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Accumulation of bone strontium measured by *in vivo* XRF in rats supplemented with strontium citrate and strontium ranelate

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ABSTRACT

Strontium ranelate is an approved pharmacotherapy for osteoporosis in Europe and Australia, but not in Canada or the United States. Strontium citrate, an alternative strontium salt, however, is available for purchase over-the-counter as a nutritional supplement. The effects of strontium citrate on bone are largely unknown. The study's objectives were 1) to quantify bone strontium accumulation in female Sprague Dawley rats administered strontium citrate ($N=7$) and compare these levels to rats administered strontium ranelate ($N=6$) and vehicle ($N=6$) over 8 weeks, and 2) to verify an *in vivo* X-ray fluorescence spectroscopy (XRF) system for measurement of bone strontium in the rat. Daily doses of strontium citrate and strontium ranelate were determined with the intention to achieve equivalent amounts of elemental strontium. However, post-hoc analyses of each strontium compound conducted using energy dispersive spectrometry microanalysis revealed a higher elemental strontium concentration in strontium citrate than strontium ranelate. Bone strontium levels were measured at baseline and 8 weeks follow-up using a unique *in vivo* XRF technique previously used in humans. XRF measurements were validated against *ex vivo* measurements of bone strontium using inductively coupled plasma mass spectrometry. Weight gain in rats in all three groups was equivalent over the study duration. A two-way ANOVA was conducted to compare bone strontium levels amongst the three groups. Bone strontium levels in rats administered strontium citrate were significantly greater ($p<0.05$) than rats administered strontium ranelate and vehicle. ANCOVA analyses were performed with Sr dose as a covariate to account for differences in strontium dosing. The ANCOVA revealed differences in bone strontium levels between the strontium groups were not significant, but that bone strontium levels were still very significantly greater than vehicle.

Introduction

Strontium ranelate, a pharmacologic agent used to treat individuals with osteoporosis, is indicated for use in Europe and Australia but not in Canada or the United States. It is thought to have a unique mechanism of action mediated by a cation-sensing receptor (e.g. calcium-sensing receptor) and the RANK/RANKL/OPG pathway [1–11] in which pre-osteoblast proliferation, osteoblast differentiation, collagen type I synthesis and bone matrix mineralisation increase while osteoclast differentiation and activation are inhibited [12]. This proposed dual mechanism of action has yielded significant positive effects on bone quality assessed by high-resolution peripheral quantitative computed tomography (hr-pQCT) and microCT, and reduced fracture incidence [3,4,13–20] in individuals with low bone mass. Both animal and human studies have shown that strontium is almost exclusively found in newly formed bone, incorporated into apatite crystals, (i.e. bone formed after commencement of strontium treatment/supplementation), and found in higher amounts in cancellous than cortical bone [21–24].

The effects of strontium ranelate on bone mineral density (BMD) have also been positive [14,15,25–28]. However, since the atomic number of strontium is greater than calcium (38 vs. 20), the substitution of strontium for calcium in bone weakens X-ray penetration during dual energy X-ray absorptiometry (DXA) scanning and results in *overestimation* of areal BMD (aBMD) [21,29–32]. Phantom experiments have shown that aBMD increased about 10% per 1% increase in strontium content [29]. Since 3 years of strontium treatment results in the presence of about 1 strontium atom for every 100 Ca

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atoms in bone [31], the observed 10% increase in aBMD may be due entirely to the presence of strontium atoms and may not reflect greater bone mass. Uncertainty in the effect of strontium on aBMD clearly warrants the use of a valid, direct assessment of bone strontium content. While such assessments can be made *ex vivo* using sophisticated techniques [22–24,33,34], an *in vivo* technique is preferable as it is less invasive and more cost-effective [35–37]. An X-ray fluorescence (XRF) system has been developed to perform non-invasive and painless *in vivo* measures of bone strontium content [35,36,38]. This method positions the region of interest in close proximity to an excitation source of ^{125}I and a detector measures the strontium X-rays produced by X-ray fluorescence (XRF) excitation [35,36]. Detection of the number of strontium X-rays emitted from a defined bone volume is directly dependent upon bone strontium. To date, bone strontium levels have been assessed in humans using this technique [35–38], but not in animals.

Although strontium ranelate is not indicated for use in Canada or the United States, other strontium preparations, available for purchase in health food stores and pharmacies as over-the-counter nutritional supplements, are marketed as products that improve bone health. While the physiological effects of these alternate strontium salts on the skeleton remain largely unknown, daily doses are recommended such that the intake of elemental strontium is similar or even equivalent to strontium ranelate. Given the paucity of data comparing the bioavailability of strontium ranelate to other strontium salts, the amount of strontium available to bone from alternative strontium salts also remains unknown. To date, there is no evidence to suggest that strontium ranelate has different or more beneficial effects on bone than any other strontium salt.

One widely available alternative strontium salt is strontium citrate. It can be purchased over-the-counter without a prescription in Canada and the United States. However, little is known about levels of bone strontium achieved after taking this salt, and no head-to-head comparisons with the currently approved osteoporosis medication, strontium ranelate, have been conducted. To this end, we conducted a vehicle-controlled animal study with the global objective of comparing bone strontium levels in rats administered equivalent elemental strontium concentrations through strontium citrate and strontium ranelate. As a second objective, we sought to verify the non-invasive XRF system for *in vivo* measurement of bone strontium content in the rat.

Materials and methods

Animal model

All protocols were approved by our institutional Animal Research Ethics Board. Female Sprague Dawley rats ($n=19$; 12 weeks old) were purchased (Charles River, Saint-Constant, P.Q.) and allowed to acclimatise and age in our animal facilities. At 18 weeks of age, rats were randomly assigned to one of three groups: strontium ranelate ($n=6$), strontium citrate ($n=7$) or vehicle ($n=6$). Baseline body mass was measured and bone strontium measures were acquired as described below. The animals were caged individually with 12 hr light/dark cycle and were allowed unrestricted cage activity and *ad libitum* access to water and food. The rat diet was a standard rat chow (8640 Teklad 22/5 Rodent Diet, Harland Teklad) with 1.13% calcium content.

Similar to previous studies [39,40], rats in the strontium ranelate group (SV Chembiotech Inc., Edmonton, AB) were dosed with 625 mg/kg/day. Based on the molecular weight of strontium ranelate, the elemental strontium dose would be 213 mg/kg/day (Table 1). To achieve an equivalent dose of elemental strontium from strontium citrate (Dr. Paul Lohmann GmbH HG, Emmenthal, Germany), we calculated that each rat should receive 676 mg/kg/day. These calculations were based on the molecular weight of strontium citrate as provided. During the study the *in vivo* XRF data showed large differences in strontium measures between the strontium citrate and strontium ranelate rats. We measured the strontium content in the strontium compounds (Section 2.5) and determined that the daily strontium dose was greater for the strontium citrate (Table 1). Starting at 19 weeks of age, each strontium group received daily strontium ranelate or strontium citrate, respectively, in suspension in flavoured (strawberry) gelatin vehicle (10 mL). The vehicle group was given flavoured gelatin with no added strontium. Because calcium and strontium competitively bind in the gut, strontium supplementation is best provided outside the normal diet [27]. Using a gelatin vehicle eliminates technical difficulties associated with oral gavage. Dosing was performed at the same time each day and the rats were observed following dosing to ensure the gelatin was consumed in entirety.

Rat body mass was measured weekly and *in vivo* bone strontium measures were acquired using X-ray fluorescence spectroscopy (XRF) at baseline and at 4 and 8 weeks after the start of dosing. We performed an ANOVA to determine if there were statistical differences ($p<0.05$) in body mass between the groups at each time point. Ten weeks after the start of strontium dosing, rats were sacrificed by CO_2 inhalation. The right limb was disarticulated at the hip with all tissues intact and was hermetically sealed in plastic bags and frozen at −20 °C.

In vivo X-ray fluorescence spectroscopy measurement

We acquired *in vivo* measures of bone strontium in each rat using a custom X-ray fluorescence spectroscopy system [35,36,38,41] at baseline, and 4 and 8 weeks after the start of dosing as described previously [35,36]. Briefly, Prostaseed ^{125}I brachytherapy seeds (Core Oncology) with an initial activity of about 13 MBq/seed were used as the excitation source. The ^{125}I seeds were inserted in a tungsten collimator, with a 5 mm internal diameter, 5.4 mm external diameter and 3 mm length. The measurements of the X-ray spectra were performed in backscattered geometry using an Si(Li) detector (EG&G ORTEC) with a 16 mm active diameter and 5.65 mm sensitive thickness. Data were acquired and processed using a DSPEC^{PLUS} multichannel analyser operating Maestro™ software (ORTEC). The spectra obtained with the XRF system were analysed using a modified in-house nonlinear least squares, Marquardt based fitting routine. The strontium Kα peaks observed at 14.16 keV were then normalised to the coherent ^{125}I peak at 35.49 keV [42,43]. The strontium Kβ peak was not used in the analysis due to significant overlap with the rubidium Kβ peak.

Prior to acquiring measurements on the rats, a cylindrical phantom was scanned with a 119.4 ppm strontium:calcium ratio (0.17 mg strontium / 1.391 g calcium) on each scanning day to calibrate the system and for comparisons between time points. For each rat, bone

Table 1
Daily strontium dose — calculated and actual doses.

	CAS#	Molecular formula	Molecular weight [g/mol]	Dose [mg/kg/day]	Calculated[a] Elemental Sr [mg/kg/day]	Actual[b] Elemental Sr [mg/kg/day]
strontium ranelate	135459-87-9	$C_{12}H_6N_2O_8SSr_2$	513.49	625	213	174.3
strontium citrate	813-97-8	$C_6H_6O_7Sr$	277.73	676	213	235.7

[a] Elemental strontium dose calculated based on molecular formula from chemical data provided.
[b] Elemental strontium dose based on measurements of strontium compounds by energy dispersive spectrometry (EDS) microanalysis after completion of the dosing study.