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Association of osteoprotegerin and bone loss after adjuvant chemotherapy in early-stage breast cancer

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Abstract

Purpose—Chemotherapy induced ovarian failure (CIOF) results in rapid bone loss. Receptor Activator of Nuclear Factor Kappa-B (RANK)-RANK ligand (RANK-L) signaling balances bone resorption and formation. Osteoprotegerin (OPG) acts as a decoy receptor for RANK, interrupting osteoclast activation and bone resorption. This study examined the relationship between OPG and bone loss in women with CIOF.

Methods—Premenopausal women with stage I/II breast cancers receiving adjuvant chemotherapy were evaluated at chemotherapy initiation, 6 and 12 months. Bone mineral density (BMD) at the lumbar spine (LS) and femoral neck (FN), follicle stimulating hormone (FSH), ionized calcium, osteocalcin, and OPG were serially measured. CIOF was defined as a negative pregnancy test, FSH levels >30 MIU/mL, and 3 months of amenorrhea.

Results—Forty women were enrolled; 31 (77.5%) met CIOF criteria. BMD significantly decreased ($p < 0.001$) in the CIOF group at both time points: LS BMD decreased from a median of 0.993 g/cm² to 0.976 g/cm² and 0.937 g/cm² at 6 and 12 months, respectively. OPG was significantly elevated at 6 months (median increase 0.30 pmol/L, $p = 0.015$) and then decreased at 12 months to levels still above baseline (median difference 0.2 pmol/L, $p = 0.70$).

Conclusions—In what was likely a compensatory response to rapid bone loss, CIOF patients' OPG levels increased at 6 months and then decreased at 12 months to values greater than baseline assessments. This phenomenon is described in other diseases, but never before in CIOF.

Keywords

Breast cancer; Osteoprotegerin; Ovarian failure; Chemotherapy; Bone loss

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1. Introduction

Chemotherapy-induced ovarian failure (CIOF) is a common toxicity of adjuvant chemotherapy in premenopausal women with breast cancer (Shapiro and Recht, 1994). CIOF leads to a variety of effects including menopausal symptoms, infertility, and bone loss (Shapiro et al., 2001). Prospective studies have confirmed that CIOF leads to bone loss soon after initiation of chemotherapy (Eastell, 2003; Fogelman et al., 2003; Hirbe et al., 2006; Saarto et al., 1997; Shapiro et al., 2001). In fact, most bone loss occurs within the first 6 months of starting systemic treatment (Shapiro et al., 2001). The mechanism for this rapid bone loss is believed to be secondary to a rapid drop in estrogen levels.

The mechanism of bone metabolism is complex since bones are constantly being remodeled. Osteoblasts are involved in bone matrix formation and osteoclasts are involved in the resorption of bone. There are three specific factors that have been found to affect osteoclast function: receptor activator nuclear factor-kappa B (RANK), receptor activator factor-kappa B ligand (RANKL), and osteoprotegerin (OPG). The RANK–RANKL interaction is important in osteoclast development and activation. RANK is expressed on osteoclast precursors in the bone, in addition to other tissues such as lymph nodes, synovium, thymus, mammary glands, lung and at low levels, in the spleen and bone marrow (Kobayashi et al., 2009). RANK is a receptor for RANKL. RANKL is produced by osteoblasts and binds to the RANK receptor. The binding of RANK to RANKL results in the formation and activation of osteoclasts. This osteoclast activation leads to subsequent bone resorption and bone loss (Boyce and Xing, 2007, 2008; Kobayashi et al., 2009; Leibbrandt and Penninger, 2009; Simonet et al., 1997; Wright et al., 2009).

OPG is a member of the tumor necrosis factor receptor super-family and is secreted by osteoblasts. OPG is also known as osteoclastogenesis inhibitory factor. As its name implies, it has potent osteoclast inhibitory activity. It achieves this by acting as a decoy receptor for the RANKL–RANK interaction, thus inhibiting the differentiation of the osteoclast precursor into a mature osteoclast. This subsequently prevents bone resorption and ultimately prevents bone loss (Boyce and Xing, 2007, 2008; Kobayashi et al., 2009; Leibbrandt and Penninger, 2009; Simonet et al., 1997; Wright et al., 2009).

Estrogen is a key factor in maintaining bone health, and the rapid drop in estrogen levels during CIOF promotes bone loss. Estrogen stimulates OPG synthesis and release from osteoblasts *in vivo* (Bord et al., 2003; Khosla, 2001). The loss of estrogen thus disrupts the delicate balance between osteoblast and osteoclast activity. This leaves the RANKL uninhibited to stimulate osteoclastogenesis, osteoclast activation, and bone resorption. This can lead to rapid bone loss in estrogen-deficient women (Theriault, 2005).

In CIOF, the degree of ovarian dysfunction appears to be variable; women can develop temporary amenorrhea (lasting for months to years) or permanent amenorrhea (premature menopause). The age of the woman is the most important factor in assessing the risk for amenorrhea. Age over 40 at the time of starting chemotherapy is associated with an increased risk of amenorrhea, regardless of regimen given (Stearns et al., 2006). Following

anthracycline and cyclophosphamide therapy, CIOF rates are reported at 44% [95% CI: 31–58%] in women 40 years old, compared to rates of 81% [95% CI: 58–94%] in women >40 years old. With the addition of a taxane to this regimen, rates approach 61% [95% CI: 52–70%] *versus* 85% [95% CI: 54–98%] in those 40 and >40 years old, respectively (Tham et al., 2007). Other than age, the impact of chemotherapy on ovarian function depends on many factors, including the type of chemotherapy, dose of chemotherapy, and length of chemotherapy (Koyama et al., 1977; Schilsky et al., 1981; Stillman et al., 1981; Warne et al., 1973).

In this study, we investigated the relationship between OPG levels and bone loss as evaluated by changes in bone mineral density and bone turnover markers. We hypothesized that during the first 6 months of CIOF, there will be a compensatory increase in OPG. This hypothesis is based on other disease investigations involving patients without CIOF associated with breast cancer (summarized in Table 1). They suggest that as a transient, compensatory mechanism to counteract bone turnover and bone loss, OPG levels may rise (Crisafulli et al., 2005; Fiore et al., 2006; Flint et al., 2009; Honsawek et al., 2009; Masi et al., 2004; Ohwada et al., 2007; Sypniewska et al., 2010; Uemura et al., 2003; Yano et al., 1999). These studies collectively posit that early in CIOF development, decreases in estradiol lead to osteoclastogenesis, which causes increased bone resorption. Initially, OPG increases in response to increased levels of RANKL as an attempt to counteract the bone resorption. However, with ongoing osteoclast stimulation, the compensatory action of OPG diminishes and there is resultant unopposed bone resorption. To our knowledge, this compensatory increase in OPG has never been reported in CIOF in breast cancer patients. This investigation endeavored to further explore this relationship.

2. Patient and methods

2.1. Eligibility

Women with the histologic diagnosis of stage I–II breast cancer were eligible for this study. This cohort was previously described in the original study by Shapiro et al. (2001). Selection criteria required women to be actively menstruating (or last menstrual period within 3 months), have a negative serum pregnancy test, and a physician recommendation for initiation of adjuvant chemotherapy. Patients taking medications or having a clinical diagnosis of a condition that could affect bone metabolism were excluded (e.g. metabolic bone disease, hyperparathyroidism, Paget's disease, rheumatoid arthritis, ankylosing spondylitis, newly diagnosed hyperthyroidism, current/recent oral contraceptive use, androgen use, anabolic steroid use, anticonvulsant use, lithium use, chronic >1000 IU of vitamin D use, glucocorticoid use, bisphosphonate use, sodium fluoride use, or calcitonin use). Thiazide diuretic usage was permitted if the dose had been stable for 3 months prior to study enrollment. Women who developed breast cancer recurrence or received subsequent care at the discretion of their primary oncologist were withdrawn from the study. CIOF was defined as negative pregnancy test, 3 or more months of amenorrhea, and a follicle stimulating hormone (FSH) level of >30 MIU/mL at the 12 month time point. Women were considered premenopausal if they did not meet these criteria (Shapiro et al., 2001, 2011).

2.2. Study design

The study was originally designed to randomize women who developed ovarian failure to nasal spray calcitonin or nasal spray placebo between months 12 and 24. However, randomization was terminated in August 1997 after an interim data analysis was presented to a mandated National Cancer Institute external monitoring committee. The monitoring committee consisted of a medical oncologist, an endocrinologist, and layperson, all of whom acted independently and were unassociated with the study. The interim analysis revealed an unexpectedly high rate of bone loss between baseline and 12 months in women who developed ovarian failure. Based on these results, the monitoring committee recommended that the placebo-controlled portion of the study be stopped. Only 7 women were randomized before this portion of the study was eliminated. The women and their physicians were notified of the monitoring committee's recommendations. After the placebo-controlled portion of the study was eliminated, women who remained on the trial discontinued calcitonin and were treated at the discretion of their primary physician (Shapiro et al., 2001).

2.3. Study assessments

Patients were evaluated at baseline (within 4 weeks before starting adjuvant chemotherapy), at 6 months, and at 12 months. Measurements completed were: bone mineral density (BMD) at the lumbar spine (LS), BMD at the femoral neck (FN), FSH, ionized calcium (iCa), osteocalcin (OC) and OPG measurements. Bone mineral density was measured by dual-energy absorptiometry using the same Quantitative Digital Radiography Machine 2000W (Hologic Inc, Waltham, MA). Each patient underwent BMD measurements on the same machine. Serum samples were collected, frozen, and stored in a -70 °F freezer. Batched samples were analyzed by standard methods in the laboratories of the Clinical Research Center at The Ohio State Medical Center. Follicle stimulating hormone was a radioimmunoassay (Endocrinology Core Laboratory, Massachusetts General Hospital, Boston, MA). Osteocalcin was a radioimmunoassay (K. Grunberg, New Haven, CT). Commercial testing kits for OPG were purchased and OPG was measured by a Sandwich ELISA that required 20 µL of serum/test (Biomedica Gruppe, Vienna, Austria) (Shapiro et al., 2001).

2.4. Statistical methods and analyses

The BMD and OPG at different time points were summarized using median and inter-quartiles range (Q1 and Q3) because they are not normally distributed. The changes of BMD, OPG and other biomarkers over time were evaluated using the one sample sign test. The correlation between the BMD and the biomarkers were evaluated using the Spearman correlation. Sensitivity analyses were conducted using the corresponding parametric method for these measures satisfying the normality assumption (with or without data transformation).

3. Results

Sixty-nine women were originally enrolled in the study. Four women did not complete the baseline evaluation, 3 women did not complete the 6 month evaluation, and 12 women did not complete the 12 month evaluation. One woman was diagnosed with Paget's disease after

study enrollment and was excluded. Forty-nine women remained to be included in the study. Thirty-four women (69%) received cyclophosphamide, methotrexate, and fluorouracil, and 15 (31%) received cyclophosphamide and doxorubicin with or without fluorouracil or paclitaxel. Eleven women (22%) received tamoxifen after chemotherapy (Shapiro et al., 2001). The characteristics of these patients are included in Table 2. Median age was 42 (range 32–52). The group with ovarian failure had a significantly increased median age compared to those who did not develop ovarian failure (44 years *versus* 38 years, $p = 0.003$). Nine women had inadequate or incomplete sample collection of OPG for analysis. Therefore, 40 women were ultimately included in the final analysis for this study.

Among the 40 women with OPG data, 31 women (77.5%) were identified as having CIOF using our previously described eligibility criteria. None of these women resumed menstruation after study completion. The data for these 31 patients are summarized in Tables 3 and 4. In the CIOF group, there were statistically significant decreases in BMD (at the LS and FN) at both 6 and 12 months (both $p < 0.001$). The LS BMD decreased from a median of 0.993 g/cm^2 (0.935–1.123, Q1–Q3) to 0.976 g/cm^2 (0.869–1.100) and 0.937 g/cm^2 (0.834–1.062) at 6 and 12 months, respectively. OPG was significantly increased at 6 months (median increase 0.30 pmol/L , $p = 0.015$), and then decreased at 12 months to levels still above baseline (although not statistically significant, median difference 0.2 pmol/L , $p = 0.70$) (see Fig. 1). There was a non-statistically significant trend that negatively correlated OPG increases to the changes of BMD at the LS ($r = -0.38$, $p = 0.057$ after removal of one potential outlier) (see Fig. 2). Significant increases in OC were also noted at 6 and 12 months (both $p < 0.001$). Additionally, iCa was significantly increased at 6 months ($p = 0.042$); marginally significant at 12 months ($p = 0.06$).

4. Discussion

This is the first report describing the association between an increase in the levels of OPG and a decrease in the BMD in women with breast cancer who experienced CIOF. This reduction in bone density is thought to be a result of the drop in estrogen levels due to chemotherapy-induced suppression of ovarian function. The change in BMD of the women with CIOF in our study was significantly decreased at 6 months and at 12 months. This was expected in women with CIOF as previous prospective studies have confirmed that CIOF leads to rapid bone loss (Eastell, 2003; Fogelman et al., 2003; Hirbe et al., 2006; Saarto et al., 1997; Shapiro et al., 2001). However, our results uniquely demonstrated that OPG significantly increases at 6 months after initiating chemotherapy in patients with CIOF. This finding was similar to a study by Jacot et al. that retrospectively evaluated vitamin D deficiency with the change in serum levels of calcium and OPG, among other variables, in 77 patients with locally advanced breast cancer treated with anthracyclinetaxane neoadjuvant chemotherapy (Jacot et al., 2012). Although there was much greater variation in measured values compared to our study, a significant increase in OPG levels from baseline to time of last treatment was observed [baseline level of 4 pmol/L (range 2.2–16.5) to 4.5 at treatment end (range 0.2–29.3); $p = 0.003$]. At 12 months in our study, OPG was slightly decreased but still remained elevated over baseline (though no longer statistically significant). These early increases in OPG are consistent with our hypothesis that OPG acts as a transient, compensatory mechanism to counteract rapid bone turnover and bone loss.

Many studies have investigated the role of OPG in other diseases, as summarized in Table 1. These studies have similarly suggested a compensatory increase of OPG when bone loss is occurring. Uemura and colleagues showed significant increases in OPG in 10 women with endometriosis who developed low estrogen after 6 months of treatment with gonadotropin-releasing hormone (GnRH) agonists (Uemura et al., 2003). GnRH agonists act on the pituitary GnRH receptors and indirectly cause downregulation of estrogen production by the ovaries via decreased luteinizing hormone and FSH secretion, which ultimately leads to hypogonadism and decreased systemic estrogen levels. The patients' OPG and BMD levels were measured in this study. After 6 months, estrogen levels were significantly decreased, BMD was significantly decreased, and OPG levels were significantly increased. Other studies have suggested a similar compensatory mechanism in celiac disease (Fiore et al., 2006), anorexia nervosa (Ohwada et al., 2007), biliary atresia (Honsawek et al., 2009), fragility fractures in elderly women with low vitamin D status (Sypniewska et al., 2010), a patient on hemodialysis (Crisafulli et al., 2005), growth hormone deficiency (Flint et al., 2009), juvenile idiopathic arthritis (Masi et al., 2004), and in postmenopausal women with osteoporosis (Yano et al., 1999).

The protective effect of OPG against bone loss has been extensively studied in mice. Mice that exhibited high levels of OPG had increased bone density (Simonet et al., 1997). If the OPG was removed, mice developed early osteoporosis (Mizuno et al., 1998). In 2001, recombinant human OPG was tested on mice in microgravity in space. It was found that it prevented bone resorption and maintained mineralization (Bateman and Countryman, 2002). These studies in mice eventually led to the development of a new targeted therapy, Denosumab (Prolia®), which was approved by the FDA for use in postmenopausal osteoporosis in 2010 (Cummings et al., 2009) and for use in metastatic bone disease (Fizazi et al., 2011; Henry et al., 2011; Stopeck et al., 2010). Denosumab is a monoclonal antibody to RANKL. Thus, it blocks osteoclast formation and activation, which helps decrease bone resorption. Essentially, denosumab acts just like OPG by occupying RANKL and preventing it from binding to RANK.

In reviewing our data, we also observed changes in ionized calcium levels. iCa levels were significantly increased at 6 months, but no statistically significant elevation was noted by 12 months. As bone resorption occurs, iCa levels rise. OPG increases in an attempt to counteract this bone resorption. It is thus effective at maintaining a stable iCa level; however, with ongoing osteoclast stimulation, the compensatory action of OPG diminishes and there is a resultant unopposed bone resorption. This could explain the transient rise in iCa into the circulation at that time. Furthermore, there were statistically significant increases in OC at both 6 and 12 months. Osteocalcin is secreted by osteoblasts and is thought to play a role in bone building. However, our data showed a statistically significant decrease in BMD at the same time. This elevated OC level could indicate that the body is attempting to build bone because of the rapid bone loss, but is not able to keep up with the rapid rate of loss.

Overall, this study had several limitations. First, its small sample size is a limitation; although even in such a small sample, we were able to detect significant changes in OPG levels. We also used a strict definition for CIOF: we adopted an FSH threshold of >30

MIU/mL in conjunction with amenorrhea for 3 months, and a negative pregnancy test. This definition has been used previously (Shapiro et al., 2001, 2011); however, other investigations have employed different criteria. This definition of CIOF may include some women who may have temporary amenorrhea, although in this study the women who had CIOF remained amenorrheic. It is unclear if these women are also at risk for long-term bone loss. Finally, this study had a relatively short 12-month follow-up period. Lengthier follow-up of these patients could better evaluate the long term effects of CIOF on bone health.

In conclusion, women with CIOF develop significant decreases in BMD in both the LS and FN at 12 months. The results of this study suggest that OPG increases significantly in the first 6 months in women with CIOF and breast cancer. This is the first report of OPG as a possible early, but transient compensatory mechanism to counteract bone loss in women with CIOF and breast cancer.

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Figure 1.

Box plot of OPG changes from baseline to 6 months and from 6 months to 12 months. OPG was significantly increased at 6 months (median increase 0.30, $p = 0.015$) and subsequently decreased at 12 months, but to levels higher than baseline (median difference -0.2 , $p = 0.70$).

Figure 2.

Scatter plot of OPG changes (from baseline to 6 months) and change of BMD at lumbar spine from baseline to 6 months. We observed a trend in the negative correlation of OPG increases with changes of BMD at the lumbar spine ($r = -0.38$, $p = 0.057$ after removal of one potential outlier).

Table 1

Summary of OPG effects on bone health in published studies.

Author (Year)	Number of patients	Study population	Study design	OPG	BMD
Uemura et al. (2003)	10	Endometriosis	Pts receiving GnRH agonists for 6 months	*↑	*↓
Fiore et al. (2006)	32	Celiac disease	Compared to control	*↑	*↓
Ohwada et al. (2007)	26	Anorexia nervosa	Compared to control	*↑	N/A
Honsawek et al. (2009)	63	Biliary atresia	Compared to control	*↑	*↓
Sypniewska et al. (2010)	92	Elderly women with fracture and low vitamin D levels	Compared to control	↑ (trend)	N/A
Crisafulli et al. (2005)	72	Hemodialysis	Compared to control	*↑	*↓
Flint et al. (2009)	54	GH deficiency	Compared to control	↑	↑
Masi et al. (2004)	124	Juvenile idiopathic arthritis	Compared to control	*↑	N/A
Yano et al. (1999)	242	Postmenopausal women with osteoporosis	Compared to control	*↑	*↓
Kudlacek et al. (2003)	1134	Normal adults aging	OPG and BMD levels measured	*↑	N/A

* Statistically significant.

Table 2
Baseline characteristics by categorization of menopausal status at the 12-month study evaluation.

	Ovarian failure (n = 35)		No ovarian failure (n = 14)		Total (n = 49)	
	Median	Range	Median	Range	Median	Range
Age, years*	44	33–52	38	32–44	42	32–52
Weight, kg	64	45–134	58	45–92	62	45–131
Body mass index	23	17–32	22	17–49	23	17–49
Body fat, %	36	14–58	34	23–49	36	14–58
Body lean, %	59	39–80	61	48–73	59	39–80
Tamoxifen, % of patients**	26		14		22	

* Only significant value identified.
p = 0.003 for the difference in age between women with who did or did not develop ovarian failure.
** Received tamoxifen after chemotherapy.

Table 3

Changes in BMD and other bone associated factors in patients with CIOF.

	Baseline median (Q1,Q3)	6 months median (Q1,Q3)	12 months median (Q1,Q3)
BMD at LS (g/cm ²)	0.993 (0.935–1.123)	0.976 (0.869–1.100)	0.937 (0.834–1.062)
BMD at FN (g/cm ²)	0.795 (0.723–0.910)	0.798 (0.705–0.878)	0.778 (0.691–0.863)
OPG (pmol/L)	3.1 (2.1–3.9)	3.1 (2.4–4.3)	2.9 (2.5–3.8)
OC (ng/mL) [normal for > or =18 years: 9–42]	6 (3.7–7.6)	9.6 (7.3–12.2)	10.6 (8.2–16.3)
iCa (mmol/L) [normal 1.1–1.35]	1.29 (1.25–1.33)	1.29 (1.27–1.32)	1.31 (1.29–1.34)

Table 4

Change of BMD and other bone associated factors in patients with CIOF over time.

	Change (0–6 m) median (Q1,Q3)	P-value	Change (6–12 m) median (Q1,Q3)	P-value
BMD at LS (g/cm ²)	−0.04 (−0.0710, −0.017)	<0.001	−0.033 (−0.063, −0.015)	<0.001
BMD at FN (g/cm ²)	−0.021 (−0.04, −0.003)	<0.001	−0.018 (−0.033, 0)	<0.001
OPG (pmol/L)	0.3 (0, 0.9)	0.015	−0.2 (−0.5, 0.6)	0.7
OC (ng/mL)	3.6 (0.7, 6.1)	<0.001	1.5 (−2.4, 8.1)	<0.001
iCa (mmol/L)	0.01 (−0.01, 0.07)	0.042	0.02 (−0.03, 0.04)	0.06