Publications

Clinics of Oncology

Review Article

Role of Bisphosphonate (Bps) in Breast Cancer: An Update

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Volume 1 Issue 2 - 2018

Received Date: 10 June 2018

Accepted Date: 02 July 2018

Published Date: 10 July 2018

2. Keywords

Bisphosphonate; breast cancer; anticancer effect; cellular pathway

1. Abstract

Bisphosphonate therapy is beneficial in breast cancer patients by reducing the development of bone metastases and also improves survival in breast cancer. However, mechanism behind this beneficial effect is still unclear. This review explored few recent studies to identify the mechanism behind Bisphosphonate anticancer effect and possible new targets for treatment of breast cancer. Anticancer effect of bisphosphonates in breast cancer may probably due to modulation of various cellular pathways i.e. elimination of DTC cells in bone marrow; modulation of regulatory T cells; altering hematopoiesis; suppress osteoclastogenesis; improve tumor microenvironment; regulating MSC-MCP-1-macrophages axis and sensitizing cancer stem cells to apoptosis. Moreover, effect of bisphosphonates therapy can also be modified by TLR-9 expression in cancer cells. Overall, the studies related to firmly establish these mechanisms of beneficial action of bisphosphonates in cancer are few. Further research is warranted in order to explore potential benefits of bisphosphonates in breast cancer therapy to clearly elucidate mechanisms in breast cancer.

3. Introduction

Breast Cancer (BC) is the most common cancer type in women worldwide and is the main cause of cancer mortality in women in the world. The majority of patients with advanced breast cancer eventually develop bone metastases [1]. Bisphosphonates (BPs) are synthetic analogues of the naturally occurring pyrophosphate. These drugs inhibit osteoclast-mediated bone resorption and they have an established role in the treatment of bone conditions that involve increased osteoclast activity, such as postmenopausal or treatment-induced osteoporosis, multiple myeloma and skeletal metastases of solid tumors [2]. Depending on their molecular structure, these drugs are divided into non-nitrogen containing BPs (Non n-BPs such as clodronate and etidronate) and nitrogen-containing BPs (n-BPs, such as alendronate, pamidronate, risedronate and zoledronate) [3]. The cellular mechanisms of action of BPs vary according to their molecular structure. Non n-BPs is metabolized into toxic, apoptosis-inducing ATP-analogs inside the cells. The primary mode of action of n-BPs is to inhibit the farnesyl pyrophosphate synthase of the mevalonate pathway, which is in the beginning of the cholesterol biosynthesis [3,4]. This results in a decreased cellular pool of prenyl groups and leads to impaired post-transcriptional prenylation of small GTPases that are required for a great variety of cellular functions, such as vesicular transportation during the bone resorption phase of osteoclast [3]. Several clinical trials have been conducted by now, to address whether adjuvant BPs prevents relapses in bone or elsewhere and affect breast cancer mortality [5-8]. In the AZURE trial, zoledronic Acid (ZA) did reduce the development of bone metastases and, for menopausal women ZA improved disease outcomes [5]. Zoledronic acid used as adjuvant therapy along with standard therapies appears to improve the 5-year survival rate for early stage breast cancer patients, and was associated with a protective effect for the bone metastases and fractures [6]. Another study found that adjuvant bisphosphonates reduce the rate of breast cancer recurrence in the bone and improve breast cancer survival [7]. Austrian Breast and Colorectal Cancer Study Group trial 12 (ABCSG-12) recently reported that the addition of ZA to endocrine therapy increased the duration of disease free survival in patients with estrogen receptor-positive breast cancer [8]. Patients with breast

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Citation: Rajesh Kumar, Pramod Kumar S, Arup Kumar M and Ajay G, Role of Bisphosphonate (Bps) in Breast Cancer: An Update. Clinics of Oncology. 2018; 1(2): 1-5

cancer metastatic to bone, treatment with IV preparations of pamidronate [9-10], zoledronic acid [11], has been shown to substantially relieve skeletal pain and reduce skeletal complications. These studies proved benefits of bisphosphonates therapy in breast cancer, but mechanism behind effect is still unclear. The aim of the present review is to explore some recent studies on bisphosphonates to identify the mechanism behind bisphosphonates anticancer effect or identify possible new targets for breast cancer.

3.1. Disseminated tumor cells in bone marrow

The presence of Disseminated Tumor Cells (DTC) in Bone Marrow (BM) is a common phenomenon observed in breast cancer patients. The presence of DTC associated with increased risks of distant metastasis, loco regional recurrence, and death in breast cancer patients [12]. In addition, it has been shown that tumor cells are able to survive chemotherapy [13] and that their persistence is strongly associated with poor outcome [14]. AZURE trial data show that Zoledronic Acid (ZA) significantly improved survival in patients who were more than 5 years postmenopausal with early breast cancer [15,16]. It has been hypothesized that anticancer effects of bisphosphonates may occur through elimination of DTC from the bone marrow. Aft et al. demonstrated that ZA (4 mg every 3 weeks intravenously) administered with chemotherapy resulted in a decreased proportion of patients with DTCs detected in the bone marrow at the time of surgery [17]. Zoledronic acid increased the proportion of DTC-free patients who remained DTC-free at 6 months versus no ZA [18], and significantly decreased DTC levels versus baseline at 12 and 24 months in DTC-positive BC patients [19]. Recent research found that survival rate is higher in ZA group (ZA plus adjuvant systemic therapy) compared to control (adjuvant systemic therapy alone) group (11% vs. 2%, $p = 0.106$). Development of metastatic or recurrent disease during follow-up is lower in ZA group compared to control group (8% vs 15%, $p = 0.205$). At 24 months, 16% of patients from the control group were still DTC positive, whereas all patients treated with ZA became DTC negative ($p = 0.032$) [20].

3.2. Immune modulation of CD4+CD25+ regulatory T cells

Regulatory T (Treg) cells comprise a subset of CD4+CD25+ T lymphocytes, and function to suppress the immune response [21]. Infiltration of Treg cells into the tumor microenvironment was shown to promote tumor cell escape from immune surveillance, and contribute to tumor growth and progression, suggesting that Treg cells play an important role in the prognosis of cancer patients [22,23]. A transgenic mouse model, it was shown that tumor-infiltrating Treg cells are the major source of Receptor Activator of NF-kB Ligand (RANKL), which facilitates metastasis of RANK-expressing breast cancer cells [24]. Zoledronic acid is able to suppress osteoclastogenesis via the inhibition of RANKL expression on osteoblasts [25]. In a mouse model of bisphosphonates-related osteonecrosis of the jaw, administration of ZA suppressed Treg-cell activity and activated inflammatory Th17 cells [26]. Zoledronic acid significantly inhibits the expansion, migration, immunosuppressive function and pro-metastatic ability of Treg cells [27]. Immuno modulation of Treg cells by ZA represents a new strategy for cancer therapy.

3.3. Hematopoiesis regulation

Development of breast cancer and its progression involves the dissemination of cancer cells to the bone marrow and increases the recruitment of tumor-supportive hematopoietic progenitor cells vice versa [28], thus opening the possibility of targeting these cell populations as part of anti-cancer therapy. Endo steal and vascular niche components are critical for maintaining hematopoietic homeostasis, modulation of either of these niches impacts hematopoietic cells, particularly hematopoietic stem cell [29]. Zoledronic acid modulates the activity of osteoclast and osteoblasts, which form hematopoietic stem cell niches. Therefore ZA may have a role in breast cancer progression by affecting hematopoietic cells. In a recent study using multiple mouse strains, observed transient changes in numbers of hematopoietic stem cells, myeloid-biased progenitor cells, and lymphoid-biased cells concurrent with changes to hematopoietic stem cell niches following ZA administration. A single, clinically relevant dose of ZA (100 μg/kg intra peritoneal injection) treatment in vivo mice bone marrow cells inhibited breast tumor outgrowth. With the decrease in the numbers of hematopoietic progenitor cells, ZA has a positive correlation with tumor suppression [30]. These findings provide novel evidence that alterations to the bone marrow play a role in the anti-tumor activity of ZA and suggest possible beneficial effects of ZA in reducing breast cancer development and progression.

3.4. Attenuates osteoclastogenesis by targeting JNK/Erk

In malignant breast cancer patients, cancer-associated bone tissues endure a series of cancer-induced pathological processes, which are triggered by the excessive activation of bone resorption by osteoclast cells, [31] and therefore contributing to the establishment of distant osteolytic lesions and influencing the normally well-balanced skeletal physiological functionality and structural integrity. Bisphosphonates such as zoledronic acid demonstrated efficacy in reducing osteoclast-induced bone loss in metastatic cancer patients [32]. The combination treatment with zoledronic

Acid (ZA) and Plumbagin (PL), a widely investigated component derived from Plumbagozeylanica, significantly and synergistically suppress osteoclastogenesis and inhibit tumor genesis both in vitro and in vivo by simulating the spatial structure of Adenosine Phosphate (ANP) to inhibit competitively phosphorylation of c-Jun N-terminal kinase/extracellular signal-regulated kinase (JNK/Erk) [33].

3.5. Microenvironment-mediated anti-tumor effect

Tumor microenvironment which includes immune cells, fibroblasts, adipose cells and endothelial cells are involved in tumor growth and metastasis [34]. Preclinical studies have shown that the microenvironment is an important regulator of cancer cell response to anticancer drug [35]. This suggests that targeting not only the cancer cells, but also the tumor microenvironment may improve treatment options for breast cancer patients. Bisphosphonates targeted tumor-associated macrophages but not tumor cells, to exert their extra skeletal effects. Therefore, it is rationale to use bisphosphonate in patients with early breast cancer [36]. Also bisphosphonates did not influence in vitro human breast cancer cell survival, but did affect human stromal cell survival, accompanied by decreased stromal TGF-β excretion and reduced TGF-β signaling in cancer cells [37]. These results suggest that bisphosphonate treatment not only modulates the bone environment, but also affects non-bone disease. Regulating the Mesenchymal Stem Cells (MSC)- Monocyte Chemo tactic Protein 1 (MCP-1)-macrophages axis MSC are non-cancerous stromal cells, which have the potential ability for self-renewal, long-term viability, and capacity for differentiation toward a variety of cell types [38]. Due to chronic inflammation in the tumor microenvironment, MSC are known to migrate to tumors, and differentiate into carcinoma-associated fibroblasts [39]. MSC may sustain cancer cell growth and survival within the microenvironment, where they can contribute to the formation of "niches" for tumor growth [40]. The macrophages in tumor microenvironment were called Tumor-Associated Macrophages (TAMs). TAMs could be induced emigration from bone marrow to the periphery by Monocyte Chemo tactic Protein 1 (MCP-1). TAMs are known to possess the tumor promoting effects, which can be recruited by MCP-1. Previous studies showed that tumor resident MSC promoted tumor growth by recruiting monocytes/macrophages through MCP-1 [41]. Thus, the MSC-MCP-1-macrophages axis may be physiologically important in tumor progression. Recent research found that ZA-treated mice showed a significant delay in tumor growth. Zoledronic acid weakened the ability of MSC to promote tumor growth by impairing TAMs recruitment and tumor vascularization. Zoledronic acid decreased MCP-1 expression of MSC, and reduced the recruitment of TAMs to the tumor sites and hence inhibited the tumor growth [42]. These results suggest that by regulating MSC-MCP-1-macrophages axis bisphosphonate can inhibit tumor progression.

3.6. Toll-like receptor 9 expression

Toll-Like Receptor 9 (TLR9) is an intracellular DNA-receptor that recognizes both microbial and host-derived DNA.43TLR9 activation initiates a rapid and a robust innate immune response, with increased secretion of inflammatory mediators [43]. TLR9 is widely expressed in breast cancers [44]. BPs induces a similar rapid inflammatory response as TLR-ligands in cells [45]. Furthermore, BPs have been shown to potentiate the pro-inflammatory effects of TLR ligands in bone marrow or peripheral blood-derived mononuclear cells [46]. Based on the similarities in the responses of TLR ligands and BPs, it can be hypothesized that TLR9 expression may affect cellular responses to BPs. Bisphosphonates have significant growth-inhibitory effects on breast cancer cells with decreased TLR9 expression in vitro and in vivo study [47]. These studies suggest that TLR9 expression can be a potential biomarker for adjuvant bisphosphonates sensitivity among breast cancer patients.

3.7. Decrease in chemo resistance by sensitizing cancer stem cells to apoptosis

Cancer Stem Cells (CSCs) are a subset of tumor cells that can self-renew and differentiate into different cancer subtypes. CSCs are considered responsible for tumor recurrence, distant metastasis, angiogenesis, and drug or radiation resistance, and also resistant to apoptosis. A recent research demonstrated that treatment with ZA resulted in a concomitant increase in apoptosis and cell cycle arrest at S-phase in CSCs.48ZA is an effective therapeutic agent as it decreases the resistance in breast MCF-7 cell lines by inducing apoptosis [48].

4. Conclusion

Previous studies suggest that bisphosphonates prevent metastasis, recurrence in breast cancer patients. It also improves disease outcome and overall survival in breast cancer patients. In this review we discussed different mechanisms underlying behind these beneficial effects. Bisphosphonate demonstrated anticancer effect by modulating various cellular pathways: by elimination of DTC cells in bone marrow; modulation of regulatory T cells; altering hematopoiesis; suppress osteoclastogenesis; improve tumor microenvironment; regulating MSC-MCP-1-macrophages axis and sensitize cancer stem cells to apoptosis. Bisphosphonate effect can be modified by TLR-9 expression in cancer cells. However, studies related to these mechanisms of bisphosphonates in cancer are few. Further studies are required using agents that target these cellular pathways to identify clear mechanism behind beneficial role of bisphosphonates in breast cancer.

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