REVIEW ARTICLE

Effects of Bisphosphonates on Bone Fracture Healing: An Overview on New Preclinical Animal and Clinical Studies

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Abstract

This review covers the bisphosphonates (BPs) characteristics as a treatment choice for osteoporosis and their discrepancies as per available literature. In addition, the current study reviewed the effects of these antiresorptive compounds on bone fracture healing in experimental animal model studies. BPs have been commonly used in treating various skeletal diseases such as Paget's disease and osteoporosis. BPs reduce bone regeneration or resorption by inhibiting the osteoclast activities and thus, maintain or enhance bone mineral density when administered to osteoporotic patients. Given the critical importance of bone resorption during the bone healing process, the use of this class of medications is doubtful and controversial. Although some studies confirmed the efficient application of BPs, concerns about the possibility of delayed or impaired bone healing, non-union, or mal-union after BP administration have been raised in some other studies. Moreover, long-term BP administration like zoledronate is associated with some side effects such as atypical femoral fracture and osteonecrosis of the jaw. As a result, it can be accomplished that dose, timing, and duration of BP administration are important factors that establish the efficacy of BPs on the healing of different types of bone defects such as tooth extraction, spinal fusion, calvarial bone, and long bones. Further studies are needed for finding more safe and efficient substitutes for BPs to minimize or eliminate their undesirable effects.

Keywords: Bisphosphonate, Bone healing, Fracture, Osteonecrosis, Osteoporosis.

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Introduction

Bisphosphonates (BPs) are the treatment of choice for some skeletal diseases such as osteogenesis imperfecta, fibrous dysplasia, multiple myeloma, bone tumors, breast and prostate cancers, Paget's disease, and osteoporosis with low bone mineral density (BMD) in postmenopausal women, male hypogonadism, Crohn's disease and in patients consuming excessive glucocorticoids (Oryan *et al.,* 2015, Kates and Ackert-Bicknell 2016; Vannala *et al.,* 2020; Oryan and Sahvieh, 2021; Otto *et al.,* 2021). Excessive osteoclast activity is a crucial pathologic feature of these conditions (Matos *et al.,* 2010). Approximately 50 % of women and 20 % of men are affected by osteoporotic fractures in their lives (Barton *et al.,* 2020). Over the last several decades, BPs as major inhibitors of bone resorption have been widely applied to treat patients who have osteoporosis (Kates and Ackert-Bicknell 2016). In veterinary medicine, BPs, particularly pamidronate and zoledronate, are consistently used to control bone pain, bone tumors, and hypercalcemia in small animals and treat bone pain, navicular disease, and sesamoiditis in horses (Suva *et al.,* 2020). However, off-label use of BPs in young Thoroughbreds to increase bone mass and strength for reducing fracture risk at the training and racing age seems to be a critical challenge due to the potential risk of further failures and reduced fracture healing (Suva *et al.,* 2020).

Despite BPs activity, pathologic bone fractures and further requirements for surgical orthopedic treatments, which need bone remodeling, are common in these diseases (Matos *et al.,* 2010). Bone fracture healing is a complex process in which the ultimate goal is the return of the injured bone to normal/pre-fracture structural and functional properties (Oryan *et al.,* 2013; Alidadi *et al.,* 2017). This process is generally divided into four phases, including early hematoma formation and inflammatory phase, repair or regenerative phase with soft callus formation followed by hard callus formation, and ultimately bone remodeling phase characterized by differentiating woven bone into lamellar bone, alignment and maturation of the newly formed bone (Oryan *et al.,* 2013; Oryan *et al.,* 2014; Miyazawa *et al.,* 2020). Inhibiting osteoclast activity due to BPs administration can inhibit bone remodeling during bone graft incorporation

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or bone fracture healing (Matos *et al.,* 2010). Probably, the callus is not absorbed, and endochondral ossification is impaired during bone fracture healing due to inhibited osteoclast activity after BP administration (Miyazawa *et al.,* 2020). Overall, the impact of BPs on the fracture healing process remains unknown and could produce adverse effects. The effects of different types of BPs have been examined in bone fracture healing. Some studies have not reported considerable changes in bone healing, whereas others have indicated a slight improvement in the amount of newly formed bone (Kiely *et al.,* 2007; Matos *et al.,* 2010). Therefore, the present communication is aimed to review the effects of BPs on the bone healing process, particularly regarding the mode, timing, and dose of BP administration.

Bisphosphonates and Their Mechanism of Action

BPs are highly stable chemical compounds with a mean half-life of alendronate that has been estimated to be at least ten years (Anderson and Freedman, 2020). BPs, as the analogs of inorganic pyrophosphate, have two side chains in their chemical structure responsible for the pharmacological properties of BPs (Kates and Ackert-Bicknell, 2016). They are classified into two main groups; nitrogen-containing BPs and non-nitrogen-containing BPs. Potent antiresorptive nitrogen-containing BPs including zoledronate, risedronate, alendronate, pamidronate, and ibandronate inhibit farnesyl diphosphate synthase involved in the mevalonate synthesis pathway. Thereby they inhibit protein prenylation required for osteoclast function (Matos *et al.,* 2010; Kates and Ackert-Bicknell, 2016; Vannala *et al.,* 2020). This action could cause a mechanical inhibition of osteoclast adhesion to the bone surface, induce osteoclast apoptosis, and reduce bone resorption (Matos *et al.,* 2010; Kates and Ackert-Bicknell, 2016; Barton *et al.,* 2020). In contrast, non-nitrogen/simple BPs such as etidronate, clodronate, and tiludronate produce metabolites within osteoclasts that switch over with the terminal pyrophosphate of adenosine triphosphate (ATP) and in this way, deprive osteoclasts of the energy source and cause apoptosis (Kates and Ackert-Bicknell, 2016; Barton *et al.,* 2020; Vannala *et al.,* 2020).

BPs could bind to hydroxyapatite crystals of the bone tissue via chelation of calcium ions by their two phosphate groups (Hokugo *et al.,* 2019; Kates and Ackert-Bicknell, 2016). During bone resorption by osteoclasts, BPs incorporated in the bone are detached and released from the binding sites into the acidic lacuna and taken-up by osteoclasts (Barton *et al.,* 2020). Non-nitrogen-containing BPs could induce osteoclast apoptosis by incorporating into ATP decreasing osteoclast resorption by reducing the number of active osteoclasts on the bone surface. On the other hand, nitrogen-containing BPs inhibit pyrophosphate synthase resulting in cytoskeletal changes in osteoclasts, and thus they inhibit osteoclast activity and provoke osteoclast apoptosis (Hokugo *et al.,* 2019; Barton *et al.,* 2020; Lechner *et al.,* 2021). Hence, the net result might be diminished bone resorption by osteoclasts (Kates and Ackert-Bicknell, 2016).

Additionally, BPs are widely applied for the treatment of metastatic bone tumors due to their antiangiogenic effects. They could inhibit vascularization, a crucial element for tissue regeneration (Lechner *et al.,* 2021; Otto *et al.,* 2021). It has been shown that this action is performed directly by inhibiting the proliferation of endothelial cells and also indirectly through reducing the vascular endothelial growth factor (VEGF) levels (Santini *et al.,* 2003; Ribatti *et al.,* 2008; Otto *et al.,* 2021).

Pros and Cons of BPs

As already mentioned, BPs are used to treat various skeletal diseases and tumors (Vannala *et al.,* 2020), by administering BPs, bone resorption is decreased, but new bone formed by osteoblasts is not equally affected; as a result, loss of bone mass becomes slow and, BMD increases for example, in osteoporotic patients (Barton *et al.,* 2020). Therefore, a secondary effect of antiresorptive BPs is improved BMD; however, this can lead to atypical bone (femoral) fractures due to becoming hard and brittle (Aki *et al.,* 2021; Miyazawa *et al.,* 2020). In other fields, like dentistry, some studies have indicated that administration of potent BPs such as zoledronate or alendronate can cause osteonecrosis of the jaw bone during tooth extraction (Ruggiero *et al.,* 2014; Lechner *et al.,* 2021; Otto *et al.,* 2021). Indeed, despite BPs being the drugs of choice for bone diseases such as osteoporosis or Paget's disease, there are enormous problems with their administration. BPs might exert short-term effects, including musculoskeletal pain, gastrointestinal disturbances, acute-phase reaction, hypocalcemia, ocular inflammation (Miyazawa *et al.,* 2020; Wilkinson, 2020; Oryan and Sahvieh, 2021). Moreover, atrial fibrillation, jaw osteonecrosis, atypical femoral fractures, and suppressed bone turnover are contributed to the well-known long-term adverse effects of BPs (Mauceri *et al.,* 2018; Wilkinson, 2020; Lechner *et al.,* 2021; Nakagawa *et al.,* 2021).

BP-related Osteonecrosis of the Jaw

BP-related osteonecrosis of the jaw (BRONJ) as a complication of intravenous and long-term administration of BPs has been reported in some studies due to diminished osteoclastogenesis (Allen and Burr, 2009; Mauceri *et al.,* 2018; Lechner *et al.,* 2021). Although the overall pathogenesis of BRONJ has not yet been fully understood, BRONJ development is often characterized by a slow start and usually presents with infarcts and thrombosis of small vascular sections of the supplying artery within the medullary canal (Lechner *et al.,* 2021). It is probable that with excessive inhibition of osteoclast function induced by BP administration, the dying osteoclasts will not be replaced, and the capillary network supplying the bone will not also be maintained, resulting in BRONJ (Gutta and Louis, 2007; Lechner *et al.,* 2021). Since this condition is caused by antiresorptive medications other than BPs, BRONJ has

recently been termed medication-related osteonecrosis of the jaw (MRONJ) (Ruggiero *et al.,* 2014; Vannala *et al.,* 2020). Nonetheless, Lechner *et al.* (2021) showed that BPs should not be regarded as the only cause of BRONJ. Other causes, such as the unresolved areas of wound healing at extraction sites, could directly contribute to the BRONJ pathogenesis (Lechner *et al.,* 2021).

Although osteonecrosis of the jaw bone is regarded as an adverse impact of long-term BP administration, many studies have produced promising outcomes of reducing or preventing this unfavorable condition (Mauceri *et al.,* 2018; Hokugo *et al.,* 2019; Otto *et al.,* 2021). For instance, in a current study, Nakagawa *et al.* (2021) revealed osteonecrosis following long-term subcutaneous administration of zoledronic acid in an osteoporotic rat model. Tooth extraction with BP administration inhibited bone remodeling, but metformin protected the jaw from the BRONJ-like lesions. In another study, Otto *et al*. (2021) showed that geranyl-geraniol (GG), a metabolite formed in the mevalonate pathway, could weaken the effects of BPs *in-vitro*, and expressed hope that it would be able to prevent BRONJ *in vivo*. They demonstrated the inhibitory effects of BPs on migration behaviour and cell viability of human osteoblasts, normal human dermal fibroblasts, human endothelial progenitor cells, and human umbilical cord endothelial cells *in-vitro*. The addition of GG attenuated the inhibitory effects of BPs on primary cell cultures. The examined BPs included clodronate, pamidronate, ibandronate, and zoledronate belonging to the potent nitrogen-containing class of BPs (Otto *et al.,* 2021). Interestingly, Hokugo *et al.* (2019) found that local application of a low potency BP such as etidronate after systemic administration of zoledronate, before tooth extraction (*i.e.*, BP-BP replacement on the bone surface) could reduce the BRONJ development and enhance the bone regeneration in mice. It is worth mentioning that zoledronate is considered the most potent member of BPs (Matos *et al.,* 2010).

BPs and Bone Healing

Bone resorption plays a crucial role in bone remodeling by removing old and poor-quality bone to allow fresh bone deposition (Oryan *et al.,* 2013). Bone remodeling, in turn, plays a vital role in bone fracture healing by allowing consolidation of the soft callus into the normal lamellar bone. Therefore, many researchers are concerned that inhibition of bone resorption by BPs may interfere with normal bone healing and cause malunion, delayed union, or non-union (Barton *et al.,* 2020). For example, the effect of BPs (zoledronic acid) on the osseointegration of cylinder titanium dental implants has been investigated in a calvarial bone defect model in rabbits (Yu *et al.,* 2021). Treatment with zoledronic acid significantly reduced bone growth rate in short and long terms compared to the control group, with no remarkable effects on BMD. They offered a declined bone resorption and remodeling due to the BP administration as the possible reason for reduced new bone formation (Yu *et al.,* 2021). A current study compared collagen sponges containing recombinant human bone morphogenetic protein (rhBMP)-2 with demineralized freeze-dried bone grafts and autogenous bone graft implanted in the femoral bone defects of rats treated with long-term and high-dose zoledronic acid injections (Moon *et al.,* 2021). They found that the highest union rate contributed to the bone defects treated with the autograft and BMP, while BP therapy significantly reduced bone turnover. They offered BMP-2 as a good substitute for autografts in patients suffering from diminished bone healing due to long-term BP treatment (Moon *et al.,* 2021).

Some studies have shown that BPs could inhibit osteoblast attachment to the bone matrix or scaffold surface and impair bone healing *in vitro* and *in vivo* (Koyama *et al.,* 2020). Hirota *et al.* (2012) fabricated a scaffold made of titanium fibers coated with thin hydroxyapatite and reported an enhanced human osteoblast attachment and proliferation *in vitro* (Hirota *et al.,* 2012) and improved bone reconstruction of the mandibular bone defects in rabbits (Hirota *et al.,* 2016). They seeded human osteoblasts onto the scaffold containing pamidronate, a nitrogen-containing BP, and evaluated cell attachment and mineralization. Then, they examined the osteoconductive properties of the scaffold by implanting it into cranial bone defects of rats. Binding pamidronate to the scaffold significantly reduced osteoblast numbers and mineralization *in vitro* and considerably diminished new bone formation *in vivo* (Koyama *et al.,* 2020).

However, it is essential to note that several studies have conversely shown positive effects of BPs on osteoblast proliferation (Im *et al.,* 2004; Maruotti *et al.,* 2012) and their inhibitory impacts on cellular apoptosis related to osteoblasts and osteocytes that might augment or facilitate the healing process in these ways (Plotkin *et al.,* 1999). Consistently, Demircan and Isler (2021) examined the effects of local (the allograft material soaked in alendronate solution at a concentration of 1-mg/mL) and systemic (3 mg/kg; 1-hour before the operation) alendronate administration in bone grafting on tibial bone healing of rats after six weeks. Antiosteclastic effects of alendronate led to better graft integration and enhanced bone formation with no significant complications such as inflammation, fibrosis, and necrosis (Demircan and Isler, 2021). Likewise, Matos *et al.* (2010) examined the effect of zoledronate during bone healing following fibular osteotomy in rabbits and showed the anabolic effect of zoledronate on fracture healing. They indicated that zoledronate did not prevent bone healing and provided an increased amount of newly formed bone after four weeks. They suggested that inhibition of bone regeneration due to zoledronate did not adversely influence the early stages of the bone healing process.

Regarding the efficacy or effects of BPs on spinal fusion, Anderson and Freedman (2020) analyzed the data about 1040 patients affected by osteopenia or osteoporosis who

underwent lumbar fusion operations by a 5-year follow-up period. Among these, 467 patients used BP before their elective operation; however, the dosing and timing of BPs were unclear. They noted that BPs neither impair spinal fusion nor increase osteoblastic activity and new bone formation, but they could improve the mechanical function of bone and reduce screw loosening (Anderson and Freedman, 2020).

Discussion and Conclusion

Despite numerous studies, many aspects are still unknown about the effects of BPs on bone healing, and the results of the studies are conflicting. Some studies have reported the positive effects of BPs on bone healing and maintain their strong position because of good efficacy in treating osteoporosis, while others have revealed the opposite effect. Given the inconsistent results achieved from different studies, it is likely that various critical points, including the timing of BP initiation after fracture, duration, and mode of the drug administration, effect or estimation of effectiveness of BPs in bone healing and the success of treatment with BPs depend on these variables. Reports are declaring that a low concentration of locally administrated BPs stimulates the proliferation and differentiation of osteoblast *in vitro* (Naidu *et al.,* 2008). Also, the short-term application of bisphosphonate improves bone regeneration due to pharmacological effects *in vivo* (Toker *et al.,* 2012). It also has been shown that BPs could enhance BMD in ovariectomized rats (Li *et al.,* 2016), and in osteoporotic patients during the first year of treatment (Tonino *et al.,* 2000; Yu *et al.,* 2021). Recently, Barton *et al.* (2020) have briefly reviewed 20 clinical and several pre-clinical studies about the timing of BP initiation after fracture due to osteoporosis. They confirmed that initiation of BP therapy after two weeks of the fracture does not increase the possibility of malunion or non-union; thus, the clinicians can initiate osteoporosis treatment with BPs promptly after the fracture (Barton *et al.,* 2020).

In another study, 132 patients who had received oral BPs for the treatment of osteoporosis and required tooth extraction were classified based on the duration of BP administration (Shudo *et al.,* 2018). The results showed that BP therapy for more than five years significantly delayed and impaired the healing process of the extraction compared to the BP administration for a less prolonged period (Shudo *et al.,* 2018). Similarly, Aki and coworkers (2021) demonstrated that long-term BP administration in osteoporotic patients with insufficient healing of femoral fracture might alter the morphological features of the fracture site compared to the normal state. By long-term use of BP, the number of osteoclasts in the bone resorption surface was reduced, the amount and ratio of woven/immature bone to mature bone was increased, and the shape of the osteons was changed (Aki *et al.,* 2021).

Additionally, Kim *et al.* (2015) investigated bone regeneration using a combination of different concentrations of alendronate and rhBMP-2 in rat calvarial defects. They indicated that low drug concentrations and rhBMP-2 could exert synergic effects on bone formation. This was confirmed by a decreased receptor activator activity of nuclear factor kappa-B ligand (RANKL), an osteoclast differentiation factor, by osteoblasts in immunohistochemistry analysis (Kim *et al.,* 2015). Yang *et al.* (2020) created bilateral femoral condyle defects in osteoporotic rabbits and treated them with porous titanium implants containing different doses of zoledronic acid (1, 10, 50, 100, and 500 µmol/L) loaded gelatin nanoparticles. These composite scaffolds stimulated osteoblast differentiation and also inhibited osteoclastogenesis *in vitro*. In an *in vivo* study, the composite scaffold composed of zoledronic acid at the low concentration of 50 µmol/L was optimal, in which bone regeneration and bone callus size were significantly improved, and bone resorption was reduced in the osteoporotic bone defects (Yang *et al.,* 2020).

Moreover, another critical point in BP administration is the selection of biomaterials or growth factors that have been used in combination with BPs. Tissue engineering could be helpful in terms of providing promotive factors of bone healing such as platelet-rich plasma or BMPs or appropriate biomaterials, particularly in nanoscales with osteoinductive properties (Oryan *et al., 2014*; Alidadi *et al.,* 2017; Alidadi, 2020; Yang *et al.,* 2020). For example, Mauceri *et al.* (2018), in one clinical study, indicated that the cancer patients benefited from erbium, chromium, yttrium, scandium, gallium, garnet (Er,Cr,YSGG) laser therapy together with autologous platelet-rich plasma (PRP) showed notable improvement in the 50 % of lesions regarding BRONJ clinically. In one study, five weeks after treating rats with alendronate, femoral defects were implanted with beta-tricalcium phosphate (β-TCP) cylinders loaded with BMP-2, and L5IP as an inhibitor for endogenous BMP antagonists for 16 weeks. Alendronate caused a reduction in the β-TCP implant turnover. It can be explained in this way that β-TCP degradation and removal are highly dependent on osteoclast resorption rather than chemical solubility (Hauser *et al.,* 2018). Thus, using such implants like calcium-phosphate bioceramics that require the osteoclast activity for their degradation might mechanically interfere with optimal bone regeneration (Hauser *et al.,* 2018). In addition, providing suitable substitutes for BPs can be regarded as an effective strategy. For instance, Reveromycin A (RMA), a polyketide isolated from *Streptomyces* spp., has been suggested as a safe and effective alternative to BPs for treating high-turnover osteoporosis (Miyazawa *et al.,* 2020). Considerable enhancement in tibial bone healing and decrease in callus areas were reported after 21 days of RMA application compared with BP (alendronate) in mice. RMA does not deposit within the bone, and this is regarded as an essential feature. Moreover, the side effects of RMA are thought to be minimal based on its short half-life and

its specific uptake by active osteoclasts (not by osteoclast precursor cells or inactive osteoclasts) (Miyazawa *et al.,* 2020).

Considering the studies reviewed here, the reasonable conclusion to be drawn is that early, local, and short-term BP therapy immediately after the fracture can be more effective. However, BP deposit within the bone and their mean half-life are prolonged (for example, at least ten years for alendronate) and; thus, stopping BP therapy before surgery does not seem to prevent its impact on the bone during the perioperative period (Anderson and Freedman, 2020).

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