Effect of Strontium Ranelate on Fracture Healing in the Osteoporotic Rats*,**

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ABSTRACT: The aim of this study was to evaluate the effect of strontium ranelate (SrR) on fracture healing in the osteoporotic rat model. Forty female Sprague–Dawley rats aged 3 months were enrolled in the study. Osteoporosis was induced by bilateral ovariectomy and subsequent daily heparin injection started 1 week after surgery and lasted for 4 weeks. Osteoporosis was confirmed by a reduction of bone mineral density (BMD). Twenty of the osteoporotic rats were assigned to the SrR group and the remaining 20 to the control group. An open right tibial midshaft transverse fracture was created and then an intramedullary fixation was performed. SrR group was treated by 450 mg/kg/day SrR per oral. Six weeks after surgical induction of fracture, all animals were sacrificed. One animal from each group died after ovariectomy. Two tibiae from the control group failed to unite. SrR-treated group showed higher mechanical strength and fracture stiffness when compared to the control group ($p = 0.006$, $p = 0.001$, respectively). SrR-treated group had mature woven bone or predominantly woven bone compared with osteoporotic control group ($p = 0.038$). SrR-treated group's callus maturity was significantly higher than control group ($p = 0.001$). SrR is associated with better fracture healing in the osteoporotic rat model. © 2010 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 29: 138–142, 2011

Keywords: strontium ranelate; fracture healing; osteoporosis; ovariectomized rat; heparin

Osteoporosis is a complex and multifactorial condition characterized by reduced bone mass and impaired micro-architecture, leading to an increased susceptibility to fractures. Osteoporosis relevant fracture is described as fragility fracture or low trauma fracture and associated with substantial disability, pain and reduced quality of life.[1] It is estimated that approximately 50% of postmenopausal women will experience osteoporotic fragility fracture and 10–20% of the hip fractures in osteoporotic patients are expected to result in death within the first year.[2,3]

An alternative system incorporating anabolism (bone forming) and catabolism (bone resorbing) may be employed due to frequent overlapping of the classical four stages of fracture healing. For the regular continuance of bone forming and resorbing activity, proper inflammatory cell response and coordination with cytokines and growth factors, adequate differentiation and competence of mesenchymal progenitors to osteoblasts and chondroblasts and balanced function and differentiation capacity of osteoblasts and osteoclasts are needed.[4] Nonunion has been reported up to 5–10% in osteoporosis related fractures.[5] Osteoporosis associated fractures cause great morbidity and mortality for the elderly population.[6]

Strontium ranelate (SrR) is a novel oral antiosteoporotic agent. In clinical trials, SrR reduces the risk of vertebral and nonvertebral fractures in postmenopausal osteoporotic women.[7–9] Preclinical studies have shown that SrR concomitantly reduce bone resorption and stimulates bone formation at remodeling sites.[10,11] It increases bone cell replication and bone formation by amplifying preosteoblastic and pluripotent mesenchymal cell replication via directly interacting with a Ca sensing receptor and trigger mitogenic signals.[12–14] SrR reduces bone resorption by decreasing osteoclast differentiation and bone resorbing activity. SrR has been shown to have dynamic activity between canaliculi and calcified bone matrix and preferably taken up by new bone.[15]

Although antifracture efficacy of SrR has been demonstrated, there is paucity of data on the effect of SrR on fracture healing in osteoporotic bone both in animal and human studies. The objective of this study is to evaluate the effect of SrR on fracture healing in an osteoporotic rat model.

MATERIALS AND METHODS

Experimental Animals

Forty female 3-month-old Sprague–Dawley rats weighing 200–250 g were obtained from experimental animals' laboratory of the Duzce University. Animals were housed one per cage in a room without any restriction of mobilization and with controlled temperature ($24 \pm 2^{\circ}C$) and relative humidity. Alternating 12-h dark and light photoperiod was performed. Animals were fed with normal calcium diet (1–1.6%). Water was available ad lubitum. Before the experimental period, animals were acclimated for 1 week settling in period. The study

* There was no conflict of interest.

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was approved by the Ethics Committee of School of Medicine, Duzce University.

Study Design

All operative procedures were performed under deep anesthesia with intraperitoneal administration of ketamin (40–90 mg/kg). Buprenorphine (0.45 mg/kg) was given to rats subcutaneously for postoperative analgesia once a day for 3 days. As a prophylaxis cefazoline sodium (20 mg/kg per day i.m.) was injected preoperatively and at 8 h postoperatively.

Osteoporosis was induced by bilateral ovariectomy and subsequent daily heparin injection (2 IU/g/day) started 1 week after surgery and lasted for 4 weeks. Following completion of heparin injection, the diagnosis of osteoporosis was confirmed by a significant reduction of bone mineral density (BMD) compared to the baseline as assessed by DXA. One week after completion of heparin injections, right tibia of each rat was exposed via an antero-medial longitudinal skin incision. Soft tissues were carefully dissected and a transverse osteotomy was performed with a ring-cutter type saw at the mid-shaft of the tibia.[16] An additional longitudinal parapatellar incision was made and patella was deflected, osteotomies were reduced and fixed with a 0.045 in. Kirschner wires. The quality of reduction and the position of the Kirschner wires were checked by radiographs. During anesthesia, the heel of the right limb was placed on a platform with the distal part of the limb in-between three points in the jaws of a specially designed forceps.[17]

Twenty osteoporotic rats were assigned to the SrR group and the remaining 20 to the controls. Animals in SrR group were given 25 g/day dry food containing 135 mg SrR that corresponded to a dose of 450 mg/kg/day for 6 weeks. Six weeks after surgical induction of fracture, all animals were sacrificed by ether inhalation. After the radiological assessment of the fracture healing, animals were randomly assigned to biomechanical testing and histological evaluation in each groups for assessment of the fracture callus.

DXA Measurement

Dual energy X-ray absorptiometry was performed using a Lunar-DPX® IQ (Madison, WI) with Small Animal software version. BMD of the lumbar spine (L1–L5) and left femur was measured.

Radiography

Radiographs were taken with a Siemens X-ray machine (Model number: 4803404, Germany). Callus maturity was evaluated with the Goldberg classification.[18] In that classification, three stages were indicates accordingly: stage 1: nonunion, stage 2: possible union and stage 3: complete union. Mean radiological scores were calculated for both groups.

Biomechanical Testing

Three point bending test was carried out using a mechanical testing machine (Zwick/Roell, 1446, Germany). The distance between the two points of support (bearing distance) was 21 mm. In a three-point cantilever bending at 2 mm/min with the fulcrum placed over the fracture callus was applied. Mechanical strength [Newton (N)] and fracture stiffness, [N/mm] were calculated regarding to the radius of the bone.

Histology

The specimens were fixed in 10% neutral buffered formalin for 4 days, then decalcified for 15 days in 10% formic acid, and embedded in paraffin. Longitudinal sections (4 μm) were cut and stained with hematoxylin–eosin. Sections were evaluated with light microscopy under 100× magnifications. All histological specimens were evaluated by a single pathologist who was blinded to the groups. Histological evaluation was performed according to the grading system of the fracture healing, described by Huo et al.[19] This grading system allows to classify the fracture healing from fibrous tissue to mature bone. Grade 1: fibrous tissue, Grade 2: predominantly fibrous tissue with some cartilage, Grade 3: equal amounts of fibrous with cartilage, Grade 4: all cartilage, Grade 5: predominantly cartilage with some woven bone, Grade 6: equal amounts of cartilage with some woven bone, Grade 7: predominantly woven bone with some cartilage, Grade 8: entirely woven bone, Grade 9: woven bone and some mature bone, Grade 10: lamellar (mature) bone.

Statistical Analysis

Statistical analyses were performed using the SPSS for Windows (release 10.0, Chicago, IL). Distributions were checked by Kolmogorow–Smirnov's test. Comparisons were done using the Student's *t*-test and Mann–Whitney *U*-test according to the distributions of the parameters. $p < 0.05$ was considered as statistically significant.

RESULTS

One animal from each group died after ovariectomy. Two tibiae from the control group failed to unite within 6 weeks postfracture and were diagnosed with nonunion. No nonunion was noted in the SrR group. There was no early or late infection.

DXA Measurement

Lumbar and femoral BMD measurements significantly decreased after the ovariectomy and heparin injections, confirming establishment of the osteoporosis model. The lumbar BMD values before and after ovariectomy and heparin injection were 0.27 ± 0.01 and 0.26 ± 0.02, respectively ($p = 0.026$). The respective data for femoral BMD were 0.3 ± 0.03, 0.27 ± 0.02, respectively ($p = 0.001$).

Radiography

Following the formation of the fracture and its stabilization by the Kirschner wire, the fracture type and fixation quality were checked by radiographs of tibia of each rat. The mean score of the callus maturity was significantly higher in the SrR-treated group compared to the control group (SrR gr: 2.7 ± 0.6, control gr: 2.2 ± 0.7; $p = 0.001$) (Fig. 1).

Biomechanical Testing

SrR-treated group showed approximately 56% higher mechanical strength when compared to the control group ($p = 0.006$). Furthermore, SrR group revealed significantly higher fracture stiffness compared to the control group ($p = 0.001$) (Table 1).

Histology

SrR-treated group had mature woven bone or predominantly woven bone compared with osteoporotic control group ($p = 0.038$) (Table 1, Fig. 2).

Table 1. The Biomechanical Test Result and Histological Scoring of the SrR and Control Groups

	SrR Group	Control Group	*p*-Value
Biomechanical test results			
Mechanical strength (N)	26.95 ± 8.4	15.02 ± 7.4	0.006
Fracture stiffness (N/mm)	2.97 ± 0.9	1.39 ± 0.8	0.001
Histological scoring	8 ± 0.8	6.6 ± 2.2	0.038

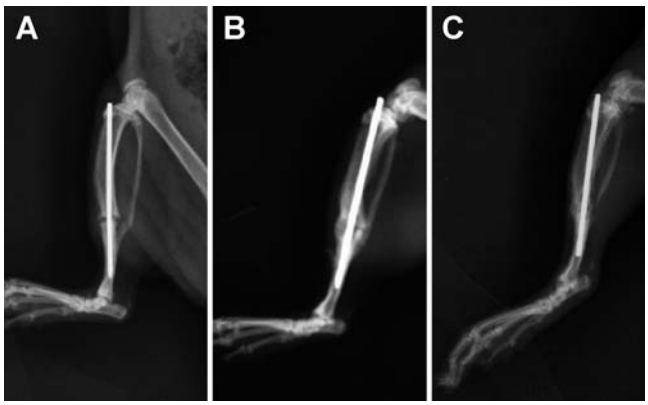

Figure 1. At 6 weeks postfracture, X-ray films of the fractured tibiae were presented as nonunion (A) and possible union (B) in the control group. Radiographic union was presented on the number C in the SrR group.

DISCUSSION

The results of the current study revealed that postfracture use of 450 mg/kg/day SrR in the heparine-induced osteoporosis on the ovariectomized rats is associated with better fracture healing as assessed by biomechanical testing, histological analysis, and radiography.

The main reason of postmenopausal osteoporosis is high bone turnover due to increased osteoclastic activity. However, osteoporosis of the elderly referred to as senile osteoporosis is due to impairment of bone formation. Mesenchymal cell pool and their ability to differentiate into the osteogenic lineage are negatively affected by ageing and osteoporosis.[20–22] Decreased bone formation may contribute to delay on the fracture healing process in this age group.[23]

The effects of antiresorptive and anabolic drugs on the fracture healing have been investigated. Parathyroid hormone (PTH) has potent anabolic effects on bones as a result of stimulation of bone formation in several animal models and human.[24–26] PTH accelerates fracture healing through stimulation of callus remodeling and formation of new cortical shell.[27] PTH related acceleration of fracture healing is occurred via enhanced differentiation of mesenchymal progenitor cells into mature osteoblasts at the fracture site by intermittent PTH treatment.[28–31] Studies with bisphosphonates and efficacy on fracture healing revealed that bisphosphonates delays structural fracture healing process by strongly suppressing callus remodeling.[32] However, these antiresorptive drugs did not impair restoration of mechanical strength of the fractured bone. SrR reveals dual effects on bone with inhibiting resorption and promoting formation. It is therefore important to improve our understanding of how SrR effects fracture healing on the osteoporotic bone.

We employed both ovariectomy and subsequent heparin injection to induce osteoporosis in our rat model, since prominent and permanent osteoporosis may not be induced by ovariectomy only.[33,34] Heparin contributes to osteoporosis by both increasing bone resorption with an increment in osteoclast number and its activity as well as reducing bone formation with a reduction in osteoblast number and its function.[35]

There are limited data on the effect of SrR on fracture healing. Cebesoy et al. evaluated the efficacy of SrR on fracture healing in healthy bone. In this study employing traumatically induced fracture model, no effect on fracture healing was reported.[36] Recently, Li et al.[37] reported that 625 mg/kg/day SrR revealed benefi-

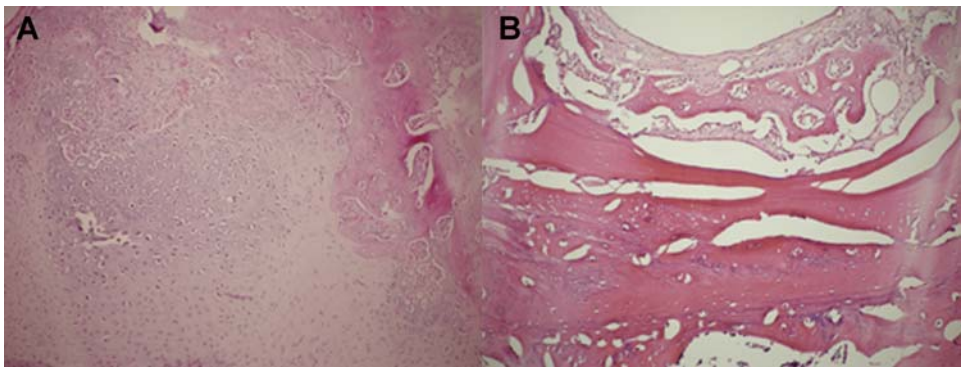

Figure 2. At 6 weeks postfracture, woven bone and some mature bone (B) was observed in SrR group, while predominantly cartilage with some woven bone (A) was revealed in control group on the histological evaluation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

cial effect on the fracture healing on the ovariectomized rats. The two main differences were observed in that study compared to the presented study. Daily SrR dose was 450 mg/kg therefore the effective daily dosage of SrR was less in the presented study. Additional heparine was given to the providing permanent and persistent osteoporosis on the ovariectomized rats. SrR-treatment group was associated with more mature woven bone as well as significantly higher fracture stiffness and mechanical strength compared to the control group.

Nonunion has been reported up to 5–10% in osteoporosis related fractures.[5,16] Nonunion causes functional disability and hence may worsen by osteoporosis.[38] In our study, two nonunions were observed in the control group; however, no nonunion was noted in the SrR group.

SrR increases bone formation by promoting differentiation of osteoblast precursors. It has a weaker antiresorptive activity by promoting release of osteoprotegerin to inhibit RANK/RANKL (NFkappaB-ligand) pathway. Vertebral antifracture efficacy of the SrR was noted to be independent of baseline bone turnover levels.[39] The weaker antiresorptive activity of SrR may not disrupt the osteoclast responsible resorption process of the remodeling phase. Anabolic activity of SrR may provide beneficial effect on the reparative and regenerative processes of the remodeling phase in the fracture healing. Further studies are warranted to delineate the precise mechanism(s) of action of SrR on fracture healing.

There are limitations of our study. First, the callus size of the fracture sites was not evaluated with the micro-CT analysis. This technique provides more detailed information on the callus volume. Second, serum SrR concentration was not determined because of the technical difficulties. However, animal consuming of the daily diet was monitored diligently.

We conclude that SrR has beneficial effect on fracture healing in the osteoporotic rat model.

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