Basic Multicellular Units and Biological Activity of Bisphoshonates

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Introduction

Bone is a dynamic tissue which provides mechanical support for stature and locomotion, protects vital organs, and acts as a metabolic organ which controls mineral homeostasis. In order to carry out these important functions, bone is subjected to modeling and remodeling process. Bone modeling is responsible for both the growth and the mechanical adaption of the bone. Conversely, bone remodeling involves the removal of old or damaged bone tissue, a process that is mediated by osteoclast (bone resorption) and subsequent replacement of new bone formed, which is mediated by osteoblasts (bone formation).

Physiological bone remodeling requires a tight coupling between bone resorption and bone formation to maintain the equilibrium and to guarantee that there is no alteration in bone mass or quality after each remodeling cycle. The remodeling process takes place within functionally and anatomically distinct sites termed basic multicellular units. These basic multicellular units require a strictly coordinated and synchronized action of the main types of bone cells: bone-lining cells, osteocytes, osteoblasts and osteoclasts. Moreover, a balance between bone resorption and bone remodeling is controlled by different molecular mechanisms, such as multiple signaling pathways. Nonetheless, an increase in osteoclast activity may occur under certain bone pathological conditions, so therapies that inhibit osteoclast, such as bisphosphonates, are effective at preventing bone loss. Therefore, these drugs are exploited in the development of new therapies for osteoporosis and other metabolic bone diseases.

The aims of this review are:
- ❖ To discuss in depth the cellular and molecular mechanisms of bone remodeling.
- ❖ To confirm and understanding of the cellular and biochemical effects that bisphosphonates could have into bone cells.

Methodology

- ❖ Scientific literature comes from reviews and original research papers, searched on PubMed and Medline databases, and also taken from histology's book.
- ❖ Papers selection: key words were introduced in order to search the most relevant papers. Papers were selected according to their date of publication (focused on the past 10 years) and impact factor of the journal.
- ❖ Key words: Bone Remodeling, Osteoblast, Osteocyte, Osteoclast, Wnt signaling, Small GTPases, Bisphosphonates

Basic Multicellular Units

Osteoblast

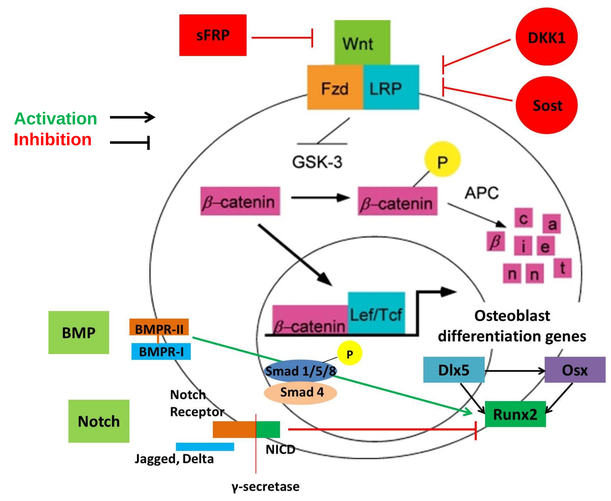


Image adapted from: Issack PS, Helfet DL, Lane JM. HSSJ 2008; 4: 66-70

Figure 1. Intracellular signaling pathways implicated in osteoblastogenesis
Osteoblasts following timely programmed steps requiring the expression of specific transcription factors, such as Runx2, Dlx5 i Osx. In turn, the expression and activity of these factors is under the control of multiple pro-osteogenic signaling pathways, such as bone morphogenetic proteins (BMPs), Wnt/β-catenin and Notch pathways.
These pathways are crucial especially in the early steps of osteoblastogenesis. The interplay between BMP and Wnt/β-catenin signaling affects bone formation. The signaling cascade activated by interaction between BMP and their receptors activates the transcription of genes which encode DKK and Sost, two inhibitors of Wnt/β-catenin signaling. Furthermore, activation of Notch signaling pathway could inhibit the transcription of Runx2 directly or indirectly. Therefore, this signaling pathway could suppress osteoblastogenic induced by BMP and Wnt.

Osteocyte

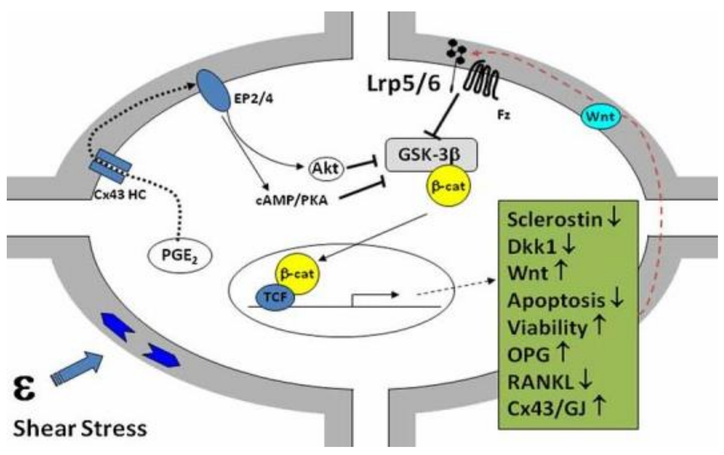


Image adapted from: Bonewald LF. J Bone Miner Res 2011; 26(2): 229-38

Figure 2. Role of connexin 43 hemichannels (Cx43) and Wnt/β-catenin signaling pathway in osteocyte function and viability
Expression of Cx43 in osteocytes is regulated by β-catenin, which has been shown to bind to Cx43 promoter, stimulating Cx43 expression and functional gap junctions between osteocytes. Therefore, the expression of Cx3 and Wnt/β-catenin signaling pathway play a role not only in osteocyte viability in response to shear stress or loading, but also in osteocyte apoptosis, so that their absence leads to cell apoptosis. In this sense, osteocytes apoptosis positively regulate osteoclast function and differentiation, and the same parameters are negatively regulated in osteoblast by osteocytes.

Osteoclast

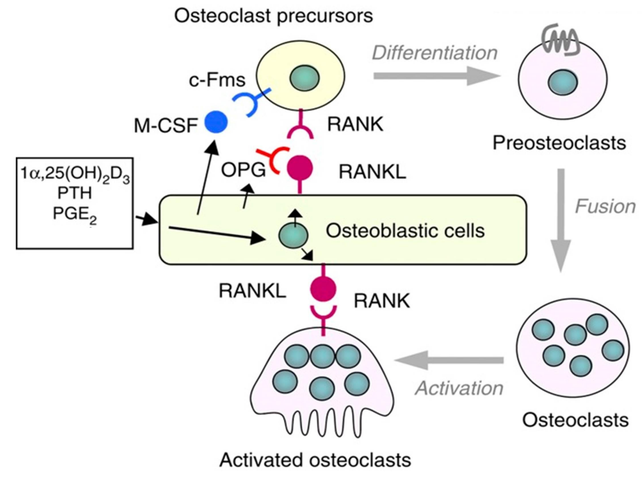


Image adapted from: Takahashi N, Udagawa N, Suda T. Bonkey Rep. 2014; 495:1-9

Figure 3. Regulation of osteoclasts differentiation and function by osteoblasts, through the RANKL/RANK/OPG pathway
Osteoclasts are members of the monocyte/macrophage lineage and are formed by multiple cellular fusions from their mononuclear precursors. Their differentiation is regulated by a number of other cells, as osteoblasts and their secreted products, especially by monocyte/macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-kB ligand (RANKL). Osteoblasts also produce another factor called osteoprotegerin (OPG), a soluble protein that inhibits osteoclastogenesis and the subsequent bone resorption by binding to RANKL and avoiding its interaction with receptor activator of NF-kB (RANK).

MESENCHYMAL STEM CELL LINEAGE — **MONOCYTE/MACROPHAGE LINEAGE**

Biological Activity of Bisphosphonates

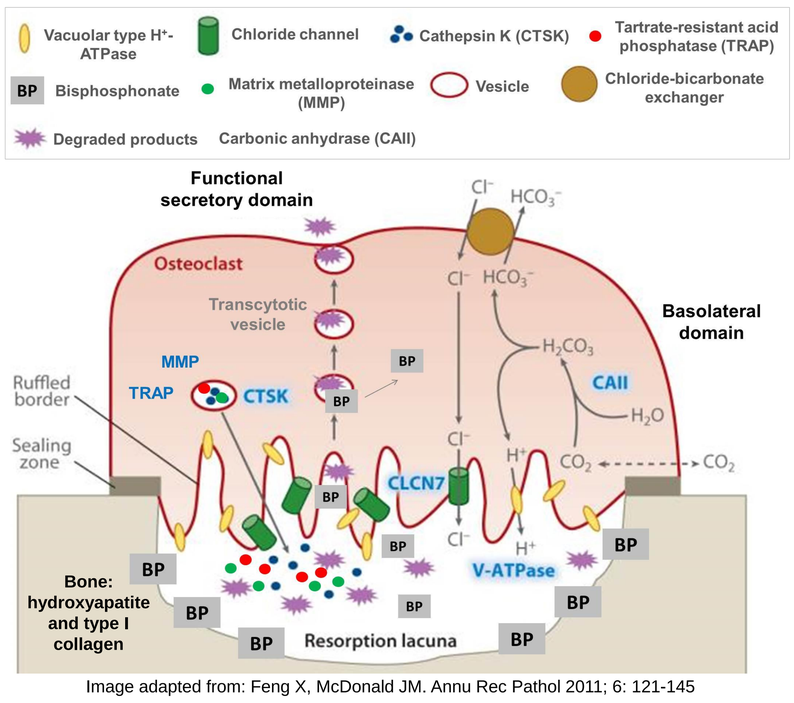
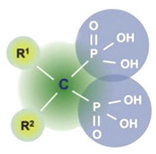


Image adapted from: Feng X, McDonald JM. Annu Rec Pathol 2011; 6: 121-145

Figure 4. Ultrastructure and function of osteoclasts
The resorbing osteoclasts are polarized and reorganize their membrane into specific and distinct membrane domains: the functional secretory domain, the basolateral domain, the sealing zone, and ruffled border. Polarization and bone resorption need a continuous membrane trafficking and modulation of the cytoskeleton.

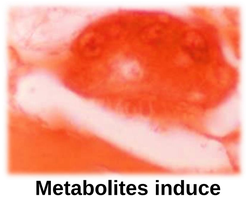
Bisphosphonates (BPs)
Chemically stable analogues of inorganic pyrophosphate



N-BPs
(alendronate, risedronate or zoledronate)
Nitrogen-containing in chemical structure

BPs simples
(clodronate or etidronate)
Non-nitrogen containing in chemical structure

Mevalonate
↓
Dimethylallyl diphosphate ⟷ **Isopentenyl diphosphate**
↓
N-BPs inhibit FPP synthase
↓
Farnesyl diphosphate (FPP)
↓
Geranylgeranyl diphosphate (GGPP)
↓
Rab geranylgeranyl transferase (Prenylation)
↓
Prenylated Rab GTPases proteins required for osteoclast function and survival

Metabolized in the osteoclast cytosol
↓
Non-hydrolyzable ATP analogues
↓

Metabolites induce osteoclast apoptosis

Mechanism of action on osteoclasts

1. Preferential binding into mineral surface of the bone
2. Concentration in areas of active remodeling
3. Internalization (endocytosis) into cytoplasm of osteoclasts by bone-resorbing osteoclast by endocytosis
4. Release the into cytosol of osteoclasts
5. Biochemical effect: inhibition of prenylated Rab GTPases proteins
6. Cellular effect: osteoclast's inactivation or apoptosis

Mechanism of action on osteocytes/osteoblasts

1. Access to osteocytes by extracellular fluid of canalicular compartment
2. Opening of the connexin 43 hemichannels
3. Activation of extracellular signal-regulated kinases (ERKs) that fosforilate their targets.
4. ERKs activate anti-apoptotic pathways
5. Inhibit apoptosis in osteocytes and osteoblasts

Conclusions

- ❖ In order to maintain the balance between bone resorption and forming, the main types of bone cells act in a synchronized way, which requires different molecular control mechanisms and multiple intracellular signaling pathways, among which RANKL/RANK/OPG and Wnt/β-catenin are the most important ones.
- ❖ Any alteration of the strict equilibrium in bone remodeling involves the development of bone pathologies, which highlight the need of using pharmacological therapies to treat these pathologies.
- ❖ Alterations in the function and regulation of osteoclasts, especially, have been shown in multiple bone pathologies, such as osteoporosis. Currently, the most widely used and effective drugs against bone resorption are bisphosphonate.
- ❖ Nowadays, although a possible mechanism of action for bisphosphonates on osteoclasts is known, it remains unclear if it could also act on other alternative pathways to modulate bone remodeling profits or losses.
- ❖ As the exact mechanism has not yet been elucidated, there is a need for studying the potential interactions between bisphosphonates and osteocytes or osteoblasts. Further studies may help to explain the efficacy of these drugs on such bone cells.

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