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## *COMMENTARIES*

# Does Rat + Bisphosphonate + Exposed Bone = An Animal Model of ONJ?

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**Commentary on:** Sonis ST, Watkins BA, Lyng GD, Lerman MA, Anderson KC. Bony changes in the jaws of rats treated with zoledronic acid and dexamethasone before dental extractions mimic bisphosphonate-related osteonecrosis in cancer patients. *Oral Oncol*. 2009 Feb;45(2):164-172.

First described just six years ago (1-3), the condition now known as bisphosphonate-related osteonecrosis of the jaw (BRONJ) has received significant attention among skeletal biologists and clinical practitioners. Despite this attention, we know very little about BRONJ due in part to the lack of a model system in which it can be studied. A recent publication by Sonis *et al*. (4), which describes changes in a rat model that have some consistencies with BRONJ, may represent a significant step forward in determining the underlying pathophysiology and viable prevention and treatment modalities for this condition.

With the goal of producing both clinical and radiographic changes consistent with BRONJ in an animal model, Sonis and colleagues divided three-month-old Sprague Dawley rats into control (n = 6 animals per group) or treated (n = 10 animals per group) conditions. All animals underwent extraction of the three molars in either the left mandible or left maxilla at different times after initiating drug treatments (8, 15, or 22 days). Groups of animals were euthanized either 14 or 28 days after extraction, resulting in group sizes of 3-5 animals within each individual group.

The most striking observation of the study is that many of the animals developed mucosal ulcerations at the molar extraction site. While all untreated animals had healed mucosa 14 days post-extraction, open wounds and exposed bone were found in 60-100% of animals treated with the various combinations of zoledronic acid (ZOL) and dexamethasone (DEX); similarly, 60% of animals treated with ZOL alone had ulcerations at 14 days post-surgery. By day 28, there were no ulcerations in ZOL-treated animals while they remained in 30-80% of the ZOL + DEX animals. It is important to note that the animals underwent a rigorous dental procedure – extraction of all 3 molars in a given jaw bone – that in general exceeds the sort of dental trauma usually experienced in humans who develop BRONJ. The extreme nature of the trauma is illustrated in the photographs provided by the authors showing an ulceration that appears severely traumatized 14 days post-surgery. Related to the rigorous dental intervention, the authors noted that tooth fractures occurred in some of the animals, yet the frequency was not provided nor was it noted whether this had any correspondence to those animals that developed ulcerations. Remnants of dental tissue could have played a role in the tissue ulceration of these animals. Ultimately it will be important to follow up with a more conservative dental intervention (*e.g.*, a single tooth extraction) to understand if the degree of dental trauma affects the outcomes in this model.

The histological evaluation in this paper was mostly qualitative although vascularity assessment was performed semi-quantitatively. Untreated control animals were said to have “occasional” bone sequestra both at 14 and 28 days post-extraction. As there were only 3 animals in each of these groups, the use of the terms “occasional” and “rare” means that at least one of the animals had a sequestrum. The formation of a sequestrum in an untreated animal suggests something inherent to the

procedure may be contributing to its formation, thus bringing into question the true effects of the drug treatments in this model. While a higher percentage of animals treated with ZOL alone or the combination of ZOL + DEX had acellular/necrotic bone, this occurred only in those animals in which the mucosa was not intact. These observations were said to be similar in nature at days 14 and 28 post-extraction. The level of inflammation, and the amount of vasculature, was said to be more notable in ZOL-only animals compared to those with ZOL + DEX. There was no presence of infection (based on histological assessment of *Actinomyces*) while there were cells that were positive for apoptosis, although the specifics of the latter finding were not presented. The absence of true quantification of these variables is unfortunate as it would have strengthened the conclusions that could be drawn regarding the model.

As with any animal study involving pharmaceutical agents, it is important to consider the doses administered with respect to those used clinically. Clinically, cancer patients are given ZOL as an intravenous infusion every 3-4 weeks at a dose of 4 mg (~ 66 µg/kg for a 60 kg individual) (5). Attempting to mimic dosing for multiple myeloma, animals in this study were dosed weekly with ZOL using a subcutaneous injection of 7.5 µg/kg. Although it is challenging to compare these rat and human doses given the difference in dosing schedule (weekly in rats versus monthly in humans), the dose administered to rats turns out to be lower than what is given to humans when calculated in multiple ways. On a µg/kg basis, the amount of ZOL administered in each dose is about 9-times lower than is used in humans (7.5 µg/kg in rats vs 66 µg/kg in humans). This difference is accentuated when accounting for the higher metabolic rate in rats, resulting in the ZOL dose being more than 30-times lower in rats compared to humans (see Table 1) (6). In those animals that received more than one injection, the cumulative dose received would more closely approximate that given to humans, but even the animals treated for 21 days still received only 33% of the clinical dose on a µg/kg basis, and about 10% of the dose on a metabolic dose basis. Thus, no matter how one performs this calculation, the ZOL dose was considerably lower than what is used clinically. Given that less significant changes were noted in the ZOL-only group, it will be important to study this model with doses of ZOL more in line with those used clinically.

**Table 1.** Zoledronic acid (ZOL) dosing in the study by Sonis *et al*. (4).

| | Rat | | | Human |
|---|---|---|---|---|
| Body weight (kg) | 0.25 | | | 60 |
| Metabolic weight (kg; actual weight $^{0.75}$) | 0.35 | | | 22 |
| Dose (µg/kg) ** | 7.5[a] | 15[b] | 22.5[c] | 66 |
| Actual dose (µg; dose / (1/body weight) | 1.88 | 3.75 | 5.63 | 3960 |
| Metabolic dose (µg/kg; actual dose / metabolic weight) | 5.4 | 10.7 | 16.1 | 180 |

**Humans are administered one dose of ZOL every month while the rats in this study were given one dose every week. Values for rats were calculated for the three different cumulative doses for animals treated with 1 dose ([a]), 2 doses ([b]), or 3 doses ([c]). Calculations are in accordance with (6).

Most animals in the study were also dosed with DEX as a daily subcutaneous injection of 1 mg/kg (for 7, 14, or 21 days). Clinically, DEX is often administered to multiple myeloma patients orally once a week at a dose of 40 mg (roughly 0.7 mg/kg for a 60 kg human). Similar to ZOL, comparing these rat and human doses of DEX is difficult not only because of different schedules (daily versus weekly) but also because of differences in route of administration (injection versus oral). On a weekly dose basis, the amount administered to rats (7 mg/kg) is 10-times higher than what is given

to humans, a difference that is likely accentuated by the lower bioavailability of oral dosing relative to subcutaneous dosing (see Table 2). After adjusting for metabolic rate differences, the DEX dose given in the current study is only about 2-times higher than what is used clinically although lower oral bioavailability in humans would make this difference larger. Thus, the DEX dose appears to be higher than what is used clinically. It will be important to determine if/how the results of the study are changed with DEX dosing more consistent with what is used clinically both for dose and route of administration.

**Table 2.** Dexamethasone (DEX) dosing in the study from Sonis *et al*. (4).

| | Rat | Human |
|---|---|---|
| Body weight (kg) | 0.25 | 60 |
| Metabolic weight (kg; actual weight $^{0.75}$) | 0.35 | 22 |
| Dose (mg/kg) | 7 | 0.67 |
| Actual dose (mg; dose / (1/body weight) | 1.75 | 40 |
| Metabolic dose (mg/kg; actual dose / metabolic weight) | 5 | 1.8 |

**Humans are administered DEX weekly while the rats in this study were given daily DEX via subcutaneous injection. Values for rats were calculated based on the cumulative dose over one week. Calculations are in accordance with (6).

Beyond studies that have assessed incidence rates and risk factors for BRONJ in various patient populations, few data exist concerning the pathogenesis of BRONJ. We know that cancer patients treated with bisphosphonates (BPs) are at the highest risk of BRONJ, suggesting that the high doses of BPs used in these patients may be a key factor in the pathophysiology (7;8). We also know that in any BP-treated population the risk of BRONJ increases 10-fold if the patients undergo dental surgery (9). Yet cases of BRONJ exist in patients treated with lower doses of BPs, such as those used for the treatment of post-menopausal osteoporosis, as well as in BP-treated patients who do not undergo dental surgery (8;9). It has been suggested in various reports that concomitant use of medications that either suppress the immune system (such as corticosteroids) (10) or inhibit angiogenesis (such as bevacizumab) (11;12) contribute to the manifestation of BRONJ, yet cases of exposed bone exist where neither of these agents have been administered. The point to be made here is that it becomes difficult if not impossible to tease out the important factors in BRONJ from clinical studies. This is why an animal model is so urgently needed and why the work of Sonis and colleagues represents a potential significant step forward.

Ultimately, progress toward understanding BRONJ will likely only be made in a pre-clinical model. In 1981, Gotcher and Jee (13) treated young rats with high doses of clodronate, an early generation non-nitrogen-containing BP, for up to 18 weeks. In the absence of any dental surgery, they noted bone protruding into the oral cavity that upon histological analysis was found to be void of viable cells and considered devitalized. Now, some 28 years later, the work of Sonis and co-authors provides a second study to support the idea that rodents may be useful as a model for BRONJ. Yet important questions remain before declaring that a useful model for BRONJ has been developed. Specifically, it is not clear that what was produced in the oral cavity of these rats is truly analogous to BRONJ. More details are needed regarding what represents “normal healing” following this rigorous dental intervention, and ideally a less rigorous intervention can be used to produce exposed bone and sequestra. Equally important will be to perform detailed analysis, including quantitative histology, on the bone and soft tissue at the site of

extraction in order to provide convincing evidence that the changes are statistically different between treatments. Finally, utilizing pharmacological agents at clinically-relevant doses and dosing schedules will be important to strengthen the validity of the model. Despite the need for more work, Sonis and colleagues are to be commended for the pursuit of a pre-clinical model for this serious and significant condition. Here's hoping that this, or another model, is generated soon so that the field can advance toward understanding the pathophysiology of BRONJ and ultimately find ways to prevent/treat this condition.

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