Metabolic Activities of Ginsenoside Rb1, Baicalin, Glycyrrhizin and Geniposide to Their Bioactive Compounds by Human Intestinal Microflora

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To evaluate the pharmacological actions of herbal medicines, metabolic activities of herbal medicine components, ginsenoside Rb1, glycyrrhizin, geniposide and baicaulin to their bioactive compounds compound K, 18β-glycyrrhetic acid, genipin and baicalein by fecal specimens were measured. Their metabolic activities were 646.1±591.4, 29.4±51.7, 926.3±569.6 and 3884.6±1400.1 µmol/h/g, respectively. The profiles of these metabolic activities of baicalin and ginsenoside Rb1 were not significantly different to those of water extracts of Scutellariae Radix and Ginseng Radix. None of the metabolic activities tested were different between males and females, or between ages. However, the difference in these metabolic activities in individuals was significant. These results suggest that the human intestinal microflora enzymes that convert herbal components to their bioactive compounds may be used as selection markers of responders to traditional medicines.

Key words intestinal microflora; metabolism; herbal medicine; component; pharmacological action

Most herbal medicines which have been used in China, Korea, and Japan are orally administered to human. Therefore, their components are inevitably brought into contact with intestinal microflora in the alimentary tract. Intestinal microflora transform these components before absorption from the gastrointestinal tract. For example, when ginsenoside Rb1, which is a major component of ginseng, is administered to humans or rats, 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (compound K) transformed by intestinal microflora, but not ginsenoside Rb1, was detected in blood. Compound K, not ginsenoside Rb1, exhibited antitumor and anti allergic actions. Glycyrrhizin of liquorice, baicalin of Scutellariae Radix and geniposide of Gardeniae Fuctus are also transformed by intestinal microflora before absorption from the gastrointestinal tract, and the transformed metabolites have exhibited pharmacological activities. Therefore, intestinal bacteria related to the metabolism of the components of herbal medicines should be an important factor for understanding the biological activities of herbal medicines.

In relation to the role of intestinal microflora on the pharmacological actions of herbal medicines, Kobashi et al. reported that some enzyme activities of intestinal bacteria were significantly different between Jitsu-syo (strong type) and Kyo-syo (weak type) Japanese, although the intestinal bacteria between Jitsu-syo and Kyo-syo Japanese were not different. We have reported that some fecal bacterial enzymatic activities related to the pharmacological actions of herbal medicines were variable among individuals. Nevertheless, studies on the metabolic activities of herbal medicine components by human intestinal microflora are not sufficient.

Therefore, we determined the metabolic activities of herbal medicinal components by human fecal microflora and investigated the relationship between the metabolic activities.

MATERIALS AND METHODS

Subjects The subjects were 92 healthy Korean males and females (average, 42.02±9.35 years; 53 males, 42.00±9.06 years; 39 females, 42.04±9.92 years). Exclusion criteria included smoking and current medication, especially regular or current use of antibiotics.

Materials Glycyrrhizin, 18β-glycyrrhetic acid (GA), baicalin, and baicalein were purchased from Sigma Chem. Co. (U.S.A.). Genipin and geniposide were purchased from Wako Pure Chem. Ind. (Japan). Ginsenoside Rb1 and compound K were isolated according to a previous method.

Specimens Preparation The human fecal specimens (about 3 g) prepared according to a previous method, were collected in plastic cups 9 h after fasting, and then carefully mixed with a spatula and suspended with cold 27 ml saline. The fecal suspension was centrifuged at 10000×g for 5 min. The supernatant was then centrifuged at 10000×g for 20 min. The resulting precipitates (about 0.3 g) were used as a metabolic enzyme source for the assay of enzyme activity. The preparation and assay of the enzyme source were performed within 24 h.

Assay of Herbal Medicine Component Metabolic Activities by Human Fecal Microflora To measure the metabolic activity of herbal medicine components (or extracts of herbal medicines), the above fecal precipitate (0.2 g) was suspended with 1.8 ml of 50 mM phosphate buffer (pH 7.0) and then used in the present experiment. The reaction mixture (1 ml) containing 0.2 ml of the fecal suspension, 0.2 ml of 0.5 mM natural glycosides and 0.6 ml of 25 mM phosphate buffer (pH 7.0) was incubated at 37°C for 1 h (or 10 h in the case of glycyrrhizin), and the reaction mixture was extracted twice with 5 ml of ethyl acetate, and then evaporated in vacuo. The ethyl acetate fraction was dissolved in methanol and then analyzed by TLC.

Thin Layer Chromatography TLC for genipin and geniposide was performed on silica gel plates (silica gel 60F-
254, Merck, Germany) with a developing solvent system of CHCl$_3$ : MeOH = 6 : 1 (v/v). TLCs for glycyrrhizin and 18β-glycyrrhetic acid were performed on silica gel plate with CHCl$_3$ : petroleum ether : acetic acid = 6 : 6 : 1 (v/v). TLC for baicalin, baicalein, ginsenoside Rb1 and compound K was performed on silica gel plate with CHCl$_3$ : MeOH : H$_2$O = 65 : 35 : 10 (lower layer, v/v). The chromatograms of these compounds were quantitatively assayed with a TLC scanner (CS-9301PC, Shimadzu Co.).

Statistics The SPSSwin 8.0 program was used for statistical analysis of the data. The differences in fecal enzyme activities between males and females and between ages were assessed by ANOVA.

RESULTS

The subjects studied in the present experiment were 92 healthy persons. To evaluate the role of intestinal microflora in the pharmacological action of herbal medicines, the metabolic activities of their main components, ginsenoside Rb1, baicalin, glycyrrhizin and geniposide of ginseng, Scutellariae Radix, liquorice and Gardeniae fructus in relation to their bioactive compounds compound K, GA, genipin and baicalein were compared using fecal specimens (Fig. 1). The metabolic activities of these compounds in relation to the bioactive compounds were 31.03—2889.87, 240.17—5015.94, 0—280.02 and 168.96—2445.01 μmol/h/g, respectively (Table 1). The activities were not different between males and females, or between ages. The average metabolic activities (mean ± S.D.) were 646.14 ± 591.39, 29.40 ± 51.74, 926.30 ± 569.61 and 3884.63 ± 1400.09 μmol/h/g, respectively.

When water extracts of ginseng and Scutellariae Radix were used instead of ginsenoside Rb1 and baicalin isolated from herbal medicines to measure the metabolic activity, the metabolic activities of these main components of herbal medicines were not different compared to those of isolated compounds (Fig. 2). The average metabolic activities of ginsenoside Rb1 and baicalin compared to compound K and

![Fig. 1. Distribution of Fecal Metabolic Activity Transforming Components Isolated from Herbal Medicines to Bioactive Compounds in 92 Subjects](image)

(A) Metabolic activity of ginsenoside Rb1 to compound K; (B) metabolic activity of glycyrrhizin to GA; (C) metabolic activity of geniposide to genipin; (D) metabolic activity of baicalin to baicalein. Closed circle, male; open circle, female.

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<th>Table 1. Metabolic Activity of Components Isolated from Herbal Medicines in Relation to Bioactive Compounds in 92 Subjects</th>
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<td><strong>Activity (μmol/h/g)</strong></td>
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<td>Compound</td>
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<tr>
<td>G-Rb1</td>
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<td>Baicalin</td>
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<td>Glycyrrhizin</td>
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baicalein were 246.0 ± 175.0 and 1860 ± 796.9 μmol/h/g, respectively. The activities were not different between males and females, or between ages.

DISCUSSION

All individuals possess their own characteristic indigenous strain of intestinal bacteria. This is due to the affinity between the intestinal lumen of the individual and the bacteria. Newly ingested bacteria cannot necessarily colonize and proliferate in the intestine. Therefore, the difference in intestinal bacteria between residents of the same environment was not significant. These results are supported by previous reports that found that intestinal microflora in feces are thought to be rather stable over time within individuals in the absence of disease and antimicrobial therapy. When some enzyme activities have been studied using fecal suspension, the contents of other intestinal segments and isolated bacterial suspension, those of fecal sample are similar to that of contents in the distal segment of the colon. Mykkänen et al. reported that spot fecal sampling produced reliable results concerning fecal enzyme activities when compared with the total collection of a single defecation. In a previous study, we also measured the differences in three fecal sample preparation methods on some activities of intestinal bacterial enzymes. However, we could not find any sign-
ificant differences between fecal specimens prepared by 3 methods: suspended fecal suspension, centrifuged fecal bacterial precipitate, and sonicated fecal bacterial suspension. Therefore, we used the fecal bacterial precipitate method for the assay of metabolic activities of herbal medicines by intestinal microflora and believe that this preparation method could be applicable to the assay of intestinal bacterial enzyme activities.

In addition, Kobashi et al. reported that some enzymes of intestinal bacteria were significantly different between Jitsu-syo and Kyo-syo Japanese, although intestinal bacteria between Jitsu-syo and Kyo-syo Japanese were not different.\textsuperscript{12, 20} Ikeda et al. reported that some intestinal bacterial enzyme activities did not appear to be associated with specific populations.\textsuperscript{20} However, these fecal bacterial enzyme activities are affected by diet,\textsuperscript{16, 21, 22} but rebound if diet or supplements were stopped for short term.\textsuperscript{21—23} These results suggest that the intestinal bacterial enzyme activities of each individual are indigenous, although they can be altered by other factors as well as diet.

Therefore, to understand the pharmacological actions of herbal medicines, the bioactive components that are absorbable from the human intestine should be investigated. Most herbal medicine components glycosides which is activated by intestinal microflora are prodrugs.\textsuperscript{1, 2} These results suggest that intestinal microflora may play an important role in the pharmacological actions of herbal medicines. In the present study, we investigated the metabolic activities of the main components from herbal medicines to bioactive compounds. These activities varied significantly between individuals. If an individual potently metabolizes ginsenoside Rb1 or ginseng extract to compound K, an antitumor agent that originates from ginseng, they may also transform the components of other herbal medicines. Therefore, we evaluated the relationship between the metabolic activities of some components (Fig. 3). The potencies of ginsenoside Rb1-metabolic individuals are significantly in proportion to those of genipose-metabolic individuals, but are out of proportion to those of other component-metabolic activities. Both ginsenoside Rb1 and geniposide may be produced due to metabolism to their bioactive compounds by β-D-glucosidases, which hydrolyze aglycone-β-D-glucopyranoside.\textsuperscript{14, 24} Glycyrrhizin and baicalin are metabolized to their bioactive compounds by β-D-glucuronidases in intestinal bacteria. Nevertheless, the glycyrrhizin-metabolic activity of individuals is out of proportion to baicalin-metabolic activity. This result is supported by previous reports which found that glycyrrhizin-metabolic β-D-glucuronidase is different to the baicalin-metabolic enzyme, although both compounds are hydrolyzed by β-D-glucuronidases. The former may recognize and hydrolyze β-D-diglucuronide, while the latter may recognize and hydrolyze β-D-monoglucuronide.

Although the baicalin- and ginsenoside Rb1-metabolic activities in an aqueous extract of Scutellariae Radix and ginseng were low compared to those of isolated compounds, their profiles were similar to those of isolated compounds. Furthermore, the profile between the baicalin- and ginsenoside Rb1-metabolic activities was also similar to that between these compound-metabolic activities in Ginseng Radix extract and Scutellariae Radix (Fig. 4).

Based on these findings, we believe that the intestinal bac-

![Fig. 4. Profile of the Relationship between Metabolic Activities of Baicalin in Scutellariae Radix and Ginsenoside Rb1 in Ginseng](image)

**G-Rb1, ginsenoside Rb1; BC, baicalin. Closed circle, male; open circle, female.**