Arch Pharm Res Vol 25, No 2, 165-169, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Fecal Metabolic Activities of Herbal Components to Bioactive Compounds

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(Received December 20, 2001)

The herbal components should be transformed to bioactive compounds by the intestinal bacteria and then expressed the pharmacological action of herbal medicines. Human fecal enzyme activities related to the metabolism of herbal components were measured. The metabolic activities of puerarin, poncirin, glycyrrhizin, ginsenoside Rb1 and ginsenoside Rb2 to their bioactive compounds were 3.5 ± 1.18, 333.1 ± 183.64, 95.7 ± 107.1, 28.6 ± 10.32 and 20.8 ± 13.3 μmol/h/g, respectively. The profile of these metabolic activities of glycyrrhizin and ginsenosides were not changed even if herbal extracts, water extract of Glycyrrhizae Radix and Ginseng Radix, instead of the isolated compounds were used. All the enzyme activities tested were not different between male and female, and between ages. However, the difference of these enzyme activities in individuals was significant. These results suggest that the metabolic activity of herbal components to bioactive compounds may be a factor of constitutional classification, and could be available for constitutional classifications, if the constitutional herbal medicines were used.

Key words: Intestinal Bacteria, Constitutional classification, Metabolism, Natural components

INTRODUCTION

Most of herbal medicines, which have been used in China, Korea and Japan, are orally administered into human. Therefore, their components are inevitably brought into contact with intestinal microflora in the alimentary tract. The intestinal bacteria transform these components before absorption from the gastrointestinal tract. Therefore, intestinal bacteria related to the metabolism of the components of herbal medicines should be an important factor to understand their biological activities of herbal medicines (Kobashi and Akao, 1997; Kim *et al.*, 1998a; Hasegawa *et al.*, 1997).

Many researchers reported that the intra- and inter-individual variations of these intestinal bacteria were not significant, but their enzyme activities were affected by dietary change and physiological factors (Reddy *et al.*, 1980, Mallet *et al.*, 1988). Therefore, to understand the pharmacological actions of herbal medicines, the intra- and inter-individual variations of these enzymatic activities are of a great importance. Because the pharmacological actions of herbal medicines were dependent on intestinal bacterial enzyme activities, and herbal medicines should be selected according to intestinal bacterial enzyme activities. We thought that the metabolic enzyme activities of natural components should be an important factor on the Korean constitutional classification.

Therefore, to understand the relationship between the metabolic activity of herbal medicinal components and the constitutional classification of traditional medicine, we first assayed the enzymatic metabolic activities of herbal medicinal components by human fecal bacteria.

MATERIALS AND METHODS

Subjects

The subjects were 92 healthy Korean persons (average, 44.7 ± 11.5 years; range, 20-69years; 53 males, 42.7 ± 10.3 years; 39 females, 47.1 ± 12.7 years). Exclusion criteria

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included smoking and current medication, especially regular or current use of antibiotics.

Materials

p-Nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucuronide, *p*-nitrophenyl-α-L-rhamnopyranoside, *p*-nitrophenyl-α-L-arabinopyranoside, *p*-nitrophenyl-β-D-xylopyranoside, glycyrrhizin, 18β-glycyrrhetic acid and puerarin were purchased from Sigma Chem. Co. (U.S.A.). Ginsenoside Rb1 and Rb2, poncirin, ponciretin and daidzein were isolated according to the previous method (Kim *et al.*, 1988b; Bae *et al.*, 1999; Bae *et al.*, 2000).

Specimens preparation

The fecal specimens were collected in plastic cups 9 h after fasting and determined the wet weight and bacterial enzyme activities within 24 h. These specimens were carefully mixed with a spatula and suspended in cooled tubes containing 20-fold saline and prepared the sample as follows. Preparation 1-Fecal suspension was centrifuged at 500 rpm for 5min. The supernatant was centrifuged at 10000 rpm for 20 min. The resulting precipitates were used for the assay of enzyme activity. Preparation 2-Fecal bacterial suspension was centrifuged at 500 rpm for 5 min. The supernatant was used for the assay of enzyme activity. Preparation 3-Suspended specimen was centrifuged at 500 rpm for 5 min, centrifuged at 10000 rpm for 20 min, sonicated for 4 min (Ultrasonic processor, HEAT system Inc.) and used for the assay of enzyme activity.

Enzyme activity assay

The α-glucosidase, α-rhamnosidase, β-xylosidase, α-arabinosidase and β-glucuronidase were assayed as follows: the reaction mixture (total volume of 0.5 ml) contained 0.2 ml of 2 mM *p*-nitrophenyl-α-D-glucopyranoside for α-glucosidase, 1 mM *p*-nitrophenyl-β-D-glucuronide for β-glucuronidase, 1 mM *p*-nitrophenyl-α-L-rhamnopyranoside for α-rhamnosidase, *p*-nitrophenyl-α-L-arabinopyranoside for α-arabinosidase or 1 mM *p*-nitrophenyl-β-D-xylopyranoside for β-xylosidase, 0.2 ml of 0.1 M phosphate buffer, pH 7.0, 0.1ml of the fecal suspension (wet weight, 4 mg). The assay mixture was incubated at 37°C for 15 min. The reaction was stopped by the addition of 0.5 ml of 0.5 N NaOH, centrifuged at 3000 rpm for 10min and measured the absorbance at 405 nm (UV-vis spectrophotometer, Shimadzu UV-1201).

Assay of intestinal bacteria enzyme activities transforming herbal components to their aglycones

Natural glycosides transforming activity was measured as follows. The fecal precipitate was suspended with 20-fold 50 mM phosphate buffer and then was used as an enzyme source. The reaction mixture containing 1 ml of the human fecal suspension and 2 mM natural glycosides was incubated at 37°C for 1 h, and the reaction mixture was extracted twice with 5 ml of ethyl acetate. The ethyl acetate fraction was analyzed with TLC.

Thin-layer chromatography

TLCs for poncirin, ponciretin, puerarin and daidzein were performed on silica gel plate (Merck, silica gel 60F-254) and developing solvent system was $CHCl_3$: MeOH=6:1 (v/v). TLCs for glycyrrhizin and 18β-glycyrrhetic acid were performed on silica gel plate with butanol : acetic acid : H_2O : $CHCl_3$=4:4:1:1 (v/v). TLCs for ginsenoside Rb1, ginsenoside Rb2 and compound K were performed on silica gel plate with $CHCl_3$: MeOH : H_2O=65:35 10 (lower layer, v/v). The chromatograms of these compounds were quantitatively assayed with a TLC scanner (CS-9301PC, Shimadzu).

Statistics

The SPSSwin 8.0 program was used for statistical analysis of the data. The difference of enzyme activities between three preparation methods was tested using Wilcoxon-signed Ranks test.

RESULTS AND DISCUSSION

The subjects tested in this experiment were 92 healthy persons. To investigate the difference of fecal enzymes according to the sample preparation methods, five fecal specimens of healthy human were randomly selected, treated according to Materials and Methods, and their enzyme activities were assessed (Table 1). Since the difference was not significant between the preparation methods, we used the fecal suspension (Preparation 1) due to the convenience for measurement of enzyme activities.

Table I. Comparison of intestinal bacteria enzyme prepared by three methods

Enzyme	Activity (μmol/h/g wet feces)		
	Preparation 1[a] (n=5)	Preparation 2 (n=5)	Preparation 3 (n=5)
β-Glucosidase	21.91± 9.21	25.28± 10.24	36.82± 10.24
β-Glucuronidase	5.89± 3.07	6.09± 2.56	8.88± 3.33
Urease	19.71± 11.43	12.71± 10.00	16.43± 15.71
Tryptophanase	0.57± 0.37	0.54± 0.71	0.50± 0.74

Values are mean± SD.
There is no significant difference by Wilcoxon-signed Ranks test.
[a]preparation 1, fecal bacterial suspension; preparation 2, fecal suspension; preparation 3, sonicated bacterial suspension.

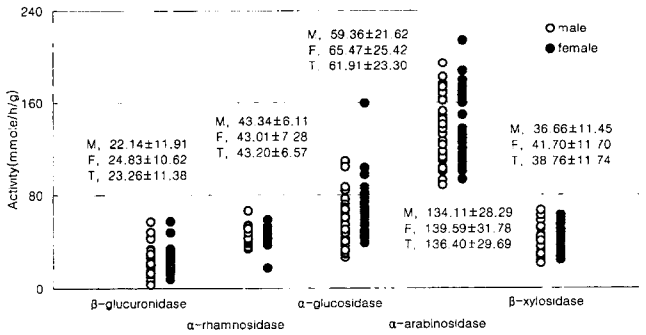

Fig. 1. Fecal Enzyme Activity of Healthy Volunteer for Synthetic Substrates. M, male; F, female; T, male and female. Values are mean ± SD.

Therefore, we measured some enzyme activities of 92 fecal specimens using synthetic substrates (Fig. 1). Among enzymes tested, α-arabinosidase activity was the highest. All these enzyme activities tested in individuals were variable. However, these enzyme activities were not significantly different between male and female, and between ages.

We also measured the metabolic activities of natural components, puerarin, poncirin, glycyrrhizin, ginsenoside Rb1 and ginsenoside Rb2 to their bioactive compounds by fecal specimens (Fig. 2). Their metabolic activities of these compounds to bioactive compounds were 3.5± 1.18, 333.1± 183.64, 95.7± 107.1, 28.6± 10.32 and 20.8 ± 13.3 μmol/h/g, respectively. These metabolic activities in individuals were highly variable compared to those hydrolyzing the synthetic substrates. When the herbal extracts were used instead of compounds isolated from herbal medicines to measure the metabolic activity, the metabolic activities of the main components of herbal medicines were weak compared to those of isolated compounds (Fig. 3). However, the profile of these metabolic activities of glycyrrhizin and ginsenosides were not changed. These activities were not different between male and female, and between ages. The metabolic activity of one isolated compound was not proportional to those of the other compounds.

All individuals possess their characteristic indigenous strain of intestinal bacteria. This is due to the affinity between the intestinal lumen of man and bacteria. Newly ingested bacteria could not necessarily colonize and proliferate in the intestine. Therefore, the difference of intestinal bacteria between residents of the same environment was not significant. These results were supported by the report of Kobashi *et al.* (1984), and Simon and Gorbach (1986) 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 were studied using fecal suspension, the contents of other intestinal segments

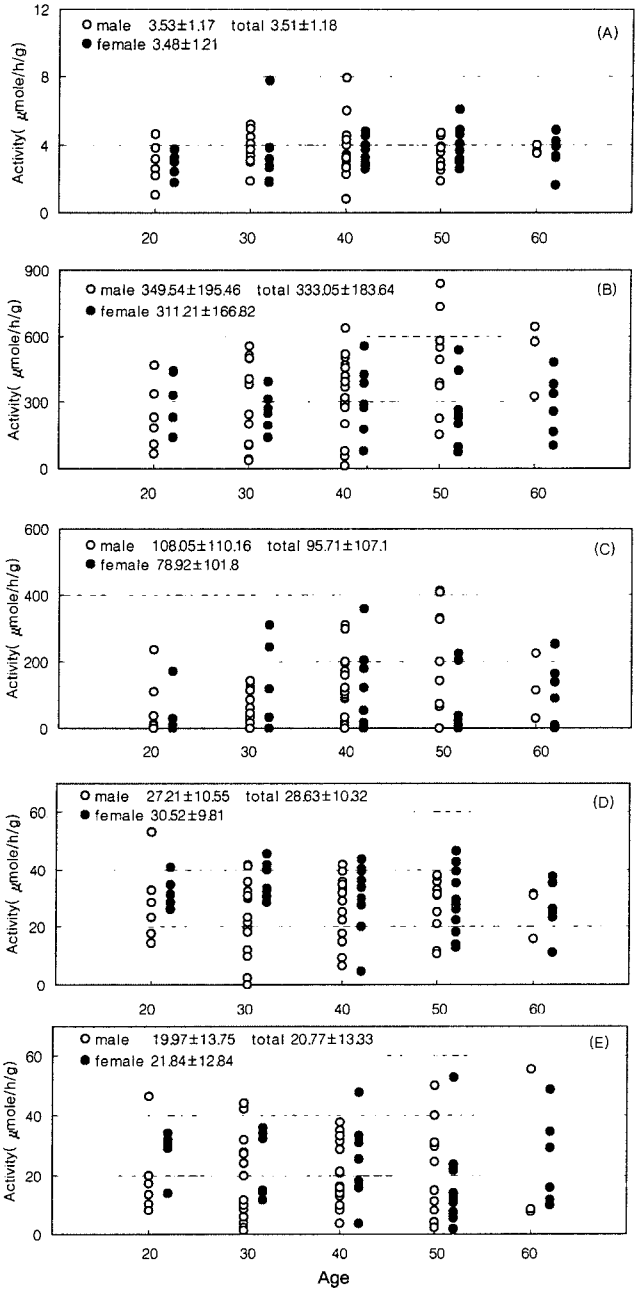

Fig. 2. Metabolic Activity Transforming Components Isolated from Herbal Medicines to Bioactive Compounds in 92 Subjects. A, activity transforming puerarin to daidzein; B, activity transforming poncirin to ponciretin; C, activity transforming glycyrrhizin to 18β-glycyrrhetic acid; D, activity transforming ginsenoside Rb1 to compound K; E, activity transforming ginsenoside Rb2 to compound K. M, male; F, female; T, male and female. Values are mean± SD.

and isolated bacterial suspension (Rumney and Rowland, 1992), those of fecal sample are similar to that of contents in the distal segment of the colon. And Mykkänen *et al.* (1998) reported that spot fecal sampling produced reliable results of the fecal enzyme activities when compared with total collection of a single defecation. We also measured

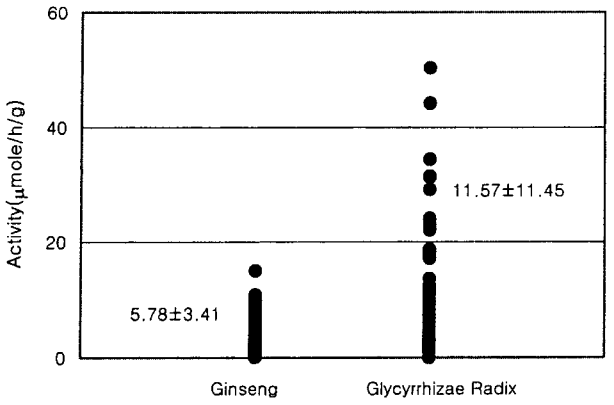

Fig. 3. Fecal Metabolic Activity Transforming Components of Ginseng and Glycyrrhizae Radix to Bioactive Compounds in 92 Subjects. Ginseng, activity transforming components of Ginseng to compound K and Glycyrrhizae Radix, activity transforming components of Glycyrrhizae Radix to 18β-glycrrhetic acid. M, male; F, female; T, male and female. Values are mean± SD.

the difference of three fecal sample preparation methods on some activities of intestinal bacterial enzymes. However, we could not find the significant difference between these methods. Therefore, we used fecal suspension method in this experiment (Preparation 1) and thought that this preparation method could be available for the assay of intestinal bacterial enzyme activities.

In addition, Kobashi *et al.* (1984) confirmed 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. Ikeda *et al.* (1994) described that some intestinal bacterial enzyme activities did not appear to be associated with specific populations. However, these fecal bacterial enzyme activities are affected by diet (Goldin *et al.* 1980, Reddy *et al.*, 1980. Mallet *et al.*, 1988), but rebound if diet or supplement for short term were stopped (Drasar *et al.*, 1976, Goldin *et al.*, 1980, Ling *et al.*, 1994). These results suggest that intestinal bacterial enzyme activities of each individual are indigenous, although they are changeable by some other factors as well as diet. Thus, we investigated the metabolic activity of natural products to bioactive compounds to understand the relationship between the intestinal bacterial enzyme activities and constitutional classifications of traditional medicine. The traditional constitutional classification is designed by Lee Je-ma in 1894 (1996). It classifies the constitution of human-being into four types; Taeyangin, Taeumin, Soyangin and Soeumin, and each type is classified by etiology, pathology and symptoms of individuals. If the constitutional classification is confused by the previous methods, he recommended a herbal medicine-treated method: herbal medicines should be orally administered to human, and then constitutional types could be classified according to their pharmacological expression. Therefore, the metabolic activities of natural products to bioactive compounds should be important for the constitutional classification of traditional medicines. When the metabolic activities of natural product components were measured to understand the constitutional classifications in traditional medicines, these activities were significantly variable between individuals. If some individuals have the potent metabolic activity of puerarin, a main component of the rhizome of *Pueraria thunbergiana* that is used for the therapy of Taeumin diseases, this group may be Taeumin group. And if some individuals have the potent metabolic activity of glycyrrhizin, a main component of the rhizome of *Glycyrrhiza uralensis* that is used for the therapy of Soeumin diseases, this group may be Soeumin group.

Based to these findings, we insist that the intestinal bacterial enzyme activities should play the important role in the pharmacological action of herbal medicines and could be a factor for constitutional classification. We recommend that these enzyme activities could be used for the constitutional classification of traditional medicines.

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