Pharmacokinetic Profiles of Active Components After Oral Administration of a Kampo Medicine, Shakuyakukanzoto, to Healthy Adult Japanese Volunteers

CHIHARU SADAKANE,1 JUNKO WATANABE,1 MIWAKO FUKUTAKE,1 HIROAKI NISIMURA,2 KAZUYA MAEMURA,2 YOSHIO KASE,3 TORU KONO1,4

1Tsumura Research Laboratories, Kampo Scientific Strategies Division, Tsumura & Co., Ibaraki, Japan
2Kampo Formulations Development Center, Production Division, Tsumura & Co., Ibaraki, Japan
3Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan
4Center for Clinical and Biomedical Research, Sapporo Higashi Tokushukai Hospital, Sapporo, Japan

Received 15 May 2015; revised 26 June 2015; accepted 2 July 2015
Published online 24 July 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24596

ABSTRACT: Shakuyakukanzoto (SKT), a traditional Japanese (Kampo) medicine, has been used by patients with muscle cramps and abdominal pains. In this trial, we analyzed plasma concentrations of active components after SKT was administered as a single oral dose of 2.5 or 5.0 g/day per person. The study was a randomized, open-label, two-arm, two-period, crossover trial conducted in healthy Japanese volunteers. Albiflorin (ALB), paeoniflorin (PAE), glycycoumarin (GCM), isoliquiritigenin (ILG), glycyrrhetic acid (GA), and glycyrrhetic acid-3-O-monoglucuronide were targeted, and the plasma concentration of each component was measured using a liquid chromatography–tandem mass spectrometry method. The pharmacokinetic parameters were calculated, and the linearity was assessed. All targeted components were detected in the plasma after oral administration of SKT. ALB, PAE, GCM, and ILG were detected at an early stage. The linearity was observed for the maximum plasma concentration of GCM, ILG, and GA and for the area under the plasma concentration–time curve of GA. In this trial, we demonstrated for the first time in humans that these components were absorbed into the blood after oral administration of SKT. The results of this pharmacokinetic trial in humans are also important and useful for understanding the mechanism of action of SKT, verifying the active components predicted in basic research, and conducting pharmacokinetics and safety studies in the future. © 2015 The Authors. Journal of Pharmaceutical Sciences published by Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3952–3959, 2015

Keywords: Clinical pharmacokinetics; Dose-response; Intestinal absorption; Intestinal metabolism; Metabolite kinetics; Shakuyaku-kanzo-to; TJ-68; randomized crossover study; healthy adult volunteers

INTRODUCTION

Shakuyakukanzoto (SKT) is a “Kampo” traditional Japanese medicine that has been approved by the Ministry of Labor, Health, and Welfare. It comprises two types of crude drugs, Glycyrrhizae radix and Paeoniae radix, and is widely used by patients with muscle cramps and abdominal pains. In clinical trials, SKT has been reported effective in alleviating muscle pains, muscle cramps, joint pain, and numbness,1–3 and its immediate potency in exerting these effects has also been studied.4 Anticonvulsant effects in a rat spasticity model5 and antiallodynic and antinoiception or analgesia effects in a mouse allodynia model6,7 have been suggested as the pharmacological effects of SKT. Glycyrrhizae radix-derived glycyrrhetic acid (GA), glycycoumarin (GCM), and isoliquiritigenin (ILG) are presumed to be active components, which are responsible for anticonvulsive, analgesic, and muscle-relaxant effects.8–10 Paeoniae radix-derived paeoniflorin (PAE) and albiflorin (ALB) are presumed to be active components that are responsible for antinociceptive effects.9 In contrast, long-term excessive ingestion of Glycyrrhizae radix is known to cause pseudoadosteronism or hypokalemia,11–14 and it has been suggested that the causal components could be GA and glycyrrhetic acid-3-O-monoglucuronide (3MGA), which is a metabolite of glycyrrhizic acid (GL) present in Glycyrrhizae radix.15–18

To evaluate the pharmacological action and safety of SKT, it is important to determine the pharmacokinetics of active components after the administration. To date, Paeoniae radix-derived components ALB and PAE and the Glycyrrhizae radix-derived components GCM, ILG, GA, and 3MGA have been detected in the blood of rats after single oral administration of SKT.19–23 In humans, the same components have been detected in the blood of human orally administered Paeoniae radix or Glycyrrhizae radix.24–26 However, the pharmacokinetics of SKT remains unclear. In Kampo medicine, there is a possibility that the absorption and metabolism of components is regulated by multiple constituent herbs and their interactions.27–29 Therefore, it is important to understand the pharmacokinetics of the components while taking SKT as a mixture of herbal medicines.
In this randomized crossover trial, we investigated the pharmacokinetics of herbal components in the blood after single oral administration of SKT (2.5 or 5.0 g/day per person) in 20 healthy adult trial volunteers. SKT-derived six target components (ALB, PAE, GCM, ILG, GA, and 3MGA) were selected with reference to the previously described pharmacokinetic trials in rats and Paeoniae radix or Glycyrrhizae radix pharmacokinetic trials in humans, and the targeted components were then measured using validated procedure for the determination of the components.

MATERIALS AND METHODS

Chemicals and Reagents

Tsumura SKT extract granules for formulation (product code TJ-68, lot H16612; Tsumura & Co., Tokyo, Japan) were used for the investigation. The drug was manufactured according to Good Manufacturing Practices and adapted to factory release testing. This herbal preparation (7.5 g) contains 2.5 g of spray-dried hot water extract of a mixture of two crude drugs: Paeoniae radix (6.0 g) and Glycyrrhizae radix (6.0 g). The standard components present in SKT, that is, ALB, PAE, GL, and GA were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); ILG was purchased from Tokyo Chemical Industry Company, Ltd. (Tokyo, Japan); 3MGA was purchased from Nagara Science (Gifu, Japan); GCM was supplied by Tsumura & Co. Atractylenolide III (Tsumura & Co.), digoxin (Sigma–Aldrich, St. Louis, Missouri), niflumic acid (Sigma–Aldrich), and 6′-O-acetyl PAE (Tsumura & Co.) were used as internal standard (IS). Figure 1 shows the chemical structures of these nine compounds. Other chemicals were purchased from commercial sources.

Liquid Chromatographic Assay of Five Components Contained in SKT

The SKT formulation (0.5 g) was suspended in 25 mL of acetonitrile (MeCN)–purified water (1:1, v/v) and ultrasonicated for 15 min. The suspension was centrifuged at 1700g for 15 min, and the supernatant was collected. The residue was re-extracted using 25 mL of methanol (MeOH)–purified water (1:1, v/v). The first and second supernatants were combined, filtered (0.22 μm), and diluted with extracted solution as the test substance solution. An aliquot (5 μL) of test substance solution was injected into a liquid chromatography–tandem mass spectrometry (LC–MS/MS) system. The LC–MS/MS system comprised an LC-20A system (Shimadzu, Kyoto, Japan) connected to an API5000 triple quadrupole mass spectrometer fitted with a TurboIonSpray electrospray ionization interface (AB Sciex, Framingham, Massachusetts). A SUMIPAX ODS Z-CLUE column (2 × 50 mm, 3 μm; Sumika Chemical Analysis Service, Ltd., Tokyo, Japan) was used for all components at 40°C. The mobile phases comprised solution A (10 mmol/L ammonium acetate buffer) and solution B (MeOH), and the flow rate was 0.35 mL/min. The analytical methods are summarized in Supplemental Table S1.

Clinical Trial Design

This clinical trial was a randomized, open-label, two-arm, two-period design (Fig. 2). With regard to inclusion criteria, the studies included healthy Japanese adults aged 20–45 years with a body mass index between 18 and 25 who were willing and able to comply with the study requirements. The exclusion criteria for this study included a history of significant liver, heart, or vessel disease and consumption of supplements that contained any SKT component or any drug within 3 days to
182.1 cm; weight, 53.8–76.3 kg; and BMI, 18.6–24.7 kg/m²) were volunteers. Twenty subjects (age, 21–42 years; height, 163.9–

Flow diagram for the subjects in this randomized crossover study.

Figure 2.

In this quantitative trial, 12-mL blood samples were collected at 0 (preadministration), 5, 15, and 30 min, and 1, 2, 4, 8, 10, 12, 14, 24, and 48 h after a single oral dose of SKT formulation (2.5 or 5.0 g). The trial subjects were hospitalized in this study. The blood was immediately centrifuged at 1700g and 4°C for 15 min to obtain plasma. The plasma fractions were stored at −20°C or lower until LC–MS/MS analysis.

Ethics Statement

The studies were conducted at the Medical Corporation Shinnanokai Shinanozaka Clinic in two periods: January and February, 2014, respectively. The studies were approved by the Medical Corporation Shinnanokai Shinanozaka Clinic and conducted in accordance with ethical norms prescribed in the Declaration of Helsinki and Good Clinical Practice guidelines. Before initiation of the studies, informed consent was obtained from all subjects.

LC–MS/MS Analysis of SKT-derived Components in the Plasma

The plasma concentrations of all components were determined using LC–MS/MS. The instrument was composed of a LC-20AD System with a QTRAP® 5500 system (AB Sciex) or Shimadzu LC system (Degasser, DGU-14A; Column oven, CTO-10A; Pump, LC-10ADvp; Autosampler, SIL-HTE) and an API4000 system. An InertSustain C18 column (3 μm, 2.1 × 50 mm; GL Sciences, Tokyo, Japan) was used for all components.

Sample Preparation

For quantification of ALB and PAE, 300 μL of the plasma sample were mixed with 15 μL of water and MeOH (1:1, v/v), 10 μL of IS solution (100 ng/mL 6′-O-acetyl PAE), and 300 μL of purified water. The mixture was loaded onto an Oasis HLB μ Elution Plate (30 μm; Waters Corporation, Milford, Massachusetts), washed with 300 μL of purified water twice, and eluted with 100 μL of MeOH. Purified water (200 μL) was added to the eluent. An aliquot (5 μL) of each sample was injected onto the analytical column.

For quantification of GCM and ILG, 300 μL of the plasma sample were mixed with 15 μL of water and MeOH (1:1, v/v), 10 μL of IS solution (100 ng/mL niflumic acid), and 300 μL of purified water. The mixture was loaded onto an Oasis HLB 3 cc, 60 mg; Waters Corporation), washed with 3 mL of purified water, and eluted with 2 mL of acetic acid–MeOH (5:1000, v/v). The eluate was dried at 40°C in a water bath under a stream of nitrogen gas. A 10-mmol/L ammonium acetate–MeOH (6:4, v/v; 150 μL) was added to the dried sample and mixed. The reconstitution sample was filtered (0.2 μm), and an aliquot (20 μL)
of the mixture was injected onto the analytical column. For quantification of GA and 3MGA, 200 μL of the plasma samples were mixed with 10 μL of 50% MeCN, 10 μL of IS solution (100 ng/mL niflumic acid), 500 μL of formic acid–pured water (1:100, v/v), and 3 mL of tert-butyl methyl ether. The mixture was centrifuged at 1800g and 4°C for 2 min. The aqueous layer was frozen in a dry-ice/MeOH bath. The supernatant was collected in a test tube and dried at 40°C in a water bath under a stream of nitrogen gas. A 10-mmol/L ammonium acetate–MeCN (1:2, v/v; 200 μL) was added to the dried sample and mixed. An aliquot (2 μL) of the mixture was injected onto the analytical column.

**Analysis Conditions**

The analytical methods are summarized in Supplemental Table S2.

**Validation for Quantitative Determination of SKT-derived Components in the Plasma**

To assure the quality of the quantitative data by LC–MS/MS of the components in the plasma, the analytical methods, including the specificity, recovery, intraday and interday reproducibility, calibration curve, stability in blood, short- and long-term stability, postpreparative stability, freeze–thaw stability, dilution integrity, matrix effect, carryover, limit of quantification, and stability in the standard solution, were validated according to the US Food and Drug Administration Guidance for Industry Bioanalytical Method Validation. In the validation data are summarized in Supplemental Table S3.

**Pharmacokinetics Analysis**

Measured values of each component in the plasma were analyzed by noncompartmental modeling using Phoenix WinNonlin (version 6.3; Certara L.P., St. Louis, Missouri) for determining various pharmacokinetic constants, including the maximum plasma concentration (C\text{max}), time to maximum plasma concentration after drug administration (t\text{max}), apparent elimination half-life (t\text{1/2}), and the area under the plasma concentration–time curve from zero to the last observation time (AUC\text{0–last}). The t\text{1/2} was divided by log(2)/ke, where ke is the terminal elimination (at least three data points on the descending linear limb) rate constant. Component plasma concentration was treated as zero when the peak was below the limit of quantitation. The plasma concentration, C\text{max}, AUC\text{0–last}, and t\text{1/2} of the target components in each group were presented as the geometric mean [95% confidence interval (CI)]. The t\text{max} data were presented as the medians with range from minimum to maximum.

**Evaluation of Proportionality of SKT Dose and Pharmacokinetic Parameters of Targeted Component**

The dose proportionality (linearity) of the orally administered SKT dose and AUC\text{0–last} or C\text{max} of each component detected in the blood was analyzed using the model shown in the following formula (Eq. (1)).

\[
\ln (\text{PK}_j) = \mu + a_j + \beta \ln (\text{dose}_i) + \varepsilon_j
\]

where PK\text{ij} is the AUC\text{0–last} or C\text{max} at dose i (i = 1, 2) in the subjects j (j = 1, 2, ..., n), μ is the overall mean, a\text{ij} is a random subject effect that describes the individual difference for subject j and assumed to be normally distributed around mean 0 with variance σ\text{a,ij}^2, dose\text{is} is the administered dose (g) of the test drug, and ε\text{ij} represents random error with mean 0 and variance σ\text{ε,ij}^2. β is a parameter to be used for dose proportionality evaluation. Inferences were made on the basis of the theoretical β of 1. Evaluation of linearity was performed using Phoenix WinNonlin and SAS 9.2 (SAS Institute, Inc., Cary, North Carolina).

**RESULTS**

**Concentration of the Six Components Presented in SKT Formulation**

The concentrations of PAE, ALB, GCM, ILG, GL, and GA were 9920, 4370, 118, 79.3, 10,400, and 5.29 μg in 1 g of the SKT formulation, respectively.

**Registered Subjects**

Twenty subjects were screened and enrolled. No adverse effect was observed in any subject treated with SKT. No irregularities were found in the measured blood or biochemical examination items before or after SKT administration (Supplemental Table S4).

**Pharmacokinetics Analysis**

**Plasma Concentrations of the Six SKT-Derived Components**

Glycycoumarin concentrations of one subject were BQL in the plasma samples collected; therefore, this subject was excluded from the analysis for GCM. Concentration of each component and metabolite were BQL in the plasma of all subjects before SKT administration. The time profiles of plasma concentrations and pharmacokinetic parameters of six SKT-derived components after oral administration of SKT formulation (2.5 and 5.0 g per person) to subjects are shown in Figure 3 and Table 1, respectively. The highest plasma concentration was found in GA followed by PAE, ALB, 3MGA, ILG, and GCM. Rapid t\text{max} values within 3 h after the administration of SKT were found for ILG, GCM, ALB, and PAE. Thereafter, the concentrations of ALB and PAE decreased, and their t\text{1/2} values were 1.76–1.81 and 1.73–1.74 h, respectively. However, the t\text{1/2} of ILG and GCM were considerably shorter than those of ALB and PAE because the concentrations of both components increased at approximately 8 h. In contrast, late t\text{max} values were found in 3MGA and GA, which are GL metabolites, and their t\text{1/2} values were 5.07–5.26 and 8.43–10.3 h, respectively.

**Dose Proportionality Between SKT Doses and the Pharmacokinetic Parameters of Target Components in the Plasma**

In clinical pharmacological studies, the test drug is considered to show linear pharmacokinetics when AUC\text{0–last} and C\text{max} proportionally increase with increasing dose. In the present study, a power model fitted mixed-effect model was used for assessing the dose proportionality between SKT doses and plasma concentration (C\text{max} or AUC\text{0–last}) of the six components. Table 2 shows the β point estimates and 90% CI analyzed using the mixed-effects model. In this study, linearity was recognized when the 90% CI of β included 1.

When the proportionality was examined between SKT dose and C\text{max}, the 90% CIs of β values of GCM, ILG, and GA included 1, whereas those of ALB, PAE, and 3MGA were less than 0.5.
When the linearity was examined between the SKT dose and AUC_{0-last}, the 90% CIs of \( \beta \) values of GA included 1. However, the 90% CIs of \( \beta \) values of ALB, PAE, and 3MGA were less than 0.7, and those of GCM and ILG were higher than 1.2.

From these results, linearity was observed in \( C_{\text{max}} \) and AUC_{0-last} for GA. GCM and ILG revealed linearity only in \( C_{\text{max}} \). Nonlinearity was observed for ALB, PAE, GCM, and 3MGA, although these components were detected in the plasma of the SKT-treated subjects.

**DISCUSSION**

This is the first study to investigate the pharmacokinetics of the six types of active components (\textit{Paeoniae} radix-derived ALB and PAE and \textit{Glycyrrhizae} radix-derived GCM, ILG, GA, and 3MGA) detected in the blood of humans in a randomized crossover trial. The SKT formulation includes five target components. However, the content of GA, a GL metabolite, was very low, that is, approximately 1:2000 of GL. It has been demonstrated that GL is not directly absorbed from the intestinal tract, that is, GL (glycoside) in the \textit{Glycyrrhizae} radix is demonstrated to be metabolized by enteric bacteria and then absorbed into the blood as either GA (aglycone) or 3MGA.\textsuperscript{34–38} 3MGA is also the secondary metabolite generated by GA absorbed into the blood.\textsuperscript{39} The data of these targeted components were determined and assured by our validated method, which was established for this trial.

Shakuyakukanzoto is reported to rapidly improve symptoms of suddenly occurring muscle cramp and abdominal pain. These pains are triggered by the sudden contraction of skeletal muscles or visceral smooth muscles. SKT has been shown to have both antimuscle cramp and analgesic effects to these symptoms. Muscle contraction is induced by increasing intracellular Ca\textsuperscript{2+} concentration via the Ca-induced Ca-release mechanism of the sarcoplasmic reticulum.\textsuperscript{40,41} The antimuscle cramp mechanism of SKT works by suppressing the Ca\textsuperscript{2+} concentration, and the active component is believed to be \textit{Glycyrrhizae} radix-derived ILG, GCM, or GA.\textsuperscript{8} Further, the analgesic effect of SKT is believed to suppress the transmission of pain to the upper central nervous system by suppressing the release of substance P from the primary afferent fiber via the activation the descending inhibitory 5-HT and NE neurons in the posterior horn of the spinal cord, and these active components are
Table 1. Pharmacokinetic Parameters of Six Shakuyakukanzoto-Derived Components

| Component | SKT Dose | C<sub>max</sub> (ng/mL) | Median (Range) | n | t<sub>max</sub> (hr) | t<sub>1/2</sub> (hr) | AUC<sub>0–last</sub> (ng·h/mL) | Median (Range) | n | GCM | AUC<sub>0–last</sub> (ng·h/mL) | Median (Range) | n |
|-----------|----------|-------------------------|----------------|---|-------------------|-------------------|---------------------------|----------------|---|-----------------|----------------|---|
| Albiflorin | 2.5 g    | 1.96 (1.70–2.08)        |                | 20| 2.00 (0.500–8.00) | 20                | 1.81 (1.57–2.08)          | 20             | 19| 1.76 (1.08–1.91) | 20             | 19|
|           | 5.0 g    | 2.14 (1.79–2.56)        |                | 20| 3.00 (0.500–10.00)| 20                | 1.73 (1.38–1.86)          | 20             | 20| 1.74 (1.38–1.91) | 20             | 20|
| Paeoniflorin | 2.5 g    | 6.06 (5.23–7.01)        |                | 20| 3.00 (0.500–10.00)| 20                | 1.73 (1.38–1.86)          | 20             | 20| 1.74 (1.38–1.91) | 20             | 20|
|           | 5.0 g    | 6.30 (5.25–7.51)        |                | 20| 3.00 (0.500–10.00)| 20                | 1.73 (1.38–1.86)          | 20             | 20| 1.74 (1.38–1.91) | 20             | 20|
| Glycycoumarin | 2.5 g  | 0.0449 (0.0373–0.0541)  |                | 19| 0.0367 (0.0219–0.0614)| 19               | 0.0367 (0.0219–0.0614)    | 19             | 19| 0.0367 (0.0219–0.0614) | 19             | 19|
|           | 5.0 g    | 0.0885 (0.0738–0.105)   |                | 20| 0.0895 (0.0698–0.119) | 20          | 0.0895 (0.0698–0.119)    | 20             | 20| 0.0895 (0.0698–0.119) | 20             | 20|
| Isoliquiritigenin | 2.5 g | 0.1357 (0.127–0.193)   |                | 20| 0.130 (0.121–0.186) | 20                | 0.130 (0.121–0.186)        | 20             | 20| 0.130 (0.121–0.186) | 20             | 20|
|           | 5.0 g    | 0.2868 (0.235–0.353)    |                | 20| 0.309 (0.241–0.398) | 20                | 0.309 (0.241–0.398)        | 20             | 20| 0.309 (0.241–0.398) | 20             | 20|
| Glycyrrhetic acid | 2.5 g | 118 (89.3–156)           |                | 20| 1540 (1230–1930) | 20                | 10.3 (2.42–7.02)          | 13             | 13| 4.73 (1.38–11.8) | 5              | 5 |
|           | 5.0 g    | 211 (169–263)           |                | 20| 2860 (2340–3480) | 20                | 10.3 (2.42–7.02)          | 13             | 13| 4.73 (1.38–11.8) | 5              | 5 |
| Glycyrrhetic acid 3-monoglucuronide | 2.5 g | 1.14 (0.907–1.43)       |                | 20| 11.4 (8.68–14.9) | 20                | 14.9 (2.56–87.0)          | 2              | 2 | 0.500 (0.250–1.00) | 19             | 19|
|           | 5.0 g    | 1.42 (1.17–1.73)        |                | 20| 15.3 (12.0–19.5) | 20                | 15.3 (12.0–19.5)          | 20             | 20| 15.3 (12.0–19.5) | 20             | 20|

GCM and ILG with rapid results suggest that the Glycyrrhizae radix-derived components ALB and PAE. However, in the linearity analysis, because an increase in the SKT dose was not accompanied by increased concentration (C<sub>max</sub> or AUC<sub>0–last</sub>) in the blood for either ALB or PAE, it was judged that there was no dose proportionality. Both components are absorbed into the intestinal tract as glycosides; however, because this absorption capacity is low, it is conjectured that the nonlinearity may have been caused by saturation of the absorption. Furthermore, portions of both components are metabolized by enteric bacteria and are absorbed into the blood as aglycone. However, their transition into the blood is considered to be slow. In the absorption phase, both components were detected in the plasma at the early stage between 5 and 15 min after the oral administration of SKT. These results suggest that glycosides are a greater contributing factor than metabolites to the immediate efficacy of SKT. The concentration of the Glycyrrhizae radix components GCM and ILG in the blood demonstrated a bimodal peak after the oral administration of SKT (Figs. 3c and 3d). The highest concentration for both occurred at the first peak: at 15 min for ILG and 30 min for GCM, which reflected extremely fast absorption. The second low-concentration peaks of these components were observed 2–3 h after the oral administration of SKT. Because this bimodality was not observed during animal tests in the case of intravenous administration, the cause is not considered to be enterohepatic circulation. This bimodality of ILG is surmised to be because of a difference in the absorption site of ILG and isoliquiritin metabolite because ILG is also produced from the glycoside isoliquiritin by enteric bacteria. The t<sub>1/2</sub> of both components could be calculated for only a few subjects because the maximum plasma concentration (C<sub>max</sub>) of these components was very low (<1 ng/mL) as compared with that of the Paeoniae radix-derived components ALB and PAE. An increase in the plasma concentration of ILG and GCM was accompanied by an increase in the dosage of SKT in the case of C<sub>max</sub>; however, this was not observed in the case of AUC<sub>0–last</sub> of both components, possibly because the t<sub>1/2</sub> value could not be calculated in a few subjects in the 2.5 and 5.0 g SKT groups.

On the contrary, intravenous injection of GA has been reported to immediately ameliorate muscle cramps. However, the t<sub>1/2</sub> of GA orally administered as SKT was extremely slow (10 h) as compared with that of ILG and GCM. GA also slowly disappeared over the next 8–10 h (t<sub>1/2</sub>) after reaching t<sub>max</sub>. These results suggest that the Glycyrrhizae radix contains two types of components. GCM and ILG with rapid t<sub>max</sub> are believed to be responsible for immediate efficacy of SKT together with Paeoniae radix-derived components ALB and PAE. GA with slow t<sub>max</sub> and t<sub>1/2</sub> is probably thought to be a component that sustains the efficacy of SKT. Thus, the contribution of SKT in providing analgesic effects and ameliorating muscle cramps may be attributed to a synergistic effect of various components with different pharmacokinetic attributes.
Another GL metabolite 3MGA, which is also considered to be a causal component in hypokalemia, showed extremely slow $t_{\text{max}}$ and $T_{1/2}$ values, similar to GA. To the best of our knowledge, this is the first report to show the blood concentration changes of 3MGA. Our study will provide foundational data to clarify the mechanisms for side effects in the future. Linearity between the blood pharmacokinetic parameters and the dose of SKT was observed in the case of GA; however, no linearity was observed for 3MGA. In a double-blind study, Kumada et al. reported that the frequency of pseudoaldosteronism or hypokalemia was extremely low when SKT was administered for 2 weeks. However, in the monitoring of side effects for other formulation yokukansan, containing, including Glycyrrhizae radix, development of pseudoaldosteronism or hypokalemia has been reported, although at a low frequency. Therefore, care must be taken when using SKT in the clinical settings.

**CONCLUSION**

In order for SKT to show anticonvulsive, analgesic, and muscle-relaxant effects, the active components presumed by the in vivo and in vitro basic researches must absorb into blood. To our knowledge, this is the first trial in humans to demonstrate that these components were absorbed into the blood after the oral administration of SKT. The results of this pharmacokinetic trial in humans are important and useful for understanding the mechanism of action of SKT, verifying the active components predicted in the basic research, and conducting ADME and safety studies in the future.

**ACKNOWLEDGMENTS**

We would like to thank Dr. Seiichi Matsui (Sumika Chemical Analysis Service Ltd., Osaka, Japan), Dr. Masataka Yagi (Sumika Chemical Analysis Service Ltd.), and Dr. Kenji Sano (Sekisui Medical Company, Ltd., Tokyo, Japan) for analysis of the SKT ingredients. We would like to thank Makoto Zushi (Product Information Management Dept., Tsumura & Co.) for analyzing the data. We are deeply grateful to Dr. Yasushi Ikarashi in Tsumura Research Laboratories for proofreading of this manuscript. This study was supported by a grant from Tsumura & Co.

C.S., J.W., M.F., H.N., K.M., and Y.K. are employed by Tsumura and Co. T.K. reports no conflict of interest. This study was funded by Tsumura and Co.

**REFERENCES**

40. Protasi F. 2002. Structural interaction between RYRs and DHPRs in calcium release units of cardiac and skeletal muscle cells. Front Biosci 7:2650–2658.