Enhancing safety and efficacy of bisphosphonates therapy by association with hydroxyapatite as adjuvant drug carrier

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Abstract
Introduction
Osteoporosis or decay of bones is one of the major causes of morbidity and sometimes mortality across the world. An enhanced bone resorption and decreased bone regeneration could be the reasons behind this disease. Bisphosphonates are one of the most potent treatment strategies available. Bisphosphonates negatively influence the bone resorption activity by decreasing osteoclast survival. However, they are associated with a wide number of side effects which might be associated with the molecular mechanism of their action. A simultaneous use of hydroxyapatite which acts by a divergent mechanism, that is, osteoinduction and osteogenesis may however potentiate the action of bisphosphonates with reduced side effects. Furthermore, hydroxyapatite offers distinguish advantage of getting converted into different drug carrier, such as ceramic-based nanoparticles. Hence, bisphosphonates delivered by a hydroxyapatite-based drug carrier may have a potentiating effect of bisphosphonates action with reduced side effects.

The Hypothesis
Among the various approaches, the use of bisphosphonates with hydroxyapatite can synergies the action of bisphosphonates without compromising the safety issues.

Evaluation of Hypothesis
Bisphosphonates activity is dependent on down regulation of osteoclast. The cytotoxic analogue generated by the bisphosphonates may be a prime cause of side effects associated with its use. Hydroxyapatite regulates all four osteoblast marker genes in hMSCs grown over calcium phosphate biomaterial and Hence it can be used as useful drug adjuvant carriers for bisphosphonates to enhance the its efficacy and reduce the risk of side effects associated with bisphosphonates.

Conclusion
Combinational therapy comprising bisphosphonates and Hydroxyapatite can be highly effective in promoting the formation of bone as well as reduce the risk of side effects associated with bisphosphonates.

Introduction
According to the International Osteoporosis Foundation (IOF), osteoporosis affects over 200 million people worldwide, 80% of which are women and 75 million people in Europe and Japan. Osteoporosis is a major public health threat for 44 million Americans, and the disease costs the U.S. healthcare system in excess of $17 billion ($47 million per day) annually compared with breast cancer at $6 billion. Although there is an increased global awareness of osteoporosis, the disease is still under diagnosed and undertreated. Osteoporosis or porous bones is a disease characterised by low bone mass and structural deterioration of tissue leading to bone fragility and an increased susceptibility to fractures of the hip, spine and wrist. Such fractures are associated with mortality and with significant short- and long-term morbidity. The treatment of osteoporosis must focus all aspects of the condition that are – bone mass should be maximised; fractures should be prevented and people who have already sustained a fracture should be rehabilitated to minimise associated pain, limitation of physical activities.

Various treatment modalities are available to manage the osteoporosis that affects bone mass, strength and turnover. Earlier, the role of oestrogen for maintenance of bone integrity and hormone-replacement therapy was recommended to prevent osteoporosis. The non-specific hormone replacement therapy has its own undesirable side effects and low compliance rates. The need of making more potent and specific treatments with large effects on bone mass resulted in the development of direct anti-resorptive agents, including the bisphosphonates and selective oestrogen receptor modulators. Bisphosphonates are first line therapy for patients with osteoporosis. Bisphosphonates are derived chemically from pyrophosphates compounds that inhibit precipitation of calcium carbonate. They are characterised by two carbon–phosphorus bonds, located on the same carbon atom (i.e. gem), which allow large variations in side chains. The different side chain combinations give each compound specific physiological and biochemical properties. Bisphosphonates have anti-resorptive activity and act on bone by binding with hydroxyapatite (HAP) and by inhibiting activation of osteoclasts. Although, bisphosphonates are generally considered safe drugs but one of the most severe adverse effects of bisphosphonates is osteonecrosis of the jaw. The area of interest was osteonecrosis of the jaw. The area of interest was osteonecrosis of the jaw.
of exposed bone in the maxillofacial region persists for at least 8 weeks\(^2,8\). Bisphosphonates treatment has also been linked to stress fractures of the femoral shaft. Renal failure has been associated with intravenous (i.v.) use of zoledronic acid, and to a lesser extent, pamidronate. At recommended doses, this adverse event happens only in minority patients depending on the underlying disease, but it can occur in up to 20–25% of patients. Several other adverse events have also been reported, such as ocular inflammation\(^6\), flu-like symptoms such as nausea, low-grade fever or fatigue; gastro-intestinal tract GIT disturbances and nephrocalcinosis were observed. Gastrointestinal disorders are the most common and more frequently reported with nitrogen-containing bisphosphonates, such as oral pamidronate and may require the drug to be given by i.v. route\(^10,11\).

**Background**

**Molecular action of bisphosphonates**

The molecular mechanism of bisphosphonates action is related to both binding with bone mineral and biochemical effect on cells, predominately osteoclasts\(^12\). Osteoclasts break down bones by a process called bone resorption. After accumulation in bone tissues, bisphosphonates are delivered to osteoclasts by a specific release mechanism. The acidic environment of the resorption space between the bone and osteoclasts triggers the release of bound bisphosphonates, resulting in their accumulation and uptake via fluid-phase endocytosis\(^13,14\). This acidic environment is generated by a proton pump in the osteoclast ruffled membrane that mediates bone resorption\(^15,16\). Upon uptake by osteoclasts, bisphosphonates exert their inhibitory activity through two main mechanisms, which are structure-dependent. The molecular target of bisphosphonates is the farnesyl pyrophosphate synthase (FPPS)\(^7,17,18\). When this enzyme is inhibited, the amount of farnesyl diphosphate (FPP) and geranylgeranyldiphosphate (GGPP) required for protein prenylation is reduced. It was shown that geranylgeranylated proteins (e.g. Rab and Rho), but not farnesylated proteins such as Ras, are essential for osteoclasts to resorb bone\(^19\). According to the recent studies not only the lack of prenylated proteins, but also the accumulation of active unprenylated proteins was responsible for the anti-resorptive effect\(^20,21\). Additionally, it was recently found that upon inhibition of FPPS, the levels of isopentanylprenylphosphate (IPP) and triphosphoric acid-1-adenosin-5′-yl ester 3-(3-methylbut-3-ethyl) ester (Apppi), a cytotoxic ATP analogue, increased. Apppi inhibits ANT and triggers apoptosis of osteoclasts by the same mechanism as non-nitrogen-containing bisphosphonates\(^22,23\).

**Reasons for side effects associated with bisphosphonates treatment**

One of the major side effects with long-term bisphosphonates treatment is stress fractures of the femoral shaft. Such stress fractures seem to occur by inhibition of bone remodelling. When a patient is undergoing bisphosphonates treatment, micro-damages in the bones are not repaired and thus accumulate, eventually leading to fracture. This occurs on compact bones at sites of high tensional stress. Renal failure is another side effect associated with the bisphosphonates, where tubular necrosis has been observed due to calcination of nephrons due to incomplete excretion. Oesophageal inflammation and ulceration have been described rarely for serious adverse effects of alendronate and, less frequently for risedronate. Most of the affected patients were elderly and had concomitant gastroesophageal reflux, because the patients took their medication before bedtime, the bisphosphonates reached the oesophagus while they were supine, resulting in erosion of oesophagus due to negligible absorption of bisphosphonates. For this reason, patients treated with these drugs are advised to take their medication in the morning. Because absorption of bisphosphonates is impaired by food and beverages containing two fold positive cations (e.g. Ca and Mg), it is recommended that patients should take these drugs on an empty stomach with 6–8 floz (130–240 mL) of water and wait 30–60 minutes before breakfast\(^24–26\) for better results. Most clinically apparent complaints vary between non-specific epigastric symptoms and severe flatulence and/or diarrhoea. Hence, bisphosphonates treatment requires a tedious dosage strategy which may lead to patient unacceptability and hence patient non-compliance.

The low oral absorption of bisphosphonates may pose another hurdle in their use. Such low oral absorption may be the result of negative charge and hydrophilic nature, which hamper their diffusion through cell membranes. Absorption is thought to occur via a paracellular pathway. Poor absorption also partly results from formation of absorbable complexes with calcium. The co-administration of bisphosphonates with the calcium chelator EDTA was shown to significantly improve their bioavailability in rats\(^27\).

Naturally occurring HAP is a mineral with a hexagonal structure that is composed of calcium phosphate groups with a general formula of Ca\(_{10}\)(OH)\(_6\)(PO\(_4\))\(_{6}\) for the unit cell. Both crystallographic and chemical studies have shown that synthetic HAP is similar to the natural occurring inorganic component found in the bone matrix and teeth and due to this structural and functional similarity, extensive research efforts have been reported on synthetic HAP as a bone and substitute replacement in several biomedical applications\(^28,29\). An investigation by Taniguchi et al. has shown sintered HAP exhibited an excellent biocompatible response to soft tissue, such as skin, muscle and
gums. It is this type of response that has made synthetic HAP an ideal candidate for orthopaedic and dental implants. It has been found that unlike bisphosphonates, HAP does not have negative osteoclast activity but has a positive bone remodelling or osteogenic activity. These properties are very important because bone tissue constantly undergoes remodelling, a process whereby bone tissue is simultaneously replaced and removed by the bone cells (osteoblasts and osteoclasts, respectively).

In a recent study, a significant improvement in the action of HAP was observed when it was converted into the nano scale range particle, a study conducted by Sun et al. addressing the influence of particle size of HAP on in vivo osteoblast cells, it was discovered that the therapeutic response was improved when smaller particle sizes (0.5–3.0 μm) were used.

The Hypothesis
Among the various approaches, the use of bisphosphonates is one of the most popular in osteoporosis treatment. However, oral administration of this class of drugs is associated with high level of side effects. Some of which are attributed to the underlying mechanism of bisphosphonates action, that is, negative effect on osteoclast activity. The simultaneous use of HAP, which acts by altogether a different action (ostegenesis and osteoinductive effect), can synergise the action of bisphosphonates without compromising the safety issues (Figure 1).

Evaluation of Hypothesis
Bisphosphonates therapy is one of the gold standards in treatment of osteoporosis. Two divergent mechanisms of action have been associated with the action of bisphosphonates. The non-nitrogenous bisphosphonates restrain bone resorption by generating cytotoxic ATP-analogues, which interfere with mitochondrial function and persuade apoptosis of osteoclasts. However, the more potent nitrogen-containing BPs bind to and inhibit crucial enzymes associated with an intracellular mevalonate pathway, thereby preventing the preylation and activation of small GTPases that are important for the bone-resorbing activity and survival of osteoclasts. Therefore, overall action of bisphosphonates activity is dependent on down regulation of osteoclast. However, the mechanism of bisphosphonates action may interfere with natural remodelling of bones and hence may cause fracture of long bones in some cases. Besides, the cytotoxic analogue generated by the bisphosphonates may be a prime cause of side effects associated with bisphosphonates treatment.

HAP also offers an excellent material for the preparation of drug carrier systems, such as nanoparticles. They can carry a wide variety of drug payload with an added advantage of biocompatibility and safety, and hence HAP can be used as useful drug carriers for bisphosphonates to enhance the efficacy and reduce the risk of side effects associated with bisphosphonates.

Discussion
Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption. They are widely used in the management of osteoporosis and other diseases of high bone turnover. BPs’ pronounced affinity for bone, but not other tissues, makes them the ideal candidates for treatment of bone diseases. BPs have been shown to increase bone mineral density (BMD), reduce bone turnover.

Figure 1: Effect of combination therapy on treatment of Osteoporosis

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markers, and reduce the risk of osteoporotic fractures.

Hydroxyapatite can positively affect bone healing, though their use has been based on unexpected observations, with a lack of clear rationale or hypothesis. Perhaps HAP has always been acting as a drug, and effects such as osteo-conduction is merely a result of ion release or localized ion concentration modification. The combinational therapeutic approaches for the management of osteoporosis regulated through bisphosphonates-hydroxyapatite which reduces the adverse effects of the bisphosphonates.

Conclusion
Compared with the existing drug therapy based on bisphosphonates, the combination therapy comprising bisphosphonates and HAP can be highly effective in promoting the formation of bone and can help to treat post-menopausal women, suffering from osteopenia. This novel combination therapy seems to be promising in the treatment of osteoporosis with high selectivity to bone tissues as well as to reduce the risk of side effects associated with bisphosphonates.

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Abbreviations list

FPP, farnesyl diphosphate; FPPS, farnesyl pyrophosphate synthase; GGPP, geranylgeranyl diphosphate; HAP, hydroxyapatite; ICMR, Indian Council of Medical Research; IOF, International Osteoporosis Foundation; IPP, isopentenyl pyrophosphate.

References


