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Bisphosphonate-osteoclasts: Changes in osteoclast morphology and function induced by antiresorptive nitrogen-containing bisphosphonate treatment in osteoporosis patients

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ABSTRACT

Osteoclasts are unique cells capable of bone resorption and therefore have become a major target in osteoporosis treatment strategies. Bisphosphonates suppress bone turnover via interference with the internal enzymatic cell system of osteoclasts leading to cytoskeletal disruption. This mechanism found its clinical relevance in reducing bone resorption, stabilizing bone mass and reducing fracture risk in osteoporosis patients. However, knowledge about specific in vivo changes in osteoclast cell morphology and function is still insufficient. We examined osteoclasts in 23 paired bone biopsies from osteoporosis patients (18 males, 5 females; age: 52.6 ± 11.5 yrs) under nitrogen-containing bisphosphonate administration with a mean treatment duration of three years. Formalin-fixed, undecalcified sections were assessed by qualitative and quantitative bone histomorphometry, where the osteoclast morphology, nuclei, distribution, location as well as resorption parameters were investigated to obtain information about cell function and viability. After three years of treatment, resorption parameters decreased significantly while the number of osteoclasts remained unchanged. Out of 23 patients, nine developed previously termed "giant-osteoclasts" with increased size, numerous nuclei (>10 nuclei/Oc) and oftentimes detachment from the bone surface. These cells frequently had pycnotic nuclei and other morphological signs suggestive of osteoclast apoptosis. Characteristic large-sized osteoclasts were uniquely found in patients treated with nitrogen-containing bisphosphonates, thus being clearly distinguishable from giant-osteoclasts in other bone disorders such as Paget disease, secondary hyperparathyroidism or osteopetrosis. The resorption indices of large-sized osteoclasts, specifically the eroded perimeter and erosion depth, revealed significantly reduced values but not an entirely inhibited resorption capability. Bisphosphonate-osteoclasts' viability and affinity to bone seem significantly disturbed while the apoptotic process may be prolonged for a yet unknown period of time in favor of maintaining a low bone turnover.

Introduction

Bisphosphonates (BP) as a treatment for osteoporosis have been in clinical use for over two decades and proven to be effective in fracture prevention [1–3]. Following early laboratory experiments by Fleisch et al. in the late 1960s [4], several bone markers and histomorphometry studies have shown the antiresorptive action of BP. Based on their effects on osteoclasts' (Oc) function, BP were reported to cause a general reduction in bone turnover, the degree of which can be perceived as a surrogate parameter for treatment efficacy [5–11]. BPs are incorporated into bone for an extended period of time and continue to affect bone metabolism long after cessation of intake [11,12]. The substance is relatively safe, however, possible osteonecrosis of the jaw in oncology patients under high-dose BP administration and atypical femoral fractures are nowadays believed to be caused by oversuppression of bone remodeling — although this is still a matter of debate [11,13–19].

BP molecules have a strong affinity to the bone surface [20] where nitrogen-containing BPs (N-BP) once assimilated by osteoclasts inhibit the mevalonate pathway enzyme farnesyldiphosphate (FPP) synthase and disrupt the cytoskeleton which affects the ruffled border and results in a loss of function [21]. Given that the osteoclasts/osteoblasts ratio in histological sections is approximately 1:6 [22] and since osteoclasts display a three- to four-fold shorter lifespan than osteoblasts, the cells capable of bone resorption are rare in histological sections [23,24]. The short time span of Oc apoptosis makes this brief stage a rarely observed event in histological sections which makes it difficult to acquire large subgroups of this cell type with special findings. Despite extensive research on both the therapeutic benefits and side effects of BP, the true nature of their effects on osteoclast morphology, vitality, life-span and fate remains inconclusive.

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Following earlier histological studies that described treatment-induced changes in the Oc phenotype, Weinstein et al. reported data on "giant osteoclast formation after long-term oral BP therapy" [25]. Weinstein's study in a clinical setting frequently showed morphological changes and apoptosis of osteoclasts, as well as an increase in Oc number following BP treatment. Similar morphological cell changes have also been observed by others and ourselves [26–29]. However, so far a number of questions regarding the affected osteoclasts remain unanswered, such as i) how the bisphosphonates affect Oc function as reflected in erosion surface and resorption depth in osteoporotic patients, and ii) whether there is discordance between osteoclast number and anti-resorptive effects. In addition, the effect of BP in a male cohort without the influence of hormonal changes, the role of intravenous BP administration, as well as the influence of different BP classes all require proper attention [30,31].

To directly explore the effects of N-BP treatment on osteoclasts' morphology and function in each individual, we conducted a histomorphometric paired-biopsy study in osteoporosis patients before and after the anti-resorptive bisphosphonate treatment.

Material and methods

Bone biopsies from the dorsal iliac crest were acquired by an experienced physician performing the Jamshidi technique from 37 patients at one institution, as described previously [32]. Patients had osteoporosis as defined by the WHO and confirmed by dual X-ray absorptiometry. For each patient, a baseline biopsy and a follow-up biopsy were available. In the treatment group, 23 patients (18 males, 5 females; age: 52.6 ± 11.5 yrs) underwent bone biopsy at two time points: at baseline (before starting BP treatment) and during BP treatment (mean follow-up of 38 ± 6 months). All patients gave written informed consent to the clinical investigator for the treatment and iliac crest biopsies. The study was approved by the local ethics committee and conducted in accordance with the guidelines of good clinical practice.

Patients with skeletal disorders and secondary osteoporosis or medication interfering with bone metabolism (other than BP) were excluded. All patients received standard calcium and vitamin D supplementation, depending on habitual daily intake. Half of the patients received intravenous nitrogen-containing BP of the 2nd or 3rd generation (ibandronate or pamidronate), whereas the other half took the recommended dosage of alendronate through oral intake. Laboratory data such as alkaline phosphatase, creatinine, phosphorus and calcium serum levels were taken and did not indicate non-compliance with the BP treatment.

For reference of normal osteoclast morphology, two additional control groups were assessed qualitatively: paired biopsies of fourteen control untreated osteoporosis patients (5 females, 9 males; age: 49.5 ± 12.8 yrs) with a median follow-up of 79 ± 49.6 months, as well as negative controls comprising single bone biopsies from 36 skeletal healthy (20 females, 16 males; age: 49.5 ± 12.8 yrs) individuals.

Histomorphometry

All biopsy specimens were embedded undecalcified in polymethylmethacrylate (PMMA) to prevent shrinking artifacts and artificial changes in cell morphology or location, which was crucial information in this study. Bone cores were 2–2.5 cm long and had a diameter of 2.5–3 mm. These biopsies contain approximately 60 mm^2 of well-preserved bone tissue, which is a prerequisite for taking valid histomorphometry data as shown in previous studies [e.g.: 32–35]. In total, all visible osteoclasts per bone biopsy were considered for both qualitative and quantitative assessments. Bone tissue from aged individuals generally shows a low number of osteoclasts, regardless of the biopsy technique [36]. In our specimens, the number of osteoclasts varied between 1 and 10 osteoclasts per section. The osteoclast perimeter per bone perimeter varied between 0.65% and 1.2%, while the osteoclast number per bone perimeter varied between 0.16/mm and 0.28/mm, which is compatible with other studies [36,25].

Longitudinal 5-μm-thick sections were taken from the specimens using a rotation microtome (Cut 4060E, MicroTech, Munich, Germany). Four consecutive sections were performed for the following standard stainings: Goldner–Masson for determination of remodeling surfaces and exclusion of mineralization defects, Giemsa for bone marrow investigation and osteoclast morphology, tartrate-resistant acid phosphatase (TRAP) for count and localization of osteoclasts- and von Kossa for evaluation of bone surface indices and resorption lacunae.

We specifically focused on osteoclasts in this study and quantitatively evaluated histomorphometric indices, including eroded perimeter per bone perimeter (E.Pm/B.Pm), osteoclast perimeter per bone perimeter (Oc.Pm/B.Pm), number of osteoclasts per bone perimeter (N.Oc/B.Pm) and erosion depth (E.De) following ASBMR standards as described previously [26,37,38]. Measurements were carried out using software for quantitative histomorphometry (OsteoMeasure, Osteometrics Inc., Decatur, GA, USA).

Osteoclasts (Oc)

The following six criteria, adapted from Weinstein et al. [25] and considering also data on normal osteoclast characteristics [39–42] were used to define the altered Oc phenotype following BP treatment. Oc with more than 10 nuclei, fulfilling also at least two of the following criteria were classified as BP-Oc: 1) more than 80 μm in the longest diameter, 2) round cell shape, 3) detachment from bone surface, 4) underlying flat lining cells, and 5) condensation of nuclei (pycnotic). At least two BP-Oc had to be diagnosed in each biopsy to be counted as a true finding.

Osteoclasts were classified as apoptotic when nuclei showed significant condensation, fragmentation, polymorphism and disintegration. Additionally disrupted or irregular cell borders gave further evidence of cell death.

In our report and discussion of unusual osteoclast morphology under N-BP treatment as described above, we refer to 'Bisphosphonate-Osteoclasts' (BP-Oc) in order to avoid confusion with 'giant or large osteoclasts' related to the characterization of other diseases, such as Paget disease of bone. Overview of differential diagnosis is presented in Fig. 1.

Based on the literature data, flat elongated cells on the bone surfaces with darkly stained nuclei and few organelles were histologically considered as bone lining cells [41,42].

Statistical analysis

The Kolmogorov–Smirnov test was used to confirm the normality of the data distribution. The Student's *t*-test for paired samples was applied to explore the differences in histomorphometric data between the baseline and follow-up. Two groups of BP-treated cases were compared using independent samples *t*-test. All analyses were performed in Statistical Package for Social Sciences (SPSS version 15.0) at the 0.05 level of significance. The data are expressed as mean ± SD.

Results

Osteoclast morphology and resorption patterns

Unusual osteoclast morphology or hypernucleation (>3–4 nuclei) was not observed in the two control groups or at baseline in the treatment group. However, in 39.1% of patients in the N-BP treatment group, there was an obvious increase in osteoclast cell size and shape, as well as in the number of nuclei (>10 nuclei/Oc) in follow-up biopsies (Fig. 2A). Six out of nine patients who developed bisphosphonate osteoclasts had received oral alendronate treatment, while the remaining three received intravenous pamidronate. The majority of these hypernucleated