INTRODUCTION:
We reported that third-generation bisphosphonates (BPs), zoledronic acid (ZOL) significantly inhibit the in vitro growth of human fibrosarcoma cell lines in a time- and dose-dependent manner by preventing prenylation of small GTPases and inducing apoptosis (53st ORS). To investigate the effect of BPs further, we examined the way to augment the effect of BPs by combination with radiation.

MATERIALS AND METHODS:
We used third-generation BPs, ZOL. Cultured cells were irradiated with 20-800cGy X-Ray using SOFTEX M-150WE (SOFTEX CO., LTD., Tokyo, Japan). The irradiation conditions selected were a distance of 1cm from the focus to the specimen, an irradiation rate of 0.5Gy/min in air. We used human fibrosarcoma cell lines, HT1080 and human synovial sarcoma, Syo-1.

Concurrent exposure to ZOL and other anti-cancer drugs
Cell growth and viability was evaluated by the MTT assay method. The IC50 values were obtained by using the nonlinear regression program CalcuSyn (Biosoft, Cambridge, United Kingdom).

(1) Growth inhibitory effects of ZOL with anti-cancer drugs
To investigate the combinable effect of ZOL with other anticancer agents, the HT1080 cells were treated with 1.5 µM, which is about IC50 concentration, of ZOL alone, an anti-cancer drug alone and the ZOL/anti-cancer drug combination in 6-well plates for 72h. The concentrations of these anticancer drugs were 1.0 × IC50 and 0.5 × IC50 of each. Proliferation of the cell lines were determined using the trypan blue dye exclusion method. The results are shown as mean ± SD.

(2) Cell cycle analysis and induction of apoptosis
Untreated HT1080 cells or HT1080 cells treated with ZOL alone, an anti-cancer drug alone and the ZOL/anti-cancer drug combination for 72 h were analyzed for alterations in the cell cycle by staining with Hoechst 33258 blue dye exclusion method of the specimen, an irradiation rate of 0.5Gy/min in air. We used human fibrosarcoma cell lines, HT1080 and human synovial sarcoma, Syo-1.

RESULTS:
(1) Cytotoxic interactions from concurrent exposure of cells to ZOL and other anti-cancer drugs
When combined with concentration of 0.5 × IC50 values, and 1.0 × IC50 values of doxorubicin, cisplatin, etoposide, 5fluorouracil, docetaxel, paclitaxel, and gemcitabine, growth inhibitory effects was augmented compared to by ZOL alone or by anti-cancer drugs alone (Figure 1). In contrast, when combined with methotrexate, P-value exceeded 0.05, indicating that combination with methotrexate produces antagonistic rather than additive effects. Only MTX, combined effect was not acquired.

(2) Effects of ZOL and other anti-cancer drugs on the cell cycle and induction of cell death
The Sub-G1 in flow cytometric analysis was also increased by combined treatment ZOL with other anti-cancer drugs excluded methotrexate. In the histogram, G1, S and G2/M phase was not so changed by combined treatment compared to by each drugs alone. (Figure 2, table 1).

DISCUSSION:
We reported that third-generation BPs, ZOL significantly inhibit the in vitro growth of human fibrosarcoma. But in clinical practice, administration of ZOL (4mg in 100ml over 15 minutes) resulting a peak systemic concentration, and the drug is rapidly cleared from the circulation within 1-2 hours. It is therefore likely that peripheral tumors are exposed to low concentration of ZOL for only a few hours. So, when using ZOL in practice, combined treatment with other agents should be encouraged, we suppose.

The aim of this study was to determine whether the anti-tumor effect of BPs can be strengthened when it was combined with other anti-tumor drugs.

In this present study, we found that combination of ZOL with etoposide has strong antitumor effect against human fibrosarcoma cells compared to the use of any of these agent alone. These results may have therapeutic application, particularly for enhancing the efficacy of a drug that cannot be administered at higher dosages because of its toxicity. Therefore, we may be able to combine ZOL with the present fibrosarcoma chemotherapy regimes. In addition, the anti-growth activities of doxorubicin, cisplatin, 5fluorouracil, docetaxel, paclitaxel and gemcitabine were also increased by ZOL. Therefore, ZOL may have therapeutic application in enhancing the efficacy of these drugs such that their effective doses against other malignant neoplasms, such as osteosarcoma, lung cancer and ovarian cancer, can be reduced. Although we did not actually determine whether the combination

Table 1: The sub-G1 in flow cytometry. A—H: similar to figure 1.

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overcomes the multi-drug resistance system, these results appear to offer benefits to fibrosarcoma patients.

In conclusion, our results indicated that the combination of ZOL with doxorubicin, cisplatin, etoposide, 5-fluorouracil, docetaxel, paclitaxel, and gemcitabine may have more effectiveness against human fibrosarcoma than the use of any of these drugs alone.