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Antiproliferative Effects of *Panax ginseng* Glycosides on DNA Synthesis in Cultured Mouse Fibroblasts

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Abstract□*Panax ginseng* ginsenosides were examined for their effects on the DNA synthesis. The DNA synthesis was measured by the [^{3}H]-thymidine incorporation into NIH3T3 cells. The ginsenoside, panaxytriol, Rh_1 and Rh_2 showed reduced [^{3}H]-thymidine incorporation. However, other ginsenosides of Rg_1, Rg_2 and Rg_3 did not inhibit DNA synthesis. Among the various ginsenosides, ginsenoside Rh_2 was found to be the most inhibitory on DNA synthesis. We suggest Rh_2 as one of the potential choice of antiproliferative drugs.

Key words□ginsenoside, thymidne incorporation, DNA synthesis.

Introduction

We have previoulsy reported that a crude root fraction of ginsenosides from *Panax ginseng* inhibited chemically induced transformation of murine fibroblasts.[1] We examined effects of root extracts of ginseng on the replicative DNA synthesis in V79 Chinese hamster lung cells and found that thymidine uptake into V79 cells was significantly reduced by the addition of ginseng extracts to the culture medium. In recent years, a number of chemical compounds have been isolated from the *Panax ginseng* that inhibit growth of malignant tumor cells and these compounds have been suggested as a potential chemopreventive or therapeutic agents for cancer treatment.[2-4] The active components of ginseng extracts that exert antineoplastic effects includes panaxytriols, glycosides of protopanaxatriols and protopanaxadiols.[5,6] Among these ginseng glycosides, aglycone molecules of Rh_1 and Rh_2 received special attentions (Fig. 1). Both ginsenosides Rh_1 and Rh_2 are known to affect the growth of melanoma cells and control of the phenotypic expression to the malignant tumor cells.[2] While effects of these chemicals on the cell growth and cell aggregation have been well investigated, the direct effect of these plant glycosides on the replicaive DNA synthesis has not been addressed. In this study, we measured the effect of ginsenosides extracted from *Panax ginseng* acting on the [^{3}H]-thymidine uptake into the cellular DNA of NIH3T3 mouse fibroblast cells.

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Materials and methods

1. Extraction of Ginsenosides

Ginsenosides were isloated from the processed red ginseng root of *Panax ginseng C.A. Meyer* as described.[7] Ginsenosides were dissolved in 98% ethanol and stored in −20℃.

2. Cell Culture

NIH3T3 mouse fibroblast cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), containing 10% fetal bovine serum, penicillin (100 units/m*l*) and streptomycin (100 μg/m*l*). Cells were subcultured in 60 mm plastic dishes at 3~4 day intervals and incubated at 37℃ in a humidified atmosphere of 5% CO_2.

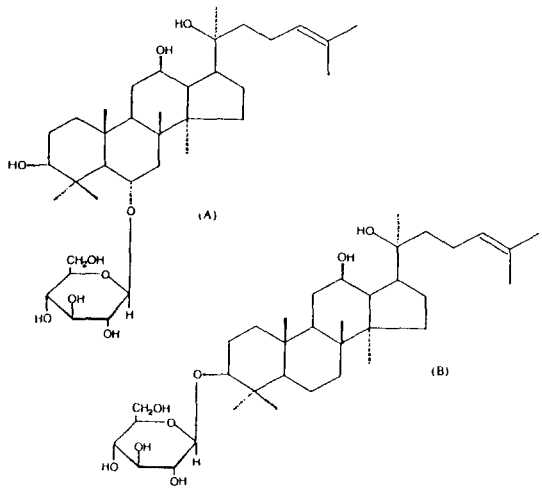

Fig. 1. Chemical structures of ginsenosides Rh_1 (A) and Rh_2 (B). Both of the ginsenosides have a dammarane skeleton resembling a steroid skeleton as an aglycon, with differences in their chemical structures at their binding site of sugar moiety.

3. Measurement of [^{3}H]-thymidine Uptake

Semiconfluent NIH3T3 cells (1×10^6 cells/plate) were treated with specified concentrations of ginsenosides for 1 hr. Cells were washed twice with DMEM followed by the addition of DMEM containing 3 μCi/m*l* of [^{3}H]-thymidine (methyl-[^{3}H]-thymidine, 83 Ci/mmole). After incubation for 24 hr, cells were removed from culture plastic dishes using trypsin-EDTA soultion(Gibco) and centrifuged at 700×g for 5 min. The supernatants were aspirated and cell pellets were resuspended in phosphate buffered saline(PBS). Cells were stored in 1 m*l* aliquots of PBS at −20℃ until assayd. Within 48 hr cells were thawed and incubated with 100 μg of proteinase K (Sigma type IV) at 37℃ for 1 hr. Samples were poured onto glass fiber filters (Whatman GF/C) held in Millipore 1225 sampling manifold. Each sample on the filters was washed twice with 5 m*l* of cold 10% trichloroacetic acid and twice with 10 m*l* of cold 95% ethanol. After drying, radioactivities of samples were counted in a liquid scintillation counter using Beckman's Ready Protein™ as a counting cocktail.

Results and discussion

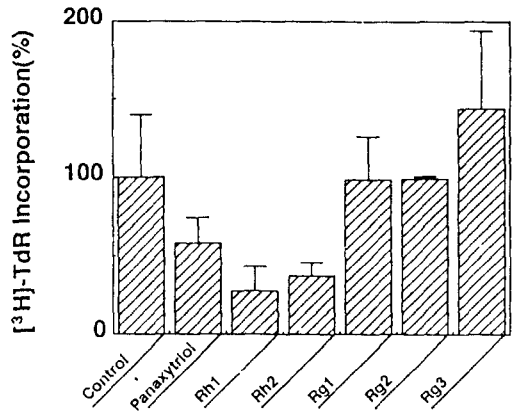

Fig. 2. Effect of ginsenosides on [^{3}H]-thymidine incorporation in NIH3T3 cells. Ginsenosides (Panaxytriol, Rh_1, Rh_2, Rg_1, Rg_2, and Rg_3) were treated at 20 μM for 1 hr before the [^{3}H]-thymidine incorporations. DNA synthesis was examined by measuring [^{3}H]-thymidine incorporation into cells. The bars indicate the mean+ standard deviations.

Rh_1 (20-S-Protopanaxatriol-6-[O-β-glucopyranoside])

Rh_2 (20-S-Protopanaxatriol-3-[O-β-glucopyranoside])

Rg_1 (20-S-Protopanaxatriol-6-[O-β-glucopyranoside])-20-[O-β-D-glucoside])

Rg_2 (20-S-Protopanaxatriol-6-[O-α-L-rhamnopyranosyl(1-2)-β-D-glucopyranoside])

Rg_3 (20-S-Protopanaxatirol-3-[O-α-L-rhamnoglucopyranosyl(1-2)-β-D-glucopyranoside])

Extracts of *Panax ginseng* have been traditionally used in medical practices in many Oriental countries. There are several lines of evidence that the extracts have antiproliferative effects in both animals and experimental cells. Yun *et al.*[8] reported that the administration of ginseng extracts reduced the size of lung adenoma initiated by dimethylbenzanthracene in mice. Reverse transformation of hepatoma cells by ginseng treatment was also reported.[9] In Fig. 2 ginsenosides were examined for their effect on the DNA synthesis, which was measured by the [^{3}H]-thymidine incorporation into NIH3T3 cells. Data show that panaxytriol and ginsenosides of Rh_1 and Rh_2, in which glucose molecule is attached to the steroid skeleton at C-6 and C-3 respectively, reduced [^{3}H]-thymidine incorporation, however, ginsenosides of Rg_1, Rg_2 and Rg_3 were not

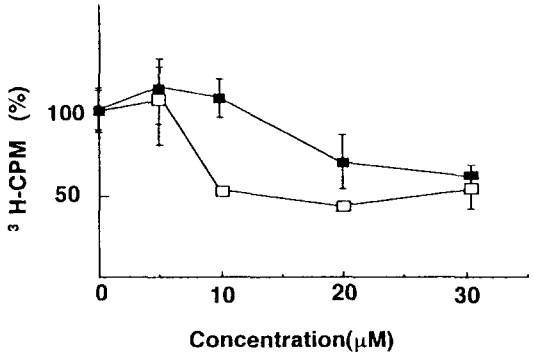

Fig. 3. Effects of ginsenosides Rh_1 and Rh_2 on replicative DNA synthesis. Cells were treated with various concentrations ginsenoside Rh_1 (■—■) and Rh_2 (□—□) for 1 hr, then cells were examined for their ability to incorporate [^{3}H]-thymidine into cells. Each point represents an average of 3 experiments.

appeared as inhibitors of DNA synthesis. These plant glycosides Rg_1, Rg_2 and Rg_3 contain two glucose molecules attached to dammarane skeleton resembling steroid skeletons. The plant ginsenoside Rh_1 and Rh_2 which were known to be involved in the control of melanoma cells[2,3] were examined for dose dependent inhibition of DNA synthesis (Fig. 3). Rh_2 molecules appeared stronger inhibitor of DNA synthesis as compared with Rh_1 inhibitor. It is further necessary to investigate the mechanism of inhibition of DNA synthesis by these ginsenosides. The present data however suggest a potential choice of *Panax ginseng* glycoside, Rh_2 as an antiproliferative drug.

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