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Short communication

Bone mineral density is reduced by telmisartan in male spontaneously hypertensive rats

Antonio Marcos Birocale[a,*], Ana Raquel Santos Medeiros[b], Leandro Dias Gonçalves Ruffoni[c], Liliam Takayama[d], José Martins de Oliveira Jr.[e], Keico Okino Nonaka[c], Rosa Maria Rodrigues Pereira[d], Nazaré Souza Bissoli[f]

[a] Department of Health Integrated Education, Federal University of Espirito Santo,Vitória, Brazil
[b] Biological and Health Sciences, Federal Institute of Espirito Santo, Vitória, Brazil
[c] Department of Physiological Sciences, Federal University of São Carlos, São Carlos, Brazil
[d] Department of Medical Clinic, Medicine College, University of São Paulo, São Paulo, Brazil
[e] Laboratory of Applied Nuclear Physics, University of Sorocaba, Sorocaba, Brazil
[f] Department of Physiological Sciences, Federal University of Espirito Santo, Vitória, Brazil

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ABSTRACT

Background: Telmisartan, an angiotensin AT1 receptor blocker, and treadmill running were compared for their effects on bone mineral density (BMD) and biomechanical properties of male spontaneously hypertensive rats (SHR). It was hypothesized that running (18 m/min/60 min/d) and telmisartan (5 mg/kg/d) would have a positive effect on bone parameters.
Methods: Three-month-old male SHRs were divided into three groups: sedentary (S), telmisartan (T), and exercise (E). At the end of an 8-week protocol, femur and lumbar vertebrae were analyzed by dual-energy X-ray absorptiometry (DXA) for bone mineral density and by the three-point bending test for biomechanical properties. Blood pressure in all groups was measured by a tail-cuff manometer.
Results: Telmisartan and treadmill running reduced blood pressure when compared to the sedentary group; however, telmisartan did not improve bone characteristics. Instead, it reduced BMD of femur total and lumbar vertebrae and worsened bone biomechanic properties. Treadmill running maintained bone characteristics and hence was effective in maintaining bone health.
Conclusion: Results showed that telmisartan negatively affected bones suggesting that caution should be taken in possible therapeutic applications for protecting bone health in hypertensive conditions. More studies are necessary to clarify the mechanisms through which telmisartan favors bone loss in this model.

Introduction

Cardiovascular diseases (CVDs) and osteoporosis are considered major public health problems that compromise quality of life and longevity because of their association with high morbidity and mortality [1]. Approximately one-third of deaths in the Western world can be a direct consequence of CVDs [2]. Men and women with osteoporotic fractures have a higher risk of mortality when compared to those without them [3]. Hypertensive individuals show changes in calcium metabolism, such as increased urinary excretion of calcium, secondary increase in parathyroid gland activity, and a tendency to low levels of serum ionized calcium. These alterations can cause higher bone reabsorption, lower bone mineral density, and increase risk of fractures [4]. Hence, there is a possible association between the degeneration of the cardiovascular system and osteoporosis (or decreased bone mineral density [BMD]) in hypertensive individuals [5].

Studies have shown that antihypertensive drugs such as thiazides [6], beta-blockers, and angiotensin-converting enzyme inhibitors [7] reduce the risk for osteoporotic fractures. These data suggests that the renin–angiotensin system may be involved in bone metabolism [8]. In animal models of bone loss induced by rosiglitazone, bone loss was prevented by an increase in the bone/tissue volume ratio, number of trabeculae, and the trabecular thickness when ovariectomized spontaneously hypertensive rats were administrated rosiglitazone along with telmisartan, an angiotensin receptor blocker (ARB) [9]. This observation indicates

* Corresponding author.
E-mail address: mbirocale@hotmail.com (A.M. Birocale).

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that the renin–angiotensin system is, in fact, somehow involved in bone metabolism.

Physical exercise (running, jumping, vibration, swimming, weight lifting) can be an effective tool in the nonpharmaceutical management of CVD(s) and osteoporosis because it can reduce blood pressure and increase bone mass, preventing age-related bone loss [10] with lower economic costs and side effects compared to pharmaceutical methods [11]. Review of epidemiological evidence on the preventive and therapeutic benefits of exercise and on the main biological mediators involved demonstrates that exercise can be considered a "polypill" to improve the population's quality of life [12]. The exact mechanism by which physical exercise prevents osteoporosis is not yet completely understood [13], but the data suggest that exercise can have a positive effect on the skeleton, and the mechanic overload generated by the physical activity was cited as being the main means for preserving and improving bone mass and strength [14].

Although the risk of BMD reduction and osteoporosis in hypertensive male rats has been documented [15–17], there are no studies that compare the pharmacological treatment with ARB (telmisartan) of osteoporosis related to hypertension with a nonpharmacological approach (physical exercise in treadmill) in spontaneously hypertensive rats (SHRs). Therefore, we hypothesize that physical exercise on treadmill increases BMD, reduces blood pressure (BP), and prevents bone loss in male SHRs, just as telmisartan does. This study was planned to evaluate changes in BMD and biomechanical bone properties in male SHRs treated with telmisartan or submitted to treadmill physical activity for an 8-week period.

Materials and methods

Adult male SHRs (3-month-olds) were placed in a controlled environment with 22 °C temperature, 55% humidity, and 12-h light/dark cycles. They were given water and a standard rat diet (Purina Labina, SP-Brazil) at will (ad libitum). All procedures were performed according to biomedical research guidelines for the ethical care and use of animals in scientific research and experimental protocol was approved by the UFES Ethical Committee for animal use. Animals were randomly assigned to three experimental groups: sedentary (S), treated with telmisartan (T), and exercised (S). The S and T groups were administered daily oral gavage for 8 weeks with vehicle (0.5% carboxymethylcellulose sodium; CMC-Na) and a telmisartan dose dissolved in vehicle (5 mg/kg/day), respectively.

Animals from the exercise groups were acclimatized on an treadmill (EP131, Insight, Sao Paulo, Brazil) by running in a progressive exercise intensity (15–18 m/min speed at 0% incline for 15–60 min/day) for 1 week and then were regularly trained at a 18 m/min speed for 60 min, 5 days per week for 8 weeks.

All animals were weighed weekly in order to measure body mass on a digital scale to adjust the drug dose and vehicle and biometric analysis, and systolic blood pressure (SBP) in conscious rats was measured using a tail-cuff manometer manufactured by IITC Life Science, Inc. (Woodland Hills, CA, USA).

After the experimental protocol, the animals were euthanized by ketamine 90 mg/kg, xylazine 10 mg/kg and left femur and tibia and the fifth lumbar vertebrae were removed using surgical tools, cleared of soft tissues, immersed in saline solution, and frozen at −80 °C for analyses.

Bone densitometry (dual energy X-ray absorptiometry [DXA]) was used to evaluate the BMD and the bone mineral content (BMC) of the fifth lumbar vertebrae (L5) and the total femur (total length, including diaphysis and epiphysis). The device Discovery-A SN: 80999 Hologic (Bedford, MA, USA) in high-resolution mode was used, with software to small animal, provided by the same company.

The length of the left femur and tibia was measured using a caliper ruler (0.05 mm of precision), and the femoral shaft's widest (in a laterolateral position) and narrowest (anteroposterior position) diameters were measured.

After the biometric analysis, bones were placed in a desiccator for 24 h in order to remove the air from the bone pores and then biomechanical analysis was initiated. The biomechanical properties were measured using the three-point bending test with a universal testing machine (Instron, model 4444, Canton, MA, USA), with a load cell with a 100 kpf capacity. The bone extremities were placed over two rolls with a 3-mm diameter and at a 21.70-mm distance and load was applied in each bone's central region until the bone fractured in accordingly with Trebackz and Zduneck [18]. A similar procedure was adopted to determine the biomechanical parameters of the fifth lumbar vertebrae by vertebral compression test using the same universal testing machine. As result of the force applied to the bone, the Instron software (series IX) generated a load deformation graphic. The biomechanical properties obtained in this graphic were: maximum force (N), rupture force (N), and stiffness (N/mm). After the bones were dehydrated in an oven at 100 °C for 24 h. At this point, the dry mass (mdri) was obtained. In order to obtain the ash mass, the bones were placed in a muffle furnace at 800 °C for 24 h. All the measures were obtained using an electro-digital scale (Chyo, model JEX 200, Instruchem, Inc., Manila, Philipinnes). The bone volume (BV) was obtained following the Archimedes Principle and then the bone density (BD) and the mineral density (MD) were obtained indirectly using the formulas.

Statistical evaluation

The data are expressed as the mean ± SEM and the statistical analysis was performed using GraphPad Prism 6. Data were compared using one-way ANOVA followed by the *post hoc* Tukey's test intragroups to analyze treatment effects. Data were considered statistically significant if $p < 0.05$.

Results

SBP mean values were reduced in treated animals and it can be observed that either treatment—use of telmisartan (T) and/or the exercise (E)—reduced SBP when compared to the sedentary group (S) (T: 134.2 ± 3.1 mm Hg or E: 167.8 ± 7.8 mm Hg *vs.* S: 243.5 ± 6.6 mm Hg with $p < 0.001$ to both comparisons). T group had a greater decrease in SBP than the E group (T: 134.2 ± 3.1 mm Hg *vs.* E: 167.8 ± 7.8 mm Hg; $p < 0.01$).

Baseline physiological parameters in animals and the effects of telmisartan and treadmill running are demonstrated in Table 1. Treatments did not cause differences in body weight at the end of treatment period. Telmisartan treatment reduced the heart weight in the T group when compared to the S and E animals. Sedentary and exercised animals groups did not show a difference between them in relation to heart weight. The use of termisartan reduced

Table 1
Effect of treatment with telmisartan and exercise training on body weight (BW), heart weight, tibial length, and heart weight/length tibial ratio. Animals groups: S (sedentary), T (telmisartan), E (exercise).

Variable	S	T	E
Initial body weight, g	242 ± 1.06	231 ± 7.23	243 ± 8.02
Final body weight, g	303 ± 3.92	279 ± 11.16	306 ± 4.98
Heart weight, g	1.3131 ± 0.028	0.8949 ± 0.058[a,b]	1.4189 ± 0.034
Tibia, cm	3.91 ± 0.006	3.89 ± 0.028	3.93 ± 0.015
Heart/tíbia g/cm	0.3366 ± 0.007	0.2301 ± 0.015 [a,b]	0.3638 ± 0.008

Values are mean ± SEM ($n = 7$): [a] $p < 0.001$ *vs.* S group; [b] $p < 0.001$ *vs.* E group.