Influence of osteoporosis on the osteocyte density of human mandibular bone samples: a controlled histological human study

Pablo S. Oliveira, Jose A. Rodrigues, Jamil A. Shibli, Adriano Piattelli, Giovanna Iezzi, Vittoria Perrotti

Authors' affiliations:

Pablo S. Oliveira, Jose A. Rodrigues, Jamil A. Shibli, Department of Periodontology and Oral Implantology, Dental Research Division, Guarulhos University, Guarulhos, Brazil;

Adriano Piattelli, Giovanna Iezzi, Vittoria Perrotti, Department of Medical, Oral and Biotechnological Sciences, University of Chieti–Pescara, Chieti, Italy.

Corresponding author:

Prof. Jamil A. Shibli Pracßa Tereza Cristina, 01 - 07023-070 - Guarulhos,SP, Brazil

Tel.: +55 11 2441 3670

Fax: +55 11 24641758

e-mail: jashibli@yahoo.com

Abstract

Objectives: Osteocytes have emerged as key regulators of skeletal and mineral homeostasis. Thenumber of these cells could be influenced by the presence of osteoporosis and osteopenia. Hence,the aim this study was to evaluate the osteocyte density in patients with osteopenia, and inpatients with osteoporosis treated with bisphosphonates.

Materials and methods: Thirty-nine patients were selected for this study and divided into three groups: (A) nine healthy patients (control), (B) 15 patients with osteopenia, and (C) 15 patients with osteoporosis. During the surgical insertion of dental implants in the lower jaw, bone samples were retrieved and processed for histological analysis of osteocyte density, measured as number of osteocytes/bone tissue area (lm2).

Results: Patients with osteopenia showed statistically higher values of osteocyte density than patients with osteoporosis ($P<0.05$) No significant differences were detected between osteopenia and osteoporosis subjects vs. healthy patients ($P>0.05$).

Conclusions: Bone metabolism diseases (osteoporosis and osteopenia) do not seem to influence the osteocyte density; this could be due to the administration of bisphosphonates in patients with

osteoporosis. This information could play a fundamental role in the diagnosis and treatment of patients in a postmenopausal stage.
Key words: alendronate, dental Implants, osteocyte density, osteopenia, osteoporosis

Introduction

Postmenopausal osteoporosis is a metabolic disease where a negative balance of bone turnover causes a steady decline of bone volume and quality (Riggs & Parfitt 2005), producing a decrease of 25% of the total bone tissue (Consensus Development Conference on Osteoporosis 1993); as a consequence of the compromised integrity of the skeleton, it predisposes to an increased risk of fractures. Osteopenia is considered a precursor of osteo-porosis; this condition is characterized by a physiological decrease of bone mineral density between 10% and 25% from the physio-logical state (Consensus Development Conference on Osteoporosis 1993). Osteoporosis and osteopenia, induced in experimental animal model, before, after or simultaneously with dental implant placement, alter the process of osseointegration and produce a significant reduction in the bone-to-implant contact (Mellado-Valero et al. 2010). However, previous histological evaluation on retrieved dental implants from human jaws from subjects with and without osteoporosis demonstrated that these metabolic diseases did not alter the osseointegration rates, at least after the initial healing period (Shibli et al. 2008). Bone tissue is characterized by a constant turnover in response to mechanical stimuli, such as loading, and osteocytes seem to play an essential role in bone mechanical adaptation to loading. The osteocyte network is also regarded as the main mechano-sensor mechanism of bone tissue (You et al. 2000; Price et al. 2011). Osteocyte density is the result of bone remodeling process (Qui et al.2003; Hernandez et al. 2004), and it shows differences between healthy and osteoporotic subjects. In healthy women, there is a significant inverse relationship between osteocyte density in superficial bone and bone formation rate (Qiu et al. 2002). Therefore, an alteration of osteocytes number or function could affect bone healing, remodeling and turnover, which are on turn regulated by osteoblasts, osteoclasts and, last but not least, osteocytes.

Bisphosphonates (BPs) are non-metabolized analogs of pyrophosphate drugs, widely used as bone stabilizers in the treatment of osseous metastases, osteoporosis and Paget's disease due to their ability to inhibit osteoclast activity (Abuid et al. 2008). These medicaments have been shown to significantly reduce the risk of bone fracture (Dhillon & Lyseng-Williamson 2008). However, some

studies suggested that high doses of BPs were directly cytotoxic to osteocytes and produce cell death and necrosis (Allen & Burr 2008).

The aim of the present prospective, controlled histological and histomorphometrical study was to evaluate the osteocyte density (number of osteocytes/bone tissue area microm^2) in healthy and patients with osteopenia, and in patients with osteoporosis treated with bisphosphonates.

Materials and methods

Study design

A total of 45 postmenopausal women (mean age of 61.6 +/- 5.97 years) from 435 patients were included in the present prospective study. The patients were referred at Oral Implantology Clinic, Guarulhos University, Guarulhos, SP, Brazil, and performed the DXA test (Dual energy X-ray absorptiometry), to evaluate their bone density as function of T-score index (World Health Organization, Switzerland 1994). The patients were submitted to anamnesis (medical and dental history), intra-oral clinical examination and preoperative laboratory tests, including complete blood count, coagulation profile, blood glucose, serum calcium and creatinine. The inclusion criteria were as follows: post-menopausal period; total edentulism in the mandible; at least 10 mm of residual bone height and 5 mm bone thickness for the implant installation determined after clinical examination using a thickness gauge, and patients with osteoporosis in oral treatment with alendronate sodium MSD 70 mg (Fosamax, Merck Sharp & Dohme Pharmaceuticals Ltd., Campinas, Brazil). Exclusion criteria were as follows: vascular diseases; chronic diseases such as rheumatoid arthritis and diabetes; smoking; chronic alcoholism; moderate or advanced chronic periodontal disease; diseases of the oral mucosa; use of glucocorticosteroids or other immunosuppressive drugs; history of radiotherapy in the head and neck region; insufficient availability of bone tissue to permit insertion of dental implants. The subjects were divided according to their DXA test results into three groups: (A)control group, including 15 healthy patients(T-score ≥ -1); (B) test group 1, including 15 patients with osteopenia (T-score -1to -2.5); (C) test group 2, including 15 patients with osteoporosis in treatment with alendronate for at least 1 year (T-score ≤ -2.5) (Hanson 1997; Looker et al. 1998). The protocol was approved by the Ethics Committee in Research of Guarulhos University, SP, Brasil (protocol #147/2007), following the World Medical Association Declaration of Helsinki requirements, and all the subjects signed a written informed consent form.

Surgical phase

Implant placement was performed only in the mandible. Briefly, after local anesthesia, a full flap was elevated and implants were inserted. Bone cores were retrieved using a trephine bur (internal wide 2.0 mm) under copious irrigation, prior to the implants insertion.

Histological processing

All the specimens were washed in saline solution and immediately fixed in 10% buffered formalin. The specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing,Rome, Italy). They were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned, along their longitudinal axis, with a high-precision diamond disk at about 150 microm and ground down to about 30 microm with a specially designed grinding machine Exakt System (EXAKT, Apparetebau GmbH, Wehrheim, Germany). One slide was obtained for each specimen. The slides were stained with acid fuchsin and toluidine blue (Piattelli et al. 1997). Histomorphometry was carried out using a transmitted light micro-scope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVCâ, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intelâ, SantaClara, CA, USA). This optical system was associated with a digitizing pad (MatrixVision GmbH, Oppenweiler, Germany) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc,Milano, Italy). Histomorphometric measurements (Barros et al. 2009; Shibli et al. 2010; Piattelli et al. 2014) were conducted at 2009; osteocytes density was obtained using the ratio of the osteocytes number, counted manually for each specimen in 10 different fields to the bone tissue area (microm^2) with the above mentioned software package (Fig. 1) .

Statistical analysis

The data were evaluated by means of Kruskal-Wallis test (Kruskal & Wallis 1952); differences among groups were assessed by Dunn's multiple comparisons test (Dunn 1961). Statistical significance was set to $P < 0.05$. All the data are presented as means +/- standard deviation (SD).

Results

A total of 45 bone samples were taken: 15samples from control group; 15 samples from osteopenia group; and 15 samples from osteoporosis group. Six of 15 samples from control group were not processed for ground section due to insufficient amount of bone remaining after collection. Therefore, 39 bone samples were evaluated for osteocyte index. Table 1 shows the osteocyte

density of the evaluated groups. The highest osteocyte density value was observed in patients with osteopenia ($P < 0.05$; Group B), followed by healthy and osteoporotic subjects (groups A and C respectively). No significant differences were detected between osteopenia and osteoporosis group vs. control patients ($P>0.05$).

Discussion

The results of the present study showed no differences in terms of osteocyte density in healthy patients when compared to osteoporosis and osteopenia. This fact could be related for osteoporotic patient with the use of bisphosphonates (alendronate), which play a key role in the inhibition of osteoclasts and in the preservation of bone matrix (Dhillon& Lyseng-Williamson 2008), while for the patients with osteopenia, this fact could be related to in initial stage of bone resorption. Similar results were observed in ovariectomized rats where both osteocyte lacunar size and osteocyte density were altered in newly formed bone after antiresorptive and anabolic pharmaceutical treatment (Tommasini et al.2012). In the other hand, Mullender et al. (2005) compared the osteocyte density from iliac crest bone in four groups of subjects: osteoporotic male, osteoporotic female, control male, and control female. It was observed that the osteocyte density was higher in healthy females than in healthy males and lower in osteoporotic females than in healthy females. These results were not observed in our study, as patients with osteoporosis presented the same osteocyte density than healthy patients, probably because patients with osteoporosis included in the present study had used bisphosphonates, as described before. Osteocytes were shown to be necessary to maintain bone mass in response to nor-mal load (Bonewald & Johnson 2008), and, probably, their presence could be associated with the ability of bone to efficiently remodel, maintain normal levels of mineralization, and repair accumulated microdamages (Ma et al. 2008). A minimum number of osteocytes seemed to be essential for their operational network (Vashishth et al. 2000). The osteocyte canaliculi arrived at the marrow spaces and were be able to recruit osteoclast precursors or induce mesenchymal stem cells differentiation (Power et al.2002). In addition, some studies (Allen &Burr 2008; Huja et al. 2009) suggested that high doses of bisphosphonates were directly cytotoxic to the osteocytes and produced cell death and necrosis. A previous animal study (Huja et al. 2009) evaluated the effects of zoledronic acid, a commonly used bisphosphonate in the treatment of bone metastases, on bone resorption. It was observed that this drug suppressed bone formation without causing osteocyte death. This could explain the number of osteocytes in patients with osteoporosis observed in our study, without any significant differences when compared to the

control group. Finally, data obtained from retrieved bone cores from human mandibles could represent more authentic information of jawbone than those obtained elsewhere (Mullenderet al. 2005). Hence, the present results could be clinically useful in the diagnosis and treatment of postmenopausal jawbone loss and, in addition, in the planning of implant prostheses rehabilitations in these patients in postmenopausal stage.

Acknowledgements: This study was supported by a scholarship to Dr. Oliveira from CAPES Foundation, Ministry of Education of Brazil, Brasilia, DF 70.040-020,Brazil (CAPES, process number99999.012539/2013-09); Prof. Shibli received grants from Sao Paulo Research Foundation (FAPESP # 2008/06972-6), The National Council for Scientific and Technological Development (CNPq # 579157/2008-3,302768/2009-2, 473282/2007-0) and Guarulhos University (PesqDOC). The authors declare that there are no conflicts of interest related to this study. The authors thank doctors Kelly C.D. Aguiar and Alessandra Cassoni for their help in the surgical procedures and study design.

References

Abuid, M.H., Warnke, P.H., Gottschalk, J., Springer,I., Wiltfang, J., Acil, Y., Russo, P.A. & Kreusch,T. (2008) “Bis-phossy jaws”–high and low risk factors for bisphosphonate-induced osteonecrosis of the jaw. Journal of Cranio-maxillofacial Surgery36:95–103.

Allen, M.R. & Burr, D.B. (2008) Mandible matrix necrosis in beagle dogs after 3 years of daily oral bisphosphonate treatment. Journal of Oral and Maxillofacial Surgery66: 987–994.

Barros, R.R., Degidi, M., Novaes, A.B., Piattelli, A.,Shibli, J.A. & Iezzi, G. (2009) Osteocyte density in the peri-implant bone of immediately loaded and submerged dental implants. Journal of Peri-odontology80: 499–504.

Bonewald, L.F. & Johnson, M.L. (2008) Osteocytes, mechanosensing and Wnt signaling. Bone 42:606–615.

Consensus Development Conference on Osteoporosis. Hong Kong. The American Journal of Medicine (1993) 95:1S–78S.

Dhillon, S. & Lyseng-Williamson, K.A. (2008) Zoledronic acid - A review of its use in the management of bone metastases of malignancy. Drugs 68:507–534.

Dunn, O.J. (1961) Multiple comparisons among means. Journal of the American Statistical Association 56:52–64.

Hanson, J. (1997) Standardization of proximal femur BMD measurements. International Committee for Standards in Bone Measurement. Osteoporosis International 7: 500–501.

Hernandez, C.J., Majeska, R.J. & Schaffler, M.B. (2004) Osteocyte density in woven bone. Bone 35:1095–1099.

Huja, S.S., Fernandez, S.A., Phillips, C. & Li, Y. (2009) Zoledronic acid decreases bone formation without causing osteocyte death in mice. Archives of Oral Biology 54: 851–856.

Kruskal, W.H. & Wallis, W.A. (1952) Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association47: 583–621.

Looker, A.C., Wahner, H.W., Dunn, W.L., Calvo,M.S., Harris, T.B., Heyse, S.P., Johnston, C.C., Jr& Linasay, R. (1998) Updated data on proximal femur bone mineral levels of US adults. Osteoporosis International 8: 468–489.

Ma, Y.L., Dai, R.C., Sheng, Z.F., Jin, Y., Zhang,Y.H., Fang, L.N., Fan, H.J. & Liao, E.Y. (2008) Quantitative associations between osteocyte density and biomechanics, microcrack and microstructure in OVX rats vertebral trabeculae. Journal of Biomechanics41: 1324–1332.

Mellado-Valero, A., Ferrer-Garcıa, J.C., Calvo-Cat-ala, J. & Labaig-Rueda, C. (2010) Implant treatment in patients with osteoporosis. Medicina Oral, Patologia Oral y Cirurgia Bucal 15:52–57.

Mullender, M.G., Tan, S.D., Vico, L., Alexandre, C.& Klein-Nulend, J. (2005) Differences in osteocyte density and bone histomorphometry between men and women and between healthy and osteoporotic subjects. Calcified Tissue International 77: 291–296.

Piattelli, A., Artese, L., Penitente, E., Iaculli, F., Degidi, M., Mangano, C., Shibli, J.A., Coelho, P.G., Perrotti, V. & Iezzi, G. (2014) Osteocyte density in the peri-implant bone of implants retrievedafter different time periods (4 weeks to 27 years). Journal of Biomedical Materials Research part B: Applied Biomaterials102: 239–243.

Piattelli, A., Scarano, A. & Quaranta, M. (1997) High-precision, cost-effective cutting system for producing thin sections of oral tissues containing dental implants. Biomaterials18: 577–579.

Power, J., Loveridge, N., Rushton, N., Parker, M. &Reeve, J. (2002) Osteocyte density in aging subjects is enhanced in bone adjacent to remodeling Haversian system. Bone30: 859–865.

Price, C., Zhou, X., Li, W. & Wang, L. (2011) Real-time measurement of solute transport within the lacunar-canalicular system of mechanically loaded bone: direct evidence for load-induced fluid flow. Journal of Bone and Mineral Research26: 277–285.

Qiu, S., Rao, D.S., Palnitkars, S. & Parfitt, A.M. (2002) Relationships between osteocyte density and bone formation rate in human cancellousbone. Bone31: 709–711.

Qui, S., Rao, D.S., Palnitkar, S. & Parfitt, A.M. (2003) Reduced iliac cancellous osteocyte density inpatients with osteoporotic vertebral fracture. Journal of Bone and Mineral Research 18:1657–1663.

Riggs, B.L. & Parfitt, A.M. (2005) Drugs used to treat osteoporosis: the critical need for a uniform nomenclature based on their action on bone remodeling. Journal of Bone and Mineral Research20: 177–184.

Shibli, J.A., Aguiar, K.C., Melo, L., d'Avila, S., Zenobio, E.G., Faveri, M., Iezzi, G. & Piattelli, A. (2008) Histological comparison between implants retrieved from patients with and without osteoporosis. International Journal of Oral and Maxillo-facial Surgery 37: 321–327.

Shibli, J.A., Grassi, S., Piattelli, A., Pecora, G.E., Ferrari, D.S., Onuma, T., d'Avila, S., Coelho, P.G., Barros, R. & Iezzi, G. (2010) Histomorphometric evaluation of bioceramic molecular impregnated and dual acid-etched implant sur-faces in the human posterior maxilla. Clinical Implant Dentistry & Related Research12: 281–288.

Tommasini, S.M., Trinward, A., Acerbo, A.S., De Carlo, F., Miller, L.M. & Judex, S. (2012) Changes in intracortical microporosities induced by pharmaceutical treatment of osteoporosis as detected by high resolution micro-CT. Bone 50:596–604.

Vashishth, D., Verborgt, O., Divine, G., Schaffler, M.B. & Fyhrie, D.P. (2000) Decline in osteocyte lacunar density in human cortical bone is associated with accumulation of microcracks with age. Bone26: 375–380.

World Health Organization. (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. World Health Organ Technical Report Series 843:1–129.

You, J., Yellowley, C.E., Donahue, H.J., Zhang, Y., Chen, Q. & Jacobs, C.R. (2000) Substrate deformation levels associated with routine physical activity are less stimulatory to bone cells relative to loading-induced oscillatory fluid flow. Journal of Biomechanical Engineering 122: 387–393.328