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Research Paper

A phase 2 trial exploring the clinical and correlative effects of combining doxycycline with bone-targeted therapy in patients with metastatic breast cancer

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ABSTRACT

Background: Bone-targeting agents (BTAs), such as bisphosphonates and denosumab, have demonstrated no discernable effects on tumour response or disease free/overall survival in patients with bone metastases from breast cancer. Doxycycline is both osteotropic and has anti-cancer effects. When combined with zoledronate in animal models, doxycycline showed significantly increased inhibition of tumour burden and increased bone formation. We evaluated the effects of adding doxycycline to ongoing anti-cancer therapy in patients with metastatic breast cancer.

Methods: Breast cancer patients with bone metastases and ≥ 3 months of BTA use, entered this single-arm study. Patients received doxycycline 100 mg orally, twice a day for 12 weeks. The co-primary endpoints were; effect on validated pain scores (FACT-Bone pain and Brief Pain Inventory) and bone resorption markers (serum C-telopeptide, [sCTx]). All endpoints (pain scores, sCTx, bone-specific alkaline phosphatase, skeletal-related events, toxicity) were evaluated at baseline, 4, 8 and 12 weeks. Bone marrow was sampled at baseline and week 12 for exploratory biomarker analysis.

Results: Out of 37 enroled patients, 27 (73%) completed 12 weeks of therapy. No significant changes were seen in pain scores or bone turnover markers. Failure to complete treatment: drug toxicity (70%) and disease progression (30%). Sixteen (43%) patients had GI adverse events.

Conclusions: Doxycycline 100 mg twice daily for 12 weeks had no significant effects on either bone pain or bone turnover markers. Its toxicity profile in this patient population would make further evaluation challenging.

Translational relevance

This is the largest study to date evaluating the effects of doxycycline in bone-metastatic breast cancer patients. Doxycycline daily for 12 weeks did not appear to significantly enhance palliative benefit nor change bone resorption markers. The toxicity profile of doxycycline in metastatic breast cancer patients will make further evaluation challenging.

1. Background

The biological behaviour of bone metastases causes an uncoupling of the actions of osteoclastic and osteoblastic cells, resulting in increased in bone turnover [1]. In clinical practice the main mechanism of action of bone-targeting agents (BTAs) (e.g. bisphosphonates or denosumab) has been through osteoclast inhibition [1,2] with resulting reduction in skeletal-related events (SREs) [3]. Despite numerous studies reporting direct anti-tumour

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and anti-metastatic activities of bisphosphonates in preclinical models, large randomized placebo-controlled trials in patients with metastatic breast cancer have shown no evidence of improvement in terms of response rate, progression free or overall survival [4,5]. One strategy to enhance the direct anti-tumour activities of BTAs in the metastatic and adjuvant settings might involve the addition of the widely available, safe, and inexpensive drug, doxycycline.

Doxycycline, a tetracycline analogue, is osteotropic with a high affinity for mineralised bone. In experimental systems, it has demonstrated anti-cancer effects including inhibition of matrix metalloproteinases, anti-angiogenesis and cytostatic effects on cancer cells [6]. Preclinical bone metastasis models have shown that doxycycline could directly inhibit tumour growth, induce bone reformation [7] as well as increase inhibition of tumour burden and increase bone formation when combined with zoledronate [8]. In addition to preclinical data, doxycycline has also undergone evaluation as an anti-cancer agent in Phase 1 trials and in breast cancer patients with newly diagnosed bone metastases prior to commencement of bisphosphonate therapy [9]. These studies suggest that the addition of doxycycline to a bisphosphonate regimen in breast cancer patients may work synergistically to enhance the direct anti-tumour effects and potentially result in increased patient benefit.

We initiated a phase II, single-arm study, where we hypothesised that in women with bone metastases from breast cancer, the addition of doxycycline to their standard BTA therapy would result in significant palliative benefits as a result of inhibition of tumour progression and osteolysis. Through the prospective collection of serum, urine and bone marrow samples, putative mechanisms of action would be explored.

2. Materials and methods

2.1. Objectives

This study was designed to evaluate the effect of adding doxycycline 100 mg orally twice a day for 12 weeks to ongoing anti-cancer therapy in women with breast cancer and bone metastases. The primary objective was to explore the potential palliative benefit of doxycycline in this population. Secondary study objectives included: effects on bone turnover markers, potential associations between bone resorption/formation markers, apoptosis and proliferation with palliative or anti-tumour response, and the ability to complete therapy, including toxicity and safety.

2.2. Study population

Patients with metastatic breast cancer with radiologically and/or biopsy confirmed bone metastases who had received ≥ 3 months of BTA therapy (e.g. bisphosphonate or denosumab) were enroled. Patients had to have an ECOG ≤ 2, a life expectancy > 3 months and no changes in systemic anti-cancer therapy for 4 weeks prior to study entry or anticipated changes in the 4 weeks after entering the study. The study was approved by the Ottawa Health Science Network Research Ethics Board and registered with clinicaltrials.gov [10].

2.3. Trial design

All study participants received doxycycline 100 mg orally twice a day for 12 weeks. Participants were provided with a paper diary to record their compliance with doxycycline. Data on self-reported bone pain was measured using 2 validated questionnaires: the Brief Pain Inventory (BPI)-worst pain score [11,12] and Functional Assessment of Cancer Therapy-Bone Pain (FACT-BP) [13,14]. Pain, analgesic use, toxicity, and occurrence of SREs (defined as radiotherapy or surgery to the bone, pathological fractures, spinal cord compression, or hypercalcemia) were assessed at baseline and weeks 4, 8 and 12. Baseline fasting serum c-telopeptide (CTX, a collagen fragment released as a result of tumour-induced bone degradation, and hence a surrogate marker of tumour-induced osteolysis), bone specific alkaline phosphatase (BSAP), parathyroid hormone (PTH) and vitamin D (25-OH-vit D) were also measured. Serum CTx and BSAP were assessed at weeks 4, 8 and 12. At baseline and week 12, bone marrow aspirate and trephine biopsy were performed from the posterior iliac crest. If tumour cells were present in the bone marrow specimen, ER, PR (by immunohistochemistry) and Her2 analysis (by FISH) and a marker of proliferation (Ki67) were measured. Patients could also optionally consent to the collection of plasma, serum and urine samples (baseline, weeks 4, 8 and 12) for future translational research studies.

All patients were advised to take calcium (1200–1500 mg/day) and vitamin D3 (800–1000 IU/day) while on study. Given that this study is pragmatic, all other assessments (e.g. scans, blood work) were at the treating physician's discretion.

2.4. Laboratory analysis

Blood was drawn in the morning following an overnight fast [15,16]. Samples were allowed to clot and were centrifuged at 4 °C for 10 min at 3400 RPM. Urine was collected as a second pass, fasting specimen. Both were frozen at −80 °C until analysis. Serum CTx, BSAP, 25-hydroxyvitamin D and PTH were measured by chemiluminescence immunoassay: CTx using CrossLaps® on an IDS iSYS automated analyzer, BSAP using Ostase®, on the Beckman Coulter Unicel DxI and 25-OH-VitD on the IDS iSYS and PTH on the Beckman Coulter Unicel DxI..

All Immunohistochemistry (IHC) testing was done on the Leica-Bond Platform. IHC for Oestrogen receptor was done using the 6F11 clone (Leica) at 1/150 with Heat Induced Epitope Retrieval (HIER) in Bond epitope retrieval solution-1 (citrate buffer, pH 6.0). IHC for Progesterone receptor was done using the PR clone 16 (Bond ready to use) with HIER in Bond epitope retrieval solution-2 (EDTA, pH 9.0). IHC for Ki67 scoring was done using the MIB-1 clone (DAKO) at 1/75 with HIER in Bond epitope retrieval solution-2. ER and PR positive is defined according to ASCO guidelines as $\geq 1\%$. Ki67 score was expressed as a percentage of cells positive (all cells available counted).

2.5. Statistical analysis

The co-primary endpoints for this study was palliative pain response at 3 months, based on the BPI questionnaire (BPI-worst), analgesic use, FACT-BP score and bone resorption markers. As with previous studies in this patient population, [17,18] complete response for palliative pain response was defined as a pain score of zero at the index site with no concomitant increase in analgesic intake (stable or reducing analgesics in daily oral morphine equivalents). Partial response was defined as either a pain reduction of 2 or more at the index site on 0–10 scale without analgesic increase, or analgesic reduction of 25% or more from baseline without an increase in pain. Pain progression was defined as an increase in pain score of 2 or more points above baseline at the index site with stable analgesic use or an increase of 25% or more in daily oral morphine equivalent compared with baseline, with pain score stable or 1 point above baseline. Pain response was also evaluated using the FACT-BP. Pain decrease was measured as a 10% decline compared to baseline, and pain progression was defined as a 10% increase in the FACT-BP compared to baseline.