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Microbial population changes in patients with medication-related osteonecrosis of the jaw treated with systemic antibiotics

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Objective. This study aimed to investigate the bacterial population in patients with medication-related osteonecrosis of the jaw (MRONJ) after treatment with doxycycline and metronidazole.

Study Design. A total of 38 patients with MRONJ (age range 55-88, mean age 73 + 8.82 standard deviation) treated with doxycycline first and with metronidazole second were enrolled in this study. Two swabs were taken at the margin of the infected MRONJ lesion after applying pressure on the marginal mucosa, and visible pus was secreted. Real-time polymerase chain reaction was used to analyze 20 periopathogenic and commensal species and the total bacterial level. Bacterial counts were compared between antibiotic treatments and with a control group of orally healthy patients who didn't have periodontal pockets of more than 3 mm (n = 29) by means of a Mann-Whitney *U* test. Comparisons between the two antibiotic treatments were performed by a paired Wilcoxon signed rank test.

Results. The total bacterial level was significantly higher in the MRONJ patients treated with systemic antibiotics compared with the control group. However, significant lower bacterial amounts were found for 12 of the 20 investigated bacteria. We couldn't establish a significant advantage of metronidazole administration after doxycycline treatment.

Conclusion. Our findings suggest that the total bacterial level in MRONJ patients is higher even when treated with systemic antibiotics. The significantly different bacterial amounts of the selected species suggest an alteration in the microbial population. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;■■:■■–■■)

Infection is believed to play an important role in the pathogenesis of medication-related osteonecrosis of the jaw (MRONJ), and recently studies have reported bacterial colonization of affected bone in MRONJ.[1-5]

Patients may be considered to have MRONJ if all the following characteristics are present: current or previous treatment with antiresorptive or antiangiogenic agents, exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that has persisted for longer than 8 weeks, and no history of radiation therapy to the jaws or obvious metastatic disease to the jaws.[6] MRONJ involves the maxilla and mandible with preference for the mandible.[7] MRONJ adversely affects quality of life, producing significant morbidity.

There are multiple risk factors, such as tooth extraction, diabetes, tobacco use, trauma to oral tori or caused by prosthetic appliances, poor oral hygiene, malnutrition and bone manipulation.[8-11]

Route of administration, type of medication and treatment duration are other important risk factors.[6,7]

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Infection naturally follows on the bone exposed to the microbial flora of the oral cavity. Almost all the MRONJ specimens are colonized with microbial biofilms.[2,4,9]

Despite the fact that they are routinely exposed to oral microorganisms that consist of more than 750 bacteria, the jaws are normally resistant to colonization. Consequently, for colonization to take place, it is needed to have a combination of patient susceptibility and the presence of potentially pathogenic microorganisms.[3,12]

The source could be the spread of odontogenic or periodontal infection. Microbial biofilms associated with teeth and periodontium that gain access to the underlying compromised bone may play a critical role in the pathogenesis of MRONJ lesions.[13-15] Bone exposure during surgery or tooth extraction works as a trigger that opens the door for bacterial invasion. This could explain the strong relationship between MRONJ and dental surgery. Furthermore, the higher sensitivity of jaws to infection, compared with other bones, strengthens this hypothesis. Jawbones come in direct contact with the external environment because of the fine layer of overlying mucosa, nonstop exposure to trauma, and presence of teeth.[16] Another fact is that MRONJ is common in cancer

Statement of Clinical Relevance

This line of investigation will be critical in the future for clinical approaches to disease intervention and for targeted antimicrobial therapeutics in patients with medication-related osteonecrosis of the jaw.

patients who are receiving chemotherapy, including immunosuppressants and corticosteroids, which give greater susceptibility to dental infection. Furthermore, medical comorbidities such as diabetes are found in MRONJ patients, and studies have reported a direct correlation with chronic periodontitis and impaired wound healing, leading to bacteria-induced bone loss.[17,18]

A biofilm is a complex community of sessile bacterial and fungal organisms attached to a surface. Biofilm organisms differ substantially from their free-floating counterparts because they are characterized by a community of cells that are attached to a substrate. They are enclosed in a matrix of extracellular polymeric substances that they have produced to connect to and to communicate with each other and with the environment. Additionally they display an altered phenotype regarding growth rate, gene transcription, and antimicrobial resistance.[3,19] A microbial biofilm applies chemical communication using signaling molecules named quorum sensing compounds as well as electrical communication via nanowires.[5,20,21] Microbial biofilms are thought to play a part in the pathogenesis of 65%-80% of all chronic infections.[4] Complex mixed-species biofilms have been reported and studied in many diseases, including dental caries, chronic periodontitis, and apical periodontitis.[14,15] Even though the presence of bacterial biofilms may not directly induce necrosis, it seems to have an important contributing role in MRONJ.

The clinical problem with MRONJ is a chronic microbial biofilm infection of bone, in the context of antiresorptive or antiangiogenic agents.[5]

Classic methods of screening and culturing for infectious disease commonly miss biofilm bacteria because these techniques are based on planktonic bacterial growth. Oral bacteria have evolved over millions of years in mixed biofilm populations, and these organisms are often unculturable. Additionally, cultures from the oral cavity have high rates of contamination, and exposure to oxygen kills a lot of the anaerobic bacteria. Biofilm identification requires direct visualization methods with advanced microscopy or DNA-and RNA-based techniques.[5]

One of the central studies that supports the concept that bone containing bisphosphonates is more sensitive to bacterial colonization was performed by Ganguli et al.[17] who reported that bacterial adhesion to pamidronate-coated hydroxyapatite (HA) was 60-fold greater than uncoated HA and 90-fold greater than clodronate-coated HA. Because clodronate is a chlorine-based bisphosphonate and not a nitrogen-based compound like pamidronate or most other bisphosphonates, these data suggest that the nitrogen moiety of the bisphosphonate compound may play a role in the pathogenesis of MRONJ. The structure of these bisphosphonate compounds could play an important role in the disease process of MRONJ.[18]

Initially it was thought that osteonecrosis was bisphosphonate related, but recently there have been reports of MRONJ with denosumab, a RANKL antibody and very strong antiresorptive.[22,23] These findings not only expand the list of drugs associated with MRONJ, they change our views on the pathophysiology of this chronic disease. Although they have different mechanism of action, denosumab and bisphosphonates both reduce the rate of bone loss, an effect that might be crucial in the etiology of MRONJ.

Several pathogenic bacteria found in the oral cavity can invade jawbones and cause bone destruction through different direct and indirect mechanisms. There are many suggested mechanisms, including direct damage to the noncellular bone matrix by bacterial acids and proteases, interaction of bacterial factors directly with bone cells, or indirectly through inflammatory agents, resulting in bone degradation and impairment of bone formation processes. Another mechanism is the invasion of osteoblasts by bacteria, producing functional disturbances and apoptosis, leading to inhibition of bone remodeling.[24] Bacterial chemical mediators of bone resorption consist of proteins such as porins and collagen-degrading enzymes, by which they obtain fundamental amino acids for growth or create an anaerobic environment in the bone for further growth and spread.[25,26]

Infection playing an important role in the pathogenesis of MRONJ could imply the need for more rational antibiotic therapy, with an efficient system of application of antibiotics to the hypovascular and hypocellular bone. That is, it should be taken into account that systemic antibiotic therapy may have a limited effect on the bacterial population associated with MRONJ lesions.[16] Here, we report the total bacterial level and the amount of selected oral bacterial phylotypes that colonize the jawbone of stage 2 and stage 3 MRONJ patients treated with systemic antibiotics and antimicrobial rinses. We also investigate the advantage of metronidazole administration after doxycycline treatment. We used real-time polymerase chain reaction (RT-PCR) analyses for 20 selected periopathogenic and commensal species.

MATERIAL AND METHODS

Sample collection

The study received approval from the Ethical Committee of the University Hospitals of the Catholic University of Leuven (S57399), was registered in the Institutional Clinical Trials database, and was conducted according to the ICH-GCP (International Conference on Harmonization Guidelines on Good Clinical Practice) principles and Helsinki guidelines. The study took place in 2015.

A total of 38 patients (male and female) with a history of intravenous bisphosphonate or denosumab therapy and bone exposure in the oral cavity for more than 8 weeks, as per the definition of MRONJ, were recruited for this