Influence of Time of Administration of a Shosaiko-to Extract Granule on Blood Concentration of Its Active Constituents

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Using a Shosaiko-to extract granule, we investigated the effects of the timing of administration (orally, before and after a meal) on the plasma concentration of its active constituents, glycyrrhizin (GL), baicalin, baicalein and glycyrrhizic acid (GA), a metabolite of GL.

The pattern of plasma concentration change of GL differed between the two times of administration, and followed a two-phase pattern when the granules were taken before meals. There was no difference neither in the area under the plasma concentration–time curve (*AUC*) between the two periods, nor *AUC* itself. GA showed no difference in plasma concentration pattern, nor was baicalin detected in the plasma following administration by either method. The plasma concentration pattern of baicalein differed between the two timings but followed that of the two-phase pattern in each case.

The plasma concentration pattern of active constituents of Shosaiko-to extract granule varied with the time of administration, but there was no significant difference in the maximum plasma concentration or *AUC* of active constituents depending on the administration. We concluded that the present clinical timing of administration of Shosaiko-to should be determined on the basis of patient compliance and other relevant factors.

Keywords glycyrrhizin; glycyrrhizic acid; baicalein; baicalin; plasma concentration; administration method

A number of extract preparations of Chinese medicines have been introduced in recent years and their consumption is steadily increasing. At the same time, many studies have been performed concerning pharmacology, absorption from the digestive tract, and pharmacokinetics of active constituents and the interaction among constituents.[1-3] The relationship between timing of administration and plasma concentration patterns of active constituents following such administration was studied here.

The administration method described on package inserts is based on ancient traditions followed for Chinese medicine and recommends taking it twice or three times a day before and between meals. In actual clinical use, however, administration after a meal is often recommended for patient compliance. We were employed subjects of normal ten men in this study, because plasma concentration pattern of active constituents differed by enterobactera, response, and they were differed between animals and human.

In the present study, we used granules of Shosaiko-to, the most frequently prescribed Chinese medicine extract, and investigated the relationship between timing of administration and the following plasma concentraion pattern of the active constituents,[4] glycyrrhizin (GL), baicalin, and baicalein.

Experimental

Reagents Shosaiko-to extract granules are a product of Tsumura. GL, glycyrrhizic acid (GA), baicalin and baicalein standards were products of Nacarai Tesque Co., Ltd. All other reagents employed were commercial special-grade products.

Subjects Ten men participated in this study after giving informed consent and were employed (weight 55—65 kg, age 32—37) who were confirmed by tests to be normal in blood, liver and urinary functions.

Administration and Blood Sampling The subjects were orally administered a daily dose (7.5 g) of Shosaiko-to extract granule with 20 ml of water. Administration was done in two ways, before breakfast (30 min prior) and after breakfast (30 min after). After breakfast the day before the experiment started, all subjects had the same meals and drinks. Smoking was prohibited. Three milliliters of blood was collected from the brachial vein in a heparinized tube. Blood samples were immediately centrifuged and the obtained plasma analyzed.

Extraction of Active Constituents and Metabolites from Plasma GL was extracted according to the method of Yasuda *et al.*[5] using an ammonia–ethanol mixture. GA was extracted according to the method of Kato *et al.*[6] using hydrochloric acid and an acetic acid buffer.

Baicalin was extracted using the method of Tomimori *et al.*[7] with 50% ethanol and 80% acetone.

Conversion of GL and Baicalin into Their Aglycons GL was converted into aglycons in the following steps: One ml of plasma was added to 5 ml of 2 N HCl and was hydrolyzed by refluxing at 120 °C for 2 h. The mixture was allowed to cool, then evaporated under reduced pressure. The residue was added to 5 ml of water and 5 ml of chloroform. After shaking for 30 min, the mixture was centrifuged at 3500 rpm for 10 min. The chloroform phase was separated into a tube with a ground glass stopper and evaporated under reduced pressure. Obtained residue was added to 0.5 ml of methanol, then filtered (Millipore Co., Ltd., pore size: 0.5 μm). The filtrate was used as the analysis sample.

Baicalin was converted into aglycons by the same method.

Method of Analysis Plasma concentrations of GL, GA, baicalin and baicalein were determined by a liquid chromatograph–mass spectrometer (LC-MS) as described below: A Hitachi L-6200 HPLC (Tokyo, Japan) was equipped with an ultraviolet detector (Hitachi L-3000) and an high performance liquid chromatography (HPLC) column packed with Cosmoseal $_5C_{18}$ (150 × 4.6 mm i.d., Nacarai Tesque Co., Ltd). Analysis was performed under the following conditions: The mobile phase was a mixture of 2% acetic acid–acetonitrile (4:3 v/v for GL, 1:2 v/v for GA and baicalein, 7:3 v/v for baicalin), and the follow up rate was 1.0 ml/min. GL and GA were measured at 254 nm, baicalin and baicalein were measured at 280 nm. The mass spectrometry was carried out by atmospheric pressure ionization. Nebulizer and vaporizer temperatures were 360 °C, and the drift voltage was 150 to 170 V.

Calibration Curves Known amounts of GL, GA, baicalin and baicalein were added to the plasma and were extracted and analyzed by methods similar to those above. The calibration curves were obtained by the peak–area method.

Results

Determination of Concentrations of GL and GA The plasma concentrations after administration of the Shosaiko-to extract granule were determined using HPLC, and peaks corresponding to GL and GA standerd were recognized.

These peak levels were very low, however, and a chromatogram showed many other peaks which seemed to originate from blood constituents which made quantitative analysis difficult. Neither of the standard calibration curves

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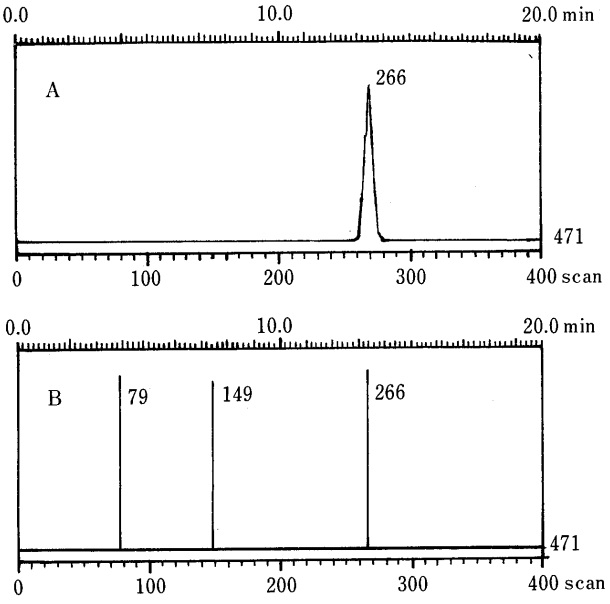

Fig. 1. Mass Chromatograms of GA Extracted from Human Plasma

(A) GA, (B) 8 h after before-meal administration.

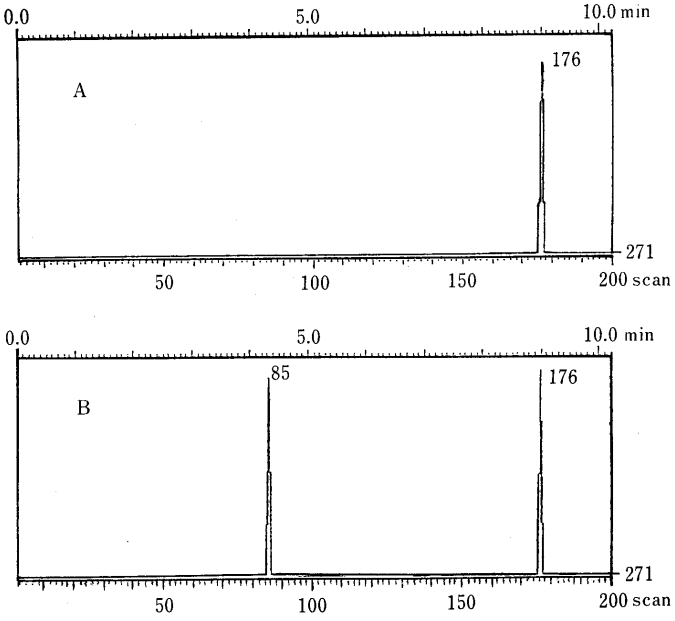

Fig. 2. Mass Chromatograms of Baicalein Extracted from Human Plasma

(A) Baicalein, (B) 12 h after before-meal administration.

of GL and GA were linear at concentrations of 1.0 μg/ml or below. Therefore, for the purpose of identification and quantitative analysis of each peak, plasma concentrations of GL and GA were measured using LC-MS.

Figure 1 shows the chromatograms obtained in the mass chromatography of GA standard and plasma samples taken 8 h after the before-meal oral administration of Shosaiko-to extract granule. The mass chromatograms of the plasma sample had a peak with a retention time of 13.4 min corresponding to the standard GA peak; this peak was quantitatively analyzed. GL was then analyzed by means of LC-MS. Glucuronic acid, the GL sugar component, was found to be decomposed under high temperature and many peaks originating from the

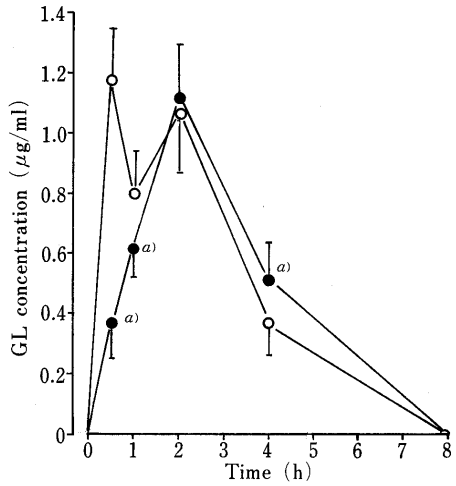

Fig. 3. Plasma Concentration of GL after Oral Administration of Shosaiko-to Extract Granule

○, before-meal; ●, after-meal. Each value represents the mean±S.D. of ten volunteers. Statistical significance by Student's *t*-test compared with before-meal, *a*) $p<0.01$.

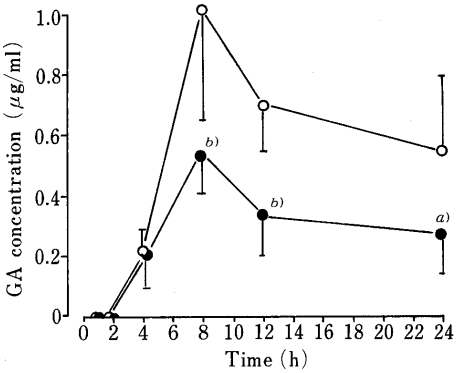

Fig. 4. Plasma Concentration of GA after Oral Administration of Shosaiko-to Extract Granule

○, before-meal; ●, after-meal. Each value represents the mean±S.D. of ten volunteers. Statistical significance by Student's *t*-test compared with before-meal, *a*) $p<0.05$, *b*) $p<0.01$.

degradation products were visible on the chromatogram. Quantitative analysis by this method was impossible.

GL was therefore converted into GA and determined using LC-MS, which revealed a peak at m/z 471 (M^+). This peak was quantitatively analyzed in the same manner as in GA described above.

Determination of Plasma Concentrations of Baicalin and Baicalein Figure 2 shows the mass chromatogram obtained in mass chromatography of the baicalein standard and plasma sample taken 12 h after the before-meal oral administration of Shosaiko-to extract granule. The mass chromatograms of the plasma sample had a peak with a retention time of 9 min corresponding to the standard baicalein peak. This peak was quantitatively analyzed. Baicalin was then analyzed by means of LC-MS. As in GL, the chromatograms showed many peaks of fragment ions originating from terminal degradation of glucuronic acid, the baicalin sugar component. Therefore, baicalin was converted into baicalein and then was quantitatively analyzed. No baicalin was detected in plasma samples.

Plasma Concentrations of GL and GA Figure 3 shows the plasma concentrations of GL which differed following

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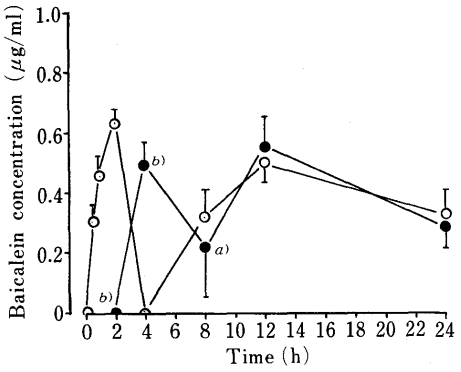

Fig. 5. Plasma Concentration of Baicalein after Oral Administration of Shosaiko-to Extract Granule

○, before-meal; ●, after-meal. Each value represents the mean±S.D. of ten volunteers. Statistical significance by Student's *t*-test compared with before-meal, *a*) $p<0.05$, *b*) $p<0.01$.

administration of Shosaiko-to extract granules before and after a meal. Following before-meal administration, two peaks were observed at 0.5 and 2 h (1.18 and 1.05 μg/ml, respectively). Concentration slowly decreased and went below the detectable limit after 8 h. In after-meal administration, a peak was recognized after 2 h (1.10 μg/ml). Concentration decreased thereafter and fell below the detectable limit after 8 h. The area under the curve (AUC_{0-8h}) of GL 3.85±0.43 (before a meal) and 3.53±0.33 h·μg/ml (after a meal), indicated no significant difference ($p>0.05$). Plasma concentration of GL was calculated hy reducing the determined concentration of GA in plasma containing GA converted from GL (total GA).

Figure 4 shows the plasma concentration of GA following administration of the granules before and after a meal, in both instances, a peak was recognized at 8 h after administration. The peak value with before-meal administration was 1.11 μg/ml while that after a meal was 0.52 μg/ml.

Plasma Concentration of Baicalein Figure 5 shows the different plasma concentrations of baicalein following the two timings of granule administration. Before-meal administration, two peaks were observed at 2 and 12 h (0.62 and 0.49 μg/ml, respectively), and thereafter the concentration gradually lowered. In after-meal administration, two peaks were observed at 4 and 12 h (0.49 and 0.52 μg/ml, respectively), and again, the concentration then gradually lowered. The AUC_{0-24} of baicalein in before-meal administration was 7.91±1.44 h·μg/ml, and that in after-meal administration was 7.75±0.61 h·μg/ml. The $AUC_{0-\infty}$ of baicalein in before-meal administration was 12.20±1.44 h·μg/ml, which that in after-meal administration was 11.38±3.10 h·μg/ml. There was no significant difference between the two timings. ($p>0.05$).

Discussion

Using a Shosaiko-to extract granule, we compared plasma concentrations of active constituents between the timing administration (before a meal and after a meal). GL concentration followed a two phase pattern in before-meal administration, while administration after a meal caused a concentration which peaked at 2 h. This suggested that the amount of food consumed may affect the GL gastric emptying rate.

Baicalein plasma concentration also followed a two-phase pattern in both instances, but the maximum concentration time was later in after-meal administration. This, together with the finding that there was no baicalin detected in plasma, suggested that there were two types of baicalein absorption: one in which baicalein was directly absorbed and the other in which baicalein was converted from baicalin by enterobacteria and then absorbed.

Plasma concentration of GA was higher in before-meal than after-meal administration, and greatly varied among individual subjects. This may reflect individual variation in conversion ratio from GL to GA by enterobacteria, and in the ability of entero-hepatic circulation of GA. Because plasma concentrations of active constituents differed depending on the time of granule consumption, yet there were no significant differences in *AUC*, we concluded that the timing of administration could be decided taking patient compliance into consideration. We will study other active constituents such as saikosaponin and attempt to establish more useful clinical administration guidelines.

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