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Bacterial profile and bone healing in rats receiving cancer therapeutic doses of bisphosphonates and corticosteroids: a pilot study

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Abstract. The microbial aetiology of bisphosphonate-related osteonecrosis of the jaw (BRONJ) remains undefined. This study investigated the oral microbiota and socket healing after zoledronic acid (ZA) and dexamethasone (DX) administration. Fourteen rats assigned randomly to experimental ($n = 8$) and control ($n = 6$) groups were injected with ZA+DX or saline, respectively, for 3 weeks prior to and 9 weeks after the extraction of left first upper and lower molars. Whole genomic DNA probes of 38 bacterial species and five *Candida* species were hybridized to DNA extracted from biofilm samples on exposed bone and adjacent teeth. Only experimental rats exhibited exposed bone at euthanasia. All BRONJ-like lesions were colonized by *Staphylococcus pasteuri*, *Streptococcus parasanguinis*, and *Streptococcus mitis*. A significant correlation was observed between the mean proportions of species colonizing BRONJ-like lesions and the teeth of experimental rats ($r = 0.818$, $P < 0.001$). Significant differences were seen in several species colonizing the teeth of control rats compared to experimental rats ($P < 0.05$). Micro-computed tomography analyses revealed higher residual bone in mandibular ($P = 0.001$) and maxillary ($P = 0.108$) tooth sockets of experimental rats. BRONJ-like lesions were colonized mainly by non-pathogenic bacteria. ZA+DX administered to rats at doses equivalent to those given to cancer patients resulted in changes to the oral biofilm and impaired bone healing following tooth extraction.

Key words: bisphosphonates; tooth extraction; oral biofilm; DNA checkerboard; bone repair.

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The use of high doses of anti-resorptive agents such as bisphosphonates and anti-angiogenic agents like sunitinib to treat cancer has been linked to osteonecrosis. Osteonecrosis of the jaw is a serious clinical problem associated with pain, infection, and bone loss.[1] Bisphosphonate-related osteonecrosis of the jaw (BRONJ) has been defined as the presence of exposed, unhealed bone for more than 8 weeks following surgery in the maxillofacial region of patients treated with bisphosphonates, but with no history of radiation to the head and neck region.[2] Bisphosphonates are a class of drugs originally developed to prevent the bone loss caused by excessive osteoclast activity in post-menopausal women. These drugs are currently prescribed to decrease skeletal complications in the management of metastases from solid tumours such as breast and prostate cancers, as well as in the treatment of metabolic bone disease like osteoporosis.[3]

Bacterial infection has been suggested as a contributing factor in the development of BRONJ, although a direct cause–effect relationship has not been demonstrated.[4] A recent study conducted on BRONJ patients showed that the affected area was heavily colonized by bacteria, with *Streptococcus*, *Eubacterium*, and *Pseudoramibacter* being the most prevalent genera.[5] Preclinical studies using animal models of BRONJ-like disease have not investigated the bacteria colonizing the teeth or the area of exposed bone.[6–8] Previous work by Mawardi et al. showed that infection of the extraction sockets with *Fusobacterium nucleatum* in mice treated with high-dose bisphosphonates resulted in delayed wound healing, leaving exposed bone.[9] The BRONJ-like lesions were proposed to arise from reduced proliferation and increased death of gingival fibroblasts, induced by the combination of pamidronate administration and infection with *F. nucleatum*.[9]

The goal of the present study was to characterize the profile of bacteria colonizing the exposed bone and adjacent teeth in a rat model of BRONJ-like disease and also to assess the impact of the bacteria on exposed bone.

Materials and methods

Rats with BRONJ-like disease

The Animal Use Protocol was approved by the necessary authorities. A total of 14 retired breeder female Sprague-Dawley rats (4–6 months old) were divided randomly into control ($n = 6$) and experimental ($n = 8$) groups. Control rats received no medication and experimental rats received zoledronic acid (ZA; 125 μg/kg twice a week) and dexamethasone (DX; 5 mg/kg once a week) for a total of 12 weeks. In a previous work by the present investigators, rats exposed to this drug combination consistently exhibited BRONJ-like lesions at the time of euthanasia, at 4 weeks postoperative.[8] The ZA and DX doses were converted from doses of ZA (8 mg/person/3 weeks) and DX (55 mg/person/week) used for humans and were within the limits described in the literature.[10,11] Doses were converted according to the National Institutes of Health (NIH) guidelines.[12] After 3 weeks, the control and experimental rats were anesthetized and their left maxillary and mandibular first molars extracted (EXO). Drug administration was continued for 9 additional weeks and all control and experimental rats were euthanized using CO_2 inhalation at 12 weeks.

Immediately after CO_2 inhalation, samples of biofilm were collected from sites of exposed bone and from the supragingival region of the adjacent teeth by rubbing with a microbrush (Microbrush International, Grafton, WI, USA) for 30 s until saturation (6 μl), as described previously.[13] The use of a microbrush has been shown to be a suitable method for oral microbial biofilm collection.[13] Given the possible inaccuracy in measuring the weight of the samples, standard-sized microbrush tips that are capable of absorbing a volume of about 6 μl were used. Individual specimens were placed in microtubes containing 150 μl of TE (10 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid (EDTA) pH 7.6) to

Table 1. Human DNA microbial species used to prepare probes for cross-reaction with species extracted from the oral cavity of rats.

Species	Reference	
Aggregatibacter actinomycetemcomitans a	ATCC	29523
Aggregatibacter actinomycetemcomitans b	ATCC	29522
Bacteroides fragilis	ATCC	25285
Campylobacter rectus	ATCC	33238
Candida albicans	ATCC	10231
Candida dubliniensis	ATCC	MYA 646
Candida glabrata	ATCC	90030
Candida krusei	ATCC	6258
Candida tropicalis	ATCC	750
Capnocytophaga gingivalis	ATCC	33624
Eikenella corrodens	ATCC	23834
Enterococcus faecalis	ATCC	51299
Escherichia coli	ATCC	10798
Fusobacterium nucleatum	ATCC	25586
Fusobacterium periodonticum	ATCC	33693
Klebsiella pneumoniae	ATCC	700721
Lactobacillus casei	ATCC	393
Mycoplasma salivarium	ATCC	23064
Neisseria mucosa	ATCC	25996
Parvimonas micra	ATCC	33270
Peptostreptococcus anaerobius	ATCC	49031
Porphyromonas endodontalis	ATCC	35406
Porphyromonas gingivalis	ATCC	33277
Prevotella intermedia	ATCC	25611
Prevotella melaninogenica	ATCC	25845
Prevotella nigrescens	ATCC	33563
Pseudomonas aeruginosa	ATCC	27853
Pseudomonas putida	ATCC	12633
Solobacterium moorei	CCUG	39336
Staphylococcus aureus	ATCC	25923
Staphylococcus pasteuri	ATCC	51129
Streptococcus constellatus	ATCC	27823
Streptococcus gordonii	ATCC	10558
Streptococcus mitis	ATCC	49456
Streptococcus mutans	ATCC	25175
Streptococcus oralis	ATCC	35037
Streptococcus parasanguinis	ATCC	15911
Streptococcus salivarius	ATCC	25975
Streptococcus sanguinis	ATCC	10556
Streptococcus sobrinus	ATCC	27352
Tannerella forsythia	ATCC	43037
Treponema denticola	ATCC	35405
Veillonella parvula	ATCC	10790

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