INTRODUCTION: Bone is the preferred site of metastases in women with breast cancer. Seventy percent of women with advanced breast cancer develop bone metastases. These can result in skeletal-related events (SREs) such as pathologic fractures, spinal cord compression and severe pain. Bisphosphonates are the current standard of care for treatment of metastatic bone disease by preventing further bone destruction[1,2]. They inhibit osteoclast recruitment, inhibit osteoclast activity and reduce the life span of osteoclasts. A new generation of bisphosphonates contains a nitrogen atom and were found to be more potent than the older bisphosphonates. Farnesyl pyrophosphate (FPP) synthase is thought to be a key enzyme in the inhibitory effect of bisphosphonates such as zoledronic acid (zol) on osteoclast activity[3]. Photodynamic therapy (PDT) has been applied successfully as a non-radiative treatment for numerous malignancies. Earlier pre-clinical studies in a metastatic rat model have shown that PDT reduced the tumour burden in the vertebrae[4]. In PDT, light of a specific wave length is delivered to a tumour after the administration of a photosensitiser. The photosensitiser accumulates in the tumour and the excited drug generates reactive oxygen species that cause cell death. The goal of this investigation was to study the effect of PDT on bisphosphonate pre-treated cancer in-vitro.

METHODS: Human breast cancer cells, MT-1, were cultured at a concentration of 0.2x10^6/ml until confluent. Cells were cultured either in Lab-Tek® Chambered Coverglass System for microscopy or 6-well plates for flow-cytometry analyses[5]. The treatment group was incubated with 10 μmol zoledronic acid (Zometa®, Novartis, Dorval, Canada) for 24 h. Thereafter, the media was changed, the cells washed once and new media was added. Prior to light application (red light; 690 nm), 1 μg/ml BPD-MA (Visudyne®, Novartis, Dorval, Canada) was added and incubated for 15 min. PDT was performed with a light dose of 1J, 5J or 10J. Fluorescence microscopy was performed with a confocal microscope (LSM 510 Meta, Zeiss). For flow cytometry analyses, the cells were trypsinized and resuspended in media at a concentration of 1x10^6/ml at either 1h or 24 h after light treatment. The following control groups were formed: cells without any treatment; cells incubated with BPD-MA only, no light treatment; cells incubated with zoledronic acid; cells treated with light without photosensitiser: cells incubated with zoledronic acid and BPD-MA, but incubated with zoledronic acid; cells treated with light without photosensitiser; cells incubated with 10 μmol zoledronic acid (Zometa®, Novartis, Dorval, Canada) for 24 h. Thereafter, the media was changed, the cells washed once and new media was added. Prior to light application (red light; 690 nm), 1 μg/ml BPD-MA (Visudyne®, Novartis, Dorval, Canada) was added and incubated for 15 min. PDT was performed with a light dose of 1J, 5J or 10J. Fluorescence microscopy was performed with a confocal microscope (LSM 510 Meta, Zeiss). For flow cytometry analyses, the cells were trypsinized and resuspended in media at a concentration of 1x10^6/ml at either 1h or 24 h after light treatment. The following control groups were formed: cells without any treatment; cells incubated with zoledronic acid; cells treated with light without photosensitiser: cells incubated with zoledronic acid and BPD-MA, but without light treatment; cells incubated with BPD-MA only, no light treatment. The cells were stained with 0.01 μmol calcine AM (Invitrogen, Burlington, Canada) and 0.01μmol Sytox® orange (Invitrogen, Burlington, Canada) for live/dead staining and analyzed using a FACSCalibur (Becton-Dickinson, Oakville, Canada). A One-way Analysis of Variance (ANOVA) was performed to analyse differences between groups.

RESULTS: Incubation of the MT-1 breast cancer carcinoma cells with bisphosphonate zoledronic acid results in a significant higher number of dying cells after PDT treatment compared with PDT treatment control (p<0.05). The incubation with zoledronic acid alone did not have an effect on cell survival after 24h compared to the control group.

DISCUSSION: In-vitro, breast cancer cells are more susceptible to PDT after they have been incubated with the bisphosphonate zoledronic acid. BPD-MA is a photosensitiser, that causes cell death mainly via apoptosis, but it also can upregulate several stress proteins involved in cell rescue[6]. Zoledronic acid inhibits farnesylpyrophosphate (FPP), which is involved in several pathways and farsenylation of cell membrane proteins like members of the Ras superfamily. It has been reported that the inhibition of FPP in some tumour cell lines induces apoptosis [5]. However, this effect was not seen in our in-vitro study. This may be due to the cell line and/or the growing conditions used. The inhibition of FPP may, however, cause a reduced effect of PDT on cell rescue as well as inhibition of cell division of surviving cells. The state-of-the-art treatment of metastatic bone disease with bisphosphonates seems to have a synergistic effect with PDT treatment. This can lead to a lower light dose for the PDT treatment, which would reduce the risk of site effects and in a reduced number of treatments. Future work must study the effect of PDT on breast cancer metastases in bone in a preclinical model pre-treated with bisphosphonates. This includes the determination of the optimal light dose. Ultimately, the application of this promising treatment combination may provide an exciting alternative for patients with skeletal metastases.

REFERENCES:

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Figure 1: Fluorescence microscopy of MT-1 cells after PDT treatment (a) and PDT treatment after pre-incubation with zoledronic acid (b) (green: live cells; orange-yellow: dead cells) The cell viability of all control groups was 92.4±1.9 % after 1 h and 93.5 ± 2.7 % after 24 h. The increased light dose for PDT treatment resulted in a lower number of surviving cells (5J-PDT: 48%; 5J-zol+PDT: 26%; 10J-PDT: 42%; 10J-zol+PDT: 14%) and the pre-treatment with zoledronic acid enhanced the process of cell-kill.

Figure 2: Example of flow cytometry analyses of MT-1 cells with a light dose of 1J.