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Denosumab-associated osteonecrosis of the jaw affects osteoclast formation and differentiation: Pathological features of two cases

YUKI MATSUSHITA[1], SAKI HAYASHIDA[1], KOTA MORISHITA[1], HIROSHI SAKAMOTO[1], TOMOFUMI NARUSE[1], YUKI SAKAMOTO[1], SHIN-ICHI YAMADA[1,2], SOUICHI YANAMOTO[1], SHUICHI FUJITA[3], TOHRU IKEDA[3] and MASAHIRO UMEDA[1]

[1]Department of Clinical Oral Oncology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8588; [2]Department of Dentistry and Oral Surgery, Shinshu University School of Medicine, Matsumoto, Nagano 390-8621; [3]Department of Oral Pathology and Bone Metabolism, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8588, Japan

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Abstract. Medication-related osteonecrosis of the jaw (ONJ) is caused by antiresorptive (bisphosphonates and denosumab) and antiangiogenic agents, with the first report of denosumab-related ONJ emerging in 2010. To date, although certain case reports on denosumab-related ONJ have been published, those of ONJ caused by a single application of the drug are scarce. In addition, only one report described the histopathological features of this condition, although not completely; only the sequestrum resected by conservative surgery was evaluated. Although conservative treatment is recommended, the effectiveness of extensive surgery in the early stages of bisphosphonate-related ONJ has been described in recent years. Here we report the clinical and histopathological features of denosumab-related ONJ caused by single application of the drug, which was treated by extensive surgery in two patients. Histopathological analysis revealed a decreased number of osteoclasts in viable bone around the sequestrum, and these appeared morphologically immature, as indicated by the presence of very few nuclei. These findings are different from those for bisphosphonate-related ONJ and may assist in elucidating the mechanism underlying denosumab-related ONJ. Furthermore, extensive surgery may be effective for the management of this condition.

Correspondence to: Dr Yuki Matsushita, Department of Clinical Oral Oncology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
E-mail: matsushita@nagasaki-u.ac.jp

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Introduction

Since Marx (1) first reported bisphosphonate (BP)-related osteonecrosis of the jaw (BRONJ), several cases have been reported worldwide (2). Anti-receptor activator of nuclear factor κ-B ligand (RANKL) antibodies, including denosumab or antiangiogenic agents, are also known to cause ONJ (2). Accordingly, the American Association of Oral and Maxillofacial Surgeons (AAOMS) changed the defined term BRONJ to medication-related ONJ (MRONJ) in 2014 (2). AAOMS basically recommends conservative treatment for the majority of MRONJ cases, excluding those of stage 3 disease or those exhibiting a well-defined sequestrum. However, the optimal treatment strategy remains controversial. In recent years, previous studies described the effectiveness of extensive surgery in the early stages of MRONJ (3,4). Our previous study also observed good outcomes of extensive surgery for MRONJ (5).

The histopathological findings of BRONJ have been evaluated in several previous studies (6-8), which revealed that the viable osteoclasts exhibit the feature of multinucleated giant cells. These giant osteoclasts are detached from the smooth bone surface and have lost their resorptive function (6-8). Furthermore, these abnormal osteoclasts may persist on the site (9). Denosumab-related ONJ was first reported in 2010 (10), with only a few previous reports regarding this being published since then (11-18). However, reports of ONJ caused by single application of denosumab are scarce. In addition, none of the above mentioned reports have described the histopathological features of this condition. Even if histopathological analysis was performed, viable osteoclasts and other bone remodeling-related cells, including osteoblasts and osteocytes were not described, since only the sequestrum, which has no viable cells, was surgically resected, according to the AAOMS recommendations for MRONJ (18).

The present study described the clinical and histopathological features of ONJ caused by single application of denosumab in two patients who were subsequently treated by extensive surgery.

Clinical findings

Case 1. A 50-year-old patient was referred to Nagasaki University Hospital (Nagasaki, Japan). The patient had undergone extraction of a fractured mandibular left second premolar 1 year previously. Three weeks after the extraction, treatment with 120 mg denosumab was administered subcutaneously for bone metastasis from breast cancer. The serum calcium level prior to denosumab treatment was 9.1 mg/dl, while that at the first visit to our department was 8.0 mg/dl. The patient had never received BP treatment. The extracted socket was already covered with oral mucosa and had remained asymptomatic for a while. However, the patient began experiencing pain with bone exposure in the left mandible 1 month prior to presentation at our department. Panoramic radiographs showed a bone defect at the site of the mandibular left second premolar. Computed tomography (CT) revealed bone sclerosis and sequestrum formation (Fig. 1A and B). Although penicillin antibiotics were administered for 2 weeks, the symptoms persisted. A final diagnosis of stage 2 MRONJ was made, and following consultation with the oncologist, marginal resection, including the sequestrum, a mandibular left first premolar, and viable bone around the sequestrum, was performed under general anesthesia. Denosumab was discontinued for 1 month prior to surgery. No recurrence occurred during a follow-up period of 7 months following the surgery (Fig. 1C).

Case 2. A 76-year-old patient was referred to Nagasaki University Hospital. Treatment with 120 mg denosumab was administered subcutaneously for bone metastasis from prostate cancer and was initiated 2 years previously. The serum calcium level prior to denosumab treatment was 8.5 mg/dl, while that at the first visit to our department was 8.7 mg/dl. The patient had never received BP treatment and had undergone root canal treatment in the maxillary left second molar 3 months previously. Although treatment was completed, the pain and swelling persisted. Therefore, the patient was referred to our department for further investigations. The maxillary left second molar was mobile and there was sequestrum formation around the tooth. Panoramic radiographs and CT revealed sequestrum separation and bone sclerosis in the left maxilla, and thickening of the mucous membrane of the maxillary sinus (Fig. 2A and B). A final diagnosis of stage 2 MRONJ was made, and following consultation with the oncologist, partial resection was performed under general anesthesia. The maxillary left first and second molars were extracted, and the sequestrum and surrounding viable bone were resected. There was no recurrence during a 6-month follow-up period after surgery (Fig. 2C).

This study was approved by the institutional review board of Nagasaki University Hospital and each patient provided informed consent for publication of this report.

Histopathological findings

The resected surgical segment was subjected to histopathological analysis, which revealed nearly identical findings in each specimen. Hematoxylin and eosin (HE) staining revealed sequestrum without viable cells, granulation tissue and viable bone with inflammation (Fig. 3A-D). In the necrotic bone, granulation tissue, containing neutrophils, lymphocytes and plasma cells, was observed. A bacterial mass was attached to the sequestrum, which revealed no osteoclasts, osteoblasts and osteocytes, with completely necrotic bone and empty osteocytic lacunae as characteristic findings (Fig. 3A and B). By contrast, in the viable bone, osteocytic lacunae, including viable osteocytes were observed, indicating the viability of the bone in this region (Fig. 3C and D). Bone resorption cavities were observed on the surface. However, the surrounding osteoclasts exhibited specific features, including being few in number despite the presence of bone resorption cavities. In case 2 in particular, barely any osteoclasts were observed. Furthermore, the existing osteoclasts had very few nuclei, giving a morphologically immature appearance. It was occasionally difficult to identify osteoclasts using HE staining. Immunohistochemistry using cathepsin K, which is regarded as a marker for osteoclasts (19), confirmed the findings of the HE staining. The cathepsin K-positive cells with very few nuclei that existed along, or were detached from, the bone surface were observed, predominantly in the case 1 specimen (Fig. 3E and F).

Discussion

Reportedly, the risk ratio for MRONJ in patients who receive anti-RANKL inhibitors for cancer treatment ranges between 0.7 and 1.9% (20,21), which is equivalent to that reported for patients who receive zoledronate treatment (22,23). Although certain previous case reports on denosumab-related ONJ have been published (10-18), only one described the histopathological features (18), which revealed complete osteonecrosis with empty osteocytic lacunae and no osteocytes, osteoblasts or osteoclasts. The authors concluded that the histological features of denosumab-associated ONJ were similar to those of BP-associated ONJ. However, the authors only evaluated the necrotic sequestrum, since only this portion was surgically resected, according to the AAOMS recommendations for MRONJ (2). By contrast, in the present report, viable bone with cells responsible for bone remodeling was observed since extensive surgery was performed, which involves resection of not only the sequestrum, but also viable, inflamed bone (3-5). Furthermore, osteocytic lacunae with osteocytes were clearly observed, permitting distinction between viable and necrotic bone. The osteoclasts in this viable region were few and revealed a decrease in the number of nuclei. It was hypothesized that the maturation of immature osteoclasts around the sequestrum in patients treated with denosumab is inhibited. Although a few immature osteoclasts were observed in the case 1 specimen, there were barely any in the case 2 specimen. This was probably a result of case 2 being older than case 1; therefore, the bone turnover rate was higher in case 1 compared with in case 2. Weinstein *et al* (6) performed a transiliac bone biopsy in patients who received BP treatment and suggested that this treatment is associated with an increase in the number of osteoclasts, which include distinct, giant, hypernucleated and detached osteoclasts that are undergoing protracted apoptosis. Additionally, Cho *et al* (8) observed a notable number of osteoclasts, which were detached from the bony trabeculae in patients with stage 3 BRONJ, treated by partial mandibulectomy. These results suggested that

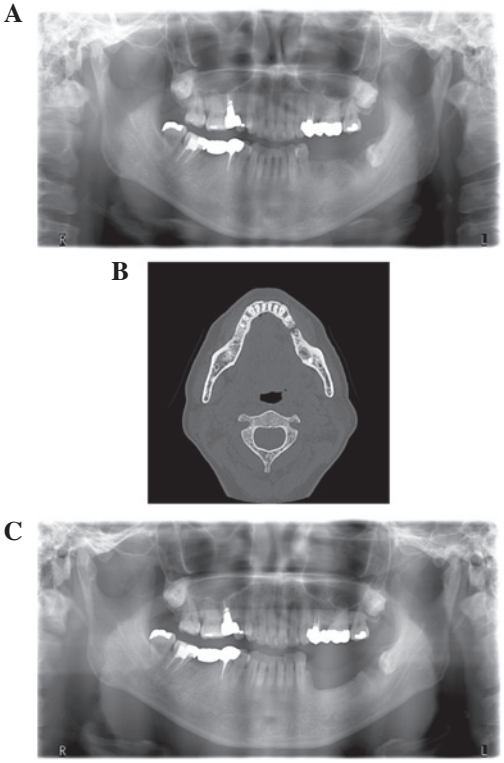

Figure 1. Imaging findings for case 1. (A) Pre-operative panoramic radiographs show bone defects at the site of the mandibular left second premolar. (B) Pre-operative computed tomography shows bone sclerosis and sequestrum formation in the left mandible. (C) Post-operative panoramic radiographs show the resected portion from the left mandible.

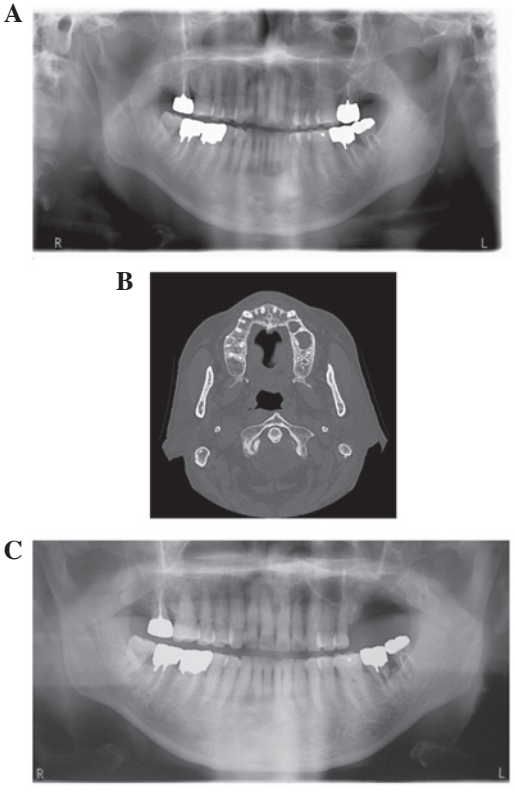

Figure 2. Imaging findings for case 2. (A) Pre-operative panoramic radiographs show sites of bone resorption in the left maxilla. (B) Pre-operative computed tomography shows bone sclerosis and sequestrum formation in the left maxilla. (C) Post-operative panoramic radiographs show the resected portion from the left maxilla.

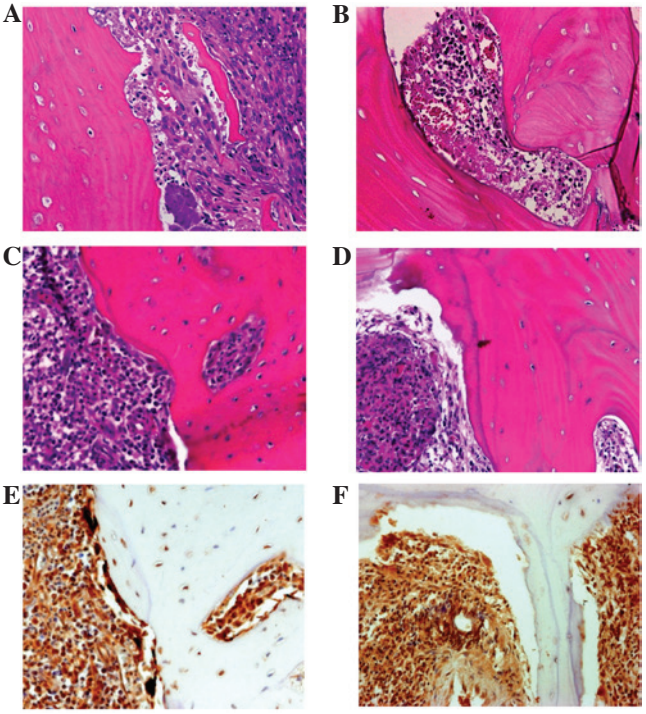

Figure 3. Histopathological features of the resected specimens. (A and B) Sequestrum with empty osteocytic lacunae, no osteoclasts and osteoblasts can be observed, along with inflammatory cell infiltration, in the specimens from (A) case 1 and (B) case 2. (C and D) Viable bone with inflammation can be observed around the sequestrum in the specimens from (C) case 1 and (D) case 2. Osteocytic lacunae containing viable osteocytes were observed. Certain osteoclasts were observed along the bone resorption cavities in (C), however, not in (D). (E) Cathepsin K staining for the case 1 and (F) case 2 specimens. Cathepsin K-positive cells with decreased nuclei, indicating immature osteoclasts, were observed in (E), however, not in (F).

osteoclasts generally remain viable following BP treatment. Therefore, evaluation of viable bone containing cells, including osteoclasts, is important for assessing the effects of denosumab on bone cells.

Denosumab is a fully human monoclonal antibody that targets RANKL (24). Generally, RANKL activates osteoclast differentiation by binding to RANK, a single transmembrane receptor expressed in osteoclast lineage cells. RANKL inhibition prevents the fusion of monocytes and macrophages to form multinucleated osteoclasts. Denosumab prevents RANKL from binding to RANK and subsequently inhibits osteoclast formation, function and survival. By contrast, BPs bind to bone minerals and these are taken up by mature osteoclasts at sites of bone resorption. These osteoclasts subsequently lose their resorptive function and persist (9). Denosumab was found to result in nearly complete disappearance of osteoclasts in an ovariectomized human-RANKL mouse model (25). From this perspective, the histopathological findings of denosumab-related ONJ in the two cases reported in the present study are acceptable. Denosumab is considered to exhibit a faster offset of action compared with BP, and its effects on bone remodeling are mostly diminished within 6 months of treatment cessation (26). Denosumab must be discontinued prior to surgery in patients with MRONJ, if systemic conditions permit. However, the effects of discontinuation remain to be elucidated. The necrotic sequestrum and inflamed bone formed in patients with ONJ are different

from normal bone, and it remains unclear how they exhibit the identical metabolism. In the present study, the serum calcium level was decreased or maintained low by denosumab treatment, indicating the effects of this drug on bone metabolism. Since it was impossible to discontinue denosumab for a long duration in these cases, the present study performed extensive surgery 1 month following discontinuation. The prognosis of each case was good during the postoperative follow-up.

In conclusion, the present study described the clinical and histopathological features of denosumab-related ONJ in two patients. More data should be collected to describe the bone metabolism at the site of denosumab-related ONJ and to elucidate the mechanisms underlying denosumab-related ONJ.

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