

Micro-SPET/CT and histology results of zoledronate treatment in a nude mouse lung adenocarcinoma skeletal metastases model: A pilot study

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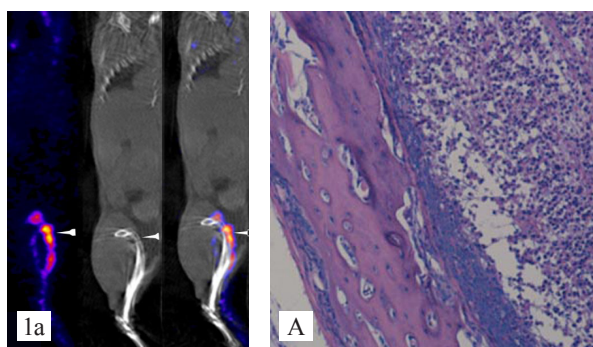
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Some studies have evaluated the efficacy of bisphosphonates for the prevention of bone metastases in breast, prostate, and lung cancers, as well as in multiple myeloma [1], but whether bisphosphonates have anti-tumor activity against bone metastases in lung cancer, and the mechanism of this possible effect, remain to be elucidated [2]. In this study, a lung cancer skeletal metastases model was established by injecting lung adenocarcinoma cell line SPC-A-1 cells into the left proximal tibias of 4 nude mice. Microscopic SPET (micro-SPET)/spiral computed tomography (CT) imaging was used to characterize bone tumors prior to initiating treatment with daily subcutaneous injections of either zoledronate or saline control. Repeat micro-SPET/CT imaging was obtained after 12 days of treatment, and animals were sacrificed at this time for histologic analysis.

Lung adenocarcinoma cell line SPC-A-1 cells in the exponential phase of growth were cultured using serum-free media. Cell concentrations were adjusted to $10^7/100\mu\text{L}$ after trypsin digestion. A $100\text{-}\mu\text{L}$ volume of this cell suspension was subcutaneously injected into the left proximal tibias of 4 nude mice (Balb/C nude mice, 6 weeks old, average weight $19.67\pm 0.67\text{g}$; Experimental Animal Center of Shanghai Medical College, Fudan University). Tumor growth was allowed to proceed for 2 weeks before treatment was initiated. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Zhongshan Hospital, Fudan University, Shanghai, China.

Bone scintigraphy was performed for each animal using a NanoSPET/CT (Bioscan, USA). The CT images were acquired before each SPET scan using standard settings: 45-kVp voltage, 0.15mA current, and 500-ms exposure. Images were reconstructed using Nucline 1.02 Software (Mediso, Hungary) for real-time images with simultaneous three-dimensional reconstructions. Micro-SPET imaging parameters were 1.0mm/pixel, 256X256 frame size, and 60s per projection with 24 projections. Acquisition times ranged from 21 to 24min. The micro-SPET raw data were reconstructed into transaxial, coronal, and sagittal slices using Invivoscope 1.44, Reconstruction Software (Bioscan, USA). Three-dimensional ordered subset expectation maximization reconstructions were created with a resolution of 0.4mm/pixel using an algorithm that used four subsets and applied an iterative calculation six times.

All 4 animals underwent micro-SPET/CT imaging 2 weeks following inoculation to characterize tumor formation. Three hours prior to imaging, animals received a caudal vein injection of 37MBq of $^{99\text{m}}\text{Tc}$ -MDP in $100\mu\text{L}$ saline. Animals were anesthetized prior to imaging with an intraperitoneal injection of $100\mu\text{L}$ 2.5% sodium barbitol. All mice were identified by imaging as having tumor invading the adjacent tibial bone. These mice were randomly assigned to one of three categories: 1 pre-intervention mouse as control group,



Figures 1a and A. Two weeks following lung adenocarcinoma cell inoculation, micro-SPET/CT imaging (1a) and histology findings (A) (H&EX200).

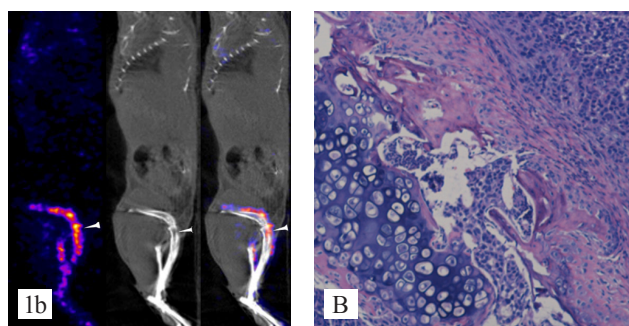


Figure 1b and B. Following the 12-day injection protocol, repeat micro-SPET/CT imaging in this saline-intervention animal demonstrated progressive bone destruction and reduced radioactive uptake compared to pre-intervention in the left tibia of the nude mouse receiving saline injections (Fig. 1b). Histology showed extensive bone destruction and numerous tumor cells within the bone marrow cavity (H&EX200) (Fig. B).

2 mice, which after initial imaging received daily subcutaneous injections of 200µL for 12 days of saline (injected-control group) and 1 mouse who received daily subcutaneous injections of 200µL of saline with 8µg (0.04g/L) zoledronate for 12 days.

Subcutaneous injections were administered in the thoracic spine. After 12 days of injections, whole-body bone scintigraphy was repeated in the 3 animals using micro-SPET/CT and then the 3 animals were harvested. The left hind limbs of all harvested animals were disarticulated and immersed in 4% formaldehyde solution for 24h. These lower extremity specimens were decalcified, embedded in paraffin and sectioned for histologic analysis. Sections were stained with haematoxyline-eosin and examined under light microscopy at 200 times amplification.

Two weeks following lung adenocarcinoma cell inoculation, spherical solid tumors (average diameter 7.93 ± 0.33 mm) were clearly observed adjacent to the left tibias in all animals. Micro-SPET/CT imaging obtained at this time point demonstrated invasion of the tibial cortex with increased radioactive uptake (Fig. 1a). On histology, corresponding to these finding on micro-SPET/CT, tumor cells were observed invading the bone marrow cavity (Fig. A).

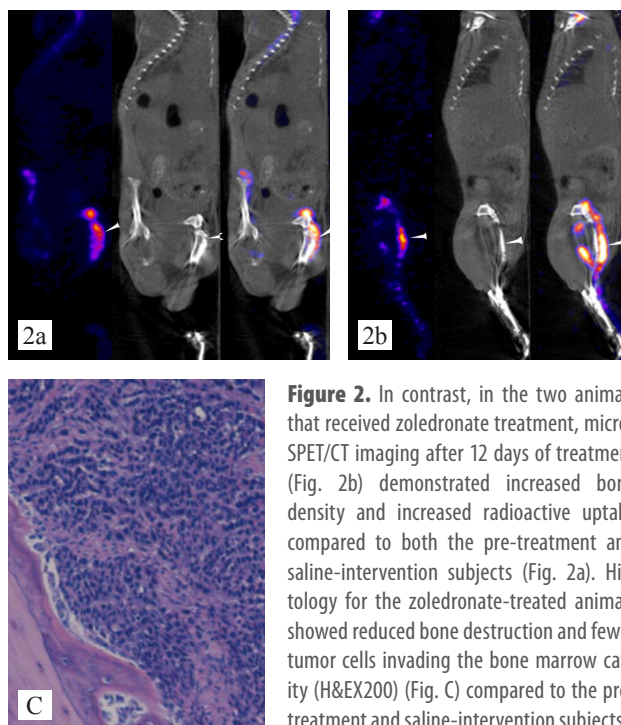


Figure 2. In contrast, in the two animals that received zoledronate treatment, micro-SPET/CT imaging after 12 days of treatment (Fig. 2b) demonstrated increased bone density and increased radioactive uptake compared to both the pre-treatment and saline-intervention subjects (Fig. 2a). Histology for the zoledronate-treated animals showed reduced bone destruction and fewer tumor cells invading the bone marrow cavity (H&EX200) (Fig. C) compared to the pre-treatment and saline-intervention subjects.

Zoledronate is a third-generation bisphosphonate. Our micro-SPET/CT and histology results suggest that treatment with zoledronate in lung cancer adenocarcinoma skeletal metastases in a nude mouse model may decrease bone destruction and bone marrow invasion.

In some cancers, zoledronate has been reported to prevent or reduce complications related to bone metastases from prostate cancer, lung cancer, and other solid tumors, maintain bone mineral density, and reduce cancer-related bone pain [3, 4]. Zoledronate have also been proposed to possess both direct and indirect anti-cancer activities on

some breast cancer, multiple myeloma and other advanced cancer [5] and also anti-angiogenic effects, and it specifically inhibits endothelial cell adhesion mediated by integrin $\alpha v \beta 3$, a key promoter of angiogenesis, which is an essential process for tumor cell invasion, mobility, and adhesion to the bone matrix [6].

The proposed treatment mechanisms of zoledronate for bone metastasis vary [7, 8].

However, it was also shown that zoledronate treatment did not significantly affect progression-free survival or overall survival in stage IIIA/B non-small cell lung cancer patients with controlled disease, with a trend toward worsening progression-free survival in the longer-term follow-up [9].

Comparing histologic sections from pre-treatment and saline-intervention controls with the zoledronate-treated sections supported our observations, that zoledronate-treated sections had reduced bone destruction and bone marrow invasion.

The limitations of our pilot study are the very small sample size and that only one representative lung cancer cell line was studied.

In conclusion, our above prodromal results, although very few, suggest that micro-SPET CT is a useful imaging technique in the experimental evaluation of lung adenocarcinoma skeletal metastases in a nude mouse model and showed that zoledronate was a possible treatment agent.

The authors declare that they have no conflicts of interest.

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