Effect of Photodynamic Therapy on Breast Cancer Metastases in Vertebrae of Rats Pre-treated with Bisphosphonates

INTRODUCTION:

Breast cancer commonly metastasizes to the spine resulting in bone osteolysis. Bone loss may result in structural instability leading to an increase in skeletal related events (i.e. pathological fracture). Bisphosphonates (BP) are considered the current standard of care for breast cancer patients with skeletal disease. BPs aim to prevent further bone loss due to metastatic involvement by inhibiting osteoclast recruitment and osteoclast activity [1]. Photodynamic therapy (PDT) is a non-radioactive treatment which has been successfully applied to various malignancies. The treatment requires the administration of a photosensitizer, which preferentially accumulates in malignant tissue, that is subsequently illuminated with light at a photosensitizer specific wavelength. This leads to the generation of reactive oxygen species causing cell death. This treatment has been shown to successfully ablate tumours within the vertebrae in a murine metastatic model [2].

Previous in-vitro study has shown that pre-treatment of breast cancer cells with the BP zoledronic acid (Zometa®, Novartis, Dorval, Canada) renders them more susceptible to PDT. The combination of these treatments was shown to have a synergistic effect in destroying MT-1 human breast cancer tumour cells. The aim of this study was to evaluate the influence of pre-treatment with BPs on the effect of PDT treatment on tumour ablation in metastatically involved vertebrae in vivo.

METHODS:

Metastases to the vertebrae were induced in fourteen 5-6 week old female athymic rats (Hsd:RH-foxn1-/-, Harlan Laboratories, Inc., Indianapolis, IN, USA) by intra-cardiac injection of 2x10⁶ human MT-1 breast cancer cells stably transfected with the luciferase gene. Four groups were formed to assess the effects of PDT and BP on tumour cells within the spine: 1. control, no treatment; 2. BP only; 3. PDT only; 4. BP and PDT combined. Seven days after tumour cell inoculation, the BP treated rats were injected subcutaneously with 60 µg/kg of zoledronic acid. This dose represents a clinically relevant dose equivalent for the treatment of breast cancer metastases. PDT treatment was administered on day 14 using the photosensitizer BPD-MA (verteporfin; Visudyne, Visudyne, Novartis, Dorval, QC, Canada) at a dose of 1.0 mg/kg injected intra-venously. Fifteen minutes later, light-ded at 690nm was administered at a dose of 75 J using a 400 µm flat cut laser fibre placed adjacent to the second lumbar vertebra (L2) under fluoroscopic guidance. The rats were euthanized 21 days after tumour cell injection, except when clinical signs (e.g. paralysis) required an earlier intervention. Institutional approval for the animal care committee was obtained for this study (University Health Network, Toronto, ON, Canada). Following sacrifice, the spines were harvested, fixed in 10% buffered formalin, decalcified, embedded in paraffin, cut into 5 µm sections and stained with haematoxylin and eosin (H&E) and mouse-anti-human epidermal growth factor receptor (hEGFr) antibody (Zymed, Laboratories Inc., San Francisco, CA, USA). A total of 45 vertebrae were evaluated using a histomorphometric program (GENIE™, Aperio Technologies Inc., Vista, CA, USA) on the mid-sagittal sections of L2 and 2-4 neighbouring vertebrae to assess tumour burden. Statistical analyses were performed using a one-way ANOVA with a Tukey post hoc test. A p-value p<.05 was considered to be statistically significant.

RESULTS:

The total tumour burden within vertebrae of rats pre-treated with bisphosphonates and/or PDT was significantly lower compared to the control rats (p<.001, Figure 1). In addition, the PDT alone treated group demonstrated significantly less tumour burden than the combined BP+PDT group. In the control and BP-only groups, large tumours were found to include regions of necrosis. The PDT treatment groups (PDT and BP+PDT) exhibited areas of necrosis throughout the entire vertebral bodies with adjacent formation of granulation tissue.

DISCUSSION:

This study evaluated BP and PDT treatment on tumour ablation in metastatically involved vertebrae in-vivo. BP, PDT and combined BP+PDT treatments all resulted in a lower overall tumour burden at 21 days post MT-1 cell injection compared to the control untreated animals. No significant difference was found in comparing the BP and PDT treatment groups alone, however a surprising increased level of tumour burden was found in comparing the combined treatment group (BP+PDT) to the PDT-only group. These findings are in contrast to previous in-vitro results, where the pre-treatment of MT-1 cells with BPs made them more susceptible to PDT. In the in-vivo scenario, the complexity resulting from the presence of multiple different cell types and their interactions may lead to a different environment for the tumour cells. Pre-treatment with BP affects both the bone and tumour cells, and as such may induce different cellular pathways in response to PDT treatment. Further investigations of combined treatments on the bone cells – osteoblasts and osteoclasts – alone and in combination with tumour cells are necessary to elucidate potential mechanism.

Tumour burden is not always equally distributed within all vertebrae in this metastatic murine model. While qualitative assessment of bioluminescent imaging indicated the presence of vertebral metastasis in all animals, quantification of tumour burden prior to treatment would allow for a more direct comparison of the treatment effects. Partly destroyed tumour cells, visible 2 days after PDT treatment [2], are no longer present at 1 week due to the remodeling process including the formation of granulation tissue in the PDT treated groups. Thus, the exact determination of the tumour burden prior to treatment was not possible in the PDT groups. However, the ability of PDT applied at day 14 to cause a similar reduction in tumour burden compared to BP treatment at day 7, suggests its ability to rapidly and effectively ablate the tumour within the bone, even in the presence of BP.

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REFERENCES:


Figure 1: Vertebral tumour burden (T13 - L4) 21 days subsequent to MT-1 cell injection in control and BP, PDT and BP+PDT treatment groups. (Note: the bars indicate the mean and standard deviation).

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