausal women and found no association between radiocalcium absorption and serum 25(OH)D. Similarly, Aloia *et al.* (15) found no association between serum 25(OH)D concentrations and radiocalcium absorption efficiency in healthy women aged 20–80 years. Although Heaney *et al.* (13) proposed that the threshold for maximum calcium absorption was 80–90 nmol/l, other evidence suggested different values. Animal studies showed that in rats and dogs, little amounts of vitamin D (10 and 20 IU respectively) were enough to reach the maximum calcium absorption (37, 38). In humans, Need *et al.* (39) described impairment of calcium absorption only with vitamin D levels lower than 10 nmol/l (4 ng/ml). In recent evaluation studies on the effect of vitamin D supplementation in calcium absorption among postmenopausal women, Gallagher *et al.* found out that it is correlated with serum 1,25(OH)₂D, but not with 25(OH)D (42, 43). They suggested that malabsorption occurs only in severe vitamin D deficiency (25(OH)D < 10 ng/ml), when the lack of substrate causes a decrease in 1,25(OH)₂D production. Our data are in agreement with these findings as our subjects were not severely deficient.

All patients in the present study had $1,25(\text{OH})_2\text{D}$ within the normal range, regardless of their vitamin D status. This finding is in agreement with the idea that in the absence of kidney failure, vitamin D deficiency must be very severe to cause deficiency in $1,25(\text{OH})_2\text{D}$ (15, 36). It seems that at low levels of $25(\text{OH})\text{D}$, secondary hyperparathyroidism enhances the conversion of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$, thereby acting as a protective mechanism. Despite the tight control of $1,25(\text{OH})_2\text{D}$ production, a significant increase in $1,25(\text{OH})_2\text{D}$ was found following cholecalciferol treatment. This has previously been described in a trial that analyzed the effect of vitamin D replacement on musculoskeletal parameters in schoolchildren (40). Although the cross-reactivity of $1,25(\text{OH})_2\text{D}$ with $25(\text{OH})\text{D}$ is estimated to be $<0.01\%$, this possibility cannot be discarded (41).

Conclusion

In summary, the correction of mild vitamin D deficiency is not linked with optimization of strontium ranelate absorption. However, the importance of vitamin D for bone health is irrefutable. Taking into account the benefits of correcting vitamin D status in osteoporotic patients, we strongly recommend the treatment of vitamin D deficiency. However, the data demonstrate that such treatment is not relevant to enhance strontium ranelate absorption during treatment for osteoporosis.

Declaration of interest

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