Bone effects of cancer therapies: pros and cons

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Purpose of review

Agents used for systemic chemotherapy can alter normal bone homeostasis through mechanisms that affect both osteoblast and osteoclast function. The identification of those agents that influence maintenance of the bone-remodeling compartment is an important component of the drug development and testing process. This brief review focuses on preclinical and clinical data illustrating the effect of several classes of chemotherapeutic agents on skeletal development and bone remodeling.

Recent findings

New preclinical data demonstrate that several classes of chemotherapeutic agents, including histone deacetylase inhibitors and proteasome inhibitors, alter osteoblast and osteoclast function. Preclinical data on retinoic acid analogues demonstrate that these agents inhibit osteoclastogenesis. In addition, a dose-dependent effect of methotrexate treatment on growth plate thickness and primary spongiosa height in rats has been demonstrated. Two recently published analyses of clinical data from trials of bortezomib in myeloma patients found increased biochemical markers of bone formation and evidence of increased bone deposition after bortezomib treatment.

Summary

Several classes of chemotherapeutic agents alter bone metabolism and negatively impact bone homeostasis. Bone mineral density (BMD) monitoring guidelines for patients receiving systemic chemotherapy are not established. Limited guidelines exist for BMD monitoring in patients receiving long-term hormonal modulation; however, the negative effect of other chemotherapeutic agents on the skeleton is underappreciated.

Keywords

bone, chemotherapy, osteoblast, osteoclast, remodeling

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Introduction

Bone is a dynamic, metabolically active tissue that serves multiple physiologic functions. In addition to structural and protective roles, bone serves as a reservoir of ions such as calcium and phosphorous and is the supportive niche for hematopoiesis. Maintenance of the bone-remodeling compartment through the interactions of osteoblasts, osteocytes, and osteoclasts is essential for maintenance of the skeleton. Cancer alters the normal homeostasis within the bone microenvironment to initiate a vicious cycle of osteoblast–osteoclast interactions that promote tumor growth [1]. Agents used for systemic chemotherapy can also alter normal bone homeostasis through several different mechanisms. The identification of those agents that influence osteoblast and osteoclast functions as well as tumor growth has become important for therapeutic intervention. Here we review recent data on the effects of several classes of chemotherapeutic agents on skeletal development and bone remodeling as a means to determine their effects on the bone microenvironment.

Glucocorticoids

Glucocorticoids are a frequent component of chemotherapy regimens for multiple myeloma and other hematologic malignancies. The effects of glucocorticoids on the skeleton have been reported extensively in the neurology and rheumatology literature. Fracture risk is related to the dose and duration of glucocorticoid treatment and long-term glucocorticoid use (greater than 3 months), even at low doses, is associated with loss of bone mineral density (BMD) [2,3••].

Glucocorticoid administration results in decreased bone formation during bone remodeling due to reduction in osteoblast precursors and premature apoptosis of mature osteoblasts. Osteoclast production is also decreased; however, the osteoclast life span is prolonged [3••,4,5].

Reduced BMD has been noted in pediatric oncology patients treated with high-dose glucocorticoids as part of regimens for acute lymphoblastic leukemia during and shortly after treatment [6]. Several studies of fracture risk

in a study population from the UK General Practice Database have evaluated fracture risk with varying doses and durations of glucocorticoid treatment [7–9]. Intermittent use of high-dose oral glucocorticoids (daily dose of >15 mg prednisone equivalent and cumulative exposure <1 g) resulted in a small increased risk of osteoporotic fracture. Patients who received several courses of high-dose glucocorticoids (daily dose >15 mg prednisone equivalence and cumulative exposure >1 g) were at a significantly increased risk of fracture. In contrast, data on the skeletal effects of pulse-dose glucocorticoid regimens similar to the schedules used in oncology regimens are limited. Fracture risk in patients from the UK General Practice Database receiving a pulsed-dose glucocorticoid schedule failed to demonstrate an association between daily glucocorticoid dose and risk of fracture [10]. A long-term evaluation of repeated high-dose methylprednisolone pulses (1 g/day for 5 days every 4 months for 3 years, then every 6 months for 2 years) in multiple sclerosis patients concluded that pulse steroid regimens were not associated with osteoporosis, though the risk of osteopenia remained significant [11]. Clinical guidelines from the American College of Rheumatology suggest primary prevention of osteoporosis with calcium and vitamin D supplementation in all patients receiving glucocorticoid treatment, regardless of dose, and secondary prevention with bisphosphonates for patients with bone mineral density T-score less than 1 [12].

Methotrexate

Methotrexate (MTX), an antifolate metabolite, is frequently used at low doses in rheumatoid arthritis and high doses as cancer chemotherapy. At high doses, MTX causes skeletal growth defects in pediatric patients and is associated with a 10–40% incidence of fracture [13]. MTX treatment increases bone resorption and inhibits bone formation by altering the differentiation of early osteoblasts [14]. Fan *et al.* [15] recently demonstrated that high-dose MTX treatment significantly reduced growth plate thickness and primary spongiosa heights in rats. In contrast, low-dose MTX did not significantly damage either the growth plate or the primary spongiosa, suggesting that the damage of bone growth by MTX is dose-dependent. This finding is consistent with clinical data from rheumatoid arthritis patients demonstrating that the effect of MTX on BMD is dose-dependent [16].

Retinoic acids

Retinoic acids are vitamin A metabolites frequently used in treatment of dermatologic conditions as well as malignancies. The association between retinoic acid treatment and advanced bone age, with or without premature epiphyseal closure, has been described [17,18]. A recent

Key points

- Clinicians frequently underrecognize skeletal effects of systemic chemotherapy.
- Systemic chemotherapy can negatively impact bone mineral density and can increase fracture risk.
- Both preclinical and clinical studies have demonstrated that systemic chemotherapy can significantly alter osteoblast and osteoclast differentiation and function.

single-institution review of 32 patients with a history of high-risk neuroblastoma treated with high-dose chemotherapy followed by single or tandem peripheral blood stem cell transplant at the Children's Hospital of Philadelphia evaluated the association between treatment with 13-*cis*-retinoic acid and advanced bone age [19•]. Twenty-nine percent of patients treated with *cis*-retinoic acids had advanced bone age compared to patients not treated with *cis*-retinoic acids, though the study was small and statistical significance was not reached.

Balkan *et al.* [20•] recently evaluated the impact of retinoic acids and isoform-specific retinoic acid receptor (RAR) agonists on osteoclastogenesis in murine bone marrow-derived monocytes and RAW264.7 cells. They demonstrated that pan-RAR agonists, all-*trans* and 9-*cis* retinoic acids, inhibited receptor activator of nuclear factor κ-B ligand (RANKL)-mediated osteoclast differentiation. Inhibition of osteoclastogenesis correlated with reductions in DNA binding and expression of transcription factors required for osteoclastogenesis.

Fenretinide [*N*- (4-hydroxyphenyl) retinamide, or 4HPR], an analogue of all-*trans* terinoic acid (ATRA), has been tested as a chemotherapeutic agent in prostate, breast, and colorectal cancer [21] and is currently under investigation in other malignancies. 4HPR induces apoptosis through a receptor-independent mechanism rather than altering cellular differentiation, and is less hepatotoxic than ATRA [22]. 4HPR has been previously shown to suppress osteoclastogenesis in murine macrophage-like cell lines [23]. Li *et al.* investigated the effect of 4HPR on myeloma cell growth and osteoclastogenesis. They demonstrated that 4HPR inhibits survival of several multiple myeloma cell lines, and that these effects are partially attenuated when myeloma cells are co-cultured with osteoclasts. In addition, 4HPR inhibited survival of mature, primary human osteoclasts, though it had minimal toxic effects on nonmalignant cells.

Hormone-modulating agents: selective estrogen receptor modulators, aromatase inhibitors, and androgen deprivation therapy

Estrogen deficiency plays a critical role in age-related bone loss and is associated with increased expression of

serum biochemical markers of bone resorption and formation [24•]. Bone expresses both estrogen receptors and aromatase. Estrogen stimulates the expression of antiresorptive factors in bone leading to decreased RANKL signaling and subsequent inhibition of osteoclastogenesis and reduced bone turnover [25•]. Selective estrogen receptor modulators (SERMs, such as tamoxifen and raloxifen) are partial estrogen receptor agonists and antagonists that exhibit antagonistic effects on breast tissue in estrogen receptor positive breast cancers, and weak agonist effects on bone [26]. The estrogen agonist effect on bone decreases osteoclast activity and increases BMD. Treatment with both tamoxifen and raloxifen increases BMD and reduces fracture risk [27].

Aromatase inhibitors suppress estrogen synthesis by inhibiting aromatase activities. They are widely used for treatment of both early and advanced postmenopausal hormone-sensitive breast cancers [28]. Reduced circulating estrogen levels results in increased osteoclast activity, accelerated bone loss and an increased fracture risk [29]. Multiple studies have demonstrated that aromatase inhibitor treatment significantly increases the rate of bone demineralization, resulting in increased fracture rates [30••]. Studies comparing fracture rates with or without associated BMD rates in patients treated with aromatase inhibitors versus tamoxifen consistently demonstrate a significantly higher fracture rate in the aromatase inhibitor arm [31,32]. This effect was also seen in a sequential dosing study comparing 5 years of tamoxifen with 2–3 years of tamoxifen followed by 2–3 years of exemestane [33]. Substudies of the ATAC trial (anastrozole versus tamoxifen) and MA.17 trials (5 years of tamoxifen followed by 5 years of letrozole versus 5 years of tamoxifen followed by placebo) evaluated changes in BMD at the lumbar spine and hip, demonstrating significant reductions at each location in patients receiving aromatase inhibitors [29,34]. In addition, patients receiving aromatase inhibitors had significant increases in markers of bone resorption and bone formation as compared with patients treated with tamoxifen.

Third-generation aromatase inhibitors (exemestane, anastrozole, and letrozole) can be divided into steroidal and nonsteroidal groups. Exemestane, a steroidal aromatase inhibitor, binds to aromatase irreversibly and has a potential androgenic effect [35,36]. The recently published Tamoxifen Exemestane Adjuvant Multinational (TEAM) Japan bone substudy evaluated changes in BMD and bone turnover markers during 2 years of adjuvant therapy with tamoxifen, exemestane or anastrozole in women with hormone-sensitive postmenopausal, early breast cancer [37•]. This substudy demonstrated that exemestane and anastrozole had comparable negative effects on BMD. Long-term follow-up data have not been reported.

Similarly, androgen deprivation-induced bone loss is of clinical concern in patients with prostate cancer treated with androgen deprivation therapy (ADT). BMD declines within months of ADT initiation, and prospective studies have demonstrated that maximal BMD loss occurs within the first year of ADT [38,39]. Patients treated with long-term androgen deprivation therapy have accelerated bone loss compared with age-matched controls [40]. Morote *et al.* [41] performed a large evaluation of BMD in prostate cancer patients stratified by duration of ADT. Hormone-naïve patients had an osteoporosis rate of 35.4% at baseline, 42.9% after 2 years of ADT, 59.5% after 6 years, and 80.6% after 10 or more years. In addition, studies of patients with newly diagnosed prostate cancer demonstrate a high baseline rate of osteopenia and osteoporosis in the absence of ADT initiation [42,43]. A recent systematic review of skeletal and cardiovascular side effects in prostate cancer patients treated with and without ADT demonstrated that patients treated with ADT had an approximately 23% increase in overall skeletal fracture risk [44].

Proteasome inhibitors: bortezomib

Bortezomib, a proteasome inhibitor active against multiple myeloma, directly alters osteoblast and osteoclast activity by decreasing RANKL and Dickkopf-1 (DKK-1, a wnt signaling inhibitor that blocks osteoblast differentiation) levels in the sera of myeloma patients [45]. In clinical studies of both newly diagnosed and relapsed myeloma patients, bortezomib therapy, either alone or in combination with other agents, demonstrated improvement in markers of osteoblastic activity and osteoclast inhibition [46–49]. Additionally, Giuliani *et al.* [47] demonstrated that bortezomib treatment increases the number of osteoblasts in the bone marrow of myeloma patients.

Bortezomib's effects on osteoblast differentiation have been extensively studied. Several clinical trials showed increased bone-specific alkaline phosphatase (ALP), a marker for osteoblast activation, in myeloma patients whose tumor responded to the drug [49,50]. Peak ALP levels in the APEX trial were noted at 6 weeks after treatment (30), a finding confirmed by Lund *et al.* [51•] in their recent study of frontline bortzomib's effects on bone formation in bisphosphonate-naïve patients.

A recently published post-hoc analysis of the phase III VISTA trial, [bortezomib, melphalan, prednisone (VMP) versus melphalan and prednisone in patients with previously untreated multiple myeloma ineligible for high-dose therapy] [52,53•] evaluated clinical bone disease events and serum markers of osteoblast activation and differentiation during treatment [54••]. Changes in serum DKK-1 and ALP, as well as radiologic data demonstrating bone healing were assessed. Unless

contraindicated, all patients with lytic bone disease or osteopenia at study enrollment were treated with bisphosphonates. Two-thirds of patients in each arm had bone lesions at enrollment, and approximately one quarter of patients in each arm had more than 10 lesions. Fewer patients developed progressive disease based on skeletal criteria in the VMP arm compared with the melphalan and prednisone arm. Among patients assessed, DKK-1 levels were correlated with the number of bone lesions at baseline. Median DKK-1 decreased in the VMP-treated subgroup and increased in the melphalan and prednisone subgroup, with a statistically significant change from baseline. ALP levels increased in both treatment arms during the study period. Increases in ALP were strongly associated with response to VMP, with a mean ALP increase that was more pronounced and longer lasting with VMP versus melphalan and prednisone. In addition, higher median maximum percentage ALP increases during treatment were present in the VMP arm among all patients as well as in patients with bone lesions. These increases in ALP were associated with response to VMP and melphalan and prednisone.

Zangari *et al.* [55•] recently reported the first prospective study of bortezomib-associated bone changes. Sixteen bortezomib-naïve myeloma patients with relapsed or progressive disease were treated with bortezomib 1.0 mg/m^2 or 1.3 mg/m^2 on days 1, 4, 8, and 11 of a 21-day cycle. Patients achieving stable disease were continued on the regimen and followed until evidence of disease progression. Serum bone biochemical markers were measured before and after each dose of bortezomib, and then daily on nontreatment days. Bone histomorphometric analysis was performed at baseline and at the end of three treatment cycles. Evaluable micro-CT measurements were obtained at baseline and the end of the study period in seven patients. After bortezomib treatment measurements of bone volume/total volume significantly increased in six of seven patients and trabecular thickness increased from baseline in five of seven patients. Histologic evaluation demonstrated a lack of osteoblast activity and osteoid formation at baseline compared to bortezomib treatment in patients who responded to therapy.

Some authors have interpreted these findings as evidence that bortezomib directly stimulates osteoblasts and inhibits osteoclasts. Lund *et al.* [51•] have suggested that biochemical markers of bone formation peak after 6 weeks of bortezomib treatment due to a direct inhibitory effect on bone resorption by osteoclasts that counteract bortezomib's initial direct osteoblast stimulatory effect. Alternatively, bortezomib's direct inhibition of myeloma cells in the bone marrow microenvironment allows normalization of osteoblast and osteoclast function, as these effects are only seen in patients whose myeloma responds to bortezomib treatment.

Histone deacetylase inhibitors: vorinostat (SAHA), and romidepsin

Histone deacetylase (HDAC) inhibitors represent a relatively new group of promising therapeutic agents that play an important role in both the regulation of gene expression and the induction of cell cycle arrest and apoptosis in cancer cells. HDACs are important for the development and maintenance of bone cells [56••], and are involved in epigenetic control of the cell cycle through deacetylation of histone and nonhistone proteins [57,58], including Runx2 [59,60], a transcription factor required for osteoblast differentiation and function, RANKL, a major mediator of osteoclast differentiation, activation, and survival, and Stat3.

Histone deacetylases are classified based on their structure and function. Class I HDACs (HDAC1, 2, 3 and 8), the primary targets of existing pan-HDAC inhibitors, are broadly expressed and generally found in the nucleus [56••]. HDAC3 binds and represses Runx2-dependent activation of osteoblast genes [61] through a mechanism involving the zinc finger protein 521 [62]. *HDAC3* conditional knockout mice demonstrate deficits in intramembranous and endochondral bone formation with fewer osteoblasts and more bone marrow adipocytes compared to wild-type or heterozygous littermates [63]. Further, knockdown of HDAC3 inhibits osteoclast formation [64•]. Class II HDACs (subdivided into class IIa HDAC4, 5, 7, 9, and class IIb HDAC6 and 10) primarily affect cytoskeletal and tubulin structure and do not appear to contribute to histone deacetylation [65]. HDAC7 directly binds and represses Runx2 activity [66], and bone morphogenic protein 2, a potent osteoblast stimulator, induces export of HDAC7 from the nucleus. In addition, HDAC7 activity inhibits osteoclast differentiation [64•]. Class III HDACs (sirtuins or Sirt1–7) are nicotinamide adenine dinucleotide-dependent protein deacetylases. Sirt-1 is present in mesenchymal stem cell (MSC) osteoblast progenitors, and activation of Sirt-1 in MSCs promotes osteoblasts and reduces adipocyte differentiation. Recent studies suggest that estrogen depletion reduces Sirt-1 protein levels *in vivo*, possibly contributing to the enhanced marrow adiposity and bone loss in animal models of postmenopausal osteoporosis and normal aging [67]. In addition, Sirt-1 activation by resveratrol, a phytoestrogen with potent Sirt-1 deacetylation activity, results in deacetylation of RANKL-induced nuclear factor κ-B (NF-κB), transcriptional activation and osteoclastogenesis [68•]. HDAC11, the only class IV HDAC, has an unclear mechanism of action [56••].

Cantley *et al.* [69•] recently evaluated the in-vitro role of class I and II HDAC inhibitors on osteoclast activity and expression of HDAC1–10 during osteoclast development. Several HDAC inhibitors targeting both class I

and II HDACs, 1179.4b and vorinostat (suberoylanilide hydroxamic acid or SAHA), significantly reduced osteoclast activity. The combination of MS-275 (a class I HDAC inhibitor) and 2664.12 (a class II HDAC inhibitor) demonstrated synergistic inhibition of osteoclast activity to a similar degree as 1179.4b, suggesting that inhibition of both class I and class II HDACs are required for in-vitro suppression of human osteoclastic bone resorption. Compound 1179.4b also reduced expression of key osteoclast transcription factors including NFATc1, required for RANKL stimulation of osteoclastogenesis, and osteoclast-associated receptor, a transcription factor induced by NFATc1, and tumor necrosis factor (TNF) receptor associated factor-6. In addition, class I HDAC8 and class II HDAC5 were elevated during late-stage osteoclast development at both the protein and mRNA levels. Taken together, these findings suggest that both class I and II HDAC inhibitors suppress human osteoclasts *in vitro*, through a mechanism related to reduced expression of key osteoclast transcription factors.

Two HDAC inhibitors, SAHA and romidepsin (FK228, FR901228, depsipeptide), are FDA-approved treatments for cutaneous T-cell lymphoma. SAHA belongs to the hydroxamic acid class of compounds and is considered a pan-HDAC inhibitor, likely inhibiting most class I, II and IV HDACs [70]. SAHA inhibits RANKL-induced osteoclastogenesis through suppression of NF-kB [71]. Analysis of SAHA's effect on bone mass and remodeling *in vivo* demonstrated a reduction in trabecular volume fraction and trabecular number in the distal femur; however, histologic and serum markers of bone resorption are not affected by SAHA [72••]. Romidepsin, a cyclic tetrapeptide that is a more selective HDAC inhibitor, more potently inhibit class I HDACs, specifically HDAC1 and 2 [73,74]. A recently reported phase II trial of romidepsin in heavily pretreated patients with refractory multiple myeloma demonstrated improvement of bone pain and resolution of hypercalcemia in several patients, though no patients achieved an objective response to single-agent treatment. These results suggest that a biologic effect associated with therapy may be present [75]. Evaluation of romidepsin's mechanism of action on osteoclasts in models of rheumatoid arthritis suggests that the compound inhibits osteoclastogenesis and bone destruction through suppression of activation of RANKL-mediated signals [76].

Other chemotherapeutic agents

A recent study evaluated the differences between bone density in pediatric patients undergoing neoadjuvant chemotherapy for bone sarcoma and compared BMD in the affected and unaffected limbs [77•]. Patients received 10 weeks of neoadjuvant methotrexate, adriamycin and cisplatin or six cycles of vincristine, ifosfamide, doxorubicine and etoposide or cisplatin. There was no significant difference in BMD of the lumbar spine in patients following completion of neoadjuvant therapy. However, patients did develop focal osteopenia in the affected limb.

Several prior studies evaluated BMD in pediatric and adult sarcoma survivors and also demonstrated reduced BMD following chemotherapy [78–80]. Kaste *et al.* [79] reported that low BMD was correlated with younger age at diagnosis. Rhabdomyosarcoma patients who received cyclophosphamide had lower BMD. However, there was no correlation between treatment with methotrexate or ifosfamide and diminished BMD.

Conclusion

These results demonstrate that many commonly used chemotherapeutic agents alter bone metabolism and negatively impact bone homeostasis. Use of bisphosphonates and the newly developed RANK-ligand inhibitor, denosumab, in the management and prevention of cancer-induced bone loss will be addressed elsewhere in this issue. Guidelines for the management of bone health in patients with breast cancer have been published by American Society of Clinical Oncology [81,82••] and suggest osteoporosis screening with dual energy X-ray absorptiometry scans at age 65 for patients with standard osteoporosis risk or at age 60 for patients at increased osteoporosis risk as well as calcium and vitamin D supplementation. Bisphosphonates are recommended for primary high-risk patients with breast cancer and T scores of -2.5 or less. Prostate cancer patients treated with androgen deprivation therapy are also at risk of accelerated bone loss. These results demonstrate that induction of a hypogonad state in patients can profoundly affect bone. Taken together, the negative and positive impact of chemotherapeutic agents remains an underappreciated by important sequelae of cancer treatments.

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Conflicts of interest

The authors declare no competing financial interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 302–303).

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