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Pharmaceutical Studies on the Medicinal Prescription Used in Traditional Sino-Japanese Medicine —Changes of the Components in Keishibukuryo-gan Prepared in the Hospital Pharmacy—

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The time courses of the amounts of the reference substances in the pills called Keishibukuryo-gan (Gui-Zhi-Fu-Ling-Wan) were determined. The pills were prepared from powdered crude drugs and honey in our hospital according to the specifications of the classical Chinese medicine, and were kept at 4°C or room temperature (*ca.* 25°C). Six compounds, namely cinnamic aldehyde, paeonol, paeoniflorin, amygdalin, prunasin and benzaldehyde, were used as reference substances. After 2 weeks, 1 month, 3 months or 6 montns, quantitative analysis of their contents in the pills was carried out by high performance liquid chromatography.

The amounts of cinnamic aldehyde, paeonol and paeoniflorin decreased after the preparation. When the pills were kept at room temperature, their amygdalin content decreased rapidly, while their benzaldehyde content increased. However, when the pills were prepared freshly from the powder which had been kept at 4°C for 6 months, the amounts of the reference substances in those pills differed only slightly from those of the pills just prepared at 0 time. As for the prunasin content, the changes were negligible or none.

Keywords——cinnamic aldehyde; paeonol; paeoniflorin; amygdalin; prunasin; benzaldehyde; "Keishibukuryo-gan"

Keishibukuryo-gan (Gui-Zhi-Fu-Ling-Wan)[1] was first described in the text book "Chin Kuei Yao Lueh" (200 A.D.)[2] and is known as one of the most important "Kampoh (traditional Sino-Japanese medicine)" prescriptions. Keishibukuryo-gan (KB) contains five kinds of crude drugs: Keishi (Cinnamomi Cortex), Bukuryo (Hoelen), Botanpi (Moutan Cortex), Tohnin (Persicae Semen), and Syakuyaku (Paeoniae Radix). In Japan today, KB is normally used in two forms, *i.e.*, decoction (KB-D) prepared from crude drugs and water[3] and, a granulated extract (KB-E) prepared in a similar manner to instant coffee. However, originally KB was to be prepared in the form of pills (KB-P): according to the instructions in the Chinese medicinal classics,[2] these five ingredients are to be powdered, then mixed with honey as a binder and made into pills.[4]

In the previous papers,[5,6] KB-P, which had been prepared in our hospital according to the original method, and KB-D were comparatively studied with reference to their chemical components and clinical effects on peripheral microcirculation. We revealed that the amounts of the chemical components and the thin layer chromatographic profiles of the two preparations were quite different from each other. Furthermore, the temperature of the palms apparently increased after KB-P administration. Thereafter, we have used KB-P, as well as KB-D and KB-E, in daily practice with Kampoh medicine.

The present study was undertaken in an attempt to reveal the changes of the amounts of several chemical components in KB-P after the preparation.

Results and Discussion

KB contains five kinds of crude drugs, and each crude drug has been known to contain a number of chemical components. In this study, six compounds, namely cinnamic aldehyde, paeonol, paeoniflorin, amygdalin, prunasin and benzaldehyde were used as reference substances. A quantitative analysis was carried out by high performance liquid chromatography (HPLC), and the measurement conditions are listed in TABLE I.

The amounts of cinnamic aldehyde, paeonol, paeoniflorin and benzaldehyde were calculated by using the previously prepared calibration curves of the respective authentic samples and an internal standard. The amygdalin and prunasin contents were calculated by using the calibration curves. Each measurement was performed three times. The recovery of each substance was above 96%.

TABLE II shows the results of the quantitative analysis of these reference substances in the crude drugs used in this experiment. The pills (KB-P) were prepared as described in "Experimental." Each pill (*ca.* 2.0 g per pill) was accurately weighed at the time of preparation, and then wrapped with polyethylene film. Then, the pills were divided into two groups, one being stored at 4°C and the other at room temperature (*ca.* 25°C). Quantitative analysis was performed at the time of preparation (0 time) and after two weeks, one month, three months, and six months.

Figure 1 shows the time courses of the reference substances after the preparation. Cinnamic aldehyde, which is a volatile substance, was shown to gradually decrease after the preparation. This change was greater in KB-P stored at room temperature. The paeonol and paeoniflorin contents decreased after the preparation, but there was no significant differences between those kept at 4°C and at room temperature. On the other hand, the amygdalin content in KB-P decreased rapidly when stored at room temperature, but very slowly when stored at 4°C. However, after six months, the amygdalin contents in these pills differed only slightly from each other. Prunasin was detected only as a trace.

It has been reported[7,8] that amygdalin in Persicae Semen was rapidly hydrolyzed to yield a stoichio-

TABLE I. Quantitative Analysis Conditions of High Performance Liquid Chromatography

Condition	Analysed component	Mobile phase	Detection wave length
A	Amygdalin Prunasin	CH_3CN–H_2O (1:6)	220 nm
B	Benzaldehyde Cinnamic aldehyde Paeonol	CH_3OH–H_2O (35:65)	260 nm
C	Paeoniflorin	CH_3OH–H_2O (35:65)	254 nm

Apparatus: Shimadzu LC-5A (Detector SPD-2A), Column: TSK ODS-120 T, Flow rate: 1.0 ml/min, Column temp.: 40°C

TABLE II. Amounts of Cinnamic Aldehyde, Paeonol, Paeoniflorin, Amygdalin, Prunasin and Benzaldehyde in Crude Drugs Used in this Experiment

Crude drug	Compound	Contents mean±S.D.
Cinnamomi Cortex	Cinnamic aldehyde	18.76±0.31[a]
Moutan Cortex	Paeonol	18.18±1.23
	Paeoniflorin	7.36±0.82
Paeoniae Radix	Paeoniflorin	33.32±0.94
Persicae Semen	Amygdalin	42.00±1.02
	Prunasin	N.D.[b]
	Benzaldehyde	N.D.

[a] mg/g crude drug, [b] not detected.

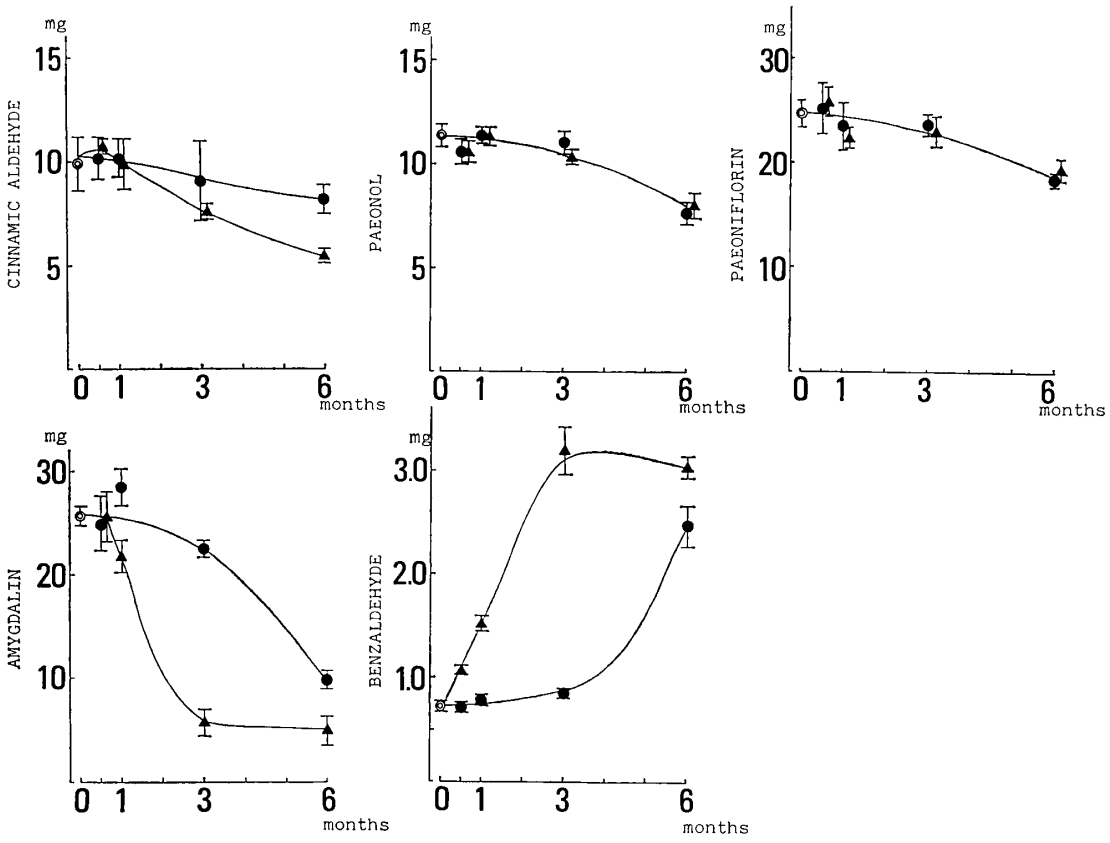

Fig. 1. Time Courses of Reference Substances in Keishibukuryo-gan (mg per 6 g pills, daily dose)

Bars represent standard deviation from the mean ($n=3$). ◎: 0 time value, ●: kept at 4°C, ▲: kept at room temperature.

TABLE III. Comparison of Amounts of Cinnamic Aldehyde, Paeonol, Paeoniflorin, Amygdalin, Prunasin and Benzaldehyde in Different Preservations of Keishibukuryo-gan (mg per 6 g pills, daily dose).

	Storage form/condition		Cinnamic aldehyde	Paeonol	Paeoniflorin	Amygdalin	Prunasin	Benzaldehyde
0 time	—	—	9.89	11.38	24.70	25.79	±	0.67
6 months	pill	4°C	8.17	7.70	18.56	9.89	±	2.49
6 months	pill	R.T.	5.45	8.05	19.63	5.03	±	3.05
6 months[a)]	powder	4°C	10.82	10.08	24.67	24.48	±	0.62

a) The powder of Keishibukuryo-gan which had been kept at 4°C for 6 months, was mixed with honey as binder to make the pills of Keishibukuryo-gan just before the quantitative analysis.

metric amount of benzaldehyde. In the present study, we observed that amygdalin in KB-P changed to benzaldehyde in the same manner. Akahori *et al.*[8] pointed out that the amygdalin in KB-P was hydrolyzed by water contained in the honey used as a binder. Therefore, we compared the amounts of the reference substances remaining in the KB-P kept for six months both at room temperature and at 4°C with those in the crude drug powder stored at 4°C. The mixed powder of KB was sealed in a bottle, stored at 4°C and six months later, KB pills were freshly prepared with this powder. As shown in TABLE III, the amount of each reference substance of the freshly made KB-P differed only slightly from that of KB-P at 0 time.

Unlike synthetic drugs, crude drugs used in Kampoh prescriptions cannot be characterized strictly by their physical properties. Although pharmacological activities of cinnamic aldehyde,[9] paeonol,[10] paeoniflorin,[11] and amygdalin[12] have been studied, it is difficult to correlate their activities directly with the effectiveness of the well known empirical properties of KB. Other compounds existing in KB-P, *e.g.*, sugars, fatty acids, tannins, *etc.*, may also take part in its pharmacological activities. Moreover,

the chemical constituents of crude drugs can vary according to such factors as the place of production, the season of harvest and the processing procedures employed. So, it is difficult to maintain a fixed quality in the ingredients.

However, it should be possible to evaluate Kampoh prescriptions chemically and pharmacologically to certain extent if well-defined materials are used and well-controlled procedures are employed in the preparation of the medications.

Therefore, in order to minimize the quantitative changes of the components in KB-P, in our hospital we prepare KB-P as follows: the mixed powder of KB is sealed in a bottle and stored at 4°C, and fresh pills are made as required.

Experimental

Materials and methods

Crude drugs——The crude drugs used in this experiment were purchased from Uchida Wakan-Yaku (Tokyo, Japan): Keishi (Cinnamomi Cortex: China, lot 072108), Bukuryo (Hoelen: China, lot 072310), Botanpi (Moutan Cortex: Nara, lot 072213), Tohnin (Persicae Semen: China, lot 162516), Syakuyaku (Paeoniae Radix: Nara, lot 072220) and honey (China).

Preparation of the pills of Keishibukuryo-gan——Keishi, Bukuryo, Botanpi and Syakuyaku were ground to fine powder (200 mesh) with a sample mill K II-1 (Fuji Powder Co., Ltd.). Tohnin was stripped of the seed coat, roasted, and then ground to fine powder. These powdered crude drugs (200 g each) were thoroughly mixed in a mortar with a pestle (total 1 kg). Honey (1 kg) was heated and boiled for 3 min. The above mixed powder was then added to the hot honey and kneaded to make pills (2.0 g each).

Reagents——Cinnamic aldehyde was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), amygdalin and prunasin from Sigma Chemical Co. (St. Louis, MO., USA) and benzaldehyde from Nakarai Chemicals, Ltd. (Kyoto, Japan). Paeoniflorin and paeonol were kindly provided by Dr. O. Tanaka (Hiroshima Univ.), *p*-Hydroxybenzoic acid methyl ester, as an internal standard, was purchased from Nakarai Chemicals. Other reagents were purchased from Wako Pure Chemicals Co., Ltd. (Tokyo, Japan).

Quantitative determinations of cinnamic aldehyde, paeonol, paeoniflorin, amygdalin, prunasin and benzaldehyde

Extraction procedure[8,13]——KB-P (2.0 g) was treated with 50%-ethanol (30 ml) at 40°C for 30 min, then agitated for 15 min, and centrifuged (1100×*g* for 10 min) to give an ethanolic extract of KB-P. The residue was extracted with 50%-ethanol (15 ml) again, and the combined ethanolic extract was adjusted exactly to 50 ml with 50%-ethanol. An aliquot of the extraction was filtered through a membrane filter (0.45 μm, Toyo Roshi Co., Ltd., Tokyo, Japan) and to 0.5 ml of the filtrate was added 0.5 ml of *p*-hydroxybenzoic acid methyl ester solution (0.013 mg/ml) as an internal standard for HPLC. This mixture was used for the quantitative analysis.

Crude drug extract was prepared by treating each crude drug (1.0 g sample) in the same way as KB-P. In order to optimize the chromatographic separation, the ethanolic extract was diluted with 50%-ethanol as required.

Apparatus——A Shimadzu LC-5A liquid chromatograph equipped with a spectrophotometric detector SPD-2A (Shimadzu) and an integrator C-R1B (Shimadzu) was used for the HPLC analysis. Column: TSK ODS-120 T (4.6 mmø×25 cm), Tosoh Co., Ltd.

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References and Notes

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3) The procedure used for the preparation in our hospital is as follows: five ingredients, Cinnamomi Cortex (4.0 g), Hoelen (4.0 g), Moutan Cortex (4.0 g), Persicae Semen (4.0 g) and Paeoniae Radix (4.0 g), are mixed with 600 ml of water, and the whole is boiled until the volume is reduced to 300 ml. The liquid is strained and a dose of 100 ml is taken an hour before each meal.
4) The instructions given in "Chin Kuei Yao Leuh" are as follows: the ingredients are powdered, kneaded with

honey, and made into pills of the size of rabbit pellets. One pill is to be taken before each meal. If no effect is observed, the dosage is to be increased to three pills.

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