J. Pharm. Pharmacol. 2000, 52: 1425–1429
Received January 11, 2000
Accepted March 23, 2000

The Anxiolytic Effect of Two Oriental Herbal Drugs in Japan Attributed to Honokiol from Magnolia Bark

HISASHI KURIBARA, EIKO KISHI, NAOKO HATTORI*, MINORU OKADA* AND YUJI MARUYAMA

*Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi-shi, Gunma 371-8511 and *Central Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan*

Abstract

An improved elevated plus-maze test in mice revealed that seven daily treatments with two differnt traditional Chinese medicines, known as Kampo medicines in Japan, Hange-koboku-to (composed of extracts of 5 plants) and Saiboku-to (composed of extracts of 10 plants), produced an anxiolytic effect, and the effect was mainly due to the presence of honokiol derived from magnolia. This study was carried out to evaluate the anxiolytic potential of honokiol, Hange-koboku-to and Saiboku-to, which were prescribed with two different magnolia samples: Kara-koboku (*Magnoliae officinalis*) (KA) or Wa-koboku (*Magnoliae obovata*) (WA).

The doses of test samples were adjusted to ensure a constant dose of honokiol at 0·2 mg kg^{-1}. Although the doses of magnolol (an isomer of honokiol), as well as those of undetermined chemicals, varied among samples, the seven daily treatments with 9 out of 10 test samples produced an anxiolytic effect almost equivalent to that produced by 0·2 mg kg^{-1} honokiol. The only exception was the sample containing the lowest amount of honokiol. Magnolia-free preparations of Hange-koboku-to or Saiboku-to did not have any anxiolytic effect.

These results confirm that honokiol derived from magnolia is the causal chemical of the anxiolytic effect of Hange-koboku-to and Saiboku-to.

It has been empirically suggested that the Kampo medicines, Hange-koboku-to (composed of extracts of 5 plants) and Saiboku-to (composed of extracts of 10 plants) (Table 1), have an anxiolytic effect as demonstrated by decreasing anxiety and nervous tension as well as improvement of sleep (Kinebuchi 1989; Murase et al 1989; Narita 1989, 1990). A behavioural study using an elevated plus-maze test in mice also revealed the anxiolytic effect of Hange-koboku-to and Saiboku-to (Kuribara & Maruyama 1996), and determined that honokiol derived from magnolia (Fujita et al 1973; Itokawa 1986) was responsible for the anxiolytic effect of Saiboku-to (Maruyama et al 1998; Maruyama & Kuribara 2000). Furthermore, our pilot study demonstrated that water extracts of three magnolia samples, two being Kara-koboku (*Magnoliae officinalis*, KA) and the other being Wa-koboku (*Magnoliae obovata*, WA), showed almost the same anxiolytic potential when the doses were adjusted for honokiol content (Kuribara et al 1999).

Since Kampo medicines are composed of specified mixtures of extracts of dried plant materials, there is a possibility that the chemicals interact to modify the individual pharmacological effects. However, as previously reported in Saiboku-to (Maruyama et al 1998) and magnolia bark (Kuribara et al 1999), if honokiol is the chemical selectively responsible for the anxiolytic effect of Kampo medicines prescribed with magnolia bark, they show almost the equivalent anxiolytic potential when the doses are adjusted for honokiol content.

To confirm this hypothesis, we evaluated the anxiolytic potential of 10 samples of Hange-koboku-to and Saiboku-to prescribed with various magnolia barks from which honokiol was derived. The effects of magnolia-free prescriptions of Hange-koboku-to and Saiboku-to were also assessed.

Correspondence: Y. Maruyama, Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi-shi, Gunma 371-8511, Japan.

Table 1. Composition of Hange-koboku-to and Saiboku-to preparations.

Plants	Content (g)	
	Hange-koboku-to	Saiboku-to
Pinelliae Tuber	6	5
Hoelen	5	5
Magnoliae Cortex	3	3
Perillae Herba	2	2
Zingiberis Rhizoma	1	1
Bupleuri Radix	7	
Scutellariae Radix	3	
Zizyphi Fructus	3	
Ginseng Radix	3	
Glycyrrhizae Radix	2	
*Dried extra/preparation	2·5/7·5	5·0/7·5

*The weight of dried extract from the mixture of the above plants

Materials and Methods

Animals

Male mice of the ddY strain (Japan SLC, Hamamatsu, Japan) were purchased at 6 weeks of age, and kept for 1 week in polycarbonate cages (width 20 cm, length 25 cm, height 15 cm) in groups of 10 with free access to a solid diet (MF: Oriental Yeast, Tokyo, Japan) and tap water. The temperature in the breeding room was $24 \pm 1^{\circ}C$; relative humidity, $55 \pm 3\%$; with a 14–10-h light–dark cycle, with lights on between 05 00–19 00 h.

All the behavioural tests were carried out between 1000 and 1400 h.

Elevated plus-maze test

The elevated plus-maze used in this study was a slight improvement on the original apparatus (Pellow et al 1985; Lister 1987). It has been previously described (Kuribara & Maruyama 1996; Kuribara et al 1999). Briefly, the closed arms with side walls (width 6 cm, length 30 cm, height 10 cm) and the centre platform (8×8 cm) were a non-transparent grey colour, whereas the open arms (width 6 cm, length 30 cm) were transparent. The plus-maze was set 40 cm above the base.

Each mouse was placed on the centre platform facing one of the closed arms randomly assigned, and the cumulative time spent in the open arms was recorded for 5 min. The criteria of the animal's entering into an open arm was crossing of the borderline separating the centre platform and the open arm with all four paws.

Activity test

To evaluate the non-specific effect of general activity on the plus-maze performance (Dawson et al 1995), the ambulatory activity of the mouse was measured for 5 min immediately after the plus-maze test with a tilting-type ambulometer equipped with a bucket-like Plexiglas activity cage 20 cm in diameter (SMA-1: O'hara & Co., Tokyo, Japan). The apparatus detected slight tilts of the activity cage generated by the mouse's ambulations (horizontal movements of comparatively longer distance).

Drugs

The drugs used were honokiol (Nacalai Tesque, Kyoto, Japan), 5 test samples of Hange-koboku-to (TJ-16) or Saiboku-to (TJ-96) and magnolia-free preparations of Hange-koboku-to and Saiboku-to. They were prepared using the same plants except for magnolia bark by Central Research Laboratories of Tsumura & Co. (Tokyo, Japan). Table 2 shows the contents of honokiol and magnolol in each sample determined using HPLC (LC-10, Shimadzu, Kyoto, Japan) with a column of TOSO TSK gel 80TM (250 mm × 4·6 mm diameter; TOSO Co., Tokyo, Japan). The mobile phase was water–methyl cyanate–acetic acid (40 : 60 : 1) at a flow rate of $1\ mL\ min^{-1}$. The column temperature was maintained at 40°C. Each sample (0·5 g) was suspended in 15 mL methanol for 30 min and centrifuged at $3000\ rev\ min^{-1}$ for 5 min. The insoluble material was suspended again in 15 mL methanol and centrifuged at $3000\ rev\ min^{-1}$ for 5 min. The supernatants obtained by the first and second methanol extractions were combined, and methanol was added to achieve 50 mL solution. Subsequently, the solution was filtered with a 0·45-μm filter to eliminate small particles, and a 10-μL sample was used for HPLC analysis.

Administration schedules

Honokiol was first dissolved in ethanol, and the resultant solution was diluted with a 10% aqueous solution of Tween-80. Subsequently, the solution was diluted with distilled water. The final concentration of both ethanol and Tween-80 in the vehicle was 0·5%. The samples of Hange-koboku-to and Saiboku-to, and their magnolia-free preparations, were suspended in the same vehicle as honokiol.

Our previous study demonstrated that a clear anxiolytic effect was produced by 7 daily treatments with $0{\cdot}2\ mg\ kg^{-1}$ honokiol whereas magnolol caused no anxiolytic effect (Kuribara et al 1998). The doses of Hange-koboku-to and Saiboku-to samples were adjusted to ensure a constant dose of honokiol ($0{\cdot}2\ mg\ kg^{-1}$). The doses of

Table 2. Contents of honokiol and magnolol in Hange-koboku-to (TJ-16) or Saiboku-to (TJ-96) preparations, and of magnolia-free preparations of TJ-16D and TJ-96D.

Samples	Kampo preparation (origin of magnolia)	Contents		Suspended (g/50 mL)[a]
		Honokiol	Magnolol	
TJ-16A	Wa-koboku (Gifu pref., Japan)	0·025	0·102	4·000
TJ-16B	Wa-koboku (Kouchi pref., Japan)	0·085	0·053	1·176
TJ-16C	Kara-koboku (Fujian prov., China)	0·013	0·052	7·692
TJ-16D	Kara-koboku (Sichuan prov., China)	0·106	0·083	0·943
TJ-16E	Kara-koboku (Yunnan prov., China)	0·138	0·137	0·725
TJ-96A	Wa-koboku (Gifu pref., Japan)	0·008	0·028	12·500
TJ-96B	Wa-koboku (Kouchi pref., Japan)	0·040	0·020	2·500
TJ-96C	Kara-koboku (Fujian prov., China)	0·007	0·025	14·286
TJ-96D	Kara-koboku (Sichuan prov., China)	0·055	0·036	1·818
TJ-96E	Kara-koboku (Yunnan prov., China)	0·086	0·077	1·163
TJ-16D (Free)		nd	nd	1·041
TJ-96D (Free)		nd	nd	1·640

nd: not detectable. [a]Weight of each test sample for making drug suspension.

magnolia-free prescriptions of Hange-koboku-to and Saiboku-to were estimated from the recovery rates of the test samples (TJ-16D, 13·5%; TJ-16D (Free), 18·1%; TJ-96D, 26·9%; and TJ-96D (Free), 27·5%). The actual calculated weight of test samples shown in Table 2 were suspended in 50 mL of vehicle, and each drug suspension was administered orally to mice at 10 mL kg^{-1} body-weight, once a day for 7 days. The behavioural tests were carried out on the day after the last (7th) administration.

Statistical analysis

The time spent by mice in the open arms in the plus-maze test and the activity count in the activity test were analysed by Student–Newman–Keuls test. Values of $P < 0{\cdot}05$ were considered significant.

Results

The effects of 5 samples of each Hange-koboku-to or Saiboku-to assessed by the plus-maze and activity tests are shown in Table 3. In the plus-maze test, as compared with the vehicle control ($7{\cdot}6 \pm 1{\cdot}1$ s), honokiol as well as the nine samples significantly prolonged the time spent by the mice in the open arms. Furthermore, the time spent in the open arms were almost identical (71·9-125·9%) for all samples, except for TJ-96C (47·4%), as well as that for honokiol. No significant change in the ambulatory activity was produced by any treatment with honokiol or test samples.

As shown in Table 4, however, the magnolia-free preparation of TJ-16D or TJ-96D, namely TJ-16D (Free) or TJ-96D (Free), respectively, did not prolong the time spent by the mice in the open arms. TJ-96D (Free) significantly accelerated the ambulatory activity.

Discussion

Seven daily treatments with honokiol prolonged the time spent by mice in the open arms in the plus-maze test without significantly changing the ambulatory activity, thus showing an anxiolytic effect. These findings support the previous studies (Maruyama et al 1998; Kuribara et al 1998, 1999). In this study, we evaluated the anxiolytic potential of 10 samples of Hange-koboku-to and Saiboku-to, Kampo medicines prepared with various Japanese magnolia barks (Wa-koboku) or Chinese magnolia barks (Kara-koboku) from which honokiol was derived.

The crude extract of magnolia contains not only honokiol but various other chemicals including magnolol (Fujita et al 1973; Itokawa 1986). According to the previous study in which the anxiolytic potential of three extracts of magnolia bark was assessed (Kuribara et al 1999), the doses of test samples were adjusted to ensure a constant dose of honokiol at 0·2 mg kg^{-1}. Thus, the doses of magnolol (from 0 mg kg^{-1} for honokiol alone to 0·816 mg kg^{-1} for TJ-16A), as well as other undetermined chemicals, varied among samples. However, it was clear that 9 out of 10 samples (i.e., all except TJ-96C), demonstrated an anxiolytic effect as high as that of honokiol. It is difficult to account for the comparatively lower anxiolytic effect of TJ-96C. However, the percentage of honokiol in

Table 3. Effects of honokiol and Hange-koboku-to (TJ-16) and Saiboku-to (TJ-96) Kampo medicines prepared with various magnolia barks, on the elevated plus-maze performance and ambulatory activity in mice.

Samples	Dose	n	Plus-maze test, time in open arm (s)	Activity test (counts/5 min)
Vehicle	10 mL kg^{-1}	50	9;7·6 ± 1·1	29·4 ± 1·4
Honokiol	0·2 mg kg^{-1}	50	9;22·8 ± 2·4† (100)	27·5 ± 1·3
TJ-16A	0·8000 g kg^{-1}	10	9;25·2 ± 10·0† (110·5)	20·4 ± 2·2
TJ-16B	0·2352 g kg^{-1}	20	9;20·4 ± 3·0† (89·5)	25·4 ± 2·1
TJ-16C	1·5384 g kg^{-1}	10	9;22·4 ± 5·4† (78·2)	27·9 ± 3·4
TJ-16D	0·1886 g kg^{-1}	10	9;22·7 ± 4·7† (99·6)	34·7 ± 3·5
TJ-16E	0·1450 g kg^{-1}	10	9;26·0 ± 6·2† (114·0)	28·9 ± 4·1
TJ-96A	2·5000 g kg^{-1}	19	9;22·3 ± 4·0† (97·8)	29·4 ± 2·7
TJ-96B	0·5000 g kg^{-1}	20	9;16·4 ± 4·0† (71·9)	24·5 ± 1·6
TJ-96C	2·8572 g kg^{-1}	20	9;10·8 ± 2·1* (47·4)	30·2 ± 2·2
TJ-96D	0·3636 g kg^{-1}	9	9;28·7 ± 5·3† (125·9)	30·9 ± 2·4
TJ-96E	0·2326 g kg^{-1}	10	9;25·5 ± 4·6† (111·8)	27·5 ± 2·9

Values in parentheses indicate percentage of honokiol value. Vehicle: aqueous solution containing 0·5% ethanol and Tween-80. TJ-16A and TJ-16B, and TJ-96A and TJ-96B were prepared with *Magnoliae obovata* Thunb (Japanese plants, Wa-koboku), and the others with *Magnoliae officinalis* Read. et Wils (Chinese plants, Kara-koboku). The dose of each sample was adjusted to ensure a constant dose of honokiol (0·2 mg kg^{-1}). Vehicle, honokiol and test samples were administered orally daily for 7 days. The plus-maze test (5 min) was carried out 24 h after the last drug administration, and was subsequently followed by the activity test (5min). The data presented are mean ± s.e.m. $*P < 0.05$ vs group treated with honokiol and $\dagger P < 0.05$ vs group treated with vehicle (Student–Newman–Keuls test).

Table 4. Effects of honokiol, Hange-koboku-to (TJ-16D) and Saiboku-to (TJ-96D) and their magnolia-free preparations (TJ-16D (Free) and TJ-96D (Free)), on the elevated plus-maze performance and the ambulatory activity in mice.

Sample	Dose	n	Plus-maze test, time in open arm (s)	Activity test (counts/5 min)
Vehicle	10 mL kg^{-1}	60	7·6 ± 1·0	30·5 ± 1·5
Honokiol	0·2 mg kg^{-1}	59	22·8 ± 2·2† (100)	28·3 ± 1·2
TJ-16D	0·1886 g kg^{-1}	20	20·4 ± 3·3**† (89·5)	32·2 ± 2·2
TJ-16D (Free)	0·2082 g kg^{-1}	10	5·4 ± 3·3 (23·7)	35·2 ± 4·7
TJ-96D	0·3636 g kg^{-1}	19	25·2 ± 3·3**† (110·5)	32·2 ± 2·1
TJ-96D (Free)	0·3280 g kg^{-1}	10	5·0 ± 2·7 (21·9)	43·3 ± 6·5*†

Values in parentheses indicate percentage of honokiol value. Vehicle: aqueous solution containing 0·5% ethanol and Tween-80. TJ-16D (Free) and TJ-96D (Free) are magnolia-free preparations of TJ-16D and TJ-96D, respectively. The experimental procedures were the same as those described in Table 2. The data presented are mean ± s.e.m. : $*P < 0.05$, and $**P < 0.01$, vs group treated with honokiol; $\dagger P < 0.05$ vs group treated with vehicle (Student–Newman–Keuls test).

sample TJ-96C was the lowest among the samples, requiring the highest dose for adjustment to achieve the required content of honokiol. This means that comparatively higher doses of undetermined chemicals were administered in the case of TJ-96C. Thus, it is probable that these undetermined chemical(s) played a role in reducing the anxiolytic effect of honokiol. Furthermore, the ambulatory activity was not modified by honokiol or by either sample except for TJ-96D (Free). It is interesting to note that the magnolia-free preparations, TJ-16D (Free) or TJ-96D (Free), revealed no anxiolytic effect. These results indicate that honokiol derived from magnolia is chiefly responsible for the anxiolytic effect of Hange-koboku-to and Saiboku-to.

Further studies are required to estimate the modification by undetermined chemicals of the anxiolytic effect of honokiol. However, taken together, the results obtained from this and previous (Kuribara et al 1999) studies, it is expected that our improved procedure for elevated plus-maze test in mice (Kuribara & Maruyama 1996; Kuribara et al 1996) may be applicable for estimation of content of honokiol in magnolia bark as well as in magnolia-containing Kampo medicines, in addition to the assessment of their anxiolytic potential.

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