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ORIGINAL ARTICLE

Oral mucosa produces cytokines and factors influencing osteoclast activity and endothelial cell proliferation, in patients with osteonecrosis of jaw after treatment with zoledronic acid

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Abstract
Objectives The intravenous injection of bisphosphonates, currently used as treatment for osteoporosis, bone Paget's disease, multiple myeloma, or bone metastases, can cause jaw bone necrosis especially in consequence of trauma. The present research aimed to clarify the mechanisms underlying bone necrosis, exploring involvement of the oral mucosa "in vivo."
Patients and methods Specimens of oral mucosa were removed from bisphosphonate-treated patients with or without jaw bone necrosis. In mucosa specimens, expression was evaluated of: cytokines involved in the inflammatory process, factors involved in osteoclast activity, i.e., receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin, a factor involved in cell proliferation, namely hydroxymethylglutaryl coenzyme A reductase, and a factor involved in angiogenesis, namely vascular endothelial growth factor (VEGF).
Results Interleukin (IL)-6 and the RANK/osteoprotegerin ratio were significantly elevated in mucosa from patients with versus without jaw necrosis, whereas hydroxymethylglutaryl coenzyme A reductase and VEGF were significantly decreased.
Conclusions Our results suggest that mucosa, stimulated by bisphosphonate released from the bone, can contribute to the development of jaw necrosis, reducing VEGF, and producing IL-6 in consequence of hydroxymethylglutaryl coenzyme A reductase reduction. In turn, IL-6 stimulates osteoclast activity, as shown by the increased RANKL/osteoprotegerin ratio.
Clinical relevance The results of this study suggest the importance of evaluating during bisphosphonate treatment the production of IL-6, RANKL, osteoprotegerin, and VEGF, in order to monitor the jaw osteonecrosis onset. To avoid repeated mucosa excisions, the determination of these factors could be carried out in crevicular fluid.

Keywords Bisphosphonates · Osteonecrosis · Jaw · Cytokines · RANKL/OPG · HMGR

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Introduction

Bisphosphonates (BPs) are currently used as first-line treatment for osteoporosis and Paget's disease of the bone and are administered routinely in patients with multiple myeloma or metastatic bone disease [1]. The affinity of BPs for bone mineral, and their inhibitory effect on osteoclast cell function, results in a strong antiresorptive action, a reduction in the risk of osteoporotic fractures, and an improvement in the overall bone health in cancer patients [2].

Nitrogen-containing BPs, but not BPs lacking nitrogen, affect the function and survival of osteoclasts by inhibiting the farnesyl pyrophosphate synthase enzyme from the mevalonate pathway, which is responsible for cholesterol and farnesyl synthesis [1, 3].

BPs can be administered orally or by intravenous injection. Oral administration of BPs is affected by poor gastrointestinal absorption. After BPs enter the circulation, approximately 50 % of the dose is incorporated into the bone. Most of the remainder is excreted unchanged in the urine, and only a negligible amount is transiently exposed to other tissues. During cycles of bone remodeling, BPs are slowly released and re-enter the systemic circulation unmetabolized [4].

In the case of the jaw bones, intravenous injection of BPs can cause bone necrosis spontaneously or in consequence of trauma. This bone lesion does not occur in the other parts of the skeleton and is, by definition, a necrotic area free of metastatic elements [5]. The mechanisms whereby BPs induce bone necrosis are, at present, unclear. One view considers that BPs reduce bone turnover, reducing osteoclast and osteoblast activity, leading to areas of necrotic bone [6]. Another hypothesis supports the involvement of bone blood supply reduction, pointing to modifications of vascular endothelial growth factor (VEGF) and platelet-derived growth factor [7, 8]. On the contrary, histological examination of samples obtained from patients with osteonecrosis of the jaw (ONJ) induced by BPs showed patent vessels in the majority of patients and no histological changes [9, 10].

Another hypothesis considers that BPs have a direct toxic action on the oral mucosa. As a result, oral pathogens are able to pass through defective or severely damaged oral mucosa and infect the bone, eventually leading to its necrosis [11].

A thorough knowledge of bone biology, and of the molecular mechanisms of normal bone remodeling, is important in order to understand bone health. Key factors in bone remodeling include the receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates bone resorption, and osteoprotegerin (OPG), which inhibits bone resorption. The ratio between these two factors regulates osteoclast formation and activity. Very recently, an "in vitro" study on osteoblasts derived from human mesenchymal stem cells showed that nitrogen-containing BPs increased the expression of OPG [12]; similarly, another study evidenced that nitrogen-containing BPs caused a moderate enhancement of OPG gene expression and a strongly increased RANKL. Interestingly, non-nitrogen-containing clodronate affected much less OPG and RANKL expression [13].

In the light of the above observations, this research aimed to explore the involvement of the oral mucosa in ONJ, examining the production of cytokines implicated in the inflammatory process, factors involved in osteoclast activity, i.e., RANKL and OPG, a factor involved in cell proliferation, namely hydroxymethylglutaryl coenzyme A reductase (HMGR), and a factor involved in angiogenesis, namely VEGF.

Patients and methods

Patients

Thirty patients with bone metastases from solid tumors or multiple myeloma and in therapeutic treatment with intravenous BP (zoledronic acid) entered the study. The patients received 4 mg intravenous zoledronic acid (Zometa®; Novartis Pharma SpA, Basel, Switzerland) every 4 weeks for 12 months. The patients did not receive radiotherapy at the head and neck region, and they did not receive any chemotherapeutical medications during BP treatment. Informed consent was obtained from all patients. The study protocol was approved by the Ethics Committee of Turin University (approval number of local ethical board, CEI/396; protocol number, 0010469). Patients treated with BPs for osteoporosis were excluded.

After 12 months of BP treatment and after 1 month from the last BP medication, the patients were observed for the absence or presence of ONJ, and then subdivided into two groups:

1. ONJ-negative group (ONJ−). This group included 11 women and 5 men, with mean age of 68.56±6.30 years.
2. ONJ-positive group (ONJ+). This group included ten women and four men, with mean age of 65.29± 5.24 years.

Surgical protocol

In the ONJ− group, tooth extraction was performed under locoregional anesthesia (mepivacaine 2 %), through luxation and avulsion with clamp. In all cases, 3–0 silk sutures were used to suture the alveolar mucosa; they were removed after 7 days. Patients were given antibiotic (amoxicillin every 8 h for 6 days) and oral anti-inflammatory treatment

(ibuprofen every 12 h for 3 days). Mucosa specimens were taken from area corresponding to the maxillary protuberance before the tooth extraction; excision was half-thickness, to avoid removing the periosteum and exposing the bone. One simple suture was placed. All specimens were placed in RNA Later solution (Qiagen, Milan, Italy), and maintained at −80 °C until use.

In the ONJ+ group, the patients were treated by the resection of the necrotic bone with primary closure of the mucosa over the bony defect using plasma rich in growth factors, as described in Mozzati et al. [14]. Mucosa specimens were excised as described above, in an area distant from the necrotic area, and before the surgical procedure for removing ONJ; the specimens were placed in RNA Later solution (Qiagen, Milan, Italy) and maintained at −80 °C until use. Panoramic radiography and computed tomography were performed before and after surgery.

Radiographic analysis

All patients were monitored through dental panoramic radiography and computed tomography (CT), to evaluate the presence or absence of ONJ.

Biological factor analysis

Mucosa specimens were analyzed to determine expression of inflammatory (interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α)), osteoclastogenic (RANKL and OPG), and proliferation parameters (HMGR and VEGF), using real-time polymerase chain reaction (PCR).

Total RNA was extracted from specimens using the NucleoSpin RNA II Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), as previously reported [15]. Real-time PCR was run with single-stranded cDNA prepared from total RNA (1 μg) using a High-Capacity cDNA Archive kit (Applied Bio Systems, Foster City, CA).

The forward (FW) and reverse (RV) primers shown in Table 1 were designed using Beacon Designer® software (Bio-Rad, Hercules, CA). Twenty-five microliters of a PCR mixture containing cDNA template equivalent to 40 ng of total RNA, 5 pmol each of the FW and RV primers, and ×2 IQ SYBR Green SuperMix (Bio-Rad, Hercules, CA) were amplified using an iCycler PCR instrument (Bio-Rad, Hercules, CA) with an initial melt at 95 °C for 10 min, followed by 35–40 cycles at 95 °C for 40 s, annealing temperature for each primer set for 40 s, and 72 °C for 40 s. A final extension of 7 min at 72 °C was applied. Each sample was tested in duplicate, and threshold cycle (Ct) values from each reaction were averaged. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as house-keeping gene. The changes in expression were defined as those detected in the mucosa specimens taken from the ONJ− or ONJ+ patients versus those detected in the mucosa specimens taken from normal subjects and calculated as $2^{-\Delta\Delta Ct}$, where $\Delta Ct = Ct_{sample} - Ct_{GAPDH}$ and $\Delta\Delta Ct = \Delta Ct_{T\ sample} - \Delta Ct_{Tnormal}$.

Table 1 Forward and reverse primers for real-time PCR analysis

Gene acc. No.	Sequence (FW or RV)	T annealing No. cycles
Human GAPDH	FW-5′-TGA AGG TCG GAG TCA ACG GAT TTG GT-3′	52 °C
NM_002046	RV-5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′	35
Human IL-1beta	FW-5′-GCA CCT TCT TTC CCT TCA TCT TT-3′	52 °C
AF043335	RV-5′-GCG TGC AGT TCA GTG ATC GTA-3′	35
Human IL-6	FW-5′-CCA GTA CCC CCA GGA GAA GAT T-3′	52 °C
M14584	RV-5′-GTC AAT TCG TTC TGA AGA GGT GAG-3′	35
Human IL-8	FW-5′-AGC TGG CCG TGG CTC TCT-3′	52 °C
NM_000584	RV-5′-TTA GCA CTC CTT GGC AAA ACT G-3′	35
Human TNF alpha	FW-5′-CTC TGG CCC AGG CAG TCA-3′	52 °C
AF043342	RV-5′-GGA GCT GCC CCT CAG CTT-3′	35
Human RANKL	FW-5′-CAC AGC ACT TCA GAG CAG AG-3′	58 °C
NM_033012	RV-5′-ACA GAC TCA CTT TAT GGG AAC C-3′	40
Human OPG	FW-5′-CAG CGG CAC ATT GGA C-3′	55 °C
NM_002546	RV-5′-CGT GCA TTA GGC CCT T-3′	30
Human HMGR	FW-5′-CTT GTG TGT CCT TGG TAT TAG AGC TT-3′	56 °C
NM_000859	RV-5′-TAA TCA TCT TGA CCC TCT GAG TTA CAG-3′	35
Human VEGF	FW-5′-AGA CGG ACA GAA AGA CAG-3′	54 °C
NM_1025366	RV-5′-AAG CAG GTG AGA GTA AGC-3′	35

FW forward, *RV* reverse, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *RANKL* receptor activator of nuclear factor kappa-B ligand, *HMGR* hydroxymethylglutaryl coenzyme A reductase, *VEGF* vascular endothelial growth factor

Mucosa specimens from normal subjects were taken during tooth extraction, after obtaining informed consent.

Statistical analysis

For each biological factor examined in the ONJ− and ONJ+ groups, descriptive analyses of data were performed. For visual representation, box plots were created by SPSS software package.

Results

Patients

Table 2 reports the number, the sex, and the age of patients enrolled in this study and the distribution of primary diseases. The analysis of the latter parameter showed that 47 % of ONJ− patients had breast cancer, 40 % prostate cancer, and 13 % multiple mieloma; 50 % of ONJ+ patients had breast carcinoma, 25 % multiple myeloma, 17 % prostate cancer, and 8 % kidney cancer. As regards the ONJ+ patients, ONJ affected the lower jaw in the 62 % of cases, and the upper jaw in the 38 % (data not shown).

Both ONJ− and ONJ+ patients, at 8 months after tooth extraction or ONJ removal, showed no signs of osteonecrosis, as evidenced by dental panoramic radiography and CT imaging.

Radiographic analysis

All patients enrolled were monitored through dental panoramic radiography; since this was found to be of limited use in assessing BP-associated ONJ, they were followed by the more informative CT imaging. This is in line with previous findings [16].

Table 2 Characteristics of ONJ− and ONJ+ patients

	ONJ−	ONJ+
Number of patients		
Women	11	10
Men	5	4
Age	68.56±6.30	65.29±5.24
Primary disease		
Breast cancer	47 %	50 %
Multiple myeloma	40 %	25 %
Prostate cancer	13 %	17 %
Kidney cancer		8 %

The percentages of primary disease refer to the total of patients enrolled in ONJ− (16) and ONG+ (14) groups

Biological factor analysis

Analysis of the biological factors involved in the inflammation process, namely IL-1β, IL-6, IL-8, and TNF-α, is shown in Fig. 1. No variation in cytokines was observed between the mucosa specimens taken from the two groups (ONJ− and ONJ+), with the exception of IL-6, which was higher in the ONG+ group than in the ONJ− group.

Figure 2 shows the variation of RANKL and OPG between specimens of mucosa taken from the ONJ− and ONJ+ patients. RANKL was higher in the mucosa from the ONJ+ patients versus ONJ− patients. On the contrary, OPG was lower in the mucosa from the ONJ+ patients than ONJ− patients. However, the RANKL/OPG ratio was very much higher in the ONJ+ than in ONJ− (9.85±9.82 vs. 2.18±2.14).

As regards factors involved in cell proliferation, i.e., HMGR, and in endothelial cell proliferation, i.e., VEGF, Fig. 3 shows that both these factors were lower in the mucosa from the ONJ+ patients than in that from the ONJ − patients.

Discussion

The present study aimed to evaluate involvement of the mucosa in inducing ONJ since the pathogenetic mechanisms underlying the development of ONJ are still unclear. With this aim, specimens of mucosa were removed from two groups of patients in order to identify any differences in gene expression profiling, in the presence or absence of ONJ; all patients were examined after 12 months of zoledronic acid treatment.

BPs have become an important component in treating both bone metastases from solid tumors and multiple myeloma. Their efficacy has been proven in large randomized clinical trials [17, 18]. Intravenous injection of BPs is now the standard of care for the treatment and prevention of skeletal complications associated with bone metastases [18]. However, the use of BPs in oncology has been the subject of debate, due to their association, in the presence of other risk factors, with the development of ONJ, a serious adverse event that can negatively affect the patient's quality of life [19, 20]. It is known that invasive dental interventions during BP treatment are one of the main risk factors for ONJ development [21]. It has also been reported that ONJ can arise spontaneously [22]. Moreover, BP treatment is also intensely debated, due both to the limited knowledge of the etiology and pathogenesis of ONJ, and to the lack of alternative therapeutic options available for patients with metastatic bone disease.

In this study, ONJ occurred especially in the lower jaw (more than 50 %) than in upper jaw. This observation is in

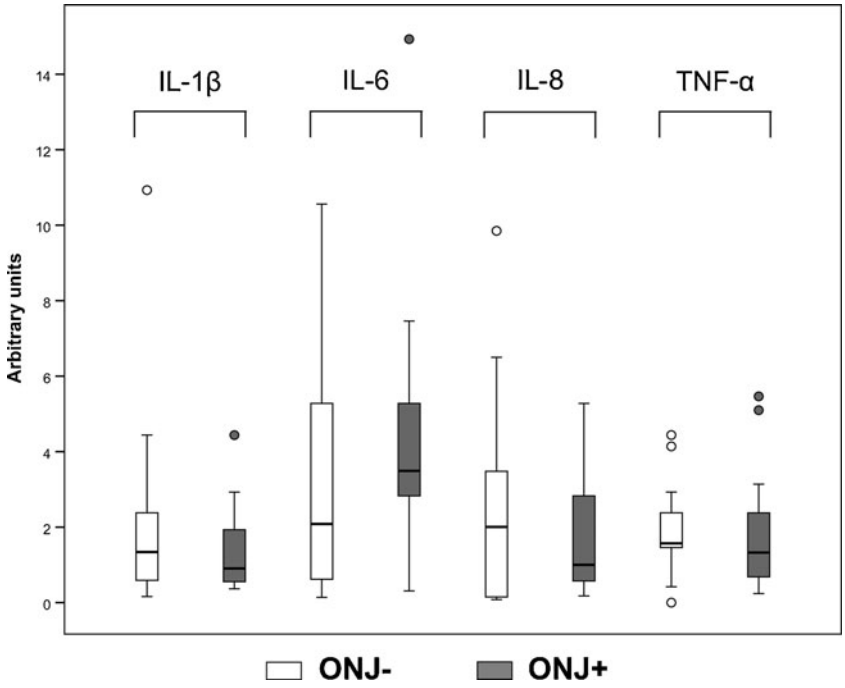

Fig. 1 Box plot graph showing cytokine mRNA content in mucosa specimens from ONJ− and ONJ+ patients

agreement with the data reported by literature, which affirm that lower jaw location is more common, because it has more areas with thin mucosa than the upper jaw [23].

It is noteworthy that the ONJ− patients, at 8 months after tooth extraction, showed no signs of ONJ; this seems to be the consequence of the fact that the inflammatory and osteoclastogenic factors were produced in different amount by mucosa in this patient group in comparison with the ONJ + group.

As regards inflammatory factors, IL-6 was found to be increased in the mucosa from ONJ+ patients, whereas the other cytokines tested, such as TNF-α, were not increased. At first sight, this appears to be in contrast with the well-known observation that BPs, and in particular the aminobisphosphonates such as zoledronic acid, have a number of side effects [24]. These include a rise in body temperature and accompanying flu-like symptoms that resemble a typical acute-phase response. These clinical features occur in

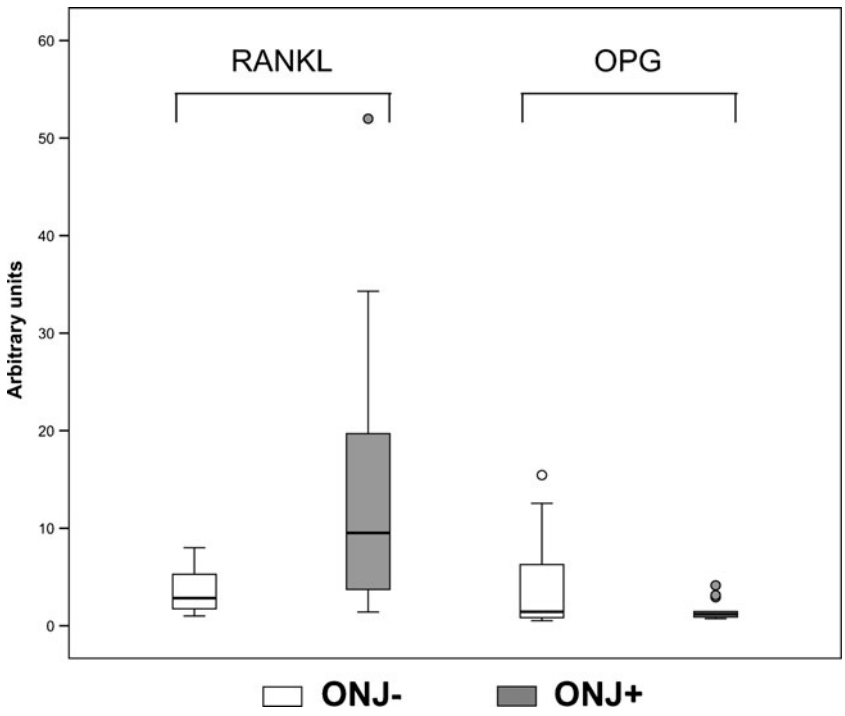

Fig. 2 Box plot graph showing RANKL and OPG mRNA content in mucosa specimens from ONJ− and ONJ+ patients

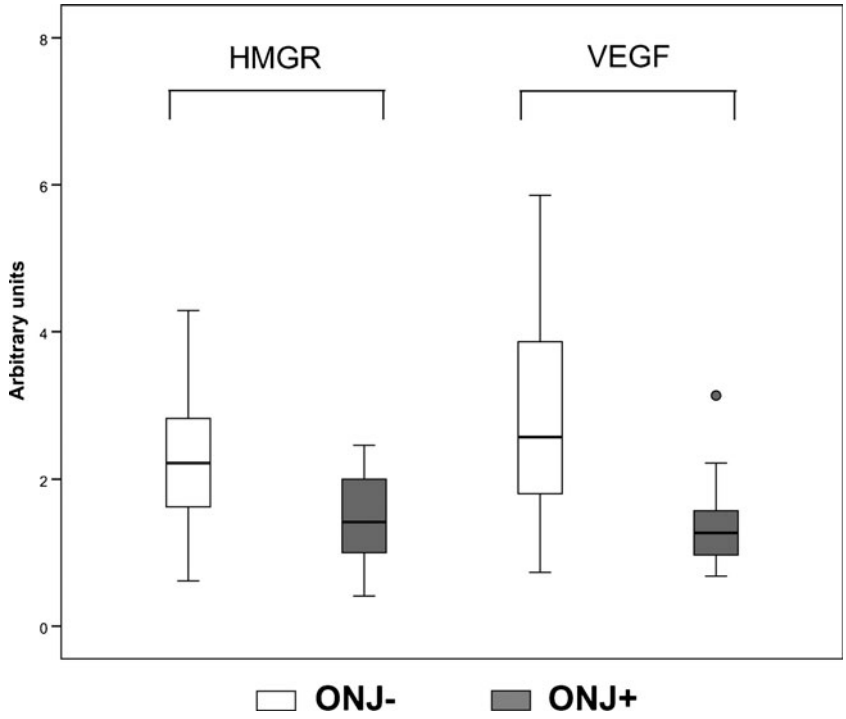

Fig. 3 Box plot graph showing VEGF and HMGR mRNA content in mucosa specimens from ONJ− and ONJ+ patients

over a third of patients receiving treatment for the first time. However, cytokine production is maximal within 28–36 h of intravenous administration and disappears 2–3 days later, despite continuing treatment. This production is characterized by increased circulating levels of IL-6, TNF, and interferon [25]. Our determinations were on mucosa from patients after the 12th dose of zoledronic acid. It is thus unlikely that the increased IL-6 expression was correlated with a general inflammatory response. It was more probably linked to local mucosal inflammation and bone necrosis, thus being the consequence of the induction of osteoclast differentiation and activity, due to the increased RANKL/OPG ratio. IL-6 is considered to be an osteoclastogenic cytokine, and thus an important mediator of bone loss [26, 27]. "In vitro" experiments have shown that zoledronic acid stimulates γδ T cells, with consequent production of cytokines; BPs appear to activate these cells by inhibiting farnesyl pyrophosphate synthase. Inhibition of the mevalonate pathway leads to an accumulation of metabolic intermediates, including isopentenyl pyrophosphate, which is a potent activator of human peripheral blood γδ T cells [28]. Moreover, this latter study found a correlation between the mevalonate pathway and γδ T cell activation by statin administration: statins inhibited the activation of human peripheral blood γδ T cells in response to endogenous nonsterol mevalonate products. In the mucosa of ONJ+ patients, HMGR expression was decreased; these results thus agree with the observation reported above, and suggest that this decrease could be responsible for inducing cells present in the mucosa to produce IL-6. In its turn, IL-6 induced osteoclast activity, as shown by the increase of RANKL and RANKL/OPG ratio.

At this point, the following question may be asked: since, in the bone, BPs inhibit osteoclast activity in order to reduce bone loss, why, in the jaw, do they induce osteoclast activity, and thus cause bone necrosis? Our answer is that BPs are known to accumulate in the bone and that, in consequence of minor trauma (dentures or dental prostheses, caries, and periodontal disease) [29], they are released from the bone into the environment. In the case of the jaw, the environment is the mucosa, where epithelial cells, fibroblasts, and lymphocytes are present. In this context, BPs induce these cells to produce cytokines by inhibiting HMGR. IL-6 in contact with alveolar bone stimulates osteoclast activity.

Moreover, other studies have reported that increased IL-6, induced by pro-inflammatory stimuli, is also accompanied by downregulation of markers of osteoblastic differentiation [30, 31]. The production of IL-6 is not correlated with the general acute-phase response, which comes about after the initial infusion of BP; rather, it may be considered the consequence of the local inflammation induced in the mucosa by the release of BP from the alveolar bone. This means that, for the first time, the mucosa comes into contact with BP, setting off cytokine IL-6 production. IL-6 induces the above-described events, i.e., the induction of osteoclasts. Moreover, the reduction of VEGF production in the mucosa specimens, evidenced in this research, could also contribute to the development of ONJ, blocking bone synthesis, and healing. This agrees with antiangiogenic effect of BPs

demonstrated by other authors with "in vitro" experiments, animal models, and human studies on BP-treated patients with advanced solid cancer and bone metastasis [32, 33]. In fact, the antiangiogenic action, with reduced capillary formation and inhibition of endothelial and vascular growth factors leading to avascular necrosis, is considered one of the mechanisms for ONJ onset. However, there is no evidence in literature for this effect of BPs in bone: the angiogenesis during bone formation seems to be unaltered by BPs. However, another recent hypothesis stated that BPs accumulate in bone in concentrations sufficient to be directly toxic to the oral epithelium, resulting in the failure of healing of soft tissue lesions [33].

In conclusion, we hypothesize that the mucosa, possibly stimulated by BP released from the bone, can cause ONJ, producing IL-6 in consequence of HMGR reduction; IL-6, in turn, stimulates osteoclast activity, evidenced by the increased RANKL/OPG ratio. ONJ may also in part be due to the reduction of VEGF expression.

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Conflicts of interest The authors declared no conflicts of interest.

References

1. Pazianas M (2011) Osteonecrosis of the jaw and the role of macrophages. J Natl Cancer Inst 103:232–240
2. Mehrotra B (2009) Bisphosphonates—role in cancer therapies. J Oral Maxillofac Surg 67:19–26
3. Dunford JE, Kwaasi AA, Rogers MJ et al (2008) Structure–activity relationships among the nitrogen containing bisphosphonates in clinical use and other analogues: time-dependent inhibition of human farnesyl pyrophosphate synthase. J Med Chem 51:2187–2195
4. Marcus R, Feldman D, Nelson D et al (2008) Bisphosphonates: pharmacology and use in the treatment of osteoporosis. In: Marcus R, Feldman D, Nelson D, Rosen C (eds) Osteoporosis, 3rd edn. Elsevier, Burlington, pp 1725–1742
5. Frei M, Bornstein MM, Schaller B et al (2010) Bisphosphonate-related osteonecrosis of the jaw combined with jaw metastasis of prostate adenocarcinoma: report of a case. J Oral Maxillofac Surg 68:863–867
6. Treister NS, Sook-Bin W (2009) Bisphosphonate-associated osteonecrosis of the jaws. In: Rosen CJ, Compston JE, Lian JB (eds) Primer on the metabolic bone diseases and disorders of mineral metabolism, 7th edn. Wiley, Hoboken, pp 505–509
7. Santini D, Vincenzi B, Dicuonzo G et al (2003) Zoledronic acid induces significant and long-lasting modifications of circulating angiogenic factors in cancer patients, and by "in vitro" research. Clin Cancer Res 9:2893–2897
8. Fournier P, Boissier S, Filleur S et al (2002) Bisphosphonates inhibit angiogenesis in vitro and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats. Cancer Res 62:6538–6544
9. Hansen T, Kunkel M, Weber A et al (2006) Osteonecrosis of the jaws in patients treated with bisphosphonates-histomorphologic analysis in comparison with infected osteoradionecrosis. J Oral Pathol Med 35:155–160
10. Hellstein JW, Marek CL (2005) Bisphosphonate osteochemonecrosis (bis-phossy jaw): is this phossy jaw of the 21st century? J Oral Maxillofac Surg 63:682–689
11. Reid IR (2009) Osteonecrosis of the jaw: who gets it, and why? Bone 44:4–10
12. Ohe JY, Kwon YD, Lee HW (2012) Bisphosphonates modulate the expression of OPG and M-CSF in hMSC-derived osteoblasts. Clin Oral Investig 16:1153–1159
13. Koch FP, Merkel C, Ziebart T et al (2012) Influence of bisphosphonates on the osteoblast RANKL and OPG gene expression in vitro. Clin Oral Investig 16:79–86
14. Mozzati M, Gallesio G, Arata V et al (2012) Platelet-rich therapies in the treatment of intravenous bisphosphonate-related osteonecrosis of the jaw: a report of 32 cases. Oral Oncol 48:469–474
15. Mozzati M, Martinasso G, Cocero N et al (2011) Influence of superpulsed laser therapy on healing processes following tooth extraction. Photomed Laser Surg 29:565–571
16. Bianchi SD, Scoletta M, Cassione FB et al (2007) Computerized tomographic findings in bisphosphonate-associated osteonecrosis of the jaw in patients with cancer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 104:249–258
17. Pavlakis N, Schmidt R, Stockler M (2005) Bisphosphonates for breast cancer. Cochrane Database Syst Rev 3:CD003474
18. Aapro M, Abrahamsson PA, Body JJ et al (2008) Guidance on the use of bisphosphonates in solid tumours: recommendations of an international expert panel. Ann Oncol 19:420–432
19. La Verde N, Bareggi C, Garassino M et al (2008) Osteonecrosis of the jaw (ONJ) in cancer patients treated with bisphosphonates: how the knowledge of a phenomenon can change its evolution. Support Care Cancer 16:1311–1315
20. Ruggiero S, Gralow J, Marx RE et al (2006) Practical guidelines for the prevention, diagnosis, and treatment of osteonecrosis of the jaw in patients with cancer. J Oncol Pract 2:7–14
21. Vandone AM, Donadio M, Mozzati M et al (2011) Impact of dental care in the prevention of bisphosphonate-associated osteonecrosis of the jaw: a single-center clinical experience. Ann Oncol 23:193–200
22. Lo JC, O'Ryan FS, Gordon NP et al (2010) Prevalence of osteonecrosis of the jaw in patients with oral bisphosphonate exposure. J Oral Maxillofac Surg 68:243–253
23. Shannon J, Shannon J, Modelevsky S et al (2011) Bisphosphonates and osteonecrosis of the jaw. J Am Geriatr Soc 340:c246. doi:10.1111/j.1532-5415.2011.03713.x
24. Russell RG, Croucher PI, Rogers MJ (1999) Bisphosphonates: pharmacology, mechanisms of action and clinical uses. Oteoporos Int 9:S66–S80
25. Santini D, Vincenzi B, Caraglia M et al (2007) A hitherto unreported high incidence of zoledronic acid-induced acute phase reaction in patients with cancer treatment-induced bone loss. Ann Oncol 18:201–202
26. Rufo A, Del Fattore A, Capulli M et al (2011) Mechanisms inducing low bone density in Duchenne muscular dystrophy in mice and humans. J Bone Miner Res 26:1891–1903. doi:10.1002/jbmr.410
27. Jimi E, Furuta H, Matsuo K et al (2010) The cellular and molecular mechanisms of bone invasion by oral squamous cell carcinoma. Oral Dis 17:462–468. doi:10.1111/j.1601-0825.2010.01781.x
28. Hewitt RE, Lissina A, Green AE et al (2005) The bisphosphonate acute phase response: rapid and copious production of proinflammatory cytokines by peripheral blood γδ T cells in response to aminobisphosphonates is inhibited by statins. Clin Exp Immunol 139:101–111
29. Woo SB, Hellstein JW, Kalmar JR (2006) Narrative review: bisphosphonates and osteonecrosis of the jaws. Ann Intern Med 144:753–761

30. Kraus D, Deschner J, Jäger A et al (2012) Human β-defensins differently affect proliferation, differentiation, and mineralization of osteoblast-like MG63 cells. J Cell Physiol 227:994–1003. doi:10.1002/jcp.22808
31. Krishnan V, Shuman LA, Sosnoski DM et al (2011) Dynamic interaction between breast cancer cells and osteoblastic tissue: comparison of two- and three-dimensional cultures. J Cell Physiol 226:2150–2158
32. Landesberg R, Woo V, Cremers S et al (2011) Potential pathophysiological mechanisms in osteonecrosis of the jaw. Ann N Y Acad Sci 1218:62–79. doi:10.1111/j.1749-6632.2010.05835.x
33. Rustemeyer J, Bremerich A (2010) Bisphosphonate-associated osteonecrosis of the jaw: what do we currently know? A survey of knowledge given in the recent literature. Clin Oral Investig 14:59–64