Effects of Herbal Drugs Prescribed in Wood Creosote Pills on the Dissolution Profile of Guaiacol

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Wood creosote pills (P4) containing wood creosote and four herbal drugs, Gambir, Phellodendri Cortex, Glycyrrhizae Radix, and Citri Unshiu Pericarpium (CUP), have been used to treat food poisoning and diarrhea through self-medication in Japan. The mean dissolution time (MDT) of guaiacol, one of the active constituents of wood creosote, from P4 (138.3±3.3 min) was significantly longer than that (42.6±4.3 min) from pills (P0) containing only wood creosote. The MDT of the variant pills prepared from P4 without CUP (54.3±12.5 min) was found to be significantly shorter than that of P4. These findings suggest that CUP plays an important role in sustaining the dissolution of guaiacol from P4. The long MDT of guaiacol is considered one of the most important factors affecting the duration of efficacy after oral administration of wood creosote pills. The present findings are considered proof that CUP has been prescribed in traditional as well as new formulations of wood creosote pills.

Key words wood creosote; dissolution time; guaiacol; Citrus unshiu

The wood creosote distilled from wood tar, not from coal tar, has long been used for the treatment of food poisoning and diarrhea. Its major and active ingredients are guaiacol and creosol, which have been shown to suppress contractions of intestinal smooth muscle and fluid secretion induced by enterotoxin.

Pills (P0) containing only wood creosote and pills (PGLR) containing wood creosote and powder of Glycyrrhizae Radix (GLR, Kanzo in Japanese) have been developed in Europe. With reference to these wood creosote pills, the pills (P4) containing wood creosote and four herbal drug powders, GLR, Gambir (GAM, Asen-yaku in Japanese), Phellodendri Cortex (PHC, Oubaku in Japanese) and Citri Unshiu Pericarpium (CUP, Chinpī in Japanese), have been developed and used in self-medication under the name “Seiro-gan” in Japan.

The GLR and PHC prescribed in P4 are considered to improve digestive problems due to their pharmacological action. However, there have been no systematic studies of the pharmaceutical role of herbal drugs prescribed in P4. In this in vitro study, effects of herbal drugs on the mean dissolution time (MDT) of guaiacol from P4 are compared using P0, PGLR and four variants of P4 each without one of the herbal drugs. Furthermore, the effects of particle size and amount of EtOH extract in various CUP prescribed in the pills on MDT of guaiacol were also examined. The MDT of guaiacol and disintegration times of pills is considered important factors affecting efficacy after the oral administration of P4.

MATERIALS AND METHODS

Wood Creosote, Herbal Drugs and Chemicals Wood creosote was supplied by Taiko Tec Co., Ltd. (Osaka, Japan), and contained 27.4±0.1% guaiacol, 19.5±0.1% creosol, and 9.9±0.1% phenol (n=3) as determined in a previous method.

Powder of GAM (Uncaria gambir Roxb. from Indonesia) was purchased from Nippon Funmatsu Yakuhin Co., Ltd. (Osaka, Japan). Powders of PHC (Cortex of Phellodendron amurense Purp. from China) and GLR (Radix of Glycyrrhiza uralensis Fisch. from China) were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan). These three powders were of the Japanese Pharmacopoeia XIV (JP XIV) standard. The powder of CUP (Pericarpium of Citrus unshiu Markovich from China preserved for 2 years: CUP2, purchased from Tochimoto Tenkaido Co., Ltd.) is the standard of the Taiko Pharmaceutical Co., Ltd. These four powders, GAM, PHC, GLR and CUP2, were used to prepare wood creosote pills (P4 and four variant pills each without one of the herbal drugs in P4) in these experiments. The particle size distributions, and major ingredients of the four herbal drugs are described in Table 1.

Chinpī (CUP and CRP) analogues: Three kinds of Chinpi (from China) preserved for 1 (CUP1), 8 (CRP8) and about 35 (CRP35) years and Chinpi (from Kagawa, Japan) preserved less than 1 year (CUPJ1) were also supplied by Tochimoto Tenkaido Co., Ltd. CUP and CRP were examined to estimate their botanical origin by referring to the HPLC-profiles as described later. The CUP2 used in P4 was divided into two parts: an orange-red outer peel, the exocarp, (CUP2op) and a white inner peel, the mesocarp, (CUP2ip).

<table>
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<tr>
<th>Table 1. Quality of Herbal Drugs Used in This Study</th>
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<tr>
<td>Ingredients (mg/g)</td>
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<tr>
<td>GAM 56.0 43.0 1.0 Catechin 302.3±8.8</td>
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<tr>
<td>PHC 14.3 81.2 4.5 Berberine 22.6±0.5</td>
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<tr>
<td>GLR 44.0 55.6 0.4 Glycyrrhizin 32.5±1.9</td>
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<tr>
<td>CUP2 87.4 9.5 3.1 Nobiletin 1.1±0.2</td>
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</table>

Powders of GAM, PHC and GLR are of JP XIV standard. Powder of CUP2 (preserved for 2 years) meets the standard of Taiko Pharmaceutical Co., Ltd. Particle size distributions were examined using the No. 100 (150 μm) and No. 200 (75 μm) screen of JP XIV. Ingredients were measured by HPLC (LC-10AT, Shimadzu Co.): Catechin: TSK gel ODS-120T, CH3CN–H2O–AcOH (7 : 91 : 2) detected at 280nm. Berberine and glycyrrhizin: Nucleosil SC18, CH3CN–3%AcOH–MeOH–triethylamine (525 : 975 : 75 : 6) detected at 250nm. Nobiletin: YMC-ODS A-312 S-5, 0.05 M NaH2PO4 (pH 7.4)–CH3CN–MeOH (10 : 7 : 3). Detected at 230nm.
They were powdered by a grinder (Wonder Blender, Osaka Chemical Co., Ltd., Osaka, Japan) and also used in wood creosote pills to examine the dissolution profiles of guaiacol. Voucher specimens of all the herbal drugs and their powders were deposited in the Research Institute of Taiko Pharmaceutical Co., Ltd.

**Chemicals** Glycerin used as a binder was purchased from Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan). Potato starch used as a diluent was obtained from Kanto Pharmaceutical Co., Ltd., Osaka, Japan. Authentic compounds such as guaiacol from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and berberine chloride, glycyrrhizin, and catechin from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) were used. Nobiletin and tangeretin were kindly supplied by Kampo & Healthcare Research Labs, Kanebo Ltd. All other chemicals and solvents were of analytical and/or HPLC grade.

**HPLC-Profile of Chinpi Analogues** Chemical constituents of Chinpi analogues were examined to estimate their botanical origin by referring to HPLC-profiles. Briefly, the 70% EtOH soluble portion of Chinpi powder (1 g/25 ml of 70% EtOH) was filtered through a 0.45 \( \mu m \) membrane filter, and the guaiacol content of the filtrate was determined by HPLC (LC-10AT, Shimadzu Co., Kyoto, Japan) under the conditions described in the legend to Fig. 2. Experiments were performed in six times and the dissolution rate (%) of guaiacol was determined by comparing the content of the sample solution with that of the pill tested.

The dissolution rate constant \( k_d \) and the maximum plateau \( \left( Y_{max} \right) \) of guaiacol were calculated up to 300 min after the beginning of the test using a non-linear least squares regression program (GraphPad PRISM). MDT was defined as the first moment of the dissolution rate–time curves of guaiacol and was calculated by trapezoidal integration using a computer program (Maikou Ni Yoru Yakubutsu Sokudoron Nyumon; Nanzando, Tokyo, Japan).

**Disintegration of Pills** Disintegration time (min) was determined using the disintegration test described in JP XIV.

**Statistics** Results were presented as the mean±S.D. and compared using Student’s \( t \)-test. Probability \( (p) \) values less than 0.05 were considered significant.

**RESULTS AND DISCUSSION**

**Dissolution Profiles of Guaiacol (Figs. 1, 2)** As shown in Fig. 1, the MDT of guaiacol from P0 containing only wood creosote was significantly \( (p<0.05) \) shorter than that of P4. Furthermore, of the four variant pills each without one herbal drug, P4-CUP2 and P4-GLR had a significantly \( (p<0.05) \) shorter MDT than the original P4. On the other hand, the MDT value of P4-GAM had a significantly \( (p<0.05) \) shorter MDT than the original P4. Consequently, it was estimated that the Chinpi from China, CUP1 and CUP2, and creosote pills to examine the dissolution profiles of guaiacol.

**Preparation of Wood Creosote Pills** Formulations of various wood creosote pills are listed in Table 2. To a mixture of four herbal drug powders, GAM (4 g), PHC (6 g), GLR (3 g) and CUP2 (6 g), were added glycerin (2 g), water (11 ml) and wood creosote (8 g) in this order. The mixture was kneaded in a porcelain mortar for 5 min and then made into pills (about 222 mg each) by hand.

**Dissolution of Guaiacol from Pills** The dissolution rate of guaiacol from the different formulations of pills was essentially determined as described in JP XIV using the paddle apparatus (Dissolution Tester NTR-6100, Toyama Sangyo Co., Ltd., Toyama, Japan). Briefly, each of 6 pills was placed in 900 ml of dissolution medium (No. 1 solution) at 37±0.5 °C and stirred with the paddle rotating at 100 rpm. At 1, 2, 3, 5, 7 and 9 h, 1 ml of sample solution was withdrawn and an equivalent volume of fresh medium was added to maintain the volume of the dissolution medium. The solution was filtered through a 0.45 \( \mu m \) membrane filter and the guaiacol content of the filtrate was determined by HPLC (LC-10AT, Shimadzu) as described in the legend to Fig. 2. Experiments were performed in six times and the dissolution rate (%) of guaiacol was determined by comparing the content of the sample solution with that of the pill tested.

The dissolution rate constant \( k_d \) and the maximum plateau \( (Y_{max}) \) of guaiacol were calculated up to 300 min after the beginning of the test using a non-linear least squares regression program (GraphPad PRISM). MDT was defined as the first moment of the dissolution rate–time curves of guaiacol and was calculated by trapezoidal integration using a computer program (Maikou Ni Yoru Yakubutsu Sokudoron Nyumon; Nanzando, Tokyo, Japan).

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**Disintegration of Pills (Fig. 3, Table 3)** The disintegration times of P0 (5±1 min) and P4-CUP2 (7±2 min) were significantly \( (p<0.05) \) shorter than those of P4 (47±5 min) and the three variants, P4-GAM, P4-PHC and P4-GLR (Fig. 3: left side). The graphs of the disintegration times of pills and MDT of guaiacol are similar in appearance. As shown in Fig. 3 (right), a correlation exists between the two. Two other
dissolution parameters, $k_d$ and $Y_{\text{max}}$, of wood creosote pills are listed in Table 3. The change in the maximum plateau of guaiacol ($Y_{\text{max}}$) in P0, P4 and the four variant forms of P4 was similar to that of MDT of guaiacol. $Y_{\text{max}}$ values were significantly ($p<0.05$) greater for P4-GLR and P4-CUP2 than P4. These findings suggest that the herbal drugs affected both the dissolution rates and the disintegration times of the pills and that CUP2 delayed the dissolution of guaiacol by lengthening the disintegration times of wood creosote pills.

**Effects of Chinpi on Dissolution Profiles of Guaiacol** (Fig. 4, Table 4) Next, effects of the quality of Chinpi on the MDT of guaiacol and disintegration times of pills were examined. The botanical origin of Chinpi was estimated by preparing Citrus unshiu cultivated in Japan, and in another paper. The dominant three flavonoids in CUPJ1 were nobiletin (peak a), 3,5,6,7,8,3',4'-heptamethoxyflavone (peak b) and tangeretin (peak c). The profile of CUPJ1 is similar to the profiles of CUP1, CUP2 and peels of C. unshiu described in a previous report. Unlike in CUPJ1, peak b was observed in only trace amounts in old Chinpi from China (CRP8 and CRP35). On referring to the reported profile, this observation suggested that the origin of CRP8 and CRP35 is C. reticulata. Although yields of EtOH extract were lower for old CRP8 and CRP35 than for CUP1, CUP2 and CUPJ1, the proportion of peaks a and c was markedly lower for old Chinpi than for new Chinpi.
Table 4. Effects of Various CRP and CUP on MDT of Guaiacol from Pills

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<tr>
<th>Quality of CRP and CUP</th>
<th>Dissolution parameter</th>
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<tr>
<td></td>
<td>MDT of guaiacol (min)</td>
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<td></td>
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<tr>
<td>under 75</td>
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<td>75—150</td>
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<td>over 150 (μm)</td>
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<td>EtOH extract yield (%)</td>
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Each value represents the mean±S.D. (n=6). Particle size distributions were examined using the No. 100 (150 μm) and No. 200 (75 μm) screen of JP XIV. P4: wood creosote pills using three herbal drugs and CUP2. P4 (CUP2re) and P4 (CUP1re): wood creosote pills using three herbal drugs and residual powders of CUP2 and CUP1 after EtOH extraction. Exp. 2 used powders of CUP1A (particle size: under 75 μm) and Exp. 3 used powders of CUP1B (particle size: 75—150 μm) P4 (CUP2j1): wood creosote pills using three herbal drugs and CUPJ1 (from Japan) P4 (CUP2op) and P4 (CUP2ip): wood creosote pills using three herbal drugs and CUP2op (outer peel of CUP2) and CUP2ip (inner peel of CUP2) P4 (CRP8) and P4 (CRP35): wood creosote pills using three herbal drugs and CRP8 (preserved for 8 years) and CRP35 (preserved for 35 years). a): p<0.05 vs. P4, b): p<0.05 vs. P4 (CUP1A), c): p<0.05 vs. P4 (CUP1B), d): p<0.05 vs. P4 (CUP2op).

The long MDT of guaiacol is considered one of the most important factors affecting the duration of efficacy after oral administration of wood creosote pills. To examine the contribution of the EtOH soluble portion and residual powders of Chinpi to the MDT of guaiacol, five experiments were carried out as shown in Table 4. In Exp. 1, the MDT of P4 was significantly shorter (p<0.05) than that of P4 (CUP2re). CUP2re is the residual powder remaining after EtOH extraction of CUP2. Since similar results were obtained in Exp. 2 and 3, it became clear that a negative correlation exists between the MDT of guaiacol from pills and EtOH extract yields of CUP. The regression equation is \( y = -3.39x + 277 \) \( r=0.79, y: \text{MDT (min)} \) and \( x: \text{EtOH extract yields (μg)} \).

Furthermore, influences of the outer (CUP2op) and inner (CUP2ip) peel of CUP2 on the MDT of guaiacol from pills were examined (Exp. 5 in Table 4). Orange-red CUP2op and white CUP2ip are equivalent to Ki-kko and Ki-ppaku in Japanese, respectively. The MDT of guaiacol from P4 (CUP2op) was significantly shorter (p<0.05) than that from P4 (CUP2ip). It became clear that the inner peel (CUP2ip), which has less EtOH soluble portion than CUP2op, has the desirable effect of prolonging the dissolution of guaiacol from wood creosote pills. Furthermore a longer MDT of guaiacol from P4 (CRP35) than P4 (CRP8) was observed as shown in Exp. 6.

The results obtained from Exp. 1—5 clearly indicated that Chinpi with a smaller amount of EtOH extract delayed the MDT of guaiacol from pills. However, the EtOH soluble portion of Chinpi may contribute to the anti-diarrhea effects of wood creosote pills, because it contains nobiletin possessing an inhibitory effect on barium sulphate transport in the small intestine of mice and providing protection to the gastric mucosal barrier. The proportion of nobiletin was markedly greater in the old Chinpi (CRP8 and CRP35) than other Chinpi preparations (Fig. 4, Table 4). The yields of EtOH extract decreased as the storage period increased. It is advisable to use Chinpi, which does not contain a large EtOH soluble portion. This corresponds to the thought in traditional Chinese medicine and pharmacy that pericarpium of Citrus fruits stored for a long time is suitable for Chinpi.

In summary, this report deal with effects of four herbal drugs prescribed in wood creosote pills on the in vitro dissolution profiles (MDT, \( k_b \) and \( Y_{\text{max}} \)) of guaiacol. The long MDT of guaiacol is considered an important factor affecting the duration of efficacy after oral administration of wood creosote pills. The herbal drug prescribed, especially Chinpi, might influence the sustainment of guaiacol release from the pills. Although a Chinpi preparation with less EtOH extract was found to delay the MDT of guaiacol, the EtOH soluble portion of Chinpi containing nobiletin may contribute to the anti-diarrhea effects of wood creosote pills. Therefore, Chinpi prescribed in wood creosote pills should be chosen to conform to suitable yields of the EtOH soluble portion and nobiletin concentration. The present findings are considered proof that Chinpi has been prescribed in traditional as well as new formulations of wood creosote.

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REFERENCES AND NOTES

4) Although the name of Chinpi is defined as Aurantii Nobilis Pericarpium in JP XIV, it was written as Citri Unshiu Pericarpium (CUP) or Citri Reticulatae Pericarpium (CRP) in this paper after its botanical origin was taken into consideration. It is named as Pericarpium Citri Reticulatae in the Chinese Pharmacopoeia 2000.