EFFECT OF STRONTIUM RANELATE ON BONE REMODELING

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ABSTRACT

Osteoporosis is a disease in which the microarchitecture of bone tissue deteriorates, with consequent loss of bone mass. Strontium ranelate (SrR) is currently used for treatment of the condition. SrR may have a dual effect: anabolic (stimulating pre-osteoblast replication) and anti-catabolic (reducing osteoclastic activity). However, its mechanism of action has not yet been completely elucidated. The aim of this study is to evaluate the effect of SrR on bone remodeling in healthy Wistar rats. Two-month old female Wistar rats were administered SrR (2 g/L) in drinking water for 30 weeks. Oriented histological sections were prepared from lower jaw and tibia and stained with H&E, and the following histomorphometric parameters were evaluated: a) in interradicular bone: bone volume, and percentages of bone-formation, quiescent and bone-resorption surfaces; and b) in tibia: bone volume, total thickness of growth cartilage, thickness of hypertrophic cartilage zone and number of megakaryocytes. No significant difference was found in the parameters between the control animals and those treated with SrR. The results would therefore show that SrR does not alter the bone parameters studied in this experimental design.

Keywords: *strontium ranelate, bone remodeling, osteoporosis.*

EFECTO DEL RANELATO DE ESTRONCIO SOBRE LA REMODELACIÓN ÓSEA

RESUMEN

La osteoporosis es una enfermedad caracterizada por el deterioro de la microarquitectura del tejido óseo y la consecuente pérdida de masa ósea. El ranelato de estroncio (RSr) es actualmente utilizado para su tratamiento ya que poseería un efecto dual: anabólico (estimulando la replicación de preosteoblastos) y anticatabólico (disminuyendo la actividad osteoclástica). Sin embargo, su mecanismo de acción aun no ha sido completamente dilucidado. El objetivo del presente trabajo es evaluar el efecto del RSr sobre la remodelación ósea en ratas Wistar sanas. Se utilizaron ratas Wistar hembras de dos meses de edad a las cuales se les administró RSr (2 gr/L) en el agua de bebida durante 30 semanas. Se realizaron cortes histológicos orientados de maxilar inferior y tibia coloreados con H&E y se evaluaron los siguientes parámetros histomorfométricos: a) En hueso interradicular: volumen óseo, porcentaje de superficies en formación, reposo y reabsorción ósea. b) En tibia: volumen óseo, espesor total del cartílago de crecimiento, espesor de la zona de cartílago hipertrofiado y número de megacariocitos. No se observaron diferencias significativas en los parámetros evaluados entre los animales control y los tratados con RSr. Por lo tanto, los resultados obtenidos indicarían que el RSr no altera los parámetros óseos estudiados en el presente diseño experimental.

Palabras clave: *ranelato de estroncio, remodelación ósea, osteoporosis.*

INTRODUCTION

Osteoporosis is a metabolic disease involving loss of bone mass and reduction of the mechanical resistance of the bone. It is considered to be the primary cause of fractures in postmenopausal women, because estrogen deficiency increases bone resorption.

Osteoporosis is currently treated with different therapies which modify the bone microarchitecture by acting on the bone remodeling process[1,2]. Most therapies use anti-catabolic drugs such as biphosphonates, which inhibit bone resorption. Anabolic drugs that stimulate bone formation, such as parathohormone, are also used. However, strontium ranelate (SrR) is the only compound which seems to have dual effect: anabolic and anti-catabolic[3,4].

The effect of SrR on bone has been researched experimentally and in dialyzed patients. In the 1970s, Gravina et al. reported that a diet containing 3% strontium carbonate for 30 days altered periodontal tissues and induced osteomalacia in the interradicular bone in experimental animals[5].

Schrooten et al. also described symptoms of osteomalacia both in experimental animals with renal failure and in dialyzed patients[6-8].

Llinas et al. propose that strontium may be able to substitute calcium in its interaction with the alkaline phosphatase enzyme, inhibiting its action and generating symptoms of osteomalacia[9].

However, in recent years it has been clinically proved that a dose of 2 gr/day of the strontium compound (SrR) reduces the risk of vertebral and non-

vertebral factures and increases bone density in the long term[10-12].

In vitro studies have shown that SrR increases the formation of extracellular collagen matrix without inducing deleterious effects in the process of mineralization[13].

Some authors suggest that SrR may activate a calcium-sensing receptor, stimulating the proliferation and differentiation of cells of the osteoblast lineage through the expression of different markers such as c-fos, egr-1, Runx2, alkaline phosphatase, bone sialoprotein and osteocalcin[14,15].

In turn, the same pathway may be involved in the disorganization of the actin cytoskeleton in the osteoclast sealing zone, leading to its apoptosis, thus reducing the bone resorption rate[15].

It has been shown in primary human osteoblast cultures that SrR increases the expression of osteoprotegerin (OPG) and suppresses the nudear factor kB ligand (RANKL) levels, inhibiting osteoclastogenesis[16,17]. Moreover, it is also suggested that there may be a stimulation pathway for osteoblast differentiation independent of the calcium receptor, with a late response (regulated by extracellular pH) in which SrR would act by indirectly by activating the FGF receptor triggering the protein kinase C (PKC) signaling cascade and mitogen-activated protein kinase (MAPK)[18].

There is sufficient evidence in the literature suggesting the existence of an association between the megakaryocytes (Mks) present in the bone marrow and homeostasis of the bone tissue. It has also been proved that Mks synthesize markers related to osteoblastic differentiation, such as osteonectin, osteocalcin, osteopontin and OPG[19-21]. Bord et al. have shown that the Mks may be involved osteoclastogenesis through the expression of OPG and RANKL[22] as well as being able to stimulate osteoblast differentiation[23]. However, it is not known whether the Mks population is affected by treatment with SrR.

Despite the data provided in the literature regarding the effects of SrR *in vitro*, little is known about its mechanism of action *in vivo*. Therefore, the aim of this study was to evaluate the effect of SrR on bone remodeling in an experimental model with healthy Wistar rats.

MATERIALS AND METHODS

Experimental animals

Fourteen healthy female Wistar rats, two months old, weighing 160 ± 10 g were divided into two groups. They were housed in galvanized wire cages, with 3 or 4 animals per cage at a temperature of 21-24°C, moisture 52-56 % and 12-hour light/dark photoperiod. They were fed *ad libitum* (standard mouse/rat feed, Cooperación, Argentina) containing 23 % protein, 1-1.4 % calcium and 0.5-0.8 % phosporous[24]. The SrR group (n=7) received 2 g/L SrR (Protos®, Servier) in drinking water. The SrR solution was renewed and its daily intake recorded. Average intake was 50 mg SrR/day/animal. The control group (n=7) received only water. After 7.5 months, the animals were weighed, anesthetized and euthanized.

The trial was performed according to The Guide for the Care and Use of Laboratory Animals (NRC 1996).

Histology

The right hemi-maxilla and tibia were taken from each animal. The tibias were measured with a Vernier type caliper and weighed on precision scales. The extracted material was fixed in 4% formaldehyde-buffer solution at room temperature and decalcified in 10% EDTA for 30 days, after which it was processed histologically and embedded in paraffin.

Longitudinal histological sections approximately 7-8 microns thick were prepared from the proximal epiphysis of the tibia, and mesio-distal sections were prepared from the first lower molar.

Hematoxylin-eosin staining

All samples were stained with hematoxylin-eosin in order to perform histological and histomorphometric studies.

Histomorphometric measurements

The subchondral trabecular bone of the tibia and the interradicular bone of the first molar were measured in given areas, as shown in Fig. 1, using Image Pro Plus 4.5 software.

Histomorphometrical parameters evaluated in the interradicular bone of the first lower molar:

- BV/TV(%): Bone volume, percentage of bone tissue present in the total area evaluated.
- Ob.S./BS (%): Percentage of bones surface covered in active osteoblasts.
- ES/BS (%): Percentage of bone surface in total resorption.
- LCS/BS (%): Percentage of bone surface covered in lining cells.

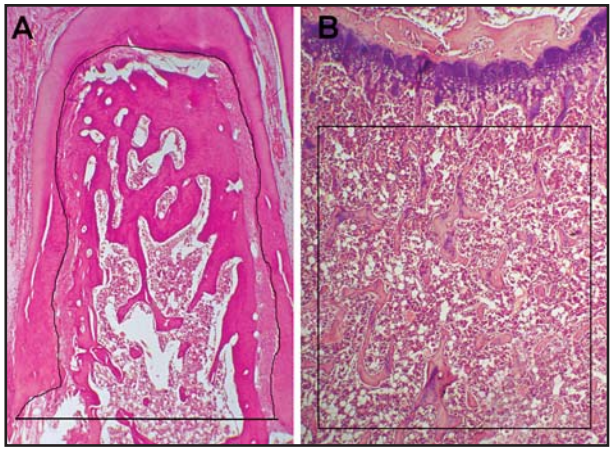

Fig. 1: Measurement area of first molar interradicular bone (a) and tibia subchondral trabecular bone (b).

In tibia subchondral bone:

- BV/TV(%): Bone volume, percentage of bone tissue present in the total area evaluated.
- GPC.Th (µm): Thickness of growth cartilage.
- HpZ.Th (µm): Thickness of hypertrophic cartilage zone.
- N.Mk/mm^2: Number of megakaryocytes per given area of bone marrow.

Statistical Analysis

Results were expressed as mean ± standard deviation. Data were analyzed with Student's t-test using the software "Primer of Biostatistics" (Mc Graw-Hill, 1992). Values for p lower than 0.05 were considered significant.

RESULTS

No significant difference was found in the final weight (g) of animals between the control group (307 ± 21) and the SrR group (311 ± 20) (p>0.05).

Interradicular bone

The histological sections of first lower molar interradicular bone were evaluated qualitatively under optical microscope, and no morphological difference was found between animals treated with SrR and control animals.

No significant difference was found in interradicular bone volume (BV/TV %) between the control group and the SrR group (control:42.1 ± 4.8%; SrR: 42.9 ± 3.4%; p>0.05; Fig. 2). Bone activity remained unchanged: bone-formation surfaces (control: 57.3 ± 10.5%, SrR: 66.4 ± 9.0%), quiescent surfaces (control: 38.5 ± 11.3%, SrR: 30.0 ± 8.7%) and surfaces in total resorption (control: 4.14 ± 3.64%, SrR: 3.58 ± 3.13%), with p>0.05 for all parameters (Fig. 3).

Tibias

No significant difference was found in weight (g) (control: 0.81 ± 0.02; RSr: 0.83 ± 0.05) or length (mm) (control: 39.09 ± 0.59; RSr: 39.39 ± 0.81) of the tibias between the control group and the SrR group (p>0.05 for both parameters).

The histological sections of tibias were evaluated qualitatively under optical microscope and no morphological difference was found between animals treated with SrR and controls (Fig. 4 A and B).

No significant difference was found in trabecular bone volume (BV/TV %) between the control group (19.8 ± 4.6) and the SrR group (21.67 ± 6.4) (p>0.05) (Fig. 5).

Growth cartilage thickness (µm) showed no significant difference between the control group (379 ± 40) and the SrR group (398 ± 8), in the proliferative and reserve zone (control: 103 ± 40, SrR: 98 ± 8) or in the hypertrophic zone (control: 138 ± 25, SrR: 150 ± 5) (p>0.05 for all parameters) (Fig. 6).

No change was found in the number of megakaryocytes (N.Mk/mm^2) between the control group (2.6 ± 0.6) and the SrR group (2.6 ± 0.3) (p>0.05) (Fig. 7).

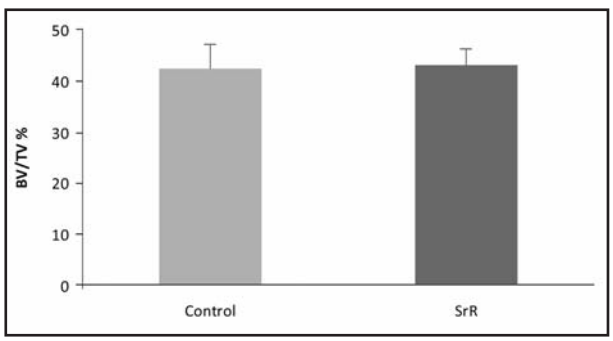

Fig. 2: Bone volume of first molar interradicular bone. Comparison between control and SrR groups, p > 0.05.

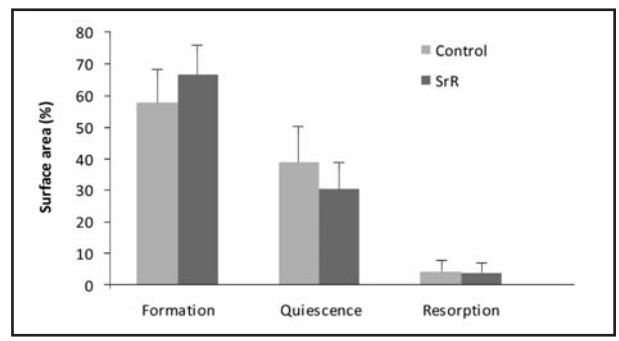

Fig. 3: Bone activity. Comparison between control and SrR groups, p > 0.05

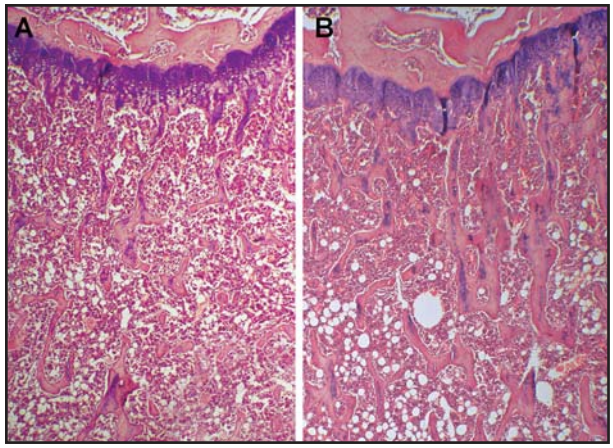

Fig. 4: Photomicrograph of histological sections of tibia, stained with H&E (original magnification: 40X). A: Control, B: SrR.

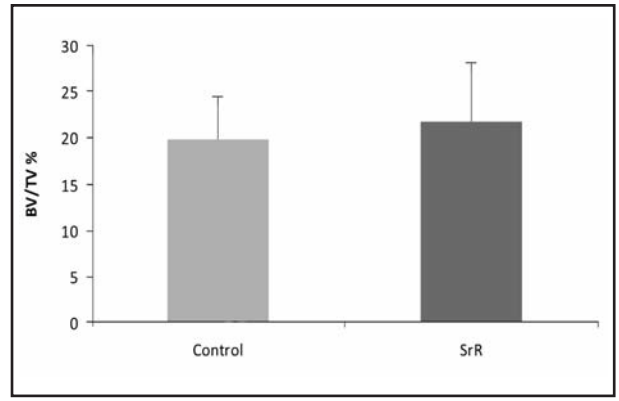

Fig. 5: Bone volume of tibia trabecular bone. Comparison between control and SrR groups, p > 0.05.

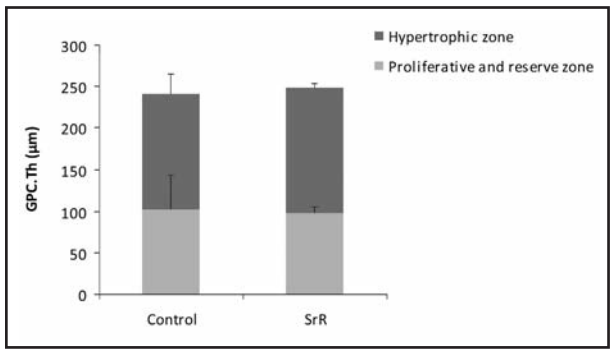

Fig. 6: Growth cartilage thickness. Comparison between control and SrR groups, p> 0.05.

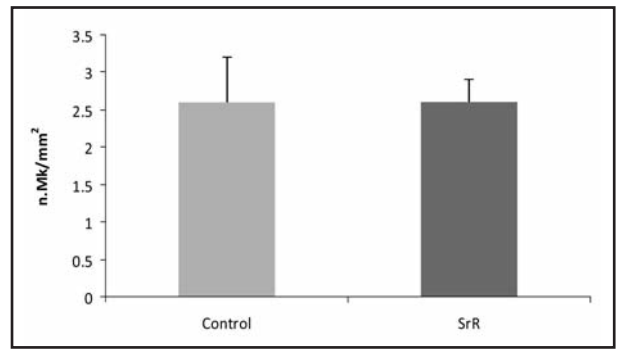

Fig. 7: Number of megakaryocytes per area unit. Comparison between control and SrR groups, p> 0.05.

DISCUSSION

This study showed that the administration of 2g/L of SrR in drinking water for 30 weeks does not modify bone volume, bone activity, growth cartilage thickness or number of megakaryocytes in bone marrow of healthy animals.

Roux et al. conducted a study on 353 patients aged 50-65 years with severe osteoporosis and proved that treatment with SrR at a dose of 2 g/day for 4 years reduces the risk of vertebral fracture[25]. Arlot et al. showed that the same dose of SrR administered for 2 and 3 years stimulates trabecular and cortical bone formation, reducing the risk of fracture[26]. *In vivo* studies by Ammann et al. showed that administration of 225-900 mg/kg/day SrR for two years modifies bone resistance, cortical and trabecular volume, microarchitecture and bone mass, improving the quality of the tissue in healthy rats[27].

It has also been reported that treatment with 625 mg/kg/day SrR of ovariectomized rats for 52 weeks prevents loss of mass and deterioration of bone quality in the vertebral column[28].

However, Cebesoy et al. found that the administration of 450 mg/kg/day SrR for 2, 3 or 4 weeks neither benefits nor harms the fracture healing process in healthy male rat tibia[29].

Based on our results and the literature, we may infer that the effect of SrR on bone remodeling depends on the dose and duration of administration.

SrR is known to be composed of two strontium atoms and one ranelic acid molecule. Upon entering the organism, the SrR molecule dissociates and the Sr atoms are released and deposited almost exclusively in the bone tissue[30,31].

Doublier et al. conducted research on iliac bone biopsies from patients treated with SrR for 2, 12, 24, 36, 48 and 60 months. They found that Sr was

located almost exclusively in newly formed bone structural units and that the process of mineralization remained within normal levels[32]. The nature of the Sr atom allows it to be captured by hydroxyapatite crystals and remain on their surface or to substitute Ca^{++} in its position in the crystals[33, 34].

The exact mechanism of action of SrR on bone remodeling has not yet been entirely elucidated. Nevertheless, as no change was recorded in the histomorphometric parameters evaluated in our study, the reduction of the risk of fracture and increase in bone density observed in patients that have undergone long-term therapy with SrR might be due to a modification in the physical properties of the mineral structure of the bone tissue caused by the incorporation of Sr to the hydroxyapatite crystals.

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