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Comparative Studies of Adriamycin and 28-Deacetyl Sendanin on *In Vitro* Growth Inhibition of Human Cancer Cell Lines

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The limonoid compound (28-deacetyl sendanin) isolated from the fruit of *Melia toosendan* SIEB. et ZUCC. was evaluated on anticancer activity. According to a standard *in vitro* cytotoxicity assay, eight human cancer cell lines and SRB assay were introduced for present evaluation. As a positive standard, adriamycin was tested in parallel. The cell lines were originated from six different organs. In view of dose-response profiles to 28-deacetyl sendanin, the most sensitive cells were SF-539 and PC-3 which were derived from CNS and prostate, respectively. In contrast, all the cell lines responded similarly to adriamycin to give rise to nearly identical dose-response profiles. By comparison of GI_{50} between 28-deacetyl sendanin and adriamycin, six cell lines were more sensitive to 28-deacetyl sendanin and two were more resistant. As a result, 28-deacetyl sendanin had more sensitive and selective inhibitory effects on *in vitro* growth of human cancer cell lines in a comparison with adriamycin.

Key words: 28-deacetyl sendanin, Limonoid, *Melia toosendan* SIEB. *et* ZUCC., Anticancer, human cancer cells, SRB assay, Adriamycin

INTRODUCTION

In our continuing effort to search for novel antitumor compounds from natural products, a limonoid, 28-deacetyl sendanin has purified from *Melia toosendan* SIEB. *et* ZUCC. directed by its activity or morphological alteration of a human breast cancer cell line, MCF-7 at lower dose but cytotoxic at higher dose (Kim *et al.*, 1993). Numerous limonoids from *Meliaceae* plant including *Melia azedarach* and *Melia toosendan* have been reported (Tang *et al.*, 1992) and have recently attracted attention by their marked insect growth regulatory activities (Champagne *et al.*, 1992; Oelrichs *et al.*, 1983). Surprisingly, in a view of their toxic nature, there is few reports describing their antitumor effect, only to the murine leukemia cell line, P388 (Pettit *et al.*, 1983).

The use of multiple *in vivo* animal model for a disease-oriented primary screening of anticancer agents was known to be not practical in the scope of requirements for adequate screening capacity and specific tumor type representation. *In vitro* models, therefore, were selected as a universal method that explores the feasibility of using established human tumor cell lines for a disease-oriented primary drug screen (Chabner, 1993). The availability of wide variety of human tumor cell lines representing many different forms of human cancer seemed to offer an attractive basis for development of a disease-oriented *in vitro* primary screen (Monks *et al.*, 1991; Wu *et al.*, 1992). *In vitro* cytotoxicity assay on such a panel might classify new drugs as to nonspecific vs. specific (e.g., with respect to either individual cell lines or groups of cell lines) (Park *et al.*, 1987). However, because this, so-called disease-oriented screening system, is so labor- and money-consuming to maintain and operate, a reduced version of the system with more specific cell line will be worthwhile in the screening of crude extract made from natural products. This limonoid's interesting activity to MCF-7 cell line prompted us to test its *in vitro* antitumor activity. In present study, we applied small number of human cell line panel (including 8 cell lines derived from 6 different organs) and standard SRB assay to the evaluation of a new candidate of anticancer agent, 28-deacetyl sendanin. Their responsiveness was compared and discussed with authentic drug, adriamycin.

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MATERIALS AND METHODS

Materials

The limonoid compound, 28-deacetyl sendanin was isolated from the fruit of *Melia toosendan*. Its structure was determined by previous report (Kim *et al.*, 1993) and shown in Figure 1. Doxorubicin·HCl (adriamycin) was obtained from Farmitalia Co., Italy. RPMI1640 media was obtained from Gibco BRL. Dimethyl sulfoxide (DMSO) and other chemicals were purchased from Sigma Co., Ltd..

Cell Culture

Eight kinds of human tumor cell lines were used and all cell lines were obtained from NCI, USA. Cell line panels included M14, NCI-H23, SF-539, PC-3, SW 620, KM12, UO-31 and ACHN which were derived from six organs (i.e., skin, lung, central nervous system, prostate, colon and renal). The cells were routinely maintained in a humidified CO_2 incubator (95% air and 5% CO_2) at 37℃ and culture media was RPMI1640 containing 10% fetal calf serum (FCS). Cell lines kept under LN_2 until use and used at early passage as possible.

Chemical Treatment

According to cell lines, different cell numbers were inoculated to 96-well microtiter plate. Culture medium was RPMI1640 containing 5% FCS. Cell suspension was made in culture medium and 100 μl was inoculated to each well of 96-well microtiter plate. Initial cell loading densities were listed at Table 1. At 24 hours after plating, 28-deacetyl sendanin was treated in concentrations of 3, 1, 0.3, 0.1, 0.03, and 0.01 μg/ml. As a positive control, adriamycin was also included in concentrations of 10, 3, 1, 0.3, 0.1, and 0.03 μg/ml. To dissolve chemicals, DMSO was used and final concentration of solvent was fixed to be 0.1% (v/v). After treatment of chemicals, cells were incubated for further

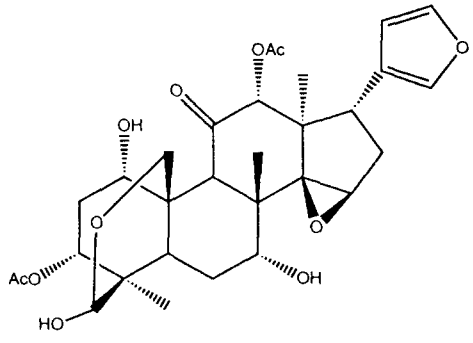

Fig. 1. Structure of 28-deacetyl sendanin, a new compound isolated from Melia toosendan SIEB. et ZUCC.

Table 1. Initial cell concentration of human cancer cell lines, and GI_{50} of 28-deacetyl sendanin (DAS) and adriamycin (ADR) against human cancer cell lines

Cell Lines	Origin	Initial Cell Density (cells/well)	GI_{50} (μg/ml)	
			DAS	ADR*
M14	Skin	15,000	0.017	0.389
NCI-H23	Lung	20,000	>3.000	0.111
SF-539	CNS	15,000	<0.010	0.545
PC-3	Prostate	10,000	<0.010	0.651
SW620	Colon	10,000	0.027	0.182
KM12	Colon	15,000	0.071	0.793
UO-31	Renal	15,000	>3.000	0.542
ACHN	Renal	15,000	0.028	0.748

*Mean GI_{50} of adrimycin was 0.495 μg/ml.

48 hours in a CO_2 incubator. Before treatment of chemicals, time zero (Tz) plate was made and cells were fixed with 50 μl of 50% TCA solution and preserved in dry state until SRB staining.

Assay Method

SRB assay was performed by previous method (Skehan *et al.*, 1990). After incubation, anchored cells were directly fixed by slow addition of 50 μl of 50% TCA solution per well. Fixation were proceeded for 1 hour at 4℃. After fixation, plates were washed 15 times with tap water and air-dried. 100 μl of SRB solution (0.4% in 1% acetic acid) was added to each well of 96-well microplate. Staining was done at room temperature for 30 minutes and residual dye was washed out with 1% acetic acid and air-dried. To each well, 100 μl of Tris solution (10 mM, pH 10.5) was added. Optical density (O.D.) was measured in microtiter plate reader at 570 nm.

Data Calculation

Growth inhibition was calculated according to the previous method (Wu *et al.*, 1992). Briefly, O.D. of treated well was subtracted O.D. at time-zero(Tz) plate and divided by calculated value of untreated control. Growth inhibition of 50% (GI_{50}) was calculated by Probit method (Litchfield and Wilcoxon, 1949).

RESULTS AND DISCUSSION

A limonoid, 28-deacetyl sendanin was treated at six different doses and its dose-responses were evaluated on each cell line. Its dose-response profiles were shown at Figure 2. At present dose range, tested cell lines showed quite different patterns of response. Cell lines were divided into three groups according to the chemosensitivity against 28-deacetyl sendanin. SF-539 and PC-3 responded sensitively to the compound. At dose range from 0.1 to 3.0 μg/ml, no growth was ob-

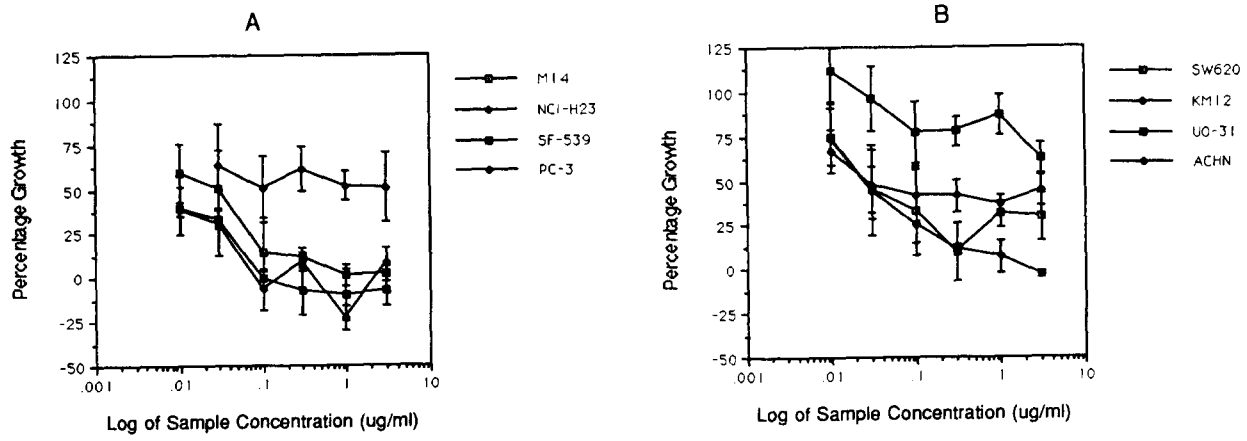

Fig. 2. Dose-response profile of *in vitro* growth inhibition induced by 28-deacetyl sendanin. All human cell lines (A and B) were inoculated at designated cell concentration and chemical exposure was done for 48 hours. Chemical concentrations were 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 μg/ml. Cell concentration was determined by SRB assay after TCA fixation. Data were expressed as mean± SD of four determinations.

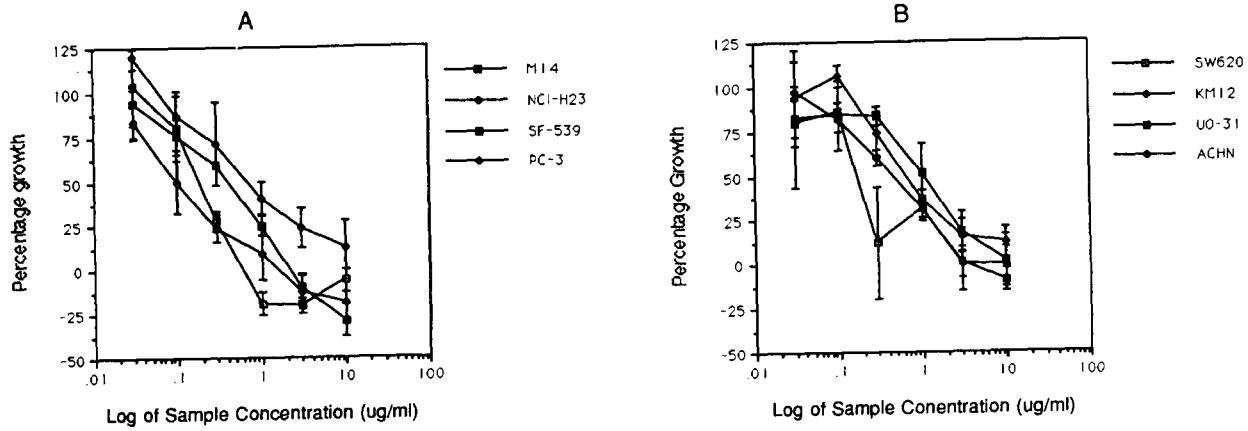

Fig. 3. Dose-response profile of *in vitro* growth inhibition induced by adriamycin. All human cell lines (A and B) were inoculated at designated cell concentration and chemical exposure was done for 48 hours. Chemical concentrations were 0.03, 0.1, 0.3, 1.0, 3.0, and 10.0 μg/ml. Cell concentration was determined by SRB assay after TCA fixation. Data were expressed as mean± SD of four determinations.

served after 48 hours. Rather slight cytotoxicity was appeared. At low doses (0.03 and 0.01 μg/ml), about 70% of growth inhibition was observed. Moderately effected cell lines included M14, SW620, KM12 and ACHN. The slopes of dose-response curves were gentle. However, KM12 was more resistant compared with other cell lines in this group. Resistant cell lines were NCI-H23 and UO-31, only about 30-40% of growth inhibition was observed at high doses (0.1 and 3.0 μg/ml). The calculated doses of 50% growth inhibition (GI_{50}) for 28-deacetyl sendanin were shown in Table 1. As shown in dose response relationship, the highest GI50 was observed in NCI-H23 and UO-31 and the lowest in SF-539 and PC-3. The difference of GI50 between sensitive and resistant cell lines was more than 300-fold. On the contrary to 28-deacetyl sendanin, human cancer cell line panel responded differently to adriamycin. As shown in Figure 3, adriamycin showed similar pattern and slope of dose response curve to nearly all the cell lines tested. The calculated GI_{50} value was shown in Table 1. The highest GI_{50} was observed in KM12, colon cancer cell line and the lowest in SNB-19, CNS cancer cell line. The difference of GI_{50} between these two cell lines was at most 25-fold. The calculated mean GI_{50} for adriamycin was 0.495 μg/ml. By comparison of GI_{50} between 28-deacetyl sendanin and adriamycin, 6 cell lines were more sensitive to 28-deacetyl sendanin and two were more resistant.

It is well known that the trend for the development of new anticancer agent are shifted to selection of cell line or organ specific chemicals (Chabner, 1993). In view of this trend, 28-deacetyl sendanin has two promising results in present experiment. First, 28-dea-

cetyl sendanin induced more potent growth inhibition in some cell lines. Sensitive cell lines showed about 30-fold lower GI_{50} than that of adriamycin. Another one is that this compound showed differential cell line specificity. In conclusion, 28-deacetyl sendanin strongly suppressed *in vitro* growth of SF-539 and PC-3 which were derived from CNS and prostate, respectively. Eventhough more studies should be done for the accurate evaluation as an anticancer drug, 28-deacetyl sendanin showed promising results in present study by showing the selective inhibition on the growth of human cancer cell lines.

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