Bisphosphonates and Skeletal Metastasis: Resorption Agonists Reduce Zoledronic Acid’s Effectiveness in Bone-Tumour Microenvironment by Modulating PTP Expression in Osteoclasts

Subramanya N. Pandruvada1, Jeffrey L. Ebersole2 and Sarandeep S. Huja1

1Division of Orthodontics and 2Center for Oral Health Research, College of Dentistry, University of Kentucky, Lexington, KY, USA

Abstract

Nitrilotriacetic bisphosphonates, e.g. zoledronic acid (ZOL), are currently used in oncological practice to reduce skeletal complications associated with bone metastases of several neoplasms. Bisphosphonates (BP) inhibit bone resorption by inducing apoptosis in osteoclasts (OC). Several studies and meta-analyses report no overall beneficial effect of prescribing ZOL for specific breast and prostate cancer treatments owing to treatment failures and development of resistance to ZOL. The bone-tumour microenvironment is a complex niche due to the presence of numerous bone resorption agonists (RA), secreted by tumour cells. The affects of these RAs on osteoclastic differention/function in the presence of BP are unknown. Our overall objective is to investigate the mechanisms of resistance developed by osteoclasts to overcome the therapeutic effects of ZOL in tumour microenvironments simulated using an in vitro model. OC were prepared using human peripheral blood derived mononuclear cells and the effect of ZOL and conditioned media from orthovactic carcinoma cells line OSCC 12 (SCC CM) on OC differentiation and function were examined. Quantification of TRAP positive (positive) revealed a 32% reduction in OC number by ZOL (10nM) when treated alone for 10 days. However, ZOL efficacy is reduced by 90% when cultured along with SCC (20nM), qRT-PCR analyses demonstrate lysis of additional treatment with ZOL + SCC CM revealed that non-receptor tyrosine protein phosphatase (PTP), PTP1B/PTPs expression levels were reduced by 6-fold in ZOL treatment. In contrast, there was a 3-fold increase of PTP13 levels with ZOL−/SCC CM treatment when compared to ZOL alone. In vitro inhibition of PTP using Orthovistic acid (OVA) for 24h made PTP13 susceptible to ZOL-induced effects. Based on these results, we propose that sustained PTP inhibition in COC is responsible for reduced efficacy of ZOL, PTP activation is known to up-regulate anti-apoptotic, pro-survival pathways. Thus simultaneous inhibition of PTPs in osteoclasts would help overcome the therapeutic effects of ZOL in tumour microenvironments. Small molecule PTP inhibitors should be evaluated to be as effective as ZOL in tumour therapies and would be to prepare the outcomes of BP treatment in tumour microenvironments.

Background

Bisphosphonates (BP), as potent inhibitors of osteoclast mediated bone resorption, have demonstrated proven clinical utility for the treatment of both postmenopausal osteoporosis and bone metastases. The third-generation bisphosphonate zoledronic acid (ZOL), is the most potent of the available containing BP. Multiple clinical trials have demonstrated that zoledronic acid effectively prevents cancer induced bone loss and increases bone mineral density above baseline levels. Interestingly, the effect of zoledronic acid seems to have a wide spectrum. Preclinical data have revealed a direct anti-tumour role for zoledronic acid which may act through the inhibition of tumour cell adhesion, invasion and proliferation as well as acting to induce apoptosis in multiple human tumour cell lines. However, no such effect has been observed in thousands of breast cancer patients. Findings from the AZURE (Adjuvant Treatment with Zoledronic Acid in Stage II/III Breast Cancer) trial reported that adjuvant use of zoledronic acid failed to improve disease free survival. Reasons for this failure are relatively unknown. Bone-tumor microenvironment is very complex with numerous resorption agonists (RA) like IL-6, TNF alpha, PTHrP.

Bisphosphonates (BP), as potent inhibitors of osteoclast mediated bone resorption, have demonstrated proven clinical utility for the treatment of both postmenopausal osteoporosis and bone metastasis. The third-generation bisphosphonate zoledronic acid (ZOL), is the most potent of the available containing BP. Multiple clinical trials have demonstrated that zoledronic acid effectively prevents cancer induced bone loss and increases bone mineral density above baseline levels. Interestingly, the effect of zoledronic acid seems to have a wide spectrum. Preclinical data have revealed a direct anti-tumour role for zoledronic acid which may act through the inhibition of tumour cell adhesion, invasion and proliferation as well as acting to induce apoptosis in multiple human tumour cell lines. However, no such effect has been observed in thousands of breast cancer patients. Findings from the AZURE (Adjuvant Treatment with Zoledronic Acid in Stage II/III Breast Cancer) trial reported that adjuvant use of zoledronic acid failed to improve disease free survival. Reasons for this failure are relatively unknown. Bone-tumor microenvironment is very complex with numerous resorption agonists (RA) like IL-6, TNF alpha, PTHrP.

Results

Figure 2: Cancer cell conditioned media enhances ZOL inhibitory effects and supports osteoclast function. PBMC derived monocytes were cultured in the presence of M-CSF and RANKL with or without cancer cell conditioned media (SCC CM) and zoledronic acid (ZOL) for 12 days. Osteoclasts were fixed and stained for TRAP activity and quantified. Bars represent mean values normalized to M-CSF/RANKL treated cells. Figure 3: PTP inhibitor Orthovistic acid down regulates cancer cell conditioned media induced increased PTP1B gene expression. PTP1B expression was significantly reduced in SCC CM treated with or without Orthovistic acid and sodium orthovanadate (OV) for 12 days. 

Conclusion

• Several studies report no overall beneficial effect prescribing Zoledronic acid to specific cancer treatments owing to development of resistance to BP.
• Receptor agonists secreted by tumor cells thwart bisphosphonates inhibitory effects in bone-tumour microenvironment.
• Sustained cytoplasmatic activation in OC by tumor cells is responsible for nullifying bisphosphonates inhibitory effects.
• Tyrosine phosphatase is an important regulator of OC function, blockade of this activity by PTP inhibitors will affect OC activity significantly.
• Addition of small molecule PTP inhibitors as adjuvants to standard anti-cancer therapy may be beneficial while treating bone loss in cancers.

Methods

Bone-Tumor Microenvironment and PTP expression

PTP Inhibitor and OC Differentiation

PTP Inhibitor and Osteoclast Function

PTP Inhibitor and OC Differentiation

Bone-Tumor Microenvironment and PTP expression

PTP Inhibitor and Osteoclast Function

PTP Inhibitor and OC Differentiation

Conclusion

• Several studies report no overall beneficial effect prescribing Zoledronic acid to specific cancer treatments owing to development of resistance to BP.
• Receptor agonists secreted by tumor cells thwart bisphosphonates inhibitory effects in bone-tumour microenvironment.
• Sustained cytoplasmatic activation in OC by tumor cells is responsible for nullifying bisphosphonates inhibitory effects.
• Tyrosine phosphatase is an important regulator of OC function, blockade of this activity by PTP inhibitors will affect OC activity significantly.
• Addition of small molecule PTP inhibitors as adjuvants to standard anti-cancer therapy may be beneficial while treating bone loss in cancers.

References

• Coleman R
• Weilbaecher KN
Weilbaecher KN

• Tyrosine phosphorylation is important regulator of OC function, blockade of this activity by PTP inhibitors will affect OC activity significantly.
• Addition of small molecule PTP inhibitors as adjuvants to standard anti-cancer therapy may be beneficial while treating bone loss in cancers.

Acknowledgements

This work was supported by National Institute of General Medical Sciences (NIGMS) grant # P20 RR 02145.