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BONE BIOLOGY

Anabolic and antiresorptive actions of locally delivered bisphosphonates for bone repair

A REVIEW

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During the last decades, several research groups have used bisphosphonates for local application to counteract secondary bone resorption after bone grafting, to improve implant fixation or to control bone resorption caused by bone morphogenetic proteins (BMPs). We focused on zoledronate (a bisphosphonate) due to its greater antiresorptive potential over other bisphosphonates. Recently, it has become obvious that the carrier is of importance to modulate the concentration and elution profile of the zoledronic acid locally. Incorporating one fifth of the recommended systemic dose of zoledronate with different apatite matrices and types of bone defects has been shown to enhance bone regeneration significantly *in vivo*. We expect the local delivery of zoledronate to overcome the limitations and side effects associated with systemic usage; however, we need to know more about the bioavailability and the biological effects. The local use of BMP-2 and zoledronate as a combination has a proven additional effect on bone regeneration. This review focuses primarily on the local use of zoledronate alone, or in combination with bone anabolic factors, in various preclinical models mimicking different orthopaedic conditions.

Cite this article: *Bone Joint Res* 2018;7:548–560.

Keywords: Bisphosphonates, Bone repair, Ceramic, Local delivery, Zoledronic acid, Carrier

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doi: 10.1302/2046-3758.710.BJR-2018-0015.R2

Bone Joint Res 2018;7:548–560.

Introduction

Osteoclast-mediated bone resorption is crucial in the maintenance and balance of bone remodelling. As bone formation is preceded by resorption, net bone loss can occur, as seen in conditions such as Paget's disease, osteoporosis, bone metastasis, multiple myeloma, and hypercalcaemia. Bisphosphonates are currently being used as one of the primary molecules against osteoclast-mediated bone loss.[1] It is known that the skeleton, and especially the spine, is a common site of metastatic disease, and about 70% to 90% of patients with advanced breast and prostate cancers develop skeletal lesions.[2] The bisphosphonates are synthetic, non-hydrolyzable analogues of inorganic pyrophosphates (PP_i), a naturally occurring polyphosphate found in serum and urine. The ability of these molecules to bind divalent cations such as Ca^{2+} has been attributed to their resemblance to pyrophosphate (P-O-P) structure,[3] but having carbon as a bridge between two phosphate groups (P-C-P), instead of P-O-P, help them to bind to bone mineral surfaces, particularly at sites for active bone remodelling,[4] thus making them more resistant to heat and enzymatic hydrolysis.[5]

The role of bisphosphonates in inhibiting the bone resorption was unravelled serendipitously by Fleish and Neuman,[6] in 1960, when studying the mechanism of calcification induced by collagen. Body fluids such as plasma and urine contain certain trace molecules that inhibit the calcification process. Based on previous reports of polyphosphates as water softeners, Fleish and Neuman[6] suspected that the molecules could be essential in the regulation of calcification under physiological conditions.[7] Successful attempts were made to inject polyphosphates and pyrophosphates into animals, and ectopic calcification in blood vessels, skin and kidneys was inhibited. However, the oral administration was found to be unsuccessful due to the hydrolysis of these molecules within the gastrointestinal tract.[8] This necessitated the search for compounds that could both inhibit

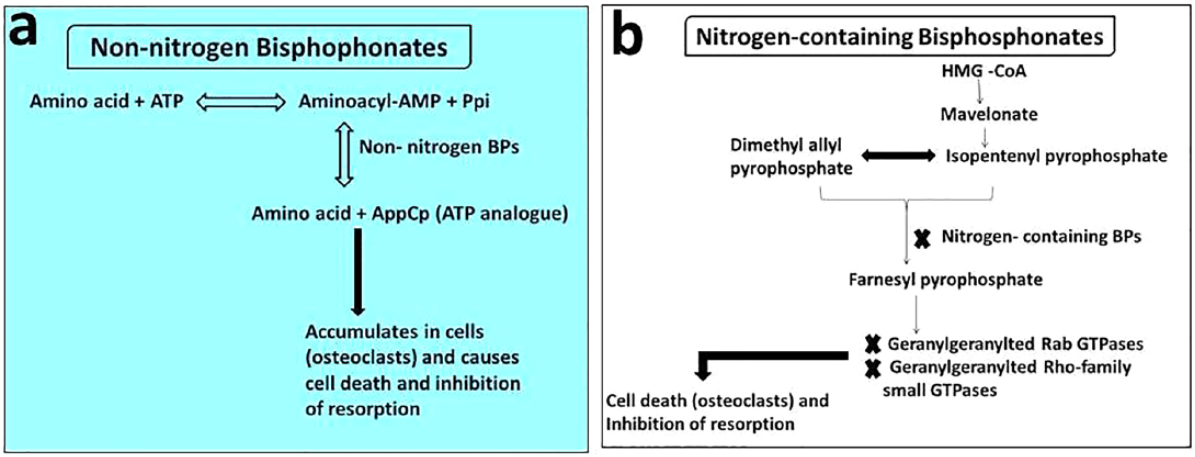

Fig. 1

Figure showing the representation of cellular mechanism of action of a) non-nitrogen and b) nitrogen containing bisphosphonates. Reproduced from **Rogers M, Frith J, Luckman S, et al.** Molecular mechanisms of action of bisphosphonates. *Bone* 1999;24:73S-9S (with permission from Elsevier) and **Roelofs AJ, Thompson K, Gordon S, Rogers MJ.** Molecular mechanisms of action of bisphosphonates: current status. *Clin Cancer Res* 2006;12(20 Pt 2):6222s–6230s.

Table I. Potency of bisphosphonates in the inhibition of farnesyl-pyrophosphate

Serial number	Bisphosphonate	Contains nitrogen	Dose	Relative potency
1.	Etidronate	No	300 mg to 750 mg	1
2.	Tiludronate	No	400 mg	50
3.	Alendronate	Yes	10 mg/day	1000
4.	Risedronate	Yes	5 mg/day	1000
5.	Ibandronate	Yes	2.5 mg/day	1000
6.	Pamidronate	Yes	90 mg/3wks	1000
7.	Zoledronate	Yes	4 mg to 5 mg/yr	1000+

*The table has been modified from **Ballantyne E**. Bisphosphonates: possible modes of action and implications for dental implant treatment. A review of the literature. *Journal of General Practices* 2014; 3:1

calcification and, at the same time, be resistant to hydrolysis. Bisphosphonates, or diphosphonates as the term then was, were shown to have a high affinity for bone mineral hydroxyapatite and did prevent calcification (including pathological), both *in vivo* and *in vitro*, even when given orally.[9] The importance of these molecules was thus recognized by the scientific community.[10,11]

Mechanism of action

Bisphosphonates can be divided into non-nitrogen-containing bisphosphonates (non-N-BPs, having no nitrogen in their structure) and nitrogen-containing bisphosphonates (N-BPs). The mechanism of action varies between the two molecules. The non-nitrogen containing moieties have been shown to be metabolized to analogues of adenosine triphosphate (ATP). These analogues interfere with mitochondrial adenosine diphosphate (ADP)/ATP translocase and result in cell death by apoptosis (Fig. 1a).[12]

The N-BPs are not metabolized to ATP analogues; instead, they act as inhibitors of farnesyl pyrophosphate synthase, a cornerstone enzyme in the mevalonate pathway.[13] The intermediates of this pathway are essential for the prenylation of intracellular proteins that control the transport of proteins to the cell membrane. This loss of prenylation in proteins leads cells to undergo apoptosis (Fig. 1b). In the case of osteoclasts, this process has been demonstrated to inhibit the maturation of cells from precursor cells.[14] Of the non-N-BP molecules, etidronate and clodronate are currently being used but with a low antiresorptive capacity. Among N-BPs, the pamidronate and alendronate contain a primary nitrogen atom in their alkyl chain, and are found to be ten to 100 times more potent than non-nitrogen containing moieties. This effect is further enhanced when tertiary nitrogen is present in the alkyl chain (ibandronate and olpadronate). Of all the antiresorptive bisphosphonates available today, those containing nitrogen in the heterocyclic ring (risedronate and zoledronate) are ten thousand times more potent than etidronate, for example (Table I).[15,16]

Administration

Bisphosphonates are administered in the patient via oral and parenteral routes. Oral administration is the most common route due to its efficacy[17-19] and general tolerability.[20-22] With oral administration, however, bisphosphonates are poorly absorbed in the gastrointestinal tract (1% to 2%), and this decreases the bioavailability of drug at the

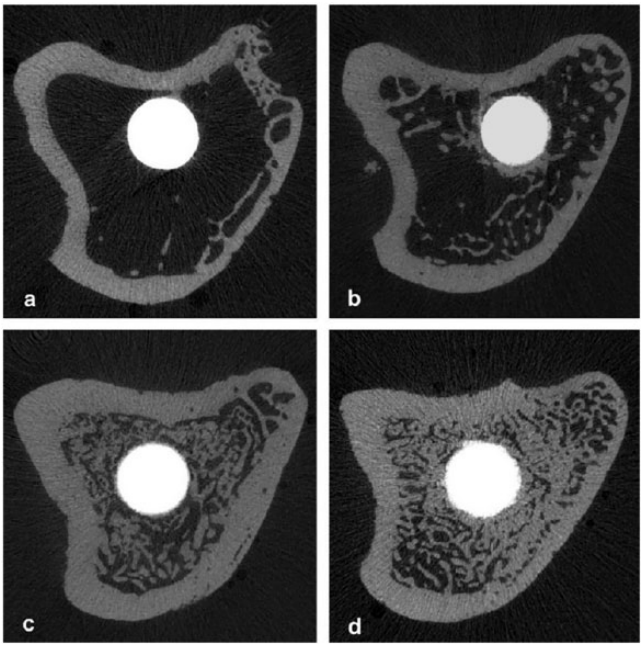

Fig. 2

Micro-CT representative images. Representative micro-CT images of tibias three months after implantation: a) control, b) pamidronate-treated, c) ibandronate-treated, and d) zoledronate-treated groups. Reproduced from **Gao Y, Zou S, Liu X, Bao C, Hu J.** The effect of surface immobilized bisphosphonates on the fixation of hydroxyapatite-coated titanium implants in ovariectomized rats. *Biomaterials* 2009;30:1790–1796 (with permission from Elsevier).

desired site. It has also been shown that N-BPs (zoledronate) lead to irritation in the upper gastrointestinal region by directly damaging the mucosa. The non-N-BPs do not cause any similar gastrointestinal irritation but they are less potent in the antiresorption and apoptosis of osteoclasts.[14] There are two ways of circumventing oral delivery, either via parenteral administration or via local administration at the desired site. In the case of parenteral administration, intravenous delivery has been shown to cause transient and sometimes severe, but self-limiting, influenza-like myalgic symptoms and is seen in 10% to 15% of zoledronate-treated osteoporotic women.[23]

Pharmacokinetics

The pharmacokinetics of bisphosphonates are complex as the potential to inhibit osteoclast-mediated bone resorption varies, as does their affinity with bone mineral hydroxyapatite. Following oral or parenteral administration, the bisphosphonates can be absorbed in the small intestines by passive diffusion via the paracellular pathway involving the epithelial extracellular space.[24] Owing to the lipophilicity and more negative charge, the bioavailability of oral administration is low (1% to 10% of total oral dose). Instead, in intravenous administration, a higher plasma concentration is reached with shorter circulation half-life. In patients with bone metastasis, 4 mg of intravenous zoledronate is given, and the maximum serum concentration of 0.1 μM to 1 μM is reached after 15 minutes. The systemic concentration decreases to less than 1% of maximum concentration at 24 hours.[25] The ability of the skeleton to take up the bisphosphonates depends on age, gender, the rate of bone turnover at the time of administration, and the type of bisphosphonate. The maximum pamidronate concentration reached about 10 μM after one hour when 60 mg was given intravenously.[26] About half of the bisphosphonates are rapidly taken up in the skeletal system following administration at therapeutic doses. The residual circulating drug is excreted by the kidneys, pre-eminently within the first hours after administration.[27,28]

Local administration using a carrier

Bisphosphonates can be incorporated into implants, or surface coated onto implants, in order to increase their local bioavailability. Several studies attempted to assess the effect of local treatment of bisphosphonate-coated implants on bone regeneration and mineralization. Due to the higher antiresorptive activity of zoledronate over other bisphosphonates, we focused this review on zoledronate. When used locally, zoledronate as an anti-catabolic molecule can reduce the osteoclast mediated bone resorption, and improve bone healing.[29]

Surface-immobilized zoledronate

Zoledronate has the most noticeable effect among all surface-immobilized bisphosphonates[30] in inhibiting resorption of bone by osteoclasts, thereby enhancing the bone mineral density (BMD) around an implant (Fig. 2). Three bisphosphonates – zoledronate, ibandronate, and pamidronate – were immobilized on hydroxyapatite-coated titanium implants in ovariectomized rats to evaluate the effect of local release of these molecules on mechanical fixation of implants, BMD, and bone-implant integration. Without understanding the physiological mechanism, the distribution of zoledronate and other bisphosphonates was maintained on the modified surface of a titanium implant, i.e. at the site where the activity of osteoclasts needs to be controlled. Implant fixation is a matter of concern in young active patients, fragile elderly patients with osteoporosis, and in revision surgery. Using the surface-immobilized zoledronate on the modified surface of a titanium implant, Gao et al[30] showed a reduced risk of aseptic loosening with bone remodelling more in the direction of bone formation and thus better mechanical fixation. Peter et al[31] attempted to examine a window (gradient) of zoledronate concentration, wherein the mechanical fixation of the implant is enhanced, to determine the optimal concentration. A titanium alloy was plasma-coated with hydroxyapatite, and a range of zoledronate concentrations (0 μg/ml, 0.2 μg/ml, 2.1 μg/ml, 8.5 μg/ml, and 16 μg/ml) were added to adsorb on top of a hydroxyapatite layer. With a low zoledronate concentration (2.1 μg/ml), there were higher pull-out forces (up to 42%) as compared with implants without zoledronate. But at higher zoledronate

concentrations, the pull-out forces were reduced by 35% compared with implants without zoledronate. This might be explained by high local doses of zoledronate impairing bone mineralization as reported previously using alendronate.[32] The study found that, with increased zoledronate concentration, there was increase in the bone density distribution around the implant. At even higher concentrations (16 μg/ml), the bone density was the same as in implants without zoledronate measured close to the implant. But at a further distance around the implant, the bone density increased also with the highest zoledronate concentration. This can be explained by the dilution of zoledronate with increasing distance. Local delivery of zoledronate led to an increase in mechanical stability due to the enhanced quality and architecture of newly formed trabecular plates. The authors speculated that this eventually could improve the long-term implant survival.[31]

The use of a combination of basic fibroblast growth factor (bFGF) and zoledronate has been shown to have a profound effect on the mechanical fixation of implants and BMD distribution, particularly where there is osteoporosis.[33] Basic fibroblast growth factor is a cytokine that triggers the proliferation of endothelial cells and osteoblasts, thus enhancing vasculature and bone formation simultaneously. Although the differentiation potential of bFGF is not in compliance with the BMP, i.e. the capacity of BMP for osteogenic differentiation is higher compared with bFGF,[34] still, a dual therapy of bFGF and zoledronate could enhance the mechanical early fixation of implants by osteoblast proliferation, vasculature ingrowth, and osteoclast apoptosis. The early resorption of newly formed bone will be inhibited around the implant as osteoclast formation will be inhibited by the presence of zoledronate.

In ovariectomized rabbits, the mechanical fixation of implant improved and the bone density increased when autologous iliac crest was placed in proximal tibial metaphyses together with hydroxyapatite-coated titanium implants coated with zoledronate. The bone density and direct contact between bone and implant was found to be highest in the dual therapy (includes both local and systemic administration) of zoledronate, followed by systemic administration, while the local administration of zoledronate showed the least bone density. This study eventually demonstrated that zoledronate enhanced osseointegration and implant fixation in autologous bone grafts, especially at the sites where bone quality was poor, as with osteoporotic patients.[35]

Enhanced fracture healing, i.e. the improvement in total mineralization content and improved strength of bone, has been demonstrated in rats when zoledronate was coated on the surface of osteosynthetic implants by Greiner et al.[36] Mean callus area, torsional stiffness, bridging, and stability of mid-tibial fractures were seen to be enhanced in the zoledronate-coated implant groups after 42 days, when compared with the control group. Local administration of zoledronate leads to a large production of callus with increased resistance to loading. After 84 days, the control and zoledronate were similar regarding maximum load and torsional stiffness. The authors speculated that the local use of zoledronate enhanced the mechanical stability of fractures most probably by delaying the bone remodelling.

The superior enhancement in mechanical fixation of implants was shown by surface-immobilizing zoledronate onto a fibrinogen-coated stainless steel implant when compared with the pamidronate immobilization. The bone density was seen to increase around the screw implants in both types of bisphosphonates as compared with uncoated but there was increased bone density in zoledronate-coated implants compared with pamidronate-coated ones.[37] Further, improved implant fixation and osseointegration was shown by Jakobsen et al[38] using zoledronate-containing Poly-D, L-Lactide (PDLLA)-coated titanium (Ti) implants. A canine zoledronate-containing PDLLA-coated Ti implant enhanced quantitatively both pre-existing lamellar bone and new woven bone around the implants, resulting in increased strength, energy, and stiffness.[38]

A local one-time, low-dose injection of zoledronate at the implantation site appears to be a simple, convenient, and easy approach for sustained drug delivery to reduce the risk of implant loosening in skeletally mature animals.[39-41] Ying et al[42] have shown that in osteoporotic rats, when 30 μg/implant of zoledronate was injected at three months after ovariectomy at the implantation site, there was significant dwindling in the rate of bone turnover around the Ti implants. Also, enhanced bone-to-implant contact and peri-implant new bone formation was observed.[42]

Low-dose impregnation of BMP and zoledronate into the beta-tricalcium phosphate (β-TCP) by Ichikawa et al[43] has shown that, when BMP was used alone, there was enhancement in the bone volume fraction (as observed in micro-CT) for three weeks, followed by significant reduction at 12 weeks due to early resorption of newly formed bone by activating osteoclasts. The addition of increased concentrations of zoledronate not only augmented the bone regeneration, but also maintained the structural integrity of the newly formed bone at 12 weeks. Thus, co-application of zoledronate and BMP could be used in critical-sized bone defects to augment the bone regeneration and also preserve the trabecular structure of newly formed bone.[43]

During the initial phases of bone healing, in osteoporotic bones, a high rate of implant failure has been observed due to the micromotion between implant and bone that enhances the osteoclast-mediated bone resorption. One study addressed this problem was addressed by coating the Ti bone screws with zoledronic acid (ZA)

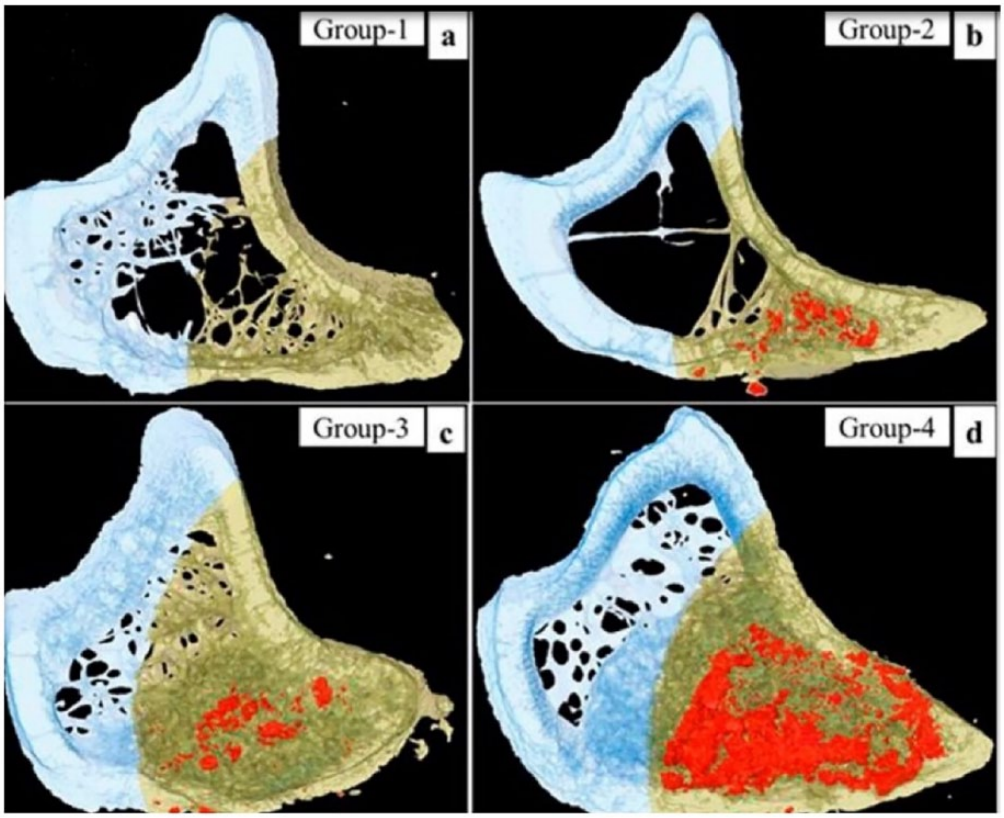

Fig. 3

Ex vivo micro-CT reconstructions in the tibia defect model. Representation of extensive bone formation (red colour) using zoledronate (0.2 mM) compared with bioactive protein fraction derived from Saos2 cell lines. (a) Group 1 (control), (b) Group 2 (gelatin-cement), (c) Group 3 (gelatin-cement + Saos2 fraction), (d) Group 4 (gelatin-cement + ZA). Reproduced from **Teotia AK, Gupta A, Raina DB, Lidgren L, Kumar A.** Gelatin-modified bone substitute with bioactive molecules enhance cellular interactions and bone regeneration. *ACS Appl Mater Interfaces* 2016;8:10775–10787 (with permission from the publisher; American Chemical Society).

(150 ng/cm^2) before implantation into compromised cancellous bone (femoral condyle), which enhanced the screw fixation. The study was based on three early timepoints (one, five, and ten days) and two late timepoints (six and 11 weeks), and it was observed after 11 weeks that both bone volume fraction and pull-out force were significantly higher in the screws that were coated with ZA than in the uncoated ones.[44]

Mixing zoledronate with ceramic carriers

In a rat tibial defect model, Teotia et al[45] demonstrated that biphasic calcium phosphate bone cement, when mixed with zoledronate (0.2 mM), results in more net bone formation than when it was mixed with only bioactive protein fraction derived from osteosarcoma cell line (Saos2) cells (Fig. 3). This showed that a ceramic material could act as a carrier to deliver a low dose of zoledronate at the bone defect site, a dose that enhanced the defect healing without giving any systemic effect. The calcium sulphate phase in the bone cement, in contrast to the calcium phosphate phase, is resorbed by dissolution and hence not affected by the presence of zoledronate. A porous scaffold is generated that improves further cell infiltration/ingrowth. In this study, the zoledronate inhibited the resorption process of osteoclasts and the bone formation was the highest in the group containing zoledronate.[45] In another study of a cranial defect model, Teotia et al[46] showed that co-delivery of BMP-2 and zoledronate, using biphasic calcium sulphate-nanohydroxyapatite cement as a carrier, could further enhance the bone ingrowth and defect healing compared with the use of the individual factors in cement, or cement alone. Further, with zoledronate, the BMP-mediated bone resorption was also inhibited.[46]

In a rat model investigating bone formation in an extraosseous site, in an abdominal muscle pouch, Raina et al[47] showed that when zoledronate (10 µg/disc) was added to a commercially available biphasic calcium sulphate-hydroxyapatite bone void filler along with BMP, there was increased mineralization when compared with that of the group containing BMP only. They suggested that zoledronate inhibited the early resorption of newly formed bone and also that there was sustained release of zoledronate and BMP owing to the greater affinity of zoledronate and BMP for the calcium ions that led to the enhanced osteogenesis (Fig. 4).[47]

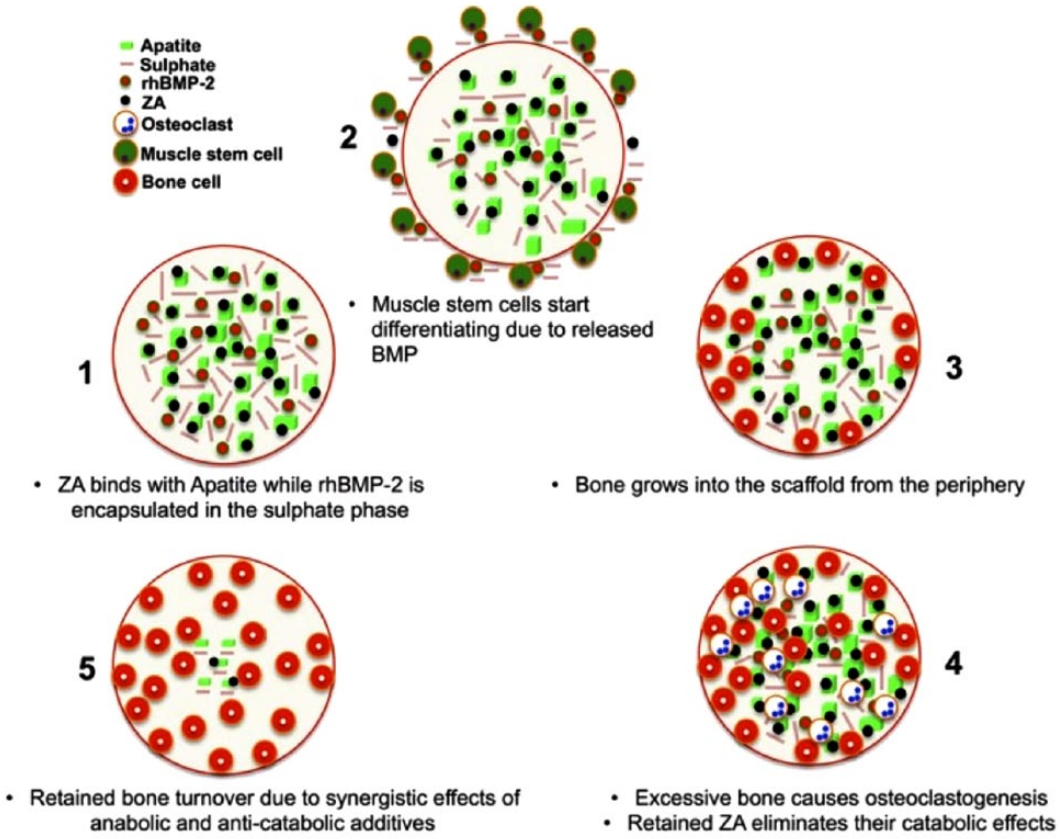

Fig. 4

Speculated mechanism of rhBMP-2 and zoledronate delivery via a calcium sulphate/hydroxyapatite biomaterial. This figure depicts a schematic controlled delivery of zoledronate and rhBMP-2 enhancing osteogenesis in a muscle pouch model. Reproduced from **Raina DB, Isaksson H, Hettwer W, et al.** A biphasic calcium sulphate/hydroxyapatite carrier containing bone morphogenic protein-2 and zoledronic acid generates bone. *Sci Rep* 2016;6:26033.

Moreover, to achieve a sustained release and long-term effect of zoledronate, a hyaluronic acid hydrogel was loaded with zoledronate-nanohydroxyapatite (1:100). Nanohydroxyapatite particles and zoledronate combinations were first evaluated *in vitro* to check their effect on osteoclast precursor macrophages (RAW264.7), where it was observed that both lower and higher concentrations of zoledronate, when mixed with nanohydroxyapatite, significantly decrease the proliferation of cells. Also, the nanohydroxyapatite-zoledronate was incorporated into hyaluronic acid hydrogel to assess its effect on peri-implant bone formation and resorption in an osteoporotic rat femoral model using a miniature screw made of radio-opaque polyetheretherketone (PEEK) coated with a 100 nm layer of titanium. Prior to the insertion of the screw, the pre-drilled unicortical holes were filled with 5 μL of hydrogels containing either nanohydroxyapatite alone or nanohydroxyapatite with 5 μg of zoledronate. A bone-reinforcing effect of hydrogel-containing nano-hydroxyapatite with zoledronate was observed as favourable bone ingrowth and fusion of granules to large mineralized regions was seen. Moreover, complete integration of these granules with the native bone matrix without any inflammatory or foreign body reactions confirmed its biocompatibility and osteoconductivity.[48]

Soaking bone grafts in zoledronate solution

For structural support in joint revision arthroplasty (allografts) and in nonunions (autografts), morcellized bone grafts are often used.[49] The temporary mechanical weakening and failure of the implants has been attributed to their mismatch with new bone formation and quick resorption.

In joint revision surgery, it is necessary to achieve new bone formation without compromising the stability of the graft and implant. This can be achieved by soaking the bone grafts in bisphosphonate solutions before implantation, to prevent resorption and enhance local bone density.[50] Belfrage et al[51] used the morcellized bone allograft, soaked in a solution of zoledronate to assess how bone resorption and bone ingrowth into an allograft is affected by different doses and routes of administration. In this study, they used three different concentrations of zoledronate (0.005 mg/ml, 0.05 mg/ml, and 0.5 mg/ml). These grafts were then rinsed in saline and packed around a Ti implant. The formation of bone was dose-dependent, with more bone formation in

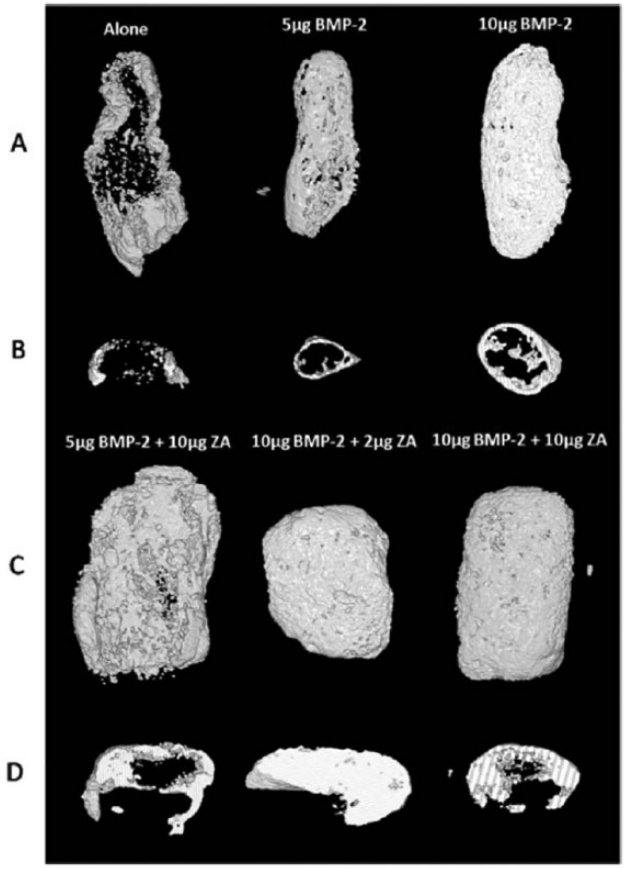

Fig. 5

3D rendering of the explants using micro-CT. The representative micro-CT constructs (a and b) and (c and d) show micro-CT slices (50 stack slices) of bone nodules formed from collagen-hydroxyapatite scaffolds loaded with saline, BMP-2 or BMP-2 + ZA at different concentrations. Reproduced from **Murphy CM, Schindeler A, Gleeson JP, et al.** A collagen-hydroxyapatite scaffold allows for binding and co-delivery of recombinant bone morphogenetic proteins and bisphosphonates. *Acta Biomater* 2014;10:2250–2258 (with permission from Elsevier).

lower concentrations (0.005 mg/ml) and no new bone formation in the highest-dose group (0.5 mg/ml). As verified by histological studies, it was found that the amount of unbound zoledronate is less important in new bone formation, but the amount of zoledronate bound to bone mineral (as defined by affinity) depends on soaking time, and concentration of zoledronate solution was essential for the ingrowth of new bone around the implant.[51] Raina et al[52] used the cryogelation technique to develop a composite scaffold soaked with zoledronate for the local delivery of zoledronate and other bioactive factors. A silk fibroin-chitosan-agarose-hydroxyapatite composite was used with or without bioactive glass for the local delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) and zoledronate. Hydroxyapatite by itself will increase the binding of zoledronate that is eventually released in a controlled way. *In vitro* studies showed that the addition of 2 μg/scaffold of zoledronate did not have any cytotoxic effects, however, free zoledronate, at the same concentration, did lead to cytotoxic effects. It was concluded that zoledronate, when bound to the scaffold, specifically targets osteoclasts. In the muscle pouch model, the bone induction/formation was further quantified by micro-CT and histomorphometry. The largest amount of mineralized tissue was found in the scaffolds that had both zoledronate and rhBMP-2. These in turn had a larger amount of bone than the scaffolds containing only rhBMP-2, owing to the BMP-induced osteoclast resorption.[52]

The shear stiffness and osseointegration were seen to improve and were found to be at their maximum when β-TCP granules were soaked in zoledronate before being grafting around Ti-coated implants. The results did not corroborate those of previous reports[53,54] which state that enhanced BMD is essential for the implant fixation. In this case, there was no significant improvement in BMD. The authors speculated that one of the reasons for this unexpected outcome could be the optimized composition of the mineral phase of the newly formed bone with a prolonged and incessant bone formation due to the local delivery of zoledronate.[55] A collagen-hydroxyapatite scaffold is hypothesized to be a better carrier for the co-delivery of an anabolic agent (rhBMP-2) and an anti-catabolic agent (zoledronate). A commercially available collagen sponge has good affinity to bind to rhBMP-2 but not to zoledronate. Murphy et al[56] devised a composite collagen-hydroxyapatite scaffold crosslinked by dehydrothermal crosslinking to deliver both rhBMP-2 and zoledronate with a small amount of hydroxyapatite present in the scaffold to increase the affinity towards zoledronate. A hind limb muscle model of rat was used for *in vivo* confirmation and the bone volume (micro-CT) was higher in the rhBMP-2/ZA group compared with rhBMP-2 only or controls (Fig. 5). This shows that co-delivery of both agents has a superior bone formation capacity than single molecule delivery, and the composite scaffold was considered superior to pure collagen BMP-2 scaffolds.[56]

Acrylic acid cement loaded with zoledronate during the preparation of polymeric bone cement was used in a rat model to improve the BMD. Surprisingly, there was no increase in the BMD around the implant but only when zoledronate was given systemically in the same model. This might be attributed to the insufficient release kinetics of zoledronate from the surface of acrylic cement, as seen under the *in vitro* elution profile with only 1% of zoledronate released in six weeks.[57]

Recently, our group carried out a study to explain the difference in the release profile of zoledronate and BMP under *in vitro* and *in vivo* conditions. It was demonstrated that the pharmacokinetic release pattern of zoledronate and BMP together was different under an *in vitro* compared with an *in vivo* microenvironment, with more BMP and more zoledronate released from a macroporous composite scaffold under *in vivo* conditions. The difference

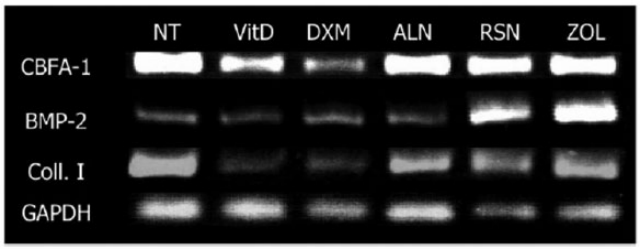

Fig. 6

Effects of bisphosphonates on BMSCs. Conventional PCR results for the expression of core binding factor alpha subunit 1 (CBFA-1), bone morphogenetic protein-2 (BMP-2), collagen 1 (Coll. I) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as loading control after 14 days of bone marrow stromal cells under different conditions: NT, non-treated; VitD, vitamin D; DXM, dexamethasone; ALN, alendronate; RSN, risedronate; ZOL, zoledronate. Reproduced from **von Knoch F, Jaquiery C, Kowalsky M, et al.** Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells. *Biomaterials* 2005;26:6941–6949 (with permission from Elsevier).

has been attributed to rapid protein encapsulation of the material reducing the release of BMP and partially preventing zoledronate from binding to apatite, thereby enhancing its initial release.[58]

Günes et al[59] have shown that the administration of zoledronate, either locally using hydroxyapatite as a carrier or systemically, could enhance the osteogenic potential of graft materials, thus improving the new bone formation. Both local and systemic treatment of zoledronate resulted in enhanced regeneration of rat calvarial bone defect, which has been explained in terms of increase in the number of osteoclasts and osteoblasts at the defect site in both treatment groups. The advantage of local delivery of zoledronate could be explained in terms of reduction in the dosage of the drug: in systemic administration, 0.1 mg/kg of zoledronate was injected into the animal model while, in local treatment, a single soak of the graft in 1 mg/ml of zoledronate solution was sufficient to accomplish similar results.[59]

One of the *in vitro* studies by Locs et al[60] has shown that the application of Poly-L-lactic acid coating on zoledronate-loaded 45S bioactive glass can achieve a sustained and long-term controlled release of the drug. Moreover, the coating has also been demonstrated to enhance the mechanical strength of such scaffolds when compared with non-coated ones.[60]

Zoledronate as a potential direct or indirect anabolic factor

Besides the primary effect of causing apoptosis of osteoclasts, the bisphosphonates have recently been suggested to be anabolic, enhancing the proliferation and differentiation of osteoblasts and inhibiting their apoptosis.[61,62] Zoledronate, risedronate, and alendronate have been studied regarding the differentiation and survival of bone marrow-derived stromal cells (BMSCs). Assessed by alkaline phosphatase activity and conventional polymerase chain reaction (PCR) and quantitative PCR (qPCR), BMSCs move towards the osteogenic lineage when treated with alendronate (10^{-8} M), risedronate (10^{-8} M), and zoledronate (10^{-8} M). The results of conventional PCR for the overexpression of the core-binding factor alpha 1 (Cbfa1) gene, collagen I (Col1) gene and BMP-2 gene are shown in Figure 6 when treating these cells with the aforementioned bisphosphonates.[63] The time-dependent and dose-dependent effects of zoledronate on important cellular components of the osteoblasts have been evaluated and it was found that osteoblast differentiation plays an important role in overall bone formation under ZA treatment.

The effect of zoledronate on increased net bone formation is dose-dependent in medium and higher doses, with enhanced bone volume and mineralization when released in a controlled way using poly(lactide-co-glycolide) (PLGA). An enhanced osteoblast number and activity, as well as an overexpression of bone formation markers, including BMP-2, osteocalcin, BMP-7, RunX2, and Col1, was found at the site of implantation (Fig. 7).[64] In another study, zoledronate was coated on PDLLA implants to evaluate the effect of release profile on primary human osteoblasts. The concentrated medium from coated implants showed no significant effects on cell viability but the higher concentrations reduced cell viability after 144 hours. In the case of soluble zoledronate (pure substance), cell proliferation was reduced with lower concentrations at 48 hours. This suggests that there is a continuous and sustained release of zoledronate when coated onto the PDLLA implants. The effect of these zoledronate-coated PDLLA implants on human osteoblasts was assessed by evaluating the ratio of Col1 to the total protein secreted by the cells and the authors found that, at low and medium doses, there is an increase in the ratio of Col1 to total cellular protein in the presence of zoledronate-coated PDLLA implants. Furthermore, the secretion of osteoprotegerin (OPG) from the osteoblasts was also reduced in the presence of zoledronate-coated PDLLA implants. At lower doses, these inhibit the Receptor activator of nuclear factor kappa-B ligand-Receptor activator of nuclear factor kappa-B (RANKL-RANK) interaction and thus reduce the osteoclast formation.[65]

The co-culturing of primary osteoblast cells and osteoclast-like cells gave further insight into the complex interplay between the two cell types and their quality of interaction. In co-cultures, there was no decrease in the soluble RANKL in the supernatant when exposed to zoledronate-coated PDLLA metallic implants but the co-culture behaved like a monoculture with a dose-dependent increase in OPG and Col1 secretion, and a decrease in tartrate-resistant acid phosphatase (TRAP)-positive cells. This co-culturing of primary osteoblasts and osteoclasts suggests that the zoledronate released

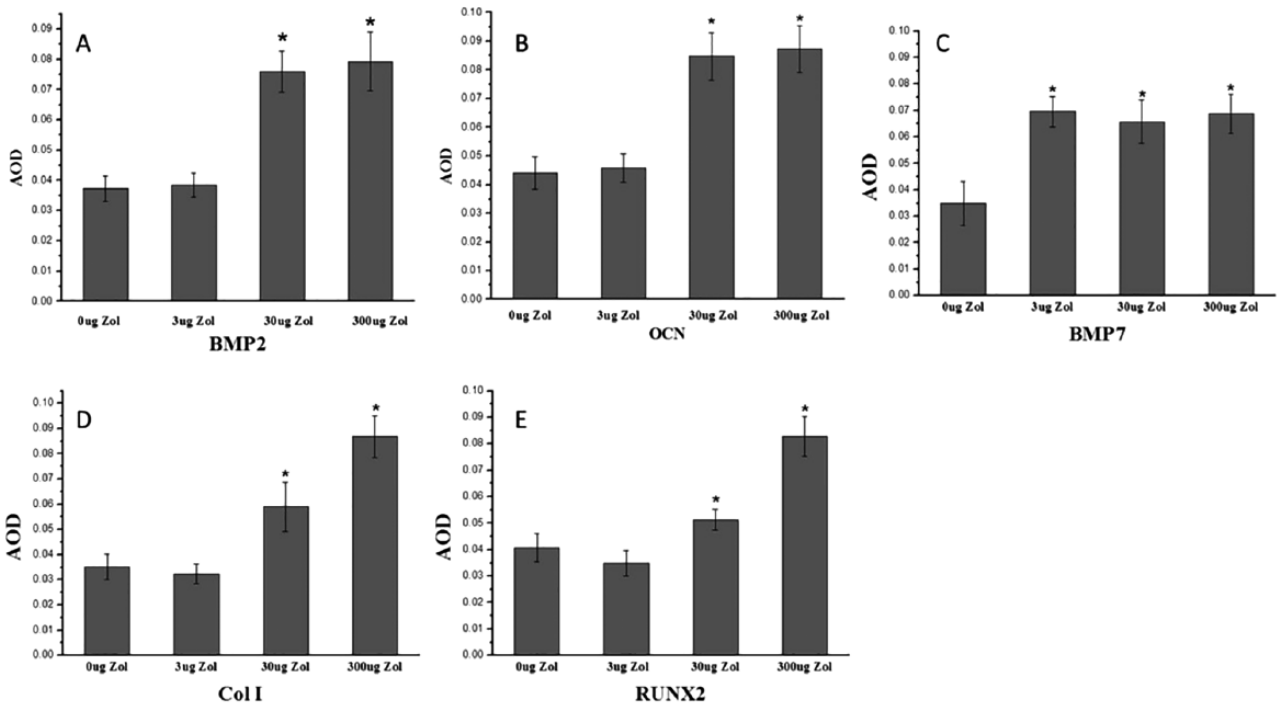

Fig. 7

Dose-dependent effects of zoledronate *in vivo* quantified using immunohistochemistry and histomorphometry. Quantification of immunohistochemistry staining for the expression of bone morphogenetic protein-2 (BMP2), osteocalcin (OCN), bone morphogenetic protein-7 (BMP7), collagen 1 (Col I) and Runt related transcription factor-2 (RUNX2) with different concentration of zoledronate. Reproduced from **Gou W, Wang X, Peng J, et al.** Controlled delivery of zoledronate improved bone formation locally in vivo. *PLoS One* 2014;9:e91317.

from the coated implants leads to a positive bone balance (enhanced bone formation than resorption) not only by directly affecting the cells but also affecting their interplay and interactions.[66]

The effects of zoledronate on the two different cell lines, osteoblasts and osteoclasts, could be explained by different mechanisms exploited by this molecule. Zoledronate and other bisphosphonates reduce the apoptotic rate of osteoblasts at a concentration that is three times lower than that of the concentration required to induce apoptosis in osteoclasts.[67] In osteoblasts, it is speculated that zoledronate enhances the phosphorylation of extracellular signal-regulated kinase (ERK) and thus leads to enhanced cell proliferation and expression of anti-apoptotic genes. This has been reconfirmed by others using the specific inhibitors of ERK activation that reverse their anti-apoptotic effect.[68] This mechanism could be explained under *in vivo* conditions by the enhanced secretion of interleukin 6 (IL-6) by the treatment of bisphosphonates leading to enhanced phosphorylation and activation of ERK (Fig. 8a). Furthermore, opening the Cx43 hemichannel via the Cx43/ERK pathway is the other mechanism suggested to inhibit the apoptosis of osteoblasts and osteocytes in order to increase their survival (Fig. 8b).[69] The other mechanism to inhibit the apoptosis of osteoblasts and osteocytes, and increase their survival, is by the opening of the Cx43 hemichannel via Cx43/ERK pathway (Fig. 8b).

Bisphosphonate-hydroxyapatite interaction

The molecular modelling method has been used to study and predict the relative interactions (interaction of different bisphosphonates with respect to each other) of different types of bisphosphonates with hydroxyapatite. The interactions were predominantly shown to be electrostatic, involving the nitrogen and phosphonate groups of bisphosphonates and the calcium ions of hydroxyapatite. The P-C-P moiety of bisphosphonates is essentially a favourable factor in increasing the coordination with calcium. Increasing the number of P-C-P moieties makes the interaction energy proportionally higher, thus increasing the pharmacological potential of bisphosphonates. Despite sharing many pharmacological features, the minute structural differences make them differ biochemically, particularly regarding the binding to bone mineral hydroxyapatite and the subsequent inhibited osteoclastic bone resorption.[70]

The difference in potency of different kinds of bisphosphonates could also be attributed to a different affinity to hydroxyapatite. Many studies have determined the affinity of different bisphosphonates to hydroxyapatite depending on the differences in their side-chain

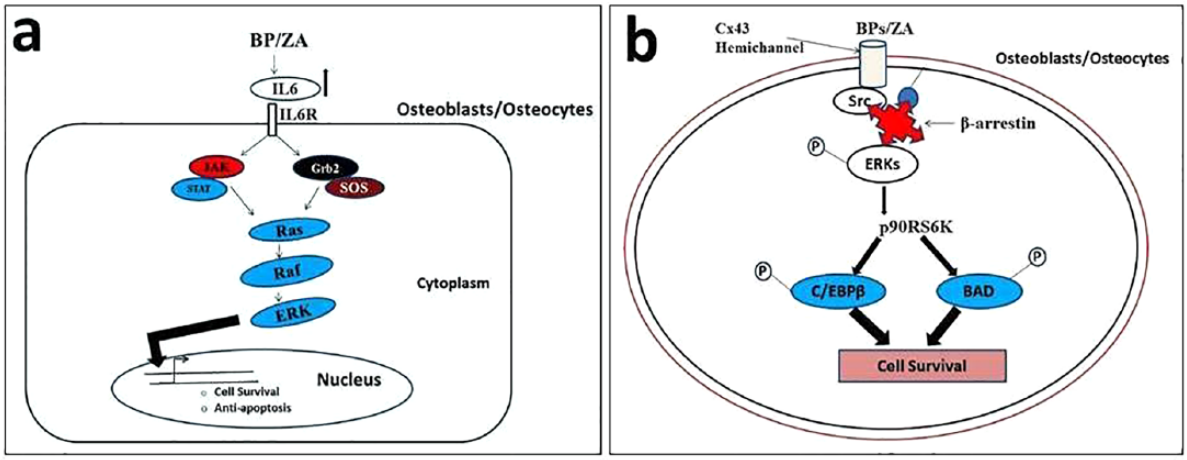

Fig. 8

Speculated mechanism of action of zoledronate *in vivo* for osteoblast survival. Probable *in vivo* effect of bisphosphonates as a factor for cell survival and osteoblastic differentiation exploiting two different mechanisms and pathways, a) and b). Figure modified from **Bellido T, Plotkin LI.** Novel actions of bisphosphonates in bone: preservation of osteoblast and osteocyte viability. *Bone* 2011;49:50–55 (with permission from Elsevier).

moieties.[70-74] In one study, R_1 groups were examined and, owing to different R_1 moiety, the difference in the affinities with hydroxyapatite was almost insignificant.[75] When the R_2 groups were studied, it was found that the affinities of different bisphosphonates related to different R_2 groups in the order of zoledronate > alendronate > ibandronate = risedronate > etidronate. The R_2 groups have also been seen to be involved in different surface properties (zeta potential and interfacial tension).[76]

Bisphosphonates as a carrier

A new insight into the utilization of bisphosphonate molecules could be their ability to deliver radiolabelled drugs for both diagnostic and therapeutic indications (radiopharmaceuticals). Without altering the ability to bind hydroxyapatite, bisphosphonates effectively chelate technetium (Tc) and rhenium (Re).[77] Despite the biological characteristics of ^{99m}Tc-labelled etidronate in bone scanning, such as greater accumulation in bone, faster blood clearance, and *in vivo* stability, it could not exceed the blood clearance rate of ^{18}F-fluoride previously used in positron emission tomography (PET).[78] The use of methylene diphosphonate (Medronate, MDP) as a bone scanning agent labelled with ^{99m}Tc, and using hydroxymethylene diphosphonate (Oxidronate, HDP) labelled with ^{99m}Tc, demonstrated a good and rapid blood clearance rate and greater affinity towards hydroxyapatite were found. Currently, these two are the only ^{99m}Tc-labelled radiopharmaceuticals used for the assessment of metastatic diseases, cancer staging, and traumatic injuries.[79,80] To kill tumour cells in metastatic bone, bisphosphonates have also been used to deliver β-particle emitting radionuclides (^{32}P, ^{89}Sr, ^{153}Sm).[81-84] Besides the sensitivity of theranostics (extent of diagnostic and therapeutic potential), the combination of bisphosphonates and a γ-emitter (single-photon emission CT, SPECT) and/or β-particle emitter and fluorescent probes in the same molecule gives a multimodal imaging approach.[85]

Search criteria

This review was based on a literature search collected from PubMed and Embase using ZA, bisphosphonates, local delivery, carrier, bone healing, osteoporotic fractures, and bone mineral density as keywords. Most of the studies selected in this review include preclinical models of bone regeneration using local zoledronate therapy.

Discussion

All of the studies that we considered show a positive effect of ZA in animal models, and no study has shown a detrimental effect. However, there are a few important drawbacks in local delivery to which we should pay attention: first, the high local dosages of ZA might prove potentially toxic to cells and cause necrosis. Second, since ZA has a strong affinity to bone and hydroxyapatite, and its clearance from the body takes a long time, this may reduce the bone remodelling at the site. This might have biomechanical implications and lead to stress shielding. Third, whether the positive results from animal studies will translate to clinical situations is unknown at this moment. It is also quite likely that the molecule may or may not show similar positive results in human studies.

The quantification of the antiresorptive activity regarding local use of more potent bisphosphonates like zoledronate is difficult in translational clinical settings. Many researchers have tried different methods to detect ZA including chromatographic techniques[86] and nuclear magnetic resonance (NMR) methods.[87] However, these methods encountered an array of difficulties as chromatography had a lower level of detection and NMR could

not differentiate between ZA and phosphate spectra. Moreover, quantification using radiolabelled ZA (^{14}C in animals, ^{3}H in clinical settings) has been considered one of the more efficient methods both *in vivo* and *in vitro*.[55,57] However, there are certain limitations associated with this technique as well, including availability of radioisotopes, high costs, and safety issues. There are indirect ways to measure the temporal reduction using biochemical markers of bone resorption such as amino- and carboxy-terminal breakdown products. Collagen-1 in serum and urine has been suggested as a reliable measure of bisphosphonate efficiency and potency. The US Food and Drug Administration (FDA) has approved one intravenous bisphosphonate injection, like zoledronate 4 mg to 5 mg,[23] to be effective using a surrogate marker. Biochemical markers of retarding bone resorption can be detected for up to a year in women with postmenopausal osteoporosis.[88] The bioavailability of bisphosphonates at the desired site is a critical limitation, owing to the hydrophilic character of a bisphosphonate limiting absorption from the gastrointestinal tract. Given orally, and in cases of intravenous administration, there are other systemic complications. If only local treatment for a bone lesion is needed, it is obvious that an *in situ* setting carrier with controlled elution of bisphosphonates will reduce systemic effects.[2]

Moreover, it was demonstrated in one of the studies that using clodronate-coated morcellized bone graft can enhance acetabular component fixation by reducing the migration, and also by precluding graft resorption.[89] The overall findings of this study were that using local bisphosphonate enhances the fixation of an implant and prevents aseptic loosening, but doesn't enhance the BMD and net bone formation that depict the negative impact of local use of bisphosphonates.[89] In one double-blind randomized study, using bone graft soaked in ibandronate, patients were found to have less mechanical failure, one of the common problems in impaction grafting.[90] However, there was no study carried out that could show that local use of ZA in clinical settings enhanced bone formation or implant fixation.

Many studies have attempted to develop coated devices to deliver zoledronate to the local site, enhance bone mineralization and reduce implant loosening, but there is still no consensus as to which characteristics are desired: sustained and optimal release profile; required degradation rate; or surface porosity for bone ingrowth of the zoledronate.[30-32] The binding of zoledronate to a hydroxyapatite has long been believed to be the Ca^{2+} ions in the matrix being released when resorbed. This makes it quite obvious that the release kinetics of zoledronate is wholly and solely dependent on the bone turnover and resorption.

It has been reported in several studies that the maximum serum concentration of zoledronate with a standard dose given is about 1 µmol/l to 3 µmol/l.[91] Little is known about the processes in the resorption pit and in the bone microenvironment. In terms of the local delivery of zoledronate, bone is formed initially in the periphery of a defect. Zoledronate is bound strongly to the matrix containing apatite. When new bone is formed, resorption is inhibited by the localized release of zoledronate (either coated onto the implant or mixed within a bone substitute matrix). Mineralized bone matrix is deposited more rapidly on the matrix that carries zoledronate. We do not know the threshold concentration of zoledronate added to a carrier, or how much is needed, and it probably differs regarding indication and anatomical site. What is the eventual fate of any bisphosphonate molecule that is released from the osteoclasts? Is it recycled, i.e. released and partly rebound to the bone matrix again, or is it metabolized? The action of bisphosphonates varies from cell type to cell type. Macrophages activating Rac and p38 lead to anti-apoptotic effect. If we use another cell type, the bisphosphonates activate the Rho pathway that might be pro-apoptotic.[5] In spite of extensive systemic usage of bisphosphonates inhibiting osteoclast-mediated bone resorption under pathological conditions, much remains to be explored regarding local applications. Some of the open aims for further studies are highlighted below:

- Optimize composite materials for controlled sustained local delivery of bisphosphonates to enhance bone regeneration and improve mechanical strength with negligible local and systemic toxicity.
- Investigate long-term effects of bisphosphonates in bone using local delivery systems compared with systemic treatment.
- Investigate the mechanism of any direct or indirect *in vitro* anabolic effect induced by bisphosphonates for further validation in different *in vivo* models.
- Explore new mechanisms for adding anticancer agents to bisphosphonates targeting bone using carriers for controlled systemic and local delivery.
- The positive effects of local bisphosphonate treatment shown in animal models paves the way for future translational studies focusing on clinically relevant areas. Of special interest are treatment and secondary prevention of fragility fractures, improved fixation of fracture devices, and implants.

References

1. **Bartl R, Frisch B, von Tresckow E, Bartl C.** *Bisphosphonates in Medical Practice: Actions, Side Effects, Indications, Strategies.* Berlin and New York, NY: Springer-Verlag, 2007.
2. **Drake MT, Clarke BL, Khosla S.** Bisphosphonates: mechanism of action and role in clinical practice. *Mayo Clin Proc* 2008;83:1032–1045.
3. **Rogers MJ.** New insights into the molecular mechanisms of action of bisphosphonates. *Curr Pharm Des* 2003;9:2643–2658.
4. **Masarachia P, Weinreb M, Balena R, Rodan GA.** Comparison of the distribution of 3H-alendronate and 3H-etidronate in rat and mouse bones. *Bone* 1996;19:281–290.

5. **Roelofs AJ, Thompson K, Gordon S, Rogers MJ.** Molecular mechanisms of action of bisphosphonates: current status. *Clin Cancer Res* 2006;12(20 Pt 2):6222s–6230s.
6. **Fleish H, Neuman WF.** Mechanisms of calcification: role of collagen, polyphosphates, and phosphatase. *Am J Physiol* 1961;200:1296–1300.
7. **Fleisch H.** *Bisphosphonates in Bone Disease: From the Laboratory to the Patient*. San Diego, CA: Academic Press, 2000.
8. **Fleisch HA, Russell RG, Bisaz S, Mühlbauer RC, Williams DA.** The inhibitory effect of phosphonates on the formation of calcium phosphate crystals in vitro and on aortic and kidney calcification in vivo. *Eur J Clin Invest* 1970;1:12–18.
9. **Russell RGG, Rogers MJ.** Bisphosphonate: from the laboratory to the clinic and back again. *Bone* 1999;25:97–106.
10. **Fleisch H, Russell RG, Francis MD.** Diphosphonates inhibit hydroxyapatite dissolution in vitro and bone resorption in tissue culture and in vivo. *Science* 1969;165:1262–1264.
11. **Russell RGG, Mühlbauer RC, Bisaz S, Williams DA, Fleisch H.** The influence of pyrophosphate, condensed phosphates, phosphonates and other phosphate compounds on the dissolution of hydroxyapatite in vitro and on bone resorption induced by parathyroid hormone in tissue culture and in thyroparathyroidectomised rats. *Calcif Tissue Res* 1970;6:183–196.
12. **Lehenkari PP, Kellinsalmi M, Näpänkangas JP, et al.** Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Mol Pharmacol* 2002;61:1255–1262.
13. **Gong L, Altman RB, Klein TE.** Bisphosphonates pathway. *Pharmacogenet Genomics* 2011;21:50-3.
14. **Grey A, Reid IR.** Differences between the bisphosphonates for the prevention and treatment of osteoporosis. *Ther Clin Risk Manag* 2006;2:77–86.
15. **Rogers MJ, Frith JC, Luckman SP, et al.** Molecular mechanisms of action of bisphosphonates. *Bone* 1999;24(Suppl):73S–79S.
16. **Ballantyne E.** Bisphosphonates: possible modes of action and implications for dental implant treatment. A review of the literature. *J Gen Pract* 2015;3:192.
17. **Brown JP, Kendler DL, McClung MR, et al.** The efficacy and tolerability of risedronate once a week for the treatment of postmenopausal osteoporosis. *Calcif Tissue Int* 2002;71:103–111.
18. **Chesnut CH III, Skag A, Christiansen C, et al.** Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. *J Bone Miner Res* 2004;19:1241–1249.
19. **Delmas PD, Recker RR, Chesnut CH III, et al.** Daily and intermittent oral ibandronate normalize bone turnover and provide significant reduction in vertebral fracture risk: results from the BONE study. *Osteoporos Int* 2004;15:792–798.
20. **McClung MR, Geusens P, Miller PD, et al.** Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *N Engl J Med* 2001;344:333–340.
21. **Miller PD, McClung MR, Macovei L, et al.** Monthly oral ibandronate therapy in postmenopausal osteoporosis: 1-year results from the MOBILE study. *J Bone Miner Res* 2005;20:1315–1322.
22. **Ste-Marie L-G, Sod E, Johnson T, Chines A.** Five years of treatment with risedronate and its effects on bone safety in women with postmenopausal osteoporosis. *Calcif Tissue Int* 2004;75:469–476.
23. **Reid IR, Brown JP, Burckhardt P, et al.** Intravenous zoledronic acid in postmenopausal women with low bone mineral density. *N Engl J Med* 2002;346:653–661.
24. **Boulenc X, Marti E, Joyeux H, et al.** Importance of the paracellular pathway for the transport of a new bisphosphonate using the human CACO-2 monolayers model. *Biochem Pharmacol* 1993;46:1591–1600.
25. **Chen T, Berenson J, Vescio R, et al.** Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases. *J Clin Pharmacol* 2002;42:1228–1236.
26. **Leyvraz S, Hess U, Flesch G, et al.** Pharmacokinetics of pamidronate in patients with bone metastases. *J Natl Cancer Inst* 1992;84:788–792.
27. **Russell RG, Watts NB, Ebetino FH, Rogers MJ.** Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. *Osteoporos Int* 2008;19:733–759.
28. **Roelofs AJ, Thompson K, Ebetino FH, Rogers MJ, Coxon FP.** Bisphosphonates: molecular mechanisms of action and effects on bone cells, monocytes and macrophages. *Curr Pharm Des* 2010;16:2950–2960.
29. **Wang G, Sui L, Gai P, et al.** The efficacy and safety of vertebral fracture prevention therapies in post-menopausal osteoporosis treatment: Which therapies work best? a network meta-analysis. *Bone Joint Res* 2017;6:452–463.
30. **Gao Y, Zou S, Liu X, Bao C, Hu J.** The effect of surface immobilized bisphosphonates on the fixation of hydroxyapatite-coated titanium implants in ovariectomized rats. *Biomaterials* 2009;30:1790–1796.
31. **Peter B, Pioletti DP, Laïb S, et al.** Calcium phosphate drug delivery system: influence of local zoledronate release on bone implant osteointegration. *Bone* 2005;36:52–60.
32. **Fratzl P, Schreiber S, Roschger P, et al.** Effects of sodium fluoride and alendronate on the bone mineral in minipigs: a small-angle X-ray scattering and backscattered electron imaging study. *J Bone Miner Res* 2009;11:248–253.
33. **Gao Y, Luo E, Hu J, et al.** Effect of combined local treatment with zoledronic acid and basic fibroblast growth factor on implant fixation in ovariectomized rats. *Bone* 2009;44:225–232.
34. **Tabata Y, Yamada K, Miyamoto S, et al.** Bone regeneration by basic fibroblast growth factor complexed with biodegradable hydrogels. *Biomaterials* 1998;19:807–815.
35. **Qi M, Hu J, Li J, et al.** Effect of zoledronate acid treatment on osseointegration and fixation of implants in autologous iliac bone grafts in ovariectomized rabbits. *Bone* 2012;50:119–127.
36. **Greiner SH, Wildemann B, Back DA, et al.** Local application of zoledronic acid incorporated in a poly(D,L-lactide)-coated implant accelerates fracture healing in rats. *Acta Orthop* 2008;79:717–725.
37. **Andersson T, Agholme F, Aspenberg P, Tengvall P.** Surface immobilized zoledronate improves screw fixation in rat bone: a new method for the coating of metal implants. *J Mater Sci Mater Med* 2010;21:3029–3037.
38. **Jakobsen T, Bechtold JE, Søballe K, et al.** Local delivery of zoledronate from a poly (D,L-lactide)-Coating increases fixation of press-fit implants. *J Orthop Res* 2016;34:65–71.
39. **Astrand J, Aspenberg P.** Topical, single dose bisphosphonate treatment reduced bone resorption in a rat model for prosthetic loosening. *J Orthop Res* 2004;22:244–249.
40. **Yaffe A, Binderman I, Breuer E, Pinto T, Golomb G.** Disposition of alendronate following local delivery in a rat jaw. *J Periodontol* 1999;70:893–895.
41. **Skoglund B, Holmertz J, Aspenberg P.** Systemic and local ibandronate enhance screw fixation. *J Orthop Res* 2004;22:1108–1113.
42. **Ying G, Bo L, Yanjun J, Lina W, Binquan W.** Effect of a local, one time, low-dose injection of zoledronic acid on titanium implant osseointegration in ovariectomized rats. *Arch Med Sci* 2016;12:941–949.
43. **Ichikawa K, Ohta Y, Mamoto K, et al.** Local co-application of zoledronate promotes long-term maintenance of newly formed bone induced by recombinant human bone morphogenetic protein 2. *Biochem Biophys Res Commun* 2016;480:314–320.
44. **Roshan-Ghias A, Arnoldi J, Procter P, Pioletti DP.** In vivo assessment of local effects after application of bone screws delivering bisphosphonates into a compromised cancellous bone site. *Clin Biomech (Bristol, Avon)* 2011;26:1039–1043.
45. **Teotia AK, Gupta A, Raina DB, Lidgren L, Kumar A.** Gelatin-modified bone substitute with bioactive molecules enhance cellular interactions and bone regeneration. *ACS Appl Mater Interfaces* 2016;8:10775–10787.
46. **Teotia AK, Raina DB, Singh C, et al.** Nano-hydroxyapatite bone substitute functionalized with bone active molecules for enhanced cranial bone regeneration. *ACS Appl Mater Interfaces* 2017;9:6816–6828.
47. **Raina DB, Isaksson H, Hettwer W, et al.** A biphasic calcium sulphate/hydroxyapatite carrier containing bone morphogenic protein-2 and zoledronic acid generates bone. *Sci Rep* 2016;6:26033.
48. **Kettenberger U, Luginbuehl V, Procter P, Pioletti DP.** In vitro and in vivo investigation of bisphosphonate-loaded hydroxyapatite particles for peri-implant bone augmentation. *J Tissue Eng Regen Med* 2017;11:1974-1985.
49. **McNamara I, Howard J, Rayment A, et al.** Mechanical properties of morcellised bone graft with the addition of hydroxyapatite. *J Mater Sci Mater Med* 2014;25:321-7.
50. **Agholme F, Aspenberg P.** Experimental results of combining bisphosphonates with allograft in a rat model. *J Bone Joint Surg [Br]* 2009;91–B:670–675.
51. **Belfrage O, Isaksson H, Tägil M.** Local treatment of a bone graft by soaking in zoledronic acid inhibits bone resorption and bone formation. A bone chamber study in rats. *BMC Musculoskelet Disord* 2012;13:240.
52. **Raina DB, Isaksson H, Teotia AK, et al.** Biocomposite macroporous cryogels as potential carrier scaffolds for bone active agents augmenting bone regeneration. *J Control Release* 2016;235:365–378.
53. **Jakobsen T, Baas J, Kold S, et al.** Local bisphosphonate treatment increases fixation of hydroxyapatite-coated implants inserted with bone compaction. *J Orthop Res* 2009;27:189–194.
54. **Jakobsen T, Kold S, Bechtold JE, Elmengaard B, Søballe K.** Effect of topical alendronate treatment on fixation of implants inserted with bone compaction. *Clin Orthop Relat Res* 2006;444:229–234.
55. **Jakobsen T, Baas J, Bechtold JE, Elmengaard B, Søballe K.** The effect on implant fixation of soaking tricalcium phosphate granules in bisphosphonate. *Open Orthop J* 2012;6:371–375.

56. **Murphy CM, Schindeler A, Gleeson JP, et al.** A collagen-hydroxyapatite scaffold allows for binding and co-delivery of recombinant bone morphogenetic proteins and bisphosphonates. *Acta Biomater* 2014;10:2250–2258.
57. **Yu NYC, Ruys AJ, Zenios M, et al.** Bisphosphonate-laden acrylic bone cement: mechanical properties, elution performance, and in vivo activity. *J Biomed Mater Res B Appl Biomater* 2008;87:482–491.
58. **Raina DB, Larsson D, Mrkonjic F, et al.** Gelatin- hydroxyapatite- calcium sulphate based biomaterial for long term sustained delivery of bone morphogenic protein-2 and zoledronic acid for increased bone formation: in-vitro and in-vivo carrier properties. *J Control Release* 2018;272:83–96.
59. **Günes N, Dundar S, Saybak A, et al.** Systemic and local zoledronic acid treatment with hydroxyapatite bone graft: A histological and histomorphometric experimental study. *Exp Ther Med* 2016;12:2417–2422.
60. **Locs J, Li W, Sokolova M, et al.** Zoledronic acid impregnated and poly (L-lactic acid) coated 45S5 Bioglass®-based scaffolds. *Mater Lett* 2015;156:180–182.
61. **Fromigué O, Body JJ.** Bisphosphonates influence the proliferation and the maturation of normal human osteoblasts. *J Endocrinol Invest* 2002;25:539–546.
62. **Plotkin LI, Weinstein RS, Parfitt AM, et al.** Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *J Clin Invest* 1999;104:1363–1374.
63. **von Knoch F, Jaquiery C, Kowalsky M, et al.** Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells. *Biomaterials* 2005;26:6941–6949.
64. **Gou W, Wang X, Peng J, et al.** Controlled delivery of zoledronate improved bone formation locally in vivo. *PLoS One* 2014;9:e91317.
65. **Greiner S, Kadow-Romacker A, Lübberstedt M, Schmidmaier G, Wildemann B.** The effect of zoledronic acid incorporated in a poly(D,L-lactide) implant coating on osteoblasts in vitro. *J Biomed Mater Res A* 2007;80:769–775.
66. **Greiner S, Kadow-Romacker A, Schmidmaier G, Wildemann B.** Cocultures of osteoblasts and osteoclasts are influenced by local application of zoledronic acid incorporated in a poly(D,L-lactide) implant coating. *J Biomed Mater Res A* 2009;91:288–295.
67. **Pazianas M, van der Geest S, Miller P.** Bisphosphonates and bone quality. *Bonekey Rep* 2014;3:529.
68. **Siddiqa A, Long LM, Li L, Marciniak RA, Kazhdan I.** Expression of HER-2 in MCF-7 breast cancer cells modulates anti-apoptotic proteins Survivin and Bcl-2 via the extracellular signal-related kinase (ERK) and phosphoinositide-3 kinase (PI3K) signalling pathways. *BMC Cancer* 2008;8:129.
69. **Bellido T, Plotkin LI.** Novel actions of bisphosphonates in bone: preservation of osteoblast and osteocyte viability. *Bone* 2011;49:50–55.
70. **Duarte LF, Teixeira FC, Fausto R.** Molecular modeling of the interaction of novel hydroxy- and aminobisphosphonates with hydroxyapatite. *ARKIVOC* 2009;2010:117.
71. **Dunford JE, Thompson K, Coxon FP, et al.** Structure-activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. *J Pharmacol Exp Ther* 2001;296:235-42.
72. **Geddes AD.** Bisphosphonates : structure-activity relationships and therapeutic implications. *Bone Miner Res* 1994;8:265-306.
73. **Rogers MJ, Gordon S, Benford HL, et al.** Cellular and molecular mechanisms of action of bisphosphonates. *Cancer* 2000;88(12 Suppl):2961-78.
74. **Jung A, Bisaz S, Fleisch H.** The binding of pyrophosphate and two diphosphonates by hydroxyapatite crystals. *Calcified Tissue Research* 197311:269-80.
75. **van Beek E, Hoekstra M, van de Ruit M, Löwik C, Papapoulos S.** Structural requirements for bisphosphonate actions in vitro. *J Bone Miner Res* 1994;9:1875–1882.
76. **Nancollas GH, Tang R, Phipps RJ, et al.** Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. *Bone* 2006;38:617–627.
77. **Kowalsky RJ, Falen SW.** *Radiopharmaceuticals in Nuclear Pharmacy and Nuclear Medicine*. Second ed. Washington, DC: APhA, American Pharmacists Association, 2004.
78. **Blau M, Nagler W, Bender MA.** Fluorine-18: a new isotope for bone scanning. *J Nucl Med* 1962;3:332–334.
79. **Ben-Haim S, Israel O.** Breast cancer: role of SPECT and PET in imaging bone metastases. *Semin Nucl Med* 2009;39:408–415.
80. **Strobel K.** Nuclear medicine diagnosis of bone metastases. *Praxis (Bern 1994)* 2009;98:1293–1297. (Article in German)
81. **Paes FM, Serafini AN.** Systemic metabolic radiopharmaceutical therapy in the treatment of metastatic bone pain. *Semin Nucl Med* 2010;40:89–104.
82. **Lewington VJ.** Bone-seeking radionuclides for therapy. *J Nucl Med* 2005; 46(Suppl 1):38S–47S.
83. **Pandit-Taskar N, Batraki M, Divgi CR.** Radiopharmaceutical therapy for palliation of bone pain from osseous metastases. *J Nucl Med* 2004;45:1358–1365.
84. **Volkert WA, Hoffman TJ.** Therapeutic radiopharmaceuticals. *Chem Rev* 1999;99:2269-2292.
85. **Palma E, Correia JDG, Campello MPC, Santos I.** Bisphosphonates as radionuclide carriers for imaging or systemic therapy. *Mol Biosyst* 2011;7:2950-2966.
86. **Maheswara Reddy L, Janardhan Reddy K, Raveendra Reddy P.** A simple RP-HPLC method for related substances of zoledronic acid in pharmaceutical products. *Arab J Chem* 2017;10:S196–S204.
87. **Sun S, Błażewska KM, Kashemirov BA, et al.** Synthesis and characterization of novel fluorescent nitrogen-containing bisphosphonate imaging probes for bone active drugs. *Phosphorus Sulfur Silicon Relat Elem* 2011;186:970–971.
88. **Black DM, Delmas PD, Eastell R, et al.** Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *N Engl J Med* 2007;356:1809–1822.
89. **Zampelis V, Belfrage O, Tägil M, Sundberg M, Flivik G.** Decreased migration with locally administered bisphosphonate in cemented cup revisions using impaction bone grafting technique. *Acta Orthop* 2018;89:17–22.
90. **Kesteris U, Aspenberg P.** Rinsing morcellised bone grafts with bisphosphonate solution prevents their resorption. A prospective randomised double-blinded study. *J Bone Joint Surg [Br]* 2006;88–B:993–996.
91. **Matsumoto S, Kimura S, Segawa H, et al.** Efficacy of the third-generation bisphosphonate, zoledronic acid alone and combined with anti-cancer agents against small cell lung cancer cell lines. *Lung Cancer* 2005:47;31-9.

Funding Statement

- VINNOVA Swedish agency for innovations systems (Grant number: 2017-00269) and Department of Biotechnology (DBT), Government of India (BT/IN/Sweden/08/AK/2017-18)

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Conflict of Interest Statement

- L. Lidgren is the board member of BoneSupport, AB, Lund, Sweden and Orthocell, Australia.