SYNERGISTIC EFFECT BY COMBINATION OF MELOXICAM, A COX-2 INHIBITOR, AND CYTOTOXIC AGENTS ON HUMAN OSTEOSARCOMA CELL LINE, MG-63.

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Introduction
Numerous studies have demonstrated the involvement of cyclooxygenase-2 (COX-2) in carcinogenesis of many types of carcinoma. Osteosarcoma, one of the most common primary malignant bone tumor, is also reported to express COX-2 constitutively (1). COX-2 is an inducible enzyme that catalyzes the synthesis of prostaglandins (PGs), and among them, PGE2 can enhance tumor growth and metastasis by stimulating angiogenesis and invasiveness, and by inhibiting apoptosis. Various types of COX-2 inhibitors have been reported to suppress carcinoma cells, however, there have been few studies that revealed efficacy of COX-2 inhibitors against bone and soft tissue malignant tumors.

Chemotherapeutic agents improved prognosis of osteosarcoma patients, however, still not a few patients of osteosarcoma result in lung metastasis after chemotherapy. New therapeutic strategies should be necessary for the patients. Several authors described synergistic effect of combination of COX-2 inhibitor and traditional cytotoxic agents against carcinoma cells (2,3).

Meloxicam, a preferential COX-2 inhibitor, has been commercially available as a NSAID. It is demonstrated that meloxicam inhibits the growth of colorectal cancer cells (4), as well as human osteosarcoma cell line, MG-63 (5). In this study, we aimed to assess synergistic effect of combination of meloxicam on cell viability of MG-63 cells.

Materials and Methods
Human osteosarcoma cell line, MG-63 (American Type Culture Collection, VA), was monolayer cultured in DMEM/10%FBS with meloxicam or various cytotoxic agents; meloxicam: at concentration ranged from 5 to 750 \( \mu \)g/ml, Cisplatin (CDDP. Bristol Pharmaceuticals K.K., Japan): from 1 to 20 \( \mu \)g/ml, doxorubicin hydrochloride (DXR. Boeringer Mannheim, Germany) at 24 hours to plot dose-response curves of each agent. Then each anti-tumor agent (CDDP: 1 or 5 \( \mu \)g/ml, DXR: 0.5 or 0.75 \( \mu \)g/ml, IFM: 1 or 5 \( \mu \)g/ml) was administrated into MG-63 cell culture together with 30 or 50 \( \mu \) M of meloxicam and MTT assay was performed again at 24 hours to evaluate synergistic effect on cell viability. Synergy was determined by isobolographic analysis (6).

TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling) staining was performed to evaluate apoptotic effect evoked by combination of meloxicam and cytotoxic agents using an In Situ Cell Death Detection Kit, POD (Roche Diagnosis, PA). All of the experiments were performed more than triplicate. The ANOVA and the follow the post hoc test (Bonferroni/Dunn) were used to compare the difference between means. P value less than 0.05 was considered as significant.

Results
Cytotoxicity of all these 3 conventional anti-tumor agents was enhanced by meloxicam significantly (p<0.05), except in the sample treated by combination of IFM and 30 \( \mu \) M of meloxicam. However, the enhancement should be further analyzed by isobologram to distinguish synergistic effect from additive effect. Isobolographic analysis clearly revealed supra-additive effect on cell viability by combination of CDDP and meloxicam (Fig.1). Combination of DXR and meloxicam also showed synergy but slightly weaker than that of CDDP. In contrast, combination of activated form IFM and meloxicam presented no synergy but slightly weaker than that of CDDP. In contrast, combination of activated form IFM and meloxicam (Fig.2). Both 30 and 50 \( \mu \) M of meloxicam showed synergy in combination of CDDP or DXR, this effect is more prominent with treatment of 50 \( \mu \) M meloxicam than 30 \( \mu \) M.

TUNEL staining revealed significant synergistic pro-apoptotic effect by CDDP-meloxicam combination compared with single administration of CDDP. Combination of DXR and meloxicam induced additive pro-apoptotic effect slightly compared with single administration of DXR. In contrast, combination of IFM and meloxicam showed no synergistic pro-apoptotic effect.

Discussion
In this study, we showed the meloxicam, a preferential COX-2 inhibitor, could enhance cytotoxicity of traditional anti-tumor agents against human osteoblastic osteosarcoma cell line, MG-63. Especially, CDDP and DXR have a remarkable synergistic effect with meloxicam by isobolographic analysis, however cytotoxic effect was not enhanced synergistically by IFM with meloxicam. Limitation of this study is that osteosarcoma is composed of heterogenous lesions among patients, suggesting that synergistic effects of meloxicam and CDDP or DXR might not be observed in other osteosarcoma cell lines. Several authors reported synergy of cytotoxic agents and COX-2 inhibitors. Bladder cancer cell line, T24, is suppressed by combination of CDDP and JTE-522, a COX-2 inhibitor, with synergy. Down-regulation of Bcl-2 expression and up-regulation of intra-cellular accumulation of CDDP by JTE-522 are suggestive of synergistic apoptosis (2), however precise mechanisms are not understood. In this study, the synergistic cytotoxic effects might be due to not only enhancement of pro-apoptotic effects but also other mechanisms. Further investigation should be necessary.

Prognosis of osteosarcoma patients has been improved by multi-drug chemotherapy in these several decades. However, considering the 50% of the patients still develop distant metastasis, new therapeutic regimen should be necessary for them. Synergistic effects of cytotoxic agents and meloxicam, commercially available COX-2 inhibitor, might be expected as a new tool for enhancement of the cytotoxic effects in osteosarcoma patients.

2: Mizutani Y et al, J Urology 172: 1474-1479, 2004

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