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Effects of swimming associated with risedronate in osteopenic bones: An experimental study with ovariectomized rats

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ABSTRACT

Physical activity and risedronate sodium have effects on metabolic bone diseases, maintaining the integrity of bone tissue. Our objective was to evaluate the effects of swimming associated with risedronate as a prophylactic means in osteopenic bone of ovariectomized rats. A total of 24 animals of the Wistar strain were used and separated into four groups containing six animals: Ovariectomy (OVX), ovariectomy and swimming (OVXS), ovariectomy and risedronate (OVXM), ovariectomy, risedronate and swimming (OVXMS). The effectiveness of the treatments were evaluated using the tibia by means of biomechanical, radiographic, histomorphometric analyzes. Statistical analysis was performed by the non-parametric Kruskal–Wallis test ($p < 0.05$). The OVXM and OVXMS groups showed higher values compared to OVX in maximum strength and rigidity. Microscopic analysis showed increased trabecular bone in the OVXM group in relation to the others, and in the OVXMS compared to OVXS. Proximal densitometry in the OVXM and OVXMS groups showed higher values than the OVX and OVXS groups. There were no significant differences in overall densitometry. In conclusion, when comparing the prophylactic means, risedronate was able to preserve bone mass significantly, unlike exercise where an improvement of bone tissue was observed, although not significant, and when swimming and risedronate are combined the result was even better.

1. Introduction

Osteopenia is characterized as a pre-osteoporosis condition and presents low bone mass and deterioration of bone microarchitecture, which predisposes to fragile bones and increases the risk of pathological fractures (Nordin, 1983). Great importance is given to disease affecting approximately one third of the female population after menopause (Gaumet-Meunier et al., 2000).

There is great debate in the literature as to which would be the ideal treatment of osteopenic conditions, and there are no well-defined protocols on the association of drugs with physical exercise. Physical activity is essential for the maintenance of bone tissue, presenting great relevance for metabolic bone diseases, such as osteoporosis, because the period of hormonal suppression is characterized by rapid mass loss of this tissue (Brockie, 2006). Various types of exercise can contribute to the improvement. Treadmill exercise increases bone mass and prevents tissue loss as a result of resorption (Iwamoto et al., 2005), high-impact activities such as jumping are also beneficial, with increased bone and cortical area strength (Umemura et al., 2008). Swimming, as well as presenting a major aerobic component and reducing the risk of musculoskeletal injury (Matsudo and Matsudo, 1992) has demonstrated efficacy in preventing post-menopausal loss of bone mass (Melton et al., 2004).

Many studies emphasize the importance of pharmacological therapy in osteoporosis, among them we can highlight that risedronate is a pyridinyl bisphosphonate (Mortensen et al., 1998). The drug risedronate sodium has its effect on decreasing the activity of osteoclasts, increasing their susceptibility to apoptosis, corresponding with a potent inhibitor effect on bone resorption (Kanis et al., 2013).

With increasing life expectancy, diseases, such as osteoporosis are increasingly present, mainly affecting women by the decline in estrogen production and consequent decrease in bone mass. Prophylactic means, such as drug therapy and exercise have great

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evidence, but the medication associated with physical activity, such as swimming, is not fully elucidated, therefore experimental studies are needed to develop means of prevention and treatment. Therefore this study aimed to evaluate the effects of low-impact exercise (swimming) and risedronate sodium as a prophylactic means in osteopenic bones of rats subjected to ovariectomy, according to the Zarrow et al. (1964) technique, through microscopic, densitometric, and mechanical analysis.

2. Materials and methods

2.1. Experimental protocol

The whole experiment was performed following the Protocol for the Use of Animals in Experiments No. 081/2012. The research utilized 24 *Rattus norvegicus albinus* rats of the Wistar variety in the young adult phase, with a weight of between 180 and 200 g. The animals were divided into 4 groups, each containing 6 animals. The OVX group with animals subjected to ovariectomy, the OVXM group in which animals underwent ovariectomy and the risedronate drug, the OVXS group containing animals subjected to ovariectomy and swimming exercise, and the OVXMN group with animals subjected to ovariectomy, the risedronate drug and swimming exercise.

The surgical technique of ovariectomy followed the methodology of Zarrowet al. (1964), and was performed bilaterally in all animals in the experiment.

2.2. Physical training

The training was conducted in a glass tank with a length of 1 m and both a height and width of 60 cm. The water, with a temperature of $32 \pm 1\,^{\circ}C$, occupied a depth of 40 cm, leaving 20 cm to the top of the container to prevent the animals from escaping. After the activity the animals were dried with the aid of a fan.

The training protocol in water was performed with the OVXS and OVXMS groups after the ovariectomy technique and the duration evolved gradually. The first week of physical activity included adaptation to the work, with five sessions of 10, 20, 30, 40 and 50 min, being one session every day. After adaptation the training lasted 60 min per day, five times a week for 12 weeks. The training program in water was based on and modified the protocol used by Nascimento et al. (2008).

2.3. Drug administration

The drug was administered to the rats in the OVXM and OVXMN groups, 30 mg/kg/month, in drinking water (Piai et al., 2005), based on Sousa et al. (2007), which discusses the possibility that the administration time was insufficient to sensitize the bone tissue. With this, there was an increase in the time of administration in the present study; one dose per month for 3 months, the first being administered within 2 days after surgery and the last dose 2 days before euthanasia, with a total of four doses. The night before administration of risedronate the food was removed so that the rats received the drug while fasting. ActonelVR, the trade name of risedronate used in this study, was manufactured by Norwich Pharmaceuticals (Nor-wich, NY).

2.4. Analyses performed

Microscopic analysis was performed with the tibias of the right side. The bones were immersed in 10% formaldehyde solution for 24 h, decalcified, dehydrated and de-paraffinized in xylene, and finally embedded in paraffin. The paraffin specimens received serial sections of 5 μm thickness in the frontal plane with a microtome Leica RM 2165 (Germany). For each animal 40 histological sections were performed, corresponding to 240 slides per group.

The head of the tibia was used for quantitative histological analysis of trabecular bone for the assessment of bone microarchitecture, with 4 slides per animal and a total of 24 per group. The slides received Masson's Trichrome staining. The analysis was performed through Axionvision software (Zeiss, Germany) which captures and quantifies the area in pixel^2.

The densitometry exam was conducted in an apparatus for X-ray densitometry (Hologic, Discovery Wi®, USA) of the Laboratory of Endocrinology and Sciences of Image and Medical Physics, Hospital das Clinicas, Faculty of Medicine of Ribeirão Preto, with the bones (tibia) positioned inside the acrylic box, with the soft parts separated. The protocol used was specific for small animals, beginning with high resolution (Sub-region Hi-Resolution), the program recognized the contour of the bone for overall bone mineral density and also evaluated bone mineral density of the proximal area of the tibia (R1). The results were expressed in g/cm^2.

After densitometric analysis the same bones were used for the mechanical analysis in the universal testing machine (EMIC®-10000N), Laboratory of Bioengineering of FMRP—USP, according to protocols used previously (Abrahão et al., 2006; Simões et al., 2008).

The tests of the tibias were for three point flexion, with distance between supports of 20 mm and 15 mm, respectively. The load cell used was 500 N and a load application speed of 1 mm/min.

2.5. Statistical analysis

For analysis of results, samples were submitted to the normality test to check if the behavior of the data was parametric or not. As the number of animals in the experiment was less than 10, the non-parametric Kruskal–Wallis (non-parametric ANOVA) was used. Where there were significant differences the post hoc Dunn test was used to specify the differences. For all analyzes a significance level of 5% was adopted.

3. Results

The results showed significant differences ($p < 0.05$) relative to the maximum strength, rigidity, quantification of trabecular bone and in the proximal densitometry. However no differences were observed in overall densitometry.

3.1. Mechanical analysis

In relation to maximum strength the OVXM group showed superior results than OVX ($p = 0.000$) and OVXS ($p = 0.006$). The result of the OVXMS group was also higher than the OVX ($p = 0.000$) and OVXS groups ($p = 0.000$). The OVXM group showed superior results than OVXMS but was not significant ($p > 0.05$). The OVXS group showed no significant values ($p > 0.05$), but expressed higher values compared to the OVX group (Fig. 1).

In the rigidity analysis the OVX group showed inferior results compared to the OVXM ($p = 0.005$) and OVXMS ($p = 0.006$) groups. The OVXM group presented higher values than OVXMS, although not significant ($p > 0.05$). The OVXS group expressed larger values than OVX and smaller values than OVXM and OVXMS however not significant ($p > 0.05$) (Fig. 2).

3.2. Histologic analysis

The OVXM group expressed qualitatively and quantitatively positive results related to trabecular bone when compared to the other groups ($p < 0.05$). The OVXMS group had a significantly higher result than OVXS ($p = 0.043$). The OVX group showed inferior to OVXS although not significantly expressed ($p > 0.05$) (Figs. 3 and 4).