The Role of Intestinal Microflora on Daidzein Excretion in Urine in Humans Treated with Chungpesagantang

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This study examined the relationship between the metabolism of the constituents of herbal medicines by human intestinal microflora and the level of metabolites excreted in the urine. This was performed by administering Chungpesagantang (CST) to volunteers and measuring their fecal metabolic activity CST and urine excretion of daidzein, one of the metabolites of CST. The metabolic activity of CST was 54.8 ± 16.7 mmol/h/g wet feces. When CST was administered orally to the subjects, the amount of daidzein excreted in the urine over 24 h was 103.7 ± 55.8 mg, which accounted for 20.2% of the puerarin, daidzin, and daidzein contained in CST. However, neither puerarin nor daidzin were excreted in the urine. The profile of daidzein excreted in the urine was found to be in proportion to that of the metabolic activity of the CST components. This suggests that the daidzein level excreted in the urine of the subjects administered CST is associated with the daidzein glycoside-hydrolyzing activity of the fecal microflora.

Key words: Chungpesagantang, Intestinal microflora, Metabolism, Daidzein, Excretion

INTRODUCTION

Functional foods and herbal medicines are commonly consumed by humans. Therefore, their components are inevitably brought into contact with the microflora of the alimentary tract and are transformed before they are absorbed (Kobashi and Akao, 1997; Kim, 2002).

All individuals have their own characteristic indigenous strains of intestinal bacteria. Newly ingested bacteria cannot necessarily colonize and proliferate in the intestine. Therefore, an individual's intestinal microflora in feces are believed to be fairly stable over time in the absence of disease and/or antimicrobial therapy (Cole et al., 1985; Rumney and Rowland, 1992; Simon and Gorbach, 1986). Ikeda et al. reported that the activity of some gastrointestinal bacterial enzymes does not appear to be associated with specific populations (Ikeda et al., 1994). These fecal bacterial enzyme activities are affected by diet (Ikeda et al., 1994; Ling et al., 1994), but this effect is reversed if the diet or supplements are stopped for a short time (Goldin et al., 1980; Reddy et al., 1980). Kobashi et al. (1984) reported that there are significant individual variations in some enzymes of intestinal bacteria. We previously reported that some fecal bacterial enzymatic activities related to the pharmacological actions of herbal medicines vary between individuals (Yim et al., 2004).

The rhizome of Pueraria thunbergiana (family Leguminosae) is frequently used as a functional food or an ingredient of herbal formulae (Lee, 1996; Jung et al., 2003). Its main components are puerarin and daidzin, which are daidzein glycosides. Kim et al. (1998) reported that puerarin and daidzin are metabolized to daidzein by human intestinal microflora. The in vitro anti-tumor, anti-inflammatory, and antioxidant activity of the metabolite, daidzein, are higher than that of puerarin and daidzin. Therefore, the components of arrowroot may need to be metabolized by intestinal microflora in order to be pharmacologically active. Nevertheless, the relationship between the metabolism of the components of herbal medicines by human intestinal microflora and the amount of absorbed or excreted metabolites has not been studied.

Chungpesagantang (CST) is a formula containing the rhizome of Pueraria thunbergiana that is frequently used in Korea to prevent stroke (Jung et al., 2003; Park, 2003). This study examined the metabolism of the isoflavone...
glycosides of CST by human fecal microflora. In addition, the amount of their metabolite, daidzein, excreted in the urine of human subjects administered CST was measured, and the relationship between the metabolic activity and the amount of metabolite excreted in the urine was determined.

MATERIALS AND METHODS

Materials

p-Nitrophenyl-β-D-glucopyranoside was purchased from Sigma Co. (St. Louis, MO, U.S.A.). 52 g CST, a herbal mixture containing 16 g arrowroot (the rhizome of Pueraria thunbergiana), 8 g Scutellaria baicalensis rhizome, 8 g Angelica tenissima rhizome, 4 g Cimicifuga heracleifolia rhizome, 4 g Angelci dahurica rhizome, 4 g Planticodon gradiforum rhizome, 4 g Raphanus satius semen and 4g Rheum palmatum rhizome, was extracted with 10 times the total weight of water for 1 h and freeze-dried (yield, 21.2%: 3.93% puerarin, 0.42% daidzin and 0.31% berberine).

Subjects

Fourteen male volunteers (age: 20-30 years; body weight: 61-77 kg; height: 169-181 cm), who were found to be healthy according to their medical history, physical examination, electrocardiography, and laboratory testing, were enrolled in this study. The exclusion criteria included smoking and current medication, particularly the regular or current use of antibiotics. All subjects were fully informed of the risks and stresses associated with the project. This study was approved by the Ethics Committee of the Medical Center of Kyung Hee University.

Urine collection and analysis of metabolites

CST extract (11 g/person) was administered orally to the volunteers and total urine was collected in a sterilized urine collector at 0, 3, 6, 9, 12 and 24 h. 50 mL of each urine sample was incubated for 20 h at 37°C with 3,000 units of α-glucuronidase (Sigma Co., St. Louis, MO, U.S.A.). The level of the metabolite daidzein was measured using HPLC (Hitachi system: column, Spherisorb ODS1 (3.0×250 mm); elution solvent, 0.5M phosphate buffer:acetonitrile (72:28); elution rate, 1.0 mL/min; detection wavelength, 280 nm).

Preparation of specimens

Fresh fecal specimens of volunteers (3 g) prepared according to a previously described method (Lee et al., 2003), were collected in plastic cups 9 h before oral administration of CST, and then carefully mixed with a spatula and suspended with 27 mL cold saline. The fecal suspension was centrifuged at 10000 g for 20 min. The supernatant was then centrifuged at 10000 g for 20 min. The resulting precipitates 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 metabolic activities of herbal medicine components by human fecal microflora

An assay of the metabolism of CST, puerarin and daidzin to daidzein by human fecal microflora was performed according to the method reported in the literature (Lee et al., 2003). Briefly, the above fecal precipitate (0.2 g) was suspended with 1.8 mL of 50 mM phosphate buffer (pH 7.0) and used as a crude enzyme solution in the present experiment.

The reaction mixture (2 mL) containing 0.4 mL of the fecal suspension, 0.4 mL of 0.5 mM CST, puerarin, daidzin, or p-nitrophenyl-β-D-glucopyranoside and 0.2 mL of 25 mM phosphate buffer (pH 7.0) was incubated at 37°C for 2 h, and the reaction mixture was extracted twice with 10 mL of ethyl acetate, and then evaporated under vacuum. The ethyl acetate fraction was dissolved in methanol and then analyzed by TLC.

Thin layer chromatography

TLC of daidzein was performed on silica gel plates (silica gel 60F-254, Merck, Germany) with a developing solvent system of CHCl3:MeOH = 6:1 (v/v) or CHCl3:petroleum ether:acetic acid = 6:6:1 (v/v). The chromatograms were quantitatively assayed with a TLC scanner (CS-9301PC, Shimadzu Co.).

Statistics

The data was analyzed using the SPSSwin 10.0 program. Correlations between the results were analyzed using the Spearman test (p <0.1).

RESULTS AND DISCUSSION

The role of intestinal microflora in the absorption and excretion of the components of CST was examined by measuring the fecal metabolism of CST constituents to daidzein. The metabolic activities of the fecal specimens of the 14 subjects were between 32.3 and 79.9 μmol/h/g wet feces (Table I). The average metabolic activity (mean ± S.D.) was 54.8 ± 16.7 μmol/h/g wet feces.

In order to understand the relationship between the metabolism of the components of CST, puerarin and daidzin, by human fecal microflora and the amount of their metabolite(s) absorbed, the amount of daidzein excreted in the urine of volunteers administered CST orally was measured (Fig. 1). The accumulated amount of daidzein excreted within 24 h of CST administration in all subjects