ALENDRONATE REGULATES CELL INVASION AND MMP-2 SECRETION IN HUMAN CHONDROSARCOMA CELL

Introduction:
Surgical resection remains the primary mode of therapy for chondrosarcoma, this mesenchymal malignancies have a poor prognosis due to the absence of an effective adjuvant therapy. Matrix metalloproteinase-2 (MMP-2) induced degradation of blood vessel basement membranes is an important pre-requisite for tumor invasion and metastasis. There is increasing evidence that bisphosphonates (BPs) have direct antitumor effects in vivo in addition to their therapeutic antiresorptive properties. BPs inhibit proliferation and induce apoptosis of many cancer cells. They also exhibit anti-invasive properties in vitro and in vivo. To our knowledge, it is the first time to study that the antitumor effects of alendronate, one of BPs, with correlation of cell invasiveness have been well investigated and analyzed in human chondrosarcoma cells (JJ012).

Materials:
Alendronate was a gift from Merck Sharp and Dohme Ltd. (Ireland). Chondrosarcoma cell line (JJ012) were cultured in DMEM/F12 supplemented with 10% FBS and treated with alendronate in different concentrations (0, 20, 40, 60, 80 and 100 µg/ml) for 12, 24 and 48 hr. The effects of alendronate on viability of the JJ cells were evaluated using the MTT assay. Gelatin zymography was done according to a protocol developed by Klemer et al. (Anal Biochem, 1994) and used to investigate the expression of MMP-2 proteins in JJ cells. Matrigel invasion assay was performed to study the effects of alendronate on the invasion ability of chondrosarcoma cell lines. Cell migration experiments were performed using Bio-Coat cell migration chambers (Becton Dickinson, France), which consist of a 24-well companion plate with cell culture inserts containing 8µm pore size filters. RT-PCR analysis was performed to determine whether MMP-2 mRNA level in chondrosarcoma cell lines was altered by alendronate treatment. All experiments were run in duplicate. All data are presented as means ± SD. Statistical significance was determined by the Student’s t-test. A P-value < 0.05 was considered to be statistically significant.

Results:
Treatment with alendronate at 24 hrs was not significant cytotoxicity in human chondrosarcoma cell lines by MTT assay (data not shown). Using a cell invasion and migration assay with Boyden chamber, it was shown that the number of invaded cells was significantly reduced in JJ cells following alendronate treatment (Fig 1). Alendronate inhibited the invasive and migration ability in a dose- and time-dependent manner (Fig 2A-D). At 100 µg/ml dose for 24 hr, alendronate reduced the invasion ability of JJ cell to 21.3% of controls (= untreated cells, set to 100%). Furthermore, the expression levels of MMP-2 protein decreased in JJ cells following alendronate treatment (Fig 1). Alendronate inhibited MMP-2 mRNA expression in JJ cells in a dose-dependent manner by RT-PCR (Fig 4).

Discussion:
Our study demonstrates alendronate could not induce significant cytotoxicity and apoptosis in human chondrosarcoma cell lines as other malignancy. But alendronate significantly reduce MMP-2 secretion and inhibit tumor cell invasion in chondrosarcoma cell lines. With an understanding of the underlying mechanism, alendronate, one of bisphosphonates, may have a potential role as for the systemic therapy of chondrosarcomas, as well as other malignant diseases.

Fig. 1. The effect of alendronate on chemo invasion of chondrosarcoma cell lines by Matrigel invasion assay. Invaded cells were stained with Giemsa.

Fig. 2A-D. Concentration- and time-dependent effects of alendronate on migration and invasion of JJ cells. A-B show the result in concentration-dependent assays at different concentration for 24 h.; and C-D show the result in time-dependent assays of 60 µg/ml for 12, 24 and 48 h. (*P < 0.05; **P < 0.01; ***P < 0.001).

Fig. 3. Effects of alendronate on the expression of MMP-2 by gelatin zymography.

Fig. 4. Effects of alendronate on the expression of MMP-2 mRNA level by RT-PCR.