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ORIGINAL ARTICLE

Influence of bisphosphonates on endothelial cells, fibroblasts, and osteogenic cells

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Abstract Bisphosphonate-associated osteonecrosis of the jaws (BP-ONJ) is a side effect primarily in patients receiving highly potent nitrogen-containing bisphosphonates. The exact etiopathology is unknown. In addition to reduced bone remodeling, there may also be an impact on soft tissues. The impact of nitrogen- (ibandronate, pamidronate, zoledronate) and non-nitrogen-containing bisphosphonates (clodronate) on human umbilicord vein endothelial cells (HUVEC), fibroblasts and osteogenic cells was analyzed employing cell viability testing and a scratch wound assay. The impact on the cell morphology of vital-stained osteogenic cells was investigated by cell visualization (confocal laser scanning microscopy). Pamidronate and zoledronate had the greatest negative impact on all cell lines, whereas the impact of ibandronate and clodronate was less distinct. The effect of clodronate on HUVEC and fibroblasts was particularly marginal. BP-ONJ could be a multifactorial event with multicellular impairments. This might result in altered wound healing. The increased impact of the highly potent bisphosphonates, particularly on non-bone cells, may explain the higher occurrence of BP-ONJ.

Keywords Bisphosphonates · Bisphosphonate-associated osteonecrosis of the jaws · Osteogenic cells · HUVEC · Fibroblasts

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Introduction

Bisphosphonates are the most important antiresorptive agents in metabolic bone diseases and bone metastases; they are frequently used in patients with multiple myeloma or bone metastases due to breast or prostate cancer and in patients with Paget's disease or osteoporosis. Bisphosphonates reduce skeletal-related events such as fractures and the need for radiation or stabilizing operations; they prevent hypercalcemic episodes, reduce pain, and, therefore, increase the quality of life in affected patients.

The side effects of bisphosphonates can be grouped into three main categories: acute phase reactions, gastrointestinal effects, and renal side effects [1]. In 2003, a new side effect, bisphosphonate-associated osteonecrosis of the jaws (BP-ONJ), was described for the first time [2] and has since increased in frequency [3]. A universally agreed-upon definition for BP-ONJ has not been established to date. According to the American Association of Oral and Maxillofacial Surgeons, BP-ONJ is defined as exposed necrotic bone in the maxillofacial region for a period of at least 8 weeks in connection with current or previous bisphosphonate therapy and a lack of head and neck radiation in the patient's history [4]. The bisphosphonates most often associated with BP-ONJ are zoledronate and pamidronate, which contain nitrogen and are administered intravenously. Ibandronate is less often associated with the condition, and the non-nitrogen-containing preparations such as clodronate are rarely implicated.

Described incidences for BP-ONJ range from 1.2% [5] to 11.4% [6] in breast cancer patients, 3.1% [5] to 17.2% [7] in multiple myeloma patients, and 2.9% [8] to 18.6% [9] in prostate cancer patients. The risk of developing BP-ONJ in osteoporosis patients treated with bisphosphonates

is lower and is estimated at one event per 20,000 to 110,000 patient-years [10].

In most patients' case history, a previous tooth extraction, a pressure denture sore, periodontal disease, or a previous dental surgical procedure is given. Some patients lack such a trigger factor. In these cases, the osteonecrosis usually occurs at the mylohyoid ridge, a region with a very thin mucosal layer protecting the mandible [11]. To date, the etiopathology of BP-ONJ and the reason it solely affects the jaws is not definitively known.

The most common theory attributes the condition to reduced bone remodeling due to bisphosphonate-induced osteoclast inhibition and the subsequent accumulation of microfractures [12]. In addition, bisphosphonates have an antiangiogenetic effect [13], resulting in an avascular necrosis. This is supported by the fact that bisphosphonates influence the number of circulating endothelial progenitor cells [14] that contribute to an increase in angiogenesis: paracrine effects, differentiation to mature endothelial cells [15]. Furthermore, bisphosphonates influence the viability of oral keratinocytes [16]. All these influences modulate the wound healing and could contribute to BP-ONJ development [10].

The aim of the present study was to analyze the responses of three different cell types involved in jaw bone remodeling and defect healing to bisphosphonates of different potencies at varying concentrations. The effects of clodronate, ibandronate, pamidronate, and zolodronate on human umbilical vein endothelial cell (HUVEC), fibroblast, and osteogenic cell viability were analyzed by employing a 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test [17]. In addition, scratch wound assays were performed to examine the effects of bisphosphonates on the ability of the three cell lines to migrate and proliferate in a monolayer cell culture. Finally, since several studies have shown that osteogenic cell attributes such as adhesion and differentiation are closely associated with characteristic cell morphologies [18–20], osteogenic cells were vital-stained to show the potential influence of bisphosphonates at different concentrations on the cell morphology.

Materials and methods

Commercially available HUVEC (Lonza, Basel, Switzerland: No. CC-2517), human gingival fibroblasts (Lonza, Basel, Switzerland: No. CC-7049), and human osteogenic cells (PromoCell, Heidelberg, Germany: No. C-12720) were cultured in an incubator with 5% CO_2 and 95% air at 37°C. Cells were passaged at regular intervals depending on their growth characteristics using 0.25% trypsin (Seromed Biochrom, Berlin, Germany).

HUVEC were cultured in an endothelial basal medium supplemented with 1 µg/ml hydrocortisone, 12 µg/ml bovine brain extract, 50 µg/ml gentamicin, 50 ng/ml amphotericin-B, 10 ng/ml epidermal growth factor, and 10% fetal calf serum (FCS) until the third passage.

Fibroblasts were grown in Stroma Cell Growth Medium (Lonza, Basel, Switzerland) with 1% penicillin–streptomycin–neomycin antibiotic mixture (PSN), 10% FCS, and 500 ng basic fibroblast growth factor per 500 ml medium.

Osteogenic cells were cultivated in a solution composed of Dulbecco's modified Eagle's medium with 1% PSN, 1% L-glutamine, and 10% FCS. Before the experiments, the osteogenic cells were positively characterized by immunohistochemical expression of alkaline phosphatase and osteocalcin (labeled streptavidin–biotin/horseradish peroxidase).

For the MTT test, cells of the respective cell line were transferred into six-well plates (HUVEC 100,000 cells/well; fibroblasts 180,000 cells/well; osteogenic cells 280,000 cells/well); after 24 h, all cell lines were incubated with bisphosphonates (clodronate, ibandronate, pamidronate, and zoledronate) in increasing amounts (0, 5, 50, 100, 200, and 500 µmol). Due to their lower cell metabolism, osteoblasts were incubated for 72 h, while HUVEC and fibroblasts were incubated for 24 h.

The cell viability of all three cell lines was evaluated with the MTT colorimetric assay (Sigma, München, Germany: M5655). Viable cells ferment tetrazolium bromide to formazan that can be measured after cell lysis photometrically at 550 nm. The experiments were performed in triplicate.

For the scratch wound assay, the cells were seeded on tissue culture plastic in Petri dishes (Nunc, Langenselbold, Germany); at 80% cell confluence, a scratch wound using a sterile pipette tip was performed and the size of the gap was measured and set to 100%. All cells were incubated with 100 µmol of the previously mentioned bisphosphonates and the closing of the scratch wound was measured for 72 h or until the wounds were closed. The experiment was performed six times for each cell line and bisphosphonate.

Vital staining of the osteogenic cells was performed with green cell tracker (Molecular Probes, Invitrogen, Karlsruhe, Germany), according to the manufacturer's staining protocol. Fifteen microliters of the stock solution was added to 100,000 cells suspended in 5 ml basal medium. After 30 min of incubation at room temperature, cells were centrifuged and further processed. Preliminary studies revealed a stable fluorescence activity over at least 72 h. All four bisphosphonates were used at 50 and 100 µmol and compared to a control. The osteogenic cells cultivated on tissue culture plastic were visualized for 72 h after adding the respective bisphosphonates by using confocal laser scanning microscopy (CLSM, Leica TCS SP2 X1, Wetzlar, Germany) in upright arrangement. A tenfold

dipping lens and a 40-fold dipping lens (HCX APO L 40×/0.8 W) allowed cell visualization without removal of the culture medium; this protected the cells against dehydration and ensured homeostasis over the whole observation period. The detection range for green fluorescence was 500 to 550 nm after blue excitation (argon laser, 488 nm). The CLSM color filter was fitted with an acousto-optical beamsplitter in a detection channel at Langpass >500 nm to optimize fluorescence detection. Synchronized CLSM reflection mode images (helium–neon laser, 594 nm) enabled simultaneous visualization of unstained cell compartments.

Statistical analysis Continuous variables are expressed as mean±SEM (standard error of mean). Comparisons between groups were analyzed by analysis of variance (ANOVA, post hoc test: Tukey) for experiments with more than two subgroups or *t*-test (two-sided). The software SPSS 16.0 for Windows was used for calculations. The *p*-values<0.05 were considered as statistically significant.

Results

MTT HUVEC

Compared to the control (0 µmol of the respective bisphosphonate), aminobisphosphonates pamidronate, zoledronate, and ibandronate particularly decreased the cell viability of HUVEC (Fig. 1). Clodronate significantly decreased the viability only at 500 µmol (p=0.035). Pamidronate had the greatest negative influence, followed by zoledronate and ibandronate. A significant difference was visible for zoledronate (p=0.007) and for pamidronate (p=0.004) at 50 µmol compared to the control (100%). The

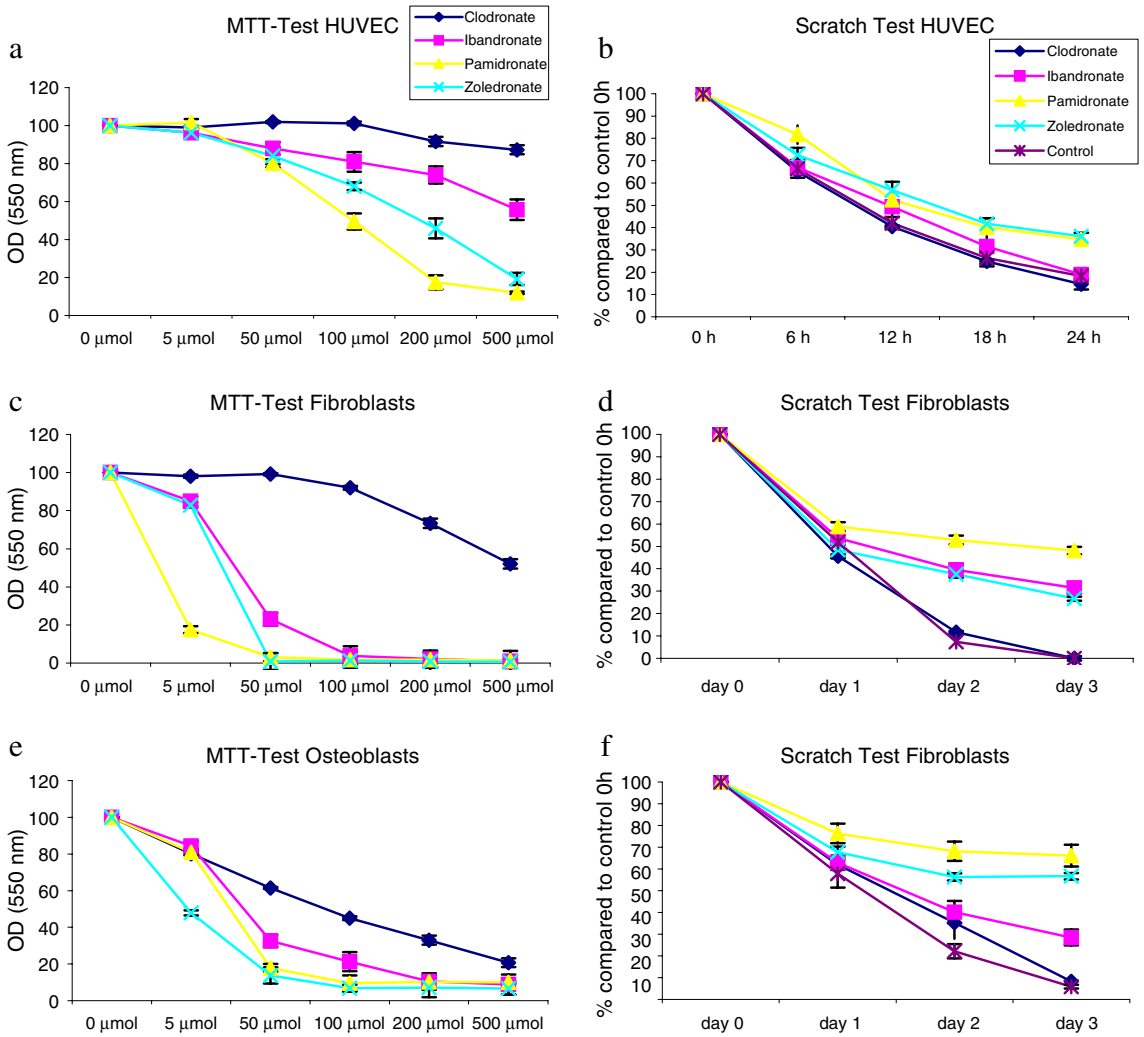

Fig. 1 **a**–**f** Viability results are shown on the *left-hand side* (**a**, **c**, **e**), scratch wound assays (50 µmol bisphosphonate) on the *right-hand side* (**b**, **d**, **f**). Results for HUVEC are shown in the *first line* (**a**, **b**), fibroblasts in the *second line* (**c**, **d**) and osteogenic cells in the *last line* (**e**, **f**)

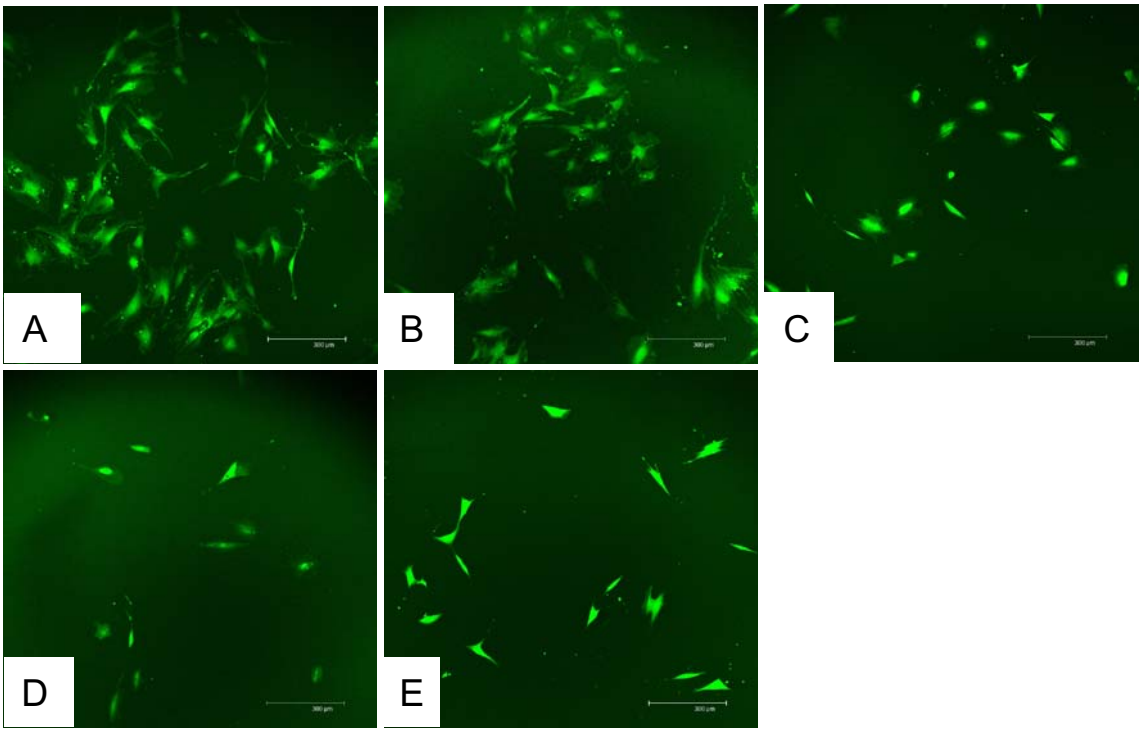

Fig. 2 Green fluorescence mode CLSM visualization of vital-stained (green cell tracker) osteogenic cells cultivated for 72 h in the presence of different bisphosphonates at 100 µmol. Original magnification: tenfold (**a** control, **b** clodronate, **c** ibandronate, **d** pamidronate, **e** zoledronate)

difference between zoledronate and pamidronate became significant at 100 µmol (p=0.038), likewise for zoledronate and ibandronate at 200 µmol (p=0.001).

Scratch wound assay HUVEC

The scratch wound assay showed significant differences between bisphosphonates and the control after 18 h (p=0.006) for zoledronate only (p=0.043). A low p-value was seen for pamidronate vs. control after 18 h (p=0.08), which became significant after 24 h (p<0.001). After 24 h, both pamidronate and zoledronate showed a significant difference compared to the control, clodronate, and pamidronate (p<0.001). No further significant differences between the bisphosphonates and the control for HUVEC could be shown (Fig. 1).

MTT fibroblasts

The aminobisphosphonates pamidronate, zoledronate, and ibandronate showed the greatest inhibition of fibroblast viability. The influence of clodronate on fibroblasts could be proved at 200 µmol (p=0.001) and higher. The aminobisphosphonates revealed differences on cell viability in lower concentrations. The cell viability was most affected by pamidronate and showed significant inhibition at 5 µmol as compared to the control, zoledronate, and ibandronate (p<0.001). Zoledronate had a greater negative impact on the viability of fibroblasts as compared to ibandronate at 50 µmol (p=0.01).

Scratch wound assay fibroblasts

A similar effect can be seen in the fibroblast wound healing assay; the influence of all aminobisphosphonates in this test became statistically significant after 2 days (p<0.001). Clodronate did not yield significant results at any time point.

MTT osteogenic cells

The viability of osteogenic cells was reduced by all bisphosphonates in a dose-dependent manner. Zoledronate was the only bisphosphonate that showed a significant difference at 5 µmol compared to the control (p<0.001). At 50 µmol, all aminobisphosphonates significantly inhibited osteogenic cell viability compared to clodronate. This difference remained significant up to 200 µmol. At 500 µmol, only zoledronate showed a statistically significant difference compared to clodronate (p=0.038). At 50 and 100 µmol, the difference between the other aminobisphosphonates ibandroante and pamidroante compared to clodronate was significant (p<0.003).

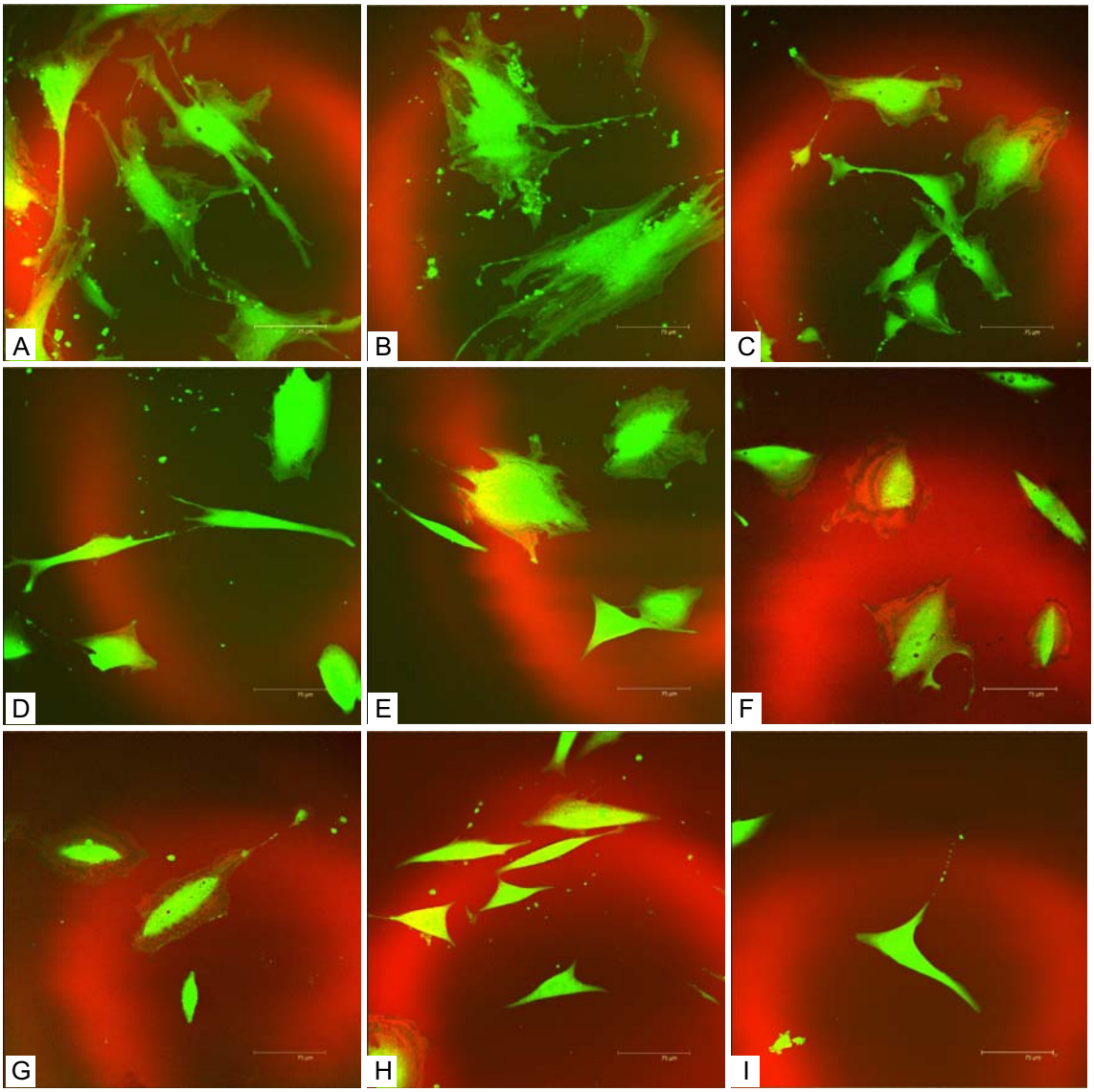

Fig. 3 CLSM visualization of vital-stained (green cell tracker) osteogenic cells cultivated for 72 h in the presence of different bisphosphonates. Merged images of green fluorescence mode and CLSM reflection mode. Original magnification: 40-fold (**a** control, **b** clodronate 50 µmol, **c** clodronate 100 µmol, **d** ibandronate 50 µmol, **e** ibandronate 100 µmol, **f** pamidronate 50 µmol, **g** pamidronate 100 µmol, **h** zoledronate 50 µmol, **i** zoledronate 100 µmol)

Scratch wound assay osteogenic cells

Pamidronate had the greatest impact on the scratch wound in an osteogenic cell monolayer culture, followed by zoledronate and ibandronate. There was no significant impact of clodronate on the migration and proliferation of the cells at any point in time. The one-way ANOVA showed significance on day 2. With the *t*-test, a significant effect at day 1 ($p=0.04$) for pamidronate was shown, compared to the control. On day 2, pamidronate and zoledronate showed a significant delay of wound closure compared to the control ($p<0.001$) and clodronate ($p=0.001$, respectively, 0.036), whereas the difference for ibandronate compared to the control became significant on day 3 ($p<0.001$). On day 3, the difference between the impact of ibandronate compared to clodronate became significant ($p=0.002$).

Cell visualization: vital staining of osteogenic cells

Visualization of vital-stained osteogenic cells is shown in Figs. 2 and 3. Figure 2a–e provides an overview at a lower magnification (tenfold) of the untreated control (Fig 2a) vs. the investigated bisphosphonates at 100 µmol (Fig. 2b–e). The normal cell population shows a sub-confluent cell layer with well-spread cells (Fig 2a). The addition of 100 µmol clodronate resulted in a similar overview (Fig. 2b). The

addition of 100 µmol ibandronate resulted in clearly reduced cell density with rather spindle-shaped and roundish cell morphologies (Fig. 2c). The addition of 100 µmol pamidronate (Fig. 2d) or 100 µmol zoledronate (Fig. 2e) led to further reduction of cell density and deprivation of cell morphologies.

Figure 3a–i provides a more detailed analysis of cellular phenotypes at higher magnification (40-fold). Figure 3a–i shows representative osteogenic cell morphologies after 72 h for the investigated bisphosphonates. Normal cellular morphologies consisting of fully spread cells with development of lamellipodias and filopodias can exclusively be identified for the control group (Fig. 3a), as well as after the addition of 50 µmol clodronate (Fig. 3b). The addition of 100 µmol clodronate resulted in a markedly smaller cellular phenotype with deprivation of filopodia (Fig. 3c). The addition of ibandronate resulted for 50 and 100 µmol in small cells with only few lamellipodia. The cells exhibit simple geometric shapes (roundish, spindle-shaped, and triangular), clear indicators for restricted cellular homeostasis (Fig. 3d, e). The addition of pamidronate resulted in similar cellular phenotypes. Furthermore, the stained cellular area seems to be restricted to the central, perinuclear region (Fig. 3f, g). Compared to the other substrates, the addition of zoledronate resulted in the most atrophied cellular phenotype with small, spindle-shaped, and triangular cells (Fig. 3h, i).

Discussion

Several theories on the development of bisphosphonate-associated osteonecrosis of the jaws are being discussed in the literature. Next to the cell death of osteoclasts and an impact on osteogenic cells with a consecutive altered bone remodeling [21, 22], important factors seem to be the bisphosphonate's antiangiogenetic potential and the development of avascular bone necrosis [23]. Another theory is the adverse impact of bisphosphonates on the integrity of the mucosal layer, due to the proapoptotic effect on keratinocytes of the gastrointestinal tract [16, 24] and the oral cavity [25]. This study's data could support a multifactorial genesis of bisphosphonate-associated osteonecrosis of the jaws.

The bioavailability of an oral dose is between 1% and 10%. Twenty percent to 80% of the absorbed bisphosphonates is taken up quickly by bone, with a higher percentage at sites of bone formation [26] and an even higher percentage at sites of bone resorption [27]. It has been described that the bone turnover in the jaws, especially at the alveolar crest, is higher compared to other parts of the skeleton [28], resulting in a higher accumulation of bisphosphonates in this region. The ability of the different bisphosphonates to chelate with Ca^{2+} is decreased at lower pH levels due to protonation of the bisphosphonate's phosphate groups. Therefore, bisphosphonates may be released faster from bone surfaces in the acid environment of an osteoclast resorption lacuna, resulting in locally high concentrations of bisphosphonates. These higher concentrations might be prevalent especially after a dental surgical procedure. The high bisphosphonate concentration could influence the cells in the immediate microenvironment such as osteoblasts, vessel cells, fibroblasts, and keratinocytes. The combined impact of bisphosphonates on the different cell types might contribute to disturbed wound healing after injury, resulting in clinically manifest BP-ONJ.

The bisphosphonates most often associated with BP-ONJ are the aminobisphosphonates, most notably pamidronate and zoledronate [2, 29], followed by ibandronate [3] or alendronate [30]. Only single cases of BP-ONJ have been published in patients taking non-nitrogen-containing bisphosphonates [31].

A reason for this distribution might be the different impact of these bisphosphonates on the different cell populations of the oral cavity as shown in these experiments. In all assays, the aminobisphosphonates ibandronate, pamidronate, and zoledronate had a distinct negative effect on all tested cell lines compared to the non-nitrogen-containing clodronate. In the comparison of the aminobisphosphonates, zoledronate and pamidronate had the biggest effect on the cell lines. Only in the fibroblast scratch wound assay did ibandronate have a greater, but not significant, impact on the migration and proliferation as compared to zoledronate.

In the cell visualization assay, zoledronate and pamidronate had the greatest effect, whereas the effect of ibandronate and especially clodronate was not as high. Since cell attributes such as adhesion and differentiation are closely associated with characteristic cell morphologies, these findings support the greater impact of pamidronate and zoledronate on osteogenic cell homeostasis.

The results presented may explain the higher occurrence of BP-ONJ in patients receiving aminobisphosphonates as compared to patients receiving non-nitrogen-containing bisphosphonates. Although it is difficult to compare an in vitro situation with an in vivo situation, these results show that it may be prudent to interrupt bisphosphonate treatment if oral surgery is necessary in order to protect the soft tissues and promote better wound healing. A better understanding of the effects of bisphosphonates on a cellular level might help to adjust therapeutic regimes in order to maintain clinical success while reducing the side effects.

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Conflict of interest None.

References

1. Diel IJ, Bergner R, Grotz KA (2007) Adverse effects of bisphosphonates: current issues. J Support Oncol 5(10):475–482
2. Marx RE (2003) Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of jaws: a growing epidemic. J Oral Maxillofac Surg 61(9):1115–1117
3. Walter C, Grotz KA, Kunkel M et al (2007) Prevalence of bisphosphonate associated osteonecrosis of the jaw within the field of osteonecrosis. Support Care Cancer 15(2):197–202
4. Advisory Task Force on Bisphosphonate-Related Ostenonecrosis of the Jaws AAoOaMS (2007) American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws. J Oral Maxillofac Surg 65(3):369–376
5. Hoff AO, Toth BB, Altundag K et al (2008) Frequency and risk factors associated with osteonecrosis of the jaw in cancer patients treated with intravenous bisphosphonates. J Bone Miner Res 23 (6):826–836
6. Aguiar Bujanda D, Bohn Sarmiento U, Cabrera Suarez MA et al (2007) Assessment of renal toxicity and osteonecrosis of the jaws in patients receiving zoledronic acid for bone metastasis. Ann Oncol 18(3):556–560
7. Boonyapakorn T, Schirmer I, Reichart PA et al (2008) Bisphosphonate-induced osteonecrosis of the jaws: prospective study of 80 patients with multiple myeloma and other malignancies. Oral Oncol 44(9):857–869
8. Wang EP, Kaban LB, Strewler GJ et al (2007) Incidence of osteonecrosis of the jaw in patients with multiple myeloma and breast or prostate cancer on intravenous bisphosphonate therapy. J Oral Maxillofac Surg 65(7):1328–1331
9. Walter C, Al-Nawas B, Grotz KA et al (2008) Prevalence and risk factors of bisphosphonate-associated osteonecrosis of the jaw in prostate cancer patients with advanced disease treated with zoledronate. Eur Urol 54(5):1066–1072
10. Rizzoli R, Burlet N, Cahall D et al (2008) Osteonecrosis of the jaw and bisphosphonate treatment for osteoporosis. Bone 42 (5):841–847
11. Walter C, Al-Nawas B, Wagner W (2008) Implantology as a reason for bisphosphonate-associated-osteonecrosis of the jaws (BP-ONJ). Clin Oral Implants Res 19(9):850
12. Mashiba T, Mori S, Burr DB et al (2005) The effects of suppressed bone remodeling by bisphosphonates on microdamage accumulation and degree of mineralization in the cortical bone of dog rib. J Bone Miner Metab 23(Suppl):36–42
13. Santini D, Vincenzi B, Avvisati G et al (2002) Pamidronate induces modifications of circulating angiogenetic factors in cancer patients. Clin Cancer Res 8(5):1080–1084
14. Allegra A, Oteri G, Nastro E et al (2007) Patients with bisphosphonates-associated osteonecrosis of the jaw have reduced circulating endothelial cells. Hematol Oncol 25(4):164–169
15. Ziebart T, Yoon CH, Trepels T et al (2008) Sustained persistence of transplanted proangiogenic cells contributes to neovascularization and cardiac function after ischemia. Circ Res 103(11):1327–1334
16. Landesberg R, Cozin M, Cremers S et al (2008) Inhibition of oral mucosal cell wound healing by bisphosphonates. J Oral Maxillofac Surg 66(5):839–847
17. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1–2):55–63
18. Kim HJ, Kim SH, Kim MS et al (2005) Varying Ti-6Al-4V surface roughness induces different early morphologic and molecular responses in MG63 osteoblast-like cells. J Biomed Mater Res A 74(3):366–373
19. Park BS, Heo SJ, Kim CS et al (2005) Effects of adhesion molecules on the behavior of osteoblast-like cells and normal human fibroblasts on different titanium surfaces. J Biomed Mater Res A 74(4):640–651
20. Klein MO, Tapety F, Sandhöfer F et al (2006) Visualization of vital osteogenic cells on differently treated titanium surfaces using confocal laser scanning microscopy (CLSM)—a pilot study. Z Zahnärztl Implantol 21(4):145–153
21. Mashiba T, Hirano T, Turner CH et al (2000) Suppressed bone turnover by bisphosphonates increases microdamage accumulation and reduces some biomechanical properties in dog rib. J Bone Miner Res 15(4):613–620
22. Rogers MJ, Watts DJ, Russell RG (1997) Overview of bisphosphonates. Cancer 80(8 Suppl):1652–1660
23. Marx RE, Sawatari Y, Fortin M et al (2005) Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: risk factors, recognition, prevention, and treatment. J Oral Maxillofac Surg 63(11):1567–1575
24. Reszka AA, Halasy-Nagy J, Rodan GA (2001) Nitrogen-bisphosphonates block retinoblastoma phosphorylation and cell growth by inhibiting the cholesterol biosynthetic pathway in a keratinocyte model for esophageal irritation. Mol Pharmacol 59 (2):193–202
25. Reid IR, Bolland MJ, Grey AB (2007) Is bisphosphonate-associated osteonecrosis of the jaw caused by soft tissue toxicity? Bone 41(3):318–320
26. Fleisch H (2003) Bisphosphonates in osteoporosis. Eur Spine J 12 (Suppl 2):S142–S146
27. Rogers MJ, Gordon S, Benford HL et al (2000) Cellular and molecular mechanisms of action of bisphosphonates. Cancer 88 (12 Suppl):2961–2978
28. Huja SS, Fernandez SA, Hill KJ et al (2006) Remodeling dynamics in the alveolar process in skeletally mature dogs. Anat Rec A Discov Mol Cell Evol Biol 288(12):1243–1249
29. Ruggiero SL, Mehrotra B, Rosenberg TJ et al (2004) Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. J Oral Maxillofac Surg 62(5):527–534
30. Marx RE, Cillo JE Jr, Ulloa JJ (2007) Oral bisphosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention, and treatment. J Oral Maxillofac Surg 65 (12):2397–2410
31. Montazeri AH, Erskine JG, McQuaker IG (2007) Oral sodium clodronate induced osteonecrosis of the jaw in a patient with myeloma. Eur J Haematol 79(1):69–71