Introduction: Breast cancer is known to cause metastatic lesions in the vertebrae, which can lead to spinal instability and/or neurological deficit[1]. Success rates with radiation therapy and surgery vary and additional treatment options are needed for vertebral metastases. Photodynamic therapy (PDT) has been applied successfully as a treatment for numerous cancers. In PDT light of a specific wavelength is delivered to the tumour after administration of a photosensitiser. The excited drug generates reactive oxygen species that cause cell death[2]. Earlier work has shown that the athymic rat model is suitable to investigate the effect of PDT on bone metastases[3]. However, the potential for neurologic sequelae makes it important to accurately define the therapeutic window for the treatment. BPD-MA (Visudyne®) has been shown to be a selective photosensitiser with the highest ratio of BPD-MA concentration between tumour-bearing vertebrae and spinal cord found 15min after drug delivery[4]. The aim of this study was to closely define the therapeutic window of photosensitiser safety and efficacy with regard to drug and light dose.

Materials and Methods: Thirty 5 to 6 week old female athymic rats (rnu/rnu) (Harlan Sprague Dawley, Indianapolis, IN) were injected intra-cardiacally with MT-1 cells transfected with the luciferase gene at a concentration of 2x10^6 in 0.2 ml RPMI1640 media. At day 14 in-vivo bioluminescence imaging (IVIS, Xenogen Corp., Alameda, CA) confirmed the establishment of metastases. A drug escalating-de-escalating scheme with stopping rules in 2 steps was used to test drug and light safety and efficacy. In the first step the photosensitiser (Visudyne®) dose was increased up 2 mg/kg bodyweight with a fixed light dose of 75J. At each drug dose the animals were clinically assessed for signs of illness (paralysis). If signs of paralysis occurred, the drug dose was lowered and the light dose increased up to 200J. Bioluminescence images were taken again 48h after PDT treatment prior to euthanasia and the percentage in increase/decrease in bioluminescence photon emission was calculated. The treated vertebrae were harvested, fixed in 10% buffered formalin, decalcified and stained with H&E and hEGF-α antibody. The hEGF-α antibody does not cross-react with rat tissue and clearly identifies the tumour within the vertebrae. This staining was used to histo-morphometrically measure the tumour burden within each vertebra and to evaluate the treatment effect due to reduced/missing staining and the presence of destroyed tumour cells (Amira™, Mercury Computer Systems, Carlsbad,CA).

The PDT treatment effect was statistically analyzed using the Kruskal-Wallis test.

Results: Metastases in the vertebrae were confirmed with bioluminescence in 80% of the rats. The bioluminescent signals decreased in the treated area over 48h with increasing drug dose as well as with an increased light-dose at a stable drug dose. Histologically the treatment dose of 0.2 mg/kg Visudyne® did not show evidence of tumour destruction. The first signs of tumour destruction within the vertebrae (presence of apoptotic and necrotic cells and inflammation) were visible at 0.5mg/kg concentration with more destruction at increasing drug dose (p = 0.036)(Fig 1).

Discussion: The effect of the photodynamic therapy on breast cancer metastases in the spine was drug and light dependent. A safe and effective drug/light dose combination for PDT treatment in this model is 0.5mg/kg and 75J for thoracic vertebrae and 1.0mg/kg and 125J for lumbar vertebrae. Flexibility in treatment planning is necessary dependent on the location of tumour relative to the proximity of the spinal cord in vertebral PDT. We observed that a lower drug and light dose can result in vertebral tumour destruction. As such, if a tumour cannot be treated at a higher dose, repeated low dose therapy may be necessary to reduce the risk of side effects. Whilst the use of this animal model is desirable in the context of evaluating PDT on the human cancer of interest, species variation in vertebral architecture and differences in light attenuation and transmission in metastatically involved vertebrae will also influence the therapeutic effect. The effect of PDT in this study was typically observed over several continuous vertebrae. This may relate in part to the parapedicular/paraverterbral fibre placement used in this study. A direct intravertebral cannulation for fibre placement (as would be used in the clinical setting) may better target and contain light to a desired level but is technically difficult in rodent vertebrae. The resultant wider transmission and attenuation of light by paraverterbral fibre placement in this animal study needs consideration in interpreting the result of the present study.


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