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Anti-tumor and anti-osteolysis effects of the metronomic use of zoledronic acid in primary and metastatic breast cancer mouse models

Ke-Wang Luo [a,b], Chun-Hay Ko [a,b], Grace G.L. Yue [a,b], Michelle Y.Y. Lee [a,e], Wing-Sum Siu [a,b], Julia K.M. Lee [a,b], Wai-Ting Shum [a,b], Kwok-Pui Fung [a,b,d], Ping-Chung Leung [a,b], Gang Li [c], Andreas Evdokiou [e,*], Clara B.S. Lau [a,b,*]

[a] Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China (CUHK), The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong
[c] Department of Orthopaedics and Traumatology and Stem Cells and Regeneration Program, School of Biomedical Sciences, Li Ka Shing Institute of Health Science, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong
[d] School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong
[e] Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, South Australia, Australia

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ABSTRACT

This study aims to determine the effect of metronomic (0.0125 mg/kg twice a week for 4 weeks) zoledronic acid (ZOL) on cancer propagation and osteolysis against both metastatic and primary breast cancer in mice model. From our results, metronomic ZOL resulted in a significant reduction of tumor burden and did not promote lung or liver metastasis. The metronomic ZOL appeared to be more effective than the conventional regimen (0.1 mg/kg once in 4 weeks) in reducing breast cancer tumor burden, and regulating its movement to lung and liver. This dosing schedule of ZOL showed great potential against metastatic breast cancer.

1. Introduction

Cancer is a major health problem worldwide, and the incidence and mortality rate showed an upward trend. Breast cancer is one of the leading causes of cancer death, which ranks the top of most frequent cancers in both sexes in 2008 [1]. The disease is characterized by a high incidence of bone metastasis causing significant morbidity including pain, fracture and spinal cord compression [2], with over 70% of patients dying of breast cancer have bone metastasis and develop severe bone destruction [3]. Breast cancer induced-bone metastasis frequently produces osteolytic bone lesions by activating local osteoclasts. During bone metastasis, parathyroid hormone-related peptide (PTHrP) is secreted by tumor cells and potentially stimulates osteoclasts. Activated osteoclasts degrade bone matrix and release growth factors including transforming growth factor-β (TGF-β), which in turn increase PTHrP secretion, and eventually promote a vicious cycle of bone destruction and tumor expansion [4]. Although many significant advances on the frontline breast cancer research and chemotherapy have been developed, the efficacies of current therapies are limited by a range of adverse side effects, toxicity and drug resistance. Therefore, novel therapeutic strategies and more effective drugs for advanced disease are still urgently needed.

Zoledronic acid (ZOL), the potent third-generation nitrogen-containing bisphosphonate, is effective in prevention and treatment of bone destruction caused by metastatic spread of primary cancer to the skeleton [5]. ZOL inhibits osteoclastic bone resorption by preventing prenylation of GTPases and ultimately induce cell death in osteoclasts [6]. In addition to the anti-resorptive efficacy of ZOL, there is an increasing number of reports describing the potential direct and indirect anti-tumor effects of ZOL in both *in vitro* and *in vivo* models. ZOL could dose-dependently inhibit proliferation of leukemia, breast cancer, prostate cancer and osteosarcoma cells *in vitro* and induce apoptosis in these tumor cells [7,8]. ZOL

* Corresponding authors. Address: E305, Science Centre East Block, Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong. Tel.: +852 39436109 (Clara B.S. Lau). Address: Basil Hetzel Institute, The Queen Elizabeth Hospital, 28 Woodville Rd, Woodville, SA 5011 Australia. Tel.: +61 8 8222 7451 (A. Evdokiou).

E-mail addresses: andreas.evdokiou@adelaide.edu.au (A. Evdokiou), claralau@cuhk.edu.hk (C.B.S. Lau).

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could also reduce tumor cell adhesion, invasion and angiogenesis activities [9]. Besides, treatment with ZOL alone or in combination with doxorubicin in animal models of breast cancer metastasis has demonstrated bone protection and inhibition of tumor growth [10]. However, previous studies showed that conventional administration of ZOL resulted in no effect on lung metastases but promoted in a xenograft mouse model that closely mimics the clinical outcome of patients with osteosarcoma [5,11]. In addition, some reports showed that long-term use of ZOL could lead to the osteonecrosis of jaw, an increased risk of oesophageal cancer, and femoral insufficiency fracture [12–14]. However, these side effects of ZOL are manageable and avoidable with standard treatment [15].

One of the strategies to potentiate the anti-tumor effects of ZOL could be administration of the drug at a metronomic way which means lower doses given more frequently on a prolonged schedule [16]. Recent clinical studies showed that the metronomic use of low dose ZOL appeared to be more effective than the conventional regimen in breast cancer patients in the long-lasting reduction of biomarkers, such as VEGF and NTx [17,18]. Metronomic administration of zoledronic acid and taxotere combination in castration resistant prostate cancer patients showed promising anti-tumor activity [19]. In addition, a recent study demonstrated that weekly administration of ZOL had greater anti-tumor effects as compared with conventional single administration in nude mice xenografted with breast cancer cells, even if the total administered dose is the same [20]. Nevertheless, very few reports on the effect of metronomic ZOL on metastatic and primary breast cancer were found. Therefore, we aimed to investigate the anti-tumor and anti-osteolysis activities of metronomic ZOL against both metastatic and primary breast cancer. In this study, the human breast cancer MDA-MB-231-TXSA-TGL cells were used. MDA-MB-231-TXSA-TGL cells, tagged with a luciferase reporter construct, were enabled for sensitive, non-invasive bioluminescence imaging tracking of the cell growth and its metastatic spread to organs after injected with the substrate of luciferase [21]. Here, we compared the anti-tumor and anti-osteolysis activities between the metronomic (0.0125 mg/kg twice a week for 4 weeks) and conventional (0.1 mg/kg once in 4 weeks) ZOL in mouse models of breast cancer development in terms of bone marrow and orthotopic mammary tissue.

2. Materials and methods

2.1. Cells and reagents

The MDA-MB231 derivative cell line, namely MDA-MB-231-TXSA-TGL [21], was cultured in DMEM medium containing 10% (v/v) fetal bovine serum and 1% (v/v) penicillin–streptomycin (Life Technologies, USA) at 37 °C in 5% CO_2 humidified incubator.

Zoledronic acid (ZOL) was purchased from Novartis Pharma Stein, Switzerland, with a Reg. No. of 031390. D-luciferin was purchased from Biosynth, Switzerland.

2.2. Intratibial breast cancer-induced osteolysis model

Four-week-old female nude mice were provided by Laboratory Animal Services Center, The Chinese University of Hong Kong (CUHK), and were housed under pathogen-free conditions, approved by Animal Experimentation Ethics Committee of CUHK. MDA-MB-231-TXSA-TGL cells (1×10^6) resuspended in 10 μl PBS, were injected into the marrow space of the proximal tibia with a 27-gauge needle coupled to a Hamilton syringe. After cancer cell implantation, mice were divided randomly into three groups ($n = 10$): control group ($1 \times$ PBS, i.p. injected twice a week for 4 weeks), ZOL-C group (0.1 mg/kg ZOL, i.p. injected once only, as conventional single dose), ZOL-M group (0.0125 mg/kg ZOL, i.p. injected twice a week for 4 weeks, as metronomic dose). During ZOL treatment, mice were imaged weekly using the *In Vivo* Imaging System (IVIS) 200 bioluminescence system (Xenogen, USA). After 4 weeks treatment, mice were sacrificed, lungs and livers were removed for bioluminescence imaging and quantification of tumor burden. Both the tibias of each animal were removed for X-ray, micro-computed tomography and histological analysis.

2.3. In vivo bioluminescence imaging

Tumor growth in live animals was assessed by the IVIS 200 system (Xenogen, USA). Mice were imaged after i.p. injected with D-luciferin solution at 150 mg/kg body weight and gas anaesthetized. Bioluminescence images were taken 30 min after the D-luciferin injection, acquired for 1–30 s and the photon emission was quantitated using the software, Living image 3.2 (Xenogen, USA), and graphed according to the average radiance (photons/s/cm^2/sr).

2.4. X-ray and micro-computed tomography (μ-CT) analysis

Tibias removed from mice were scanned with X-ray (MX-20, Faxitron X-ray, WI, USA) and a high resolution microtomographic system, μ-CT 40 (Scanco Medical, Switzerland). The tibia specimens were measured at room temperature and placed inside the X-ray chamber of the X-ray machine. The voltage and exposure time of the X-ray were 32 kV and 10 s, respectively. Then the samples were exposed to the μ-CT. Each three-dimensional image data were consisted of approximately 500 micro-CT slide image (8 μm/slide) starting from the growth plate of tibial interface and moving down the tibia. The bone density was expressed as percentage of BV/TV, which was generated and compared with each groups using the formula: (Bone volume/Tissue volume) × 100% [20].

2.5. Histology

Lungs and livers were fixed in 10% buffered formalin for 7 days at room temperature. As for the tibia, after fixed in 10% buffered formalin, the tibia was decalcified in decalcification buffer (14% EDTA w/v in distilled water, pH 6.8–7.2) for 21 days. Then samples were paraffin embedded, sectioned longitudinally at 5 μm, and stained with H&E. Stained sections were examined and photographed using an Olympus IX71 microscope (Japan) and were analyzed using SPOT advanced (version 3.5.6) software. Tumor burden, defined as the tumor area, was calculated from the section of the lung or liver and expressed as an average tumor area per group in absolute units (mm^2).

2.6. Mouse mammary tumor model

Female nude mice (6–8 weeks of age) were provided by Laboratory Animal Services Center, CUHK. MDA-MB-231-TXSA-TGL cells (5×10^6) resuspended in 0.2 ml PBS, were subcutaneously (s.c.) inoculated at the mammary fat pad of each nude mouse. After the tumor size reached 80 mm^3, the tumor-bearing nude mice were randomly assigned into three groups ($n = 16$): control group ($1 \times$ PBS, i.p. injected twice a week for 4 weeks), ZOL-C group (0.1 mg/kg ZOL, i.p. injected once only, as conventional single dose), ZOL-M group (0.0125 mg/kg ZOL, i.p. injected twice a week for 4 weeks, as metronomic dose). Tumor size and body weight of each mouse were measured twice a week during the 4-week treatment period. At day 28, mice were injected (i.p.) with D-luciferin solution at 150 mg/kg body weight, and then sacrificed, and the lungs and livers were removed for bioluminescence imaging and quantification of tumor burden. Tibias of mice from different groups were removed for micro-CT analysis.

2.7. Statistical analysis

All data were expressed as mean ± SEM. Statistical analysis was performed using one way ANOVA (unless otherwise specification), with p-value (p) < 0.05 as considered statistically significant.

3. Results

3.1. Effect of ZOL on tumor burden, metastasis, and cancer-induced bone destruction in metastatic breast cancer

To evaluate the efficacy of ZOL against tumor growth within bone, cancer-induced bone destruction and tumor metastasis, an intratibial breast cancer-induced osteolysis model was employed. In this model, the MDA-MB-231-TXSA-TGL cells were injected into the tibial marrow cavity of nude mice directly. After ZOL treatment, no significant body weight loss was found in ZOL-treated groups (data not shown). As shown in Fig. 1A, an increase of photon emission (expressed as average radiance) from day 7 onwards associated with an increase in tumor burden of bone. Unlike ZOL-C, treatment with metronomic ZOL (ZOL-M) resulted in the slowing down of tumor growth and a significant difference was shown at day 28 (Fig. 1B). Metronomic use of ZOL resulted in a reduction of tumor burden in the bone, while the conventional ZOL had no effect.