Short Communication

Antitumor Activity of A Novel 9-Aminoanthracycline, Amrubicin, Alone and in Combination with Celecoxib against Human Malignant Mesotheliomas *In vitro*

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Abstract

Pleural mesothelioma is an asbestos-related malignancy that is highly resistant to current therapeutic modalities. Survival of patients with malignant mesothelioma, especially in the advanced stages, is very poor despite recent advances in chemotherapeutical modalities including the combination of cisplatin and antifolate. In this study, we evaluate the effects of amrubicin, a novel 9-aminoanthracycline, and the cyclooxygenase-2 inhibitor celecoxib for malignant mesothelioma. Seven cell lines derived from malignant mesothelioma were tested. Dose-dependent inhibition of proliferation was observed in all seven cell lines, and the adjunct use of celecoxib was shown to significantly enhance efficacy of amrubicin. Thus, the use of amrubicin is promising, and its combination with a selective COX-2 inhibitor may be of use in the treatment of malignant pleural mesothelioma.

ABBREVIATIONS

COX-2: Cyclooxygenase 2; IC_{50}: 50% inhibiting dose; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

INTRODUCTION

Malignant mesothelioma is a fatal cancer of increasing incidence associated with asbestos exposure. Malignant mesothelioma responds poorly to surgery, chemotherapy, and radiotherapy, and has an appalling prognosis [1]. Most patients have unresectable disease and patient survival is very poor (median survival 7-11 months after diagnosis), especially in advanced-stage patients, regardless of a recent advance in chemotherapeutic modalities that combines cisplatin and pemetrexed [2]. New strategies based on better understanding of the biology are clearly needed to improve the treatment efficacy of this disease.

Anthracyclines have a potent antitumor activity and have been widely used in the treatment of cancers such as acute leukemia, malignant lymphoma, and solid tumors. Amrubicin hydrochloride, a completely synthetic anthracycline derivative characterized by a 9-amino group and a simple sugar moiety, exhibited both greater efficacy in human tumor xenografts and lower cardiotoxicity than doxorubicin in preclinical studies [3-5]. In previous phase II trials, amrubicin has been shown to have a response rate of approximately 20% against non-small-cell lung cancer, and a response rate exceeding 75% against untreated extensive-disease small-cell lung cancer [6,7]. Previous studies have demonstrated that both amrubicin and its 13-hydroxyl metabolite amrubicinol interact with DNA and inhibit DNA topoisomerase II by stabilizing the cleavable complex [8]. Amrubicinol is 5-100 times more active than amrubicin [9].

Accumulating evidence suggests that an increase in the expression of cyclooxygenase-2 (COX-2), a key inducible enzyme involved in the production of prostaglandins and other icosanoids, might play a significant role in carcinogenesis in

addition to its well-known role in inflammatory reactions [10-15]. Elevated expression of COX-2 is also associated with the progression of established human cancers including breast, colon, esophagus, liver, lung, pancreas, prostate, cervix, and head and neck. Although there is often broad variation in COX-2 expression in tumors, overexpression is generally associated with a more malignant phenotype. In many cancers, COX-2 overexpression is associated with aggressive tumor behavior, worse prognosis and the development of metastatic disease [16,17]. Edwards showed that COX-2 was expressed in malignant mesothelioma and that increasing levels of COX-2 protein were a poor prognostic factor [18].

In this study, we examined whether a novel 9-aminoanthracycline, amrubicin, can inhibit the proliferation of malignant mesothelioma cells, which are notoriously resistant to chemotherapy, and also investigated whether its efficacy was enhanced by the adjunct use of celecoxib.

MATERIALS AND METHODS

Cell lines

Seven malignant pleural mesothelioma cell lines, ACC-MESO-1 and -4, and Y-MESO-8A, -8D, -9, -12, -14, were established in our laboratories at Aichi Cancer Center [19-22]. All mesothelioma cell lines were maintained in RPMI-1640 medium (Sigma-Aldrich, Irvine, UK) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and 1× antibiotic-antimycotic (Invitrogen) at 37°C in a humidified incubator with 5% CO_2. Samples and clinical data were collected after obtaining appropriate institutional review board approval and written informed consent from all patients.

Agents

Amrubicin and its active *in vivo* metabolite amrubicin-13-OH (amrubicinol), provided by Dainippon Sumitomo Pharmaceutical Co., Osaka, Japan (Nippon Kayaku Co., Ltd.), were dissolved in water. Celecoxib, a selective COX-2 inhibitor, came from Astellas Pharma Inc. (Tokyo, Japan).

MTT assay for chemosensitivity

To evaluate chemosensitivity, an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed using the Cell Titer 96™ kit (Promega Corp., Madison, WI). Briefly, cells were plated in 96-well plates and exposed continuously for 4 days to a range of concentrations of amrubicinol and/or celecoxib. At least three independent experiments were carried out in quadruplicate.

RESULTS AND DISCUSSION

The colorimetric cell proliferation assay was first performed to examine the *in vitro* antitumor effects of amrubicinol as a single agent. Significant growth suppression was observed at clinically achievable concentrations of amrubicinol (range: 0.14-0.64 µM), showing dose-dependent inhibition of the proliferation of seven malignant pleural mesothelioma cell lines (Figure 1).

The effects of the COX-2 inhibitor celecoxib on the growth of the mesothelioma cell lines were also examined (Figure 2). Celecoxib, at relatively high concentration, induced dose-dependent inhibition of the proliferation of mesothelioma cell lines. Interestingly, the use of 200 µM celecoxib, which is a concentration clinically achievable with injection to thoracic cavity, inhibited cell growth by over 80%.

The inhibitory effects of celecoxib at 0, 10, 100 µM were evaluated in combination with a low concentration of amrubicinol (0.02 µM) in Y-MESO-9 cells: significant growth inhibition was observed. Amrubicinol alone resulted in a reduction of 15%; however, 45% and 80% growth inhibition was observed in combination with celecoxib 10 µM and 100 µM, respectively (Figure 3A).

An isobologram [23] was constructed, based on the dose-response curves in Y-MESO-9, to examine the synergistic effects of amrubicinol and celecoxib. A supra-additive effect was observed with 10 µM of celecoxib (Figure 3B), chosen because this is equivalent to the concentration in human plasma following daily consumption of 800 mg celecoxib [24]. The mechanism of
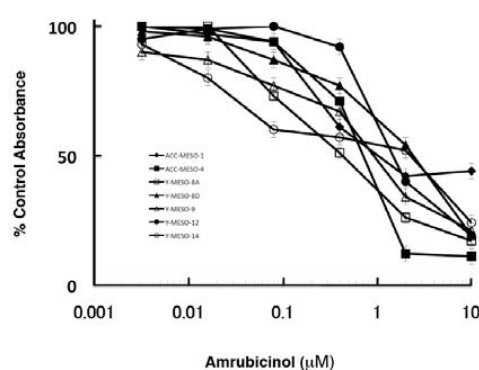

Figure 1 Sensitivity to amrubicinol measured by MTT assay. Dose-dependent inhibition of the proliferation of seven mesothelioma lines in response to increasing amrubicinol concentration.
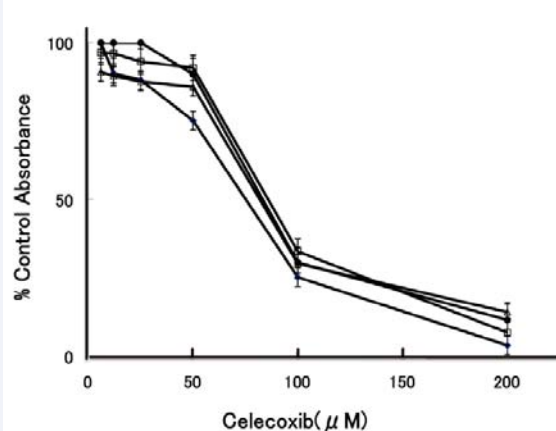

Figure 2 Sensitivity to a COX-2 inhibitor, celecoxib, measured by MTT assay. Dose-dependent inhibition of the proliferation of mesothelioma lines in response to increasing celecoxib concentration.
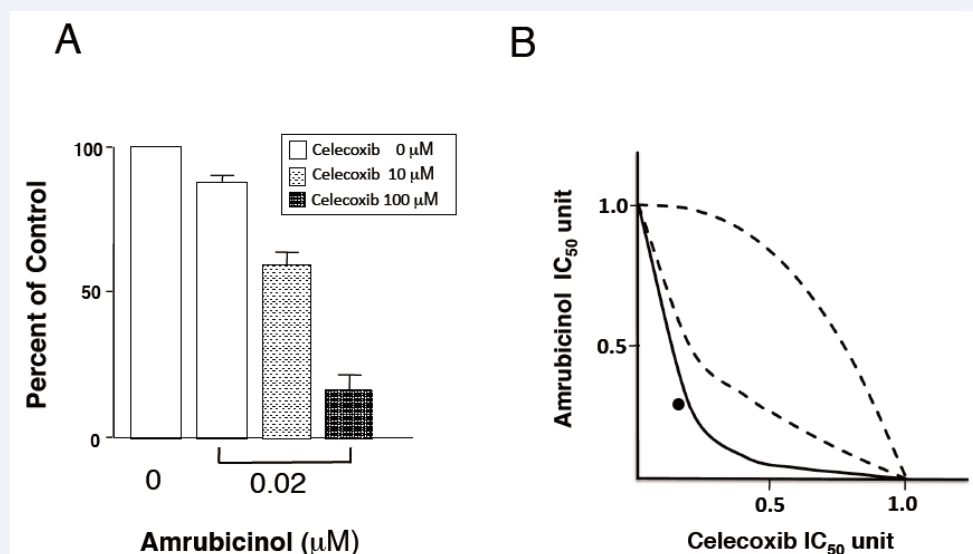
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Figure 3 Sensitivity of Y-MESO-9 cells to amrubicinol in combination with celecoxib (A), as well as IC$_{50}$ isobologram of the use of celecoxib as an adjunct with amrubicinol (B).
- A.   Inhibition of Y-MESO-9 cells by amrubicinol alone or in combination with celecoxib.
- B.   Supra-additive enhancement of chemosensitivity to amrubicinol is seen as a result of the adjunct use of celecoxib at 10 µM (●) in Y-MESO-9 cells. Solid and broken lines represent Mode I and Mode II in the IC$_{50}$ isobologram, constructed from dose-response curves of celecoxib and amrubicinol. The area delineated by Mode I and Mode II lines represents the envelope of additivity, while the area below this envelope represents the supra-additive area and the area above it the sub-additive area.

this enhancement needs to be studied in more depth. Similar results were obtained using Y-MESO-8A cells (data not shown).

CONCLUSION

Pleural mesothelioma is a fatal malignancy most often associated with asbestos exposure, and current approaches, ranging from aggressive surgical treatment to chemotherapy, have not improved the prognosis of this disease. In this study, significant growth suppression was observed with amrubicinol at a clinically achievable concentration. Our study also showed that a COX-2 inhibitor, celecoxib, could inhibit proliferation of mesothelioma cells *in vitro* in a dose-dependent manner, and, when used in combination, celecoxib reduced the IC$_{50}$ values of amrubicinol. Thus, amrubicin and COX-2 inhibitor in combination hold promise in the treatment of mesothelioma. Edwards reported significantly increased COX-2 expression in mesothelioma, so our findings are of great clinical interest. It is especially encouraging to think that it might be possible to enhance the anticancer activity without compromising quality of life by treatment with this type of selective inhibitor, although further studies are necessary to generalize our findings.

ACKNOWLEDGMENT

Conflict of Interest

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