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Research Article

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Anti-proliferative effects of protopanaxadiol-type ginsenosides on human colon epithelial cancer cells

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ABSTRACT

Ginsenosides are the major active components in ginseng. In this study, we prepared 4 kinds of protopanaxadiol-type ginsenosides with different sugar residues, and observed their anti-proliferative activities on human colorectal cancer cell lines HCT-116 and HT-29. The results suggested that the native protopanaxadiol-type saponinRc had a dose-dependent inhibitory effects both on HCT-116 and HT-29 cells. With the number of sugar residues decreased, the anti-proliferative effect of intermediate product Mb increased significantly. However, the biotransformation products Mc and Rg3 with two sugar residues had slight inhibitory effects on both two human colon cancer cells. HT-29 cells were more sensitive to all these ginsenosides than HCT-116 cells. When the glucosidic linkages of ginsenosides were specifically cleaved by glycosidases, their anti-proliferative effects changed notablely. Our results suggested that the number of sugar residues might mainly influence the anti-proliferative functions of ginsenosides. Our results provide some useful information about the correlation between the anti-proliferative activities and the structures of ginsenosides. The protopanaxadiol-type ginsenosidesRc, Mb and Mc might be exploited as potential drug candidates for application in medicine or pharmaceutical industry.

Keywords: ginsenoside, anti-proliferative activities, colorectal cancer

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INTRODUCTION

Ginsenosides are the major bioactive components of ginseng. Some ginsenosides exist in large amount in ginseng, such as Rb1, Rb2 and Rc, termed major ginsenosides. It has been reported that they had many pharmacological activities, such as anti-tumor and anti-oxidant activities, neuroprotection effects, and potential synergistic effects of combinations [1-4]. In recent years, some major ginsenosides can be biotransformed into the other ginsenosides[†] These authors contributed equally to this articleby hydrolyzing the glucosidic linkage used glycosidases[5]. All these ginsenosides have the same aglycone, but the number of sugar residues was different. The products are naturally present in ginseng at low levels, termed rare ginsenosides. It was found that these rare ginsenosides had more biological properties. For example, Rg3, a kind of rare ginsenoside from the Panax ginseng, has been reported to inhibit tumor metastasis and cancer cell growth of several tumors of rat and human [6-7]. The anti-proliferatvie activities of Rb1 and its metabolites, Rb2 and its metabolites have been reported in our previous publication [8]. The results implied that the bioactivities of these ginsenosides might be correlated with the structures of ginsenosides. However, the precise mechanism is not clear yet, and due to the lack of the studies about ginsenosideRc and its metabolites. In this study, we carried out a program to compare the inhibitory effects of protopanaxadiol-type saponinsRc and its metabolites, and rare ginsenosides Rg3 on two human colorectal cancer lines HCT-116 and HT-29. Our results suggested that the number of sugar residues might play an important role for their anti-proliferative activities. This study could provide some new information for anti-proliferative activity-structure relationship of ginsenosides.

EXPERIMENTAL SECTION

Materials: Standard ginsenosides were purchased from Chengdu Mansite Biotechnology (Chengdu, China). The ginsenosidesRc and its metabolites, and Rg3 were prepared as described in previous publication [9,10]. All metabolites were identified by TLC, HPLC and ^{13}C-NMR spectrometry. Dulbecco's modified Eagle's medium/F-12 (DMEM/F12) medium, Iscove's modified dulbecco's medium (IMDM) and fetal bovine serum (FBS) were purchased from Gibco. Penicillin/streptomycin was from the Tian Jin Hao Yang biological manufacture Co., Ltd. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma. All the plates used in this study were purchased from Nunc (Rochester, NY, USA). All chemicals used were of analytical grade or better.

Cell Culture: The human colorectal cancer cell lines HCT-116 and HT-29 were obtained from the American Type Culture Collection. HCT-116 cells were grown in IMDM medium with 10% heat-inactivated FBS, 100 units/ml penicillin and streptomycin. HT-29 cells were maintained in DMEM/F12 medium containing 10% heat-inactivated FBS. The cells were maintained in a humidified chamber of 95% air and 5% CO_2 at 37^{o}C.

Cells Proliferation: The cells were seeded at 1×10^4 cells/well in 96-well plates. After 24 h, the cells were treated with different concentrations (50, 100, 150, 200, 250 μM) ofginsenosides for 72 h. Control cells were treated similarly without drugs. Then the media were removed, MTT solution (0.5 mg/ml) was added to each well. The plate was incubated for 4 h in a humidified atmosphere at 37^{o}C, and the media were carefully aspirated. One hundred microliter of dimethyl sulfoxide was added, and the plate was measured at 570 nm in a microplate reader (Bio-Rad). All experiments were performed in triplicate at least.

Statistical analysis: The results were expressed as mean ± standard deviation (SD). All the data were analyzed by SPSS 17.0 Software. Statistical significance was compared between the treatment and the control groups by one-way ANOVA. The differences were considered significant when * $P< 0.05$; ** $P< 0.01$. All experiments were performed in triplicate.

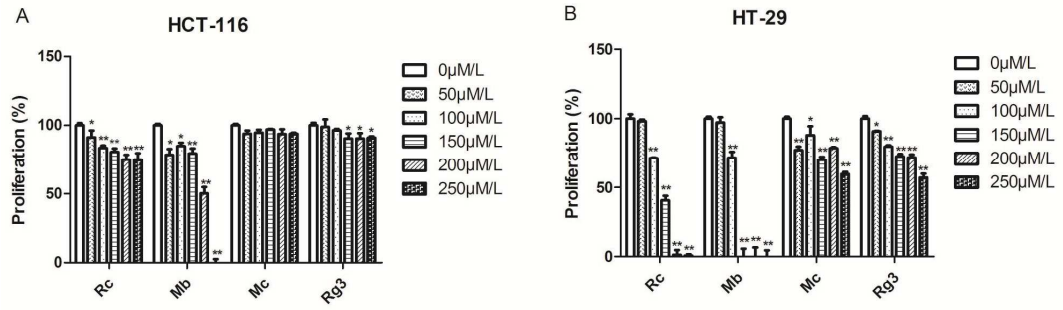

Fig.1 Effects of ginsenosides on HCT-116 and HT-29 cell proliferation

*Cells were treated with 4 kinds of ginsenosides at different dosages (0, 50, 100, 150, 200, 250 μM) for 72 h. The anti-proliferative effects of the ginsenosides were determined by the MTT assay. A. Effects of Rc, Mb, Mc and Rg3 on HCT-116 (A) and HT-29 (B) cell proliferation. The data are shown as means ±SD. * P< 0.05; ** P< 0.01.*

RESULTS

Effects of ginsenosides on cells proliferations: Two cell lines representing human colon cancer cells were treated with four preparedginsenosides at concentrations of 50-250 μM, and cell viability was determined by MTT assay.

As shown in Fig. 1A, protopanaxadiol-type saponinRc had a slight dose-dependent anti-proliferative effect on HCT-116 cells. Even at the high dose of 250 μM, the inhibitory rate of Rc was 25.1%. After hydrolyzed by glycosidases, the intermediate product Mb with three sugar residues exhibited stronger inhibitory effect than Rc. The anti-proliferative activities of Mb at low concentrations (50-150 μM) were similar to Rc. With treatment of 200 μM, the inhibitory rate of Mb was 49.83%. Mb inhibited the growth of HCT-116 cells completely at the high dose of 250 μM. Then the sugar residues of Rc were decreased from four to two, giving rise to Mc. Meanwhile, the anti-proliferative activities of Mc declined dramatically. We observed almost no inhibitory effect of Mc on HCT-116 cells. Another rare ginsenoside with two sugar residues Rg3 also displayed no anti-proliferative effect on HCT-116 cells even at the high concentration.

As shown in Fig. 1B, HT-29 cells were more sensitive to the ginsenosides than HCT-116 cells. Rc had a significant dose-dependent anti-proliferative effect on HT-29 cells.At the high doses of 200 and 250 μM, the inhibitory rate of

Rc was 98.7% and 100.0%, respectively. With the number of sugar residues decreased, the inhibitory effect of the metabolite Mb increased dramatically.At the low doses (50-100 μM), the anti-proliferative activities of Mb were similar to Rc. When the concentration of Mb was 150-250 μM, there was almost no survival cells observed. However, the metabolite Mc with two sugar residues, just exhibited a moderate anti-proliferative activity on HT-29 cells. Even at the high concentration of 250 μM, the inhibitory rate of Mc was 41.3%. The other rare ginsenoside Rg3 showed similar inhibtory effect, with the inhibitory rate was 42.8% at the dose 250 μM. All these results were consistent with that of the cell proliferation assay on HCT-116 cell.

DISCUSSION

Ginsenosides, exist in *Panax* ginseng, have many biological properties. The major protopanaxadiol-type saponinRc has been reported to enhance the capsaicin-induced inward current in a concentration-dependent and reversible manner [11], exert a antinociceptive effect in the substance P-induced pain model [12], and protect human erythrocytes against free-radical-induced hemolysis [13]. However, the anti-proliferative activity of Rc and its metabolites on human colon cancer cells were not clear yet.

With hydrolysed by glycosidases, the major ginsenosides could be transformed into the rare ginsenosides, such as Mb, Mc and Rg3. All these ginsenosides have the same aglycone, but the number of sugar residues was different. In our study, the protopanaxadiol-type saponins with three or four sugar residues, such as Rc and Mb, could significantly inhibit human colon cancer cells growth, especially on HT-29 cells. The rare ginsenosides with two sugar residues, such as Mc and Rg3, had weaker anti-proliferative effects on these cancer cells.

Many studies have been reported biological activities of some rare ginsenosides. For example, Mc has been tested the cytotoxic activity *in vitro* on HL-60 human leukemia cell line [14], but not in the cells used in this study. Our results suggested that the same ginsenoside displayed different anti-proliferative effects on various cell lines. Generally speaking, HT-29 cells were more sensitive to ginsenosidesRc, Mb, Mc and Rg3 than HCT-116 cells, indicating that the anti-tumor effects of them *in vitro* were depends on cancer cell lines.

In recent years, the structures of ginsenosides, especially the aglycone portion were elucidated by many biologists [5,15]. However, the relationship between the carbohydrate portionofginsenoside and their anti-tumor activity was not clear. In this study, when we decreased the sugar residues of ginsenosides, their inhibitory effects changed dramatically. When the number of sugar chains of ginsenosides were hydrolysed from four to one, the anti-proliferative activities of Rc, Mb, Mc and Rg3 were significant, more significant, moderate and moderate on HT-29 cells, respectively. We also found the similar results on HCT-116 cell proliferation. These data implied that the number of sugar chains might play an important role in the biological properties of ginsenosides.

We further investigated the anti-proliferative effects of Mc and Rg3, which both have two sugar residues. The difference of them was the sugar residues substituted, arabinose(*f*)(1→6)-glucose- for Mc at C-20, glucose(1→6)-glucose- for Rg3 at C-3. The experimental results showed that Mc and Rg3 had similar inhibitory activities both on HCT-116 and HT-29 cells. These results suggesting that the anti-tumor activity mechanism of ginsenosides might be correlated with their numbers of sugar residues, but neither position nor kinds of the carbohydrate portion. Our study may provide new insight into the relation between the number of sugar residues of ginsenosides and their anti-proliferative activities. The ginsenosidesRc and Mb have promising prospect for application of human colon cancer in pharmaceutical industry.

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