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Honokiol, a Putative Anxiolytic Agent Extracted from Magnolia Bark, has no Diazepam-like Side-effects in Mice

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Abstract

Use of the elevated plus-maze experiment and activity and traction tests in mice have revealed that seven daily treatments with 0·2 mg kg^{-1} and higher doses of honokiol, a neolignane derivative extracted from Magnolia bark, had an anxiolytic effect without change in motor activity or muscle tone. Diazepam, 1 mg kg^{-1}, had the same anxiolytic potential as 0·2 mg kg^{-1} honokiol but induced muscle relaxation. The aim of this study was to determine whether honokiol had diazepam-like side-effects.

Mice treated with 1–10 mg kg^{-1} diazepam, but not those treated with 0·1–2 mg kg^{-1} honokiol, for 12 days showed withdrawal symptoms characterized by hyperactivity and running-fit when they were challenge-administered intraperitoneal flumazenil (10 mg kg^{-1}) 24 h after the last treatment with diazepam. Oral diazepam (0·5–2 mg kg^{-1}, 10 min before) dose-dependently prolonged hexobarbital (100 mg kg^{-1}, i.p.)-induced sleeping, disrupted learning and memory, and inhibited (+)-bicuculline (40 mg kg^{-1}, i.p.)-induced death. Honokiol (0·2–20 mg kg^{-1}, p.o., 3 h before) had no such effects. The prolongation by diazepam (1 mg kg^{-1}) of hexobarbital-induced sleeping was not modified by honokiol (0·2–20 mg kg^{-1}).

These results suggest that honokiol is less likely than diazepam to induce physical dependence, central depression and amnesia at doses eliciting the anxiolytic effect. It is also considered that honokiol might have no therapeutic effect in the treatment of convulsion.

Since the discovery of the anxiolytic effect of chlordiazepoxide and diazepam in the early 1960s, benzodiazepine anxiolytics have been used for treating anxiety disorders (Shader & Greenblatt 1995). Such benzodiazepine anxiolytics result in full allostic modulation of $GABA_A$ receptors (Lüddens et al 1995). However, concern about the long-term safety and risk of dependence on synthetic anxiolytics has led to increasing scientific interest in the biological approach to the treatment of anxiety disorders. Benzodiazepine anxiolytics frequently cause central depressant symptoms such as ataxia, over-sedation, amnesia, ethanol and barbiturate potentiation, and tolerance and dependence that are characterized by withdrawal symptoms after the termination of long-term use. Such unwanted side-effects limit the clinical application of benzodiazepine anxiolytics (Woods et al 1992, 1995; Schweizer et al 1995; Woods & Winger 1995). To avoid the occurrence of unwanted symptoms, drugs capable of partial allostic modification of $GABA_A$ receptors or selective agonistic action on the anxiety-related $GABA_A$ receptors are proposed (Haefely et al 1990; Doble & Martin 1992; Gardner et al 1993; Costa & Guidotti 1996).

Kuribara et al (1998) and Maruyama et al (1998) recently reported results from elevated plus-maze, activity and traction tests indicating that oral honokiol (0·2–2 mg kg^{-1}, 7 daily treatments), a neolignane derivative of Magnolia bark (Fujita et al 1973), had an anxiolytic effect without eliciting any significant change in motor activity or muscle tone. Our findings suggest that although the anxiolytic effect of honokiol is different from that of benzodiazepine anxiolytics, in combination with diazepam or flumazenil honokiol was likely to increase

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$GABA_A$ receptor function (Kuribara et al 1998). Thus, further experiments are required to elucidate the characteristics of the effect of honokiol.

To clarify the pharmacological differences between honokiol and benzodiazepine anxiolytics, and to reveal the specificity of the effect of honokiol, we conducted the following experiments on mice: physical dependence test; hexobarbital-induced sleeping test; learning and memory test; and bicuculline-induced death test. The effects of honokiol were compared with those of a benzodiazepine anxiolytic diazepam.

Materials and Methods

Animals

Experiments were performed on male BALB/c strain mice (Halan, Indianapolis, IN), 6 weeks, 22–27 g. Groups of five mice were housed in standard polycarbonate cages (15 cm width × 25 cm length × 12 cm height, with wood-chip bedding) and solid diet and tap water were freely available except during a short period of behavioural tests. The conditions of the breeding room were controlled (temperature 23 ± 1°C, relative humidity 55 ± 3%, and a 12-h light–dark cycle with lights on 0600–1800 h).

All experimental procedures were conducted according to The Guide for the Care and Use of Laboratory Animals and Animal Welfare Act, and the experimental protocol (97031-34-01-A) was approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Drugs

The drugs used were honokiol (Nacalai Tesque, Kyoto), diazepam and flumazenil (Hoffmann-La Roche, Nutley, NJ), and hexobarbital-Na and (+)-bicuculline (Sigma, St Louis, MO). Honokiol was first dissolved in a very small amount of ethanol, and the solution was diluted with Tween 80 (0·1%) in physiological saline solution so that the final ethanol concentration was 0·4%. Diazepam, flumazenil and bicuculline were suspended in Tween 80-physiological saline solution, and hexobarbital was dissolved in physiological saline. The concentration of each drug solution or suspension was adjusted so that each volume administered was constant at 0·1 mL/10 g.

Physical-dependence test

The drug administration schedule was similar to that reported by Cumin et al (1982). Groups of 10 mice each were given either Tween 80-physiological saline (vehicle), honokiol (0·1, 0·2, 0·5, 1 or 2 mg kg^{-1}, p.o.) or diazepam (0·5, 1, 2, 5 or 10 mg kg^{-1}, p.o.) daily for 12 days. All mice were challenge-administered intraperitoneal flumazenil (10 mg kg^{-1}) 24 h after the last drug treatment and the occurrence of the abstinence symptoms hyperreactivity (vocalization induced by a light pushing of the back), tremor, clonic convulsion, tonic convulsion, tail-flick or -reaction, and running-fit (wild running evoked by a key-ring sound) was observed for 30 min. During the measurement of tremor, clonic and tonic convulsions, and tail-flick no external stimulus was presented to the mouse.

Hexobarbital-induced sleeping test

Groups of five mice were pretreated with either honokiol (0 (i.e. Tween 80-saline), 0·2, 2 or 20 mg kg^{-1}, p.o., 3 h before), diazepam (0 (i.e. Tween 80-saline), 0·5, 1 or 2 mg kg^{-1}, p.o., 10 min before) or a combination of honokiol (0·2, 2 or 20 mg kg^{-1}, p.o., 3 h before) and diazepam (1 mg kg^{-1}, p.o., 10 min before). Subsequently, they were given hexobarbital (100 mg kg^{-1} in the salt form, i.p.). The latency times of loss and recovery of righting reflex were recorded, and the difference was considered as the sleeping time.

Learning and memory test

The experimental apparatus was the elevated plus-maze used for assessment of the anxiolytic effect of honokiol (Kuribara et al 1998; Maruyama et al 1998) with a minor change—the floor of the open arms was not transparent.

The experimental procedure was almost the same as that described by Itoh et al (1990, 1991). Briefly, in the training trial (1st day), each mouse was placed at the end of one open arm, which was randomly selected, facing away from the center platform. The latency time of transfer from the open arm to either of the closed arms (the transfer latency) was recorded. The criterion for entry of the mouse on to the closed arm was the crossing with all four paws of the borderline separating the closed arm from the platform. After the measurement of transfer latency, the mouse was allowed to move freely in the plus-maze for 2 min. Then, the mouse was gently returned to its home cage. On the next day, the retention trial was conducted. The mouse was placed again in the same position as in the training trial, and the transfer latency was recorded.

Groups of 10 mice each were treated with either honokiol (0 (i.e. Tween 80-saline), 2 or

20 mg kg^{-1}, p.o., 3 h before) or diazepam (0 (i.e. Tween 80-saline), 0·5, 1 or 2 mg kg^{-1}, p.o., 10 min before) in the training or retention trial.

Bicuculline-induced death test

Groups of 10 mice were pretreated with honokiol (0 (i.e. Tween 80-saline), 0·1, 0·2, 0·5, 1 or 2 mg kg^{-1}, p.o., 3 h before) or diazepam (0 (i.e. Tween 80-saline), 0·5 or 1 mg kg^{-1}, p.o., 10 min before). Subsequently, they were given bicuculline (40 mg kg^{-1} i.p.), and the number of dead mice was recorded. In the control groups pretreated with Tween 80-saline bicuculline caused clonic convulsion in all mice within 5 min of administration; this was generally followed by tonic convulsion within the next 3 min, except for a few mice. All of the mice suffering tonic convulsion died within 1 min. If mice did not suffer tonic convulsion by 3 min after the first occurrence of clonic convulsion, they survived. Therefore, to avoid the distress caused by clonic convulsions, mice that survived for 8 min after the administration of bicuculline were killed by exposure to a lethal concentration of carbon dioxide.

Statistical analysis

The duration of sleeping in the hexobarbital-induced sleeping test and the transfer latency in the learning and memory test were analysed by the Fisher PLSD test. In the physical dependence and bicuculline-induced death tests, the number of mice with the symptoms or the number of dead mice was analysed with the chi-square test. Values of $P < 0.05$ were considered indicative of significance.

Results

Physical-dependence test

As shown in Table 1, the administration of flumazenil to mice pretreated with Tween 80 resulted in slight excitation characterized by hyper-reactivity in 3 out of 10 mice. The challenge administration of flumazenil to mice pretreated with honokiol (0·1–2 mg kg^{-1}) was followed by almost the same symptoms as those observed for the Tween 80-pretreated mice. After diazepam pretreatment, however, challenge with flumazenil was followed by dose-dependent precipitated withdrawal symptoms. Occurrences of hyper-reactivity, tremor and running-fit were significantly higher in groups pretreated with 1 and 2 mg kg^{-1} and higher doses, of diazepam. Although some mice pretreated with 10 mg kg^{-1} diazepam suffered tonic and clonic convulsions after challenge with flumazenil, all the mice survived.

Hexobarbital-induced sleeping test

Table 2 shows the hexobarbital-induced sleeping time of mice pretreated with honokiol (0, 0·2, 2 and 20 mg kg^{-1}, p.o., 3 h before), diazepam (0, 0·5, 1 and 2 mg kg^{-1}, p.o., 10 min before) and a combination of honokiol (0·2, 2 or 20 mg kg^{-1}, p.o., 3 h before) and diazepam (1 mg kg^{-1}, p.o., 10 min before). Control mice pretreated with Tween 80-

Table 1. Number of mice suffering symptoms after challenge-administration of flumazenil.

Pretreatment (12 days)		Challenge	Hyper-reactivity*	Tremor	Clonic convulsion	Tonic convulsion	Tail flick or tail reaction	Running-fit†
Tween 80-saline		Tween 80-saline	1/10	0/10	0/10	0/10	0/10	0/10
Tween 80-saline		Flumazenil	3/10	0/10	0/10	0/10	1/10	0/10
Honokiol	0·1 mg kg^{-1}	Flumazenil	4/10	0/10	0/10	0/10	0/10	0/10
	0·2 mg kg^{-1}	Flumazenil	4/10	0/10	0/10	0/10	0/10	0/10
	0·5 mg kg^{-1}	Flumazenil	4/10	0/10	0/10	0/10	0/10	0/10
	1·0 mg kg^{-1}	Flumazenil	5/10	0/10	0/10	0/10	0/10	0/10
	2·0 mg kg^{-1}	Flumazenil	5/10	0/10	0/10	0/10	0/10	0/10
Diazepam	0·5 mg kg^{-1}	Flumazenil	7/10	0/10	0/10	0/10	0/10	0/10
	1·0 mg kg^{-1}	Flumazenil	10/10‡	0/10	0/10	0/10	0/10	3/10
	2·0 mg kg^{-1}	Flumazenil	10/10‡	5/10‡	0/10	0/10	2/10	5/10‡
	5·0 mg kg^{-1}	Flumazenil	10/10‡	7/10‡	0/10	0/10	1/10	8/10‡
	10·0 mg kg^{-1}	Flumazenil	10/10‡	5/10‡	3/10	1/10	1/10	10/10‡

Tween 80, honokiol and diazepam were administered orally once a day for 12 days and the challenge with intraperitoneal flumazenil (10 mg kg^{-1}) was performed 24 h after the last administration. The figures presented are the numbers of mice that showed the symptoms during the observation period of 30 min. *Vocalization induced by light pushing of the back. † Wild running evoked by a key-ring sound. ‡ $P < 0.05$, significantly different from result from mice pretreated with Tween 80-saline and challenged with flumazenil.

Table 2. Effects of oral honokiol and diazepam on sleeping induced by intraperitoneal hexobarbital.

Treatment		Sleeping time (s)
Tween 80-saline		2552±209
Honokiol	0·2 mg kg^{-1}	2454±254
	2·0 mg kg^{-1}	2334±129
	20·0 mg kg^{-1}	2480±12
Tween 80-saline		2902±84
Diazepam	0·5 mg kg^{-1}	3688±91*
	1·0 mg kg^{-1}	4476±336*
	2·0 mg kg^{-1}	5370±480*
Honokiol	0·2 mg kg^{-1} + diazepam, 1 mg kg^{-1}	4144±457
	2·0 mg kg^{-1} + diazepam, 1 mg kg^{-1}	4466±232
	20·0 mg kg^{-1} + diazepam, 1 mg kg^{-1}	5040±472

Honokiol and diazepam were administered 3 h and 10 min, respectively, before challenge with 100 mg kg^{-1} hexobarbital. *$P < 0.05$, significantly different from result from Tween 80-saline-treated control; n = 5 in each experiment.

saline 3 h or 10 min before fell asleep (loss of righting reflex) within 3 min, and continued sleeping for 40–50 min (mean sleeping times 2552±209 and 2902±84 s, respectively). Pretreatment with honokiol (0·2–20 mg kg^{-1}) had no significant effect on the hexobarbital-induced sleeping. Diazepam dose-dependently prolonged the sleeping time and honokiol did not change the diazepam-induced prolongation of the hexobarbital-induced sleeping.

Learning and memory test

Table 3 shows the transfer latencies in the training (1st day) and retention (2nd day) trials of mice treated with honokiol or diazepam. Treatment with 2 mg kg^{-1} honokiol before the training trial did not change the transfer latency in this trial, but prolonged the transfer latency in the retention trial. There was no significant difference between control and honokiol (2 mg kg^{-1})-treated groups in the gross times of shortening of the transfer latency caused by the training (difference between transfer latencies in the training and retention trials 33·5±7·4 and 21·3±5·6 s for the Tween 80-saline and 2 mg kg^{-1} honokiol-treated groups, respectively). Pretraining treatment with honokiol (20 mg kg^{-1}) did not change the transfer latency in either the training or retention trial. Treatment with 2 or 20 mg kg^{-1} honokiol before the retention trial did not cause any significant change in the transfer latency in the retention trial.

Treatment with 0·5 mg kg^{-1} diazepam before the training trial shortened the latency in the training trial. Pretraining treatment with diazepam dose-dependently prolonged the latency in the retention trial. No significant change in the latency resulted when diazepam was administered before the retention trial.

Table 3. Effects of oral honokiol and diazepam on transfer latency in training and retention trials.

Treatment		Training (s)	Retention (s)
Administration before the training trial			
Tween 80-saline		59·1±9·8	25·6±3·3
Honokiol	2 mg kg^{-1}	60·3±6·9	39·0±4·8*
	20 mg kg^{-1}	50·6±7·5	18·1±3·0
Tween 80-saline		59·1±10·1	32·5±9·5
Diazepam	0·5 mg kg^{-1}	27·2±6·1*	60·5±13·6*
	1·0 mg kg^{-1}	41·8±9·3	109·2±16·9*
	2·0 mg kg^{-1}	69·6±17·9	114·3±22·8*
Administration before the retention trial			
Tween 80-saline		62·8±12·2	25·2±2·8
Honokiol	2 mg kg^{-1}	51·2±10·3	31·9±4·0
	20 mg kg^{-1}	59·7±7·4	26·8±6·3
Tween 80-saline		71·7±10·4	31·9±9·9
Diazepam	0·5 mg kg^{-1}	60·8±18·9	24·2±3·6
	1·0 mg kg^{-1}	85·3±13·2	37·1±5·8
	2·0 mg kg^{-1}	63·9±15·7	30·0±3·7

Honokiol and diazepam were administered 3 h and 10 min, respectively, before the trials. *$P < 0.05$, significantly different from result from Tween 80-saline-treated control; n = 10 in each experiment.

Bicuculline-induced death test

Within 5 min of administration of bicuculline, all mice suffered clonic convulsion, followed by tonic convulsion in almost all mice. All mice suffering tonic convulsion died within 1 min of the first episode of tonic convulsion. As shown in Table 4, 0·1–2 mg kg^{-1} honokiol did not significantly protect against bicuculline-induced death, whereas 0·5

Table 4. Effects of oral honokiol and diazepam on death induced by bicuculline.

Treatment		Death
Tween 80-saline		9/10
Honokiol	0·1 mg kg^{-1}	9/10
	0·2 mg kg^{-1}	9/10
	0·5 mg kg^{-1}	8/10
	1·0 mg kg^{-1}	7/10
	2·0 mg kg^{-1}	7/10
Tween 80-saline		10/10
Diazepam	0·5 mg kg^{-1}	4/10*
	1·0 mg kg^{-1}	0/10*

Honokiol and diazepam were administered 3 h and 10 min, respectively, before intraperitoneal administration of bicuculline (40 mg kg^{-1}). *$P < 0.05$, significantly different from result from Tween 80-saline-treated control.