THE THIRD-GENERATION BISPHOSPHONATE, ZOLEDRONIC ACID INHIBITED LUNG METASTASIS WITH SUPPRESSION OF VEGF PRODUCTION IN MURINE OSTEOSARCOMA CELLS

Naoyuki Horie1, Hiroaki Murata1, Tomoya Sakabe1, Takaaki Matsui1, Shinya Kimura2, Taira Maekawa2, Toshikazu Kubo1
1Orthopaedics, Kyoto Prefectural University of Medicine, Kyoto, Japan; 2Transfusion Medicine and Cell Therapy, Kyoto University Hospital, Kyoto, Japan

Introduction: We already reported the inhibitory effects of the third-generation bisphosphonates (BPs) against murine osteosarcoma cell lines in vitro and in vivo (51st -53rd ORS)(1-2). And then, to investigate the anti-metastatic effects of BPs, we mainly focused on the angiogenesis of osteosarcoma. The aim of this study was to determine whether BPs can inhibit VEGF production and tumor metastasis.

Materials and Methods: We used third-generation BPs, ZOL (Novartis), and murine osteosarcoma cell line, LM8, which was established from the murine Dunn osteosarcoma cell line and has high metastatic potential to lung.

(1) In vitro effect of ZOL on cell viability and VEGF production;

The cell viability was determined by MTT assay after the cells were seeded with various concentrations of ZOL for 48 hours. And also, to extract LM8 produced VEGF proteins, the concentrations of VEGF of the culture supernatants were determined by using ELISA (R&D Systems, Minneapolis, MN).

(2) In vivo Effect of ZOL on LM8 cells;

The LM8Luc, stably expressing luciferase (Luc) LM8 cells, were generated. The mice injected 1 x 107 LM8Luc cells into the lateral lumbar were divided into three groups, namely, (i) untreated mice, (ii) mice treated with 80 μg/kg ZOL once a week and (iii) mice treated with 80 μg/kg ZOL three sequential days a week. Each group contained 6 mice. ZOL was administered intraperitoneally initiated from day 1. We analyzed primary tumor lesion and lung metastasis by using the in vivo imaging system (Xenogen). After 4 weeks treatment, all mice were killed humanely for histological analysis. Sections (4 μM thick) of the resected tissues were stained with hematoxylin-eosin (H.E.). To describe the number of vascular endothelial cells, immunohistochemical staining was performed by using antibodies against α smooth muscle actin (SMA). The SMA positive area was calculated per all field from 10 random microscopic fields (x 200 magnification).

Results: (1) Effect of ZOL on cell viability and VEGF production;

ZOL inhibited the growth of LM8 cells in a dose-dependent manner. To examine the indirect effects such as angiogenic property of ZOL on osteosarcoma cells, we investigated the concentrations of VEGF in the culture supernatant of ZOL-treated and untreated LM8 cells. The treatment reduced the elaboration of VEGF in a dose-dependent manner, with 50% reduction being achieved with 2.55 μM of ZOL, although the cell viability was reduced slowly with 50% reduction being achieved with 7.36 μM (Fig 1).

Fig 1. Effect of ZOL on LM8 cell viability and VEGF production.

(2) Effect of ZOL on primary tumor lesion;

We evaluated the efficacy of ZOL by measuring the photon counts of the primary lesion. There was no significantly effect of ZOL between groups (i) and (ii) after 4 weeks treatment. However the growth of group (iii) was significantly suppressed than that in group (i) after 3 weeks treatment (Fig 2A). And also, many necrosis and calcification area was found from the ZOL treated group in the H.E. staining section (Fig 2B). Since the VEGF production had been inhibited by ZOL in vitro (Figure 1), we investigated whether ZOL inhibits the angiogenic property of osteosarcoma cells in vivo. The SMA positive area was significantly inhibited in the ZOL treated group (Fig 2C).

Fig 2. A: Effect of ZOL on primary tumor. B: Histological findings of primary tumor lesion. C: Immunohistochemical findings of SMA.

(3) Effect of ZOL on pulmonary metastasis lesion;

The growth of the lung metastasis of group (iii) was significantly inhibited by ZOL after 4 weeks treatment (Fig 3A). Interestingly, although there was no significantly effect of ZOL on primary tumor lesion in group (ii) (Fig 2A), the growth of the lung metastasis of group (ii) was significantly inhibited by ZOL (Fig 3A). That is to say, the inhibitory effect on tumor metastasis of ZOL might be stronger than the inhibitory effect on primary tumor growth of ZOL.

Fig 3. A: Effect of ZOL on pulmonary metastasis. B: Histological findings of lung metastasis section.

Discussion: In clinical practice, intravenously administration of ZOL could achieve maximally 1-3 μM in serum levels, despite 1 mM in bone resorption lesion. In the present in vitro study, less than 2.55 μM of ZOL inhibited the VEGF production from murine osteosarcoma cells significantly. VEGF is one of the most potent angiogenic factors and the production is essential for the tumor cells metastasis. To summarize, less than the concentration of ZOL achieved in clinical administration, ZOL inhibited the essentials of tumor metastasis. Then, in the in vivo study, ZOL also inhibited the lung metastasis in both of the ZOL treated groups significantly. To detect the reason of inhibitory effects on tumor metastasis in vivo, the SMA expression level was investigated. Because, in the resected section, the SMA expression was significantly suppressed in the ZOL treatment group, the suppression of angiogenesis might lead to the decline of tumor cell contact with endothelial cells and cascade of tumor metastasis inhibition.

In conclusion, we could find that ZOL inhibited the essentials of tumor metastasis, such as VEGF, less than the physiologic concentration of ZOL achieved in clinical, which leads to the suppression of lung metastasis in vivo. Because lung metastasis is one of the poor prognostic factors for osteosarcoma patients, our present studies indicate that ZOL may be very beneficial in treating osteosarcoma for improving the prognosis.


Poster No. 518 • 6th Combined Meeting of the Orthopaedic Research Societies