Rheumatology 2008;47:iv17–iv19 doi:10.1093/rheumatology/ken165

Rebalancing bone turnover in favour of formation with strontium ranelate: implications for bone strength

J. E. Fonseca[1,2]

This review updates our current knowledge on the mechanism of action of strontium ranelate and analyses the way it rebalances bone turnover and how it influences bone biomechanics. Strontium ranelate is able to increase pre-osteoblast replication, osteoblast differentiation, collagen type I synthesis and bone matrix mineralization probably through a calcium-sensing receptor (CaR)-dependent mechanism. Paralleling this anabolic effect there is inhibition of osteoclast differentiation and activity mediated by an increase in osteoprotegerin (OPG) and a decrease in RANK ligand (RANKL). The overall effect is a rebalanced bone turnover in favour of improved bone geometry, cortical thickness, trabecular bone morphology and intrinsic bone tissue quality, which translates into enhanced bone strength.

Key words: Strontium ranelate, Osteoblast, Osteoclast, Collagen type I, Calcium-sensing receptor, RANK ligand, Osteoprotegerin, Plastic energy, Bone strength.

Introduction

Bone is composed of cells and an extracellular matrix, mainly occupied by type I collagen, which confers ductility to bone, meaning that it allows bone to absorb energy through plastic deformation, without total failure (fracture) of bone structure [1, 2]. On the other hand, collagen type I acts as a scaffold for bone mineralization and directs the deposition of calcium hydroxyapatite crystals, a critical determinant of bone stiffness and strength [1, 2]. In fact, the efficacy of bone as a structural element of our body depends on the balance between these different bone components and their biomechanical properties. However, this balance is permanently being challenged by bone formation and resorption, in a process called bone remodelling, which is mediated by three distinct cell types: the osteoblasts, or bone-forming cells, the osteoclasts, or bone-resorbing cells, whose functions are intimately linked, and the osteocytes, which are osteoblasts entrapped within bone lacunae [3]. In order to physiologically balance bone formation and resorption in healthy individuals, there is a constant cross-talk between these three key players in bone metabolism [4]. Resorption is much faster than formation: an area of bone can be resorbed in 2–3 weeks but it takes at least 3 months to rebuild it [4]. Consequently, when resorption is increased, such as after menopause, bone formation is not able to fully compensate this effect and changes in geometry, cortical thickness, trabecular bone morphology and intrinsic bone tissue quality occur, causing degradation of bone biomechanical properties. Ideally, to reverse this process, treatments should be able to rebalance bone remodelling, providing bone with adequate amounts of organic and mineralized matrix in order to restore bone biomechanical properties.

Strontium ranelate is a treatment of osteoporosis which, unlike any other drug, has a dual effect on bone remodelling, being able to stimulate bone formation by osteoblasts, a property shared with bone-forming agents, and to inhibit bone resorption by osteoclasts, as do anti-resorptive agents [5]. Strontium ranelate is composed of two atoms of stable strontium (Sr) combined with ranelic acid, which acts as carrier [6].

This review will update our current knowledge on the mechanism of action of strontium ranelate and will analyse to what extent this drug is able to rebalance bone turnover and to restore biomechanics in osteoporotic bone.

Pre-osteoblast replication, osteoblast differentiation, collagen synthesis and matrix mineralization

In rat calvarial cultures, strontium ranelate enhances the replication of pre-osteoblasts and increases collagen type I synthesis [7]. In addition, in primary osteoblasts derived from mouse calvaria, strontium ranelate promotes bone nodule formation, increasing the differentiation from early progenitor cells to mature osteoblasts, as reflected by the expression of osteoblastic markers such as ALP, bone sialoprotein and OC [8]. Moreover, bone marrow stromal cells, exposed to a differentiating medium for 21 days, when treated with strontium ranelate showed an increase in the formation of mineralized colony-forming unit-osteoblasts and in the expression of the *Runx2* gene, but not of OC [9]. On the contrary, in more mature osteoblastic OB-6 cells strontium ranelate induced only minimal effects on *Runx2* expression but had a positive effect on OC expression [9]. *Runx2* is a master gene so critical for osteoblast differentiation that mice deficient in this gene fail to form osteoblasts [10], while OC is a known negative regulator of osteoblast differentiation, normally expressed during the latter stages of differentiation [11]. From these experiments it can be concluded that strontium ranelate stimulates the replication of pre-osteoblast, increases collagen type I production and induces the differentiation of the osteoblast into a more mature, mineralizing phenotype. For the appropriate clinical interpretation of these conclusions it is important to highlight that most of these effects occur with a concentration of strontium ranelate as low as 0.1 mM, which is close to the active concentration in the blood of post-menopausal osteoporotic women treated with strontium ranelate (0.12 nM) [8, 12].

Despite our current knowledge on the cellular effects of strontium ranelate on osteoblasts, the precise molecular mechanisms involved remain elusive. Since Sr is a divalent cation that closely resembles Ca in its atomic and ionic properties, it has been hypothesized that strontium ranelate could act as an agonist of the extracellular Ca-sensing receptor (CaR) that is expressed at all stages of osteoblast development and exerts several actions on bone cells *in vitro* that could be the basis for the known anabolic effects of Ca in bone [13, 14]. Indeed, preliminary data have demonstrated that strontium ranelate activates CaR [15]. It was shown more recently in human embryonic kidney 293 (HEK293) cells transfected with bovine CaR (HEK–CaR) that Ca and strontium ranelate concentration-dependently activated the CaR,

[1]Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa and [2]Serviço de Reumatologia e Doenças Ósseas Metabólicas, Hospital de Santa Maria, Lisbon, Portugal.

Submitted 31 January 2008; revised version accepted 1 April 2008.

Correspondence to: J. E. Fonseca, Rheumatology Research Unit, Instituto de Medicina Molecular, Edifício Egas Moniz, Faculdade de Medicina da Universidade de Lisboa, Av. Professor Egas Moniz, 1649-028 Lisboa, Portugal. E-mail: jefonseca@netcabo.pt

and that within physiologic Ca concentrations, strontium ranelate induced further CaR activation [16]. Interestingly, strontium ranelate was less potent than Ca in stimulating inositol phosphate accumulation but was comparable with Ca in stimulating extracellular signal-regulated kinase (ERK) phosphorylation and a non-selective cation channel, suggesting that Ca and strontium ranelate have differential cellular effects [16]. Reinforcing the existence of at least partially separate molecular mechanisms from Ca, strontium ranelate activation of mitogen-activated protein (MAP) kinase and protein kinase C (PKC) pathways is delayed in comparison with activation mediated by $CaCl_2$, possibly due to the synthesis of an autocrine growth factor, and its proliferative effect on osteoblasts appears to be mainly dependent on PKC but not on MAP kinases [17]. Finally, rat primary osteoblasts proliferation and the expression of genes involved in osteoblast proliferation and differentiation, such as c-Fos and Egr-1, were shown to be stimulated by strontium ranelate, but the effect was attenuated by overexpressing the dominant negative CaR, demonstrating that the mechanism was CaR-mediated [16]. Therefore, strontium ranelate is a full CaR agonist in primary osteoblasts and its proliferative effect on these cells can be, at least partially, explained by a CaR-dependent mechanism. Nevertheless, the strontium ranelate concentration needed to attain this effect was higher than that measured in patients treated with 2 g of strontium ranelate per day [12]. Despite the lack of direct evidence, it has been hypothesized that only CaR expressed at sites where the levels of either Ca or strontium ranelate might be higher than in blood, such as bone, would be stimulated by therapeutic doses of strontium ranelate, thus not affecting CaR in peripheral tissues, such as kidney and parathyroid [16].

Overall, the data presented so far suggest that strontium ranelate induces pre-osteoblast proliferation, enhances osteoblast differentiation and activity, leading to collagen type I synthesis and mineralization, through molecular mechanisms possibly involving CaR activation.

RANK ligand/osteoprotegerin ratio

One of the key elements in the cross-talk between osteoblasts and osteoclasts is the RANK ligand (RANKL) and osteoprotegerin (OPG) system. RANKL, expressed by pre-osteoblasts and osteoblasts, activates its receptor RANK, present on pre-osteoclasts and osteoclasts, inducing the differentiation of mature osteoclasts and increasing osteoclast survival [18]. OPG, a soluble receptor for RANKL produced by osteoblasts, inhibits RANKL-induced osteoclastogenesis [18]. Thus, the OPG/RANKL ratio is decisive for bone resorption and a decreased ratio has been described in clinical settings where bone loss occurs, such as in post-menopausal women. On the contrary, drugs that could increase this ratio would contribute to a reduction in bone resorption. Interestingly, strontium ranelate enhances OPG expression and down-regulates RANKL expression both in primary human osteoblastic cells *in vitro* and in murine rat calvaria cells, [19]. These observations could mean that when strontium ranelate stimulates osteoblasts two types of signals are produced simultaneously: anabolic pathways are activated in pre-osteoblasts and osteoblasts, and an anti-catabolic message is sent to pre-osteoclasts and osteoclasts throughout an increase in the OPG/RANKL ratio.

Osteoclast differentiation, activity and apoptosis

Apart from possible interference with the OPG/RANKL system, strontium ranelate has been shown to interfere directly with osteoclasts. As a matter of fact, strontium ranelate was able to decrease osteoclast markers in differentiating chicken bone marrow cultures and murine osteoclasts, to reduce bone resorption in mouse organ cultures of cranial bone and mouse osteoclasts, to diminish differentiation of human monocyte into osteoclast and to enhance osteoclast apoptosis [20–22]. These observations were recently confirmed in osteoclasts derived from murine spleen cells in a series of experiments where the number of mature osteoclasts and their resorbing activity were strongly decreased after strontium ranelate exposure, in a process associated with the disruption of the actin cytoskeleton of the osteoclast-sealing zone [8]. As the CaR is directly involved in both osteoclast differentiation and apoptosis [23] and strontium ranelate is able to stimulate this receptor [15, 16] it seems likely that stimulation of CaR is the underlying major mechanism for the cellular effects of strontium ranelate on these cells. Accordingly, there is preliminary evidence for a CaR-mediated strontium ranelate-induced osteoclast apoptosis [24].

Taken together these results indicate that strontium ranelate is able to decrease osteoclast differentiation and activity and enhance osteoclast apoptosis through a CaR-dependent mechanism.

Bone structure and biomechanics

Strontium ranelate has been studied in various rat and animal models, including intact animals, immobilization-induced osteopenia and ovariectomy-induced osteoporosis [25–29]. In these *in vivo* experiments, strontium ranelate increased markers of bone formation and decreased markers of bone resorption, promoting bone gain as reflected in increased external diameter of long bones, enhanced bone mass evaluated by DXA and microarchitecture improvement assessed by histomorphometry [25–29]. These observations are in accordance with the results of strontium ranelate in clinical trials that have shown an uncoupling of bone markers, increase in DXA results [12] and histomorphometric bone improvement [30]. Furthermore, these positive effects on bone were obtained without affecting bone mineralization [31].

The bone gain induced by strontium ranelate treatment was predictive of increased bone strength that was indirectly confirmed by the reduction in fracture risk in clinical trials [12, 32] and directly documented in a series of animal experiments. In effect, the occurrence of fractures is a multifactor event and only animal models provide objective information on all the structural determinants of bone strength: bone geometry, cortical thickness and porosity, trabecular bone morphology and intrinsic properties of bone tissue [33]. The first set of experiments was performed in intact female rats [25] treated over a 2-yr period with doses that reached plasma levels close to the value observed in patients treated with 2 g/day [12]. In the strontium ranelate group, there was an increase in the external diameter and cortical thickness of long bones, as well as an improvement in vertebral bone mass, trabecular bone volume, trabeculae number, trabecular thickness, connectivity and cortical thickness, reflecting improved bone geometry, cortical properties, porosity and trabecular bone morphology [25]. Moreover, 3-point bending tests performed on the mid-shaft femur reveal a dose-dependent increase in maximal load and total energy without modification of bone stiffness, while L4 vertebral body compression tests produced similar results, with most of the energy being absorbed at the cost of an increase in plastic, but not elastic energy [25]. Consequently, new bone formed following strontium ranelate treatment is able to withstand greater deformation before fracture (increased ductility), while keeping normal bone elastic properties. A subsequent study analysed the biomechanical effects of strontium ranelate in a setting closer to clinical practice. Six-month-old ovariectomized rats treated over a 1-yr period with strontium ranelate showed dose-dependent prevention of bone strength alteration, but only partial prevention of microarchitecture bone changes, suggesting that direct effects on intrinsic bone quality might be an additional explanation for the improvement in bone strength in mature rats [34]. In addition, some of the strontium ranelate may be absorbed in the hydrated bone layer, as suggested by the fact that it is rapidly eliminated after treatment withdrawal, potentially

allowing it to influence intrinsic bone tissue quality [35]. To test this hypothesis, nanoindentation tests were performed on trabecular bone and cortex in vertebrae of rats treated with several different doses of strontium ranelate [36]. In agreement with previous results, strontium ranelate increased maximal load, total energy and plastic energy, and enhanced elastic modulus, hardness and dissipated energy in trabecular bone, but these effects were only present in wet conditions [36]. These effects totally disappeared in dry conditions suggesting that the presence of strontium ranelate in the hydrated bone layer could structurally modify the bone matrix and directly improve intrinsic bone tissue quality, and thus potentially decrease the propagation of microcracks and enhance bone strength [36].

Altogether, structural and biomechanical results have shown that strontium ranelate is able to positively influence bone geometry, cortical thickness and porosity, trabecular bone morphology and intrinsic bone tissue quality, globally resulting in increased bone strength.

Conclusion

We have seen that strontium ranelate is able to increase pre-osteoblast replication, osteoblast differentiation, collagen type I synthesis and bone matrix mineralization probably through a CaR-dependent mechanism. Paralleling these anabolic effects, there is inhibition of osteoclast differentiation and activity and enhanced osteoclast apoptosis apparently mediated by an increase in OPG/RANKL ratio and by a CaR-mediated mechanism. The overall effect is a rebalanced bone turnover in favour of improved bone geometry, cortical thickness, trabecular bone morphology and intrinsic bone tissue quality, which translates into enhanced bone strength.

Rheumatology key messages

- Strontium ranelate induces pre-osteoblast replication, osteoblast differentiation, collagen type I synthesis and bone matrix mineralization.
- Strontium ranelate inhibits osteoclast differentiation through an increase in OPG and a decrease in RANKL.
- Strontium ranelate rebalances bone turnover in favour of enhanced bone strength.

Disclosure statement: J.E.F. has received consulting fees and speaker fees from Servier.

References

1 Caetano-Lopes J, Canhão H, Fonseca JE. Osteoblasts and bone formation. Acta Reumatol Port 2007;32:103–10.
2 Seeman E, Delmas PD. Bone quality-the material and structural basis of bone strength and fragility. N Engl J Med 2006;354:2250–61.
3 Ducy P, Schinke T, Karsenty G. The osteoblast: a sophisticated fibroblast under central surveillance. Science 2000;289:1501–4.
4 Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature 2003;423:349–55.
5 Marie P. Strontium ranelate: a dual mode of action rebalancing bone turnover in favour of bone formation. Curr Opin Rheumatol 2006;18:S11–5.
6 Marie P. Strontium ranelate: a novel mode of action optimizing bone formation and resorption. Osteoporos Int 2005;16:S7–10.
7 Canalis E, Hott M, Deloffre P, Tsouderos PJ, Marie P. The divalent strontium salt S12911 enhances bone cell replication and bone formation in vitro. Bone 1996;18:517–23.
8 Bonnelye E, Chabadel A, Saltel F, Jurdic P. Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. Bone 2008;42:129–38.
9 Zhu LL, Zaidi S, Peng Y *et al.* Induction of a program gene expression during osteoblast differentiation with strontium ranelate. Biochem Biophys Res Commun 2007;355:307–11.
10 Karsenty G, Ducy P, Starbuck M *et al.* Cbfa1 as a regulator of osteoblast differentiation and function. Bone 1999;25:107–8.
11 Karsenty G. Transcriptional regulation of osteoblast differentiation during development. Front Biosci 2003;3:834–7.
12 Meunier PJ, Roux C, Seeman E *et al.* The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med 2004;350:459–68.
13 Chattopadhyay N, Yano S, Tfelt-Hansen J *et al.* Mitogenic action of calcium-sensing receptor on rat calvarial osteoblasts. Endocrinology 2004;145:3451–62.
14 Dvorak MM, Siddiqua A, Ward DT *et al.* Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calciotropic hormones. Proc Natl Acad Sci USA 2004;101:5140–5.
15 Coulombe J, Faure H, Robin B, Ruat M. In vitro effects of strontium ranelate on the extracellular calcium-sensing receptor. Biochem Biophys Res Commun 2004; 323:1184–90.
16 Chattopadhyay N, Quinn SJ, Kifor O, Ye C, Brown EM. The calcium-sensing receptor (CaR) is involved in strontium ranelate-induced osteoblast proliferation. Biochem Pharmacol 2007;74:438–47.
17 Caverzasio J. Strontium ranelate increases osteoblast replication through activation of an original cellular mechanism. Calcif Tissue Int 2007;80(Suppl 1):S74.
18 Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in bone cell biology. J Mol Med 2001;79:243–53.
19 Marie PJ. Strontium ranelate: New insights into its dual mode of action. Bone 2007;40:S5–8.
20 Baron R, Tsouderos Y. In vitro effects of S12911-2 on osteoclast function and bone marrow macrophage differentiation. Eur J Pharmacol 2002;450:11–7.
21 Takahashi N, Sasaki T, Tsouderos Y, Suda T. S 12911-2 inhibits osteoclastic bone resorption in vitro. J Bone Miner Res 2003;18:1082–7.
22 Mentaverri R, Hurtel AS, Kamel S, Robin B, Brazier M. Extracellular concentrations of strontium directly stimulates osteoclast apoptosis. J Bone Min Res 2003;18:M237.
23 Mentaverri R, Yano S, Chattopadhyay N *et al.* The calcium sensing receptor is directly involved in both osteoclast differentiation and apoptosis. FASEB J 2006;20:2562–4.
24 Mentaverri R, Hurtel AS, Wattel A *et al.* Calcium-sensing receptor mediates ranelate-induced osteoclast apoptosis. Bone 2005;36(Suppl 2):S403.
25 Ammann P, Shen V, Robin B, Mauras Y, Bonjour JP, Rizzoli R. Strontium ranelate improves bone resistance by increasing bone mass and improving architecture in intact female rats. J Bone Miner Res 2004;19:2012–20.
26 Delannoy P, Bazot D, Marie PJ. Long-term treatment with strontium ranelate increases vertebral bone mass without deleterious effect in mice. Metabolism 2002;51:906–11.
27 Hott M, Deloffre P, Tsouderos Y, Marie PJ. S12911-2 reduces bone loss induced by short-term immobilization in rats. Bone 2003;33:115–23.
28 Marie PJ, Hott M, Modrowski D *et al.* An uncoupling agent containing strontium prevents bone loss by depressing bone resorption and maintaining bone formation in estrogen-deficient rats. J Bone Miner Res 1993;8:607–15.
29 Buehler J, Chappuis P, Saffar JL, Tsouderos Y, Vignery A. Strontium ranelate inhibits bone resorption while maintaining bone formation in alveolar bone in monkeys (*Macaca fascicularis*). Bone 2001;29:176–9.
30 Arlot ME, Jiang Y, Genant HK *et al.* Histomorphometric and μ-CT analysis of bone biopsies from postmenopausal osteoporotic women treated with strontium ranelate. J Bone Miner Res 2008;23:215–22.
31 Boivin G, Farlay D, Simi C, Meunier PJ. Bone strontium distribution and degree of mineralization of bone in postmenopausal osteoporotic women treated with strontium ranelate for 2 and 3 years. Osteoporosis Int 2006;17(Suppl 2):S86.
32 Reginster JY, Seeman E, De Vernejoul MC *et al.* Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. Clin Endocrinol Metab 2005;90:2816–22.
33 Ammann P. Strontium ranelate: a physiological approach for an improved bone quality. Bone 2006;38(2 Suppl. 1):15–8.
34 Marie P, Ammann P, Shen V, Bain S, Robin B, Dupin-Roger I. Evidence that strontium ranelate increases bone quality in rats by improving bone strength and architecture. Bone 2003;32:S80 (OR29W).
35 Farlay D, Boivin G, Panczer G, Lalande A, Meunier PJ. Long-term strontium ranelate administration in monkeys preserves characteristics of bone mineral crystals and degree of mineralization of bone. J Bone Miner Res 2005;20:1569–78.
36 Ammann P, Badoud I, Barraud S, Dayer R, Rizzoli R. Strontium ranelate treatment improves trabecular and cortical intrinsic bone tissue quality, a determinant of bone strength. J Bone Miner Res 2007;22:1419–25.