Inhibitory Effect of *cis*-Dehydromatricaria Ester Isolated from *Solidago altissima* on the Growth of Mammalian Cells

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A cell growth inhibitory substance was isolated from *Solidago altissima*, which is known as a naturalized weed. The data of infrared spectrum, proton and carbon-13 nuclear magnetic resonance spectrum, and mass spectrum were identical with those of *cis*-dehydromatricaria ester. The compound inhibited both tumor and normal mammalian cells.

Keywords *cis*-dehydromatricaria ester; polyacetylene compound; *Solidago altissima*; cell growth inhibitory activity

In previous papers,[1] we reported that panaxytriol, which is a polyacetylene compound in red ginseng, is a tumor growth inhibitory substance. The root of prolific *Solidago altissima*, which is known as a naturalized weed, also contains polyacetylene compounds. It has been reported that one of the polyacetylene compounds in *Solidago altissima*, *cis*-dehydromatricaria ester (*cis*-DME), inhibited the growth of a rice seedling.[2a] This experiment was performed to examine whether or not polyacetylene compounds in *Solidago altissima* inhibit the growth of mammalian cells *in vitro*.

Results and Discussion

The roots of *Solidago altissima* were extracted with ethyl acetate (AcOEt). The crude AcOEt extract inhibited *in vitro*-cell growth of human gastric adenocarcinoma MK-1 cells. In order to isolate active compounds from the AcOEt extract, the extract was first applied to chromatography on a silica gel column. Finally, an active compound was crystallized from *n*-hexane, giving pale yellow needles, mp 112—115 °C.

From infrared (IR), proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and mass (MS) spectrum, the substance was identified as *cis*-DME, previously described by several investigators.[2]

$$\overset{1}{CH_3}(\overset{2}{C}\equiv\overset{7}{C})_3\overset{8}{CH}=\overset{cis\ 9}{CH}\overset{10}{C}O\overset{11}{O}CH_3$$

cis-dehydromatricaria ester (*cis*-DME)

It is difficult to examine the effect of *cis*-DME on *in vitro*-cell growth because of its water-insolubility. In order to make *cis*-DME water-soluble, the solid complex (*cis*-DME/CD) of *cis*-DME with α-cyclodextrin (CD) was prepared. The *cis*-DME/CD was added to a RPMI-1640 culture medium, and sonicated for 3 min. The solution was sterilized through a 0.22 μm filter. The concentration of *cis*-DME in the RPMI-1640 culture medium was determined by high performance liquid chromatograhy (HPLC).[3]

The effect of *cis*-DME on cell growth was examined *in vitro* using various kinds of cultured cells. MK-1 human gastric adenocarcinoma cells, L-929 mouse fibroblast-derived tumor cells, B-16 mouse melanoma cells, and MRC-5 human embryo-derived fibroblasts were used as target cells. The concentration (0.5 mg/ml) of CD in the culture medium had no effect on the cell growth.

As shown in Table I, the concentrations of *cis*-DME required to give 50% growth inhibition (ED_{50}) were 0.59, 0.98, 1.87 and 2.70 μg/ml against MK-1, L-929, B-16 and MRC-5 cells, respectively. The activity of *cis*-DME against tumor cells was nearly equal to that of panaxytriol isolated from red ginseng, as indicated in our previous reports.[1] Although the ED_{50} of panaxytriol against human fibroblasts, MRC-5 cells, was over 40 μg/ml, that of *cis*-DME was 2.70 μg/ml. Namely, tumor-specificity in cell growth inhibition of *cis*-DME is lower than that of panaxytriol.

TABLE I. Effect of *cis*-DME on Cell Growth *in Vitro*

Cell line[a]	ED_{50} (μg/ml)[b]
MK-1	0.59±0.18
L-929	0.98±0.12
B-16	1.87±0.13
MRC-5	2.70±0.14

Fifty microlitres of cell suspension (1×10^5 cells) and 50 μl of *cis*-DME solution were plated in flat-bottomed microtiter wells and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Percent growth inhibition

$$=\left(1-\frac{\text{no. of viable cells in medium with } cis\text{-DME}}{\text{no. of viable cells in medium without } cis\text{-DME}}\right)\times 100\,.$$

a) MK-1 (human gastric adenocarcinoma); L-929 (mouse fibroblast-derived tumor); B-16 (mouse melanoma); MRC-5 (human fibroblast). *b*) ED_{50} is the concentration of *cis*-DME required to obtain a 50% growth inhibition. Mean±S.D. of three experiments.

Conclusion

In this study, *cis*-DME isolated from *Solidago altissima* inhibited the growth of several kinds of mammalian cells *in vitro*. This paper is the first report concerning the effect of *cis*-DME on the growth of mammalian cells.

Experimental

The melting point was taken on a Yanagimoto micromelting point apparatus and is uncorrected. The IR spectrum was recorded with a Hitachi 270-30 spectrometer, the ultraviolet (UV) spectum with a Shimadzu UV-240 spectrometer, ^{1}H- and ^{13}C-NMR spectra with a JEOL JNM-GX400 spectrometer (with tetramethylsilane as an internal standard, $CDCl_3$ solvent) and MS with a Hitachi M-2000. For the sonication, an Astrason W-385 sonicator was used. HPLC was done with a Shimadzu LC-6A high performance liquid chromatograph. Column chromatography was carried out on Silica gel 60 (100—200 mesh, Nakarai). Thin-layer chromatography (TLC) was performed on Kiesel gel 60 plates (E. Merck). The spots were detected by spraying the plates with concentrated H_2SO_4 and by heating.

Extraction and Isolation of *cis*-DME A root of *Solidago altissima* (1.3 kg) was extracted with AcOEt (6000 ml) for 24 h at room temperature. The AcOEt extract was evaporated and a residue (26.0 g) was fractionated by column chromatography (SiO_2, 350 g) using chloroform as the eluant. Fractions containing *cis*-DME (3.8 g) were further fractionated by column chromatography (SiO_2, 120 g) using ether–*n*-hexane (2:8, v/v) to give crude *cis*-DME. The crude *cis*-DME was crystallized from *n*-hexane, giving

pale yellow needles (210 mg).

cis-DME: UV $\lambda_{max}^{methanol}$ nm (ε): 208 (3.29×10^4), 244 (4.14×10^4), 254 (5.20×10^4), 285 (5.43×10^3), 302 (1.12×10^4), 322 (1.64×10^4), 345 (1.25×10^4). IR ν_{max}^{KBr} cm^{-1}: 2250—2190 (C≡C), 1720 ($COOCH_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.01 (3H, s, H-1), 3.78 (3H, s, H-11), 6.15 (1H, d, J=11 Hz, H-8), 6.27 (1H, d, J=11 Hz, H-9). ^{13}C-NMR ($CDCl_3$) δ: 4.72 (C-1), 51.68 (C-11), 58.47, 64.89, 70.04, 72.19, 80.76, 86.09 (acetylenic carbons), 124.44 (C-8), 132.56 (C-9), 164.52 (C-10). MS *m/z* (%): 173 ($(M+1)^+$, 100), 149 (36), 142 (14), 132 (84), 113 (34).

Preparation of Solid Complex The *cis*-DME (25 mg) was dissolved in acetone (0.2 ml) and was then added to CD in an aqueous solution (146 mg/1.5 ml). The mixture was vigorously shaken for 24 h at room temperature and was evaporated. The obtained crystalline powder was dried under vacuum for 24 h at 40 °C. The complex was dissolved in a RPMI-1640 culture medium. The concentration of *cis*-DME in the culture medium was determined by HPLC.

Antitumor Activity Fifty microliters of cell suspension (1×10^5 cells) on RPMI-1640 culture medium containing 20% fetal calf serum (GIBCO Lab, N.Y., U.S.A.) and 50 μl of *cis*-DME solution were plated in flat-bottomed microtiter wells and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Acknowledgments The authors wish to thank Miss Y. Iwase, Analytical Center of the Faculty of Pharmaceutical Sciences, Fukuoka University, for NMR spectral measurements. The authors also thank Dr. J. Tadano and Mr. S. Kawasaki, of this medical school, for MS spectral data.

References and Notes

1) H. Matsunaga, M. Katano, H. Yamamoto, M. Mori and K. Takata, *Chem. Pharm. Bull.*, **37**, 1279 (1989); M. Katano, H. Yamamoto, H. Matsunaga and K. Hirano, *Oncologia*, **21**, 70 (1988); M. Katono, H. Matsunaga and H. Yamamoto, *J. Jpn. Surg. Soc.*, **89**, 971 (1988); M. Katano, H. Yamamoto and H. Matsunaga, Proc. of 5th International Ginseng Symposium, Korea Ginseng and Tobacco Research Institute, Seoul, Korea, 1988, p. 33.

2) *a*) H. Sakai and K. Yoneda, *J. Chem. Ecol.*, **8**, 185 (1982); *b*) Y. Kimura, M. Mori, A. Suzuki and A. Kobayashi, *Agric. Biol. Chem.*, **45**, 2915 (1981); *c*) A. Kobayashi, *Kagaku To Seibutu*, **14**, 64 (1976).

3) HPLC conditions are as follows: column 4 × 125 mm cartridge column LiChrospher RP-18, 5 μm (Kanto Kagaku, Tokyo, Japan); mobile phase, H_2O: CH_3CN (30 : 70, v/v); detector, UV 250 nm.