Pharmacokinetics of Ephedrine and Pseudoephedrine after Oral Administration of Kakkonto to Healthy Male Volunteers

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The pharmacokinetics of serum ephedrine and pseudoephedrine as the indexed reference ingredients of Kampo Kakkonto (ge-gen-tang in Chinese) were compared after the oral administration of 2.5 g and 3.75 g of Kakkonto extract. The mean maximal serum concentrations of ephedrine and pseudoephedrine obtained after the administration at 3.75 g were 1.50- and 1.58-fold higher (p<0.05), respectively, than those obtained after the administration of 2.5 g, whereas the time required to reach the maximal concentration did not significantly differ between the two doses. The mean areas under the concentration-time curves of ephedrine and pseudoephedrine obtained after the 3.75 g of Kakkonto were 1.31- and 1.48-fold higher (p<0.05), respectively, than those obtained after the 2.5 g dose, while no significant differences were observed in the mean residence time and elimination rate constant of the terminal phase between the doses. Disposition profiles showed that the kinetic behavior of ephedrine and pseudoephedrine were largely linear at the doses examined.

Key words: Kakkonto, Chinese herbal medicine, bronchodilation, nasal decongestant, pharmacokinetics

Introduction

Many herbal remedies (Kampo) have been marketed to treat acute and chronic diseases. Kampo extracts were originally imported from China and developed into new remedies and extract formulations in Japan. Only a few herbal remedies are regulated by quantitative tests of their indexed ingredients according to the guidelines of Japanese Pharmacopoeia. Recent reports indicate that the pharmacokinetic behavior of drugs can be altered by the concurrent administration of herbal medicines1-3. Some herbal remedies have been pharmacologically and pharmacokinetically evaluated in animals with respect to their indexed compounds4-8. Human studies have confirmed the absence of major chemicals extracted from Saibokuto (ten herbs used to treat severe asthma) in either the blood or urine9, indicating inhibited absorption. Yufune and Cyong have reported that the area under the concentration-time curve (AUC) and the maximum concentration (Cmax) of ephedrine after oral administration of Kakkonto are significantly greater than those of Shosaikoto even when these Kampo remedies contain a similar amount of ephedrine10.

Herbal preparations are frequently administered 3 times daily after meals (t. i. d.). In Japan, together with western medicines if needed although the national regulatory agency has approved the administration of these remedies 2 or 3 times per day between or before meals. To enhance patient compliance, newer western drugs tend to be administered at a reduced frequency in terms of doses per day, i. e., twice (b. i. d.) or once (q. d.) a day.

The main function of Kakkonto in Japan is usually to treat the common cold. Kakkonto consists of crude extracts of Pueraria root, Jujube, Ephedra herb, Glycyrrhiza, Cinnamon bark, Peony root, and Ginger. Ingredients vary in herbal medicines depending upon the place and season of harvest. One method of ensuring quality and quantity is to regulate the amounts of ingredients required to generate specific

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concentrations of indexed substances\textsuperscript{11}. The main ingredients of the Ephedra herb (Mao) include ephedrine and pseudoephedrine. We examined the serum concentration profiles of ephedrine and pseudoephedrine, as indexed substances of Kakkonto extracts, at two doses simulating t.i.d. and b.i.d. administration. Understanding the pharmacokinetic behavior of indexed substances will help to ensure the safety and efficacy of herbal remedies.

Methods

1. Materials
   
   An extract of Kakkonto (Kanebo Pharmaceuticals, Tokyo, Japan) contained 14.43 and 5.73 mg each of granulated ephedrine and pseudoephedrine per daily dose (7.5 g) of granules, respectively. Reference standards of ephedrine and pseudoephedrine as well as their deuterium-labeled isotopes (d\textsubscript{3}-ephedrine and d\textsubscript{3}-pseudoephedrine) were a gift from Kanebo Pharmaceuticals. The acylation agent heptafluorobutyryl imidazole (HFBI) was purchased from GL Sciences, Tokyo, Japan. All other chemicals were of analytical grade and supplied by Wako Pure Chemical Industries, Osaka, Japan.

   Blank sera were donated by the Hokkaido Red Cross Blood Center, Sapporo, Japan.

2. Clinical study
   
   This open two-way crossover randomized study involved 10 healthy male volunteers (age: mean 24.3 y, range 23-26 y; body weight: mean 64.0 kg, range 52-74 kg), who provided written informed consent to participate. All were healthy according to a medical history, complete physical examination and laboratory findings before and after participation. Laboratory parameters did not significantly differ before and after the study except for slight changes in LDH and total cholesterol (p<0.05), but both values remained within normal ranges. The present study did not attempt to explain these changes.

   The volunteers were randomly assigned to two groups. After an overnight fast, each group was given 2.5 or 3.75 g Kakkonto extract with 200 mL of tap water, 1 hour after a standard breakfast, which usually did not include any source of either ephedrine and pseudoephedrine, in a cross over fashion. No other food or drink was consumed until a standard lunch 4 hours later. Caffeine-free drinks were allowed ad libitum. Breakfast and lunch contained 592 and 699 kcal on day 1 and 533 and 699 kcal on day 2, respectively. The regimen was repeated after an interval of 2 weeks.

   Blood samples (approximately 5 mL) were obtained by direct venipuncture before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after drug ingestion. Serum was separated by centrifugation and stored frozen at −20°C.

   The ethical committee of Otaru Kyokai Hospital, Otaru, Japan approved the protocol.

3. Extraction procedure
   
   Serum ephedrine and pseudoephedrine concentrations were measured using several methods with modifications\textsuperscript{12〜19}. Briefly, the d\textsubscript{3}-form of each drug (100 \(\mu\)L : 1 ng/mL) was mixed with 1 mL serum in water, 3 mL 0.2 N HCl saturated with NaCl, and 5 mL n-hexane : ethyl acetate (9 : 1) for 5 min. The mixture was separated by centrifugation for 10 min, then the aqueous phase was mixed with 2 mL 1 N NaOH and 5 mL n-hexane : ethyl acetate (9 : 1) and similarly separated by centrifugation. The organic phase was mixed with 100 \(\mu\)L HCl and evaporated to dryness under vacuum at 50°C. The residue was acylated with 30 \(\mu\)L HFBI at 50°C for 30 min, and then shaken with 1 mL n-hexane : ethyl acetate (9 : 1) and 1 mL of 1 N NaOH saturated with NaCl for 5 min. The aqueous phase obtained by centrifugation was stored at −20°C and the organic phase was evaporated to dryness under vacuum at 50°C in 1.5 mL tubes. The residue was dissolved in 5 to 10 \(\mu\)L n-hexane : ethyl acetate (9 : 1) and 1 \(\mu\)L portions were injected into an Automass 200 gas chromatograph-mass spectrometer (JEOL, Tokyo, Japan) system (GC-MS).

4. GC-MS conditions
   
   Samples were separated by GC using an HP-5, 0.32 mm I.D. x 30 m, capillary column (J & W Scientific, CA, USA) at a helium flow rate of 1.2 mL/min. The temperatures of the injection port, column, transport line and detector were set at 280, 60, 280, and 200°C, respectively. The initial column temperature was maintained for 2 min then raised at a rate of 40°C/min to 300°C for 3 min.

   Mass spectra were measured as positive ions in electron impact mode at an electron energy of 70 eV. Deuterium labeled ephedrine and pseudoephedrine in samples were analyzed by selected ion monitoring at m/z 254 and 257, respectively. Ephedrine and
pseudoephedrine were completely resolved under these conditions with retention times of 7.07 and 7.12 min, respectively.

5. Standard curves

Known amounts of ephedrine and pseudoephedrine up to 200 ng/mL were added to blank serum. The concentrations of both drugs were estimated from the peak height ratios with the corresponding deuterium labeled isotopes as internal standards. Standard curves were constructed for each set of serum samples. Linear standard curves for both drugs were obtained with correlation coefficients of over 0.999. Coefficients of variation were <10% for 20 ng/mL of both drugs.

6. Pharmacokinetic analysis

The C_{max} and time to reach C_{max} (t_{max}) were calculated from the observed data. The area under the serum concentration-time curve from 0 to 12 hours (AUC_{0–12}) was calculated using the trapezoidal rule. The area obtained by dividing the concentration at 12 h after administration by the terminal elimination rate constant (k) estimated by least-squares regression analysis of 3 or 4 points of the terminal concentration-time profile was added to the AUC_{0–12} to calculate area to infinity (AUC). The mean residence time (MRT) was also calculated.

7. Statistical analysis

Normally distributed data were analyzed using Student's paired t-test, and other distributions were analyzed using the Wilcoxon signed-rank test. A p value of <0.05 was considered significant. Results are expressed as mean values with standard deviation (SD).

Results

All volunteers completed the study. Figures 1 and 2 show the serum concentration profiles of ephedrine and pseudoephedrine after administering the 10 volunteers with two doses of Kakkonto extract. Table shows the pharmacokinetic parameters. The mean C_{max} values of ephedrine and pseudoephedrine were 1.49- and 1.58-fold higher after the 3.75 g, than after the 2.5 g dose (p<0.05) although the t_{max} did not significantly differ (p>0.05). The mean AUC value of ephedrine and pseudoephedrine following the two doses of Kakkonto were significantly higher (both p<0.01) after the 3.75 g dose relative to the dose ratio, while the mean MRT and k did not significantly differ.

Discussion

Granular preparations of Kampo extracts are now widely prescribed as routine therapy in Japan as well as in China. Kampo preparations should be clinically evaluated to ensure their efficacy and safety, particularly in relation to the pharmacokinetics of their ingredients. Several studies have shown higher rate constants during the elimination phase compared with values during the absorption phase after the oral administration of ephedrine and pseudoephedrine.
The present study showed that the absorption and elimination rate constants were almost identical in 2 volunteers at both doses possibly because of relatively low absorption rate constants in these volunteers. Thus, we used moment analysis rather than compartment analysis to evaluate the pharmacokinetic profiles of ephedrine and pseudoephedrine.

Although the administration of Kampo extracts before or between meals, b. i. d. or t. i. d. is approved in Japan, patients frequently take them t. i. d. after meals. The timing of meals relative to the time of oral drug dosage can play an important role in influencing rates and/or extent of bioavailability\(^{24-26}\). The bioavailability of cefetamet pivixil\(^{27}\) and hydralazine\(^{28}\) is enhanced by food, whereas meal times exert negligible effects on other drugs including adinazolam\(^{29}\) and cibenzoline\(^{30}\). Pharmacokinetic or pharmacodynamic differences in Kampo remedies after oral administration b. i. d. and t. i. d. or before, between, and after meals have not been reported. We compared the pharmacokinetics of ephedrine and pseudoephedrine as reference compounds for Kakkonto extract after administration as b. i. d. and t. i. d. after meals. We did not perform compartment model analysis in the present study because the absorption and elimination constants were almost identical in 2 volunteers, possibly due to the flip-flop phenomenon.

Quality control of the ingredients of Ephedra herbs is regulated based on total alkaloid contents calculated by summation of ephedrine and pseudoephedrine contents according to the 15th Edition of the Japanese Pharmacopoeia\(^{11}\). Ephedrine has been used as a bronchodilator, nasal decongestant, and to treat urinary incontinence\(^{31}\), whereas pseudoephedrine is used to relieve nasal and sinus symptoms caused by the common cold, hay fever, and other respiratory allergies\(^{32}\). Differences in the pharmacokinetic and pharmacodynamic behavior of ephedrine and pseudoephedrine have never been compared. The clinical effects of Mao may be attributed more to ephedrine than pseudoephedrine because the present study found that the concentration profiles of ephedrine were almost 3-fold higher. The reported elimination half life of ephedrine administered as 50 mg of oral racemic ephedrine hydrochloride is 4.8 h with \(C_{\text{max}}\) values of 100 ng/mL\(^{22}\); those of \(d\)- and \(l\)-ephedrine are about 8 and 10 h with \(C_{\text{max}}\) values of 100 and 70 ng/mL under the same administration conditions\(^{23}\). These results confirmed the linear kinetic profile of ephedrine at a dose of 50 mg of racemic ephedrine hydrochloride.

In conclusion, the pharmacokinetic profiles of Kakkonto taken orally as b. i. d. or t. i. d. were similar with no unusual increases in serum ephedrine and pseudoephedrine concentrations. These valuable findings are important as Ephedra sinica is a major constituent of the Kakkonto herbal remedy.

**Acknowledgement**

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**References**

1) Makino T, Inagaki T, Komatsu K, Kano Y. Pharmacokinetic interactions between Japanese traditional medicine (kampo)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>2.5 (ng/mL)</th>
<th>3.75 (ng/mL)</th>
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<tr>
<td>C(_{\text{max}})</td>
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<td>8.1±1.9</td>
<td>12.8±3.4(*)</td>
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<td>t(_{\text{max}}) (h)</td>
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<td>2.8±0.7</td>
<td>3.0±0.6</td>
<td>3.4±1.1</td>
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<td>AUC (ng·h/mL)</td>
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<td>312.9±86.7(*)</td>
<td>66.8±30.3</td>
<td>99.2±36.7(*)</td>
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<td>MRT (h)</td>
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<td>6.8±1.5</td>
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<td>k (1/h)</td>
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<td>0.1±0.0</td>
<td>0.2±0.1</td>
<td>0.2±0.1</td>
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Data represent the mean±standard deviation (SD).
C\(_{\text{max}}\) : maximum concentration
\(t_{\text{max}}\) : time to maximum concentration
AUC : area under the concentration-time curve
MRT : mean residence time
\(k\) : elimination rate constant at terminal phase
\(*\) p<0.01 compared to the data obtained after oral administration of Kakkonto at a dose of 2.5 g


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