Intravenous bisphosphonate therapy does not thicken cementum or change periodontal ligaments of cancer patients

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Objective. To test the hypothesis that intravenous (IV) bisphosphonate (BP) therapy thickens or alters the micromorphology of cementum and periodontal ligament (PDL) in cancer patients.

Study Design. Thirty-two teeth extracted from 24 cancer patients and separated into test (patients who have undergone IV BP therapy, n = 16) and control (patients naive to BP therapy, n = 16) groups were studied. Cementum thickness was measured in 3 different areas of the dental root with polarized light microscopy. PDL was assessed by optical light microscopy and the immunohistochemical expression of periostin.

Results. No significant difference was detected in cementum thickness (apical, $P = .06$; medium, $P = .16$; cervical, $P = .18$) between groups. The numbers of fibroblasts in PDL ($P = .56$), incremental lines of cementum ($P = .51$) and the immunohistochemical patterns of periostin expression in PDL ($P = .68$) did not differ between groups.

Conclusion. IV BP therapy does not thicken cementum or change the micromorphology of PDL. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;123:591-599)

Bisphosphonates (BPs) are among the most important classes of antiresorptive agents that have been introduced in the clinical setting for the treatment of a series of metabolic and malignant bone diseases, including bone metastases, tumor-associated hypercalcemia, and multiple myeloma.[1,2]

Oral BPs are often used for benign systemic diseases such as osteoporosis, Paget's disease, and pediatric inherited skeletal disorders and are considered one of the top prescribed drugs worldwide.[2,3] Intravenous (IV) BPs, such as pamidronate and zoledronic acid (ZA), are used routinely in patients with cancer-related conditions such as multiple myeloma, bone metastasis from solid tumors, and hypercalcemia of malignancy.[4,5]

In spite of the medical benefits of these drugs, recent studies have shown a series of side effects, such as BP-related osteonecrosis of the jaw (BRONJ).[4,6] This condition is defined by the American Association of Oral and Maxillofacial Surgeons as the presence of exposed necrotic bone in the maxillofacial region that does not heal within 8 weeks after clinical identification in a patient currently or previously treated with BPs who has never undergone head and neck radiotherapy.[6,7] Several hypotheses have been proposed to describe the etiology of BRONJ, including inhibition of bone resorption by selectively affecting osteoclasts, suppression of bone remodeling, anti-angiogenic effects, infection, and cytotoxicity, among others.[4,8,9]

More recently, a series of dental and periapical alterations have been reported in association with the use of IV BPs. However, the implications of such observations have not been completely understood. To better understand the significance of these changes, several studies have been conducted in animal models[10,11] or in uncontrolled studies based on human teeth extracted from patients with BRONJ.[12,13] In this context, the most commonly observed dental abnormality was hypercementosis, followed by pulp necrosis,

Statement of Clinical Relevance

The present study represents the first attempt to quantitatively characterize possible tooth changes related to intravenous bisphosphonate therapy. Results revealed that intravenous bisphosphonate therapy does not thicken cementum or change the micromorphology of the periodontal ligament.

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pulp stones in the pulp chamber,[2] and interference in root formation and tooth eruption.[10] Additionally, relatively common radiographic findings of tooth-bearing areas in people who have undergone IV BP treatment include osteosclerosis, thickening of cementum and the lamina dura, and widening of the periodontal ligament PDL.[12]

The aim of the present study was to test whether or not IV BP therapy can lead to thickening or alteration of the micromorphology of cementum and PDL of extracted teeth of cancer patients. We combined optical light microscopy observations, polarized light microscopy analyses, and the immunohistochemical expression of periostin, a protein highly expressed by PDL fibroblasts, which is implicated in the maintenance of periodontal integrity.[14]

MATERIALS AND METHODS

Patients

This study was approved by the Ethics Committee of Piracicaba Dental School (protocol 081/2015), University of Campinas São Paulo, Brazil. Thirty-two teeth were extracted from 24 cancer patients and separated into test (n = 16) and control (n = 16) groups. The test group was composed of 16 teeth extracted from 8 patients who had undergone IV BP for the treatment of bone metastases and multiple myeloma from solid tumors. All extractions were indicated due to periodontal disease. Samples from test and control groups were further divided into 2 subgroups: Subgroup 1, polarized light microscopy (n = 8), and subgroup 2, optical light microscopy and immunohistochemistry (n = 8). All patients in the experimental test group received IV BPs at different doses for at least 3 months. All patients underwent periodontal treatment before dental extraction, with the goal of minimizing the risk of BRONJ development in those patients exposed to IV BPs. The periodontal status of all teeth used in this study was classified before the extraction according to the clinical attachment loss (CAL) as slight (1−2 mm), moderate (3−4 mm), or severe (>5 mm), following the periodontal disease classification system of the American Academy of Periodontology.[15] After extraction, all teeth were stored in 10% neutral-buffered formalin solution for 24 hours and further submitted to sample processing.

Micromorphological analysis

For the micromorphological study of cementum and remaining PDL, an optical light microscope (OLM) (DM 5000; Leica, Heerbrugg, Switzerland) was used. The specimens were decalcified in Ana Morse solution (equal volumes 20% sodium citrate and 50% formic acid) at 4°C for 3 weeks, with changes every 2 days.[16] The samples were embedded in Paraplast Plus (Leica Biosystems, Richmond, IL, USA) to produce 5 μm-thick sections on a microtome (Leica, Nussloch, Germany). The sections were deparaffinized with xylol, hydrated in progressive concentrations of ethylic alcohol from 100% to 50%, and stained with hematoxylin for 12 minutes. They were then rinsed under running water and counterstained in eosin for 5 minutes. The specimens were then dehydrated in ethylic alcohol (100%) and cleared in xylol, and the histologic glass slides were mounted for evaluation of the following morphologic structures: number of incremental lines of cementum, presence of inflammatory cells, and number of fibroblasts in the remaining PDL. Hematoxylin and eosin stained slides were evaluated in a descriptive manner to classify inflammation as present or absent in the remaining PDL. In addition, the number of cementum incremental lines and the number of PDL fibroblasts were counted with the use of Image J software (v1.47; National Institutes of Health, Bethesda, MD, USA). Three areas (20× magnification) of the cementum and remaining PDL of each sample were randomly selected, and an average of the values obtained in these 3 areas was obtained, resulting in 1 value per sample. Two-way analysis of variance (test group vs control group; *t* test, parametric) was done at the 5% level of significance.

Polarized light microscopy

Axial sections of 200 μm through the middle of the tooth samples were performed with a low-speed saw (Isomet; Buehler, Lake Bluff, IL USA) under constant water irrigation. Sections were hand-grounded and polished with silicon carbide paper of 600,800 and 1,200 grits to a final thickness of 100 μm. Sections from each sample were investigated after immersion in water and analyzed under the polarized light microscope (DM 5000; Leica, Wetzlar, Germany).[17] The mean thickness was measured in 3 areas of the dental root (apical, medium, and cervical thirds) using LAS software version 4.5 (Leica). Comparisons between test and control groups were performed among homologous teeth to a faithful comparison by using statistical analyses based on the mean thickness values in the apical, medium, and cervical thirds of the cementum. Two-way analysis of variance (test group vs control group; *t* test) was done at the 5% level of significance.

Immunohistochemical analysis

The remaining PDL was analyzed through immunohistochemical expression of the primary anti-periostin