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Oxytocin promotes bone formation during the alveolar healing process in old acyclic female rats

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ABSTRACT

Objective: OT was reported to be a direct regulator of bone mass in young rodents, and this anabolic effect on bone is a peripheral action of OT. The goal of this study was to investigate the peripheral action of oxytocin (OT) in the alveolar healing process in old female rats.

Materials and methods: Females Wistar rats (24-month-old) in permanent diestrus phase, received two ip (12 h apart) injections of saline (NaCl 0.15 M – control group) or OT (45 µg/rat – treated group). Seven days later, the right maxillary incisor was extracted and analyses were performed up to 28 days of the alveolar healing process (35 days after saline or OT administration).

Results: Calcium and phosphorus plasma concentrations did not differ between the groups. The plasma biochemical bone formations markers, alkaline phosphatase (ALP) and osteocalcin were significantly higher in the treated group. Histomorphometric analyses confirmed bone formation as the treated group presented the highest mean value of post-extraction bone formation. Tartrate-resistant acid phosphatase (TRAP) was significantly reduced in the treated group indicating an anti-resorptive effect of OT. Immunohistochemistry reactions performed in order to identify the presence of osteocalcin and TRAP in the bone cells of the dental socket confirmed these outcomes.

Conclusions: OT was found to promote bone formation and to inhibit bone resorption in old acyclic female rats during the alveolar healing process.

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1. Introduction

Postmenopausal osteoporosis is a systemic skeletal disease resulting in increased bone fragility and fracture risk subsequent to a decrease in bone mass and the degradation of bone microarchitecture.^{1,2} Despite the availability of several treatment options, annual hip fracture rates are predicted to

exceed six million by 2050 and this is a question of scientific, social and economic importance.^{1,3,4} Additionally, there are important age-related changes in the maxillofacial skeleton, particularly in association with tooth loss and periodontal disease. Osteoporosis and periodontal disease increase with age and postmenopausal women without hormone replacement therapy had the highest maxillary implant failure rate.⁵ Bone metabolism combines bone resorption (osteoclasts) and

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formation (osteoblasts) synchronously. With ageing in humans, this balance is impaired and is mainly related to the decrease in the secretion of ovarian steroids, due to significant modulation of the remodelling cycle exerted by oestrogen.^{6,7} Thus, the aim of treatments for osteoporosis is to increase the density, by acting on the decrease in bone resorption or increase in bone formation.^{4,8,9}

Studies have been developed to find new bone formation therapies and oxytocin (OT), a primitive neurohypophyseal hormone, has been reported to be an anabolic bone mass regulator.^{10–13} OT plasma levels are significantly lower in postmenopausal women who develop osteoporosis than in their healthy counterparts.¹² Ovariectomized rodents with changes in bone remodelling and consequent osteopenia, have significantly decreased OT levels when compared with sham-operated controls.¹³ In young rodents, it was verified that deletion of OT or OT receptors (Oxtr) causes reduced bone formation and suggested that OT is indispensable for basal skeletal homeostasis in both sexes.¹³ In adult male albino rats (150–200 g body weight) intramuscular OT injection was found to have growth promoting effects on bone.¹⁰ Subcutaneous OT injection reversed bone loss in eight-week-old OVX mice and reduced marrow adiposity.^{12,13} Transcriptomic analysis of the Oxtr pathway as a potential regulator of osteoblast/adipocyte balance of human multipotent adipose-derived stem cells (hMADS) was identified by Elabd et al.¹² They verified that OT and carbetocin (a stable OT analogue) negatively modulated adipogenesis while promoting osteogenesis in both hMADS cells and human bone marrow mesenchymal stromal cells. It was found that intracerebroventricular OT infusion did not affect serum markers of osteoblast (osteocalcin) and osteoclast (C-telopeptide) function, or influence *ex vivo* osteoblast or osteoclast formation. However, an important finding was that intraperitoneal injection of OT in mice increased bone mineral density as well as osteoblast formation, and this anabolic effect was attributed to a peripheral skeletal action of OT.¹³

The aim of this study was to analyze peripheral action of OT in the alveolar bone healing process of old acyclic female rats, 28 days after tooth extraction.

2. Materials and methods

2.1. Animals and treatment

Sixteen female Wistar rats (24-month-old) were housed in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) with a 12/12 h light/dark cycle. Animals were provided with standard rat chow and water *ad libitum*. The study protocol and all procedures involving animals were in compliance with the principles of laboratory animal care¹⁴ and national laws on animals, and the study itself was authorized by the Animal Research Ethics Committee of the São Paulo State University, Brazil (Protocol No. 2009-005746).

The rats underwent colpocytological examination to determine the cycle phase. A cotton swab impregnated with saline solution (0.15 M) was inserted in the vagina. A slide smear was carried out and immediately analyzed using a light microscope at 40 $\times$ magnification. The estrous cycle phases were determined according to cell quantification: epithelial,

cornified and leukocytes in the colpocytological examination. Animals in the diestrus cycle phase were divided into two groups (8 rats in each group) and were assigned to plastic cages (4 rats/cage). Two ip injections of saline (NaCl 0.15 M – control group) or OT (45 $\mu\text{g}/\text{rat}$ – treated group) were given with a 12 h interval between them.¹³

2.2. Tooth extraction

Seven days after receiving OT, the rats were anaesthetized (Xylazine – 10 mg/kg bw/ip – Dopaser Laboratories Calier S.A., Barcelona, Spain; and Ketamine – 80 mg/kg bw/ip – Fort Dodge Saúde Animal Ltda, Brazil) and after antiseptis (Polyvinylpyrrolidone iodide; Indústria Química e Farmacêutica Rioquímica Ltda, Brazil) the right maxillary incisor was luxated with the aid of a tapered instrument and extracted with a small forceps. The movement of extraction was smooth and followed the curvature of the rat incisor, so that root fracture would not occur. The atraumatic surgical technique was used, which allows tooth extraction without postoperative complications.¹⁵ The dental sockets were sutured with silk thread (Ethicon 4.0, Johnson and Johnson, São Paulo, SP, Brazil). The extractions were performed in such a way that at the end of the experimental period it was possible to obtain pieces with reference to a 28 day period of the alveolar healing process.

2.3. Collection of materials

At 35 days after application of OT/saline solution or 28 days post-extraction, the animals were anaesthetized (Xylazine + Ketamine, as previously described), blood was collected from the external jugular vein by cannula¹⁶ and put into a centrifuge tube. The plasma was separated by centrifugation (3000 rpm/10 min/ 1°C). A sample was acidified with acetic acid 20% in the proportion of 1/100 for Tartrate-resistant acid phosphatase (TRAP) determination. Calcium, phosphorus, total alkaline phosphatase (ALP) concentrations were determined in accordance with the manufacturer's instructions (Labtest kits, São Paulo, Brazil). Plasma osteocalcin and TRAP levels were determined with a quantitative sandwich type of enzyme linked immunoassay (ELISA) technique using rat kits purchased from Uscn Life Science Inc. (Wuhan, China).

After euthanasia with an overdose of anaesthetic, the right maxilla was removed. The pieces obtained were post fixed in 4% formaldehyde and demineralized in 10% EDTA (Merck, Darmstadt, Germany). Using a cryostat (Micron Zeiss, Berlin, Germany), the pieces were sliced longitudinal to the long axis of the dental socket to obtain 06 μm thick slices, which were mounted on previously gelatinized slides.

2.4. Histomorphometric analysis

The histomorphometric analysis of the bone mass and of the middle thirds of the rat alveolus^{15,17} was performed with two slices from each animal stained with haematoxylin and eosin (Haloquímica, São Paulo, SP, Brazil). The analyses were performed without the examiner knowing to which group the slices belonged. Stained sections were examined by light microscopy under 10 $\times$ objective lenses, and images were obtained with a digital camera (JVC TK-1270 Color Video

Camera) mounted on the microscope, and analyzed with Leica Qwin Color/RGB software.

2.5. Immunohistochemistry reaction

For the immunohistochemistry reactions, primary antibodies anti osteocalcin and anti TRAP (goat anti osteocalcin polyclonal and goat anti TRAP polyclonal – Santa Cruz Biotechnology, CA, USA) were used, and biotinylated rabbit anti-goat antibodies (AffiniPure rabbit anti goat IgG – Pierce Biotechnology, Rockford, IL, USA) were the secondary antibodies. The immunohistochemistry reaction signal was amplified with the Avidin–Biotin system (Kit ABC Vectastain Elite, ABC, Vector Laboratories, Burlingame, CA, USA) and the reaction was revealed using diaminobenzidine (Sigma, Saint Louis, MO, USA) as the chromogen. Haematoxylin was used for counterstaining. The analysis was performed in order to identify the labelling characteristics of each protein studied by this methodological approach.

2.6. Statistical methods

Differences between groups were tested using the Student's *t*-test as the criteria of normality. Equal variances between

groups were met with a significance level of 0.05 using GraphPad Prism 3.02 (GraphPad Software, Inc.; La Jolla, CA, USA). All data are reported as means with their standard errors of the mean (SEM).

3. Results

The plasma concentration of calcium (control = 10.70 ± 0.6580 ; treated = 10.41 ± 0.6663 mg/dL) (Fig. 1A) and phosphorus (control = 4.880 ± 0.3216 ; treated = 5.629 ± 0.3932 mg/dL) (Fig. 1B) was not significantly changed by treatment with oxytocin. However plasmatic concentrations of biochemical bone formation markers¹⁸ ALP (control = 73.20 ± 4.510 ; treated = 130.8 ± 11.37 U/L) (Fig. 1C) and osteocalcin (control = 0.7280 ± 0.02956 ; treated = 1.117 ± 0.04839 ng/mL) (Fig. 1D) were significantly increased after OT treatment. The plasmatic levels of bone resorption marker, TRAP,¹⁸ (control = 2.404 ± 0.3724 ; treated = 0.5867 ± 0.07200 U/L) were significantly decreased (Fig. 1E) in the treated group. At 28 days after tooth extraction, histomorphometric evaluation (Fig. 1F) showed a significant increase in trabecular bone volume of the middle third of the alveoli of treated animals ($44.36 \pm 2.697\%$) compared with those of

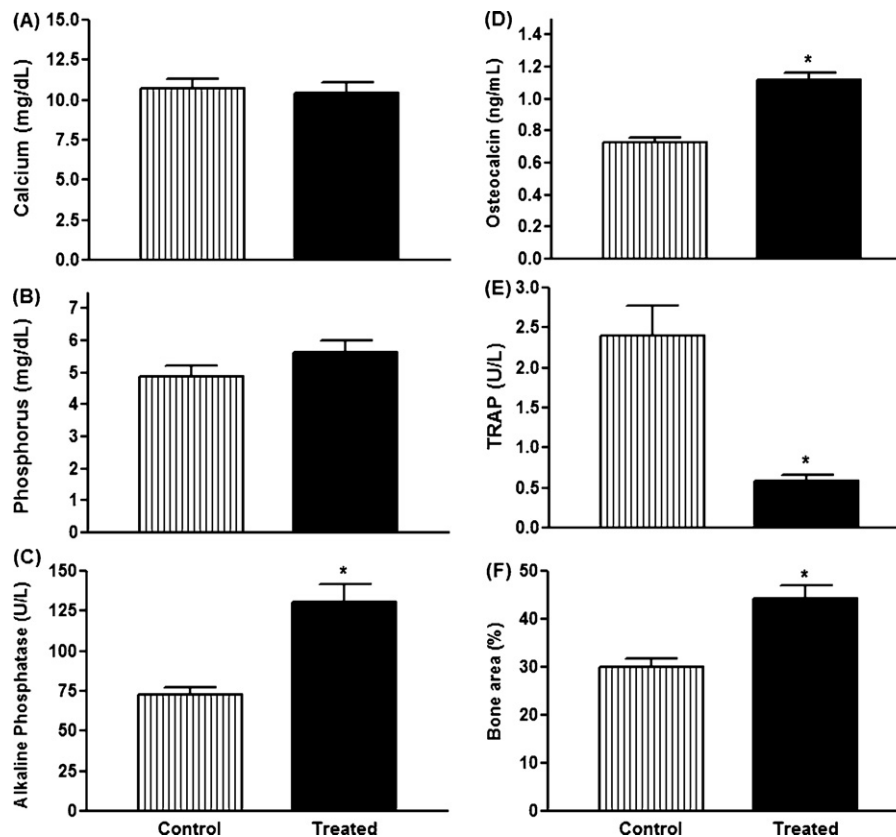


Fig. 1 – Plasma concentrations of calcium (A), phosphorous (B), ALP (C), osteocalcin (D), TRAP (E) and area of bone formation in the middle third of the alveoli at 28 days of healing process (F). Results were obtained 35 days after treatment (28 days post tooth extraction) with two ip injections of saline solution (0.15 M/control group) or OT (45 µg/rat/treated group) administered with an interval of 12 h between them. Calcium and phosphorous plasma levels did not differ between the groups. Biochemical bone formation markers (ALP and osteocalcin) increased, as the biochemical bone resorption marker (TRAP) decreased. Histomorphometric evaluation showed a significant increase in trabecular bone volume in the middle third of the alveolus. Values are means ± SEM, *n* = 8 rats per group. Statistics: Student's *t*-test; *P* < 0.05. *Different from control.

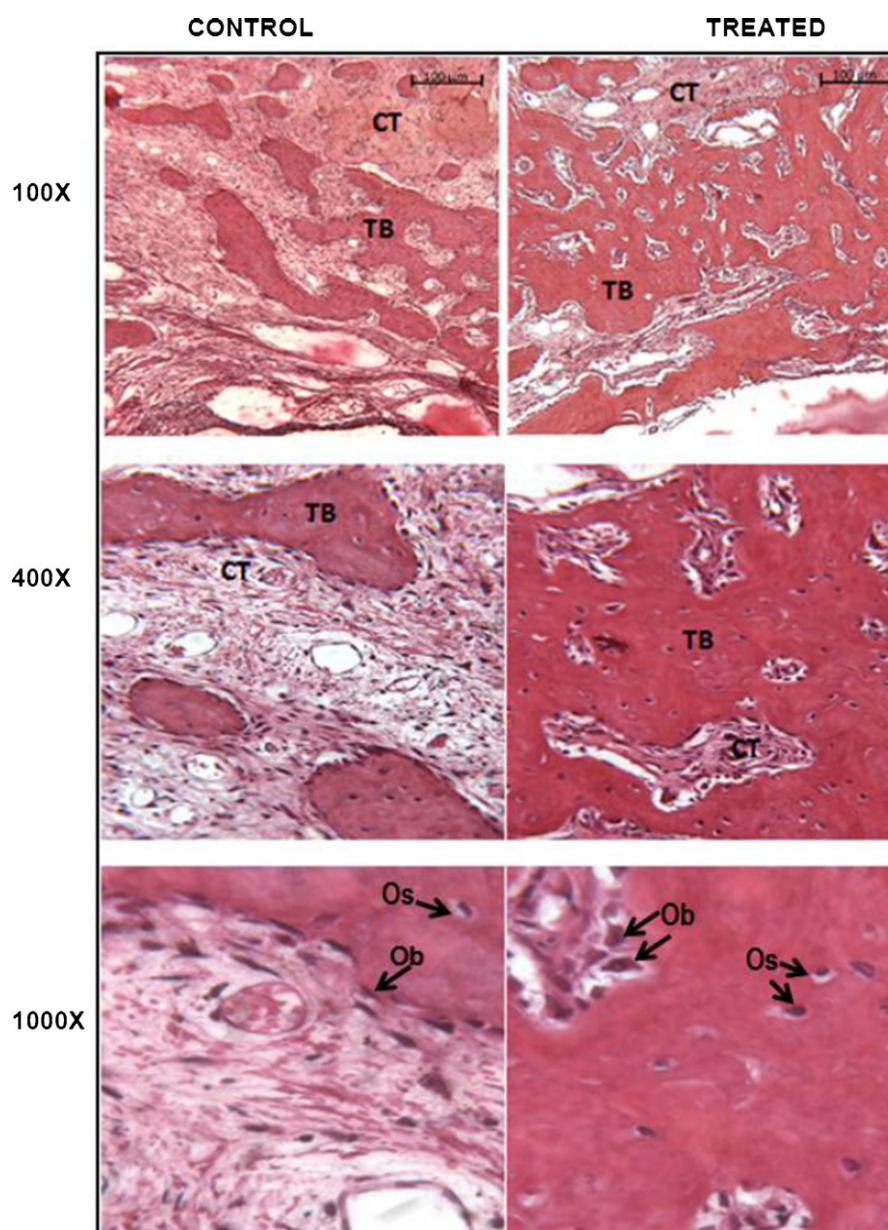


Fig. 2 – Histological sections (6 μ m thick) of the middle third of rat alveolus at 28 days of healing process. Results were obtained 35 days after treatment (28 days post tooth extraction) with two ip injections of saline solution (0.15 M/control group) or OT (45 μ g/rat/treated group) administered with an interval of 12 h between them. Control (A – 100 $\times$; C – 400 $\times$; E – 1000 $\times$) showed areas with thin bone trabeculae (BT) and connective tissue (CT); osteoblasts (Ob) predominated over the osteocytes (Os); treated (B – 100 $\times$; D – 200 $\times$; F – 1000 $\times$) showed larger amount of bone matrix with greater presence of osteoblasts and osteocytes. Histological sections stained with haematoxylin and eosin.

control group animals ($29.95 \pm 1.807\%$). Histological examination of the healing process in the alveolar middle third of the control group (Fig. 2A, C, and E) showed lower bone formation in comparison with the OT treated group (Fig. 2B, D, and F), with much larger areas of thin bone trabeculae and areas occupied by connective tissue. In these animals, the bone had formed osteoblasts predominantly over the osteocytes, and trabeculae were more isolated. In the OT treated group (Fig. 2B, D, and F) there was a larger area of bone formation occupying most of the interior of the alveoli. In this region, there was a large amount of bone matrix with greater presence of osteocytes. The formation

of islands of bone tissue was observed to a greater and more developed extent, with the presence of osteoblasts and osteocytes in greater quantity. There was presence of granulation tissue to a lesser extent (presence of tissue supplied with blood vessels, scattered collagen fibres, fibroblasts and amorphous ground substance). It was also found that the trabecular bone in this group was more compact, i.e., a greater fusion of the trabeculae when compared with the group that did not receive OT, in which the trabeculae were found to be more isolated.

Immunohistochemistry reactions against osteocalcin and TRAP were performed in the paraffin slices in order to identify

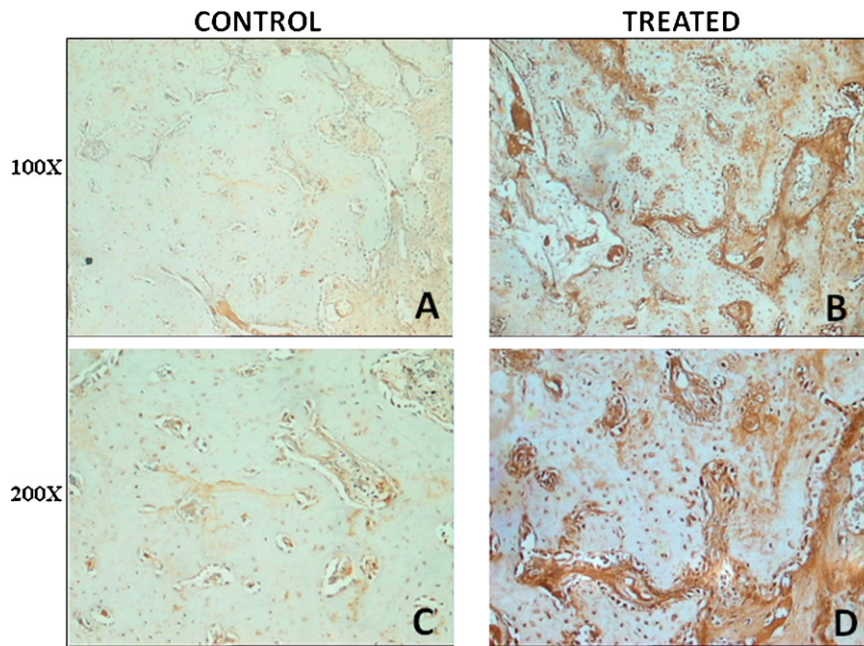


Fig. 3 – Histological sections (6 μm thick) of the middle third of rat alveolus at 28 days of healing process (35 days after OT treatment) showing the osteocalcin immunolabelling. Note that in A (100 $\times$) and C (200 $\times$), almost no positive labelling for osteocalcin either in mineralized bone matrix or in bone cells could be observed. Otherwise in B (100 $\times$) and D (200 $\times$) (treated groups), a greater labelling of this protein was observed, showing that in the treated groups there was an increase in mineralized bone matrix labelled with this protein, the main bone mineralization marker. Osteoblasts were also positive for this protein.

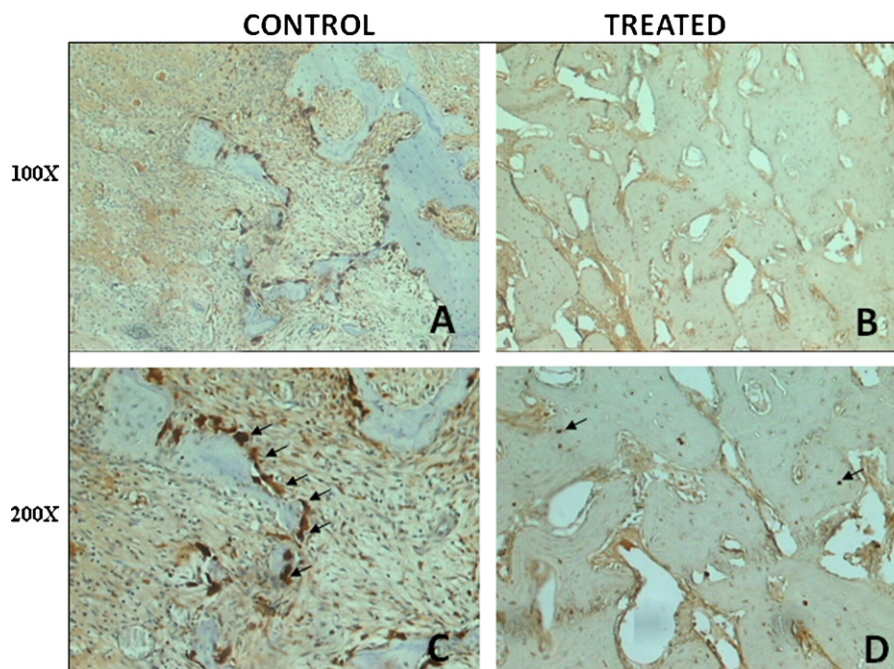


Fig. 4 – Histological sections (6 μm thick) of the middle third of rat alveolus at 28 days of healing process (35 days after OT treatment) showing TRAP immunolabelling. Note that in A (100 $\times$) and C (200 $\times$) (control groups), it was possible to observe a large number of osteoclasts with positive TRAP labelling, representing an intense bone resorption activity. In B (100 $\times$) and D (200 $\times$) (treated groups), there were no osteoclasts present close to the bone trabeculae.

the cell responses during alveolar bone healing. Osteocalcin labelling showed that there was a decrease in the presence of this protein in osteoblasts and in the mineralized matrix in the control group (Fig. 3A and C). Indeed, in the treated group (Fig. 3B and D), there was greater labelling of this protein showing that the bone presented a better condition of mineralization when compared with the control group. With regard to TRAP labelling, there was a large quantity of osteoclasts with positive label for this enzyme in the control group (Fig. 4A and C), showing intense bone resorption activity in these animals. Otherwise, in the treated groups, there was an important decrease in the expression of cells with positive TRAP labelling, showing that there was a less resorption activity in the treated animals (Fig. 4B and D).

4. Discussion

These results indicate that in old female rats (24-month-old), OT promoted increase in bone formation and decrease in bone resorption during the alveolar bone repair process. The mechanisms involved in this response are not fully understood, but this can occur due to changes in intracellular events essential for the development of osteoblasts and osteoclasts.

Old female rats have many interesting characteristics for analysis of bone loss after acyclicity of the estrous cycles.¹⁹ In this study they were subjected to tooth extraction for analysis of the alveolar bone regeneration process, as this model may be considered a good indicator of bone damage.^{17,20,21}

Although the major sites of OT gene expression occur in the central nervous system, it is also synthesized in peripheral tissues, e.g., the uterus, placenta, amnion, corpus luteum, testis and heart.²² Exogenous OT exerts this effect peripherally since the neuropeptide does not cross the blood–brain barrier.^{11,23} It was reported that intraperitoneal injection of OT negatively modulated adipogenesis while promoting osteogenesis in young rodents (3–6-month-old).^{11,12}

The peripheral action of OT in old rats analyzed in this study promoted significant increases in ALP and osteocalcin. These indicate new bone formation, since ALP and osteocalcin are excellent biochemical markers of bone formation activity.¹⁸ It has been observed that higher serum-osteocalcin levels are relatively well correlated with increases in bone density during treatment of osteoporosis with anabolic bone formation drugs.²⁴ Furthermore, the ALP produced in the bone formation phase is an excellent indicator of bone formation activity.²⁴ Thus, our results suggest that OT stimulates osteoblast activity and neobone formation in old rats (24-month-old).

Although analyses of the bone formation markers after OT treatment signalled changes in the alveolar bone regeneration process, histomorphometric and immunohistochemistry analysis, considered reliable markers, were also tested. The significant increase in alveolar bone formation and the greater labelling of osteocalcin observed in OT treated rats suggests that mineralization had taken place with utmost fidelity.

The immunohistochemical approach allows preservation of the tissue cytoarchitecture, which is an important aspect to consider when the alveolar healing process is evaluated.^{25,26} Osteocalcin, an indicator of bone turnover, is synthesized by

osteoblasts and becomes incorporated into the bone matrix by binding to hydroxyapatite in a calcium-dependent fashion.^{27,28} The greater labelling of this protein in the treated group showed that the bone presented a better condition of mineralization when compared with the control group.

The greatest alveolar bone formation observed in our study after OT administration could be collected from the interaction of the hormone with its receptor in bone cells,²⁹ resulting in intracellular events, such as those described by Copland et al.³⁰ Thus, OT could increase the release of intracellular calcium and prostaglandin E₂ synthesis, with consequent positive bone balance.³¹ The interaction between OT and Oxtr in osteoblasts may also involve other intracellular events (MAP kinase phosphorylation and c-Fos expression), as described in other OT target cells.^{30,32} These intracellular pathways are essential for osteoblasts and osteoclasts.

In fact the action of OT has been reported to be a direct regulator of bone mass mediated mainly through its stimulation of osteoblast formation, with variable effects on osteoclasts.¹³ Oxtr expression by fully differentiated human osteoclasts and by their precursors has been demonstrated.³³ Furthermore, it has been reported that the receptor is functional, and that the hormone may affect osteoclastogenesis, since it increases the number of pre-osteoclasts.^{13,33}

Despite the fact that OT may favour osteoclastogenesis, it can inhibit the resorptive function of mature osteoclasts through a calcium signalling mechanism activated by Oxtr, since OT treatment induces an increase in intracellular calcium.¹³ This could explain the significant decrease in the levels of TRAP, a bone resorption marker,¹⁸ observed in the OT treated rats. Osteoclast origin TRAP is presumably released into circulation during bone resorption and after the removal of osteoclasts from their place of work.^{34,35} The significant decrease in TRAP levels after OT treatment suggests that in old female rats oxytocin acts by inhibiting bone resorption during the alveolar healing process. In agreement with this, the immunohistochemical analysis against TRAP demonstrated a greater quantity of osteoclasts with positive label for this enzyme in the control group. Otherwise, in the treated groups, there was an important decrease in the expression of cells with positive TRAP labelling.

Since the interaction between oxytocin and its receptor triggers intracellular events that are important for the development of osteoblasts and osteoclasts, OT could be associated with the disruption of balance between osteoblasts and osteoclasts that causes senile and endocrine-mediated osteoporosis.²⁸

The positive bone balance and the decreased bone resorption observed during the alveolar healing process of old acyclic rats suggest that OT could be studied for use as an ally in the recovery of dental or alveolar trauma and in maxillary implants. Further studies with OT should explore the possibilities of using OT as an anabolic bone hormone as therapy for postmenopausal osteoporosis in humans.

5. Conclusion

The results presented indicate that in old acyclic female rats, peripheral OT plays a role in achieving a positive bone balance

and a decrease in bone resorption during the alveolar healing process.

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Competing interests

No conflict of interest.

Ethical approval

Animal Research Ethics Committee of the Univ Estadual Paulista (UNESP), Brazil (Protocol Number 2009-005746).

REFERENCES

1. Siris ES, Bilezikian JP, Rubin MR. Pins and plaster aren't enough: a call for the evaluation and treatment of patients with osteoporotic fractures. *Journal of Clinical Endocrinology and Metabolism* 2003;**88**:3482–6.
2. Freemantle N, Cooper C, Roux C, Díez-Pérez A, Guillemin F, Jonsson B, et al. Baseline observations from the possible EU[®] study: characteristics of postmenopausal women receiving bone loss medications. *Archives of Osteoporosis* 2010;**5**(1–2):61–72.
3. Roux C, Briot K, Horlait S, Varbanov A, Watts NB, Boonen S. Assessment of non-vertebral fracture risk in postmenopausal women. *Annals of the Rheumatic Diseases* 2007;**66**:931–5.
4. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA. Effect of parathyroid hormone on fractures and bone mineral density in postmenopausal women with osteoporosis. *New England Journal of Medicine* 2001;**344**:1434–41.
5. Glowacki J. Impact of postmenopausal osteoporosis on the oral and maxillofacial surgery patient. *Oral and Maxillofacial Surgery Clinics of North America* 2007;**19**:187–98.
6. Fujita M, Urano T, Horie K, Ikeda K, Tsukui T, Fukuoka H, et al. Estrogen activates cyclin-dependent kinases 4 and 6 through induction of cyclin D in rat primary osteoblasts. *Biochemical and Biophysical Research Communications* 2002;**299**:222–8.
7. Syed F, Khosla S. Mechanisms of sex steroid effects on bone. *Biochemical and Biophysical Research Communications* 2005;**328**:688–96.
8. Roberts MD, Santner TJ, Hart RT. Local bone formation due to combined mechanical loading and intermittent hPTH-(1–34) treatment and its correlation to mechanical signal distributions. *Journal of Biomechanics* 2009;**42**:2431–8.
9. Poole KE, Treece GM, Ridgway GR, Mayhew PM, Borggreffe J, Gee AH. Targeted regeneration of bone in the osteoporotic human femur. *PLoS One* 2011;**6**(1):e16190.
10. Elabd SK, Sabry I, Hassan WB, Nour H, Zaky K. Possible neuroendocrine role for oxytocin in bone remodeling. *Endocrine Regulations* 2007;**41**:131–41.
11. Camerino C. Oxytocin thinks globally and acts locally: the oxytocinergic regulation of bone mass. *IBMS BoneKEY* 2009;**6**(8):295–300.
12. Elabd C, Basillais A, Beaupied H, Breuil V, Wagner N, Scheideler M, et al. Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis. *Stem Cells* 2008;**26**(9):2399–407.
13. Tamma R, Colaianni G, Zhu LL, DiBenedetto A, Greco G, Montemurro G, et al. Oxytocin is an anabolic bone hormone. *Proceedings of the National Academy of Sciences* 2009;**106**(17):7149–54.
14. National Research Council, guide for the care and use of laboratory animals. Department of Health and Human Services, Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources; 1985.
15. Okamoto T, Russo MC. Wound healing following tooth extraction: histochemical study in rats. *Revista da Faculdade de Odontologia de Araçatuba* 1973;**2**:153–69.
16. Harms PG, Ojeda SR. A rapid and simple procedure for chronic cannulation of the rat jugular vein. *Journal of Applied Physiology* 1974;**36**:391–2.
17. Carvalho ACP, Okamoto T. *Cirurgia bucal: fundamentos experimentais aplicados à clínica*. São Paulo: Panamericana; 1987.
18. Seibel MJ. Biochemical markers of bone turnover: part I: biochemistry and variability. *The Clinical Biochemist Reviews* 2005;**26**:97–122.
19. Gore AC. Gonadotropin-releasing hormone neurons, NMDA receptors, and their regulation by steroid hormones across the reproductive life cycle. *Brain Research Reviews* 2001;**37**:235–48.
20. Hsieh YD, Devlin H, Roberts C. Early alveolar ridge osteogenesis following tooth extraction in the rat. *Archives of Oral Biology* 1994;**39**:425–8.
21. Hsieh YD, Devlin H, Mccord F. The effect of ovariectomy on the healing tooth socket of the rat. *Archives of Oral Biology* 1995;**40**:529–31.
22. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function and regulation. *Physiological Reviews* 2001;**81**:629–83.
23. Wahl RU. Could oxytocin administration during labor contribute to autism and related behavioral disorders? A look at the literature. *Medical Hypotheses* 2004;**63**(3): 456–60.
24. Epstein S. Serum and urinary markers of bone remodeling: assessment of bone remodeling: assessment of bone turnover. *Endocrine Reviews* 1988;**9**:437–48.
25. Luvizuto ER, Dias SMD, Okamoto T, Dornelles RCM, Okamoto R. Raloxifene therapy inhibits osteoclastogenesis during the alveolar healing process in rats. *Archives of Oral Biology* 2011;**56**:984–90.
26. Luvizuto ER, Dias SMD, Queiroz TP, Okamoto T, Garcia Jr IR, Okamoto R, et al. Osteocalcin immunolabeling during the alveolar healing process in ovariectomized rats treated with estrogen or raloxifene. *Bone* 2010;**46**:1021–9.
27. Dickson IR. Bone. In: Royce P, Steinmann B, editors. *Connective tissue and its heritable disorders*. New York: Wiley-Liss; 1993. p. 249–85.
28. Prakash D, Behari J. Synergistic role of hydroxyapatite nanoparticles and pulsed electromagnetic field therapy to prevent bone loss in rats following exposure to simulated microgravity. *International Journal of Nanomedicine* 2009;**4**:133–44.
29. Zingg HH, Laporte SA. The oxytocin receptor. *Trends in Endocrinology and Metabolism* 2003;**14**:222–7.
30. Copland JA, Ives KL, Simmons DJ, Soloff MS. Functional oxytocin receptors discovered in human osteoblasts. *Endocrinology* 1999;**140**(9):4371–4.
31. Jee WS, Ma YF. The in vivo anabolic actions of prostaglandins in bone. *Bone* 1997;**21**:297–304.
32. Soloff MS, Jeng YJ, Copland JA, Strakova Z, Hoare S. Signal pathways mediating oxytocin stimulation of prostaglandin

- synthesis in select target cells. *Experimental Physiology* 2000;**85**:51S–8S.
33. Colucci S, Colaianni G, Mori G, Patono N, Grano M, Zallone A. Human osteoclasts express oxytocin receptor. *Biochemical and Biophysical Research Communications* 2002;**297**:442–5.
34. Azria M. The value of biomarkers in detecting alterations in bone metabolism. *Calcified Tissue International* 1989;**45**:7–11.
35. Christenson RH. Biochemical markers of bone metabolism: an overview. *Clinical Biochemistry* 1997;**30**:573–93.