Short Communication

Pharmacokinetics of Daikenchuto, a Traditional Japanese Medicine (Kampo) after Single Oral Administration to Healthy Japanese Volunteers

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ABSTRACT:

The pharmacokinetics of daikenchuto (TJ-100), a pharmaceutical-grade traditional Japanese medicine, were investigated in healthy Japanese volunteers after a single oral administration of 2.5-, 5-, and 10-g 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 liquid chromatography-tandem mass spectrometry. The results indicated that HAS, an ingredient derived from Zanthoxylum piperitum fruit, exhibited the highest plasma concentration among the six ingredients investigated. The plasma concentrations of HAS, HBS, 6S, and 10S reached the maximum concentration (approximately 400, 80, 0.14, and 0.6 ng/ml, respectively, after a 5-g administration of TJ-100) within 30 min after administration, and the mean half-life was approximately 2 h. Thus, these compounds were rapidly absorbed and eliminated. The plasma concentration of GRB1 reached the maximum concentration (2 ng/ml after a 5-g administration of TJ-100) at approximately 4 h after administration and the half-life of GRB1 was approximately 40 h. 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 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 spp. 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 a prokinetic effect largely attributed to the activity of Zanthoxylum piperitum fruit (Jin et al., 2001; Kawasaki et al., 2007; Tokita et al., 2007a,b), contraction and relaxation of intestinal smooth muscle (Kito and Suzuki, 2006), an increase in intestinal blood flow due to the activity of ginger ingredients (Murata et al., 2002; Kono et al., 2008), an 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 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 four healthy volunteers (Iwabu et al., 2010). In that study, we constructed a liquid chromatography-tandem mass spectrometry 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.

On the basis of 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 concentrations of the compounds have enough strength to establish the validated determination methods; 2) the measurement at least one compound per botanical raw material is enabled; and 3) pharmacological activities of the selected compounds have been reported previously. 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. (Ami, Ibaraki, Japan). 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 high-performance liquid chromatography-grade acetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Asiaticoside was purchased from Extrasynthese (Genay, France), and nonivamide was from Enzo Life Sciences, Inc. (Farmingdale, NY). Water was purified using a pure water supply system (Milli-Q, Nihon Millipore Ltd., Tokyo, Japan). An Oasis HLB µElution Plate (Nihon Waters K.K, Tokyo, Japan) was used as the solid-phase extraction plate. The isolation, synthesis, and identification of the six authentic standards of the TJ-100 ingredients were described in a previous article (Iwabu et al., 2010). The structural formulas of the authentic standards are provided in Supplemental Fig. 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. The safety endpoint was evaluated in 19 subjects. Participants fasted from 12 h before and 4 h after administration. All foods and drinks (including spices) containing ginseng, Japanese pepper, and ginger were strictly prohibited for 3 days before dispensing of 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 h after administration and were immediately centrifuged (1700 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 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 Concentrations of TJ-100 Ingredients. The concentrations of six TJ-100 ingredients were determined according to the method reported by Iwabu et al. (2010) with modification. In brief, 200 µl of the plasma samples were mixed with 200 µl of 0.1 M hydrochloric acid, 10 µl of internal standard 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 high-performance liquid chromatography system (Agilent Technologies, Santa Clara, CA) with a Sciex API 5000 (AB SCIEX, Tokyo, Japan). A YMC-Pack ODA-AQ column (3 µm, 150 × 2.0 mm i.d.; YMC Co., Ltd., Kyoto, Japan) was used for analyzing HAS, HBS, GRB1, and GRG1; a YMC-Pack ODA-AQ column (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 U.S. Food and Drug Administration Guidance for Industry Bioanalytical Method Validation (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf). These ana-

![FIG. 1. A semilogarithmic 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; ■, 5 g; △, 10 g. Values are means of the results ± S.D. n = 15 or 16.](image-url)
lytical methods and their validation data are summarized in Supplemental Tables 1, 2, and 3.

Pharmacokinetic Analysis. Pharmacokinetic parameters were estimated using WinNonlin (version 5.2; Pharsight, Mountain View, CA). Experimentally observed values of maximum concentration ($C_{\text{max}}$) and time to maximum concentration ($t_{\text{max}}$) after TJ-100 administration were used for the analysis. The area under the plasma concentration-time curve from zero to time $t$ (AUC$_{0-t_{\text{last}}}$) was calculated from time 0 to the last detected time. Apparent elimination half-life ($t_{\text{1/2}}$) was calculated divided by $\ln(2)/k_e$ where $k_e$ is the terminal elimination rate constant. $C_{\text{max}}$ and AUC$_{0-t_{\text{last}}}$ are presented as the mean ± S.D. The apparent elimination half-life and $t_{\text{max}}$ are presented as the median with range.

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

$$\ln(PK)_{ijk} = \beta_0 + \beta_k \ln(Dose_i) + \eta_i \times a_k \times \epsilon_{ijk}$$

$\ln(PK)_{ijk}$ is the natural logarithm of the AUC$_{0-t_{\text{last}}}$ or $C_{\text{max}}$ in group $i$ ($i = A, B, C$) during period $j$ ($j = 1, 2, 3$) in the subject ($k = 1, 2, \ldots, n_i$). The Dose$_i$ is the administered dose ($g$) of the test drug in group $i$ during period $j$. $\eta_i$ is the term to express the fixed period effect, and $a_k$ is the term to express the subject-specific effect, which is assumed to be normally distributed around mean 0 with intersubject S.D. of $\sigma_d$. $\epsilon_{ijk}$ is the error term with mean 0 and S.D. $\sigma$. $\beta_0$ is the parameter to be used for the dose proportionality evaluation, and $\beta_k$ is the intercept term. When the value of $\beta_1$ differs significantly from 1, the hypothesis of dose proportionality is rejected. Evaluation of linearity of the dosage-exposure relations analysis was conducted using SAS 9.1.3 (SAS Institute, Inc., Cary, NC).

Results and Discussion

Study Subjects and Adverse Effects. Sixteen healthy subjects (age, 20–37 years; height, 150.7–189.7 cm; weight, 44.5–72.5 kg; and body mass index, 18.5–24.1 kg/m$^2$) 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 to be physiological changes; thus, a causal relationship with TJ-100 was denied.

Pharmacokinetics in Human Subjects. The plasma concentration-time profiles in the human subjects for each dose (2.5, 5, and 10 g) are shown in Fig. 1. The pharmacokinetic parameters of the six main TJ-100 ingredients are summarized in Table 1.

HAS, which is a constituent of Z. piperitum fruit, exhibited the highest plasma concentration among the six compounds measured. The plasma concentration of HAS reached the maximum concentration within 30 min after administration and its median half-life was 1.6 to 1.7 h, indicating its rapid absorption and elimination. The plasma concentration of HBS showed a pattern parallel 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., 2007a,b).

Various in vitro experimental systems have demonstrated that HAS exerts its pharmacological effects in the range of 0.1 to 100 μM (Jin et al., 2001; Koo et al., 2007). In this study, the maximum concentration of HAS in plasma was 0.76 to 2.66 μM. Taken together, these results 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 a 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

### Table 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>n</th>
<th>AUC$<em>{0-t</em>{\text{last}}}$</th>
<th>$C_{\text{max}}$</th>
<th>$t_{\text{1/2}}$</th>
<th>$t_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(ng · h/ml)</td>
<td>(ng/ml)</td>
<td>h</td>
<td>h</td>
</tr>
<tr>
<td>HAS</td>
<td>2.5 g/day</td>
<td>15</td>
<td>349 ± 136</td>
<td>209 ± 100</td>
<td>1.65 (1.01–2.48)</td>
</tr>
<tr>
<td></td>
<td>5 g/day</td>
<td>16</td>
<td>658 ± 223</td>
<td>391 ± 136</td>
<td>1.71 (1.04–3.26)</td>
</tr>
<tr>
<td></td>
<td>10 g/day</td>
<td>16</td>
<td>1290 ± 329</td>
<td>664 ± 165</td>
<td>1.62 (1.46–2.32)</td>
</tr>
<tr>
<td>HBS</td>
<td>2.5 g/day</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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<tr>
<td></td>
<td>5 g/day</td>
<td>16</td>
<td>130 ± 81.4</td>
<td>80.4 ± 45.9</td>
<td>1.17 (0.681–1.76)</td>
</tr>
<tr>
<td></td>
<td>10 g/day</td>
<td>16</td>
<td>234 ± 88.4</td>
<td>131 ± 52.6</td>
<td>1.09 (0.925–1.70)</td>
</tr>
<tr>
<td>6S$^a$</td>
<td>2.5 g/day</td>
<td>16</td>
<td>0.0306 ± 0.0211</td>
<td>0.0762 ± 0.0542</td>
<td>0.618 (0.280–0.956)</td>
</tr>
<tr>
<td></td>
<td>5 g/day</td>
<td>16</td>
<td>0.0751 ± 0.0871</td>
<td>0.142 ± 0.109</td>
<td>0.312 (0.286–0.793)</td>
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<tr>
<td></td>
<td>10 g/day</td>
<td>16</td>
<td>0.173 ± 0.0907</td>
<td>0.262 ± 0.125</td>
<td>0.429 (0.302–0.526)</td>
</tr>
<tr>
<td>10S$^b$</td>
<td>2.5 g/day</td>
<td>15</td>
<td>0.397 ± 0.372</td>
<td>0.290 ± 0.157</td>
<td>1.46 (0.463–4.66)</td>
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<tr>
<td></td>
<td>5 g/day</td>
<td>16</td>
<td>0.821 ± 0.522</td>
<td>0.636 ± 0.341</td>
<td>0.851 (0.559–3.45)</td>
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<td></td>
<td>10 g/day</td>
<td>16</td>
<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>GRB1</td>
<td>2.5 g/day</td>
<td>15</td>
<td>1.33 ± 0.653</td>
<td>0.0504 ± 0.0189</td>
<td>37.8 (12.2–101)</td>
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<tr>
<td></td>
<td>5 g/day</td>
<td>16</td>
<td>2.27 ± 0.839</td>
<td>0.0744 ± 0.0229</td>
<td>41.0 (21.3–330)</td>
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<td></td>
<td>10 g/day</td>
<td>16</td>
<td>3.45 ± 1.39</td>
<td>0.123 ± 0.0466</td>
<td>40.1 (20.3–598)</td>
</tr>
<tr>
<td>GRO1$^c$</td>
<td>2.5 g/day</td>
<td>15</td>
<td>0.191 ± 0.241</td>
<td>0.0198 ± 0.0320</td>
<td>27.8 (2.07–5.35)</td>
</tr>
<tr>
<td></td>
<td>5 g/day</td>
<td>16</td>
<td>0.209 ± 0.236</td>
<td>0.0209 ± 0.00910</td>
<td>4.40 (2.06–1390)</td>
</tr>
<tr>
<td></td>
<td>10 g/day</td>
<td>16</td>
<td>0.113 ± 0.0802</td>
<td>0.0230 ± 0.0117</td>
<td>14.3 (2.69–171)</td>
</tr>
</tbody>
</table>

$^a$ The elimination half-life was calculated from 2 subjects at the dose of 2.5 g, 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 2.5 g, 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 2.5 g, 3 subjects at the dose of 5 g, and 7 subjects at the dose of 10 g.
GRB1 was gradually eliminated with a half-life of approximately 4 h followed by maximum plasma concentrations. Thereafter, generate robust evidence on the pharmacological activity of TJ-100. Strong basis for future absorption, distribution, metabolism, and excretion studies to help create a consistent kinetic profile and to generate robust evidence on the pharmacological activity of TJ-100.

Conversely, the plasma concentration of GRB1, which is one of the constituents of processed ginger, gradually increased, with a t\text{max} of approximately 4 h followed by maximum plasma concentrations. Thereafter, GRB1 was gradually eliminated with a half-life of approximately 40 h. 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 those 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 approximately 1 to 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 the clinical effect of TJ-100. Calculation of the 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(Dose)\text{0–last} is graphically displayed in Fig. 2. The β1 of HAS, HBS, 6S, and 10S for AUC(Dose)\text{0–last} was close to 1 for doses ranging between 2.5 and 10 g of TJ-100. These results suggested that the pharmacokinetics of HAS, HBS, 6S, and 10S were linear within the dose range of 2.5 to 10 g/day of TJ-100. However, a 90% confidence interval of the \beta1 value for GRB1 did not include 1. Whereas the AUC(Dose)\text{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 was not conducted because only a few subjects had detectable plasma concentrations. The result of dose proportionality for Cmax was similar to that of AUC(Dose)\text{0–last}. The present study provides a strong basis for future absorption, distribution, metabolism, and excretion studies to help create a consistent kinetic profile and to generate robust evidence on the pharmacological activity of TJ-100.

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Department of Surgery, Kochi Medical School, Nankoku, Kochi, Japan (M.M., H.K., K.I., K.H.); Tsumura Laboratories (J.W.) and Pharmaceutical and Quality Research Department (K.A.), Tsumura & Co., Ami, Ibaraki, Japan; and Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical University, Hokkaido, Japan (T.K.).

Authors Contributions

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

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

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

Wrote or contributed to the writing of the manuscript: Munekage, Kitagawa, Ichikawa, Watanabe, Aoki, Kono, and Hanazaki.

References


Address correspondence to: Dr. Kazuhiro Hanazaki, Department of Surgery, Kochi Medical School, Oko-cho kohasu, Nankoku-shi, Kochi 783-8505, Japan. E-mail: hanazaki@kochi-u.ac.jp