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Osteoporosis Affects Functional Activity and Gene Expression of Osteoblastic Cells Derived from Rat Alveolar Bone

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Recent studies suggest that osteoporosis, in addition to the damage caused in long bones, may cause deterioration in the jaws, especially in alveolar bone sites, with effects in the progress of periodontal disease as well as in bone healing. The aim of this study was to evaluate the effect of osteoporosis in the metabolism of rat alveolar bone osteoblasts. There were used 10 female rats divided in two experimental groups (Sham and OVX), which were ovariectomized and after 8 weeks euthanized to collect mandibular bone samples in order to isolate osteoblastic cells. The cells were cultured in 24-well plates to perform the in vitro experiments. After 7, 10 and 14 days, there were evaluated cell proliferation by MTT assay, *in situ* detection of alkaline phosphatase (ALP) as well as mineralized nodules and expression of genes associated to bone remodeling. Results showed that at 7, 10 and 14 days cell proliferation was lower for OVX group. In situ detection of ALP was higher at 7 days and lower at 10 and 14 days in OVX group. At 17 and 21 days, OVX group had a significative decrease of mineralization nodules. There was a downregulation in the expression of *Alp*, *Bglap* and *Runx2* genes and an upregulation of *Opg* in OVX group, whereas *Opn* and *Rankl* modulation was similar between the evaluated groups. Our results suggest that osteoporosis has a deleterious effect on alveolar bone cells from ovariectomized rats, which might affect the treatment of diseases associated to the jaw bones.

Key Words: Osteoporosis, osteoblasts, alveolar bone.

Introduction

Osteoporosis is a systemic skeletal disease characterized by an imbalance between osteoclast bone resorption and bone formation by osteoblasts, with a consequent decrease in bone mass and an alteration of its microarchitecture, which leads to increased fragility and risk of fracture (1). There is a high vulnerability of elderly women to osteoporosis due to menopause transition, when there is a reduction of estrogen hormone, resulting in an accelerated bone resorption that exceeds its formation (2). This disease is considered a world public health problem with an increasing prevalence (3), since it is estimated that 1 in 5 men and 1 in 3 women will experience bone fractures within their lifetime (4). Excessive reactive oxygen species and inflammatory responses which occur in osteoporosis have been shown to stimulate osteoclast differentiation as well as induce osteoblast apoptosis and suppress its proliferation and differentiation (5).

Recent studies suggest that osteoporosis may cause deterioration in mandible bones besides long bones (6-8). Estrogen deficiency may promote bone mineral density (BMD) reduction and contribute to the imbalance of RANK-RANKL-OPG system, which also affects alveolar bone, increasing serum inflammatory mediators (IL-1, IL-6, and TNF), leading to bone loss (1,3) and affecting other processes such as alveolar healing after tooth extraction (9).

Although the relationship between osteoporosis and long bones is well established in the literature, its effects on mandible bone osteoblasts are not well known. Thus, the aim of this study was to evaluate the effect of osteoporosis on alveolar bone osteoblasts from ovariectomized rats, evaluating cell viability, detection and quantification of alkaline phosphatase and mineralized nodules, as well as the expression of genes associated with osteogenesis. The hypothesis is that osteoporosis condition might influence osteoblast functional activity, which could lead to alterations in mandible bone alveolar matrix formation and in its metabolism.

Material and Methods

The experimental protocol was approved by the Ethics Committee for Animal Experimentation, University of São Paulo (approval number No. 2016.1.594.58.7). Ten Wistar female rats weighting approximately 300 g were submitted to bilateral ovariectomy to induce osteoporosis (n=5) or sham operated to serve as control (n=5). Sample size calculus was a priori performed considering α=0.05, power of 95% for *Rankl* gene expression data for PTFE membrane (1.002±0.0713) and P(VDF-TrFE)BT membrane (0.796±0.0197) from Scalize et al. (10) investigation. The minimal sample size obtained was 6, being 3 for each group. In the present study, we selected n=5 for each group to compensate eventual losses. Sample size

was calculated with software G* Power 3.0.10 (Franz Faul, Kiel University, Kiel, Germany). The animals were selected from the Central Vivarium of the University and were kept in a polyethylene box. The temperature was maintained between 23 and 24 °C with a 12-h cycle of light per day. Throughout the experiment, the animals received a selected solid diet and water ad libitum.

Ovariectomy and Sham Surgery

The rats were weighted and anesthetized by an intramuscular injection of xylazine (10 mg/kg) and ketamine (75 mg/kg) (Agibrands do Brasil LTDA, Campinas, SP, Brazil). After trichotomy and antisepsis, the ovaries were excised. Suture was performed with silk thread 4.0 (Ethicon, Johnson & Johnson, São José dos Campos, SP, Brazil). Each animal received an intramuscular injection of 0.1mL/100g body weight of veterinary pentabiotic (Pentabiotic Veterinário Pequeno Porte-Fort Dodge®, Campinas, SP, Brazil) followed by 0.2 mL/100 g of injectable Banamine® (Injetável Pet-Schering-Plough, Cotia, SP, Brazil). The success of ovariectomy was evaluated by the atrophy of uterine horns. Euthanasia was performed after 8 weeks and mandibles were collected to isolate osteoblastic cells as described below.

Cell Culture

The hemimandibles were ground and the alveolar bone fragments were transferred to a sterile centrifuge tube for digestion with collagenase type I (Gibco) at the concentration of 1mg/mL and placed in a water bath at 37 °C under constant stirring. After thirty minutes of digestion, the supernatant was extracted and transferred to another centrifuge tube containing an equal amount of culture medium. This collagenase digestion procedure was performed six times. Supernatants from the first two digests were discarded and the last four were centrifuged at 2000 rpm for 5 min. Cells and fragments were suspended and placed in culture bottles with α-MEM (Gibco), supplemented with 10% fetal bovine serum (Sigma-Aldrich, ST Louis, MO, USA), 10^{-7}M dexamethasone (Sigma), 2 mL of gentamicin and 0.3µg/mL fungizone (Gibco). After reaching confluence, cells were enzymatically released from the culture flasks using 1 mM EDTA (Gibco) and 0.25% trypsin (Gibco). Cells were centrifuged at 2000 rpm for 5 min and homogenized in supplemented medium for osteogenic differentiation (MTS) containing α-MEM (Gibco), 10% fetal bovine serum (Sigma), 10^{-7}M dexamethasone (Sigma) 5 g/mL ascorbic acid (Sigma), 7 mM β-glycerophosphate (Sigma), 0.3 g/mL fungizone (Gibco) and 50 g/mL of gentamicin (Gibco). The first passage was seeded in 24-well plates at a cell density of $2x10^4$ cells per well. Throughout the culture period, the cells were maintained in a humid environment with 5% CO_2 and 95% atmospheric air, and the culture medium was changed every 3 days.

Cell Proliferation

Cell proliferation was assessed by MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) at 7, 10, and 14 days. To this end, cells were incubated with 10% MTT (5 mg/mL) in culture medium at 37 °C for 4 h. The medium was then aspirated from the well, and 1mL of isopropanol (0.04N HCl in isopropanol) was added to each well. The plates were placed on a shaker for 5 min and 200 µL of this solution was transferred to a 96-well plate. The optical density was read at 570 nm (µQuant, BioTek Instruments, Winooski, VT, USA).

In Situ Detection of Alkaline Phosphatase

Analysis of in situ detection of alkaline phosphatase was performed after 7, 10 and 14 days. Cells were washed twice with PBS at 37 °C. There were dissolved 320 mg of Trizma® Pre-set crystals (Sigma-Aldrich) in 20 mL of deionized water. From this solution, 18 mL were added to 2 mL of dimethylformamide (Merck) with 8 mg Naphthol (Sigma-Aldrich). The next step was adding 7 mg of Fast Red reagent (Sigma-Aldrich) to this final work solution. Finally, cells were incubated in 1mL of this solution in a humidified atmosphere at 37 °C with 5% CO_2 for 30 min and then removed from the wells for subsequent qualitative and quantitative analysis.

Mineralized Matrix Formation

Mineralized matrix formation was detected after 17 and 21 days by means of Alizarin Red S (Sigma-Aldrich) staining for areas rich in calcium. Attached cells were fixed in 10% formalin at 4 °C, for 2 h. After fixation, specimens were dehydrated through a graded series of alcohol, which was followed by staining with 2% Alizarin Red S, pH 4.2, for 10 min. Calcium content was evaluated with a colorimetric method formerly described (11).

Quantitative Gene Expression (Real Time PCR)

To evaluate the quantitative expression of runt-related transcription factor 2 (*Runx2*), alkaline phosphatase (*Alp*), osteocalcin (*Bglap*), osteoprotegerin (*Opg*), receptor activator of nuclear factor kappa-β ligand (*Rankl*) and osteopontin (*Opn*) genes, after 10 days of culture the medium was removed from the wells to add Trizol LS (Life Technologies, Grand Island, NY, USA) at room temperature under agitation. The samples were transferred for eppendorf vials and stored in freezer -20 °C for 48 h. After this period, it was performed RNA extraction utilizing SV Total RNA Isolation System kit (Promega, Madison, WI, USA), following manufacturer's instructions. Following, total RNA was quantified from 1 µL sample inNanoVue

equipment (GE Healthcare Life Sciences, Piscataway, NJ, USA) in different wave lenght (260, 280, 230 e 320 nm) and the integrity of each sample was evaluated utilizing a chip able to verify RNA samples in the concentration of 25 to 500 ng/μL, with Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). There were considered viable samples those with RIN higher than 8. After RNA integrity confirmation, cDNA was created from 1 μg de total RNA in Mastercycle Gradient (Eppendorf, Hamburg, Germany) by means of reverse transcriptase enzyme, using High-capacity cDNA Reverse Transcription kit (Applied Biosystems, Fort City, CA, USA), following manufacturer's instructions. Table 1 lists the oligonucleotide primers used in qRT-PCRs primers for mRNAs (Taqman probes, Applied Biosystems) for target genes utilizing StepOne Plus (Life Technologies). To each reaction was added 5μL of TaqMan Universal PCR Master Mix-No AmpErase UNG (2X), 0.5 μL of TaqMan probes for genes of interest (20X TaqMan Gene Expression Assay Mix) and 11.25 μg of cDNA. The reactions were performed with 2 min at 50 °C, 10 min at 95 °C, forty cycles of 15 s at 95 °C and 1 min at 60 °C. Results were analyzed with Ct value (cycle threshold - or threshold cycle) and all samples were subjected to reactions for the mRNA detection of Gapdh (Glyceraldehyde-3-Phosphate Dehydrogenase) constitutive gene expression, which was used to normalize the expression levels of the target gene samples. Normalization and relative quantification of gene expression were performed by 2-ΔΔCT methods.

Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed by means of analysis of variance (ANOVA) or non-parametric Kruskal-Wallis, with the level of significance set at 5% ($p \leq 0.05$) using the software GraphPad Prism 5.0e. (GraphPad Software, Inc., La Jolla, CA, USA)

Table 1. TaqMan probe for the genes of interest

Gene symbol	Gene name	Sequence
Alp	Alkaline phosphatase	Rn01516028_m1
Rankl	Receptor activator of nuclear factor kappa-β ligand	Rn00589389_m1
Bglap	Bone gamma-carboxyglutamate protein	Rn00566386_g1
Opn	Secreted Phosphoprotein 1 (osteopontin)	Rn01449972_m1
Runx2	Runt-related transcription factor 2	Rn01512298_m1
Opg	Osteoprotegerin	Rn00563499_m1
Gapdh	Glyceraldehyde 3-phosphate dehydrogenase	Rn99999916_s1

Results

Cell Proliferation

Cell proliferation was analyzed at 7, 10 and 14 days with a statistically significant decrease in all periods ($p=0.017$, $p=0.001$; $p<0.001$, respectively) in OVX group compared to sham group (Fig. 1).

In Situ Detection of Alkaline Phosphatase

Figure 2 shows that at 7 days a significant increase in alkaline phosphatase in situ detection was observed in qualitative and quantitative analysis ($p<0.001$) in OVX

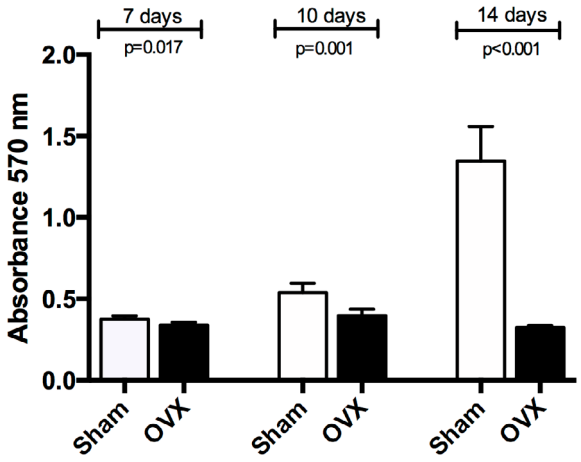

Figure 1. Viability of alveolar bone osteoblastic cells from sham and osteoporotic (OVX) groups in experimental periods of 7, 10 and 14 days. Statistical test for $p<0.05$.

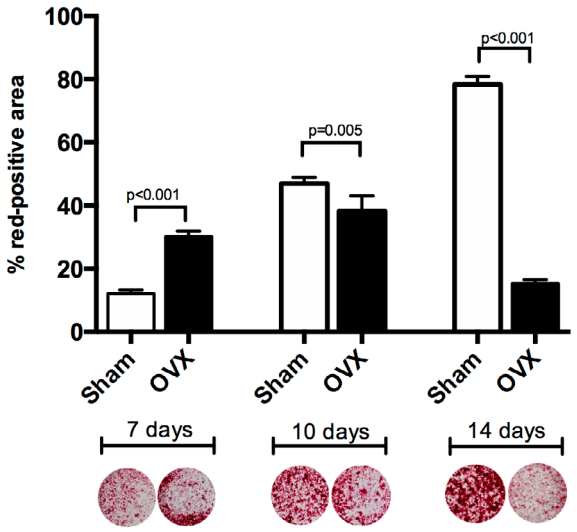

Figure 2. Qualitative and quantitative In situ detection of alkaline phosphatase in alveolar bone osteoblastic cells from sham and osteoporotic (OVX) groups, in the experimental periods 7, 10 and 14 days. Statistical test for $p<0.05$.

group when compared to the sham group. At 10 and 14 days, this activity decreased significantly (p=0.005 and p<0.001, respectively).

Mineralized Matrix Formation

Figure 3 shows that the evaluation of mineralized calcium nodules showed a significant decrease in the mineralization of cells from osteoporotic group compared to sham group at 17 and 21 days in both qualitative and quantitative analysis (p=0.007 and p=0.007, respectively).

Quantitative Gene Expression

Runx2, *Alp* and *Bglap* genes were downregulated in OVX group when compared to sham group (p=0.037, p=0.028 and p=0.028, respectively). The opposite was observed with *Opg* gene, which showed an upregulation in OVX group when compared to sham group (p=0.028). *Rankl* and *Opn* genes did not show statistically significant difference between the groups (p=0.828, p=0.342, respectively) (Fig. 4).

Discussion

In the present investigation there was observed that the model of osteoporosis utilized affected viability, mineralization and gene expression of mandible alveolar bone osteoblasts, evaluated by means of biochemical and molecular assays. We induced osteoporosis by ovary resection, which is a well-known and recognized method to use in animal models (12) and conducted the investigate of possible alterations at 8 weeks after ovariectomy, knowing that each part of the mandible as well as other body parts are expected to show completely different bone changes in response to osteoporosis (13).

The MTT assay showed a lower cell proliferation in OVX group in all experimental periods. These results differ from other investigations that used cells from different sites such as bone marrow, when osteoblastic cells showed higher or similar cell proliferation in OVX groups when compared to sham group (14). Nevertheless, Semeghini et al. (15) also showed lower cell proliferation of calvaria osteoblastic cells from female ovariectomized whereas bone marrow cells from sham and OVX groups had similar proliferation.

Cell differentiation can be evaluated by alkaline phosphatase, which is a bone marker protein that increases and stimulates mineralization (16). In the present investigation, its activity was initially higher for OVX group, which may be explained by a higher energy metabolism caused by a higher generation of ATP that occurs in the presence of osteoporosis (17), suggesting a compensatory function as an important method occurring in chronic diseases like osteoporosis. The same authors also observed a higher *Alp* activity in mandibular osteoblasts of OVX group. In later periods alkaline phosphatase levels were significative lower for OVX groups, suggesting that in later periods of an osteoporotic condition, *Alp* activity declines, facilitating osteoclast activation and generating the imbalance of bone formation and resorption (18-20).

The selected genes for rt-PCR analysis are involved in the modulation of bone remodeling mechanism (21). It was observed that *Alp*, *Bglap* and *Runx2* genes were downregulated in OVX group, corroborating the reduced extracellular matrix formation observed in the present study. In agreement to Cheng et al. (22), the expression of *Runx2* is critical for osteoblast differentiation skeletal morphogenesis and may modulate the expression of early and late essential bone biomarkers in a situation of osteoporosis through overexpression of miRNAs like MiRNA-365a-3. The present results suggest that downregulation of *Runx2* significantly downregulated the expression of *Alp* and *Bglap*, weakening the capability of mineralization.

We observed a significative higher expression of *Opg*, in contrast with *Rankl* and *Opn* genes that did not show statistically significant difference between the groups. Osteoprotegerin and *Rankl* are produced by osteoblast lineage cells and represent a key link between bone formation and resorption during bone remodeling. *Rankl* stimulates osteoclastic activity and differentiation and inhibits osteoclast apoptosis whereas osteoprotegerin inhibits osteoclast formation by binding to *Rankl* (23). The higher expression of *Opg* in Ovx groups have also

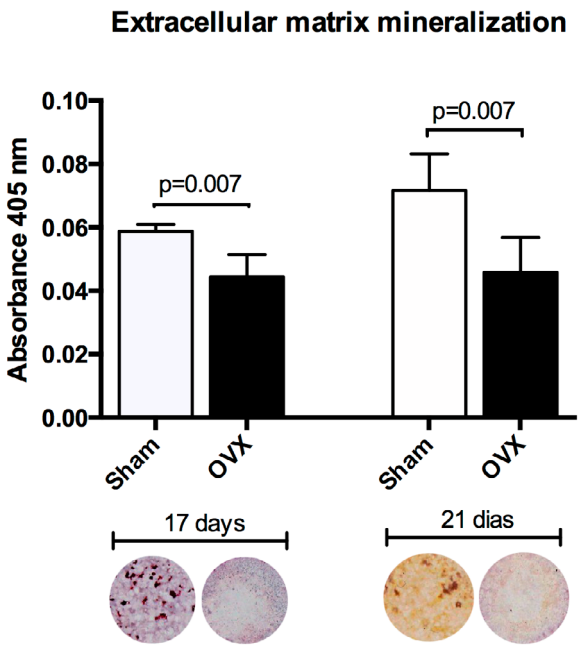

Figure 3. Qualitative and quantitative analysis of mineralization nodules of alveolar bone osteoblastic cells from sham and osteoporotic (OVX) groups after 17 and 21 days of cell culture. Statistical test for p<0.05.

been observed in a previous report (14) with the use bone marrow osteoblastic cell source. In agreement to Yu et al. (17), the removal of the ovaries reduces estrogen concentrations, leading to a need for initial enhancement of osteoblast function in order to compensate for the effects of the decrease in estrogen. Thus, we suggest that the modulation of *Opg* and *Rankl* might differ with other time periods or further development of osteoporosis. On the other hand, the lack of difference for *Opn* expression in the present investigation might be due to the fact that we performed the evaluation with 10 days of culture, possibly not demonstrating its effects in activities such as adhesion

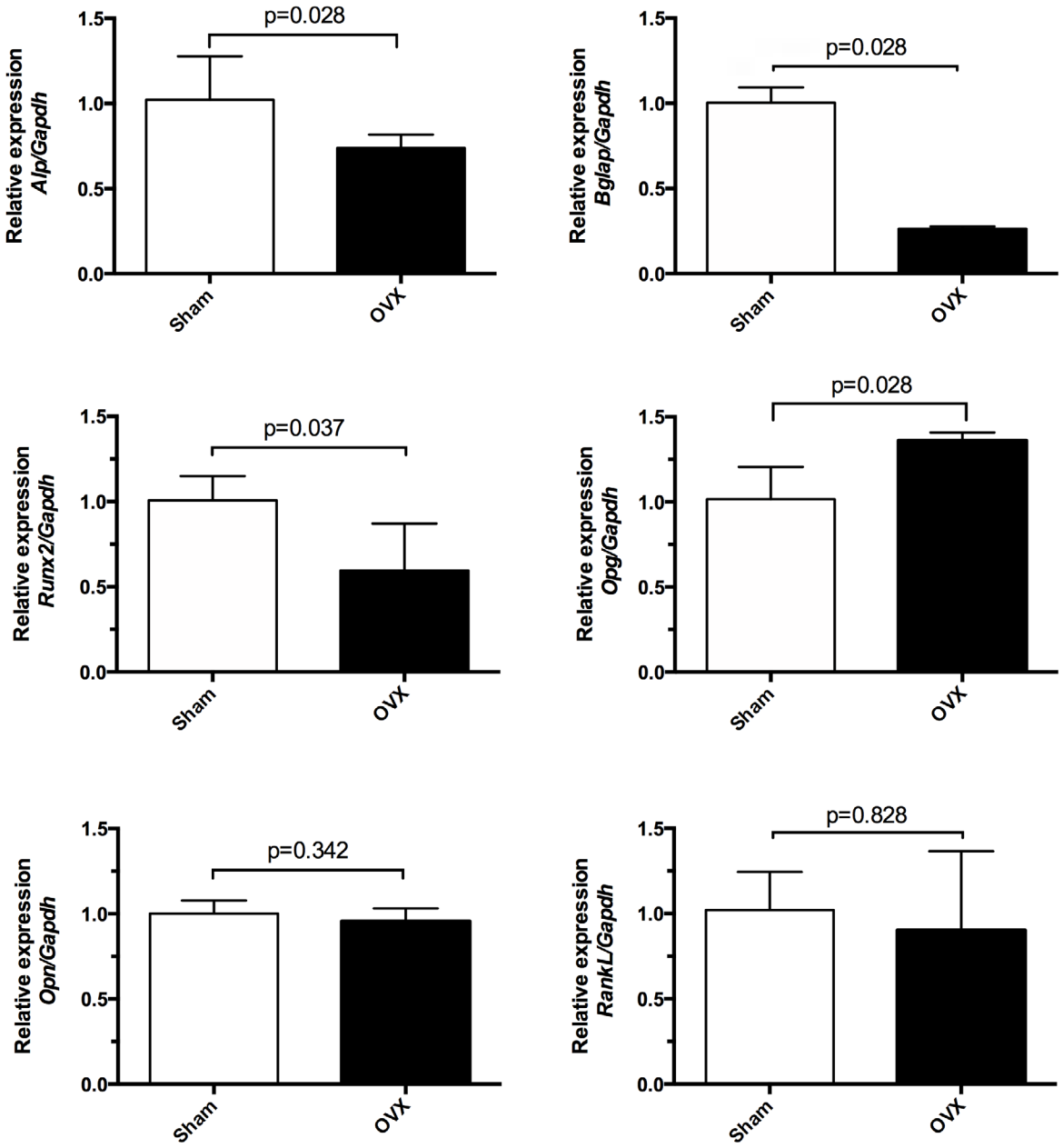

Figure 4. Analysis of gene expression of the transcription factor related to runt 2 gene (*Runx-2*), Alkaline Phosphatase (*Alp*), Osteocalcin (*Bglap*), Osteoprotegerin (*Opg*), Nuclear factor activator receptor ligand kappa B (*Rankl*) and Osteopontin (*Opn*) in alveolar bone osteoblastic cells of sham and ovariectomized (OVX) groups. Statistical test for $p<0.05$

and mineralization promoted by osteopontin.

The evaluation of gene expression in one time period could be faced as a limitation of the present study, as well as the possible variability among animals that might exist. A future perspective is to perform in vivo experiments including histological and microtomographic analysis to confirm the present data.

Our results suggest that osteoporosis has a deleterious effect on alveolar bone cells from ovariectomized rats and further in vivo and clinical investigations are thus warranted to confirm this association.

Resumo

Estudos recentes sugerem que a osteoporose, além dos danos provocados em ossos longos, pode causar deterioração dos ossos maxilares, especialmente na região do osso alveolar, com efeitos na progressão da doença periodontal assim como no reparo ósseo. O objetivo deste estudo foi avaliar o efeito da osteoporose no metabolismo de osteoblastos do osso alveolar mandibular de ratos. Foram utilizadas 10 ratas fêmeas divididas em dois grupos experimentais (Sham e OVX), que foram ovariectomizadas e após 8 semanas, eutanasiadas para coletar amostras do osso mandibular e isolar as células osteoblásticas. As células foram cultivadas em placas de cultura de 24 poços para serem realizados os experimentos in vitro. Após 7, 10 e 14 dias foram avaliados a proliferação celular pelo ensaio de MTT, detecção in situ de fosfatase alcalina (ALP) assim como de nódulos mineralizados e expressão quantitativa de genes associados à remodelação óssea. Os resultados mostraram que aos 7, 10 e 14 dias a proliferação celular foi menor para o grupo OVX. A detecção in situ de ALP foi maior aos 7 dias e menor aos 10 e 14 dias no grupo OVX. Aos 17 e 21 dias o grupo OVX apresentou uma diminuição dos nódulos mineralizados. Houve uma repressão na expressão dos genes *Alp*, *Bglap* e *Runx2* e uma indução do gene *Opg* no grupo OVX, enquanto que a modulação dos genes *Opn* e *Rankl* foi similar entre os grupos experimentais. Nossos resultados sugerem que a osteoporose tem um efeito deletério no metabolismo de células do osso alveolar em ratas ovariectomizadas, o que pode afetar o tratamento de doenças associadas aos ossos maxilares.

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References

1. Anbinder AL, Moraes RM, Lima GMG, Oliveira FE, Campos DRC, Rossoni RD, et al. Periodontal disease exacerbates systemic ovariectomy-induced bone loss in mice. Bone 2016;83:241-247.
2. Kolte RA, Kolte AP, Potey AM. Risk assessment of osteoporosis in pre- and postmen-opausal periodontal healthy and chronic periodontitis women with digital panoramic radiographs. J Indian Soc Perodontol 2017;2:461-465.
3. de Franca NA, Camargo MB, Lazaretti-Castro M, Peters BS, Martini LA. Dietary pat-terns and bone mineral density in Brazilian postmenopausal women with osteoporosis: a cross-sectional study. Eur J Clin Nutr 2016;70:85-90.
4. Akesson K, Marsh D, Mitchell PJ, McLellan AR, Stenmark J, Pierroz DD, et al. Capture the Fracture: a Best Practice Framework and global campaign to break the fragility frac-ture cycle. Osteoporos Int 2013;24:2135-2152.
5. Shen CL, Han J, Wang S, Chung E, Chyu MC, Cao JJ. Green tea supplementation bene-fits body composition and improves bone properties in obese female rats fed with high-fat diet and caloric restricted diet. Nutr Res 2015;35:1095-1105.
6. Mashalkar VN, Suragimath G, Zope SA, Varma SA. A cross-sectional study to assess and correlate osteoporosis and periodontitis among postmenopausal women: a dual energy x-ray absorptiometry study. J Midlife Health 2018;9:2-7.
7. Wang CJ, McCauley LK. Osteoporosis and periodontitis. Curr Osteoporos Rep 2016;14:284-291.
8. Ayed MS, Alsharif AF, Divakar DD, Jhugroo C, Alosaimi B, Mustafa M. Evaluating the possible association between sustemic osteoporosis and periodontal disease pro-gression in potmenopausal women. Dis Mon 2019;65:193-215.
9. Arioka M, Zhang X, Li Z, Tulu US, Liu Y, Wang L, et al. Osteoporotic Changes in the Periodontium Impair Alveolar Bone Healing. J Dent Res 2019; 98:450-458.
10. Scalize PH, Bombonato-Prado KF, de Sousa LG, Rosa AL, Beloti MM, Semprini M, et al. Poly(Vinylidene Fluoride-Trifluorethylene)/barium titanate membrane promotes de novo bone formation and may modulate gene expression in osteoporotic rat model. J Mater Sci Mater Med 2016;27:180.
11. Gregory CA, Gunn WG, Peister A, Prockop DJ. An Alizarin red-based assay of miner-alization by adherent cells in culture: comparison with cetylpyridinium chloride extrac-tion. Anal Biochem 2004;329:77-84.
12. Thorndike EA, Turner AS. In search of an animal model for postmenopausal diseases. Front Biosci 1998;3:c17-26.
13. Lee C, Lee JH, Han SS, Kim YH, Choi YJ, Jeon KJ, et al. Site-specific and time-course changes of postmenopausal osteoporosis in rat mandible: comparative study with fe-mur. Sci Rep 2019;9:14155.
14. Oliveira GR, Vargas-Sanchez PK, Fernandes RR, Ricoldi MST, Semeghini MS, Pitol DL, et al. Lycopene influences osteoblast functional activity and prevents femur bone loss in female rats submitted to an experimental model of osteoporosis. J Bone Miner Metab 2019;37:658-667.
15. Semeghini MS, de Azevedo FG, Fernandes RR, Assis AF, Dernowsek JA, Rosa AL, et al. Menopause transition promotes distinct modulation of mRNAs and miRNAs ex-pression in calvaria and bone marrow osteoblastic cells. Cell Biol Int 2018;42:12-24.
16. Aubin JE. Advances in the osteoblast lineage. Biochem Cell Biol 1998;76:899-910.
17. Yu SJ, Liu HC, Ling-Ling E, Wang DS, Zhu GX. Proliferation and differentiation of os-teoblasts from the mandible of osteoporotic rats. Exp Biol Med 2012;237:395-406.
18. He YX, Zhang G, Pan XH, Liu Z, Zheng LZ, Chan CW, et al. Impaired bone healing pattern in mice with ovariectomy- induced osteoporosis: a drill-hole defect model. Bone 2011;48:1388-1400.
19. Chen CH, Wang L, Serdar Tulu U, Arioka M, Moghim MM, Salmon B, et al. An osteo-penic/osteoporotic phenotype delays alveolar bone repair. Bone. 2018;112:212–219.
20. Jin P, Li M, Xu G, Zhang K, Zheng LI, Zhao J. Role of (-)-epigallocatechin-3-gallate in the osteogenic differentiation of human bone marrow mesenchymal stem cells: An en-hancer or an inducer?. Exp Ther Med 2015;10:828-834.
21. Jiang LB, Tian L, Zhang CG. Bone marrow stem cells-derived exosomes extracted from osteoporosis patients inhibit osteogenesis via microRNA-21/SMAD7.Eur Rev Med Pharmacol Sci 2018;22:6221-6229.
22. Cheng F, Yang MM, Yang RH. MiRNA-365a-3p promotes the progression of osteopo-rosis by inhibiting osteogenic differentiation via targeting Runx2. Eur Rev Med Phar-macol Sci 2019;23:7766-7774.
23. Zheng ZG, Zhang X, Zhou YP, Lu C, Thu PM, Qian C, et al. Anhydroicaritin, a SREBPs inhibitor, inhibits Rankl-induced osteoclastic differentiation and improves di-abetic osteoporosis in STZ-induced mice. Eur J Pharmacol 2017;809:156-162.

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