Pharmacokinetics of daikenchuto, a traditional Japanese medicine (Kampo) after single oral administration to healthy Japanese volunteers

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Running title: Pharmacokinetics study of daikenchuto

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  Abstract: 199
  Introduction: 377
  Results and Discussion: 855

ABBREVIATIONS: TJ-100, daikenchuto; HAS, hydroxy-α-sanshool; HBS, hydroxy-β-sanshool; 6S, [6]-shogaol; 10S, [10]-shogaol; GRB1, ginsenoside Rb1; GRG1, ginsenoside Rg1; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry
ABSTRACT

The pharmacokinetics of Daikenchuto (TJ-100), a pharmaceutical grade traditional Japanese medicine, were investigated in healthy Japanese volunteers after single oral administration of 2.5, 5 and 10g doses. Six ingredients [hydroxy-α-sanshool (HAS), hydroxy-β-sanshool (HBS), [6]-shogaol (6S), [10]-shogaol (10S), ginsenoside Rb1(GRB1) and ginsenoside Rg1(GRG1)] of TJ-100 were determined by using LC-MS/MS. The results indicated that HAS an ingredient derived from Zanthoxylum fruit, exhibited the highest plasma concentration among the 6 ingredients investigated. The plasma concentrations of HAS, HBS, 6S and 10S reached maximum concentration (approximately 400, 80, 0.14 and 0.6 ng/mL, respectively after 5 g administration of TJ-100) within 30 min after administration and the mean half-life was approximately 2 hr. Thus, these compounds were rapidly absorbed and eliminated. The plasma concentration of GRB1 reached maximum concentration (2 ng/mL after 5 g administration of TJ-100) at approximately 4 hr after administration and the half life of GRB1 was approximately 40hr. The plasma concentration of GRG1 was extremely low (< 0.023 ng/mL). The pharmacokinetics of HAS, HBS, 6S, and 10S, were linear within the range of 2.5 g to 10 g/day of TJ-100. On the other hand, the kinetics of GRB1 and GRG1 were not proportional to dosage, and plateauing was observed.
INTRODUCTION

Daikenchuto (TJ-100) is a pharmaceutical grade traditional Japanese medicine consisting of Japanese pepper (zanthoxylum fruit), processed ginger, and ginseng with maltose as an additive. In Japan, TJ-100 is routinely used in the modern medical care system as a prescription drug for the treatment of various gastrointestinal disorders, including postoperative ileus, postoperative intestinal paralysis and adhesive bowel obstructions [Itoh et al., 2002; Kono et al., 2009]. Results from a recent clinical pharmacological study have shown that TJ-100 accelerates intestinal transit in healthy humans [Manabe et al., 2010]. Furthermore, basic pharmacological studies have suggested that TJ-100 and its ingredients have a wide variety of biological effects, including prokinetic effect largely attributed to the activity of zanthoxylum fruit [Tokita et al., 2007; Kawasaki et al., 2007; Jin et al., 2001], contraction and relaxation of intestinal smooth muscle [Kito et al., 2006], increase of intestinal blood flow due to the activity of ginger ingredients [Kono et al., 2008; Murata et al., 2002], anti-inflammatory effect [Kono et al., 2010], and suppression of bacterial translocation possibly mediated by ginseng ingredients [Yoshikawa et al., 2008].

Estimating the factors involved in the absorption, distribution, metabolism, and excretion (ADME) of the multiple constituents in a Kampo medicine is an ongoing challenge, and obtaining a consistent picture of its mechanism of action is difficult as well. To solve these problems, we have conducted preliminary clinical trials to obtain the profiles of compounds absorbed in the plasma and urine after a single oral administration of 15 g of TJ-100 in 4 healthy volunteers [Iwabu et al., 2010]. In that study, we constructed an LC-MS/MS analysis to detect as many compounds as possible, and successfully identified 44 ingredients of TJ-100. Twenty-three ingredients and their metabolites were detected in the plasma, indicating that a large number of TJ-100 ingredients were actually absorbed and metabolized.

Based on the results, we have developed assay methods for six compounds, hydroxy-α-sanshool, hydroxyl-β-sanshool, [6]-shogaol, [10]-shogaol, ginsenoside Rb1 and ginsenoside Rg1 using the following criteria: 1) Plasma concentration of the compounds have enough strength to establish...
the validated determination methods, 2) Enables the measurement at least one compound per botanical raw material, 3) Pharmacological activities of the selected compounds have been previously reported.

In this study, we focused on the pharmacokinetics of the ingredients of TJ-100.

MATERIALS AND METHODS

Chemicals and Reagents. TSUMURA Daikenchuto Extract Granules was manufactured by TSUMURA & CO. Fifteen grams of TSUMURA Daikenchuto Extract Granules contains 1.25 g of a dried extract prepared from a mixture of three herbs (5.0 g of processed ginger, 3.0 g of ginseng and 2.0 g of Japanese pepper), and 10.0 g of maltose. Extra pure grade acetonitrile and HPLC grade acetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Asiaticoside was purchased from Extrasynthese (Genay, France) and nonivamide from Enzo Life Sciences, Inc. (Farmingdale, NY). Water was purified using a pure water supply system (MILLI-Q, Nihon Millipore Ltd., Japan). Oasis HLB μElution Plate (Nihon Waters K.K, Japan) was used as the solid phase extraction (SPE) plate. The isolation, synthesis and identification of the 6 authentic standards of TJ-100 ingredients were described in the previous paper [Iwabu et al., 2010]. The structural formulas of the authentic standards are provided in Supplemental Figure 1.

Clinical Trial Design, Administration, and Sample Collection. This was a randomized, open label, three-arm, three-period study (Japic CTI-101114) conducted at the Kochi Medical School. Nineteen subjects participated in this study but three subjects were excluded from the pharmacokinetic analysis because they did not meet the eligibility criteria. Safety endpoint was evaluated in 19 subjects. Participants were fasted from 12 hours before and 4 hours after administration. All foods and drinks (including spices) containing ginseng, Japanese pepper, and
ginger were strictly prohibited from 3 days prior to dispensing the study medication until completion of each treatment phase. Blood samples (7 mL each) were collected from the medial cubital vein into evacuated tubes containing heparin just before and at 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24 and 48 hours after administration and were immediately centrifuged (1700 \( \times \) g, 10 min).

Plasma fractions were stored at -20°C until analysis. All study procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki, consistent with the Good Clinical Practice guidelines, and approved by the Institutional Review Board at Kochi Medical School Hospital. Subjects gave their written informed consent before participating in the study.

**Determination of plasma concentration of TJ-100 ingredients.** The concentrations of six TJ-100 ingredients were determined according to the method reported by Iwabu et al. [Iwabu et al., 2010] with modification. In brief, 200 µL of the plasma samples were mixed with 200 µL of 0.1 mol/L hydrochloric acid, 10 µL of IS solution (schizandrin: 10 ng/mL for HAS and HBS, nonivamide: 10 ng/mL for 6S and 10S, and asiaticoside: 100 ng/mL for GRB1 and GRG1) and 10 µL of water/acetonitrile (7:3, v/v). The resulting mixture was loaded onto an Oasis HLB µElution 96-well plate (30 µm), which was washed with 200 µL of methanol and 200 µL of water. The plate was then washed with 200 µL of water and eluted with 50 µL of acetonitrile. A 40-µL portion of the eluate was mixed with 60 µL of water and a 20-µL portion was injected into the Agilent 1200 series HPLC system (Agilent Technologies, Inc.) with Sciex API 5000 (AB Sciex Pte. Ltd.). YMC-Pack ODA-AQ(3 µm, 150 × 2.0 mm I.D., YMC Co., Ltd) was used for analyzing HAS, HBS, GRB1 and GRG1; YMC-Pack ODA-AQ(3 µm, 50 × 2.0 mm I.D., YMC Co., Ltd) was used for analyzing 6S and 10S. The analytical methods were validated according to the FDA Guidance for Industry Bioanalytical Method Validation. These analytical methods and their validation data are summarized in Supplemental data (Supplemental Table1, 2, and 3.)
Pharmacokinetic Analysis. Pharmacokinetic parameters were estimated using the WinNonlin version 5.2 (Pharsight, Corporation, Mountain View, CA). Experimentally observed values of maximum concentration (C_{max}) and time to maximum concentration (t_{max}) after TJ-100 administration were used for the analysis. The area under plasma concentration-time curve from zero to time t (AUC_{0-last}) was calculated from time zero to last detected time. Apparent elimination half-life (t_{1/2}) was calculated divided by \log_2/k_e where k_e means the terminal elimination rate constant. C_{max} and AUC_{0-last} are presented as mean ± SD. Apparent elimination half-life and t_{max} are presented as median with range.

Evaluation of Linearity of Dosage-Exposure Relations. The dose-proportionality was analyzed via a power model fitted as a linear mixed effect model which included the period effect and random subject effect (equation 1).

\[
\ln(PK)_{ijk} = \beta_0 + \beta_1 \ln(Dose_{ij}) + \pi_j + a_k + \epsilon_{ijk}.
\]

The \ln(PK)_{ijk} means natural logarithm of the AUC_{0-last} or C_{max} in group i (i=A,B,C) during period j (j=1,2,3) in the subject (k=1,2,…ni). The Dose_{ij} is the administrated dose (g) of the test drug in group i during period j. The \pi_j is the term to express the fixed period effect, and the a_k is the term to express the subject specific effect which is assumed to be normally distributed around mean 0 with inter-subject standard deviation of \sigma_a. The \epsilon_{ijk} is the error term with mean 0 and standard deviation \sigma. The \beta_1 is the parameter to be used for dose-proportionality evaluation and \beta_0 is the intercept term. When the value of \beta_1 significantly differs from 1, the hypothesis of dose-proportionality is rejected. Evaluation of linearity of dosage-exposure relations analysis were conducted using SAS9.1.3 (SAS Institute Inc., 2004).

RESULTS AND DISCUSSION

Study subjects and adverse effects.
Sixteenth healthy subjects [age: 20-37 years, height: 150.7-189.7 cm, weight: 44.5-72.5 kg, and body mass index (BMI): 18.5-24.1 kg/m²] were included in the pharmacokinetic analysis in this study. There was an uneven gender ratio in the study (13 men and 3 women). Six adverse experiences were observed in four patients, all of which were deviations from normal reference laboratory values. However, these deviations were judged by the principal investigator as physiological changes, thus causal relationship with TJ-100 was denied.

**Pharmacokinetics in human subjects.**

The plasma concentration–time profiles in human subject for each dose (2.5, 5 and 10 g) are shown in Figure 1 and the pharmacokinetic parameters of the 6 main TJ-100 ingredients are summarized in Table 1.

HAS, which is a constituent of zanthoxylum fruit, exhibited the highest plasma concentration among the six compounds measured. The plasma concentration of HAS reached maximum concentration within 30 min after administration and its median half-life was 1.6-1.7 h, indicating its rapid absorption and elimination. The plasma concentration of HBS showed the parallel pattern to the plasma concentration of the HAS. It has been reported that both HAS and HBS have unique biological activities and play an important role in the efficacy of TJ-100 [Hayakawa et al., 1999; Tokita et al., 2007 a; Tokita et al., 2007 b]. Various in vitro experimental systems have demonstrated that HAS exerts its pharmacological effects in the range of 0.1 to 100 μM [Koo et al., 2007; Jin et al., 2001]. In this study, the maximum concentration of HAS in plasma was 0.76 to 2.66 μM. Taken together, these result suggest that HAS absorption into the blood circulation may contribute to the pharmacological effects of orally administered TJ-100. These findings are intriguing in light of the fact that several studies strongly suggest the localized effect of TJ-100, that is, TJ-100 ingredients mainly affect the mucosal, nervous, and smooth
muscle cells in the intestinal lumen. For example, intraduodenal and intrajejunal TJ-100 administration induced phasic contractions in the duodenum and proximal jejunum, respectively, and those contractions migrated distally [Shibata et al., 1999; Jin et al., 2001]. No motor response was obtained proximal to the site of administration, suggesting that direct contact with the intestinal mucosa is essential for TJ-100 to exert its effects. The results of this pharmacokinetic study underscores the importance of clarifying whether HAS has systemic biological effects.

The plasma concentrations of 6S and 10S, the constituents of processed ginger, were significantly lower than those of zanthoxylum fruit constituents. The values of $C_{\text{max}}$ of 6S were observed within 15 min after administration and its elimination half-life was 0.3-0.6 hr. Meanwhile, 10S reached maximum plasma concentrations within 30 min after administration and its elimination rate was slightly lower than that of 6S at 0.8-1.5 hr. Although the plasma concentrations of 6S and 10S were extremely low, it is possible that their intestinal concentrations were sufficient to exert an effect in the intestinal lumen. Conversely, the plasma concentration of GRB1, which is one of the ginseng constituents, gradually increased with a $t_{\text{max}}$ of approximately 4 hr followed by maximum plasma concentrations. Thereafter, GRB1 was gradually eliminated with a half-life of approximately 40 hr. The $t_{\text{max}}$ and the half-life of GRB1 indicate a relatively slow increase and gradual disappearance. The plasma concentrations of GRB1 are therefore expected to increase to levels higher than the concentrations observed in this study when TJ-100 is administered repeatedly. From the present data, we anticipate that five half-lives can be measured once a steady-state blood concentration of GRB1 is achieved after about 1-2 weeks of repeated administration. Although there are a few adverse reports related to the long term use of TJ-100 in Japan,
repeated-dose pharmacokinetic trials will be required to clarify this point. In addition, the information on plasma concentration of GRB1 after repeated TJ-100 is useful to discuss the possible involvement of the ingredient in TJ-100’s clinical effect.

The calculation of elimination half-life of GRG1 was possible in only a few subjects because the plasma concentration of GRG1 was low and less than the lower limit of quantitation (0.01 ng/mL) in many of the subjects.

Analysis of Linearity in Dosage-Exposure Relations.

The dose-proportionality of AUC\textsubscript{0-last} is graphically displayed in Figure 2. The $\beta_1$ of HAS, HBS, 6S, and 10S for AUC\textsubscript{0-last} was close to one for doses ranging between 2.5 g and 10 g of TJ-100. These result suggested that the pharmacokinetics of HAS, HBS, 6S, and 10S were linear within the dose range of 2.5-10 g/day of TJ-100. However, a 90% confidence interval of the $\beta_1$ value for GRB1 did not include 1. While the AUC\textsubscript{0-last} value of GRB1 was very limited because the last observation time points were only 50% of the estimated half-life, the result inferred a plateauing phenomenon of GRB1. The analysis for GRG1 has not been conducted because only a few subjects had detectable plasma concentrations. The result of dose-proportionality for C\textsubscript{max} was similar to that of AUC\textsubscript{0-last}.

The present study provides a strong basis for future ADME studies to help create a consistent kinetic profile and to generate robust evidence on the pharmacological activity of TJ-100.
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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Munekage, Kitagawa, Ichikawa, Kono, Hanazaki

Conducted experiments: Munekage, Kitagawa, Ichikawa, Kono, Hanazaki

Performed data analysis: Munekage, Kitagawa, Ichikawa, Watanabe, Kono, Hanazaki

Contributed to the writing of the manuscript: Munekage, Kitagawa, Ichikawa, Watanabe, Aoki, Kono, Hanazaki
REFERENCES


FOOTNOTES

This study was supported by a grant from TSUMURA & CO., Tokyo, Japan.
LEGENDS FOR FIGURES

Figure 1 A semi-logarithmic plot of plasma concentrations of main TJ-100 ingredients in human healthy volunteers. The results of quantification of main TJ-100 ingredients in human plasma are shown. A: HAS, B: HBS, C: 6S, D: 10S, E: GRB1, F: GRG1. 2.5 g (closed circle), 5 g (closed square), 10 g (closed triangle). Values are means of the results ± S.D. (n = 15 or 16).

Figure 2 Relations between dosage and $AUC_{0\text{-}last}$ of HAS, HSB, 6S, 10S and GRB1 after oral administration of TJ-100 in healthy volunteers (n = 16). Each data represents geometric mean of $AUC_{0\text{-}last}$. Solid line represents predicted curve given by power model fitting. HAS (closed circle), HSB(open circle), 6S (closed square), 10S (open square), GRB1(closed triangle).
Table 1. Pharmacokinetic Parameters for Components of TJ-100

<table>
<thead>
<tr>
<th>Dose (g/day)</th>
<th>N</th>
<th>AUC(0-last)* (ng·hr/mL)</th>
<th>C_{max}* (ng/mL)</th>
<th>t_{1/2}† (hr)</th>
<th>t_{max}† (hr)</th>
</tr>
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<tbody>
<tr>
<td>HAS</td>
<td>2.5</td>
<td>15</td>
<td>349±136</td>
<td>209±100</td>
<td>1.65(1.01-2.48)</td>
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<tr>
<td></td>
<td>5</td>
<td>16</td>
<td>658±223</td>
<td>391±136</td>
<td>1.71(1.04-3.26)</td>
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<tr>
<td></td>
<td>10</td>
<td>16</td>
<td>1290±329</td>
<td>664±165</td>
<td>1.62(1.46-3.22)</td>
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<tr>
<td>HAS</td>
<td>2.5</td>
<td>15</td>
<td>66.3±35.0</td>
<td>42.2±25.5</td>
<td>1.14(0.478-1.71)</td>
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<td></td>
<td>5</td>
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<td>130±81.4</td>
<td>80.4±45.9</td>
<td>1.17(0.681-1.76)</td>
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<td></td>
<td>10</td>
<td>16</td>
<td>234±88.4</td>
<td>131±52.6</td>
<td>1.09(0.925-1.70)</td>
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<tr>
<td>HAS</td>
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<td>15</td>
<td>0.0396±0.0211</td>
<td>0.0762±0.0542</td>
<td>0.618(0.280-0.956)</td>
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<td>16</td>
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<td>0.142±0.109</td>
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<td>0.429(0.302-0.526)</td>
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<td>0.290±0.157</td>
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<td>0.636±0.341</td>
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<td>1.48±0.760</td>
<td>1.20±0.742</td>
<td>0.812(0.460-5.42)</td>
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<tr>
<td>HAS</td>
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<td>0.0504±0.0189</td>
<td>37.8(12.2-101)</td>
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<td>0.123±0.0466</td>
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<td>15</td>
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<td>0.0230±0.0117</td>
<td>14.3(2.69-171)</td>
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</table>

* : Data represent the means ± S.D.
† : Data represent the median (range)

a : The elimination half-life was calculated from 2 subjects at the dose of 2.5g, 10 subjects at the dose of 5 g, and 12 subjects at the dose of 10 g.

b : The elimination half-life was calculated from 10 subjects at the dose of 2.5g, 15 subjects at the dose of 5 g, and 16 subjects at the dose of 10 g.

c : The elimination half-life was calculated from 2 subjects at the dose of 2.5g, 3 subjects at the dose of 5 g, and 7 subjects at the dose of 10 g.
Figure 1
Figure 2