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Animal models of osteonecrosis of the jaw

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Despite numerous publications that associate bisphosphonate treatment with osteonecrosis of the jaw (ONJ)¹⁻⁵, the exact role of bisphosphonates in the pathophysiology of this condition remains unclear^{6,7}. This is due, in part, to the absence of an established animal model of ONJ. An animal model will be essential to help determine the pathophysiology of ONJ and to establish prevention and management strategies to be tested clinically⁸.

A primary goal in establishing an animal model should be to mimic the clinical presentation of ONJ. Thus, the ideal model would be one in which the animal develops persistent exposed bone in the oral cavity in response to bisphosphonate treatment and without radiation exposure^{5,8}. Given that most ONJ cases have common co-factors in addition to bisphosphonate treatment (e.g., dental trauma, periodontal disease, immunosuppression)^{1,3,8} it most likely will be necessary to combine factors in addition to bisphosphonates in order to mimic the human condition. While a key component of the ONJ working definition is that the exposed bone should exist for >8 weeks^{5,8}, this timeframe is likely to differ in an animal model. Therefore, the exposed bone should persist longer than in an appropriate control group. While achieving a model of exposed bone should be the ultimate goal, animal studies in which exposed bone does not occur should also be undertaken. These studies can provide much needed insight into the effects of bisphosphonates (with or without co-factors) on jaw tissue physiology.

Studies in dogs have documented that bone turnover rate in the jaw exceeds that of the long bones by 6-10-fold under normal conditions, and may increase by an additional 10-

fold following dental extraction^{11,12}. Suppression of turnover, the main mechanism through which bisphosphonates exert their biological effect, is hypothesized to play an integral role in ONJ pathophysiology^{2,3,9,10}. If suppression of intracortical remodeling is a permissive factor in ONJ, then species that undergo intracortical remodeling are most likely to manifest the condition. Working under this assumption, our laboratory has performed studies to evaluate dogs, which undergo intracortical remodeling throughout the skeleton (including the jaw)¹¹, as a potential animal model for ONJ.

As part of a separate study aimed at understanding the effect of bisphosphonate-induced turnover suppression on microdamage and bone mechanics^{13,14}, skeletally-mature, intact female beagles were treated daily for either one or three years with oral doses of vehicle or alendronate (0.20 or 1.0 mg/kg/day). These doses approximate, on a mg/kg basis, those used for post-menopausal osteoporosis and Paget's disease, respectively. There was no sign of exposed oral bone in any of these animals during the course of the study. To determine whether regions of necrosis were present within the bone matrix, four segments of the mandible were stained en bloc with basic fuchsin, which passively diffuses to stain all empty spaces within the tissue (lacunae, canaliculi, Haversian canals, and microcracks)^{15,16}. Histological sections (100-200 μm thick) were cut from the stained blocks and assessed using bright field and confocal microscopy. Regions of bone matrix that were void of basic fuchsin stain and >500 μm^2 were considered necrotic. This minimal size reduces the variability of the analysis as it excludes regions smaller than a single osteon or interstitial region.

Following treatment durations of 1 to 3 years, regions of matrix necrosis were observed in the mandible of 25% of those dogs treated with the lower dose of alendronate (Figure 1). At the higher dose, incidence ranged from 17% (1 year) to 33% (3 years). Similar regions were noticeably absent from all vehicle-treated animals at both 1 and 3 years. There was no difference in the number of animals having necrotic regions or the size of the regions between the two different alendronate doses. These regions of necrosis occurred predominately in

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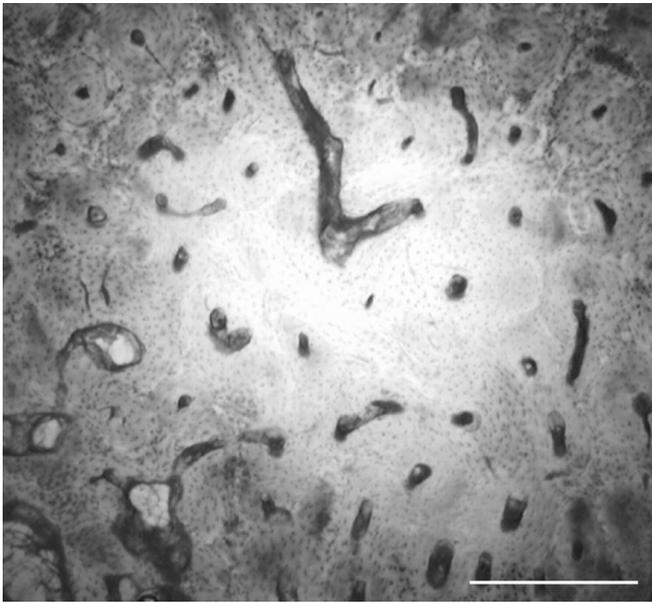


Figure 1. Photomicrograph of matrix necrosis in the mandible of a bisphosphonate-treated dog. Following three years of daily oral bisphosphonate treatment, matrix necrosis was assessed using basic fuchsin en bloc staining. Necrotic bone matrix can be observed using brightfield microscopy as a region that is void of basic fuchsin stain (white). This is contrasted with adjacent tissue, which is heavily stained with fuchsin (dark), evidence of stain permeation throughout the osteocyte-canalicular network. Scale bar = 500 μm .

the alveolar bone and were clearly void of patent canaliculi when viewed using confocal microscopy. The absence of necrotic regions in any of the vehicle-treated animals suggests a direct role of bisphosphonate treatment in this matrix necrosis. We hypothesize this may represent an early stage of ONJ with the eventual manifestation to exposed bone upon the addition of physiological stressors (e.g. dental extraction). Preliminary results from a follow-up study in dogs administered intravenous zoledronate, at dosing regimens consistent with those used for cancer patients, show focal regions of non-viable osteocytes (assessed using lactate dehydrogenase histochemistry) in the alveolar bone after three months of treatment. No such regions were present in vehicle or oral alendronate animals. Collectively, these dog studies provide provocative findings of non-viable mandible bone matrix following bisphosphonate treatment, although it remains unclear if these changes are part of the ONJ pathophysiology.

The matrix necrosis observed in these dogs is not confined to the mandible. Using similar staining techniques as described above, matrix necrosis has also been observed in the ribs of bisphosphonate-treated, but not vehicle-treated, animals. The rib is a high turnover site, which has been shown to experience significant turnover suppression with bisphosphonates¹⁷. Existence of matrix necrosis in the rib is therefore consistent with the proposed role of turnover suppression in the manifestation of matrix necrosis. It also suggests that the

mechanisms underlying matrix necrosis with bisphosphonate treatment, if found to be part of the ONJ pathophysiology, could be studied at sites other than the mandible.

If intracortical bone turnover suppression is a factor in the development of osteonecrosis in the jaw, then rabbits might be a smaller, less expensive model in which to study this process. Rabbits are known to have significant bone remodeling in their long bones. However, our cursory examination of mandibles from non-bisphosphonate treated rabbits revealed that young, but skeletally-mature (>1 year) rabbits have very few secondary osteons, suggesting a low intracortical bone turnover state.

Rodents are a standard animal model for many skeletal conditions. If suppression of intracortical turnover plays a role in the manifestation of ONJ, the generalized absence of normal intracortical remodeling in rodents would limit their usefulness as a model for ONJ. However, intracortical turnover has been shown to occur in the long bones of skeletally-mature C3H mice¹⁸. This suggests that some strains of mice, if shown to have intracortical turnover in the mandible, could potentially serve as models for ONJ. In addition, if rodents were to manifest exposed bone in response to bisphosphonates (with or without additional intervention), it would provide clear evidence that suppression of intracortical turnover is not an underlying part of the ONJ pathophysiology.

The rapid emergence of osteonecrosis of the jaw in association with bisphosphonate treatment has resulted in more questions than answers. Given the clinical complexities of this condition, there is a clear need for an animal model in which to study all aspects of ONJ including the direct cause/effect relationship to bisphosphonates. Based on studies in our laboratory, the dog shows promise as an animal model given the defined regions of matrix necrosis and non-viable osteocytes that exist only in bisphosphonate-treated animals. However until the dog, or another species, is shown to manifest exposed oral lesions in the presence of bisphosphonates, rigorous studies should be conducted in multiple species.

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