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Bisphosphonate-related osteonecrosis of the jaw: a mechanobiology perspective

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ABSTRACT

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a dramatic disintegration of the jaw that affects patients treated with bisphosphonates (BPs) for diseases characterized by bone loss. These diseases are often metastasizing cancers (like multiple myeloma, breast cancer and prostate cancer (Aragon-Ching et al., 2009)) as well as osteoporosis. BRONJ is incompletely understood, although it is believed to arise from a defect in bone remodeling—the intricate process by which sensory osteocytes signal to osteoclasts and osteoblasts to resorb and form bone in response to stimuli. Further, tooth extraction and infection have been overwhelmingly linked to BRONJ (Ikebe, 2013). Because bone cells are highly networked, the importance of multicellular interactions and mechanotransduction during the onset of these risk factors cannot be overstated. As such, this perspective addresses current research on the effects of BPs, mechanical load and inflammation on bone remodeling and on development of BRONJ. Our investigation has led us to conclude that improved in vitro systems capable of adequately recapitulating multicellular communication and incorporating effects of osteocyte mechanosensing on bone resorption and formation are needed to elucidate the mechanism(s) by which BRONJ ensues.

1. Introduction

BRONJ is specifically defined as necrotic bone in the oral cavity that does not heal within eight weeks of onset. Additionally, the affected person must have been exposed to a BP and must not have undergone radiation therapy in the craniofacial region or have suffered previous metastasis to the jaw (Migliorati et al., 2013; Saia et al., 2010; Advisory Task Force on Bisphosphonate-Related Osteoencrosis of the Jaws, 2007; Ruggiero et al., 2014). A position paper published by the American Association of Oral and Maxillofacial Surgeons in 2014 suggests replacing the nomenclature of BRONJ with medication-related osteonecrosis of the jaw (MRONJ) to incorporate cases of osteonecrosis following exposure to other antiresorptive and antiangiogenic treatments. These include the antiresorptive human monoclonal antibody, Denosumab, and antiangiogenic tyrosine kinase inhibitors (Ruggiero et al., 2014). Denosumab prevents osteoclast resorption by inhibiting receptor activator of nuclear factor kappa-B ligand (RANKL), which binds to RANK on the surface of osteoclasts to promote differentiation and activation (Qaisi et al., 2016). Tyrosine kinase inhibitors may exaggerate suppression of bone remodeling by BPs, counteract mucosal healing and increase risk of infection in the jaw. Research shows tyrosine kinase inhibitors, including sunitinib and imatinib, can promote osteonecrosis of the jaw with and without supplementary BP therapy (Ruggiero et al., 2014; Viviano et al., 2017). For the purposes of this review, which explores osteonecrosis associated with only BP therapy, we will continue to use the original terminology.

BPs mitigate bone resorption by osteoclasts and remodeling as a whole. They are used to treat the following conditions characterized by excess bone loss: tumor bone metastasis, osteoporosis, malignancy-associated hypercalcemia and Paget's disease (Feller et al., 2009; Zara et al., 2015; Manzano-Moreno et al., 2015; Heymann, 2010; Landesberg et al., 2011). An increase in BP prescriptions has led to an increased need to interpret the mechanism(s) by which BRONJ develops. From a research standpoint, mechanical trauma (tooth extraction) and inflammation derived from infection have been strongly associated with BRONJ (Ikebe, 2013; Otto et al., 2012; Abu-Id et al., 2008; Aragon-Ching et al., 2009). These two risk factors are closely linked because extraction sockets may become exposed to oral bacteria, causing infection. BPs, mechanical load and inflammation likely contribute to the disease by disturbing normal bone turnover. Limited studies have been performed to discern effects of these risk factors on bone remodeling in isolation, and very little is known about their effects in tandem. Mechanobiology is the study of the coordination of biological mechanisms by mechanical or physical stimuli (Epari et al., 2010). Physical forces

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are translated into biochemical signals that prompt cellular responses by a process called mechanotransduction. In bone, mechanotransduction includes four phases: 1) mechanocoupling (which involves the stretching of bone cells and generation of fluid movement within the bone canaliculae by mechanical loads), 2) biochemical coupling (or the conversion of a mechanical signal into a biochemical reaction by way of cellular pathways), 3) transmission of the signal from the sensor to the effector cell and 4) the effector cell response (Huang and Ogawa, 2010; Duncan and Turner, 1995; Turner and Pavalko, 1998). As current research seeks to elucidate the mechanism(s) by which BRONJ develops, the study of the disease from a mechanobiology perspective will support this resolve. Although we have chosen to present experimental studies on the effects of cofactors on bone cell communication and functional activity, a systematic review of clinical trials, case series and retrospective studies on BRONJ published between 2003 and February 2014 validates our decision to focus on tooth extraction and infection. Fliefel et al. found that, within 3198 cases of BRONJ, 61.7% were caused by tooth extractions, and 5% were associated with periodontal disease (inflammation) (Fliefel et al., 2015).

2. Bisphosphonates

BPs are chemotherapeutical antiresorptive compounds that mediate the morphology and activity of bone cells in several ways; these actions characterize them as risk factors for BRONJ (Zara et al., 2015; Donetti et al., 2014). BPs commonly used in therapy are made up of a central carbon atom attached to a hydroxyl group, which gives BPs the ability to bind to calcium. On either side of the carbon atom is a phosphonate group responsible for the drug's affinity for hydroxyapatite (Fig. 1). As such, BPs are preferentially taken up by bone (Russell, 2011). If a nitrogen or amino group is present, the drug is termed “nitrogen-containing.” Nitrogen-containing BPs (NBPs) are more potent in their antiresorptive capabilities than non-nitrogen-containing BPs by 10 to 10,000 times (Drake et al., 2008). NBPs prevent osteoclast survival and bone-resorbing ability by binding to and hindering enzymes of the intracellular mevalonate pathway. This in turn inhibits prenylation—attachement of isoprenoids for anchorage to cell membranes—of small GTPases. Buildup of unprenylated small GTPases then causes inappropriate activation of signaling pathways (Jobke et al., 2014). The most potent NBP, zoledronic acid (Zafar et al., 2016), is frequently associated with clinical cases of BRONJ.

2.1. Effects of bisphosphonates on osteoclasts

BPs act on osteoclasts to inhibit bone resorption. They prevent osteoclast formation, alter phenotype, prohibit function and promote apoptosis (Sharma et al., 2013; Gong et al., 2011; Bagan et al., 2013). They form chelates with calcium ions and bind to hydroxyapatite on the exterior of bone, prompting release of a soluble factor that prevents precursor cells from fusing to form osteoclasts (Zara et al., 2015). Osteoclasts are multicellular and contain the following: 1) ruffled borders (to which proteins and acids are localized for the degradation of bone (Stenbeck, 2002)), 2) intracytoplasmic vesicles (by which the products of bone degradation are transported intracellularly (Galvão et al., 2011)) and 3) sealing zones (ring shaped actin-rich structures that encircle the sites at which the plasma membranes adhere to the bone (Teti et al., 1991; Matsumoto et al., 2013)). BPs cause an increase in size and number of nuclei, disrupt the cells' ruffled borders, prevent formation of intracytoplasmic vesicles and promote detachment of sealing zones (Jobke et al., 2014). Further, NBPs obstruct the mevalonate pathway of cholesterol synthesis which restricts the enzyme, farnesyl diphosphate synthase. As described above, small GTPases cannot be prenylated (Manzano-Moreno et al., 2015), and osteoclasts are unable to break down bone.

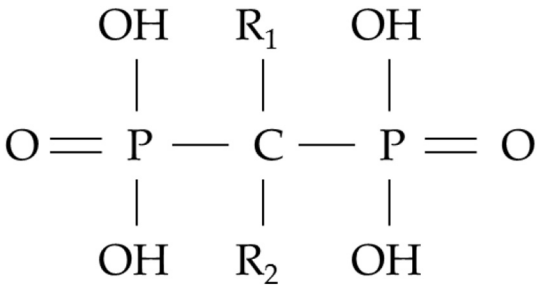

Fig. 1. Chemical structure of a BP. If a nitrogen or amino group is present, the drug is termed “nitrogen-containing.”

2.2. Effects of bisphosphonates on osteoblasts and osteocytes

While the effects of BPs on osteoblasts and osteocytes are much less studied, there is evidence that both contribute to the onset of BRONJ—the osteoblasts through altered mineralization and the osteocytes through mechanotransduction. BPs administered at high doses ($\geq 10^{-5}$ M) have been shown to arrest the osteoblast cell cycle and induce apoptosis, thereby reducing proliferation of the osteoblast lineage. Low doses ($\sim 10^{-9}$–10^{-6} M) have been reported to exert positive effects on osteoblasts (Zara et al., 2015; Manzano-Moreno et al., 2015). Importantly, BPs promote connexin 43 (Cx43)-required osteocyte survival (Plotkin et al., 2008). However, it is likely that osteocyte survival is also BP dose-dependent with cell death occurring at high concentrations (Pazianas et al., 2014). Evidence infers that BPs gain access to osteocytes by way of the canalicular network. Fluorescent BP analogues have been shown to target osteocyte lacunae, specifically lacunar walls that neighbor osteocytes recently embedded near the surface of bone. However, it is unknown whether this process is BP type-dependent (Roelofs et al., 2010).

2.3. Effects of bisphosphonates on tissue properties of bone

In addition to acting on individual cells, BPs can significantly alter the tissue properties of bone. For example, BP therapy has been shown to decrease apatite crystal size and perfection that can lead to compromised mechanical characteristics, like elastic modulus and contact hardness (Bala et al., 2012). Several studies on the properties of the jawbone following BP treatment, including cortical porosity, bone mineral density (BMD) and crack surface density (Cr. S. Dn), have been carried out in beagle dog models. One study indicated that exposure to BP treatment impacted jaws but not tissue mineralization. After three months of exposure, average tissue mineralization was unchanged but jaws displayed significantly decreased cortical porosity and significantly increased areal BMD when compared to controls. However, other studies reported significantly higher Cr. S. Dn. in the basal alveolar regions of experimental samples treated with high-dose BP for one year as well as matrix necrosis occurring between one and three years of high-dose therapy (Allen, 2011).

Kim et al. investigated the role of microdamage in conjunction with BP on BRONJ development. Rats were administered injections of BP or saline for six weeks. Tooth extractions were performed, and treatments were sustained for eight weeks before sacrifice. The number and length of microcracks in the BP group were greater than those in the control group; 68.4% of the rats that were injected with BP were sorted into a BRONJ group. BRONJ faction samples showed significantly greater crack density (Cr. Dn.) as well as Cr. S. Dn when compared to samples from the non-BRONJ group. The authors conclude there is a significant relationship between the aggregation of unrepaired microcracks and the onset of BRONJ. This aggregation represents a plastically yielded and mechanically compromised environment. In addition to affecting bone cell morphology and activity, high-dose/long-term BP treatment likely decreases bone's mechanical integrity and propagates microcrack formation, supporting BRONJ development (Kim et al., 2016).

3. Signaling and bone remodeling

Understanding BRONJ requires comprehension of the osteocyte's role in sensing trauma and relaying damage signals within the disease environment. Crosstalk with neighboring osteocytes and bone lining cells occurs through gap junctions and communication with osteoclasts and osteoblasts through soluble signals (Florencio-Silva et al., 2015). As such, it is ideal to approach BRONJ as a mechanotransduction problem.

The process of bone remodeling is incredibly intricate. Osteocytes, the most abundant bone cells, function as mechanosensors, coordinating their activity when stimulated by biological and mechanical cues. Through mechanotransduction, osteocytes regulate bone resorption by osteoclasts and bone formation by osteoblasts. In their quiescent state, dendritic osteocytes are networked via gap junctions within the mineralized bone matrix. Here, they reside within fluid-filled lacunae, or cavities. Mechanical loading prompts osteocyte activity by fluid flow-induced shear stress. Osteocytes respond to this stress by relaying signals to alter gene expression. Mechanical cues are communicated via gap junctions to bone lining osteoblasts, resulting in regulation of mineral (hydroxyapaptite) formation. Importantly, expression levels of cyclooxygenase-2 (COX-2), RANKL and osteoprotegerin (OPG) are altered, affecting bone formation and osteoclastogenesis. Specifically, an increase in COX-2 leads to synthesis of prostaglandin E2 (PGE2) downstream, which upregulates bone formation. A change in expression of OPG, a decoy receptor for RANKL, influences osteoclast differentiation which requires activation of RANKL by RANK (Hesse et al., 2014; Temiyasathit and Jacobs, 2010; Haugh et al., 2015). Further, osteocyte secretory factors, semaphorins 4D, 3B and 3A, signal osteoblast progenitor recruitment and promote proliferation and differentiation (Fukuda et al., 2013; Negishi-Koga et al., 2011; Sutton et al., 2008). And, osteocyte death likely prompts release of chemoattractants that call upon osteoclast precursor cells to instigate resorption of damaged bone (Bivi et al., 2012). Several mechanisms involved in osteocyte mechanotransduction are illustrated in Fig. 2. Finally, mechanotransduction pathways can also be activated within osteoblasts to prompt remodeling-related gene expression. In a study by Watabe et al., mandibular osteoblasts were exposed to low intensity pulsed ultrasound, which had been determined to activate mechanotransduction pathways in osteoblast-like cell lines. It was found that mandibular osteoblast signals that encode the antiapoptotic protein, Bcl-2, as well as RANKL were distinctly upregulated. Additionally, it was shown that in order to upregulate expression of Bcl-2, RANKL, β-catenin, which governs osteoclast differentiation, and phospho-Akt (p-Akt), mandibular osteoblasts require mechanotransduction (Watabe et al., 2011; Wang et al., 2014).

Bone turnover is not fully understood, and we are constantly making discoveries that add to the complexity of the process. For example, it has been shown that osteocytes *themselves* can remodel their perilacunar/canalicular environment, removing mineralized matrix via molecular mechanisms not unlike those employed by osteoclasts (Qing et al., 2012). And, while still in its infancy, study of bidirectional signaling may reveal an important role for osteoclast/osteoblast coupling in remodeling. Osteoclast/osteoblast coupling includes matrix-derived signals such as transforming growth factor beta 1 (TGFβ-1) and insulin-like growth factor 1 (IGF-1) which are released during resorption and affect mesenchymal stem cell migration and differentiation into osteoblasts. Membrane-bound factors including ephrin also mediate communication between osteoclasts and osteoblasts (Matsuo and Otaki, 2012), but the importance of ephrin is currently controversial. Eph/ephrin binding is a contact-dependent process that mediates cell function. As such, arguments against ephrins affecting coupling include the lack of evidence that osteoclasts and osteoblasts are in direct contact (Florencio-Silva et al., 2015). However, Zhao et al. offered compelling data to suggest ephrins enable bidirectional signaling—initially called forward and reverse signaling—in bone. Unlike other methods of activation, the ephrin ligand from one cell makes contact with the ephrin receptor from another to transmit signals in both cells. Specifically, ephrin/Eph binding from osteoclasts (ephrin) to osteoblasts (Eph) drives new bone formation while simultaneous Eph/ephrin binding from osteoblasts (Eph) to osteoclasts (ephrin) ceases bone resorption (Zhao et al., 2011).

3.1. Roles of gap junction protein and cell adhesion complex

Expression of the gap junction gene, Cx43, found within osteocytes and osteoblasts, is enhanced by mechanical loading. BPs and mechanical strain have been demonstrated to prompt opening of Cx43 hemichannels, which may serve as transducers of extracellular signals, play a role in the release of PGE2 and prevent osteocyte and osteoblast apoptosis. Specifically with regard to osteocyte survival, mechanical forces engage integrins α5 and β1. Kinases are triggered to promote osteocyte survival. More recently, it has been shown that interaction of the Cx43 C-terminus domain with these integrins activates P13K, leading to mechanically-induced hemichannel opening (Fig. 2) (Plotkin and Bellido, 2013). Moreover, a lack of Cx43 increases osteocyte apoptosis and decreases OPG expression and, accordingly, local levels of sclerostin, a protein which inhibits bone formation. Thus, Cx43 also helps regulate gene expression associated with bone resorption and formation. Because of the sites at which bone resorption and formation occur in a Cx43-deficient environment, Bivi and coworkers suggest that Cx43 enhances formation and resorption because of modeling, the process of bone formation not preceded by resorption, rather than remodeling (Bivi et al., 2012). Clearly, bone remodeling is a complex and nuanced process that we have yet to fully appreciate, especially in relation to diseases like BRONJ.

4. Mechanical trauma

While long term effects may be disuse and unloading, the immediate response to tooth extraction is overload. It has been estimated that average extraction forces are 10 kg ± 4 kg with hard removal reaching forces as high as 32 kg (314 N) (MacGregor and Tomlinson, 1979–1980). Short term loading, especially following BP therapy, may have long term effects, including BRONJ, on bone tissue of the oral cavity. In fact, recent studies have demonstrated that BRONJ can be induced reproducibly in animal models with surgical tooth extractions in the presence of a BP (Howie et al., 2015; Vidal-Gutiérrez et al., 2017). Several theories exist to explain abnormal bone remodeling within the jaw. For example, it has been hypothesized that BP treatment prior to tooth extraction may initially prevent bone resorption within the resulting cavity, effectively delaying the early stages of socket healing (Hikita et al., 2009). Aguirre et al. showed that following extraction, BPs decrease resorption of interdental alveolar bone as well as reduce bone formation and vascularity in rats. The researchers suggest suppressed bone removal will ultimately culminate in necrosis (Aguirre et al., 2010). On the other hand, bone mass requires strain stimuli for its preservation. According to Hansson and coworkers, strains are reduced following tooth extraction, and this condition is no longer met. As such, resorption occurs, and mandibular dimensions—both vertical and horizontal—are decreased. Theoretically, resorption should persist until strains return to those experienced prior to extraction or reach a new homeostatic level (Hansson and Halldin, 2012).

Recent work by Gong et al. showed that, following tooth extraction, zoledronic acid prevents bone resorption by osteoclasts by downregulating the RANKL/OPG ratio and RANKL/RANK/OPG pathway and inhibits osteoblast mineralization by downregulating Wnt-3a and the canonical Wnt/β-catenin pathway (Gong et al., 2017). Jacobs et al. also reported that mechanical forces in combination with BPs affect the OPG/RANKL system, osteoclastogenesis and bone apposition factors. They showed tensile strain increases OPG synthesis and expression, causing a decrease in osteoclast activation but also suggested mineralization occurs at the location of tension during tooth movement. They

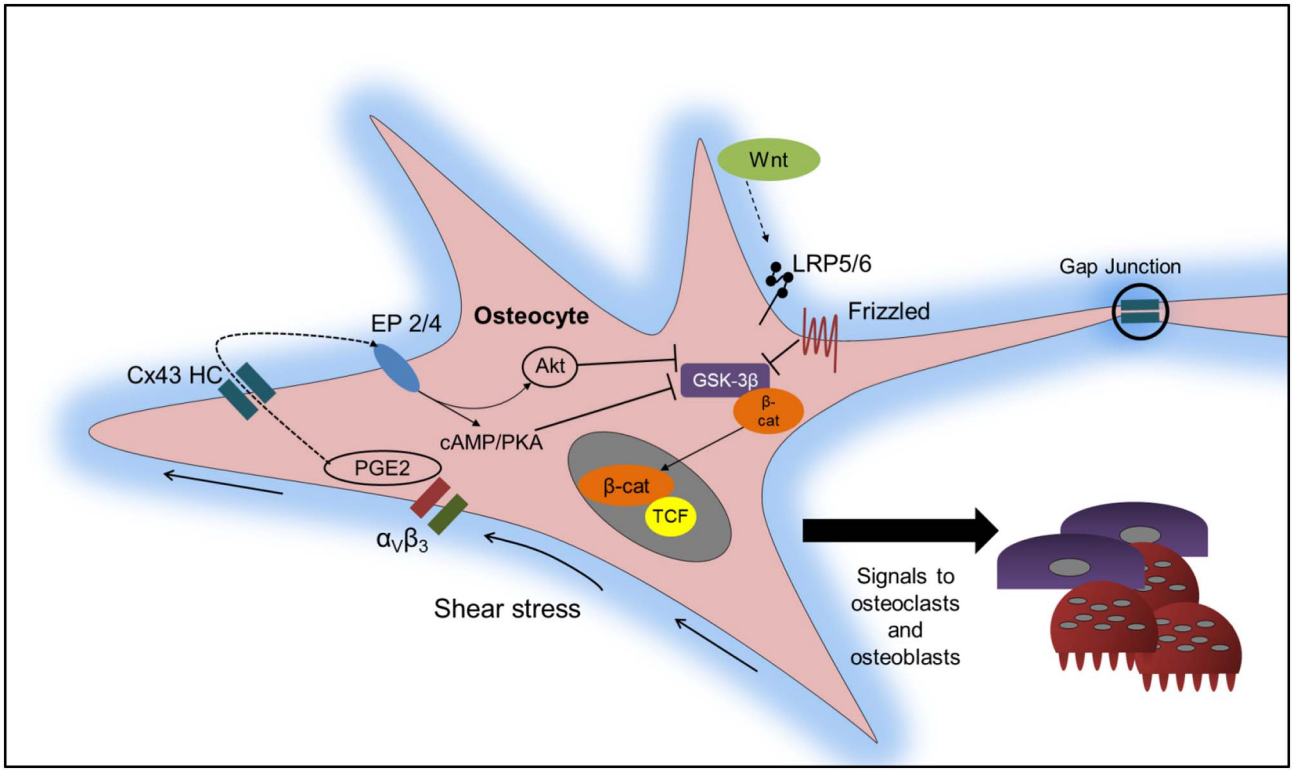

Fig. 2. Mechanisms involved in osteocyte mechanotransduction. The Wnt/β-catenin pathway initiates with binding of Wnt to the complex made up of frizzled and lipoprotein receptor 5/6 (LRP5/6) co-receptors which leads to a cascade of events including the eventual phosphorylation of GSK-3β (Bonewald and Johnson, 2008). β-catenin accumulates and is translocated to the nucleus; here, it forms a complex with the T cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors to mediate expression of key genes associated with bone cell function (Krishnan et al., 2006; He et al., 2004). In osteocytes, the α5β1 integrin interacts with gap junction gene, Cx43, to control opening of Cx43 hemichannels (Cx43 HC) activated by fluid flow. Osteocytes perceive mechanical load at αVβ3 integrin attachment locations (Marie et al., 2014). Via release of PGE2, shear stress triggers Akt and cAMP/PKA signaling, which together inactivate GSK-3. This results in further build-up of nuclear β-catenin (Bonewald, 2011). In effect, osteocytic gene expression is altered, mediating osteoblast and osteoclast activity.

conclude that, in the presence of a BP, high strength tensile strain may encourage bone loss by way of the OPG/RANKL pathway, while moderate strength tensile strain may contribute to formation of new bone (Jacobs et al., 2015).

While confusion exists surrounding the impact of load on bone exposed to BP, strong evidence indicates a link between mechanical trauma and BRONJ. Thus, it is feasible that immediate short term overload is a critical component of the disease; it is this response that needs to be modeled in vitro. To aid in the elucidation of BRONJ, the models must be capable of explaining the mechanisms by which osteocytes respond to mechanical trauma in a BP environment and subsequently relay signals to effector cells responsible for turnover. An important minipig osteonecrosis model developed by Otto et al. illustrated that all tooth extraction sites (24/24) in animals pretreated with BP showed signs of osteonecrosis of the jaw. However, the authors conclude that tooth extraction is not mandatory for manifestation of the disease because osteonecrosis also developed within infected areas of the oral cavity (Otto et al., 2017). Thus, multicellular interactions and remodeling in the presence of infection and associated inflammation must also be explained.

5. Inflammation

Inflammation associated with infection increases susceptibility to BRONJ, as is noted by Lesclous et al. who studied histopathological and clinical characteristics of individuals with the condition. In patients who had been diagnosed with and treated for chronic refractory osteomyelitis, a similar and overlapping disease (Tolstunov et al., 2012), necrotic bone was resected and examined. Empty osteocyte lacunae were identified, and bacteria and polymorphonuclear leukocytes were discovered in medullary spaces. Marrow spaces in perinecrotic bone showed signs of inflammation, including inflammatory cells and marrow fibrosis. The authors reason that NBPs themselves may activate inflammation. Following initial infusion of the drug, T cells mediate an immune response called an acute-phase reaction. Because NBPs inhibit the mevalonate pathway, they may prompt production of interferon-γ (IFNγ) followed by tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) by macrophages and monocytes. Stimulation of TNF-α then incites increased intercellular adhesion molecule 1 (ICAM-1) expression by monocytes, which in turn controls leukocyte attachment to endothelial cells and mononuclear cell transendothelial migration (Lesclous et al., 2009; Endo et al., 2017). Additionally, it was discovered that the severity of osteonecrosis as well as osteocyte apoptosis and the number of empty lacunae increase with heightened bone marrow inflammation. These apoptotic osteocytes may trigger inflammatory processes which can affect bone remodeling and, especially, osteoclast activity. More multinucleated cells positive for tartrate-resistant acid phosphatase (TRAP)—TRAP is expressed by actively resorbing osteoclasts and inflammatory macrophages – within marrow spaced were observed. These cells did not express the calcitonin receptor that when acted upon by calcitonin, prevents resorption (Masi and Brandi, 2007). Possibly, they differentiate in response to strong marrow inflammation and participate in clearing necrotic tissue.

Graves et al. argued that the proximity of inflammation infiltrate to bone determines its impact on remodeling. For example, when the subepithelial connective tissue is inflamed, gingivitis occurs. However, as the inflammation migrates closer to the bone, osteoclastogenesis is promoted and bone loss occurs. At this location, inflammation that prompts osteoclastogenesis and resorption may also lead to uncoupling of osteoclasts and osteoblasts, effectively preventing new bone

formation (Graves et al., 2011). These conclusions are particularly intriguing because they seem to suggest that inflammation works to counteract the effects of BPs on osteoclasts.

Presumably, inflammation also plays a significant role in osteocyte activity. Bakker et al. treated MLO-Y4 osteocytes with BRONJ-related cytokines—TNF-α and IL-1β—and reported an inhibition in upregulation of pulsatile fluid flow-stimulated nitric oxide. This is probably caused by a decrease in intracellular calcium concentration. The authors noted a decrease in F-actin and a reduction in elastic modulus. Possibly, treatment with these cytokines compromises cell stiffness, effectively altering the osteocytes' mechanosensitivity. If so, osteocyte-mediated osteoclast and osteoblast activity can be diminished (Bakker et al., 2009). For example, generation of RANKL, interleukin-6 (IL-6), TNFα and fibroblast growth factor 23 by osteocytes is heightened by IL-1β and TNFα. And, osteocyte-mediated osteoclastogenesis is increased by recombinant IL-1β, IL-6 and TNFα, while osteoblast-mediated osteoclastogenesis is increased by interleukin-8, chemokine (C-C motif) ligand 20 and TNFα. Therefore, heightened levels of inflammatory cytokines, like those implicated in BRONJ, may interrupt regular bone turnover (Pathak et al., 2016). Evidently, inflammation plays a large part, however complicated, in bone turnover as well as crosstalk among the bone cells responsible for turnover.

Differing from conclusions made by Lesclous and Graves, Ikebe predicted trauma via tooth extraction in combination with inflammation promotes release of NBPs in the mouth cavity, causing BRONJ. He hypothesized that, during turnover, osteocytes are exposed to NBPs that have accumulated in the alveolar bone. Micronecrosis ensues, and osteoclasts cannot resorb the necrotic bone which builds gradually. During tooth extraction, inflammatory cytokines are produced, inflammatory reactions take place and NBPs are increasingly released from the bone. The BPs may prohibit remodeling by preventing angiogenesis and cellular recruitment, effectively blocking formation of granulation tissue. Bacterial infection persists, enhancing osteonecrosis, and due to uncoupling between osteoclasts and osteoblasts, turnover is inhibited. Finally, liberated NBPs prevent mucosal keratinocyte proliferation, causing exposure of necrotic bone (Ikebe, 2013). To test this hypothesis, bone resorption and formation as functions of BP and disease co-factors—load and inflammation—must be simulated and quantified in vitro.

6. Conclusion

As we learn more about bone, we recognize its elegant intricacy. As such, to grasp and exploit bone's innate ability to remodel, we must move toward models that more accurately represent physiologic complexity. Specifically, to interpret the mechanisms by which BRONJ develops, we must first address the complexity of bone cell mechanotransduction within the BRONJ environment. Multiple factors, including BP, load and inflammation, act upon multiple cell types to engender a defect in bone remodeling. Improved mechanotransduction models need to be developed with the knowledge that cofactors do not affect osteocytes, osteoclasts and osteoblasts in isolation; rather, communication between cells makes up an aggregate response that is more physiologic. At its extreme, this cumulative response results in oversuppression of bone turnover and compromised tissue properties. Investigating BRONJ methodically using multicellular in vitro systems will help elucidate the pathways by which it occurs and may offer novel therapeutic strategies.

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