SYNERGISTIC EFFECT BY COMBINATION OF COX-2 INHIBITOR AND CYTOTOXIC AGENTS ON HUMAN CHONDROSARCOMA CELL LINE

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Introduction: Numerous studies have demonstrated the involvement of cyclooxygenase-2 (COX-2) in carcinogenesis of many types of carcinoma. Chondrosarcoma, the common primary malignant bone tumors, is also reported to express COX-2 constitutively. COX-2 catalyzes the synthesis of prostaglandins (PGs), PGE2 which can enhance tumor either in its growth or metastasis by stimulating angiogenesis, invasiveness, inhibiting apoptosis. There have been various types of COX-2 inhibitors that were reported to suppress carcinoma cell. Surgical resection remains the primary mode of therapy for chondrosarcoma, these mesenchymal malignancies have a poor prognosis due to the absence of an effective adjuvant therapy. New therapeutic strategies should be necessary for the patients. Synergistic effect of combination of COX-2 inhibitor and traditional cytotoxic agents against carcinoma cells has been reported. Meloxicam, a COX-2 inhibitor, has been commercially available as a NSAID, was reported to inhibit the growth of colorectal cancer cells as well as human osteosarcoma cell line. In this study, we aimed to assess synergistic effect of combination of meloxicam on cell viability of chondrosarcoma cell.

Materials and Methods: Human chondrosarcoma cell line (JJ012) was cultured and treated with meloxicam or various cytotoxic agents; meloxicam: at concentration ranged from 50μM, cisplatin (CIS): from 0 to 50μM, doxorubicin hydrochloride (DOX): from 0 to 100μM, and ifosphamide (IFM): from 0 to 100μM. Cell survival in each sample was analyzed by MTT assay at 48 hours to plot dose-response curves of each agent. Then each anti-tumor agent (CIS: 0-50μM, DOX: 0-100μM, IFM: 0-100μM) was administrated into JJ cell culture together with 50μM of meloxicam and MTT assay was performed again at 48 hours to evaluate synergistic effect on cell viability. Flow cytometry was performed to evaluate apoptotic effect by combination of meloxicam and cytotoxic agents. Western blot analysis was used to identify the pathway of cell cycle arrest. All experiments were run in duplicate. Student's t-test is used for statistics evaluation.

Results: The cell viability of chondrosarcoma cells were decreased significantly in the combination of meloxicam and DOX or CIS, but no significant effect in IFM (Fig 1). Combination of CIS and meloxicam showed synergy but slightly weaker than that of DOX. Flow cytometry revealed the inhibition of proliferation by inducing cell cycle arrest at G2/M phase (Fig 2). Western blots identify the significant expression in p21 and p27 of chondrosarcoma cells after combination of meloxicam and DOX or CIS (Fig 3).

Discussion: In this study, we showed the meloxicam, a COX-2 inhibitor, could enhance cytotoxicity of traditional anti-tumor agents against human chondrosarcoma cell line. Especially, CIS and DOX have a remarkable synergistic effect with meloxicam. Synergy of cytotoxic agents and COX-2 inhibitors has been reported in bladder cancer line, T24. Down-regulation of Bcl-2 expression and up-regulation of intra-cellular accumulation of CIS by JTE-522 (COX-2 inhibitors) are suggestive of synergistic apoptosis in T24, however precise mechanisms are not understood. In our study, the inhibition of JJ cells proliferation after combined treatment by inducing cell cycle arrest at G2/M phase. The significant expression of p21 and p27 demonstrated related change of CDK during cell cycle. The results of commercially available COX-2 inhibitor, might be expected as a new tool for enhancement of the cytotoxic effects in chondrosarcoma patients. Further investigation should be necessary.