Effects of strontium ranelate on cortical bone collagen integrity

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ABSTRACT

الأهداف: التحقق من تأثير سترونتيوم رانيلات على عملية ربط أواصر كولاجين العظام لدى الجرذان التي أُجريت لها عملية استئصال للمبايض.

الطريقة: أُجريت هذه الدراسة في مختبرات جامعة ميرسين، جامعة ميرسين، ميرسين، تركيا وذلك خلال الفترة من يناير إلى مايو 2008م. وشملت الدراسة 28 جرذي أنثى بالغ من النوع ألبينو ويستر (12 أسبوع)، ويبلغ وزنها 200–250 غرام، وقد قُسموا (7 جراذ في كل مجموعة) إلى مجموعة العملية الجراحية الزائفة، والمجموعة التي اسُتأصلت مبايضها، ومجموعة التدخل، ومجموعة العلاج. لقد قمنا بإعطاء الحيوانات في مجموعة التدخل سترونتيوم رانيلات (500 ملغ/كلغ/يومياً عن طريق الفم) وذلك لمدة 120 يوم مباشرةً بعد استئصال المبايض. فيما أعطينا الحيوانات في مجموعة العلاج سترونتيوم رانيلات (500 ملغ/كلغ/يومياً عن طريق الفم) وذلك لمدة 120 يوم بعد مرور 90 يوم من عملية استئصال المبايض. ولقد قمنا بتحليل مستويات القلق، والإرهاق، وخشونة قشرة عظم الفخذ، بالإضافة إلى تقييم بنية الكولاجين بعد نهاية التجربة.

النتائج: أشارت نتائج الدراسة إلى انخفاض قيم القلق، والإرهاق، والخشونة لدى الجرذان في المجموعة التي أُجريت لها عملية استئصال المبايض، ومجموعة التدخل، ومجموعة العلاج وذلك عند المقارنة مع مجموعة العملية الجراحية الزائفة. ولقد قمنا بملاحظة تنظيم الألياف في مجموعة العملية الجراحية الزائفة، في حين تم استبدال الألياف الموازية بالكامل بمجموعة تنظيم عشوائية في المجموعة التي أُجريت لها عملية استئصال المبايض، ومجموعة التدخل، ومجموعة العلاج.

خاتمة: أظهرت الدراسة بأن سترونتيوم رانيلات لم يقم بإصلاح عملية ربط أواصر كولاجين العظام لدى الجرذان التي أُجريت لها عملية استئصال المبايض.

Objective: To investigate the effect of strontium ranelate on bone collagen cross-linking in an ovariectomized rat model.

Methods: Twenty-eight adult (12 weeks) albino female Wistar rats weighing between 200-250 g (n=7 per group) were divided into sham-operated, ovariectomized, prevention, and treatment groups. Animals in the prevention group were treated with strontium ranelate (500 mg/kg/day orally) for 120 days, starting immediately after ovariectomy. Animals in the treatment group were treated with strontium ranelate (500 mg/kg/day orally) for 120 days, starting 90 days after ovariectomy. At the end of the experimental period, stress, strain, and toughness of the cortical femur was measured, and collagen ultrastructure was evaluated. The study was conducted in Mersin University Biophysics Laboratory, Mersin University, Mersin, Turkey from January to May 2008.

Results: Stress, strain, and toughness values decreased in ovariectomized, prevention, and treatment groups when compared with the sham-operated group. In this study, fiber organization was observed in the sham-operated group, whereas the parallel packing of fibrils was completely replaced by a random arrangement in the ovariectomized, prevention, and treatment groups.

Conclusion: Strontium ranelate treatment did not repair collagen cross-linking in ovariectomized rats.

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Most of the treatments of osteoporosis have focused on preservation of skeletal mass by inhibiting osteoclastic bone resorption or reversing bone loss by stimulating osteoblastic bone formation.[1] Strontium ranelate, which consists of 2 atoms of stable strontium and an organic acid, ranelate, is a new orally administrated drug for the treatment of postmenopausal osteoporosis. Strontium ranelate (Protelos®) has a dual effect on bone metabolism.[2,3] In vitro studies have suggested that strontium ranelate enhances osteoblastic cell replication and activity.[4] Simultaneously, the strontium ranelate dose dependently decreases preosteoclast differentiation and osteoclastic activity.[4-6]

The bone is an anisotropic and viscoelastic material. It can adapt to changes in its physiologic or mechanical environment. The bone matrix consists of 2 phases. The mineral phase provides the stiffness, and the collagen phase provides the ductility and ability to absorb energy. The resistance of mechanical forces and fractures depends on both the quantity and quality of bone. Previous studies showed that collagen plays an important role in the bone resistance to fracture.[7,8] Changes in collagen fibers affect biomechanical properties of bone and increase fracture risk. Collagen cross-links decrease in osteoporosis, and this may increase bone fragility.[7,8]

The aim of the study was to investigate the effect of strontium ranelate on bone collagen cross-linking in osteoporotic rats. For this purpose, we measured intrinsic properties of cortical femur because of their relation to collagen integrity, and we evaluated collagen ultrastructure by transmission electron microscopy.

Methods. *Animals.* Twenty-eight adult albino female Wistar rats weighing 200-250 g were used in this study. The animals were acclimatized for one week to our laboratory conditions prior to experimental manipulation, and were exposed to a 12-hours light and 12-hours dark cycle at a room temperature of 22°C. They had free access to standard laboratory chow and water ad libitum. Under ketamine (50 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and Xylazine (8 mg/kg, Rompun, Parke-Davis/Pfizer, Istanbul, Turkey) anesthesia, 21 animals were ovariectomized by ventral incisions. The ovariectomized rats were randomly assigned into 3 groups. These groups were designed as the ovariectomized group, the prevention group (prevention of ovariectomy), and the treatment group (treatment of established ovariectomy).

Ovariectomized group. Animals in this group (n=7) were administered placebo (saline) via oral gavage.

Prevention group. Animals in this group (n=7) were treated with strontium ranelate (500 mg/kg/day orally) for 120 days, starting immediately after ovariectomy. They were sacrificed at the end of this 120 days treatment.

Treatment group. Animals in this group (n=7) were treated with strontium ranelate (500 mg/kg/day orally) for 120 days, starting 90 days after ovariectomy.

Sham-operated group. Sham operations were performed in 7 animals. Animals in this group were administered placebo (saline) via oral gavage.

Strontium ranelate was obtained as a characterized drug from Servier Pharmaceuticals, Istanbul, Turkey. The Institutional Animal Care and Use Committee at Mersin University Medical Faculty approved the experiments described in this study. This study was conducted in Mersin University Biophysics Laboratory, Mersin University, Mersin, Turkey from January to May 2008. All procedures on laboratory animals were performed according to the NIH Guiding Principles in the Care and Use of the Animals.

On the day following the last day of treatment, animals were sacrificed under over dose anesthesia. Bone mineral density (BMD) of the femurs were measured using dual energy x-ray absorptiometry (Norland XR 45, Norland Scientific Instruments, Fort Atkinson, WI, USA), with a scan speed of one mm/s, and a resolution of 0.5x0.5 mm. Before taking the measurements, the instrument was calibrated by means of a Norland phantom. The BMD (mg/cm^2) was determined by the analysis of the femoral diaphysis. A cross-sectional area was measured by CT (ARSTAR 40, Erlangen, Germany) and the length of femoral shaft was measured with a digital clipper.

Bone biomechanics. Biomechanical measurements were performed at the femoral diaphysis of the left femur. Bones were resected, cleaned, wrapped in gauze soaked in isotonic saline, and frozen at -20°C until testing. Tensile test was performed to measure intrinsic parameters of diaphysial femur. After thawing the samples at room temperature, bones were tested using a biomaterial testing machine (MAY 03,BIOPAC, Santa Barbara, CA, USA). For the tensile test, the femur was mo unted horizontally in the machine with the use of colacryl. The tensile loading speed in all tests was 2 mm/min. Data were transferred to the computers translating to the numerical signals by 16 bit A/D converter for

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off-line analysis. The sampling rate was chosen as 1,000 samples/s. During mounting and testing of the specimens, Ringer's solution was regularly applied to prevent the bones from drying. Each specimen was subject to a small initial preload (5 N) before actual testing. Load-displacement data were recorded using BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, CA, USA). Load-displacement recordings were normalized by a cross-sectional area, and this curve was converted to a stress-strain curve. Stress-strain curves for each specimen were generated, and the ultimate stress, ultimate strain, and toughness were determined from these curves.[9]

Histological investigating. Femurs were fixed with 2.5% glutaraldehyde, decalcified with 10% EDTA, postfixed with 1% osmium tetroxide, dehydrated in graded alcohol series, cleared with propylene oxide, and embedded in Epoxy resin. Thin sections (50-70 nm) were cut by Leica UCT-125 (Leica Microsystems GmbH, Vienna, Austria), and contrasted with uranyl acetate and lead citrate. Sections were examined and photographed by JEOL JEM-1011 electron microscope (United Kingdom).[9]

Statistical analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) 10.0 software. After obtaining normal distribution (Kolmogorov-Smirnov), data were expressed as mean ± standard deviation (SD) and Tukey significant difference test to compare different groups. Significance was set at $p<0.05$.

Results. *Bone mineral density.* In the ovariectomized group, BMD was significantly lower than the other groups. There were no significant differences between the sham, prevention, and treatment groups for BMD values (**Table 1**).

Bone biomechanical properties. From the stress-strain curve, ultimate stress, ultimate strain, and toughness were calculated. It is observed that compared to the sham group; stress, strain, and toughness measured values decreased in all groups. These variations were significant for the stress and toughness data. However, the decrease in strain values in the prevention and treatment groups was significantly different compared with the sham group. These values were shown in **Table 1**.

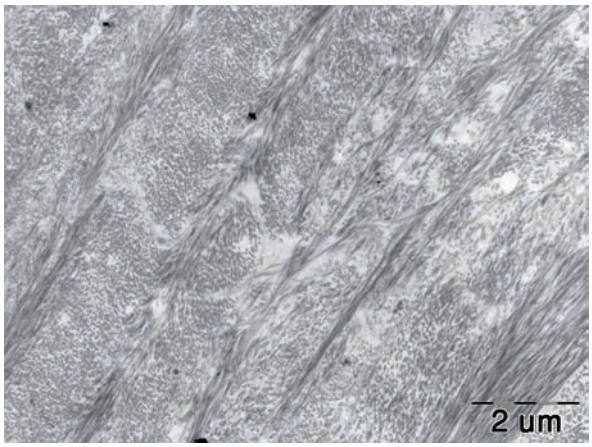

Figure 1 - Bone tissue with normal appearance. Collagen fibers arranged in lamellar fashion (X12,000).

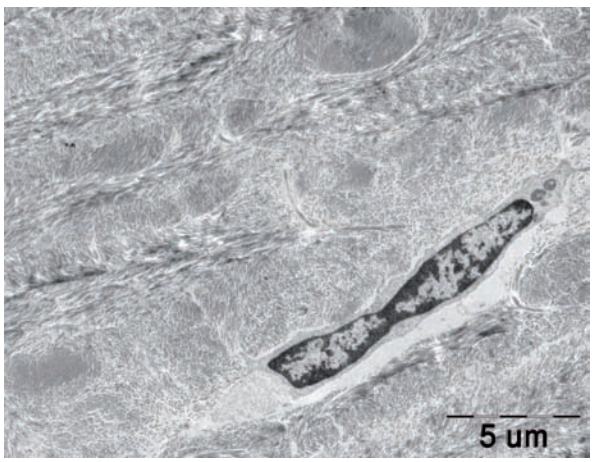

Figure 2 - Bone tissue with mild disorganization in lamellae (X6000).

Table 1 - Bone mineral density, stress, strain, and toughness values in sham, ovariectomized, prevention, and treatment groups.

Variables	Sham group	Ovariectomized group	Prevention group	Treatment group
Bone mineral density (g/cm^2)	0.19 ± 0.01	0.14 ± 0.008*†‡	0.22 ± 0.021	0.21 ± 0.017
Stress (MPa)	15.71 ± 6.29	5.97 ± 2.72*	11.65 ± 3.06*	10.14 ± 3.44*
Strain	0.45 ±0.16	0.35 ± 0.085	0.30 ± 0.093*	0.15 ± 0.003*†
Toughness (MPa)	6.20 ± 2.35	1.48 ± 0.28*	3.00 ± 0.31*	0.90 ± 0.28*†

*Significantly different from the sham group $p<0.05$; †Significantly different from the prevention group $p<0.05$; ‡Significantly different from the treatment group $p<0.05$

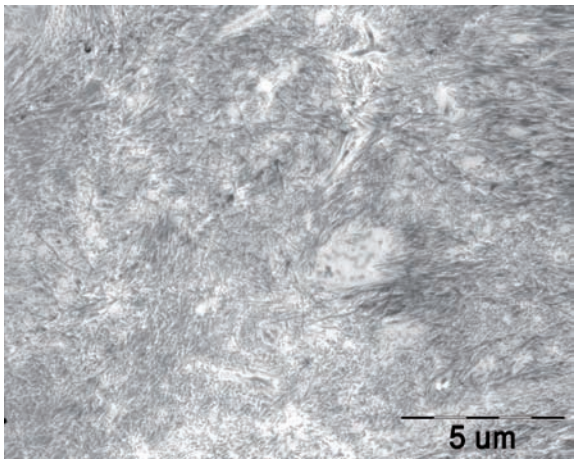

Figure 3 - Bone tissue collagen fibers with completely disordered lamellar organization (X7,500).

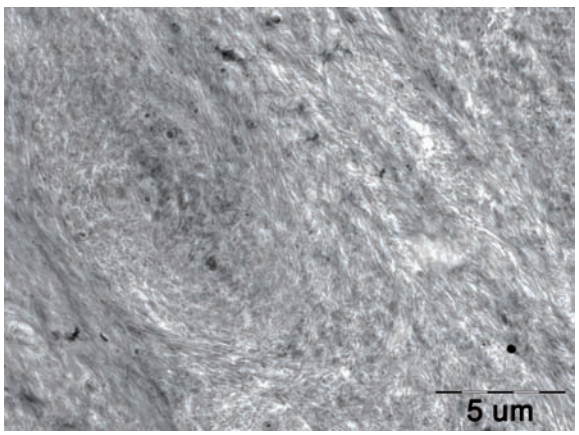

Figure 4 - Bone tissue collagen fibers with completely disordered lamellar organization (X6,000).

Collagen cross-linking. Severe alterations in bone collagen fibrils were detected at the ultra-structural level. Regular collagen fiber organization was observed in the sham-operated group (**Figure 1**), whereas the parallel packing of fibrils was completely replaced by a random arrangement in ovariectomized rats (**Figure 2**). Irregular organization of collagen fibers was observed in the prevention group (**Figure 3**) and the treatment group, similar to the ovariectomized group (**Figure 4**).

Discussion. In this study, the effect of strontium ranelate on collagen integrity of osteoporotic cortical bone was investigated in an ovariectomized rat model. Although this drug has led to increase in the BMD levels, on the other hand, it has not improved collagen integrity in osteoporotic bone. The mechanical properties of the bone reflect the inherent material properties of its constituents, and the way in which they are arranged and interact. In all connective tissues, collagen has mechanical functions, providing elasticity and structure for the component tissues. In bones, a large body of evidence indicates that type I collagen molecules are involved in mechanical properties of bone.[8] Several studies indicate that collagen plays a substantial role in its toughness (capacity to absorb energy), while the mineral content is mainly involved in determining bone stiffness.[10] There are many studies on the effect of strontium treatment on bone mechanical properties. In these studies, some authors reported that the effect of strontium on bone is dose dependent.[11] Similarly, Fuchs et al[12] showed that a low dose (25-150 mg/kg/day) of strontium does not have any effect on bone mechanical parameters. On the contrary, Morohashi et al[13,14] demonstrated that a large amount of strontium, more than approximately 400 mg/kg/day, disturbs calcium metabolism, and decreases bone formation. However, Ammann et al[15] reported that the plasma level of strontium obtained in animals treated with 625 mg/kg/day was close to the value observed in patients treated with 2 g/day. We used 500 mg/kg/day strontium, but found a decrease in bone stress, strain, and toughness in both treatment and prevention groups. Compared with the sham group, stress, strain, and toughness values were significantly decreased. Their decreased values were 25% and 35% for stress, 33% and 66% for strain, and 51% and 85% for toughness in the prevention and treatment groups. The decline in stress, strain, and toughness parameters were lower in the prevention group compared with the treatment group.

The decrease in stress, strain, and toughness may be related to deformation of collagen integrity. Collagen fiber organization was evaluated by transmission electron microscopy. We observed regular collagen fiber organization in the sham group, but there was no preferential organization of the collagen fibers in ovariectomized, prevention, and treatment groups. Collagen fibers were not tightly packed and were observed in randomly oriented bundles. Studies on rat femora suggest that the decline in bone's mechanical properties with age and osteoporosis may be dependent on the stability and cross-linking of the collagen. Only a few studies on the osteoporosis-related changes in collagen and its correlation with the toughness of bone have been reported in the literature,[16-18] and on literature review, there are no studies investigating the effects of strontium ranelate treatment on collagen networks and toughness of the cortical bone.

Osteoporosis is a chronic disease, and treatment is very important. The aim of treatment of osteoporosis is

to reduce the risk of fractures in patients. Many different types of drugs are used to treat osteoporosis. According to our data, strontium ranelate increases BMD, but does not repair collagen cross-linking. Treatment with strontium ranelate has no effect on bone quality, and is therefore, not suitable for osteoporosis treatment. The suitability of the drug, the patient lifestyle, the patient's preference, tolerability, and convenience should all be considered. The BMD measurement is most widely used for assessment of the efficacy of osteoporosis treatment, but BMD alone is not a good predictor for fracture risk. We suggested that in addition to BMD, the measurements of bone turnover markers, bone collagen markers, magnetic resonance imaging analysis, and micro-CT can be considered in the treatment of osteoporosis.

In conclusion, our biomechanical and histological results suggest that strontium ranelate treatment has no effect on collagen cross-linking repair. An important limitation of this study was the measurement of bone biomechanical parameters. These parameters cannot be measured by the same methods in osteoporotic women, because it is not possible to break bones in humans. However, it may be suggested that collagen cross-linking may be represented differently using methods mentioned previously, before or after treatment to determine the effectiveness of treatment. Many investigations are needed on this issue to provide information regarding clinical studies.

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