Preparation of Goreisan Suppository and Pharmacokinetics of trans-Cinnamic Acid after Administration to Rabbits

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Goreisan suppository is prepared as a hospital preparation, and successfully used for the treatment of diarrhea and vomiting in young children with common cold. While clinical efficacy of the suppository has been reported, few studies have been carried out to clarify the preparation procedure and pharmacokinetics of the suppository. In this study, trans-cinnamic acid (CA) was used as a representative substance of goreisan constituents, and assayed by HPLC-UV. We investigated the properties of goreisan suppositories prepared using various sizes of pulverized goreisan extract granules, in vitro dissolution profiles using the reciprocating dialysis tube method, and pharmacokinetics in rabbits compared with those for goreisan enema. Mass and content uniformity tests on the suppositories of three size fractions, 0–75, 75–150, and 150–300 μm, showed good acceptance for all kinds of suppository. Storage stability at 4°C was maintained until 4 months. In vitro dissolution of CA from the suppository was proportional to time until 45 min, and slower than that from the enema. Finally, 80% of CA had dissolved at 60 min. Pharmacokinetic study in rabbits revealed that the area under the plasma concentration–time curve from 0 to 120 min ($ \text{AUC}_{0–120 \text{ min}}$) of the suppository was twice that of the enema. Moreover, from a study in rabbits using CA injection and CA suppository, we revealed that CA was rapidly and well absorbed from the rectum, showing 84% absolute bioavailability. Thus, we illustrated the defined preparation procedure of the suppository and the superiority of the suppository over the enema. This study will support evidence that the suppository is fast-acting and efficacious in clinical use.

Key words  goreisan; suppository; pharmacokinetics; trans-cinnamic acid (CA); enema; dissolution

Goreisan, a five-ingredient powder with Poria, is an herbal formula consisting of Polyporus, Alismatis Rhizoma, Sclerotchum Poriae Cocos, Atractylodes macrocephala Rhizoma, and Cinnamomi Cortex, and its herbal extract granule is accepted as a Kampo medicine in Japan.1) Goreisan is used generally for patients with the following health conditions and symptoms: low urination, edema, diarrhea, vomiting, dizziness, and hangover. In particular, goreisan is highly effective for preventing diarrhea and vomiting in young children with common cold.2,3) In terms of oral use of this herbal medicine, however, it is hardly accepted by infants and young children owing to its unpleasant taste and odor.4) Therefore, goreisan extract granules are changed to suppositories by pharmacists in hospital pharmacies.5,6) The goreisan suppository is reported to be effective for clinical use in children.5,6)

There are, however, some issues to clarify for the goreisan suppository, concerning its properties and pharmacokinetic behavior. The goreisan suppositories are prepared from powdered goreisan extract granules and suppository bases. In the preparation procedure, there is a difference in size fractionation of powdered goreisan extract granules among hospitals. Size fractionation would influence the properties of the suppositories, such as content uniformity and dissolution behavior. On the other hand, goreisan suppository is known to be fast-acting.3) However, the pharmacokinetics of goreisan suppository is unclear. Besides, enema of goreisan is also used for the treatment of acute gastroenteritis in children with vomiting.7,8) The enema is prepared by suspending powdered goreisan extract granules in warm water or normal saline. However, it is unknown whether there are differences between the goreisan suppository and the enema in terms of their pharmacokinetics.

Although the clinical efficacy of goreisan rectal preparations has been reported, data on the physical properties and pharmacokinetics of the preparations are still lacking because goreisan contains many active pharmaceutical ingredients that include well-known and unknown substances. In general, Kampo, namely Japanese traditional medicine, contains many constituents. Actually, it is impossible to determine all constituents of the medicine. Similarly, although the goreisan extract granule contains many constituents, the granule is well regulated as an ethical medicine.9) In this study, we selected trans-cinnamic acid (CA) as a representative substance of goreisan constituents, which is one of the principal active pharmaceutical ingredients,9) because it can be accurately detected using HPLC. For instance, Kampo medicine maouto suppository is evaluated using l-ephedrine as an indicative substance.10)

In this study, we investigated the effects of the size of powdered goreisan on the content uniformity and dissolution behavior of goreisan suppositories by using the detection of CA. Then, the pharmacokinetics of CA after administration of the goreisan suppository and goreisan enema in rabbits was investigated to obtain fundamental basic information on its clinical efficacy. At present, no report focusing on the rectal absorption of CA appears to be available. Therefore, we also elucidated the rectal absorption of CA in rabbits using pure CA suppository and injection.

MATERIALS AND METHODS

Materials  Goreisanryo extract fine granules (Kotaro Pharmaceutical Co., Ltd., Osaka, Japan) were used as the goreisan.

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stroke speed was 40 rpm and stroke width was 55 mm. Sam -
(No. 27, Wako Pure Chemical Industries, Ltd.). Up and down
(pH 7.4), and the membrane was 20 cm of dialysis membrane
humidity (30–50%). The animals were fasted for 24 h prior to
access to a normal diet (ORC-4, Clea Japan, Tokyo, Japan)
pling was conducted according to the experimental plan, and
±
The supernatant was filtered with a membrane filter (0.45
solution (IS solution, 0.7 mg/mL) was added to the solution.
- hydroxypropyl benzoic acid were purchased from
Tokyo, Japan. HPLC-grade acetonitrile and methanol (MeOH)
saline was obtained from Otsuka Pharmaceutical Co., Ltd., Osaka, Japan) was used as a suppository base. Normal
Hard fat Witepsol H-15 (Vosco, Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) was used as a suppository base. Normal saline was obtained from Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan. HPLC-grade acetonitrile and methanol (MeOH) were purchased from Honeywell International Inc. (U.S.A.). CA and p-hydroxypropyl benzoic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as a standard and an internal standard (IS), respectively. All other chemicals were of reagent grade.

Preparation of Goreisan Suppository The goreisan suppository was prepared using a fusion method. Briefly, goreisan extract granules were ground and sieved with a 50 mesh sieve (opening: 300µm). Using 100 and 200 mesh sieves (opening: 150 and 75µm, respectively), the obtained gorei-
san powders were classified in three sizes of 0–75, 75–150, and 150–300µm, named L, M, and H, respectively. Then, the goreisan powder was mixed well with suppository base Witepsol H-15 melted at 60°C at a weight ratio of 1:1. The resultant mixture was poured into plastic containers (1.75mL, Kanaze Co., Ltd., Osaka, Japan), and was allowed to solidify at room temperature and stored at 4°C until use. The suppository weighted 2g and contained 1g of powdered goreisan.

Preparation of Goreisan Enema Goreisan extract gran-
ule was ground and sieved with a 50 mesh sieve (opening: 300µm). Then, one gram of the powdered goreisan was sus-
pended in 10mL of normal saline by hand.

Preparation of CA Formulations CA suppository was prepared by the fusion method. Namely, 10mg of CA was mixed well with 1g of Witepsol H-15 melted at 60°C. Then, the mixture was poured into a plastic container (1.75mL) and was allowed to solidify at room temperature and stored at 4°C. The CA suppository contained 10mg of CA. CA injection was prepared by dissolving 1mg of CA into normal saline to make a final volume of 10mL, and then filtered with a membrane filter (0.22µm, Milex-GV, Millipore, U.S.A.).

Determination of CA in the Suppositories and Goreisan Granules A suppository or one gram of goreisan granules was dissolved in 40mL of MeOH–water (1:1, v/v) mixture at 50°C.11 One milliliter of p-hydroxy propylbenzoic acid MeOH solution (IS solution, 0.7mg/mL) was added to the solution. Then, the resultant solution was centrifuged (3500rpm, 5min). The supernatant was filtered with a membrane filter (0.45µm, Minisart RC15, Sartorius Stedim Biotech GmbH, Germany) and was introduced onto HPLC.

In Vitro Dissolution Test Dissolution of CA from the suppository and enema was investigated at 37±0.5°C using the reciprocating dialysis tube method with tapping12 (HZ-11D, Miyamoto Riken Industries Co., Ltd., Osaka, Japan). The dissolution medium was 1L of 50mM phosphate buffer (pH 7.4), and the membrane was 20cm of dialysis membrane (No. 27, Wako Pure Chemical Industries, Ltd.). Up and down stroke speed was 40rpm and stroke width was 55mm. Sam-
pling was conducted according to the experimental plan, and CA concentration was assayed using HPLC. The test was repeated three times.

Animals Japanese white rabbits (male, Japan SLC Inc., Shizuoka, Japan) weighing 2.2±0.3kg were housed with free access to a normal diet (ORC-4, Clea Japan, Tokyo, Japan) and water, and were maintained on a 12-h light/12-h dark cycle in a room with controlled temperature (22–24°C) and humidity (30–50%). The animals were fasted for 24h prior to experimentation, but continued to have free access to water during this time. The study was performed in accordance with the guidelines of the Experimental Animal Ethics Committee of the University of Shizuoka.

Pharmacokinetic Study in Rabbits Rabbits were admin-
istered with a suppository or enema containing 1g of goreisan by insertion into the anus, which was then closed using a clip to prevent leakage. For CA rectal absorption study, rabbits were administered with a CA suppository (10mg) rectally, and intravenously injected with CA (100µg) into a marginal ear vein according to crossover design with at least one week washout period. After administration, blood samples were collected from the auricle vein of the other ear at designated times. Plasma samples were isolated from the whole blood by centrifugation and stored at −20°C until determination of the concentration of CA.

Assay of CA in the Plasma of Rabbits The plasma concentration of CA was determined using HPLC.11 Briefly, 5µL of IS solution (1µg/mL), 50µL of 3% acetic acid solution, and 1mL of MeOH–acetone mixture (1:1, v/v) was added to the plasma sample of rabbits (100µL). After centrifugation at 9000rpm for 10min, the supernatant fluid was separated and dried using a rotary evaporator. Then, the residue was solved into a mobile phase, and was introduced onto HPLC.

Determination of CA Using HPLC HPLC (LC-10ADVp, Shimadzu Co., Ltd., Kyoto, Japan) was performed using the an analytical column (Inertsil ODS-100V, 3µm, 2.1mm×150mm, Tosoh Co., Tokyo, Japan) with a mobile phase (MeOH–acetone–0.6% acetic acid aqueous solution = 25:20:55, v/v) delivered at a flow rate of 0.2mL/min. The wavelength of detection was 272nm (SPD-10AVp, Shima-
dzu Co., Ltd.). CA concentration was determined using peak area for dissolution test and peak area ratio of CA and IS for the other studies.

Data Analysis The linear trapezoidal method was used to calculate the area under the plasma concentration–time curve from 0 to 120min (AUC0-120min) after intravenous and rectal administration in rabbits. In the case of rectal administration, the peak plasma concentration (Cmax) and the time to the peak plasma concentration (Tmax) were obtained by inspection. In the case of injection, the plasma concentration at time 0 (C0) was calculated using linear regression from the first three points of the log plasma concentration–time curve. Results are expressed as mean±S.D. The differences of pharmacokinetic parameters between the two groups were tested by Student’s t-test. The p values <0.05 were considered statistically sign-
ificant.

RESULTS

Properties of Goreisan Suppositories Since there is
inter-hospital difference in the preparation procedure, we
examined the effect of size fraction on the properties of sup-
postories. To investigate the influence of size fractionation of
goreisan powder on the properties, four kinds of goreisan sup-
postory were prepared using L, M, H, and non-fractionized
goreisan powders. The weight and CA content of the goreisan
powders were classified in three sizes of 0–75, 75–150,
and 150–300µm, respectively.

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Content uniformity test of JP16 was also conducted for each sample, resulting in its adoption. On the other hand, raw material, that is, goreisanryo extract fine granules, contained $191\pm2.0\ \mu g$ (mean $\pm$ S.D., $n=3$) of CA per gram. Therefore, the suppositories contained 99.5–107% of goreisan powder. These results indicated that the preparation procedure was suitable to maintain the quality of the suppositories. Besides, there was no significant difference in weight and CA content among the four kinds of suppository prepared at different size fractions. This indicated that size would not influence the preparation procedure.

Stability during Storage  In hospital preparation, storage life is important for planning the production cycle. Therefore, we investigated the stability during storage at 4°C using determination of the CA content in the suppositories. The results are presented in Fig. 1. The residual CA percent against the initial content was $99.0\pm3.9\%$ after storage for 4 months. A relatively high level of CA remained in the suppositories, indicating that the storage life was at least 4 months.

In Vitro Dissolution from Suppository  In general, the particle size of drug powder influences the dissolution rate. Therefore, we examined L-sup, M-sup, and H-sup for the dissolution behavior of CA. Dissolution profiles are presented in Fig. 2. Dissolution profiles were linear with respect to time until 45 min, finally reaching about 90% dissolution. The dissolution profiles of CA from the three kinds of suppository were similar to each other, being almost superimposable. There was no influence of the size fraction on the dissolution behavior, indicating no need for fractionation in the preparation procedure.

Pharmacokinetics of CA after Rectal Administration of Goreisan Suppository and Enema  After rectal administration of goreisan suppository or enema at a dose of 1 g/g body to four rabbits, the plasma concentration of CA was determined over 120 min. The mean plasma concentration–time profiles are summarized in Table 2. No significant difference in $C_{\text{max}}$ and $T_{\text{max}}$ was observed.
between the two formulations. $AUC_{0-120\text{ min}}$ obtained after suppository and enema administration were 1.30 and 0.586 µg·h/mL, respectively. Thus, pharmacokinetic analysis revealed that the suppository showed significantly higher bioavailability than the enema.

To confirm the difference of the $AUC_{0-120\text{ min}}$, the in vitro dissolution behavior of CA from the enema was examined. The suppository gradually melted within 10 min from the start of the test due to external heat condition (37°C), and dispersed in the dialysis tube. The goreisan powders, which were released from the suppository or the enema, were retained in the dialysis tube and some sank at the lower position in the tube. The dissolution profile is shown in Fig. 4. The dissolution rate of CA from the enema was faster than that of the suppository. Totals of 63 and 80% of CA were dissolved in the medium at 30 and 60 min, respectively. It appears that there is no state of release from the base in the case of the enema compared with the suppository. The faster dissolution would cause rapid absorption and elimination of CA in plasma.

**Bioavailability of CA after Rectal Administration**

To confirm the rectal absorption and bioavailability of CA after rectal administration, we carried out pharmacokinetic study using pure CA suppository and CA injection. The average plasma level–time curves of CA after intravenous and rectal administration are shown in Fig. 5. The plasma levels are presented as the plasma concentration divided by the dose because there were differences in the dose between the formulations due to difficulty in manufacturing the injection with a high dose. The plasma levels of CA from the CA suppository peaked at 5 min, and then gradually returned to the baseline level after 60 min. In contrast, plasma CA levels after injection decreased rapidly, reaching the baseline at 45 min. This indicated that CA was continuously absorbed until about 60 min. Pharmacokinetic parameters are summarized in Table 3. $T_{\text{max}}$ of the suppository was about 6 min, indicating rapid absorption of CA from rectum. $C_{\text{max}}$ of the suppository showed about 28% of $C_0$, and bioavailability calculated from $AUC_{0-120\text{ min}}$ obtained after rectal administration and $AUC_{0-120\text{ min}}$ obtained after intravenous administration was about 84%. These observations indicated that CA was well absorbed from the rectum. Moreover, the absorption rate of CA from the rectum was evaluated by a deconvolution method from the time-courses of CA level after dosing of injection and suppository, and is presented in Fig. 6. Half of the total uptake amount was rap-

### Table 2. Pharmacokinetic Parameters of Goreisan Rectal Formulations in Rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suppository</th>
<th>Enema</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>2.52±0.66</td>
<td>2.09±1.3</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.292±0.14</td>
<td>0.167±0.0</td>
</tr>
<tr>
<td>$AUC_{0-120\text{ min}}$ (µg·h/mL)</td>
<td>1.30±0.36*</td>
<td>0.586±0.27</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. ($n=4$). The asterisk shows a significant difference between the two formulations at *$p<0.05$ (Student’s $t$-test).

### Table 3. Pharmacokinetic Parameters of CA Concentration Divided by Dose after Intravenous and Rectal Administration in Rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Injection</th>
<th>Suppository</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$ (ng/mL/dose)</td>
<td>144±75</td>
<td>—</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL/dose)</td>
<td>—</td>
<td>41.0±16</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>—</td>
<td>0.104±0.036</td>
</tr>
<tr>
<td>$AUC_{0-120\text{ min}}$ (ng·h/mL/dose)</td>
<td>20.4±10</td>
<td>17.1±7.5</td>
</tr>
</tbody>
</table>

Plasma concentration of CA was divided by dose to compare the two formulations directly. Each value represents the mean±S.D. ($n=4$).
idly absorbed in the first 12 min. Thereafter, the CA absorption continued to about 60 min. It appears that CA shows good absorbability from the rectum.

DISCUSSION

This study clarified the effect of size fractionation of powdered goreisan extract granules on product uniformity of goreisan suppository and in vitro CA dissolution from the suppository. The three kinds of suppository prepared from the goreisan powders L, M, and H were adopted after JP16 mass and content uniformity tests (Table 1), and then showed similar dissolution profiles of CA (Fig. 2). These results indicate that the size fraction of powdered goreisan does not affect the quality of the suppositories. According to the manufacturer’s information on goreisan extract granules, the dissolution of CA from goreisan extract granules is quite rapid, reaching 100% dissolution at 5 min when a dissolution test was conducted in water at a speed of 100 rpm using the JP16 paddle method. Thus, size fractionation of powdered goreisan in the range of 0–300 μm was not needed before mixing with suppository base.

Then, we elucidated the pharmacokinetics of CA after rectal administration of the goreisan suppository as well as the goreisan enema, indicating that $AUC_{0–120 min}$ of the suppository was twice that of the enema (Fig. 3, Table 2). In some cases, goreisan enema is used instead of suppository. However, $AUC_{0–120 min}$ of the enema was quite different from that of the suppository, suggesting that they differ in their clinical efficacy. Of course, further research is expected to clarify the difference between the two formulations. In addition, compared with the enema, the suppository is advantageous in that no cumbersome handling is required. Therefore, pharmacists should be able to prepare the suppository easily if rectal formulation of goreisan is needed.

Moreover, we revealed the rectal absorption of pure CA in rabbits. To our knowledge, there is no report of an investigation of the rectal absorption of CA. To clarify the pharmacokinetics of CA suppository, we determined the time-course of CA suppository, we determined the time-course of CA in goreisan suppository because intestinal absorption of CA upon rectal administration of the goreisan suppository as well as the goreisan enema was rapid and continued up to 60 min, reaching 80% of absolute bioavailability. These results support the clinical evidence of hospital preparations, to achieve safe and effective pharmacotherapy using them.

In conclusion, we confirmed that there is no need for fractionation under 300 μm of powdered goreisan extract granules in the preparation procedure. The residual content of CA in the suppository was maintained at 100% during 4 months of storage at 4°C. The in vitro dissolution rate of CA from the suppository was proportional to time until 45 min, reaching to 80% dissolution. Although the suppository and the enema showed similar $C_{max}$ and $T_{max}$, the $AUC_{0–120 min}$ of the suppository was twice that of the enema. Rectal absorption of CA was rapid and continued up to 60 min, reaching 80% of absolute bioavailability. These results support the clinical evidence that goreisan suppository is fast-acting and highly effective for diarrhea and vomiting in children.

REFERENCES

11. Nishimura N, Doi N, Uemura T, Taketani T, Hayashi G, Kasai...


