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Antiproliferative effects of protopanaxadiol ginsenosides on human colorectal cancer cells

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Abstract. Ginsenosides are the main biologically active components of ginseng. In this study, seven types of protopanaxadiol ginsenosides were assessed for their antiproliferative activity on the HCT-116 and HT-29 human colorectal cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The experimental results indicated that the native protopanaxadiol ginsenosides Rb1 and Rb2 inhibited the proliferation of the colorectal cancer cells in a dose-dependent manner. The deglycosylation products F2 and CO (from ginsenosides Rb1 and Rb2, respectively) significantly inhibited the growth of the human colorectal cancer cell lines, whereas product C-K (from Rb1 and Rb2) exerted no antiproliferative effects on the cancer cell lines assessed in this study. HT-29 cells were more sensitive to these ginsenosides compared to HCT-116 cells. In addition, the antiproliferative activity of ginsenosides was found to be correlated with the number and type of sugar residues. The potent growth inhibitory effect of protopanaxadiol ginsenosides on cancer cells may be used in the pharmaceutical industry.

Introduction

Ginsenosides are the main active components of ginseng and have various pharmaceutical activities, such as antitumor, antioxidant and neuroprotective activities (1-4). Protopanaxadiol ginsenosides Rb1 and Rb2 are abundantly found in ginseng. Protopanaxadiol ginsenosides Rd, F2 and compound K (C-K) are the biotransformation products of Rb1, while CO, CY and C-K are the biotransformation products of Rb2. It has been demonstrated that certain biotransformation products of Rb1 and Rb2 have significant pharmacological activities, such as C-K possessing antiallergic and anti-inflammatory activities (5-8). All these protopanaxadiol ginsenosides have the same aglycone (protopanaxadiol); however, the sugar chains are different (Fig. 1). The structure-activity relationship of ginsenosides has been investigated, but not fully elucidated (9,10). Ginsenosides with fewer sugar residues appear to be more active. In this study, we investigated the activities of seven protopanaxadiol ginsenosides on the inhibition of proliferation of the HCT-116 and HT-29 human colorectal cancer lines. The results may provide information on the antiproliferative activity-structure relationship of ginsenosides.

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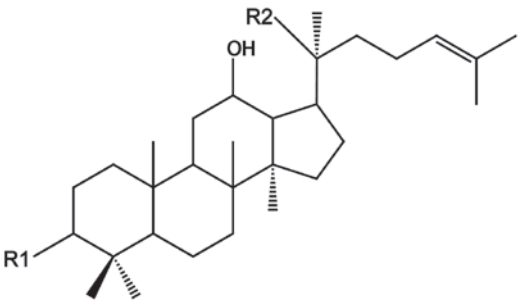
Materials and methods

Materials. Standard ginsenosides were purchased from Chengdu Mansite Biotechnology Co., Ltd. (Chengdu, China). The ginsenosides Rb1 and Rb2 and their biotransformation products were prepared as described in our previous study (11). The metabolites were identified by thin layer chromatography, high-performance liquid chromatography and ^{13}C-NMR spectrometry. Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 (DMEM/F12), Iscove's modified Dulbecco's medium (IMDM) and fetal bovine serum (FBS) were purchased from Gibco-BRL (Carlsbad, CA, USA). Penicillin/streptomycin were purchased from the Tianjin Hao Yang biological manufacture Co., Ltd. (Tianjin, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). The plates used in this study were purchased from Nalge Nunc International (Rochester, NY, USA). Chemicals used were of analytical grade or higher.

Cell culture. The HCT-116 and HT-29 human colorectal cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). HCT-116 cells were grown in IMDM with 10% heat-inactivated FBS and 100 U/ml penicillin and streptomycin. HT-29 cells were maintained in DMEM/F12 containing 10% heat-inactivated FBS. The cells were maintained in a humidified chamber of 95% air and 5% CO_2 at 37˚C.

MTT assay. Cells were seeded at a density of $1x10^4$ cells/well in 96-well plates. After 24 h, the cells were treated with each ginsenoside at different concentrations (50, 100, 150, 200 and 250 μM) for 72 h. Control cells were treated similarly without



Ginsenoside	R1 (C-3)	R2 (C-20)
Rb1	-O-Glc$^{2-1}$Glc	-O-Glc$^{6-1}$Glc
Rd	-O-Glc$^{2-1}$Glc	-O-Glc
F2	-O-Glc	-O-Glc
C-K	-OH	-O-Glc
Rb2	-O-Glc$^{2-1}$Glc	-O-Glc$^{6-1}$Ara (p)
CO	-O-Glc	-O-Glc$^{6-1}$Ara (p)
CY	-OH	-O-Glc$^{6-1}$Ara (p)

Glc: β-D-glucopyranosyl; Ara (p): α-L-arabinopyranosyl

Figure 1. Structures of related ginsenosides.

the addition of ginsenosides. Subsequently, the media were removed and MTT solution (0.5 mg/ml) was added to each well. The plate was incubated in a humidified atmosphere at 37˚C for 4 h and the media were carefully aspirated. Dimethyl sulfoxide (100 μl) was added and the absorbance was measured at 570 nm by a microplate reader (Bio-Rad, Hercules, CA, USA). The experiments were performed in triplicate.

Statistical analysis. The results were expressed as mean ± standard deviation. Data were analyzed by SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was compared between the treatment and the control groups by one-way analysis of variance. The differences were considered statistically significant when $P<0.05$ and $P<0.01$ (Fig. 2). The experiments were performed in triplicate.

Results

Effects of Rb1 and its biotransformation products on cell proliferation. Ginsenoside Rb1 and its biotransformation products Rd, F2 and C-K were assessed for their *in vitro* antiproliferative activity on two human colon cancer cell lines. The MTT assay results demonstrated that Rb1 exhibited no antiproliferative activity on HCT-116 cells at a low concentration (50 μM). Starting from a dose of 100 μM, Rb1 exhibited a marginal dose-dependent inhibitory effect. At a dose of 250 μM, the inhibitory rate of Rb1 was 27.0% (Fig. 2A). The

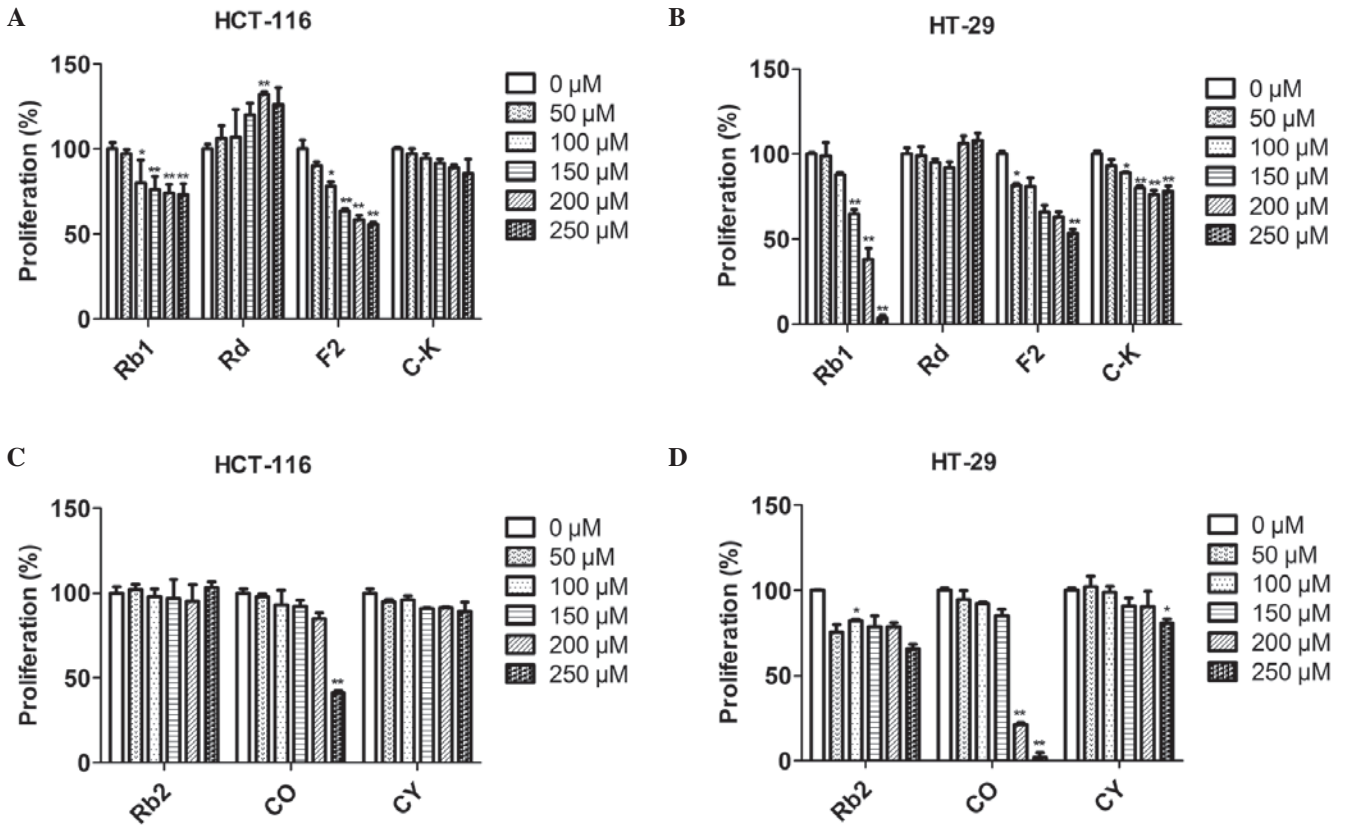


Figure 2. Effects of ginsenosides on HCT-116 and HT-29 cell proliferation. Cells were treated with seven types of ginsenosides at various concentrations (0, 50, 100, 150, 200 and 250 μM) for 72 h. The antiproliferative effects of the ginsenosides were determined by the MTT assay. (A) Effects of Rb1, Rd, F2 and C-K on HCT-116 cell proliferation. (B) Effects of Rb1, Rd, F2 and C-K on HT-29 cell proliferation. (C) Effects of Rb2, CO and CY on HCT-116 cell proliferation (D) Effects of Rb2, CO and CY on HT-29 cell proliferation. Error bars and all data are expressed as mean ± standard error in triplicate. $^{*}P<0.05$, $^{**}P<0.01$.

HT-29 cell line was more sensitive to Rb1. Treated with 100 μM or higher concentration of Rb1, the HT-29 cell growth was inhibited in a dose-dependent manner. At the highest dose of 250 μM, the inhibitory rate was 96.4% (Fig. 2B). Ginsenoside Rd significantly increased HCT-116 cell proliferation, whereas it exerted no obvious effect on HT-29 cell growth. Ginsenoside F2 exhibited a moderate antiproliferative activity on HCT-116 cells, with an inhibitory rate of 44.2% at 250 μM of F2. A similar inhibitory effect was observed on HT-29 cells, with an inhibitory rate of 46.4% at 250 μM. Ginsenoside C-K exerted almost no antiproliferative activity on HCT-116 cells and a marginal inhibitory effect on HT-29 cells, with an inhibitory rate of 22.0% at the highest dose of 250 μM.

Effects of Rb2 and its biotransformation products on cell proliferation. Ginsenoside Rb2 and its metabolites CO and CY were assessed for their *in vitro* antiproliferative activity. As shown in Fig. 2C and D, Rb2 exerted no antiproliferative effect on HCT-116 cells, even at the highest concentration of 250 μM. Rb2 exhibited a marginal inhibitory activity on HT-29 cells. The antiproliferative effect of Rb2 was not significantly altered at low concentrations (50-200 μM). Treatment at the highest dose of 250 μM achieved an inhibitory rate of 34.1%. Ginsenoside CO, the deglycosylation product of Rb2, exerted no inhibitory effect on HCT-116 cell proliferation at low concentrations (50-200 μM), whereas it exerted a significant antiproliferative effect at the highest dose of 250 μM, with an inhibitory rate of 58.7%. HT-29 cells were more sensitive to ginsenoside CO compared to HCT-116 cells. At low doses (50-150 μM), there was no antiproliferative effect on HT-29 cells. However, following treatment with 200 and 250 μM of CO, the inhibitory rate was 78.7 and 98.0%, respectively. The CY hydrolytic product of Rb2 exerted no inhibitory effect on HCT-116 cell proliferation. Similar results were observed on HT-29 cells at the range of 50-200 μM. CY exhibited a marginal antiproliferative effect on HT-29 cells, with an inhibitory rate of 19.1% only at the highest dose of 250 μM. C-K, with one sugar residue at C-20 of protopanaxadiol, is also a hydrolytic product of Rb2 and it exerted no inhibitory effect on HCT-116 or HT-29 cell growth (Fig. 2A and B).

Discussion

Ginsenosides are the major active components of ginseng. It was reported that certain protopanaxadiol ginsenosides, such as Rb1 and Rb2, are transformed into rare bioactive ginsenosides through the hydrolysis of sugar residues, which is usually enabled by glycosidases (11). In this study, ginsenoside Rb1 and Rb2 and their biotransformation products Rd, F2, C-K (from Rb1), CO and CY (from Rb2), were selected for assessment of their antiproliferative activity on human colon cancer cells. It was observed that the seven ginsenosides possessed the same aglycone but different sugar residues. Therefore, the association between the sugar residues in protopanaxadiol ginsenosides and their antiproliferative activity was elucidated in this study.

Our results demonstrated that the number of sugar residues significantly affected the inhibitory activity. As shown in Fig. 1, ginsenoside Rb1 and Rb2 have four sugar residues, Rd and CO have three, F2 and CY have two and C-K has one. Taking Rb1 and its deglycosylation products as an example, when the sugar residues decreased from four to one, the antiproliferative activity was significantly altered. Similar effects were observed on HCT-116 and HT-29 cells. Moreover, Rb2 and its metabolites also exhibited different inhibitory effects on the two human colon cancer cells tested. Ginsenoside F2, found in ginseng in minute amounts, was produced by deglycosylation of Rb1 with the catalyzing action of glycosidase. Ginsenoside F2 exerted a more potent inhibitory effect compared to that of Rb1. Similar to F2, the minor ginsenoside CO, which was produced by deglycosylation of Rb2, exerted more potent inhibitory effects compared to those of Rb2. These results indicated that the number of sugar residues may affect the antiproliferative activity of ginsenosides. However, the detailed mechanism needs to be further investigated.

In addition, our results demonstrated that the antiproliferative activity of ginsenosides was also correlated with the type of sugar residue. Ginsenoside Rb1 and Rb2 have a similar structure, with two sugar residues substituted at C-3 and C-20, respectively. The only difference between them is the disaccharide substituted at C-20 [glucose-β-(1→6)-glucose- for Rb1 and arabinose (p)-α-(1→6)-glucose-β- for Rb2]. It was observed that ginsenosides at the same dose but with different terminal sugar residues exhibited different antiproliferative activities. Ginsenoside Rb1 exerted a significant inhibitory effect on HT-29 cell growth in a dose-dependent manner. However, the antiproliferative activity of Rb2 was significantly lower compared to that of Rb1, whereas no dose-dependent inhibitory effect was exerted on HT-29 cells by Rb2. These results indicate that the terminal sugar residue at the C-20 site may affect the inhibitory effects of ginsenosides to a certain extent.

In conclusion, we investigated the inhibitory effects of seven protopanaxadiol ginsenosides on HCT-116 and HT-29 human colorectal cancer cell proliferation. Our results indicate that the number and type of sugar residues may affect the antiproliferative activity of ginsenosides, which may provide useful information on the association between ginsenoside structure and antiproliferative activity.

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