Curr Osteoporos Rep (2016) 14:219–225
DOI 10.1007/s11914-016-0318-z

CRANIOFACIAL SKELETON (WE ROBERTS, SECTION EDITOR)

Osteonecrosis of the Jaw—a Bone Site-Specific Effect of Bisphosphonates

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Published online: 29 July 2016

Abstract A known complication that can occur in patients using bisphosphonates (BPs) is osteonecrosis of the jaw (ONJ). ONJ features bone exposure that may be associated with severe pain, swelling, local infection, and pathological fracture of the jaw. Current literature indicates that a complex combination of factors is necessary to induce ONJ. Several hypotheses about the pathophysiology of ONJ were previously reported. Here, we review these hypotheses and introduce new ideas and suggestions on this topic, focusing on bone site-specific cells, and the effect that BPs and other anti-resorptive drugs have on those cells. Gaining more insight into bone site-specific effects may help to better understand the pathogenesis ONJ, and contribute to the development of new bone site-specific anti-resorptive drugs.

Keywords Bisphosphonates · Osteonecrosis · Jaw · ONJ · Osteoclast · Microenvironment · Bone marrow

This article is part of the Topical Collection on *Craniofacial Skeleton*

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Introduction

Bisphosphonates (BPs) have been used for decades to treat bone-degrading diseases such as osteoporosis and bone metastasis. BPs increase bone density and reduce fracture risk by inhibiting bone resorption by osteoclasts. First-generation BPs do this by causing osteoclast apoptosis whereas the newer and more potent BPs containing nitrogen, such as alendronate (ALN) and zoledronic acid (ZA), also inhibit osteoclast activity [1–4]. By virtue of their high affinity for calcium and therefore rapid localization to the bone, BPs primarily act on osteoclasts because they are taken up by those cells during bone resorption [5]. A rare though severe side effect of BP use is osteonecrosis of the jaw (ONJ).

Osteonecrosis of the Jaw

ONJ was described as the first long-term complication of BP use [6–8]. With an incidence of up to 5–20 % after intravenous administration, the risk is much higher than after oral use, which has an incidence of around 0.04 % [9–11]. In 64 % of the cases, ONJ is preceded by a dental extraction or other oral trauma [12], and periodontitis often (84 %) accompanies ONJ [13]. In 2007, BP-related ONJ was defined as an area of exposed bone that did not heal within 8 weeks and was not the result of radiotherapy in the craniofacial region [14]. Recently, the International Task Force on Osteonecrosis of the Jaw updated the definition of ONJ from being a result of exposure to BPs to exposure to an anti-resorptive agent, indicating that BPs are not the sole initiators of ONJ [15•].

Clinically, apart from exposed or necrotic bone, ONJ can be recognized but not diagnosed by symptoms and radiographic observations such as pain, osteolysis, bone sequestration, and infection or soft tissue inflammation [15•, 16]. The presence

and severity of these symptoms are used to determine the stage of the disease according to the staging system first introduced by Ruggiero and co-workers [17] and later adopted by the American Association of Oral and Maxillofacial Surgeons (AAOMS) [16]. Several of the mentioned clinical manifestations were shown to be inducible in animal models which are partly listed in Table 1 and which will be discussed below.

Several hypotheses about the pathophysiology of ONJ were previously reviewed [11, 15•, 34, 35]. Briefly, BPs may inhibit cells other than osteoclasts such as epithelial cells. For instance, a toxic effect on soft tissue could secondarily lead to bone exposure, and a negative effect on endothelial cells and/or angiogenesis may cause avascular necrosis [36, 37]. Microbial infections may lead to bone degradation directly, which may be worsened by a decreasing pH due to the inflammation [35, 38]. A reduced pH may also lead to release of BPs from the bone [39] and therefore be toxic to other cells. Furthermore, suppression of bone turnover, and the inability to heal microdamage, may lead to the accumulation of necrotic tissue [40, 41]. Finally, more recent studies show that single nucleotide polymorphisms (SNPs) in several genes may lead to a genetic predisposition to ONJ [42, 43]. Altogether, a complex combination of factors seems to be necessary to induce ONJ, and more research is required to distinguish the roles of osteoclasts and bone loss in the pathogenic mechanism.

Animal Models

To gain more insight into the pathophysiology of osteonecrosis of the jaw, animal disease models were developed. Since an association between the RANKL inhibitor denosumab and ONJ was only first shown in 2010 [44, 45], the majority of animal models use BPs to induce ONJ. Since BPs alone did not usually lead to apparent signs of ONJ [46, 47], other inducing factors such as tooth extraction, immunosuppressive drugs, or periodontal disease were applied. Those combinations of treatments had variable outcomes on the manifestations of ONJ such as exposed bone, necrotic bone, bony sequestrum, and inflammation. An extensive overview

Table 1 Overview of animal models of ONJ

1st author year	Species	Treatment	Frequency	Comorbidity	Exposed bone	Necrotic bone	Sequestrum	Inflammation	Ref
Abtahi 2013	Rat	sc ALN 200 ug/kg	1/day 3 weeks	DEX, extraction	10/10	10/10	6/10	10/10	[18]
Aghaloo 2011	Rat	ip ZA 66 ug/kg	3/week 15 weeks	Periodontal disease	4/19	9/19, area: ±60 %	6/19	Only with ligature	[19]
Aghaloo 2014	Mouse	ip OPG-Fc 10 mg/kg	1/week 12 weeks	Periapical disease	3/10	10/10, area: 17.7 %	ns	At sites of periapical disease	[20]
Aguirre 2012	Rat	iv ZA 80 ug/kg	1/month 18 weeks	Periodontal disease	Yes	Yes, apoptotic osteocytes	ns	Bacterial colonization	[21]
Allen 2008	Dog	Oral ALN 1 mg/kg	1/day 3 years	–	No	4/12	ns	ns	[22]
Allen 2013	Dog	iv ZA 60 ug/kg	1/2 weeks 9 months	DEX, extraction	No	No	No	Yes	[23]
Bi 2010	Mouse	ip ZA 125 ug/kg	2/week 15 weeks	DEX, docetaxel, extraction	10/10	10/10	Yes	10/10	[24]
de Molon 2014	Mouse	ip ZA[a] 200 ug/kg	3/week 12 weeks	Peri-radicular infection (natural)	8/38 hemimaxillae	10/38 hemimaxillae, area: 16 %	ns	Infection necessary for necrosis	[25••]
Hokugo 2013	Rat	iv ZA 70 ug/kg	1/month 2 months	VitD deficiency, extraction	ns	±55 % viable osteocytes	Yes	Yes	[26]
Howie 2015	Rat	iv ZA 80 ug/kg	1/week 13 weeks	Extraction (twice)	Yes	26.7 % empty lacunae	Yes	No bacterial colonization	[27]
Huja 2011	Dog	iv ZA 100 ug/kg	1/month 4 months	Extraction, mini-implant	No	Osteocyte death in maxilla, area: <1 %	ns	ns	[28]
Kikuiri 2010	Mouse	iv ZA 125 ug/kg	2/week 8 weeks	DEX, extraction	Yes	Yes, area: ±5 %	Yes	Bacterial colonization and inflammation	[29]
Kuroshima 2012	Mouse	sc ZA 50 ug/kg	2/week 13 months	Excision palatal mucosa	ns	Empty lacunae	ns	PMN influx, no bacterial colonization	[30]
Kuroshima 2013	Mouse	sc ZA 50 ug/kg	2/week 7 weeks	MEL, extraction	13/16 sockets	Area: ±10 %	ns	PMNs but not macrophages	[31]
Pautke 2012	Mini-pig	iv ZA 50 ug/kg	1/week 16 weeks	Extraction	5/5	29/30 extraction sites	Yes	Bacterial colonization	[32]
Takaoka 2015	Rat	iv ZA 35 ug/kg	1/2 weeks 29 weeks	Diabetes, extraction	6/6	Empty lacunae	Yes	Bacterial colonization	[33]

When more than one treatment or doses are described in one paper, only the most relevant treatment for these signs of ONJ was mentioned in the table. Except in "frequency," #/# indicates the number of mice that show signs of the indicated pathology per total number of mice, unless indicated otherwise

ALN alendronate, *DEX* dexamethasone, *MEL* melphalan, *ns* not specified, *PMN* polymorphonuclear cell, *VitD* vitamin D, *ZA* zoledronic acid

[a] Similar results with RANK-Fc and OPG-Fc

of ONJ manifestations is supplied in Table 1. Interestingly, the comorbidities of pro-inflammatory [19, 21, 25••] as well as anti-inflammatory [18, 23, 24, 29, 31] conditions induced symptoms such as exposed and necrotic bone.

Most models focus on the jaw alone, since osteonecrosis only occasionally occurs in other bones. Thus, it is appropriate to address the micro-environmental differences between the jaw and other bones, in both physiological and pathological circumstances.

Jaw Versus Other Bones

Long bone is produced via a cartilage intermediary, i.e., endochondral bone formation, whereas the jaw is primarily of intramembranous origin. The human mandible [48] as well as the murine calvaria [49] were shown to contain more collagen than long bones. Calcium content is also higher in the human mandible [48], and so it may adsorb more BPs than the long bones [50]. Besides the differential composition for various bones in the skeleton, the cells degrading and forming the osseous tissue, as well as the bone marrow composition, were shown to be bone site-specific.

Osteoclasts

Different bones have osteoclasts with distinct characteristics (reviewed in [51, 52]). Mechanistically, resorption by long-bone osteoclasts is mainly carried out by cathepsins whereas calvaria osteoclasts may also utilize matrix metalloproteinases (MMPs) [53]. Interestingly, MMP-9 was shown to play an important role in osteoclast-mediated stimulation of angiogenesis [54], indicating that it would be of interest to compare this interaction in different bones. Zenger et al. [55] showed higher expression of cathepsin K as well as tartrate-resistant acid phosphatase (TRACP) in long bone compared to calvaria osteoclasts. Long-bone osteoclasts also use different ion exchangers to maintain intracellular pH [56]. In vitro assays show different rates of osteoclastogenesis and RANKL/OPG ratios [57] as well as different osteoclastogenic potential [58] using mandibular and long-bone marrow. Furthermore, in response to BPs, jaw osteoclasts were less apoptotic compared to long-bone osteoclasts, indicating that the former may be less sensitive to BPs [59•]. Interestingly, in a mouse model for ONJ, RANKL inhibitors were more potent than ZA in reducing osteoclast numbers in the jaw [25••].

Osteoblasts

Similar to osteoclasts, bone-forming osteoblasts and their precursors in the bone marrow also differ among bone sites. Rat mandibular-bone-marrow stromal cells were shown to have a higher osteogenic potential than those from tibia in vitro [60, 61]. Also in vitro, human jaw-derived bone marrow stromal cells demonstrated more calcium accumulation than iliac crest-derived cells whereas the latter induced more bone formation in mice under osteogenic conditions [62]. Matsubara and colleagues on the other hand showed similar osteogenic potential of human alveolar and iliac bone marrow stroma, but showed lower adipogenic and chondrogenic potential of alveolar BMSCs [63].

Next to differences in osteoblastogenic potential of stromal cells, osteoblast signaling towards other cells was shown to be bone site-dependent. Murine calvaria osteoblasts more potently induced osteoclastogenesis than those from long bone [64]. Human maxillary BMSCs were more potent inducers of angiogenesis than those derived from iliac crest [65]. On the other hand, disrupted *Vhl* gene expression in osteoblasts, and the resulting Hifα overexpression increased long-bone volume, but did not affect calvaria bones, indicating that osteoblast-stimulated angiogenesis plays an important role mainly in endochondral bone formation [66].

Even though BPs primarily affect osteoclasts, inhibiting effects on osteoblast viability and proliferation were also shown in vitro [67–69]. Surprisingly, anti-apoptotic effects of low concentrations of BPs were also reported in vitro and in vivo [70, 71]. In animal models, BPs generally reduced bone formation markers [21, 22, 27, 28, 33, 47, 72, 73] strongly indicating that either directly or indirectly they can affect bone cells other than osteoclasts.

Bone Marrow

Besides differential osteoblast and osteoclast induction potential, the composition of the bone marrow was also shown to differ among bone sites. Long bones contain more marrow fat than flat bones, and murine long-bone marrow contains more osteoclast precursors than jaw bone marrow [57]. Mesenchymal stem cells from the mandible have higher proliferation rates than those from long bones [74].

Despite its high specificity for bone-resorbing osteoclasts, BP uptake has also been noted in monocytes of rabbit bone marrow [75]. Monocyte/macrophage marker RNA expression in the bone marrow was also altered by ZA in rat long bones [76]. In vitro, non-bone cells cultured next to bone-resorbing osteoclasts were shown to internalize BP released from the bone [77]. In addition, the current authors showed that long-term treatment with ZA reduced the number of jaw bone marrow cells (unpublished results). Since macrophages are highly endocytic cells [78], they may be a major cell type in the bone marrow taking up BPs. Therefore, the effect of BPs on macrophage functioning would be an interesting direction for future research [79•, 80].

Collectively, the close interaction between different cell types in the bone microenvironment, and the effects that BPs can have on those cells, should all be considered when formulating a hypothesis explaining the pathophysiology of osteonecrosis of the jaw.

Hypotheses

Since both BPs and denosumab were shown to induce ONJ, the most comprehensive hypothesis would explain both relations and, at the same time, would also explain why ONJ is rare in other bones. Denosumab and BPs both inhibit osteoclasts and their activity, pointing towards suppression of bone turnover as a likely underlying mechanism. However, indirect effects through coupling with osteoblasts or other cells in the environment should not be ruled out. Coupling and direct effects on non-osteoclastic cells may be differently influenced by RANKL inhibition due to denosumab versus induction of apoptosis by BPs. Therefore, a direct comparison of denosumab and BPs on osteoclast numbers, osteoclast activity, osteoblast activity, and bone turnover in the jaws versus other bones would be of great interest. Although de Molon et al. [25••] did study the effect of both RANKL inhibitors and BPs in a mouse model of ONJ of the jaw, they did not assess the effects on other bones.

Suppression of Bone Turnover

Impaired osteoclast activity and the potential inability to heal microdamage may lead to an accumulation of necrotic bone tissue [40, 41]. This hypothesis does not explain though how a specific bone is exposed or why other bones are not affected. The effect may be stronger in the jaw if more BPs accumulate there, but that effect would not explain why denosumab is also related to ONJ. Also, in mice, we did not find any evidence that BPs more potently inhibit bone turnover in the jaw than in the long bones; on the contrary, it seemed to be the other way around (unpublished results).

Toxicity to Non-osteoclasts

A potentially toxic effect of BPs to other cell types in the bone may lead to necrosis and exposed bone. In vitro, BPs were indeed shown to inhibit proliferation of endothelial cells and angiogenesis [36, 81], reduce the wound healing capacity of murine epithelial cells [82], and furthermore, reduce the viability of periodontal ligament fibroblasts [83, 84] and macrophages [77, 85]. Osteoblasts and osteocytes were reported to be both positively and negatively affected by BPs, which was reviewed by Bellido and Plotkin [86], and they attributed the different findings to the concentration of the drug. It is therefore uncertain whether the low concentration of BPs in vivo to which non-osteoclasts are exposed would cause the severe toxicity that could lead to ONJ. However, we previously showed that jaw bone marrow cells internalized more BPs than long-bone marrow cells in vitro [59•] and that ZA reduced the number of jaw bone marrow cells in vivo (unpublished results). Also, repeated administration of BPs was shown to replace bone-bound BPs, indicating that previously administered BPs can be released into the environment, increasing the local concentration [26]. BP release from the bone and a minor effect on the healing capacity might be enough to lead to the onset of ONJ in the event of an extraction or other trauma [11], which may be enhanced by an infection [35, 87]. Due to the constant pathogenic challenge in the jaw, this hypothesis would explain why especially the jaw is affected by osteonecrosis, yet it does not explain how denosumab could cause ONJ. Bone site-specific effects of RANKL inhibition may exist though, since the RANKL/OPG ratio was shown to be different in long-bone and jaw bone marrow [57].

Infection

Evidently, soft tissue toxicity and infection are two closely related phenomena possibly explaining the pathophysiology of ONJ. Unlike long bones, the jaw is constantly exposed to pathogens present in dental plaque, particularly the *Actinomyces* species commonly found in ONJ [88]. Furthermore, there is a high coexistence of ONJ and periodontitis [13], all of which implies a role for infection in ONJ. The ability to overcome infections may be inhibited by the negative effect of BPs on immune cells such as macrophages [79•]. In addition, the decreased pH associated with inflammation may enhance the release of BPs from adjacent bone, making them available for uptake by other cells [35], thereby inducing soft tissue destruction leading to necrotic bone exposure. Conversely, disruption of the mucosa to expose bone increases the risk of infection [15•, 34, 46]. Remarkably, Leclous et al. [87] found that a higher bone marrow inflammatory infiltrate correlated with more severe ONJ. Finally, since different osseous sites may have distinct compositions of immune cells, bone site-specific responses are likely to occur.

Similar to the ONJ-hypothesis of BP toxicity to other cells, the infection hypothesis explains the difference between bone sites, but does not explain how denosumab is related to ONJ. Yet, inhibition of RANKL may affect the immune cells expressing RANK, such as T cells, B cells, dendritic cells, and macrophages [89, 90]. The effect of denosumab on those immune cells in different bones requires further investigation.

Conclusion

The multifactorial pathogenesis of osteonecrosis of the jaw seems to rely on interactions between bone cells and their unique site-specific environment. These interactions and the superimposed effects of anti-resorptive drugs on specific bones require further investigation. Gaining additional insight into bone site-specific cells may help in the development of more specific, anti-resorptive drugs.

Compliance with Ethical Standards

Conflict of Interest Vincent Everts, Jenny Vermeer, and Greetje Renders declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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