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Review Article

The Effects of Bisphosphonates Family with Focus on Zoledronate Drug on Bone Healing

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Abstract

Fracture and bone diseases injure bone structure and a proper material or method is needed to heal the injured site. Successful bone healing is one of the significant worldwide concerns and the orthopedic surgeons emphasis on this notable issue. Metabolism and catabolism are two consequential responses for bone healing and a well balance between them leads to a more efficient fracture healing. One of the drugs that could interfere with these responses is bisphosphonates (BPs). They are a group of drugs with anti-catabolic effect and slow down the process of osteoclastic function and catabolic reactions and result in a reduction of bone degradation and increase the mechanical strength of callus. This drug family also helps the process of healing by inhibiting the apoptosis of osteoblasts and osteocytes. Besides, this family does not just have desirable effects on bone regeneration but it may have some side effects and limits the capacity of healing. Therefore, in this article, we stated both the positive and negative effects of BPs with focus on the most potent one in clinical usage, named zoledronate (ZOL) on bone healing. Zoledronate is effective on the recruitment, proliferation, and migration of osteoblasts in addition to its anti-resorptive activity and results in enhanced bone remodeling and regeneration. Based upon the efficient effects of zoledronate on bone regeneration, it could be used as one of the efficacious treatment regimens in this field.

Keywords: *Bone Healing; Bisphosphonates; Anti-Catabolic Effect; Zoledronate*

Introduction

Finding a functional method to enhance bone healing is one of the significant concerns of orthopaedic surgeons and researchers [1]. Some conditions such as trauma, accidents, non-union bone defects, burns, osteotomies, bone tumor resections, arthritis, osteomyelitis, compound or pathologic fractures, chronic bone infections, osteoporosis, bone cancer, osteoarthritis, and myeloma-related bone diseases cause massive bone defects and need to regenerate and reconstruct by efficient methodologies [2-6]. Bone tissue could not repair itself spontaneously in the critical bone defects and need intervention [7]. There are many materials and approaches that the orthopedic surgeons and researches test to overcome this insufficiency. Natural and synthetic biomaterials, statins, grafts, intramedullary pins, plates, screws, growth factors, stem cells with different sources, glycosaminoglycans, and some drugs such as denosumab, SERMs, bazedoxifene and raloxifene have been used in the hope to enhance bone regeneration [2,8-13]. Some of these approaches and materials have specific disadvantages which limit their utilization in bone healing and regenerative medicine [2,14]. Therefore, selection of more powerful and

effective materials or drugs with minimum harmful effects on bone healing is noteworthy. In this article, we focused on the effectiveness of bisphosphonate drugs (BPs) on the healing process of bone tissue and also specified their side effects.

Bone healing

Bone regeneration starts by hematoma or blood clot formation and continues by acute inflammation which is characterized by infiltration of platelets and polymorphonuclear cells and then proceeds to chronic inflammation in which macrophages, lymphocytes, and plasma cells infiltrate into the defect site [15]. For the first few days, the inflammatory mediators such as histamine, serotonin, bradykinin, thromboxane, plasmin, and leukotrienes such prostaglandins are released by platelets, mast cells, basophils, and blood plasma to result in vasodilatation and further cell infiltration from the blood vessels to the defect area. At the later phases of the inflammatory stage, the inflammatory cells release growth factors and cytokines to establish the migration, proliferation, and differentiation of the osteoprogenitor cells [15,16]. Some of these growth factors and cytokines influence on the mesenchymal cells and lead them to migrate, proliferate, and differentiate into chondroblasts, chondrocytes, osteoblasts, and osteocytes to form fibrocartilaginous, cartilaginous, and bone tissues during the proliferative and maturation stages of bone healing [17,18].

Osteoclasts are the multinuclear giant cells that are derived from the myeloid lineage and can mediate bone loss in osteoporosis or osteoarthritis. They also initiate bone remodeling during tooth eruption, bone growth, and fracture healing [19]. The necessary mediator of osteoclast formation, activation, and survival is the receptor activator of nuclear factor (NF)-kB ligand (RANKL) which binds to RANK, as its receptor, on the surface of osteoclast precursor cells and results in activation and differentiation of osteoclasts and finally induce bone resorption [20,21]. The rate of bone formation after fracture is determined by bone resorption based on the activity of the osteoclasts and new bone formation based upon the activity of osteoblasts [22]. The intercellular communication between the osteoclasts and osteoblasts is vital for bone homeostasis which needs to induce osteoclastogenesis [20]. There is a complex set of regulated signaling pathways that reabsorb the damaged matrix of bone tissue and control formation and remodeling of the new bone matrix. In fact, this pathway is primarily mediated by anabolic osteoblasts to form the new bone, and then it is continued by osteoclasts activity resulting in resorption of the original bone tissue [22]. As a general rule, the anabolic response starts with the early acute inflammatory stage of the bone healing to further regain the bridging in the fracture site by osteoblasts in the proliferative phase and finally results in optimum mechanical integrity. Further, the catabolic response starts in the fracture site and the new bone is remodeled by osteoclasts. Ultimately, the equilibrium between the anabolic and catabolic responses should be established. An increased catabolic response or failure in anabolic response or both may disrupt the healing process and delayed union or non-union are occurred (Figure 1) [20,23].

A successful fracture healing is characterized by regeneration and remodeling of the defect area with formation and remodeling of the well-vascularized newly formed bone to result in adequate stability and lead to optimum skeletal functions [18,23,24]. Atrophic non-union due to diabetes, infection, smoking, some drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and local factors like devascularized periosteum, reduce regeneration and new bone formation. Manipulating the anabolic and catabolic processes by biological, pharmacological, and even mechanical interventions would result in enhanced fracture healing [23,25]. One of the effective methods for bone healing is a drug delivery system with a drug that is not cytotoxic or allergic and is effective on mesenchymal cell recruitment, proliferation, and differentiation to osteoblast cells [26].

Bisphosphonates

This drug family has some contradictory effects on bone healing; these include increasing bone mineral density, reducing the rate of bone resorption, and stabilizing osteolytic lesions [27]. On the other hand, bisphosphonates improve the stability of endoprostheses and prevent pain, hypercalcemia, and pathologic fractures when they are utilized for bone diseases with excessive activity of osteoclasts including skeletal metastases of malignant tumors, prostate cancer, breast cancer, Parget's disease, hypercalcemia of malignancy, multiple myeloma, osteolytic, metastatic bone diseases, osteoporosis, and osteogenesis imperfecta [21,28-31]. BPs have been used to treat human osteoporosis and resulted in enhanced bone healing with proper mineralization and remodeling and minimum bone resorption [22].

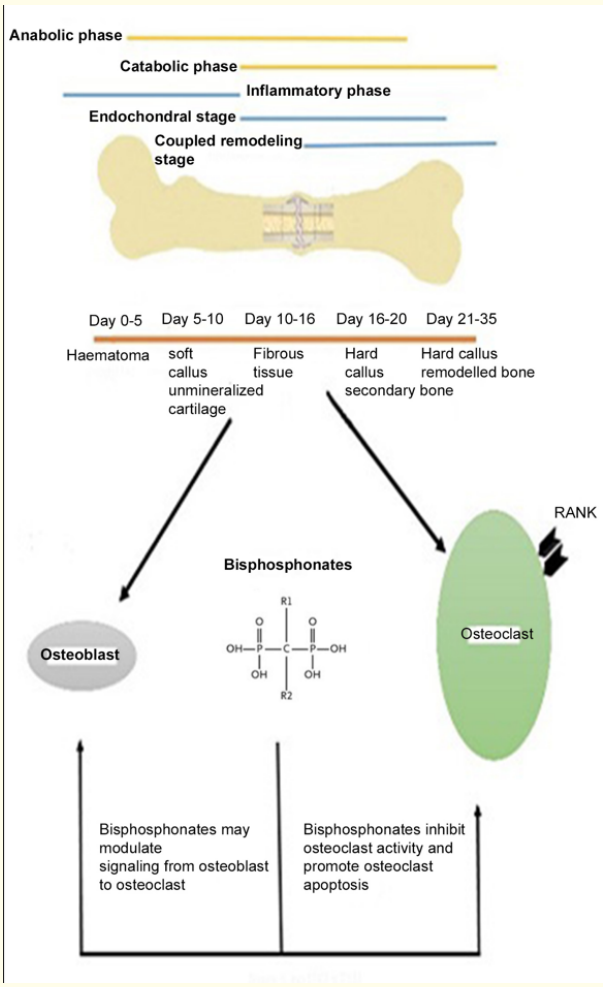

Figure 1: *This is a bone healing process that bisphosphonates could intervene it by affecting on osteoclasts. Bisphosphonates induce apoptosis in the osteoclast and cause a decrease in bone turnover due to the reduction of bone resorption. It also causes a decrease in the number of new bone multicellular units. Decreasing in osteoclast recruitment and adhesion is also another effect of this family.*

Structure and function

Carbon substitutes pyrophosphate analogs and a P-C-P instead of P-O-P exists in BPs structure which is related to endogenous pyrophosphates and is resistant to chemical and enzymatic hydrolysis [30,32]. If R1 is a hydroxyl, it tightly binds to the bone mineral to assist the R2 side which is BPs to do its pharmacological activity. Drugs with a P-C-P binding in this family allow a large amount of possible variations which result in prevention of osteoclastic bone resorption either by esterifying phosphate group or changing the two lateral chains of the carbon atom [30]. Based on the attachment of R1 or R2 to the central carbon atom, BPs are classified into two groups. The first group is the non-nitrogen containing BPs including etidronate, tiludronate, and clodronate, and the R2 counterpart contains CH3 or CL at a nitrogen-free ring structure [30,33]. The non-nitrogen containing BPs are built by the phosphate chain of adenosine triphosphates [34]. This group induces apoptosis by combining with ATP in the osteoclast and ultimately reduces the number of active osteoclasts to

decrease bone resorption [22]. The intracellular metabolites create the inactive and cytotoxic analogs of ATP based on inhibition of the mitochondrial ADP/ATP translocase, leading to osteoclasts apoptosis and inhibiting the osteoclastic bone resorption [30].

The second group includes zoledronate (ZOL), alendronate, ibandronate, risedronate, and pamidronate which are the members of nitrogen-containing BPs. Two drugs of this family including alendronate and pamidronate have an aliphatic side chain with a single nitrogen atom and zoledronate has a second nitrogen atom in the ring structure which is the imidazole derivative. This group interferes with farnesyl pyrophosphate (FPP) synthase, protein prenylation, and the mevalonate biosynthetic pathways to affect osteoclast activity [21,22,29,30]. They bind to the enzyme in the 3-hydroxy-2-methylglutaryl-CoA (HMG-CoA) reductase pathway by the mevalonate pathway to block the prenylation of the GTPases which damages the survival and function of osteoclasts [18].

Inhibition of the mevalonate pathway causes cytoskeletal disorders in the osteoclasts and results in inhibition of its activity, induces cell apoptosis and prevents bone resorption [22,25]. Prenylation affects the signaling proteins and cell processes, cell morphology, cytoskeletal arrangement, and vesicle trafficking verification which are important for the osteoclast function to result in osteoclastic apoptosis [29]. The geranylgeranylated proteins that have a role in the formation and function of osteoclasts are inhibited by nitrogen-containing BPs, leading to inhibition of the osteoclasts mediated bone resorption, mevalonate pathway, and damage to the protein prenylation. Inhibition of the mevalonate pathway occurs due to the prevention of key enzyme, farnesyl diphosphate synthase (FPPS) (Figure 2) [30,35]. Farnesyl pyrophosphate synthase is the major enzyme target for the nitrogen-containing BPs and inhibition of the FPPS leads to the prevention of biosynthesis of isoprenoid compounds, especially farnesol and geranylgeraniol which are necessary for the post-translational prenylation of GTP-binding proteins like rho, rab, and rac. These proteins have a role in the intracellular signaling events within osteoclasts [19].

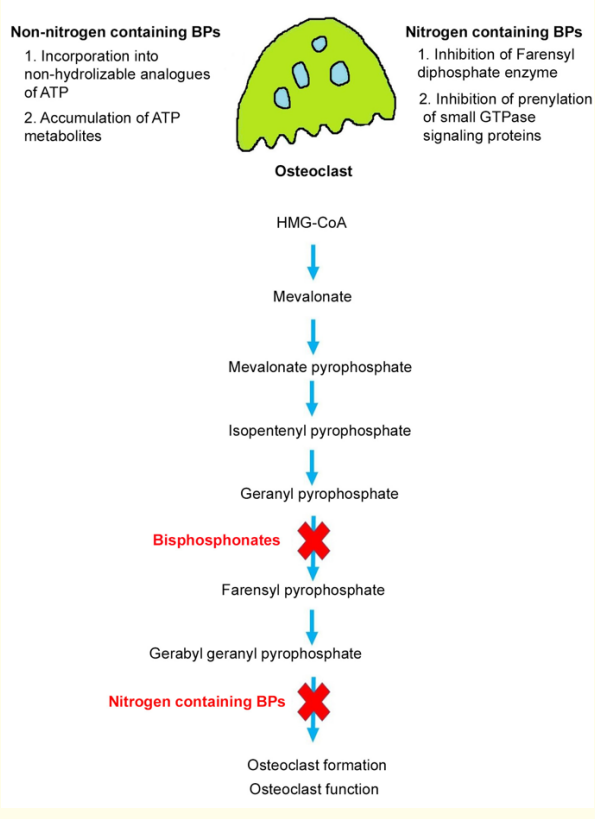

Figure 2: *The mechanism of nitrogen and non-nitrogen containing bisphosphonates on osteoclasts.*

The nitrogen-containing BPs inhibit the osteoclast precursors from migration toward the inflammatory osteolytic lesions and differentiation to osteoclasts and also prevent the catabolic activity of the mature osteoclasts [33]. Finally, both the non-nitrogen containing BPs and nitrogen-containing BPs reduce the osteoclastic bone resorption, but the anti-resorptive activities of nitrogen-containing BPs are more powerful than the non-nitrogen containing BPs [22,36]. Zoledronate, risedronate, pamidronate, alendronate, and ibandronate are the amino BPs that have faster efficacy, greater potency, and more persistent inhibitory effects on bone turnover than the non-amino BPs including clodronate and etidronate [37]. Prevention of the bone mass loss at the sites of active remodeling occurs due to inhibition of the osteoclast activity with the intermediation of risedronate, zoledronate, alendronate, and pamidronate. In fact, they have the calcium-chelating abilities and could efficiently target at this site [18]. Clodronate and etidronate prevent the mitochondria key function, resulting in loss of energy production in osteoclasts by generating a toxic analog of adenosine triphosphate. On the other hand, alendronate and zoledronate inhibit the key enzymes of the pathway of the cholesterol/mevalonate biosynthesis (Table 1) [19].

• Reduce osteoclast recruitment and number
• Reduce osteoclast adhesion
• Increase the apoptosis of osteoclast
• Reduce osteoclast activity (reduction of enzyme activity and acid extrusion)
• Alter cytoskeleton of osteoclast
• Reduce the release of cytokines by macrophage
• Increase number and differentiation of osteoblast
• Prevent the mevalonate pathway
• Reduce post-translational prenylation of GTP-binding proteins

Table 1: *Effects of bisphosphonates on cells and molecules.*

Bisphosphonates have a high affinity to hydroxyapatite (HA) in the bone structure and inhibit the dissolution of HA *in vivo* and *in vitro* [22]. They also have a high affinity to calcium particles of bone tissue and are involved in the anabolic phase of remodeling during new bone formation [28]. Based on their high affinity to calcium, they inhibit calcium phosphate dissolution and bone resorption and maintain structural integrity of the mineralized bone. They tend to gather in the mineralized bone matrix, especially at the interface of osteoid mineralized bone and at the edge of trabeculae to inhibit maturation and recruitment of the osteoclasts [22,30].

Advantages and disadvantages of BPs administration

Many studies showed the beneficial effects of this family on bone healing in mice, rats, rabbits, ovine, dogs, and humans. Etidronate was the first drug used in human medication, but nowadays at least eleven BPs are administrated for various clinical applications [19]. In this section, we discussed the positive and negative effects of BPs on fracture healing.

Advantages: BPs recruit and differentiate the osteoclasts and regulate the osteoclast-mediated bone resorption activity [19]. They raise the stability of fracture and perform faster effects when they are used by local delivery [18]. Low concentrations of BPs inhibit apoptosis of the osteocytes and osteoblasts, stimulate formation of the mineralized bone nodules, and have beneficial effects on protein synthesis, based on *in vitro* findings [38]. It has been reported that the osteoclastic activity was reduced and fracture healing was improved by systemic bisphosphonates therapy [29,39]. Bisphosphonates have anti-catabolic effects and slow down the bone remodeling process which is a sequence of osteoblast-mediated bone formation and resorption. In addition, by changing the balance between resorption

and regeneration based on the osteoclasts and osteoblasts function, BPs improve the callus strength and fracture healing in combining with the anabolic treatment [38,40]. This family also prevents premature resorption of new bone formation [23]. High doses of BPs inhibit bone resorption and enhance bone regeneration, remodeling, and mineralization [32]. The extent of bone regeneration depends on the susceptibility to BPs treatment and their regenerative capacities [40]. BPs also inhibit formation of the osteoclast-like cells in the human bone marrow cell cultures in a dose-dependent manner [19]. They inhibit bone resorption and increase bone mineral content [41]. A bisphosphonate with a heterocyclic ring such as zoledronate has more than 10,000 times potential than etidronate and when it is administrated in orthopedic surgery and dentistry it increases the density of bone tissue around the orthopedic screws, increases the mechanical performance of the orthopedic implant, accelerates the fracture healing, and improves the stability of joint replacements [42]. Non-nitrogen bisphosphonates (non-NBPs) are rapidly metabolized, so they do not make bone necrosis [33].

They also have anti-tumor potential by negatively regulating tumor cells, macrophages, and endothelial cells [43]. BPs reduce skeletal-related events in cases with bone metastatic breast cancer and cancer therapy-induced bone loss because of their anticancer activity based on stimulating immunity and inhibiting invasion and tumor cell adhesion. Their antitumor effect on osteosarcoma cell lines, when they were administered intravenously or transplanted intraosseously, was directly via suppressing lung metastases, reducing primary tumor growth, and prolong survival in murine models [44]. The development of neointimal hyperplasia and atherosclerosis were inhibited by BPs because of their effect on the phagocytic capabilities of monocytes and macrophages which were inactivated with BPs. Reduction in local inflammation and prevention in the growth of vascular smooth muscle cells occurred following BPs administration [28]. Overall, all BPs decrease the risk of vertebral fracture in osteoporotic patients and risk of the hip fracture in non-vertebral fractures except ibandronate [45].

Increase in fracture bridging with/without retention of the cancellous bone structures within the callus was seen after BPs treatment [22]. It has been reported that a larger fracture callus was formed after treatment of the ovariectomized rats by alendronate [46]. The delay of healing in the animals with a high dose of risedronate was much more than the animals used a low dose of this drug and no interfering was observed with the callus formation [47]. A study was done in 80 elderly patients with acute fragility of distal fractures and 40 patients received 35 mg alendronate, orally once a week for six months. It was stated following radiologic and clinical studies that alendronate was not responsible for the delayed fracture healing [48].

When alendronate was used in the treatment of the osteotomized rat model it increased bone mineral density [49]. A dose of 100 mM of zoledronate caused toxicity in the tested cell types including squamous carcinoma cells, prostate adenocarcinoma cells, gingival fibroblasts, and periodontal ligament cells. However, etidronate was not toxic at 1 - 100 mM in these cell types and even decreased the cytotoxicity of zoledronate in some cell-types such as gingival fibroblasts [36]. After subcutaneous administration of clodronate and etidronate, the number and size of osteoclasts and the thickness and number of the trabeculae in the metaphysis of the long bones were elevated [30]. Ibandronate and pamidronate demonstrated a greater increase in endosseous implant stability than the control group [50]. Pamidronate disodium and zoledronate, the most potent BPs in clinical usage, were used in cancer treatment. Pamidronate disodium inhibited bone resorption, but by discontinuing the treatment for 3-5 years, the incidence rates of fractures were comparable to the normal bone [27]. Zoledronate and alendronate were administrated for 3 years and risedronate was given for 5 years and they resulted in maintaining their anti-fracture effects for more than 10 years [27].

It has been stated that the local delivery of BPs in bone implantation had a generally positive effect on implant fixation and bone volume fraction. In fact, as early as 2 or 3 weeks after implantation, the administration of this family in rats resulted in increased bone volume fraction and pull-out force. Likewise, the increase in implant fixation was related to the amount of bone formed at the border of the implant beside that the scaffold preserved the initial osteoid formation, but the new bone formation was not related to the local delivery of BPs [51]. Administration of this drug family in the treatment of periodontitis, after mucoperiosteal surgery in rat, prevented bone

loss, reduced bone resorption and resulted in skeletal relapse after mandibular distraction and maxillary expansion, and induced post orthodontic relapse, pulpal mineralization of the re-implanted teeth after an avulsion, and root resorption [52].

BPs were used in both systemic and local approaches, but the best result was obtained with the local delivery; besides the local delivery was not associated with undesirable systemic side effects [53]. Local application of this family raised the osseointegration of implants, especially in osteoporotic bone [18]. Also, the half-life of BPs in the circulation is short but, because of their long maintenance in the bone tissue, the half-life of elimination of this family from the skeletal system may last up to 10 years [22]. By implanting the BPs-loaded scaffolds in bone tissue, the drugs are released into the acidic lacuna to be phagocytosed by the osteoclasts [22]. In this approach, the bone graft substitutes immerse into the BPs solution and a high concentration of a drug is present in the defect region [53]. Likewise, there are some strategies in enhancing the effect of local delivery of BPs including controlled short-term release and co-delivery with some bone anabolic factors [18]. Injectable bone cement, biodegradable polymer coatings, and calcium phosphates-containing implant coatings are the most delivery strategies for this drug family [18]. The orthopedic implants coated by BPs resulted in enhanced fracture healing, increased mechanical fixation of implants and improved the joint replacements stability, the fixation, and bone volume surrounding the screws [52].

Disadvantages: Oral administration of the NBPs injures the gastrointestinal and esophageal tissues and causes esophagitis, vomiting, diarrhea, vasculitis, nausea, hypocalcemia, gastric ulcer, pyrexia, pain, and hypophosphatemia but the non-NBPs are unlikely to be associated with gastrointestinal injuries or jaw osteonecrosis and are not associated with inflammatory or necrotic side effects [36,53]. It has been reported that some cases treated with a high dose of nitrogen-containing BPs showed osteonecrosis jaw, but there is no report of osteonecrosis in long bones [40]. There is no idea to show why just this family causes necrosis in the jaws or why the greatest number of cases result in periodontitis and tooth extractions, or why this disease has a high recurrence rate after exposure of the jaw bones to oral surgery [54,55].

BPs are anti-resorptive agents that may have adverse effects at some stages of bone regeneration such as inhibition of the hard callus or mature lamellar bone remodeling [48]. The common route of BPs administration is intravenously or orally, but various side effects occur following its systemic administration, for example, they may increase the jaw osteonecrosis risk following double drug exposure [38,56]. The intraperitoneal injection of NBPs induced chronic inflammation in the liver, lungs, bones, and spleen, in mice. BPs also induced necrosis at the injection site when they were administrated subcutaneously in rat and caused necrosis and inflammation when injected intradermally in the ear-pinnas of mice [36]. Long-term treatment of BPs increased the number of multinucleated osteoclasts [30]. Thus, long term administration of BPs might induce osteoporosis and some metabolic bone disorders which cause excessive bone resorption [23]. It has also been reported that some BPs inhibited osteoblast proliferation and reduced bone mineralization [38]. Medium to high doses of this family might cause a reduction in bone formation and inhibit the activity of the pre-existing bone structure [38].

Some adverse effects such as atrial fibrillation, inhibition of angiogenesis, toxic effects on kidneys, lowering OH of the environment, atypical fractures of the femur shaft, toxic action on epithelial cells in wound healing, increase in body temperature, inhibiting bone metabolism, inflammation, necrosis of the jaw bones, and irritation of the digestive tract have been attributed to BPs therapy [31]. This family might over suppress bone turnover and limits the healing consequence of micro damages. Long-term BP administration may interrupt callus formation, bone remodeling, and diminish bone strength [27,45]. BPs inhibit the formation and aggregation of the calcium phosphate crystals and are effective on osteoclasts, osteoblasts, chondrocytes, and endothelial cells [41]. Therefore, they may inhibit remodeling by preventing the osteoclastic activity and differentiation by binding to the bone minerals and also inactivating monocyte-macrophage origins which are effective in bone resorption during healing [21,28,29,40].

BPs caused deduction in the remodeling of the fractured callus besides a delay in changing the woven bone to mature lamellar bone in the fracture region [22]. Administration of increased dose interval of ibandronate led to subtraction of a delay in changing the woven bone to the mature lamellar bone [57]. The ibandronate reduced the mean stress at failure point as evaluated by the four-point bending,

decreased the progress of fracture union, deduced the bone mineral density, and diminished presence of undifferentiated mesenchymal and cartilage-like tissues in the osteotomy site [49]. Prolonged administration of BPs increased the duration of the healing process and reduced the quality of the newly regenerated bone tissue [18].

In a case series survey which was started in 2005 and finished in 2012, 422 cases of atypical femoral fracture were investigated, using bisphosphonates. The patients who were treated with alendronate, showed delayed fracture-healing, reduced or absent osteoblastic function, severe suppression of bone remodeling and bone turnover, and resembled adynamic bone disease [41]. BPs inhibit bone turnover by interfering with osteoclasts. As therapeutic medication-related osteonecrosis of the jaw bone which occurred after tooth extraction without primary wound closure or with periodontal disease, zoledronate demonstrated the highest osteonecrosis. After administration of a high concentration of BPs, bone turnover was suppressed on the 'stressed area' of alveolar bone. Suppression of angiogenesis was seen as a BPs effect and activation of BPs was bounded by reducing the local pH rates. BPs suppressed the level of intracortical remodeling in the alveolar bone [58].

BPs increased biofilm formation and bacterial colonization, and the microbial colonization in bisphosphonate-related osteonecrosis of jaw cases was higher than the bone necrosis which occurred in the absence of BPs. Pamidronate raised adhesion to the hydroxyapatite and led to colonization, in bone infections with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Pamidronate protonated the amine group and formed two additional hydrogen bonds with hydroxyapatite, and its reactive cationic amino group attracted bacteria by direct electrostatic interaction, so the adherence by pamidronate was higher than other BPs. Consequently, the administration of BPs led to failure in dental implants or BP-coated joint prostheses, increased osteomyelitis, and complicated bone surgery in patients [31,59,60].

Zoledronate

For the first time, zoledronate was prescribed to treat bone metastases and multiple myeloma, in 2002. This amino-bisphosphonate was used by the IV route and resulted in enhanced new bone formation and initiated a positive effect on remodeling and improved bone mass in the metaphysis. Comparison between zoledronate and other family members of BPs depicted that this drug was more effective on bone healing, bone mineralization, and proliferation of human osteoblast-like cells, and bone remodeling [29,38,40,51]. Though zoledronate has a positive effect on bone healing but some studies stated that using this drug may cause some negative impression on the healing process.

In vivo investigations regarding beneficial effects of zoledronate on bone healing

Some investigations focused on the effects of zoledronate on bone regeneration and healing in humans and animal models *in vivo*. It has been shown that zoledronic acid enhanced attachment of the osteoblasts to the titanium implant surfaces and resulted in proliferation and migration of these osteogenic cells [61]. Also, it was reported that this drug accelerated the healing potential of mandible fracture and also thoroughly new bone formation on the implant surface on-growth and gap in-growth [62,63]. A low dose of zoledronate increased new bone formation when it was used with an implant as a local delivery vehicle [56]. Moreover, zoledronate combined with ifosfamide, in a preclinical study, enhanced tissue repair and tumor regression [44]. A combination of zoledronate therapy, bone morphogenetic protein 2 (BMP-2), and allograft showed retaining of the bone tissue in a bone conduction chamber and enhanced fracture healing, in a rodent study [39]. The effect of zoledronate was compared to autologous bone, allograft, and allograft with BMP-7 which was found that subcutaneous administration of 0.1 mg/kg of this drug resulted in mature bone formation in the fracture site after 6 weeks and showed the allograft combined with BMP-7 and/or zoledronate had more potentials effect than autograft alone [23]. Intravenous injection of 0.08 mg/kg zoledronate led to the comparable new bone formation to the recombinant human bone morphogenic protein-2 (rhBMP-2) after 4 and 8 weeks [64].

Zoledronate raised the femoral fracture healing strength in the rat when used as a single-dose [20]. In the Greiner and colleagues' study, zoledronate was incorporated into poly L, D lactic acid (PDLLA) coating of the implant and resulted in promotion of fracture heal-

ing with greater mechanical strength, callus area, and faster fracture bridging of the closed tibial fracture in rat [65]. When the Fe foam implantation coated by zoledronate was compared to the strontium coated Fe foam on ovariectomized rats, it was depicted that bone formation was similarly improved in both groups after 6 weeks of implantation [66]. Bone preservation and bone filling were observed in the extraction site of the first molar of rats that were treated with 16 µg zoledronate, after 21 days [52]. Systemic IV administration of 76 µg/kg zoledronate, once every 4 weeks over 24 weeks, led to a higher proportion of the nonattached osteoclasts in the extracted maxillary incisor tooth compared to the control group and this treatment strategy resulted in decreased local bone resorption [67]. The effects of intraperitoneal administration of 0.1 mg/kg zoledronate was studied on intact mandible in rat, after eight weeks. Limited inflammation in the posterior mandible and no inflammation was seen in the femur and anterior mandible of the zoledronate treated rats [37]. One µg/ml of the zoledronate-loaded hydrogel had a successful potential in enhancing osteosynthesis following implant fixation in the defect site of ovariectomized rats, after 58 days [68].

The bone defect which was treated by 0.1 mg/kg Zol-rhBMP-2 showed new bone formation, bridged the fracture gap, formed bone marrow cavity, and mature osteoid tissue, after 6 weeks [20]. Subcutaneous administration of 0.1 mg/kg Zol improved fracture healing, trabecular microarchitecture, the volume of absolute bone fraction, bone microarchitecture, bone mineral content, and callus formation, 6 weeks after administration in ovariectomized rats [25,69]. Reduction in graft resorption, promotion in osteogenesis, and bony integration with the defect margins were seen in the graft-local Zol pre-treated animals, after 6 weeks. In addition, increased blood vessel proliferation, enhanced immature woven bone formation, and elevated osteocytes and osteoblasts counts were observed in the ZOL-treated group [70].

Matos and colleagues showed that 0.04 mg/kg intraperitoneal administration of zoledronate increased the amount of trabecular bone and decreased periosteal fibrosis, after 1, 2 and 4 weeks in rabbits [29]. 150 ng/cm^2 of this drug onto the Titanium bone screw showed increased bone volume fraction in the trabecular region, after 6 weeks, and increased bone volume fraction and the pull-out force, in the femoral condyle, after 11 weeks [51]. Ma., *et al*. used hydroxyapatite-zoledronate (0.005 mg ZOL/ml saline) on femoral head of rabbits and depicted increased bone matrix, enhanced hematopoietic tissue formation, improved expression of the osteocalcin, osteopontin, and osteoprotegerin markers, elevated new bone formation and inhibited bone resorption, after 4 weeks post-surgery [56]. The results of 0.1 mg/kg IV delivery of zoledronate after ovariohysterectomy in rabbit reflected insignificant differences with the sham and OVX-saline groups with an increase in vascularization, vascular endothelial growth factor, ossification, mononuclear cell infiltration measures, and osteoclast counts [71]. The IV administration of 0.1 mg/kg Zol showed that the allograft (rhBMP-2 implant) with zoledronate treatment can enhance bone healing in dogs, after 4 weeks [39]. 16 µg Zol was used to treat dogs with mini-screw implants and showed more trabecular bone formation and maintaining the stability of the implants, after 8 weeks [42].

In vivo investigations regarding harmful effects of Zoledronate on bone healing

Zoledronate inhibits the proliferation, migration, and adhesion of vascular smooth muscle cells without inducing apoptosis or necrosis [72]. It has been shown that zoledronate resulted in a delay in cartilage hypertrophy, angiogenesis, and remodeling of callus, cartilage, and bone in mice [40]. Zoledronate is not metabolized, but accumulates in bone tissue, suppresses bone remodeling, and causes osteonecrosis of the jaw [33]. Long-term Zoledronate therapy decreased bone formation due to the uncoupling balance between osteoblastic and osteoclastic activities [20]. Another investigation used 0.1 mg/kg Zol in 200 ml saline, IV, once 4 weeks before fracture and once at the time of fracture. The fractures were performed on tibial and mandibular bones and it was demonstrated that the tibial fracture included endochondral ossification, reduced callus and cartilage formation during the early stages of repair, delayed in cartilage hypertrophy, decreased in angiogenesis during the soft callus phase and later stages of repair, and finally delayed callus, cartilage, and bone remodeling. The endochondral and intramembranous ossification were depicted in the mandibular fracture in addition to delayed callus, cartilage, and bone remodeling, and reduction in osteoclast number during the soft and hard callus phases [40]. 0.06 mg/kg Zol (IV) was used 6 weeks apart in the first group or once a week for 6 weeks in the second group and it was demonstrated that bone regeneration was delayed and

consisted of fibrous, cartilaginous, and woven bone tissues in the first group after 2 weeks. The callus feature was nearly the same as those in the 2nd and 4th weeks post-operation, after 6 weeks. Less amount of bone remodeling was detected in the first group in comparison to the second group, after 4 weeks [73]. 5 mg/kg Zol created a large amount of newly formed woven bone like a structure typical of immature bone with an irregular arrangement of collagen fibers, high cellularity, less mineral content, and periosteal callus formation on the ovariectomized rats, after 8 weeks. Besides, the activation power of the bone lining osteoblasts was more and the bone remodeling was less advanced than the group treated with hydrogel [38].

Discussion

Bone formation occurs via anabolic response and the bone resorption takes place through the catabolic response. The first step of bone regeneration is characterized by endochondral ossification and production of non-mineralized cartilage and remodeling starts by the new lamellar bone formation following osteoclastic resorption [29]. There are some acceptable reasons to select BPs as appropriate treatment strategies for bone healing. Much research in this field stated that this family has potential in preventing loss of bone mass by inhibiting osteoclast activity [18]. On the other hand, BPs lead to some side effects such as osteonecrosis of mandible, atypical femur fracture, and inhibition of bone metabolism which have been explained in this article [27,31,45]. BPs have cytotoxic effects on the endothelial cells, osteoclasts, and oral mucosa cells which have an effective role in jaw osteonecrosis. Osteoclasts are the keys of jaw osteonecrosis, but it is not clear that why just the jaw osteonecrosis develops by BPs and other bones remain intact. It might happen due to the aberrant behavior of osteoclasts after BPs uptakes such as secretion of cytokines around the environment and their inhibitory effects on bone resorption functions of osteoclasts [67].

Some oppose studies propounded that using BPs is doubtable because of their side effects such as injuring the GI tract and inhibiting the remodeling of mature lamellar bone and hard callus formation [36,48]. The contradiction between these studies may have been occurred because of the route and dosage of administration or duration of the utilization of this family. It has been stated that systemic administration of this family has many side effects including inflammation in the visceral organs and necrosis in the region of injection. But local delivery of the drugs of this family including loading these drugs on the scaffolds supports bony integration in addition to promoting bone formation during fracture healing [38].

Zoledronate is one of the most powerful drugs among this family that has been used for bone healing based on its potential in increasing new bone formation [38]. It also controls further loss of bone tissue in postmenopausal women [74]. It has also been shown that local delivery of Zol on peri-implants increase the rate of early bone formation and has anti-resorptive action [38]. However, some negative effects of this drug on bone healing depending on its utilization protocol has been claimed. As zoledronic acid prevents the secretion of platelet-derived growth factor BB (PDGF-BB) and blocks osteoclasts formation, it results in suppression of osteogenesis and angiogenesis *in vitro*. Therefore, Gao., *et al*. stated that the prevention of angiogenesis and osteogenesis might partly have been resulted from the suppression of PDGF-BB secretion at earlier stages of bone healing [75]. It has been stated the bone-associated connective tissue, cartilage, and bone tissues were formed by osteoblasts that were influenced by BMPs [53]. On the other hand, it has been claimed that a high dosage of zoledronate is cytotoxic for osteoblasts and the differentiation of osteoblasts is suppressed after exposure to the high dosage of this drug because of down-regulation of BMP-2 [53]. BMPs are the main extracellular signaling molecules that are responsible in differentiation of the osteoblast precursors into the mature osteoblasts [53].

Based on the speculation that this drug has sufficient effect to inhibit reduction of mechanical strength and also recover the structure of long bones or vertebrates following ovariectomy in rat, further investigation on the effects of ZOL on bone restoration is indicated [74]. Besides, there are some disagreements regarding the effectiveness of ZOL on bone healing. Some studies indicated that this drug had not promoted bone healing and was not effective on bone formation and mineralization of the trabecular and woven bones [29,76,78]. Some other studies claimed that Zol delayed bone remodeling and reduced the number of osteoclasts [40]. After all, many studies showed the

beneficial effects of Zol on bone healing, particularly when it is used locally. But it is still needed to design more experiments on the effects of the drugs of this family particularly zoledronate on bone regeneration.

Conclusion

Bisphosphonates are the drug family that promote bone healing and also reduce the risk of bone fracture. Zoledronate is the most powerful drug and has been studied more than the other family members. Zoledronate prevents bone resorption and inhibits osteoclastic activity which leads in the reduction of bone mass loss. Furthermore, zoledronate has some negative effects on bone healing which may depend on the route of administration or dosage of the drug. Therefore, different effects of zoledronate on bone healing should be studied in more detailed by reliable experiments.

Conflict of Interests

There was no conflict of interest.

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