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Bisphosphonates: Focus on Inflammation and Bone Loss

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Bisphosphonates are pharmacological compounds that have been used for the prevention and treatment of several pathological conditions including osteoporosis, primary hyperparathyroidism, osteogenesis imperfecta, and other conditions characterized by bone fragility. Many studies have been performed to date to analyze their effects on inflammation and bone remodelling and related pathologies. The aim of this review is, starting from a background on inflammatory processes and bone remodelling, to give an update on the use of bisphosphonates, outlining the possible side effects and proposing new trends for the future. Starting from a brief introduction on inflammation and bone remodelling, we collect and analyze studies involving the use of bisphosphonates for treatment of inflammatory conditions and pathologies characterized by bone loss. Selected articles, including reviews, published between 1976 and 2011, were chosen from Pubmed/Medline on the basis of their content. Bisphosphonates exert a selective activity on inflammation and bone remodelling and related pathologies, which are characterized by an excess in bone resorption. They improve not only skeletal defects, but also general symptoms. Bisphosphonates have found clinical application preventing and treating osteoporosis, osteitis deformans (Paget's disease of bone), bone metastasis (with or without hypercalcaemia), multiple myeloma, primary hyperparathyroidism, osteogenesis imperfecta, and other conditions that feature bone fragility. Further clinical studies involving larger cohorts are needed to optimize the dosage and length of therapy for each of these agents in each clinical field in order to be able to maximize their properties concerning modulation of inflammation and bone remodelling. In the near future, although ''old'' bisphosphonates will reach the end of their patent life, ''new'' bisphosphonates will be designed to specifically target a pathological condition.

Keywords: bisphosphonates, bone, inflammation, osteoporosis, pain, rheumatoid arthritis, complex regional pain syndrome, ankylosing spondylitis

INTRODUCTION

The bone undergoes a continuous resorption and formation, respectively carried out by osteoclasts (OCLs) and osteoblasts (OBs). The balance between these 2 processes is responsible for the adult skeletal homeostasis.[1] A disruption to this balance has been linked to rheumatic diseases affecting the joint, such as rheumatoid arthritis (RA) and spondyloarthropathies, leading to bone loss or bone accumulation in a process that also involves inflammatory mediators such as cytokines and factors found within the local bone microenvironment of the affected joint.[2] Bisphosphonates (BPs) represent an interesting class of pharmacological agents that have

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been studied not only for their effects on bone remodelling, but also for their effects on inflammatory conditions. These agents inhibit the formation, aggregation, and dissolution of calcium phosphate crystals; they display a high affinity for bone mineralized matrix and are able to inhibit bone resorption processes, which represent their most important biological effect.[3] Furthermore, experimental studies have shown how BP treatment increases callus volume, trabecular bone volume and bone mineral content, although it delays the callus maturation and remodelling.[4–9] The ability of BPs to attach to bone surfaces is due to the strong negative charge in the BP nucleus that binds to the positively charged surface of hydroxyapatite.[10] BPs are cleared from the circulation within hours after intravenous administration, becoming incorporated into the bone and remaining within it for years.[11,12] BPs are widely used in the clinic for treatment of osteoporosis, malignant diseases affecting the skeleton, Paget's disease of bone, and other pathological conditions.[13] They include alendronic acid, clodronic acid, etidronic acid, ibandronic acid, neridronic acid, pamidronic acid, risedronic acid, tiludronic acid, and zoledronic acid (ZA). They are available in the European Union as tablets and solutions for infusion under various trade names and as generic medicines (see European Medicines Agency, 2011. Questions and answers on the review of BPs and atypical stress fractures EMA/288359/2011).

REVIEW CRITERIA AND AIM

A PubMed search was performed by using the following key words (separately or combined): ''BPs'' combined with ''Inflammation'', ''Bone'', and ''Remodelling''. Selected articles, including reviews, were published between 1990 and 2011 and chosen on the basis of their content. The focus was on specific aspects concerning the effect of BPs on inflammation and bone remodelling and related pathologies.

Inflammation

Inflammation is a complex set of interactions among soluble factors and cells that can arise in any tissue in response to infection and traumatic, post-ischemic, toxic or autoimmune injury. The reaction can be either local or systemic, acute or chronic, and must be strictly regulated because a deficiency or excess of the inflammatory response can cause morbidity and shorten lifespan. Acute inflammation represents the initial physiologic reaction to tissue injury. It is mediated by the release of several mediators (Table 1) and it usually precedes the immune response to foreign agents. In contrast to acute inflammation, which is made manifest in vascular changes, oedema, and largely by neutrophil infiltration, the chronic phase is characterised by infiltration of lymphocytes, mononuclear leukocytes, tissue destruction, angiogenesis, fibrosis, and specific mediators (Table 2). Inflammation can affect every organ and tissue and many bone-related pathologies are of inflammatory aetiology.[14]

Bone remodelling

Bone remodelling can be defined as a cycle necessary to maintain the skeleton structure and is mediated by the bone-forming OB, the bone-degrading OCL, the osteocyte, and the bone-lining cell.[15] Bone tissue constitutes the skeleton and, when analyzed macroscopically in a longitudinal section, it can be distinguished in 2 types, that is, cortical or compact bone and trabecular or cancellous bone. The first type is a rather dense tissue, although it is penetrated by blood vessels through a canalicular network. It is primarily found in the shaft of long bones. The second type is porous and primarily found near joint surfaces at the end of long bones and within vertebrae. It has a complex three-dimensional structure consisting of struts and plates. The orientation of trabeculae coincides with the direction of stress trajectories. The bone adapts its structure according to the applied load.[16] In fact, a gain in bone mass is observed after excess bone loading although immobility, space flight, and long-term bed rest result in bone loss; a gain in bone mass also occurs during growth, when the refined trabecular bone observed during childhood changes into a coarser trabecular morphology which is visible in maturity, after fracture healing and in relation with implant incorporation.[16] In a state of homeostatic equilibrium, continuous bone resorption and bone formation, namely remodelling, accounts for the ability of the bone to adapt to mechanical load allowing the old bone to be continuously replaced by new tissue.[16] This balanced equilibrium is necessary to maintain the mechanical integrity of the bone without causing changes in its morphology and is defined as remodelling state.[17,18] The skeleton undergoes renewal by remodelling in approximately 10 years.[19] The remodelling process can be divided in 6 cycling phases, that is, quiescence, activation, resorption, inversion, formation, and mineralization.[19] The remodelling process is based on the separate actions of bone-resorbing cells called OCLs, and bone-forming cells called OB. However, during the modelling process, which takes place principally in the child, formation and resorption are not balanced, causing changes in the microarchitecture because the new bone forms at a location different

Table 1. Acute inflammation mediators.

	Mediator	Origin	Effects
Protein	Complement system	Liver	C5a: is chemotactic for neutrophils; increases vascular permeability; releases histamine from mast cells; C3a: has properties similar to the C5a ones, but less active; C567: is chemotactic for neutrophils; C56789: has cytolytic activity; C4b, 2a, 3b: opsonizes bacteria
	Coagulation system	Liver	Induces hemostasis; thrombin: converts fibrinogen to fibrin and fibrinopeptides; fibrinopeptides: induces vascular permeability and it is chemotactic for leukocytes
	Fibrinolytic system	Liver	Induces hemolisys; plasmin: activates complement components C3 and C5 and splits fibrin into fibrin split products; fibrin split products: induces vascular permeability
	Kinin system	Liver	Induces smooth muscle contraction; stimulates arteriolar dilatation; increases permeability of venules (very potent); induces pain
Lipid	Eicosanoid	Ubiquitous	PG: PGE2: vasodilates, intensifies bradykinin induced pain, induces fever; PGD2: vasodilates; PGI2: vasodilates and is a powerful inhibitor of platelet aggregation; TXA2: vasoconstricts and stimulates platelet aggregation; LT: LTB4: is a potent neutrophil and macrophage chemotactic agent, and causes aggregation and increased adherence of polymorphonuclear neutrophils to vascular endothelia; LTC4, LTD4, LTE4 (identified as the SRS-A): constricts extravascular smooth muscle, vasoconstricts, and increases vascular permeability
	Acetyl glycerol ether Phosphocholine (PAF)	A variety of cell types (such as neutrophils, basophils, platelets, and endothelial cells)	Activates platelets; increases vascular permeability directly and from the effect of histamine and serotonin released from platelet activation; induces chemotaxis; stimulates leukocyte aggregation and adhesion; stimulates target cells to synthesize eicosinoids and augments their effects
Other	Histamine	Mast cells, basophil and eosinophil leukocytes, and platelets	Induces arteriolar dilatation and increases permeability of venules in the immediate transient phase; is chemotactic for eosinophils
	Serotonine (5-hydroxytryptamine, 5-HT)	Mast cells and platelets	It is vasoactive (dilatation and constriction) and increases permeability of venules; it regulates hemostasis
	NO	Vascular Endothelia (constitutive) and macrophages (inducible)	Induces vasodilation
	Endotoxin	Bacteria	Triggers complement activation, which causes vasodilation and increases vascular permeability; activates coagulation and fibrinolytic; elicits T-cell proliferation

LT, leukotrienes; NO, nitric oxide; PAF, platelet activating factor; PG, prostaglandin.

Table 2. Chronic inflammation mediators.

Mediator	Origin	Effects
IL-1	Helper CD4+ T lymphocytes, monocytes, macrophages, and endothelial cells	Induces fever; induces sleepiness; decreases appetite; induces hemo dynamic effects (hypotension, decreased vascular resistance, increased heart rate); induces leukocytosis; induces hepatic synthesis of acute phase proteins (fibrinogen, complement components, CRP); increases synthesis of surface adhesion molecules; increases adhesion of leukocytes to endothelium; increases elaboration of PGI2 and platelet activating factor; increases thrombogenicity of the endothelial surface; increases proliferation and collagen synthesis; increases collagenase and protease synthesis; increases synthesis of PGE2
IL-6	—	Induces fever, acute phase protein and PGE2
IL-8	—	Is a chemotactic agent and an activator of neutrophils
IL-17	—	Induces fever; induces sleepiness; decreases appetite; induces hemodynamic effects (hypotension, decreased vascular resistance, increased heart rate); induces leukocytosis; induces hepatic synthesis of acute phase proteins (fibrinogen, complement components, CRP); increases synthesis of surface adhesion molecules; increases adhesion of leukocytes to endothelium; increases elaboration of PGI2 and platelet activating factor; increases thrombogenicity of the endothelial surface; increases proliferation and collagen synthesis; increases collagenase and protease synthesis; increases synthesis of PGE2
TNF-α	—	Induces fever; induces sleepiness; decreases appetite; induces hemodynamic effects (hypotension, decreased vascular resistance, increased heart rate); induces leukocytosis; induces hepatic synthesis of acute phase proteins (fibrinogen, complement components, CRP); increases synthesis of surface adhesion molecules; increases adhesion of leukocytes to endothelium; increases elaboration of PGI2 and platelet activating factor; increases thrombogenicity of the endothelial surface; increases proliferation and collagen synthesis; increases collagenase and protease synthesis; increases synthesis of PGE2
GM-CSF	—	Induces macrophage and leucocyte activation
PDGF	—	Induces fibroblast proliferation and chemotaxis

GM-CSF, granulocyte and monocyte colony stimulating factor; PG, prostaglandin; PDGF, platelet-derived growth factor.

from the destroyed one. The remodelling process begins at a quiescent bone surface with the OCL appearance. They are large multinucleated cells that are formed by fusion of mononuclear precursors of hematopoietic origin.[20] They attach to the bone tissue matrix and form a ruffled border at the bone/OCLs interface that is completely surrounded by a "sealing" zone. Thus the OCLs create an isolated microenvironment. Subsequently, the OCLs acidify the microenvironment and dissolve the organic and inorganic bone matrices.[21] As soon as this resorptive process stops, OBs make their appearance at the same surface site. The OBs derive from mesenchymal stem cells found in the bone marrow, periosteum, and soft tissues. OBs deposit osteoid, which becomes a mineralized form of new bone. Some of the OBs are encapsulated in the osteoid matrix and differentiate into osteocytes. Remaining OBs continue to synthesize bone until they eventually stop and transform into quiescent lining cells that completely cover the newly formed bone surface. These lining cells are highly interconnected with the osteocytes in the bone matrix through a canalicular network.[22,23] It seems that OCLs and OBs closely collaborate in the remodelling process in what has been called a "Basic Multicellular Unit", indicating that a coupling mechanism may exist between formation and resorption.[24] Many mediators such as hormones, chemotactic agents, and cytokines participate in remodelling. These mediators attract OCLs and OBs in remodelling sites, they modify cell proliferation and differentiation, and they induce bone matrix deposition. Frequently, the pathophysiology of

bone disease is characterized by an alteration of the activity of these mediators.[25–31]

Inflammation and Bone Loss

It is widely accepted that chronic inflammatory process and associated immune system activation can induce bone modifications,[32] like in an excess of subchondral bone resorption.[14] Molecular bases of osteoclastogenesis and bone resorption depend on imbalances of receptor activator of nuclear factor-κB ligand (RANKL)-RANK—osteoprotegerin (OPG) system. RANKL is produced by OBs and, together with macrophage colony stimulating factor (M-CSF), induces the differentiation of OC precursors and their activation by binding to its surface-receptor, i.e. RANK. When bone-resorption is not necessary, RANK is inhibited by the binding with OPG.[32] Many proinflammatory cytokines, such as M-CSF, interleukin (IL)-6, IL-11 (stimulates Ig production, induces proliferation of myeloid precursors and megakaryocytes), tumor necrosis factor alpha (TNF-α), IL-1, hormones, such as parathyroid hormone (PTH), eicosanoids, such as PGE2, and other mediators, such as NO and reactive oxygen species, enhance RANKL expression. Instead, other molecules, such as OPG, IL-18, IL-12, IL-4, interferon-γ, act as bone resorption inhibitors.[33] For instance, an in vitro study has showed how IL-12 potentially inhibits OCL formation in M-CSF and RANKL treated mouse bone marrow cells and IFN-γ may be involved in OCL formation inhibition.[34] IL-18 inhibits osteoclastogenesis by stimulating T-cell production in response to GM-CSF[35] and it can also increase OPG expression in OBs without altering RANKL production.[36] IL-4 inhibits OCL differentiation through inhibition of the RANKL-induced activation of NF-κB pathway.[37–39] A mechanism for IL-4 inhibition of bone resorption has been also reported. It occurs through prevention of RANKL-induced nuclear translocation of p65 NF-κB subunit and intracellular Ca2+changes.[37] In this process, activation of STAT6, which subsequently acts as a transcriptional repressor of NF-κB–activated genes, plays a key role.[38]

Many pathologies are characterised by inflammation and bone modifications. For instance, RA is characterized by destruction of articular cartilage and by excessive subchondral osteoclastic bone resorption.[40] In the inflammatory state, macrophages (which differentiate into OCLs) accumulate in the rheumatoid synovial membrane.[41] Many osteoclastogenic mediators, including IL-1,[42] IL-6,[43] IL-11,[44] IL-13,[45] IL-17,[46] TNF-α,[47] PGE2,[48,49] and PTH,[50] have an active role in RA pathophysiology. Rheumatoid synovial fibroblasts producing RANKL, and T-cell–producing RANKL, have been shown to promote OCLs formation, without the participation of other cells.[51,52] OCLs release enzymes, matrix metalloproteinases (MMPs) and chemotactic factors to promote bone destruction,[53] and increase the risk of osteoporosis and bone fractures.[54]

For years, osteoarthritis (OA) was thought to be characterized only by focal destruction of articular cartilage, but nowadays, it is known to affect the joint in all its major tissues, that is, cartilage, synovial membrane, and subchondral bone.[55] An abnormal expression of OPG and RANKL was observed in the osteoarthritic subchondral bone OB subpopulations in humans and their ratio was significantly reduced in the subpopulation displaying resorptive properties.[56] Joint inflammation may accelerate joint damage. Cytokines, produced by the synovium and chondrocytes, especially interleukin IL-1 and TNF-α, play a significant role in the degradation of cartilage and subchondral bone,[57] and prostaglandins and leukotrienes may also be involved as PGE2 increases in human OA-affected tissue.[58] C-reactive protein (CRP) levels may be modestly elevated in OA patients' serum.[59] MMPs have been implicated in the excessive matrix degradation that characterizes the cartilage and subchondral bone degeneration of OA.[60] The expression of several MMPs and members of the A Disintegrin and Metalloprotease with Thrombospondin (ADAMTS) family of proteinases is high in OA patients' cartilage (eg, MMP-3, MMP-13, MMP-14, ADAMTS-1, ADAMTS-4, and ADAMTS-5).[61]

Ankylosing spondylitis (AS) is a chronic inflammatory arthritis and autoimmune disease. It mainly affects the joints in the spine and sacroiliac joint in the pelvis and can cause eventual spine fusion 8.[62] TNF-α and IL-6 are overexpressed in patients affected by AS.[63] These proinflammatory cytokines have an important role in the pathogenesis of the disease because the use of TNF-α inhibitors reduces autoimmune response and improves the symptoms of the pathology.[64–66] Serum RANKL/OPG ratio is higher in AS patients than in healthy subjects, and this imbalance is probably involved in the pathogenesis and clinical courses of the frequently associated osteoporosis in AS.[67]

Periodontitis represents a specific inflammatory response to microbial residents of the subgingival biofilm. Emerging evidence strongly suggests that the inflammatory response of the host induces tissue destruction. Hence, although bacteria are necessary for disease appearance, periodontitis does not develop unless it is associated with inflammatory response and host susceptibility.[68] The inflammatory reaction induced by the large bacterial bioburden brings to the activation of the monocyte/macrophagic system and T lymphocytes with release of MMP and RANKL, whereas gingival fibroblasts release M-CSF[69] and

modulate negatively OPG synthesis and imbalance RANKL/OPG axis toward the bone resorption.[70–73] Periodontitis-associated osteoclastogenesis also depends on upregulation of cyclooxygenase-2 (COX-2, enzyme which synthesizes prostaglandin) expression in periodontal fibroblast, cementoblast, and OB and the consequent increased production of PGE2.[74]

The major inflammatory bowel diseases (IBD) are Crohn disease and ulcerative colitis.[75] Crohn disease is a chronic, relapsing, transmural inflammatory disease affecting the whole gastrointestinal tract.[76,77] Ulcerative colitis is a chronic, relapsing, nontransmural mucosal inflammatory disease, but it affects only the colon.[78,79] Celiac disease is a chronic intestinal disorder due to an immune reaction to the fraction of gluten called gliadin and therefore, when this protein is ingested, it leads to villous atrophy and inflammatory alterations of small bowel mucosa from the duodenum to the distal ileum.[80,81] These diseases are immune/inflammatory based, and the abnormal production of pro-inflammatory mediators is pathophysiological. In particular, many studies report higher level of RANKL in IBD and celiac disease.[82,83] Furthermore, the alteration of intestinal absorption that characterizes these pathologies reduces calcium collection and deposition.[84]

Periprosthetic osteolysis is one the major causes of orthopedic implant failure.[85] Wear particles, such as titanium or polyethylene particles, derived from the wear of orthopedic implant surfaces, could activate macrophages able to secrete cytokines and stimulate osteoclastic bone resorption, causing osteolysis around orthopedic implants.[86] Different pro-inflammatory mediators, such as IL-1, TNF-α, inducible nitric oxide synthase (iNOS), COX-2, TNF-like weak inducer of apoptosis are increased and contribute to upregulate and secrete RANKL.[85,87,88]

Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver, characterized by the slow progressive destruction of the small bile ducts within the liver, cholestasis, and high risk of fibrosis and cirrhosis.[89] IL-1β, IL-6, TNF-α, and complement components are overexpressed in PBC patients.[90] Although osteoporosis is common in PBC,[91] high levels of OPG and low levels of RANKL have been detected in subjects affected by this pathology. Probably, it represents a compensatory mechanism to the negative balance of bone remodelling.

Complex regional pain syndrome (CRPS) is a chronic progressive disease characterized by severe pain, swelling, and changes in the skin. Based on the presence of nerve lesion after the injury, CRPS is divided as follows: type 1 (reflex sympathetic dystrophy) without demonstrable nerve lesions; and type 2 (causalgia) with nerve damage.[92] Proinflammatory cytokines (IL-1β, IL-6, TNF-α)[93] and neuropeptides[94] are directly involved in CRPS pathophysiology. The main role of inflammation on CRPS pathogenesis is supported by the effectiveness of antibody anti–TNF-α in reduction of CRPS symptoms.[95]

Bisphosphonates

BPs are a class of drugs that prevent the loss of bone mass. They are called BPs because they have 2 phosphonate groups (Figure 1). BPs inhibit the digestion of bone by encouraging OCLs to undergo apoptosis and slowing bone loss[96,97,98]). BPs have an anabolic activity on OBs inducing an increase in bone matrix.[99]

BPs are classified in nitrogenous BPs (NBPs) and nonnitrogenous BPs. The nonnitrogenous BPs (etidronate, clodronate and tiludronate) are metabolized in the cell to compounds that replace the terminal pyrophosphate moiety of ATP, forming a nonfunctional molecule that competes with ATP in the cellular energy metabolism. The OCLs initiate apoptosis and die, leading to an overall decrease in the bone breakdown.[100]

NBPs (pamidronate, neridronate, olpadronate, alendronate, ibandronate, risedronate, zoledronate) act on bone metabolism by binding and blocking the enzyme farnesyl diphosphate synthase in the mevalonic acid metabolic pathway.[101] This pathway plays a key role in lipid modification (prenylation) of small guanosine-5′-triphosphate proteins, such as Ras, Rho, Rac, involved in osteoclastogenesis, cell survival, and cytoskeletal dynamics. In particular, the cytoskeleton is vital to maintain the ''ruffled border'' that is required between a resorbing OCLs and a bone surface[102,103]). The uses of BPs include the prevention and treatment of osteoporosis, osteitis deformans (Paget disease of bone), bone metastasis (with or without hypercalcaemia), multiple myeloma, primary hyperparathyroidism, osteogenesis imperfecta, and other conditions that

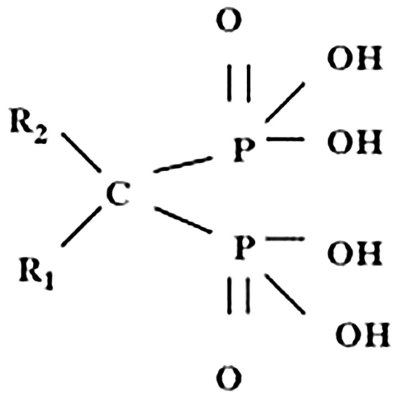

FIGURE 1. Molecular structure of bisphosphonates.

feature bone fragility.[104] Several clinical studies have also investigated the effects of BP therapy on AS reporting, sometimes, conflicting results. For instance, a study assessed the therapeutic potential of intravenous pamidronate (60 mg infusions/6 months) in 35 cases of nonsteroidal anti-inflammatory drug (NSAID) refractory or intolerant cases of AS.[105] This study showed good efficacy with an early feel good response observed in 62% patients within 48 hours of the first infusion and fever, arthralgia and myalgia observed in 6 cases after the first infusion, and in a case after the second infusion. Improvements in many functional indexes and scores were observed after 6 months. A reduction in the tender and swollen joint counts of 54% patients with peripheral arthritis was observed.[105] Another clinical study in 90 AS patients showed how BMDs values of both L-spine and femur showed tendencies to the most increase in the group treated with concurrent BP and anti-TNF-α agent if compared with conventional treatment and BP and anti–TNF-α agent; in this study, the gain of bone mass was associated with the reduction of inflammation.[106] Maksymowych et al[107] designed 2 studies to assess the efficacy of pamidronate in AS patients. The first open study assessed an intensive regime of Pamidronate (60 mg pamidronate at 1, 2, 14, 28 and 56 days) in 9 AS patients showing significant improvements: the mean Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) decreased by 44.2%, mean Bath Ankylosing Spondylitis Functional Index (BASFI) by 47%, mean Bath Ankylosing Spondylitis Global Index (BASGI) by 42%, mean erythrocyte sedimentation rate (ESR) by 49.4%, and CRP by 66.9%; a decrease in mean tender and swollen joint count by 98.2% and 93.8%, respectively was also observed.[107] A second randomized double blind trial compared 60 mg versus 10 mg intravenous pamidronate adminsitered for 6 months in 84 NSAID refractory AS patients. Seventy-two patients completed therapy showing a 34.5% decrease in the mean BASDAI in the 60 mg group and a 15% decrease in the 10 mg group at 6 months; significantly, reductions were also observed in the 60 mg group for the BASFI, BASGI, and Bath Ankylosing Spondylitis Metrology Index. Significantly, more patients achieved a reduction of >25% in the BASDAI in the 60 mg group versus the 10 mg group (63.4% vs. 30.2%).[108] Another open study tested the efficacy of pamidronate (administered monthly for 6 months at a 60 mg dosage and infused in 500 mL glucose over 2 hours) in 35 patients affected by AS or spondylarthropathies. It showed a progressive decline in the BASDAI from baseline reaching significance at 3 months. No significant changes were observed in BASFI, CRP levels, and ESR during the study, we did not find significant changes between baseline and subsequent visits. No clinical amelioration of peripheral arthritis was observed. Pamidronate infusions were well tolerated with side effects in only 10 cases.[109] A further study assessed the efficacy of monthly pamidronate infusions (60 mg over 4 hours) for 6 months (1 infusion per month) in 21 AS patients. In the fifteen patients, who completed all 6 infusions, no improvement was reported.[110] An interesting study also evaluated bone markers in 15 AS patients (mean disease duration of 14.8 years) who received 6 infusions of pamidrinate per month (30 mg starting dose followed by 60 mg). The study revealed a significant fall in degradation products of type-1 collagen C-terminal telopeptides ($P = 0.001$), serum bone GLA protein ($P = 0.02$), bone-specific alkaline phosphatase ($P = 0.02$), and a significant improvement was seen in the BASDAI score; but not in Bath Ankylosing Spondylitis Metrology Index, CRP, or ESR.[111] An interesting case report also showed how pamidronate could bring benefit if started when incomplete response is observed with anti–TNF-α therapy in AS. In this report, the patient underwent 3 monthly infusions of pamidronate along with continuing adalimumab displaying complete disappearance of the back pain after the second pamidronate infusion.[112] BP therapy also found application for treatment of psoriatic arthritis (PA). For instance, the effect of ZA (3-monthly infusions for 1 year) on articular bone in patients with PA (n = 6) was investigated using magnetic resonance imaging (MRI) and compared with placebo (n = 16). ZA reduced the progression of MRI bone edema, indicating probable suppression of osteitis concordant with reduction in clinical measures of disease activity.[113] A recent study has also investigated by means of MRI scan the effect of ZA (3-monthly infusions for 1 year) on articular bone in patients with erosive PA compared with placebo and to patients receiving no infusions. MRI scans from 6 patients who received ZA, 6 patients who underwent placebo, and 10 who received no infusions showed a decrease in bone edema score in the ZA group (15.5–8.5) and an increase in the non-ZA group (indicator of osteitis suppression), whereas no difference between groups in change in MRI erosion score was observed.[113] Only a few nonrandomized and randomized clinical studies are available on the link between BPs and fracture risk in Cystic fibrosis, AN autosomal recessive disease characterized by vertebral fractures with an estimated prevalence of 14% among CF patients.[114,115] A Cochrane review analyzing data from five randomized controlled trials of BP therapy in 145 adults with cystic fibrosis to assess the effect of oral etidronate, oral alendronate, intravenous pamidronate (30 mg IV every 3 months), and zoledronate (2 mg

every 3 months) has also been performed. After 6 months, bone mineral density (BMD) values at the lumbar spine and femur were increased, but the fracture risk was not significantly decreased.[116]

In the past few years, BPs have demonstrated a specific antiphlogistic effect.[117] Apoptosis induction in synovial macrophages has been suggested as one of the most important mechanisms of antiarthritic/anti-inflammatory effects of BPs.[118] BPs, in particular NBPs, induced a significant depletion of synovial lining macrophages[118–120] and, selectively, on macrophage-like type A synoviocytes, which are among the most important sources of cytokines, resulting in a local reduction of pro-inflammatory factors, such as IL-1, IL-6, and TNF-α[121–123]; B lymphocytes, plasma cells, and fibroblast-like type B synoviocytes are not affected. BPs demonstrated to inhibit macrophage migration and adhesion.[3,124,125]

BPs and Inflammation

Many clinical trials verify the antiphlogistic effects of BPs in inflammatory disease.

Initially, BPs were used in RA to reduce the bone-lytic effect of glucocorticoids. Lems et al[126] observed the progressive improvement of BMD, bone toughness index, in lumbar spine, and of bone-turnover markers in patients with RA, treated with low-dose prednisone, if BPs were added to the therapy. The inhibition of osteoclastogenesis by BP treatment, in patients affected by RA, was verified by other specific studies as follows: dual x-ray absorptiometry quantified vertebral strength maintaining[127]; RANKL decreased[128]; serum and urinary bone resorption markers decreased.[129] Interestingly, many proinflammatory parameters, such as ESR and concentrations of serum CRP, IL-1, IL-6, and TNF-α, significantly improved in RA patients, subject to BPs.[43,121,130,131] This evidence suggests an additional and effective anti-inflammatory effect of BPs in RA treatment.

Anti-OCLs activity of BPs was observed in OA treatment. A significant decrease of tartrate-resistant acid phosphatase 5b (TRACP 5b), a marker of OCLs function, was observed in OA patients, treated with BPs.[132] In OA patients, BPs induced symptomatic improvements, such as a decrease in pain and functional improvements concerning limb extension and joint mobility.[133] BPs were able to preserve the structural integrity of subchondral bone in knee OA.[134] Interestingly, a reduction in the urinary level of a marker of cartilage degradation, C-terminal cross-linking telopeptide of type II collagen, was also observed.[135,136] It suggests that inflammatory response, concerning in particular MMP and other lytic enzymes, which are terminal effectors of cartilage and bone matrix degradation, is partially inhibited by BPs. BPs effectively managed AS. BPs increased BMD and reduced bone resorption.[137] BPs improved the most important activity and functional indices used to evaluate the progression of AS.[105] BPs may constitute a reasonable alternative treatment in patients who are refractory to NSAID[108] or anti–TNF-α therapy.[138] Unfortunately, no evidence of anti-inflammatory effect of BPs in AS treatment was found. BPs have demonstrated to be potent inhibitors of bone resorption and inductors of new bone formation in periodontitis as follows: treatment revealed a significant improvement in the clinical parameters, such as gingival index, probing pocket depth, and clinical attachment level.[139] Also BMD[140] and bleeding on probing[141] also benefited from BPs administration. BPs reduced gingival fibroblasts-induced RANKL secretion, the major stimulus of OCLs proliferation in periodontitis.[142] However, BPs role on periodontitis inflammation is not clear. BPs prevented PGE2 synthesis by inhibiting the expression of COX-2,[143] but also IL-6 increase was detected.[142] Probably, IL-6 secretion depends on acute proinflammatory effect induced by BPs. BPs do not reduce inflammation in IBD because it is principally sustained neither by resident fibroblasts nor macrophages, but by T lymphocytes. BPs induce apoptosis on resident macrophages (partially committed to osteoclastic lineage)[118] and modify fibroblasts secretion-pattern,[142] but they do not interact with pro-inflammatory lymphocytes. Although the pathophysiologic mechanism, which leads to IBD, is not completely understood, an important suppressor effect to IBD immune reaction is induced by CD4+CD25+TR cells.[144] Totsuka et al[145] discovered that the suppression induced by CD4+CD25+TR cells was abrogated if RANKL pathway was blocked by antibodies anti-RANKL. So the decrease of RANKL improves bone condition, but it blocks lymphocyte suppression. Probably, it is another cause which limits BPs effectiveness in IBD treatment with antiosteoporotic effect[146–150] but not to anti-inflammatory effect. The same is true for periprosthetic osteolysis. The proinflammatory cytokine profile, produced by macrophages and the nuclear factor κβ pathway expression in osteolysis, does not change in subjects with arthroplasty-associated aseptic failure who were treated with BPs.[151] Probably, the huge amount of wear debris particles, released by the prosthesis, induced a massive inflammatory reaction,[152] although BPs acted downstream on OCLs differentiation and function. BPs improved the BMD and the longevity of different type of arthroplasties (eg, hip, knee, femoral).[153–156] Being an autoimmune disease, PBC presents a relevant contribution to inflammation in its

pathophysiology. At the present, no clinical trial on BPs anti-inflammatory effect is ongoing, but the literature contains many trials on the effective treatment of PBC-dependent osteoporosis. Moreover, a recent study of BPs treatment for osteoporosis showed a slight gain in BMD in the unremodelled bone.[157] NBPs, better than nonnitrogenous ones, are safe and improve significantly BMD, decreasing bone resorption in PBC patients.[158–160] Therefore, because one of the severest symptoms of CRPS is osteoporosis, BPs have been employed in CRPS therapy.[161] Interestingly, their therapeutic effect is not only the inhibition of osteoclastogenesis, but also pain management.[162,163] Although, many studies have showed that BPs reduce pain and their effect probably depends on their anti-inflammatory action,[164,165] no molecular evidence has been reported (Figure 2).

Adverse effects

The administration of BPs is associated with the risk of manifestation of several side effects[166] (Figure 3). BPs have been associated with adverse events in the upper gastrointestinal tract, acute phase response, hypocalcaemia, and secondary hyperparathyroidism, musculoskeletal pain, osteonecrosis of the jaw (ONJ), ocular events, renal toxicity, atrial fibrillation, atypical fractures of the femoral diaphysis, hepatitis, and esophageal cancer.[167,168] However, there is lack of agreement on the relation between oral BPs and the risk of esophageal cancer. For instance, Cardwell et al[169] performed a cohort study showing that oral intake of BPs are not significantly associated with incident esophageal or gastric cancer if compared with nonuser controls as assessed at 4.5 year follow-up. On the other hand, another research group[170] designed a case–control sudy showing that the risk of esophageal cancer increased with 10 or more prescriptions for oral BPs and with prescriptions over about a 5-year period as assessed at 7.6 year follow-up. Comparing the time-dependent results, these 2 studies signal no increased risk of cancer detectable within the first 3 years of exposure to oral BPs.

It has been pointed out that there is no awareness of possible risks associated to BPs therapy for longer than 5 years; and however, in the case of osteoporosis, it has been reported that oral BPs reduce the incidence of devastating osteoporotic fractures in patients with osteoporosis, but after 5 years, the overall fracture risk is the same in patients who keep taking BPs as in patients who discontinue them and therefore it suggests that these drugs are no longer necessary after 5 years.[171]

Generally, intravenous BPs (pamidronate, ibandronate, and ZA) are more potent than oral BPs (alendronate, risedronate, and ibandronate), and the frequency and severity of some of the BPs-associated adverse events are dose and potency dependent.

NBPs can cause nausea, vomiting, epigastric pain and dyspepsia, oesophagitis, oesophageal erosions, and ulcerations in the upper gastrointestinal tract.[172] To avoid gastrointestinal symptoms, the patients are advised to take NBPs orally, with at least 1 glass of water, not to chew or allow the pill to dissolve in their mouth, and to stay in the upright position for 30 minutes

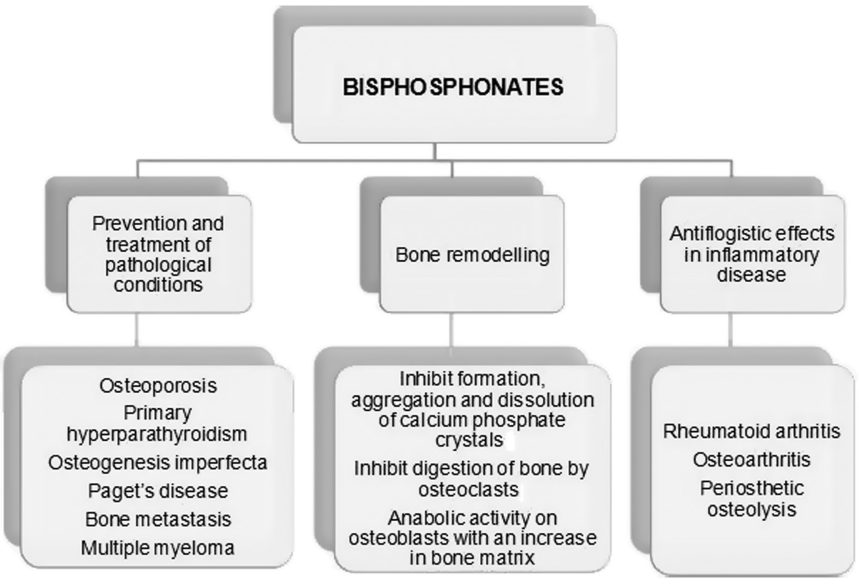

FIGURE 2. Therapeutical effectiveness of bisphosphonates.

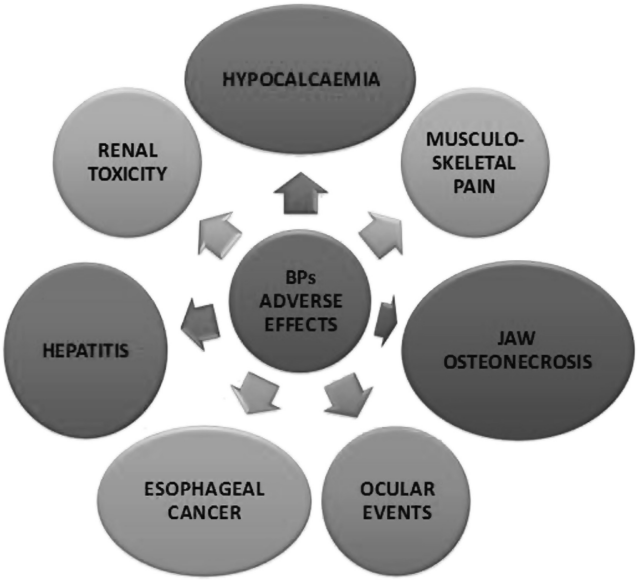

FIGURE 3. Common adverse effects associated with bisphosphonate therapy.

after the drug intake, without eating or drinking.[173] Intravenous NBPs can give fever and flu-like symptoms after the first infusion, which is thought to occur because of their potential to activate human γδ T cells.[174,175] Barrett's esophagus should be a contraindication for BPs because it increases the risk of oesophageal cancer.[176]

Many cases of renal toxicity have been reported with BP treatment, in particular, intravenous BPs.[177,178] Patterns of nephrotoxicity with these drugs include toxic acute tubular necrosis and collapsing focal segmental glomerulosclerosis.[179] High affinity of BPs for metal ions, including calcium and their tendency to form soluble or insoluble complexes and aggregates with metal ions, especially when drugs were infused rapidly in high quantity, was considered to be a possible cause of renal toxicity.[166] The so formed complexes could also be retained within the kidney leading to renal injury.[166] The risk of BP-associated renal toxicity depends on the malignant disease, which affected the patient. Many pathologies, for example, pre-existing chronic kidney disease, multiple myeloma, hypercalcaemia, hypertension, diabetes mellitus, advanced age, have an intrinsic risk of nephrotoxicity.[180,181]

The most common ocular adverse reaction of therapy with BPs is nonspecific conjunctivitis, which usually is self-limited.[182] A few cases of other ocular side effects, such as eyelid edema, optic or retrobulbar neuritis, periorbital edema, cranial nerve palsy, and ptosis, have been reported. Uveitis and scleritis are the most serious ocular side effects of BP therapy and require the discontinuation of BP treatment.[183,184] Many diseases (e.g., AS, Behcet syndrome, psoriasis, Reiter syndrome, IBD, polychondritis, Wegener granulomatosis, RA, systemic lupus erythematosus, sarcoidosis, and syphilis) and drugs (e.g., rifabutin, trimethoprim-sulfamethoxazole, diethylcarbamazine, metipranolol and cidovir) are associated with scleritis and uveitis, so the appearance of ocular adverse effects could be marginally attributed to BPs.[184] In this situation BPs may play the role of precipitating factor for other substances.[184]

Intravenous BPs are known to cause a dose-dependent Acute Phase Response (APR).[185] Clinically, this systemic reaction is characterized by fever, sometimes with rigours, and influenza-like symptoms such as fatigue, malaise, myalgia, arthralgia and bone pain.[185,186] The APR is maximally expressed 28–36 hours from the fourth BP induced administration and subsides 2–3 days later, despite continuous treatment.[185] The BP-induced APR is mediated by interleukin 6,[187] TNF-α, and other proinflammatory cytokines released by receptor-activated γδ T cells and macrophages.[186,188] The BP-induced APR is usually benign and self-limiting and can be treated with antipyretics.

BP-dependent inhibitors of bone resorption induces, 6 weeks after the administration, serum calcium and phosphorus decrease; and PTH significantly increases in a dose-dependent fashion. Calciuria and phosphaturia also decrease.[189] The increased PTH acts antagonizing the effect of BPs within the bone and conserves calcium by increasing tubular reabsorption of calcium in the kidneys and stimulating the production of 1,25-dihydroxyvitamin D by the kidneys. Therefore, under normal conditions, hypocalcemia induced by BPs often resolves although the BP therapy is continued; evidence suggests that symptomatic hypocalcaemia is common after intravenous BP therapy.[190] Many risk factors for severe BP-induced hypocalcaemia have been identified as follows: (1) pre-existing hypoparathyroidism; (2) parathyroid dysfunction during thyroidectomy in a patient receiving chronic BP therapy; (3) vitamin D deficiency; (4) renal failure.[190] Treatment with adequate vitamin D and calcium about 2 weeks before BP therapy may help avoiding or attenuating the BP-induced hypocalcaemia and secondary hyperparathyroidism.[166]

Severe and sometimes incapacitating bone, joint, and/or muscle (musculoskeletal) pain can affect patients who are taking BPs. The severe musculoskeletal pain may occur within days, months, or years after the beginning of BP therapy. Some patients have reported a complete relief of symptoms after discontinuing BPs, whereas others have reported slow or incomplete resolution.[191,192] A mechanism BP-related bone pain has been hypothesized. In cases of BP-induced secondary

hyperparathyroidism, BPs cause a relatively smaller reduction in bone turnover due to the antagonistic effect of the high PTH. In these cases, the bone turnover that is higher than we had expected after treatment with BP therapy, may result in a relatively higher bone uptake of BPs and higher than the mean concentration of the drug in the bone microenvironment. This fact may result in an inflammatory response within the bones which is mediated by a localized relatively increased release of proinflammatory cytokines including IL-6 which are induced by BPs. On the other hand, increased levels of IL-6 and consequent higher BP concentration in bone are caused by high PTH in secondary hyperparathyroidism, and high PTH may exert a synergistic effect in increased release of IL-6.[193,194]

BPs have been associated with ONJ.[195,196] Many cases of BP-associated ONJ occurred after high-dose intravenous administration used for treatment of many types of cancer. ONJ is a severe bone disease that affects the jaws, including the maxilla and the mandible. Damage and death to areas of jawbone occur as a result of multifactorial pathological events[197] that are not completely clarified yet. Many cases are preceded by a dental surgical procedure that involves the bone, so it has been suggested that intravenous BP treatment should be postponed to any dental procedure.[198]

Controversial results on BP use and increased risk of atrial fibrillation in women were reported.[13,199–201] It has been hypothesized that the inflammatory response to BPs or fluctuations in calcium blood levels may be responsible for atrial fibrillation.[202] The current opinion is that BPs do not induce atrial fibrillation but may aggravate atrial fibrillation in patients predisposed to it from other causes (such as patients with heart failure, coronary artery disease, or diabetes).[202]

Previous studies in women affected by osteoporosis have shown that BP intake was related to unusual fractures in the femur (thigh bone) and in the shaft (diaphysis or subtrochanteric region) of the bone, rather than at the head of the bone, which represents the most common site of fracture. However, the results showed that these unusual fractures are extremely rare if compared with the common hip fractures and the overall reduction in hip fractures caused by BPs, by far outweighed the unusual shaft fractures.[203] Probably, unusual fractures resulted in oversuppression of bone turnover by BPs. It is hypothesized that microcracks in the bone are unable to heal and eventually unite and propagate, resulting in atypical fractures. Rarely, such fractures tend to heal poorly and often require some form of bone stimulation, for example, bone grafting as a secondary procedure.[203,204]

A few cases of hepatitis, developing several months[205,206] or years[207] after starting BP therapy and resolving several months after discontinuing BPs, have been reported. Liver biopsy revealed lesions suggestive of a drug effect,[208] even if some doubts remain due to a rapid clearance of BPs from the circulation and soft tissues and to the lack of metabolic degradation.

CONCLUSIONS

BPs are universally recognized as effective antiosteoporotic drugs. Their selective activity on bone has made BPs an important instrument for primary and secondary osteogenesis impairment. Evaluating inflammation-based or inflammation-associated pathologies with excess of bone resorption, it seems that BPs improve not only skeletal defects but also general symptoms. However, a careful analysis reveals that only the pathologies with skeletal localization demonstrate molecular and macroscopic anti-inflammatory effects.

Indeed, RA, OA, and periosthetic osteolysis reveal both inhibition of bone resorption and reduction of inflammation when treated with BPs. We think that BPs are able to modify the release of proinflammatory cytokines in these pathologies because they act on local effector cells, such as resident macrophages, OCLs, progenitors, and fibroblasts.[116–121] In these pathologies, the contribution of lymphocytes, generally untargeted by BPs, is minor with respect to systemic inflammatory or autoimmune disease, such as IBD and PBC.

IBD and PBC are lymphocyte T helper–mediated disease, therefore, the inflammation component of these pathologies is not susceptible to BPs. IBD and PBC patients benefited from BP therapy only in secondary osteoporosis treatment.

Periodontitis, AS and CRPS have particular behaviors when are treated with BPs. In periodontitis, the infection-derived inflammation leads to bone resorption. The bone damage is responsive to BP treatment, whereas the acute inflammation is not. Probably it is due to lymphocyte T immune response. In in vivo models of periodontitis and in bone implants, BPs have also shown interesting activities leading us to believe that these applications will be a fixed point in BP development in the next future.[209,210] In a model of induced periodontitis in monkeys, alendronate, at low concentration, was able to inhibit the bone loss and improve the probing pocket depth measurement. The same product was able to prevent alveolar bone loss following mucoperiosteal flap surgery in rat mandible.[211]

AS is an autoimmune disease lymphocyte T dependent with bone effects. Although BPs are not able to switch off the activity of lymphocytes T, their specific bone activity allows to manage AS and the frequently

associated bone loss. Clinical studies, in patients affected by AS,[212] have shown that intravenous neridronate treatment has induced a relevant improvement on pain (measured using visual analog scale), some clinical parameters and an increase in bone density.

In CRPS patients, BP treatment improves osteoporosis but also inflammatory symptoms, such as pain and swelling. Even if the real mechanism of action is not known, the analgesic activity and the inhibition of the phlogistic phenomenons, induced by these compounds, and the inhibition of the local synthesis of some mediators (IL-6,TNF-α,PGE2), may have a role. Moreover, the effects on the microcirculation and the inhibition of the lactic acid production lead to an inhibition of the nociceptors and mechanoreceptors with a consequent analgesic effect. It has been postulated that BPs can bind to a bone surface inhibiting the growth of adjacent nonbone cells widening the range of potential target cells for these drug reducing rates of cancer recurrence and the pathogenesis of ONJ.[208] A recent study has reported on the use of BPs in many types of cancer. Initially, BPs were used in cancer associated with bone metastasis.[213] However, researchers found that BPs were able to inhibit tumoural cell proliferation.[214,215] It is hypothesized that the blockage of the cycle of mevalonate induces an increase in isopentenyl diphosphate (IPP). IPP could behave as a phosphorantigen and trigger the release of cytokines and interferon by γδ T-lymphocytes. In particular, the interferons are powerful antitumoral, antinfective and anti-inflammatory agents. The interests in BPs has increased thanks to the unexpected therapeutic effects that have been discovered during their traditional application.

It is clear that BP action is not only directed at the bone cells, but also at several inflammatory cytokines.[216] Even if the mechanism, by which BPs control the immune-inflammatory responses, are not completely[216] elucidated, preliminary clinical data show some encouraging results on the inhibition of the phlogosis and the bone and articular damage, making possible a more extended use of the BPs in other clinical fields. In conclusion, nowadays many BPs exist and they are all characterized by peculiar binding properties and exert their functions at the cellular level. It is important to optimize the dosage and length of therapy for each of these agents in each clinical field in order to be able to maximize their properties concerning modulation of inflammation and bone remodelling. Further clinical studies involving larger cohorts need to be designed in order to further determine their efficacy analyzing side effects that may also occur in the long term and making sure that they do not outweigh the benefits. We should not exclude the possibility that in the near future, while ''old'' BPs will reach the end of their patent life, ''new'' BPs will be designed to specifically target a pathological condition.

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