Urinary Pharmacokinetics of Baicalein, Wogonin and Their Glycosides after Oral Administration of Scutellariae Radix in Humans

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Baicalin, baicalein, wogonoside and wogonin are flavone constituents of Scutellariae Radix with various beneficial biological activities. The purpose of this study was to investigate the urinary pharmacokinetics of these flavones after oral administration of Scutellariae Radix commercial powder. Ten healthy male volunteers received a dose of 5.2 g commercial powder (comparable to 9 g crude drug), respectively. The concentrations of baicalin, baicalein and wogonin in the commercial powder as well as their metabolites in urine were assayed by HPLC method. The glucuronides and sulfates of baicalein and wogonin in urine were hydrolyzed with β-glucuronidase and sulfatase, respectively. Our results showed that the mean cumulated renal excretion of baicalein glucuronides and sulfates were 43.1±4.5 μmol (2.9% of dose) and 64.8±6.3 μmol (4.3% of dose), respectively, whereas wogonin glucuronides and sulfates were 21.6±2.0 μmol (5.9% of dose) and 20.7±1.7 μmol (5.7% of dose), respectively. The result indicated that the renal excretion of conjugated metabolites of wogonin (11.6% of dose) were higher than that of baicalein (7.2% of dose). The baicalein sulfates was predominant than the corresponding glucuronides, whereas wogonin sulfates was comparable to the corresponding glucuronides.

Key words baicalin; baicalein; wogonin; Scutellariae Radix; pharmacokinetics

Flavonoids are a class of naturally occurring phenolic plant constituents with low toxicity. They have been reported to exhibit a wide range of biological activities. Therefore, their use as potential therapeutic agents against a variety of diseases is of interest.1) Scutellariae Radix contains a large number (over 40 compounds) of flavonoids and is widely used in clinical Chinese medicine as a remedy for treatments of inflammation, fever, cough, dysentery, jaundice, hepatitis and hypertension.2) Scutellariae Radix is a major component of a Kambo medicine “Sho-saiko-to” which is effectively used to treat chronic hepatitis in Japan.3) A herbal mixture, also containing Scutellariae Radix, called PC-SPES (Fan and Wang, U.S. patent pending 08/697.920) has been used for patients with prostate cancer. Baicalin with its aglycone, wogonoside is used to treat chronic hepatitis in Japan.3) A herbal mixture, also containing Scutellariae Radix, called PC-SPES (Fan and Wang, U.S. patent pending 08/697.920) has been used for patients with prostate cancer. Baicalin with its aglycone, wogonoside is used to treat chronic hepatitis in Japan.3) A herbal mixture, also containing Scutellariae Radix, called PC-SPES (Fan and Wang, U.S. patent pending 08/697.920) has been used for patients with prostate cancer. Baicalin with its aglycone, wogonoside is used to treat chronic hepatitis in Japan.3) A herbal mixture, also containing Scutellariae Radix, called PC-SPES (Fan and Wang, U.S. patent pending 08/697.920) has been used for patients with prostate cancer. Baicalin with its aglycone, wogonoside is used to treat chronic hepatitis in Japan.3) A herbal mixture, also containing Scutellariae Radix, called PC-SPES (Fan and Wang, U.S. patent pending 08/697.920) has been used for patients with prostate cancer. Baicalin with its aglycone, wogonoside is used to treat chronic hepatitis in Japan.3) A herbal mixture, also containing Scutellariae Radix, called PC-SPES (Fan and Wang, U.S. patent pending 08/697.920) has been used for patients with prostate cancer. Baicalin with its aglycone, wogonoside has been extensively explored to show anti-inflammatory,5,6) antiviral,7—9) free radical scavenging,4,10) antioxidant10—11) and anticancer activities.12—14) In addition, baicalin was also shown to induce apoptosis in prostate cancer cell line.15)

The fate of many flavonoids are understood that aglycones are absorbed directly from the gut wall, whereas flavonoid glycosides are usually absorbed only after being hydrolyzed by enterobacterial enzymes to corresponding aglycones. Baicalin was transformed, in part, into baicalein prior to absorption,16) and subsequently metabolized into its conjugated metabolites during the first pass. The predominant metabolites of baicalin are baicalein 7-O-glucuronide (baicalein) and baicalein 6-O-sulfate after the administration of “Sho-saikotō” in humans.17)

In recent decades, herbal extract preparation in granule or powder form has been commercialized for routine clinical use and is the only dosage form of Chinese medicine covered by the national health insurance in Taiwan. However, till now there was no information available on the pharmacokinetics of the flavonoids in the commercial powder of Scutellariae Radix. Recently, assay methods for the conjugated metabolites of flavonoids in serum or urine have been developed in our laboratory.18—21) The main object of this study was to investigate the urinary pharmacokinetics of the bioactive flavones in Scutellariae Radix after oral administration of a commercial powder to ten healthy male volunteers.

MATERIALS AND METHODS

Chemicals Baicalin, baicalein and wogonin were obtained from Wako (Osaka, Japan). β-Glucuronidase (type B-1 from bovine liver), sulfatase (type H-1 from Helix pomatia), methyl paraben and propyl paraben were purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). Acetonitrile, methanol and ethyl acetate were LC grade and purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, U.S.A.). L(+)-Ascorbic acid was obtained from RdH Laborchemikalien GmbH & Co. KG (Seelze, Germany). Other reagents were HPLC grade or analytical reagent grade. Milli-Q plus water (Millipore, Bedford, MA, U.S.A.) was used throughout this study.

Instrumentation The HPLC apparatus included a pump (LC-6AD, Shimadzu, Japan), an UV spectrophotometric detector (SPD-6A, Shimadzu, Japan) and chromatopac (C-18A, Shimadzu, Japan) with an automatic injector (Series 200 Autosampler, Perkin Elmer, U.S.A.). The Inertsil ODS-2 column (4.6×250 mm, 5 μm) was equipped with a guard column (4.6×50 mm, 5 μm) (GL Science Inc., Tokyo, Japan).

Extraction of Commercial Powder The commercial powder (500 mg) was extracted two times with 50 ml 70% aqueous methanol by ultrasonic shaking for 2 h and filtered. Sufficient amount of methanol was added to the combined filtrates to make 100 ml and then frozen at −30°C for later analysis.

Acid Hydrolysis of the Methanol Extract The methanol extract of commercial powder was added with an
equal volume of 5% HCl, then divided into three aliquots (2.0 ml each). Each aliquot was wrapped with aluminum foil and the hydrolysis was carried out at 100°C in a water bath for 1.5 h. The hydrolysates obtained were added with sufficient methanol to make 2.0 ml and then frozen at −30°C for later analysis.

Quantification of Baicalin, Baicalein, Wogonoside and Wogonin in Commercial Powder The assay method and validation were reported in our previous study.19) Two hundred microliters of sample was added with 200 µl ethyl paraben solution (20.0 µg/ml in methanol) as internal standard and 20 µl were subjected to HPLC analysis. The mobile phase consisted of acetonitrile−0.005% phosphoric acid (36:64, v/v) for simultaneous assay of baicalin, baicalein and wogonin in urine. The detection wavelength was set at 270 nm and the flow rate was 1.0 ml/min. The contents of baicalin, baicalein and wogonin were determined directly, whereas wogonoside was calculated by subtraction of the free form wogonin from the total wogonin after acid hydrolysis.

Subjects Ten healthy Chinese males, 20—22 years old and weighing 58—69 kg, provided their informed consents. Routine biochemical tests indicated that their hepatic and renal functions were in good condition. They did not smoke or drink and had taken no medication for at least 2 weeks and throughout the experiment.

Commercial Powder Administration and Urine Collection After overnight fasting, each volunteer was administered orally with 5.2 g commercial powder and 200 ml water. Food was withheld for 3 h after drug administration. Urine samples were collected before and 0—2, 2—4, 4—6, 6—8, 8—10, 10—12, 12—24, 24—36 and 36—48 h after dosing.

Hydrolysis of Glucuronides of Baicalein and Wogonin in Urine The concentrations of glucuronides of baicalein and wogonin in urine were determined after β-glucuronidase treatment. For enzymolysis, 200 µl urine sample was mixed with 100 µl β-glucuronidase (889 units/ml in pH 5 acetate buffer), 20 µl ascorbic acid (200 mg/ml) and incubated at 37°C for 7 h under anaerobic condition and protected from light.21) After hydrolysis, the urine was acidified with 50 µl 0.1 N HCl and partitioned with 350 µl ethyl acetate (containing 8.0 µg/ml of propyl paraben as internal standard). The ethyl acetate layer was evaporated under N2 to dryness and reconstituted with an appropriate volume of mobile phase prior to HPLC analysis.

Hydrolysis of Sulfates of Baicalein and Wogonin in Urine The concentrations of sulfates of baicalein and wogonin in urine were determined after sulfatase treatment. For enzymolysis, 200 µl urine sample was mixed with 100 µl sulfatase (847 units/ml in pH 5 acetate buffer), 20 µl ascorbic acid (200 mg/ml) and incubated at 37°C for 2 h under anaerobic condition and protected from light. The later procedure was the same as enzymolysis with β-glucuronidase described above.

HPLC Analysis of Baicalin and Wogonin in Urine Hydrolysates The assay method and validation for baicalin and wogonin in urine hydrolysate were reported in our previous study.19)

HPLC Analysis of Baicalin in Urine One hundred microliters of urine with appropriate dilution was added with an equal volume methyl paraben solution (5.0 µg/ml in methanol) as internal standard and then filtered through 0.45 µm millipore membrane prior to HPLC analysis. A mobile phase consisting of acetonitrile−0.1% phosphoric acid (22:78 v/v, pH 2.23—2.26) was used. The detection wavelength was set at 268 nm and flow rate was 1.0 ml/min.

For calibration preparation, 200 µl urine spiked with various concentrations of baicalin, respectively, was added with an equal volume of methyl paraben solution (5.0 µg/ml in methanol) to afford urine standards with concentrations in the range of 1.3—40.0 µg/ml. The calibration curve was plotted by linear regression of the peak-area ratios (baicalin to internal standard) against concentrations of baicalin.

Validation of Assay Method for Baicalin in Urine The system suitability was evaluated by the intraday and interday precision and accuracy of triplicates. The accuracy of this method was further assessed with recovery study by spiking baicalin into blank urine and phosphate buffer (pH 6) to afford 5.0, 10.0 and 20.0 µg/ml in triplicates, respectively, and the concentrations obtained in blank urine to the corresponding ones in buffer were compared. The LOQ (limit of quantification) represents the lowest concentration of analysis in a sample that can be determined with acceptable precision and accuracy, whereas LOD (limit of detection) represents the lowest concentration of analysis in a sample that can be detected (S/N>=3).

Data Analysis The concentrations of baicalin, conjugated metabolites of baicalein and wogonin in urine were multiplied by the respective urinary volume collected in each time interval to obtain the total amount excreted in the sampling time. The fraction excreted in urine was calculated as percentage of the dose which included the intake of baicalin with baicalein and wogonoside with wogonin in Scutellariae Radix, respectively. The apparent elimination rate constants were estimated by Sigma–Minus method in which the slope of the regression line obtained when the natural logarithm of the metabolite amount not yet excreted was plotted versus time and then transformed into half-lives.

RESULTS

Flavone Contents in the Commercial Powder Quantification of the commercial powder indicated that each administered dose contained baicalin 1380.1 µmol (616.0 mg), baicalein 194.3 µmol (52.5 mg), wogonoside 254.5 µmol (117.2 mg) and wogonin 112.5 µmol (32.0 mg).

Determination of Baicalin in Urine Typical chromatograms of baicalin in urine are shown in Fig. 1. Good linear relationship was obtained for baicalin in the concentration range of 1.3—40.0 µg/ml (Y=0.056X+0.118, r=0.999) in urine. The precision and accuracy of this method for deter-
mination of baicalin in urine indicated that all coefficients of variation (CVs) were below 8.1% and the relative errors were below 9.0%. The LOQ was 1.3 \( \mu g/ml \) and LOD was 0.6 \( \mu g/ml \). The recoveries of baicalin from urine were 105.9, 101.1 and 108.4% for the concentrations 5.0, 10.0 and 20.0 \( \mu g/ml \), respectively.

**Determination of Glucuronides and Sulfates of Baicalein and Wogonin in Urine**

No free form of baicalein or wogonin was detected in urine. The renal excretion, percentages excreted and the elimination half-lives of the conjugated metabolites of baicalein and wogonin are shown in Table 1. Total amounts (the percentages of the dose) of 25.8\% (1.7\%), 43.1\%±4.5 \( \mu mol \) (2.9\%) and 64.8\%±6.3 \( \mu mol \) (4.3\%) for baicalin, baicalein glucuronides and sulfates were excreted in urine, respectively, whereas those for wogonin glucuronides and sulfates were 21.6\%±2.0 \( \mu mol \) (5.9\%) and 20.7\%±1.7 \( \mu mol \) (5.7\%), respectively. The mean cumulated urinary excretion during 48 h and the mean urinary excretion rates of baicalin, baicalein glucuronides, baicalein sulfates, wogonin glucuronides and wogonin sulfates in each time interval of ten individuals were shown in Fig. 2 and Fig. 3, respectively.

**DISCUSSION**

Quantitation of the commercial powder of Scutellariae Radix indicated that baicalin and wogonoside were the major flavone constituents, whereas their aglycones baicalein and wogonin were less abundant. The commercial products of Chinese herbal extract were generally prepared by hot water extraction and spray-drying in Taiwan, therefore they usually contain more polar constituents. The assay methods of baicalin, baicalein and wogonin in commercial powder as well as conjugates of baicalein and wogonin in urine were developed and validated in our laboratory.\(^{19,21}\) Because authentic wogonoside standard was not available; therefore, the total content of wogonoside with wogonin was determined after acid hydrolysis of the commercial powder, then the wogonoside was calculated subsequently by subtraction of the free form wogonin from the total wogonin in the hydrolysate. The result indicated that the content of wogonoside was about two times of the aglycone wogonin.

Biological activities of flavonoids depend on their absorption. The chemical structures and physicochemical properties of flavonoids determine their rates and extents of intestinal absorption and the nature of the metabolites circulating in the plasma. Two metabolites of baicalin and baicalein in human plasma after oral administration had been identified.
as baicalein 7-O-glucuronide (baicalin) and baicalein 6-O-sulfate.\textsuperscript{17} Contrasted with our previous report\textsuperscript{19} using a mixed enzyme of glucuronidase/sulfatase to hydrolyze urine, the present study determined individual concentrations of baicalein glucuronides, baicalein sulfates, wogonin glucuronides and wogonin sulfates by using $\beta$-glucuronidase and sulfatase to hydrolyze urine separately. The enzymatic hydrolysis procedure was conducted with the addition of ascorbic acid, under anaerobic condition and protected from light. The optimum hydrolysis time was 7 h for $\beta$-glucuronidase\textsuperscript{19} and 2 h for sulfatase as determined in this study.

Because there are two phenolic groups of baicalein available for glucuronidation, therefore baicalein glucuronides may include 7-O-glucuronide and 6-O-glucuronide. Baicalin (baicalein 7-O-glucuronide) was quantitated prior to urine hydrolysis in this study. If one subtracts baicalin from the total concentration of baicalein glucuronides, the obtained concentration will be 6-O-glucuronide. It was found that the HPLC analysis of baicalin had to be carried out in a mobile phase with pH below 2.3, the peak would split otherwise. The validation of baicalin assay in urine indicated that the precision and accuracy were satisfactory and almost quantitative recoveries of baicalin from urine were obtained.

Besides, no free form of baicalein and wogonin was detected in urine prior to hydrolysis. This result is correspondent to our other study in which baicalein sulfates/glucuronides were found exclusively circulating in the blood and negligible baicalein was detected after oral dosing of baicalin and baicalein to rats (data not shown).

Baicalin and wogonoside are glycoside compounds which are relatively polar and thus not lipophilic enough to permeate through enterocytes, whereas the aglycones (baicalein and wogonin) are relatively nonpolar and absorbable from gut wall.\textsuperscript{22} The early renal excretion of baicalin, glucuronides and sulfates of baicalein and wogonin between 0—2 h (Fig. 3) could be accounted by the rapid absorption of the aglycones, baicalein and wogonin, in Scutallarieae Radix by the small intestine. Regarding to the absorption of glycosides, baicalein and wogonoside, their hydrolyzed metabolites baicalein and wogonin gradually formed after fermentation by enterobacteria were absorbed later in the colon. Therefore, baicalin and wogonoside could be considered as the natural slow-released prodrugs of baicalein and wogonin, respectively, whereas their aglycones, baicalein and wogonin, served as immediate-released drugs. The result showed that the excretion of wogonin conjugated metabolites (11.6% of dose) was more than that of baicalein (7.2% of dose), indicating that wogonin was absorbed in a greater extent. From their retention times on the reversed-phase HPLC chromatogram,\textsuperscript{19} it could be speculated that wogonin eluted later than baicalein should be more lipophilic and thus more permeable through enterocytes. The low urinary recovery of the conjugated metabolites of baicalein and wogonin from the dose might be explainable by that the remaining fraction of dose was excreted in bile and/or degraded to other metabolites by the enterobacteria. The urinary excretion of baicalein sulfates was higher than corresponding glucuronides, whereas that of wogonin sulfates was comparable to corresponding glucuronides. The apparent elimination half-life of baicalein conjugates was around 8 h, whereas those of wogonin conjugates were about 10 h, indicating pretty long residence time for these metabolites in humans. The inter-individual variation of urinary pharmacokinetics of these flavones was quite large. This phenomena is in good agreement with a recent report of flavonoid glycoside rutin in healthy volunteers.\textsuperscript{23}

In conclusion, this study showed that the commercial powder of Scutellariae Radix provided a rich source of antioxidant flavones including baicalin, baicalein, wogonoside and wogonin for absorption in humans. Their urinary pharmacokinetics indicated that the sulfates and glucuronides of baicalein and wogonin should make major contribution to the efficacy of flavones in Scutellariae Radix. Therefore, we suggest that it would be more appropriate to focus on the conjugated metabolites of the flavones in Scutellariae Radix indicating baicalin for future in vitro pharmacological studies.

REFERENCES


