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Inhibition of cell proliferation and DNA synthesis by ISP-1 in melanoma cells

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Objectives

The occurrence of malignant melanoma is undergoing a dramatic increase in persons with light-color skin in all parts of the world. Melanoma is a highly aggressive tumor which frequently resists chemotherapy, therefore, the search for new agents for its treatment is of great importance. In this study, we investigated their anti-cancer potency against melanoma cell lines in order to develop new agents for melanoma treatment.

Materials and Methods

○ Materials

ISP-1 (Sigma St. Louis, MO) was prepared as 5 mM stock solution in methanol. Sphingolipid reagents were from Avanti (Alabaster, AL). All organic solvents were analyticlal grade and purchased from Merck (Dramsdadt, Germany).

○ Methods

Monolayer cultures of B16F10 murine melanoma cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/㎖ penicillin, and 100 ㎍/㎖ streptomycin. Cells were treated with ISP-1 at concentrations of 1 and 10 μM for 96hr Morphological changes were observed under microscope (magnification, 100x). Densities were counted at the indicated time. [^{3}H]-thymidine (1 μCi/ml) was added to the medium, and cells were incubated for 4h. Radioactivities were determined using a liquid scintillation counter. Lipids for sphingolipid analysis were extracted from samples spiked with N-oleoyl-D-*erythro*-sphingosine (C_{17} ceramide) as an internal standard. Ceramide was resolved by TLC, complexed with fatty-acid free bovine serum albumin (BSA), and deacylated by ceramidase. The released sphingosine was derivatized with o-phthalaldehyde (OPA) and measured by high performance liquid chromatography (HPLC).

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Results and Discussion

ISP-1 is a potent and specific serine palmitoyl transferase (SPT) inhibitor and is known to have an immunosuppressive activity. Treatment of B16F10 melanoma cells with ISP-1 resulted in growth suppression in a time-and concentration dependent manner. From the morphological observation, melanoma cells showed a cell growth inhibition from control cells compared with ISP-1-treated cells, and then decreased DNA synthesis. The levels of sphingosine (So) and ceramide (Cer) were reduced down to the over value in control when cells were treated with ISP-1. Moreover, So and Cer concentrations were significantly correlated, indicating that SPT inhibition suppresses the synthesis of both these sphingolipids concomitantly. Inhibition of cell growth by ISP-1 in melanoma cell lines have high pharmacological value and implies that ISP-1 might be developed as new agent for melanoma treatment.