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Differences among Decoction Methods in the Composition of Alkaloids in Decoctions Produced from Sen-uzu, *Aconitum* Root

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Differences between the decoction methods amounts of alkaloids in decoctions from *Aconitum* root was investigated in the differing decoction apparatus (electric decoction unit, enameled pot or an earthen pot conditions), filtrations and temperatures (25, 40 or 97°C). Aconitine-type alkaloids (aconitine, mesaconitine and hyaconitine) and total alkaloids were measured by HPLC and titration, respectively. The filtrations and temperatures conditions did not affect the amount of aconitine-type and total alkaloids. The amount of mesaconitine in the decoction with an enameled pot was significantly lower than in the other apparatuses, but the amount of total alkaloids was the same as in the other apparatus. It is recommended producing decoction be done with an enameled pot followed by immediate filtration from the standpoint of decreasing the toxicity of *Aconitum* root which constitutes several Chinese medicines.

Keywords—*Aconitum* root, Sen-uzu, decoction, aconitine-type alkaloid, aconitine, mesaconitine, hyaconitine

Introduction

Decoctions of Chinese traditional medicines and domestic herbs are widely used in therapy of various diseases in Japan. Nevertheless, there have been only a few reports of study on chemical changes of herbal constituents in the decoctions. Chikazawa et al. investigated the contents of total alkaloids in the decoctions of different Chinese medicines containing either of the *Aconitum* roots preparations, "Shujibushi" and "Uzu"¹⁾. But in fact the decoction methods including apparatus, temperature and boiling time for prescribed Chinese medicines are not often directed to the recipients with experimental grounds.

The *Aconitum* raw roots, "Sen-uzu", is a dried preparation of *Aconitum carmichaeli* DEBX. roots and used as a crude drug which constitutes some of Chinese medicines such as "Uzu-to", "Dai-uzu-sen" and "Sho-katuraku-tan". The crude drug has been found to contain various pharmacologically active alkaloids, especially toxic ones of aconitine type, aconitine, mesaconitine and hyaconitine (the LD₅₀ i.p. in mice is 0.31, 0.21 and 1.10 mg/kg, respectively²⁾). Therefore, release and decomposition of these alkaloids in the process of decoction of the *Aconitum* preparations are crucial for both the

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effectiveness and toxicity of them.

We previously reported that 22 of 100 subjects that had received Chinese herbal medicines claimed any of adverse effects³⁾. Reasonable directions of decoction methods should be attached to particular medicines for promotion of patient care. The present experimental results show the essential role of the type of decoction apparatus according to the composition of effective alkaloids for the decoction of the *Aconitum* roots.

Materials and Methods

Materials:

The roots of *Aconitum carmichaeli* DEBX. (Aconiti Tuber), Sen-uzu (川烏頭) (Sichuan Prov., 四川省; China) was provided by Matsuura Yakugyo-Co., Ltd. (Nagoya, Japan). Roots were chopped into small cubes of about 5 mm across before decoction. A Sep-Pak C18 column was purchased from Waters Co., Ltd. (Tokyo, Japan). All chemicals used were of the highest grade available.

Decoction methods:

Three types of decoction pot, an electric decoction unit (Torobi IK-230, 1000 ml, Tochimoto Tenkaido Co. Ltd., Osaka, Japan), an earthen teapot ("Dobin", 140 mm in the maximum diameter, 1000 ml), and an enameled teapot ("Horo" pot, 128 mm in diameter, 1000 ml) were used for decoction. An electric decoction unit was installed with a 100 watts heater. Enameled and earthen teapots were directly set on LP gas cookers, uncovered, and warmed with fixed, moderate flame enough to boil the contents in 10 min. The decoction unit was used with a cover as specified. A tea filter (40 mesh) made of stainless steel or a tea bag (about 150 mesh) made of fibers was used for filtration after decoction as specified in the results. Decoction of the roots was usually continued for 60 min, started at 25°C, and terminated by filtration with a tea filter unless otherwise specified. One g of the dried raw roots was added to 100 ml of distilled water in each of an electric decoction unit and to 300 ml or water in each of an enameled or an earthen pot to make decoction sample. The sample volume was adjusted to 100 ml with distilled water after filtration.

Extraction of alkaloids:

Each decoction sample (100 ml in final volume) was shaken with a mixture of 5 ml of 5% NH_4OH and 50 ml of diethylether for 10 min. The combined diethylether fraction obtained after three repeated extraction was evaporated to dryness at 40°C with a rotary evaporator. The residue was shaken three times in dilute HCl (each time with 20 ml of acid for 10 min). To the combined solution, 25 ml of 5% NH_4OH and 30 ml of CHCl_3 were added and the mixture was shaken for 10 min. To the combined CHCl_3 fraction obtained after three repeated extraction, 10 g of nonhydrated Na_2SO_4 was added. CHCl_3 was allowed to evaporate after 10 min of mixing, leaving residue A.

Assay of total alkaloids in decoction:

Mixture of 5 ml of ethanol, 30 ml of distilled water, 5 drops of methyl red solution and a sample of residue A was titrated with 0.01 N HCl. Total alkaloids were expressed as the amount of benzoylaconine, assuming that 6.038 mg of this alkaloid is neutralized by 1.000 ml of 0.01 N HCl⁴⁾.

Assay of aconitine-type alkaloids:

Mixture of 15 ml of 0.001 N HCl and a sample of residue A was separated on a Sep-Pak C18 column with elution by 10 ml of methanol. After evaporation of methanol, the eluate was mixed with 2 ml of acetonitrile and filtered to be subjected to HPLC. Aconitine, hypaconitine and mesaconitine were analysed by an HPLC apparatus (Yanaco L-5000, Tokyo, Japan) connected with a UV detector (Yanaco m-315, Tokyo, Japan) and a data processor (Shimadzu C-R6A, Kyoto, Japan) by the method of Kitagawa et al.⁵⁾ A reversed phase column (Nucleosil 5C18, 4.6 × 250 mm) was applied with mobile phase consisting of 0.05 M KH₂PO₄ (pH2.35), tetrahydrofuran and CH₃CN (41:7:1, v/v) at a flow rate of 0.8 ml/min and UV at 235 nm.

Content of alkaloids in the roots:

One g of the raw *Aconitum* roots was milled by a mixer (Shibata, Tokyo, Japan) and shaken with 100 ml of methanol for 20 min. After the methanol was evaporated, each sample was subjected to assay of total or aconitine-type alkaloids as described above on extraction with diethylether in alkaline solution.

Statistical analysis:

Results were expressed as means ± SD of repeated experiments and statistical significance of the difference of means was evaluated by one-way analysis of variance (1-way ANOVA) procedure as the method of Scheffe⁶⁾. The least significant difference test was used as a multiple-comparison method to compare data among groups, and considered statistically significant when $p < 0.05$.

Results**Total and aconitine-type alkaloids in the decoction with an electric decoction unit:**

When decoction of the *Aconitum* roots was carried out with an electric decoction unit, the amount of total alkaloids in the decoction increased until about 50 min after start of heating showing rapid (less than initial 20 min) and slow phases of the time course (Fig. 1). The content of total alkaloids in the methanol extract was estimated at 7.71 mg for 1 g of dried raw roots. Accordingly the amount of total alkaloids in the decoction in 60 min with an electric unit, 4.63 mg, was approximately 60% of the content. The contents of three aconitine-type alkaloids, aconitine, mesaconitine and hypaconitine, in the methanol extract were 5.60 ± 0.59 , 82.81 ± 7.92 and $5.40 \pm 0.69 \mu\text{g}$ for 1 g of roots, respectively. The amounts of these three alkaloids in the decoction in 60 min with an electric unit were 3.39 ± 0.31 (60.54% of its content in the methanol extract), 51.35 ± 4.38 (62.01%) and 3.36 ± 0.74 (62.22%) μg for 1 g of roots, respectively.

All of the total and aconitine-type alkaloids in the decoction were unchanged in concentrations for 24 h when it was placed at room temperature after 60 min of decoction.

Total and aconitine-type alkaloids in the decoctions with different types of decoction apparatus:

The amount of total alkaloids in the decoction was not significantly different between the types of apparatus (Fig. 2). The amount of mesaconitine in the decoction, however, was significantly lower

Fig. 1. Time Course of the Change in Total Alkaloids (%) in the Decoction of the *Aconitum* root with the Electric Decoction Unit.

Each value represents percent of the amount of total alkaloids in the decoction at each time indicated to that in the methanol extract as the mean $\pm$ S.D. of 3 experiments. See the Materials and Methods for other experimental conditions.

Fig. 2. Amounts of Aconitine-type and Total Alkaloids in the Decoctions with Different Types of Decoction Apparatus.

Filtration was done with the tea filter immediately after 60 min of decoction. Values are the means $\pm$ SD of 3-6 experiments. * p < 0.05, ** p < 0.01 (one-way ANOVA by Scheffe's method).

Fig. 3. Amounts of Aconitine-type and Total Alkaloids in the Decoctions with the Electric Decoction unit and Different Filters Indicated.

a) Filtration was done immediately after 60 min of decoction. b) Filtration was done 1 h after 60 min of decoction. Values are the means $\pm$ SD of 3-6 experiments.

Fig. 4. Amounts of Aconitine-types and Total Alkaloids in the Decoctions with Different Temperatures at the Start of Decoction in the Electric Unit. Filtration was done with the tea filter immediately after 60 min of decoction. Values are the means $\pm$ SD of 3-6 experiments.

Fig. 5. Profiles of the Change of Water Temperature with the Three Types of Decoction Apparatus.

○, electric decoction unit; ■, enameled pot; □, earthen pot. Water was heated in the three types of apparatus to boiling as usual for 60 min. Values are the means of 3 experiments. The SD is not shown in each point since it is too small to indicate.

Fig. 6. Amounts of Aconitine-type and Total Alkaloids in the Decoctions with Different Boiling Temperatures in the Enameled Pot. Filtration was done with the tea filter immediately after 60 min of decoction. Values are the means $\pm$ SD of 3-6 experiments.

with an enameled pot than that with the other types of apparatus. And the amount of hyaconitine was significantly higher with an enameled pot than that with an earthen pot.

Total and aconitine-type alkaloids in the decoctions with different filtration conditions:

To consider influence of the fine powder of the *Aconitum* roots, a tea filter and a tea bag were used in the filtration conditions. There was found no significant difference in the amount of either total or aconitine-type alkaloids between the decoctions produced with the two types of filters and those with and without 1 h standing (without heating) before filtration, when commonly an electric decoction unit was used (Fig. 3). There was tendency to increase (not significant) in the amounts of aconitine and mesaconitine in the both decoctions produced with two types of filter when filtration was done 1 h after the end of decoction.

Effect of initial temperature at decoction on total and aconitine-type alkaloids in the decoctions:

All the total and aconitine-type alkaloids in the decoctions were not significantly different in their amounts with different temperatures from 25 to 97°C at start of decoction when an electric decoction unit was used (Fig. 4).

Time courses of change of temperature of water in different types of decoction apparatus:

It was found in model experiments that the rate of increase of the temperature of water was initially largest in an enameled pot (Fig. 5). After 10 min of heating the mean of temperatures determined at intervals of 5 min was very precisely 99, 98 and 92°C for an enameled pot, an earthen pot and an electric decoction unit, respectively.

Total and aconitine-type alkaloids in the decoctions with an enameled pot in different boiling temperatures:

Raising the boiling temperature (the state that a small bubble forms in the decoction and its temperature is stable) from a range, 96–97°C, to a higher range, 98–99°C, in an enameled pot by regulation of heater resulted in slight increases (not significant) in all of total and each aconitine-type alkaloids in the decoction (Fig. 6).

Discussion

It has been shown in the present experiments that decoction apparatus affects the amounts of aconitine-type alkaloids in the decoction of the *Aconitum* roots. Both filtration conditions and temperatures were apparently unimportant for the extents of decoction for 60 min in terms of the contents of total and aconitine-type alkaloids. But the amount of mesaconitine was significantly decreased in the decoction for 60 min with an enameled pot against that in the decoctions with an electric decoction unit and an earthen pot. The amount of hypaconitine was contrarily highest in the decoction with an enameled pot (significantly increased against that with an earthen pot). These results may be attributed to the temperature during decoction maintained at higher degrees and/or to the heat conduction of the apparatus. However, the amount of mesaconitine in the decoction with an enameled pot could not be explained in terms of the difference of temperature during decoction. The amount of aconitine-type alkaloids in the decoction should be estimated from the changes of release and decomposition of the alkaloids. Mesaconitine may be decomposed by heat when an enameled pot is used, because the temperature in the decoction fluid rapidly increased to 99°C. Hikino et al. have reported that mesaconitine is decomposed rapidly at 100°C²⁾. Decomposition of aconitine-type alkaloids may be explained on the basis of fact that was not observed significant decreasing of total alkaloids with an enameled pot compared with other types of apparatus. But the amount of aconitine-type alkaloids is a small fraction of the total alkaloids, and the decomposition of mesaconitine is uncertain in this experiment. We considered that the decoction of mesaconitine might affect by differences of the heat conduction of apparatus and unknown factor(s).

The aconitine-type alkaloids and non-aconitine-type alkaloids such as hygenamine^{7,8)} and coryneine⁹⁾ are confirmed as active components. The components have been demonstrated to exert several pharmacological activities in animals, affecting their respiration, ileum, heart rate, circulation and behavior⁵⁾. According to detailed study of the aconitine-type alkaloids using animal tissues *in vitro*, the effect of mesaconitine and hypaconitine on the heart is biphasic: temporary contraction followed by its inhibition¹⁰⁾. The LD₅₀ values of the aconitine-type alkaloids were very low. The aconitine-type alkaloids are the effective and toxic components of *Aconitum* roots, so differences in their decoction may cause adequate therapeutic effect to be unsatisfactory or given rise to adverse effects. Chikazawa et al. reported that the amounts of aconitine-type alkaloids were decreased by processing the raw roots with boiling under pressure⁴⁾. Decrease of the aconitine-type alkaloids in the decoctions of the *Aconitum*-containing medicines may be preferable from the standpoint of reduction of toxicity of them.

In these studies, we found the suitable decoction method from *Aconitum* roots, which decreases the amount of aconitine-type alkaloids and maintain the amount of total alkaloids. When patients are prescribed a Chinese medicine that contains *Aconitum* roots, directions about preparation should specify that an enameled pot should be used, decoction should be for 60 min, and filtering should be done within 1 hour after decoction is ended. In a similar way, for each Chinese medicine, if adverse effects are to be prevented as much as possible and if therapeutic effects are to be suitable, decoction conditions should be established, and patients should be provided with these heeded details about preparation.

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