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# Transformation of Ginseng Saponins to Ginsenoside $Rh_2$ by Acids and Human Intestinal Bacteria and Biological Activities of Their Transformants

**Eun-Ah Bae, Myung Joo Han, Eun-Jin Kim[1], and Dong-Hyun Kim[1]**

*Department of Food and Nutrition, and [1]College of Pharmacy, Kyung Hee University, Hoegi #1, Dongdaemun-Ku, Seoul 130-701, Korea*

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When ginseng water extract was incubated at 60°C in acidic conditions, its protopanaxadiol ginsenosides were transformed to ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$. However, protopanaxadiol glycoside ginsenosides $Rb_1$, $Rb_2$ and Rc isolated from ginseng were mostly not transformed to ginsenoside $Rg_3$ by the incubation in neutral condition. The transformation of these ginsenosides to ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ was increased by increasing incubation temperature and time in acidic condition: the optimal incubation time and temperature for this transformation was 5 h and 60°C resepectively. The transformed ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ were metabolized to ginsenoside $Rh_2$ and $\Delta^{20}$-ginsenoside $Rh_2$, respectively, by human fecal microflora. Among the bacteria isolated from human fecal microflora, *Bacteroides* sp., *Bifidobacterium* sp. and *Fusobacterium* sp. potently transformed ginsenoside $Rg_3$ to ginsenoside $Rh_2$. Acid-treated ginseng (AG) extract, fermented AG extract, ginsenoside $Rh_2$ and protopanaxadiol showed potent cytotoxicity against tumor cell lines. AG extract, fermented AG extract and protopanaxadiol potently inhibited the growth of *Helicobacter pylori*.

**Key words**: Ginseng, Ginsenoside $Rg_3$, Intestinal bacteria, Ginsenoside $Rh_2$, *Helicobacter pylori*, Cytotoxicity

## INTRODUCTION

Ginseng (the roots of *Panax ginseng* C.A. Meyer, Araliaceae) is frequently taken orally as a traditional herbal medicine in Asian countries. The major components of ginseng are ginsenosides, which are glycosides with a dammarane skeleton aglycone (Shibata *et al.*, 1963; Tanaka *et al.*, 1972). These ginsenosides have been reported to show various biological activities, including anti-inflammatory activity (Wu *et al.*, 1992) and anti-tumor effects (inhibition of tumor-induced angiogenesis and the prevention of tumor invasion and metastasis) (Sato *et al.*, 1994; Mochizuki *et al.*, 1995). To explain these pharmacological actions, it is thought that ginseng saponins must be metabolized by human intestinal bacteria after oral ingestion (Akao *et al.*, 1998a, 1998b; Kanaoka *et al*, 1992, 1994). For example, ginsenosides $Rb_1$, $Rb_2$ and Rc are transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901, compound K) by intestinal bacteria (Hasegawa *et al.*, 1997; Karikura *et al.*, 1991). This transformed IH-901 induces an anti-metastatic or anti-carcinogenic effect by blocking tumor invasion or preventing chromosomal aberration and tumorigenesis (Wakabayashi *et al.*, 1998; Lee *et al.*, 1999). Han *et al.* (1982) reported that ginsenosides $Rb_1$, $Rb_2$ and Rc were transformed to ginsenoside $Rg_3$ by the mild acid treatment such as with stomach acid. Furthermore, this ginsenoside $Rg_3$ is a main component of Red ginseng and heat-processed ginseng (Kitagawa *et al.*, 1983; Kown *et al.*, 2001). We reported that the ginsenoside $Rg_3$ was transformed to ginsenoside $Rh_2$ by human intestinal bacteria (Bae *et al.*, 2002). This transformed ginsenoside $Rh_2$ showed more potent cytotoxic activity than ginsenoside $Rg_3$ or ginsenoside Rc. Nevertheless, studies on the effects of acids and temperature on the transformation of ginsenosides and the metabolism of the acid-treated ginsenosides by human intestinal bacteria are not yet comprehensive. Therefore, we investigated

Correspondence to: Prof. Dong-Hyun Kim, College of Pharmacy, Kyung Hee University, Hoegi #1, Dongdaemun-ku, Seoul 130-701, Korea
Tel: 82-2-961-0374, Fax: 82-2-957-5030
E-mail: dhkim@khu.ac.kr

the effects of mild acids and temperature on the transformation of ginsenosides, transformation of acid-treated ginsenosides to ginsenoside $Rh_2$ by human intestinal bacteria and *in vitro* anti-*Helicobacter pylori* and cytotoxic activities of the biotransformed ginseng.

## MATERIALS AND METHODS

### Materials and bacterial strains

General anaerobic medium (GAM) was purchased from Nissui Pharmaceutical Co., Ltd., (Japan). Tryptic soy (TS). The other chemicals were of analytical reagent grade. Ginsenoside $Rb_1$, $Rb_2$ and Rc were isolated from ginseng according to the previous method (Bae *et al.*, 2000). 20(*S*)-Ginsenoside $Rg_3$, 20(R)-ginsenoside $Rg_3$, $\Delta^{20}$-ginsenoside $Rg_3$, 20(*S*)-ginsenoside $Rh_2$, 20(*R*)-ginsenoside $Rh_2$, $\Delta^{20}$-ginsenoside $Rh_2$, 20(*S*)-protopanaxadiol and 20(*R*)-protopanaxadiol were prepared by the previously reported method (Bae *et al.*, 2002). Intestinal bacteria previously isolated in our lab were used. The isolated bacteria were anaerobically cultured at 37°C for 24 h in GAM broth.

### Mild acid treatment of ginsenosides

Ginsenosides or ginseng water extract (2 g) were treated in mild acidic conditions (0.1% or 1% acetic acid, citric acid, lactic acid, tartaric acid or HCl) at 37°C, 60 °C, or 80°C for 1, 2, 5, or 10 h. The reaction mixtures were neutralized with sodium hydroxide and extracted with *n*-BuOH, and the transformant was analyzed by TLC.

### Assay of metabolic activity of ginsenosides by human intestinal bacteria

The reaction mixture containing 100 μL of each ginsenoside at 0.1 mM (0.1% acid-treated ginseng [AG]) and 100 μL of suspended human feces (or bacterial suspension cultured in GAM broth) (2 mg) was incubated for 24 h at 37°C. The reaction mixture was extracted with BuOH, evaporated and assayed by TLC: TLC plates, silica gel $60F_{254}$ (Merck Co., Germany); developing solvent, $CHCl_3$-MeOH-$H_2O$ (65:35:10 v/v, lower phase). The plates were stained by spraying with MeOH-$H_2SO_4$ (95:5 v/v), followed by heating. The stained TLCs were then analyzed by a TLC scanner (Shimadzu model CS-9301PC, Japan).

Each isolated bacterium was cultured in 50 mL GAM broth and collected at 5000×g for 30 min. Each collected bacterial pellet was suspended in 50 mM phosphate buffer and used as a crude enzyme solution.

### *In vitro* cytotoxicity assay

The *in vitro* cytotoxicity was tested against L1210 (mouse lymphocytic leukemia cell line), P388 (mouse lymphoid neoplasma cell line) and HepG2 (human liver carcinoma) by MTT [3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay according to the method of Carmichael *et al.* (1987).

### Assay of anti-*Helicobacter pylori* activity

A growth inhibition assay of *Helicobacter pylori* was performed according to the previous method (Bae *et al.*, 2000).

## RESULTS

### Transformation of ginseng by acids

When ginseng and ginseng saponin BuOH fraction were incubated in boiling water, two additional spots (diastereomeric ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$) were mainly observed on TLC compared to the non-treated ginseng BuOH fraction. To understand why ginsenosides were transformed to these compounds, we isolated protopanaxadiol glycosides, ginsenosides $Rb_1$, $Rb_2$ and Rc, from ginseng, incubated these ginsenosides at 60°C and measured the transformed ginsenosides level of diastereomeric ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ (Table I). However, ginsenosides were not transformed under neutral condition. Therefore, upon checking the pH of ginseng homogenized with water, we found it to be acidic at pH 5.0-6.0. These ginsenosides were incubated with 0.1% acids at 60°C and the levels of transformed ginsenosides were measured. By the incubation in acidic conditions, HCl, acetic acid, lactic acid and tartaric acid all transformed these ginsenosides to diastereomeric ginsenoside $Rg_3$. The tested acids, except HCl, transformed ginsenosides to diastereomeric ginsenoside $Rg_3$ with a yield of more than 80% by the assay of *Fusobacterial* biotransformation (Bae *et al.*, 2002), which did not transform 20(*R*)-ginsenoside $Rg_3$, but transformed 20(*S*)-ginsenoside $Rg_3$. The production of 20(*R*)-ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ was increased when the incubation temperature was raised. However, the treatment with HCl at 80°C decreased the content of these transformed ginsenosides compared to that at 60°C.

The effect of incubation temperature on the transformation of ginsenosides by acids was investigated (Table II). The transformation rate increased with increasing temperature. However, temperatures above 60°C did not increase the yield of ginsenoside $Rg_3$. The yield of ginsenoside $Rg_3$ was increased with increasing incubation time, but not at more than 5 h-incubation (data not shown). Exceptionally, ginsenoside $Rh_2$ was produced by the treatment of HCl at 80°C for 5 h, but not by the other tested acids. The optimal incubation temperature and time for the transformation of ginsenosides to ginsenoside $Rg_3$ was 60°C and 5 h, respectively.

The effect of acid concentration (0.1 and 1%) on the

**Table I.** Ginsenoside contents of ginseng treated with acids

| Acid | Transformed ginsenoside (μg/mL) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2h | | | | | | 5h | | | | | |
| | $Rg_3$ | $Rg_3$ | $Rh_2$ | $Rh_2$ | ppd | ppd | $Rg_3$ | $Rg_3$ | $Rh_2$ | $Rh_2$ | ppd | ppd |
| None | 21.4 | 4.1 | <1 | <1 | <1 | <1 | 25.4 | 4.6 | <1 | <1 | <1 | <1 |
| Acetic acid | 56.5 | 17.8 | <1 | <1 | <1 | <1 | 71.7 | 21.9 | <1 | <1 | <1 | <1 |
| Citric acid | 92.7 | 23.1 | 1.2 | <1 | <1 | <1 | 92.3 | 21.9 | 1.2 | <1 | <1 | <1 |
| Lactic acid | 89.7 | 26.6 | 1.2 | <1 | <1 | <1 | 89.5 | 23.0 | 1.1 | <1 | <1 | <1 |
| Tartaric acid | 75.5 | 24.2 | 1.1 | <1 | <1 | <1 | 91.7 | 37.7 | 1.2 | <1 | <1 | <1 |
| HCl | 85.8 | 35.3 | 1.6 | <1 | <1 | <1 | 54.5 | 13.2 | <1 | <1 | <1 | <1 |

Ginseng water extract (6 mg) was treated with 1 mL of 0.1% acids, heated at 60 °C for 2 h or 5 h, and extracted with BuOH, after which the transformants were assayed by TLC assay systems.
$Rg_3$, ginsenoside $Rg_3$; $\triangle Rg_3$, $\triangle^{20}$-ginsenoside $Rg_3$; $Rh_2$, ginsenoside $Rh_2$; $\triangle Rh_2$, $\triangle^{20}$-ginsenoside $Rh_2$; ppd, protopanaxadiol; $\triangle$ppd, $\triangle^{20}$-protopanaxadiol.

transformation of ginsenosides to ginsenoside $Rg_3$ was also investigated (Table III). This transformation by 1% acids was not greater than that of 0.1% acids. However, the transformation of ginsenosides to $\Delta^{20}$-ginsenoside $Rg_3$ by 1% acids was significantly greater than that by 0.1% acids.

## Biotransformation of acid-treated ginseng and ginsenosides by human intestinal bacteria

When acid-treated ginsenosides, which mainly consist of diastereomeric ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$, were incubated with previously isolated human intestinal bacteria for 24 h, most of the bacteria did not transform these ginsenosides. However, *Fusobacterium* K-60, *Bifidobacterium* K-506 and *Bacteroides* HJ-15 converted 20(*S*)-ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ to ginsenoside $Rh_2$ and $\Delta^{20}$-ginsenoside $Rh_2$, respectively (Table IV). However, among them, some bacteria, such as *Fusobacterium* K-60, converted 20(*S*)-ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ to protopanaxadiol via ginsenoside $Rh_2$ and to $\Delta^{20}$-protopanaxadiol *via* $\Delta^{20}$-ginsenoside $Rh_2$ (data not shown).

When AG extract was incubated with human fecal suspension, diastereomeric ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ were decreased and ginsenoside $Rh_2$, $\Delta^{20}$-ginsenoside $Rh_2$, protopanaxadiol and $\Delta^{20}$-protopanaxadiol were produced as metabolites (Table V). The main metabolites were ginsenoside $Rh_2$ and $\Delta^{20}$-Ginsenoside $Rh_2$. When the transforming activity of AG extract to ginsenoside $Rh_2$ was assayed in five specimens of human feces, the activity was detected in 4 specimens. However, these activity levels were varied dependent on the individual samples. The average of the activities transforming 20(*S*)- and 20(*R*)-ginsenoside $Rg_3$ to 20(*S*)- and 20(*R*)-ginsenoside $Rh_2$ were 0.36±0.15 and 0.005±0.0026 nmol/h/mg wet weight of feces, respectively.

**Table II.** Effect of temperatures on the transformation of ginsenosides by acids

| Acid | Temp (°C) | *Transformed Concentration* (μM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ginsenoside $Rb_1$ | | Ginsenoside $Rb_2$ | | Ginsenoside Rc | |
| | | $Rg_3$ | $\triangle^{20}$-$Rg_3$ | $Rg_3$ | $\triangle^{20}$-$Rg_3$ | $Rg_3$ | $\triangle^{20}$-$Rg_3$ |
| None | 37 | <1 | <1 | <1 | <1 | <1 | <1 |
| Acetic acid | | 2.0 | <1 | 1.4 | <1 | 1.0 | <1 |
| Citric acid | | 7.1 | <1 | 3.8 | <1 | 2.5 | <1 |
| Lactic acid | | 7.9 | <1 | 4.6 | <1 | 2.5 | <1 |
| Tartaric acid | | 5.6 | <1 | 5.2 | <1 | 2.9 | <1 |
| HCl | | 36.1 | 5.8 | 38.7 | 10.3 | 33.5 | 6.9 |
| None | 60 | <1 | <1 | <1 | <1 | <1 | <1 |
| Acetic acid | | 56.2 | 8.1 | 38.5 | 6.3 | 21.4 | 5.5 |
| Citric acid | | 62.8 | 17.5 | 4.2 | – | 39.0 | 11.6 |
| Lactic acid | | 60.4 | 5.9 | 85.0 | 10.4 | 64.6 | 22.1 |
| Tartaric acid | | 74.7 | 21.5 | 64.5 | 13.9 | 45.7 | 11.8 |
| HCl | | 54.8 | 9.6 | 42.1 | 3.4 | 31.8 | 3.6 |
| None | 80 | <1 | <1 | <1 | <1 | <1 | <1 |
| Acetic acid | | 87.6 | 11.5 | 68.7 | 12.9 | 30.9 | 8.4 |
| Citric acid | | 43.1 | 11.0 | 67.9 | 17.1 | 71.9 | 28.6 |
| Lactic acid | | 57.2 | 16.2 | 63.7 | 9.2 | 46.1 | 9.9 |
| Tartaric acid | | 52.4 | 13.3 | –[a] | – | 59.5 | 12.1 |
| HCl | | 42.5 | 12.9 | – | – | 42.1 | 10.9 |

Each ginsenoside (100 μM) was treated with 0.1% acid, heated at various temperatures for 2 h, and extracted with BuOH, followed by assay of the transformed ginsenosides $Rg_3$ and $Rh_2$ by TLC assay systems. However, ginsenoside $Rh_2$ was not detected.
$Rg_3$, ginsenoside $Rg_3$; $\triangle Rg_3$, $\triangle^{20}$-ginsenoside $Rg_3$.
[a] not determined.

## Biological activities of biotransformed ginseng and ginsenosides

Ginseng BuOH fraction, ginsenosides $Rb_1$, $Rb_2$, Rc,

**Table III.** Effect of acid concentration and incubation time on the transformation of ginsenosides by acids

| Acid | Concn (%) | Int. Time (h) | Transformed Concentration (μM) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ginsenoside $Rb_1$ | | | | Ginsenoside $Rb_2$ | | | | Ginsenoside Rc | | | |
| | | | $Rg_3$ | $Rh_2$ | $\triangle Rg_3$ | $\triangle Rh_2$ | $Rg_3$ | $Rh_2$ | $\triangle Rg_3$ | $\triangle Rh_2$ | $Rg_3$ | $Rh_2$ | $\triangle Rg_3$ | $\triangle Rh_2$ |
| None | 0 | 2 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| | | 5 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| Acetic acid | 0.1 | 2 | 56.2 | <1 | 8.1 | <1 | 38.5 | <1 | 6.3 | <1 | 21.4 | <1 | 5.5 | <1 |
| | | 5 | 82.1 | <1 | 16.9 | <1 | 62.8 | <1 | 11.8 | <1 | 26.5 | <1 | 6.4 | <1 |
| | 1 | 2 | 61.5 | <1 | 9.6 | <1 | 48.9 | <1 | 13.4 | <1 | 29.0 | <1 | 4.6 | <1 |
| | | 5 | 82.2 | <1 | 13.3 | <1 | 62.0 | <1 | 24.0 | <1 | 28.9 | <1 | 4.4 | <1 |
| Citric acid | 0.1 | 2 | 62.8 | <1 | 17.5 | <1 | 42.2 | <1 | 17.1 | <1 | 39.0 | <1 | 11.6 | <1 |
| | | 5 | 74.5 | <1 | 17.7 | <1 | 54.6 | <1 | 18.6 | <1 | 42.6 | <1 | 14.7 | <1 |
| | 1 | 2 | 60.2 | <1 | 16.9 | <1 | 51.5 | <1 | 18.5 | <1 | 52.1 | <1 | 16.3 | <1 |
| | | 5 | 53.4 | <1 | 18.4 | <1 | 59.8 | <1 | 21.2 | <1 | 42.6 | <1 | 11.9 | <1 |
| Lactic acid | 0.1 | 2 | 60.4 | <1 | 5.9 | <1 | 85.0 | <1 | 10.4 | <1 | 64.6 | <1 | 22.1 | <1 |
| | | 5 | 74.4 | <1 | 11.2 | <1 | 95.7 | <1 | 9.6 | <1 | 73.9 | <1 | 25.4 | <1 |
| | 1 | 2 | 66.1 | <1 | 5.5 | <1 | 84.6 | <1 | 8.6 | <1 | 65.2 | <1 | 12.2 | <1 |
| | | 5 | 86.0 | <1 | 5.5 | <1 | 82.1 | <1 | 6.8 | <1 | 60.8 | <1 | 16.2 | <1 |
| Tartaric acid | 0.1 | 2 | 74.7 | <1 | 21.5 | <1 | 64.5 | <1 | 13.9 | <1 | 45.7 | <1 | 11.8 | <1 |
| | | 5 | 71.8 | <1 | 18.5 | <1 | 61.8 | <1 | 15.6 | <1 | 46.3 | <1 | 11.4 | <1 |
| | 1 | 2 | 69.5 | <1 | 20.6 | <1 | 58.2 | <1 | 15.3 | <1 | 43.7 | <1 | 9.6 | <1 |
| | | 5 | 68.7 | <1 | 19.8 | <1 | 54.3 | <1 | 18.8 | <1 | 47.7 | <1 | 12.3 | <1 |
| HCl | 0.1 | 2 | 54.8 | <1 | 9.6 | <1 | 42.1 | <1 | 3.4 | <1 | 31.8 | <1 | 3.6 | <1 |
| | | 5 | 41.1 | <1 | 7.1 | <1 | 42.7 | <1 | 8.4 | <1 | 35.5 | <1 | 4.8 | <1 |
| | 1 | 2 | 45.2 | <1 | 12.6 | <1 | 44.1 | <1 | 13.1 | <1 | 33.9 | <1 | 8.0 | <1 |
| | | 5 | 32.2 | 1.6 | 14.3 | <1 | 32.2 | 1.2 | 14.3 | <1 | 32.8 | 1.2 | 9.3 | <1 |

Each ginsenoside (100 μM) was treated with 1ml of 0.1 or 1% acid, heated at 60°C for 2 h or 5 h, and extracted with BuOH, followed by assay of the transformed ginsenosides $Rg_3$ and $Rh_2$ by TLC assay systems. However, ginsenoside $Rh_2$ was not detected.
$Rg_3$, ginsenoside $Rg_3$; $\triangle Rg_3$, $\triangle^{20}$-ginsenoside $Rg_3$; $Rh_2$, ginsenoside $Rh_2$; $\triangle Rh_2$, $\triangle^{20}$-ginsenoside $Rh_2$.

**Table IV.** Ginsenoside $Rh_2$ content of acid-treated ginsenosides fermented by human intestinal bacteria

| Microbe | Transformed ginsenoside (M) | | | | | |
|---|---|---|---|---|---|---|
| | Acid-treated $Rb_1$ | | Acid-treated Rb2 | | Acid-treated Rc | |
| | $Rg_3$ | $Rh_2$ | $Rg_3$ | $Rh_2$ | $Rg_3$ | $Rh_2$ |
| None | 30.2 | <1 | 42.5 | <1 | 32.3 | <1 |
| *Bifidobacterium* K-103 | 24.1 | <1 | 21.8 | 2.7 | 22.8 | <1 |
| *Bifidobacterium* K-506 | 23.7 | 3.0 | 23.5 | 1.6 | 17.4 | 6.4 |
| *Bifidobacterium* K-525 | 24.9 | 1.1 | 25.1 | <1 | 23.7 | <1 |
| *B. longum* KCTC 3215 | 25.5 | <1 | 24.9 | <1 | 26.4 | <1 |
| *B. breve* 1192 | 32.0 | <1 | 28.4 | <1 | 26.7 | <1 |
| *Fusobacterium* K-60 | 5.6 | 20.2 | 9.8 | 17.3 | 7.9 | 16.8 |
| *Eubacterium* A44 | 22.8 | <1 | 21.5 | <1 | 23.9 | 2.7 |
| *Bacteroides* HJ15 | 7.7 | 16.8 | 6.1 | 23.2 | 6.3 | 27.4 |
| *Bacteroides* JY6 | 6.9 | 16.2 | 8.3 | 8.5 | 8.7 | 16.6 |
| Human intestinal microflora | 7.2 | 17.5 | 6.1 | 22.8 | 6.3 | 27.1 |

Each ginsenoside (50 μM) was treated with 0.1% lactic acid, heated at 60°C for 2 h, adjusted at pH 7 with 1N-NaOH, metabolized by human intestinal bacteria or suspended fresh feces (final concentration, 1% w/v), and extracted with BuOH, followed by assay of the tr ansformed ginsenosides $Rg_3$ and $Rh_2$ by TLC assay systems.

**Table V.** Ginsenoside $Rh_2$ content of acid-treated ginseng water extracts fermented by human intestinal bacteria

| Treated acid | Bacterium | Transformed ginsenoside (M) | |
|---|---|---|---|
| | | $Rg_3$ | $Rh_2$ |
| None | *Bacteroides* HJ-15 | <1 | <1 |
| Acetic acid | | 2.5 | 23.3 |
| Citric acid | | 2.2 | 18.2 |
| Lactic acid | | 2.8 | 19.7 |
| Tartaric acid | | 2.4 | 22.3 |
| HCl | | 2.5 | 21.7 |
| None | *Bifidobacterium* K-506 | <1 | <1 |
| Acetic acid | | 11.1 | 14.9 |
| Citric acid | | 8.3 | 9.9 |
| Lactic acid | | 9.8 | 12.9 |
| Tartaric acid | | 8.5 | 10.3 |
| HCl | | 7.1 | 8.3 |
| None | Human intestinal microflora | <1 | <1 |
| Acetic acid | | 16.2 | 10.6 |
| Citric acid | | 14.9 | 5.4 |
| Lactic acid | | 17.4 | 7.7 |
| Tartaric acid | | 15.6 | 7.7 |
| HCl | | 12.3 | 7.0 |

Ginseng water extract (3 mg) was treated with 0.1% acids, heated at 80°C for 3 h, adjusted at pH 7 with 1 N NaOH, metabolized by human intestinal bacteria or suspended fresh feces (final concentration, 1% w/v), and extracted with BuOH, followed by assay of the transformed ginsenosides $Rg_3$ and $Rh_2$ by TLC assay systems.

**Table VI.** Anti-*Helicobacter pylori* activity of ginseng and transformed ginseng

| Agent | MIC (mg/mL) | |
|---|---|---|
| | HP43504 | HP82548 |
| Ginseng extract | 1 | >1 |
| Acid-treated Ginseng extract[a] | 1 | 1 |
| Fermented Acid-treated Ginseng extract | 0.5 | 1 |
| Ginsenoside $Rb_1$ | >0.1 | >0.1 |
| Ginsenoside $Rb_2$ | >0.1 | >0.1 |
| Ginsenoside Rc | >0.1 | >0.1 |
| Ginsenoside $Rg_3$ | >0.1 | >0.1 |
| $\Delta^{20}$-Ginsenoside $Rg_3$ | >0.1 | >0.1 |
| 20(S)-Ginsenoside $Rh_2$ | >0.1 | >0.1 |
| $\Delta^{20}$-Ginsenoside $Rh_2$ | >0.1 | >0.1 |
| 20(S)-Protopanaxadiol | 0.05 | 0.05 |
| *$\Delta^{20}$-Protopanaxadiol* | 0.05 | 0.05 |
| Ampicillin | 0.001 | 0.002 |

[a] Ginseng heated at 60°C for 2 h with 0.1% lactic acid.

**Table VII.** Cytotoxicity of ginseng and ginsenosides against tumor cell lines

| Agent | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | L1210 | P388 | HepG2 |
| Ginseng extract[a] | >200 | >200 | >200 |
| Acid-treated Ginseng extract[a,b] | 115 | 140 | >200 |
| Fermented Acid-treated Ginseng extract[a] | 98 | 98 | 160 |
| Ginsenoside $Rb_1$ | >200 | >200 | >200 |
| Ginsenoside $Rb_2$ | >200 | >200 | >200 |
| Ginsenoside Rc | >200 | >200 | >200 |
| Ginsenoside $Rg_3$ | 34 | 33 | 58 |
| $\Delta^{20}$-Ginsenoside $Rg_3$ | 34 | 36 | 38 |
| 20(S)-Ginsenoside $Rh_2$ | 22 | 33 | 25 |
| $\Delta^{20}$-Ginsenoside $Rh_2$ | 23 | 23 | 20 |
| 20(S)-Protopanaxadiol | 18 | 33 | 28 |
| Cisplatin | 3.9 | 5.5 | 17.8 |

[a] Unit of final concentrations is mg/mL.
[b] Ginseng heated at 60°C for 2 h with 0.1% lactic acid.

$Rg_3$, and $Rh_2$, and $\Delta^{20}$-ginsenoside $Rg_3$ and $Rh_2$ did not inhibit HP growth (Table VI). However, AG (ginsenoside $Rg_3$-enforced ginseng) and fermented AG (ginsenoside $Rh_2$-enforced ginseng) inhibited HP growth. Their MICs were 500-1000 mg/mL.

Ginseng BuOH fraction and ginsenosides $Rb_1$, $Rb_2$ and Rc did not show cytotoxicity against tumor cell lines (Table VII). However, AG, fermented AG, ginsenoside $Rg_3$, ginsenoside $Rh_2$ and protopanaxadiol exhibited potent cytotoxicity against tumor cell lines, with $IC_{50}$ values of 115->200, 98-160, 34-58 and 20-33 μM, respectively.

## DISCUSSION

The ginsenoside $Rg_3$ is an important component of Red ginseng and heat-processed ginseng, although it is not contained in dried ginseng. Han *et al.* (1982) reported that the ginsenosides $Rb_1$ and $Rb_2$ could be transformed to ginsenoside $Rg_3$, when these saponins were incubated in mild acidic conditions. When the pH of these ginseng samples was measured, it was acidic (pH 5.0-6.5). It was suggested that, when ginseng is steamed, ginsenosides of ginseng can be transformed to ginsenoside $Rg_3$. Nevertheless, the effects of acids and temperature on the transformation of ginsenosides have not been studied.

Therefore, we incubated protopanaxadiol saponins from ginseng under mild acidic conditions and assayed the contents of the main transformants, diastereomeric ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$. The composition of these ginsenosides was affected by acids and temperature. High temperature and diluted HCl significantly

increased the ratio of 20(*R*)-ginsenoside $Rg_3$ and $\Delta^{20}$-ginsenoside $Rg_3$ to 20(*S*)-ginsenoside $Rg_3$, compared to that for lactic acid and citric acid. When Schizandrae Fructus, which is widely used as an ingredient of herbal formulae, was incubated with ginseng, ginsenosides were also transformed to ginsenoside $Rg_3$ (data not shown). These results suggest that this transformation of ginsenosides to ginsenoside $Rg_3$ occurs frequently in herbal formulae containing ginseng, and that the pharmacological action of ginseng could alter according to the ingredients of herbal formulae.

When AG was incubated with human intestinal microflora, its ginsenoside $Rg_3$ was mainly transformed to ginsenoside $Rh_2$. When ginsenosides were treated by acids and incubated with intestinal bacteria, these compounds were mainly transformed to ginsenoside $Rh_2$ *via* ginsenoside $Rg_3$. These results are supported by our previous report that 20(*S*)-ginsenoside $Rg_3$ is potently metabolized to 20(*S*)-ginsenoside $Rh_2$, but that 20(*R*)-ginsenoside $Rg_3$ is nearly not metabolized by human intestinal microflora and intestinal bacteria isolated from human feces (Bae *et al.*, 2002).

Ginseng is frequently taken orally as a traditional medicine. The ginsenosides contained in ginseng have been reported to show various biological activities including antitumor activity. To explain the biological activities of these saponins *in vivo*, these ginsenosides are likely to be metabolized to ginsenoside $Rh_2$ or compound K by human intestinal bacteria. When ginsenosides $Rb_1$ and $Rb_2$ were orally administered, they could be transformed to ginsenoside $Rg_3$ in the stomach. This ginsenoside $Rg_3$, which is in red ginseng, heat-processed ginseng and AG, and which is metabolized from ginsenosides $Rb_1$ and $Rb_2$ in the stomach, should be metabolized to ginsenoside $Rh_2$ or 20(*S*)-protopanaxadiol in the human intestine. However, if orally administered ginsenosides $Rb_1$ and $Rb_2$ were not transformed to ginsenoside $Rg_3$ in the stomach, they should be metabolized to compound K (IH 901) in the human intestine.

To determine the active compounds of ginseng when orally administered in humans, we measured some biological activities, such as anti-HP and cytotoxic activity against tumor cell lines of ginseng and ginsenosides. Ginsenoside $Rh_2$-enforced ginseng exhibited the most potent cytotoxicity against tumor cell lines. We found that the cytotoxicity of ginseng against tumor cell lines was increased when it was treated with acids or when it was biotransformed by intestinal bacteria. The cytotoxicity of ginsenosides against tumor cell lines was increased when 20(*S*)-ginsenoside $Rg_3$ was metabolized to either 20(*S*)-ginsenoside $Rh_2$ or 20(*S*)-protopanaxadiol by human intestinal microflora. The anti-*Helicobacter pylori* activity of ginseng was also increased when it was treated with acids or when it was biotransformed by intestinal bacteria.

Based on these findings, it is suggested that the ginsenosides found in ginseng may be prodrugs, which can be transformed to active compounds by acids and /or intestinal bacteria, for *Helicobacter pylori* infection and tumors.

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