JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 261–266

Received March 1, 2010
Accepted August 10, 2010
DOI
10.1111/j.2042-7158.2010.01191.x
ISSN 0022-3573

Research Paper

Effect and mechanism of senkyunolide I as an anti-migraine compound from *Ligusticum chuanxiong*

Yi-Han Wang[a], Shuang Liang[a], De-Sheng Xu[b], Xiao Lin[a], Chun-Yong He[a], Yi Feng[a] and Yan-Long Hong[a]

[a]Engineering Research Center of Modern Preparation Technology of Traditional Chinese Medicine, Ministry of Education, Shanghai University of Traditional Chinese Medicine, Shanghai, China and [b]Shuguang Hospital, affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

Abstract

Objective To evaluate the analgesic and anti-migraine activities of senkyunolide I from *Ligusticum chuanxiong*.

Methods Mice were orally administered various doses of senkyunolide I, and their pain levels were assessed in a hot-plate test and by application of acetic acid. The levels of 5-hydroxytryptamine (5-HT), 5-hydroxytryptophan (5-HTP), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE) and dopamine (DA) in plasma and brain were assessed, and the monoamine turnover rates (5-HT/5-HTP, 5-HIAA/5-HT and NE/DA) were also calculated.

Results Mice given senkyunolide I at 16 and 32 mg/kg had significantly elevated pain thresholds in the hot-plate test, and a dose of 32 mg/kg also reduced the number of abdominal writhing responses caused by acetic acid. Significant improvements were observed in the neurotransmitter levels of the drug-treated rats compared with the saline-administered controls. Compared to the rats with nitroglycerin-induced migraines, the levels of nitric oxide in the plasma and whole brain of rats given senkyunolide I were lower.

Conclusions The present study suggests that senkyunolide I may be an active component of *L. chuanxiong*, traditionally used to treat migraine. The mechanism of pain relief in migraine model rats may be through adjusting the levels of monoamine neurotransmitters and their turnover rates, as well as decreasing nitric oxide levels in the blood and brain. Therefore, senkyunolide I may be developed as a potential treatment for migraine pain.

Keywords acetic-acid-induced writhing test; hot-plate test; migraine; monoamine neurotransmitters; nitric oxide; senkyunolide I

Introduction

The rhizome of *Ligusticum chuanxiong* Hort (chuanxiong) is a well-known traditional Chinese medicine that has been used for thousands of years for its hemodynamic and analgesic effects. It is commonly used for the treatment of migraine and various cardiovascular diseases. Some studies have shown that the ethanol extract of chuanxiong (CXE) has significant anti-migraine activity, and ligustilide may be the active component, but this is still uncertain and would require more study to confirm.[1] However, ligustilide is very thermolabile and photo-unstable, restricting research and further use.[2,3]

Our previous study demonstrated that senkyunolide I (Figure 1) is one of main migration components found in both rat plasma and cerebrospinal fluid after the normal and migraine model rats were orally administered with CXE.[4] This implies that senkyunolide I may be an active component in chuanxiong's traditional use in the treatment of migraines. Senkyunolide I is a lactone compound with the same nuclear structure as that of ligustilide, and some studies have shown that it may be a degradation product of ligustrazine *in vitro* and *in vivo*.[5,6] Although senkyunolide I may be an important component of chuanxiong, only one study has been carried out to show that it can reduce the damage of red blood cells caused by Con A.[7] Therefore, it would be of great interest to investigate the pharmacology of senkyunolide I for its potential therapeutic benefits in pain management. Our present investigation was therefore designed to evaluate the analgesic and anti-migraine activities of senkyunolide I *in vivo* using classic animal models.

Correspondence: Yi Feng and Yan-Long Hong, Engineering Research Center of Modern Preparation Technology of TCM, Ministry of Education, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China.
E-mail: fyi@vip.sina.com; hfuir@163.com

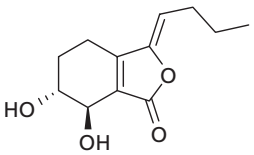

Figure 1 Chemical structure of senkyunolide I.

Materials and Methods

Plant material and chemicals

Slices of chuanxiong (no. 2007-12-15) were purchased from Shanghai Kangqiao Medicinal Materials Electuary Company (Shanghai, China) and identified as *Ligusticum chuanxiong* Hort. by Professor Zhi-Li Zhao of the Shanghai University of Traditional Chinese Medicine (Shanghai, China). Senkyunolide I (purity: 96.2%) was prepared by chromatography and identified by comparing the physical and spectroscopic data with those in the literature.[8] Dopamine (DA), norepinephrine (NE), 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were purchased from Sigma-Aldrich Company (St Louis, MO, USA). Bucinnazine hydrochloride tablets (no. 160901) were purchased from Shenyang First Pharmaceutical Company Ltd (Liaoning, China) and diluted to a concentration of 2.3 mg/ml with physiological saline for administration. Ergotamine caffeine tablets (no. 070401) were purchased from Shanghai Xingyi Pharmaceutical Company Ltd (Shanghai, China) and diluted in physiological saline (ergotamine 1.23 mg/ml, caffeine 123 mg/ml). Nitroglycerin injections (no. 20080122) were purchased from Beijing Yimin Pharmaceutical Company Ltd (Beijing, China). Nitric oxide (NO) detection kits (nitrate reductase, no. 20080420) and protein quantification kits (Coomassie Brilliant Blue G250, no. 20080420) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Other reagents were purchased from Sinopharm Chemical Reagent Company Ltd (Shanghai, China).

Animals

Kunming mice (female, 18–22 g, certificate no. 2007-0005) and Sprague–Dawley rats (both, 230–270 g, certificate no. 2008-0016) were purchased from the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China). The animals were housed in standard laboratory conditions (at a temperature of 20 ± 1°C and a 12 h light/dark cycle) with food and water available. All animal treatment protocols were strictly in accordance with the International Ethical Guidelines and the National Institute of Health Guide Concerning the Care and Use of Laboratory Animals, as well as with those of the Experimental Animal Administration of the university.

Hot-plate test[9,10]

The hot-plate test was carried out with Kunming mice and was performed at 55.0°C (ranging from 54.5 to 55.5°C) using a Model 501 Thermostatic Bath (Shanghai Laboratory Instrument Works Company Ltd, Shanghai, China). Mice were preadapted to the testing apparatus by putting each animal in a plastic box on the cold plate for 1–2 min on the day before testing. On the day of testing, mice were placed on the heated smooth surface, and the latency to licking, shaking of the limbs or jumping was measured. In the first session, there was a selection of appropriate subject mice. The mice were tested twice on each of the 2 days prior to the experiment, and this calculated mean pain threshold of each mouse was considered their basic pain threshold level. Mice that had a basic pain threshold lower than 5 s or higher than 30 s were excluded. The mice were then randomly divided into five groups to receive p.o. saline, bucinnazine hydrochloride (23 mg/kg) or senkyunolide I (8 mg/kg, 16 mg/kg or 32 mg/kg). The hot-plate tests were performed on mice individually at 20, 120, 180, 240 and 360 min after drug administration by the method described above. If no response occurred within 60 s, the mice were removed from the hot-plate to avoid tissue injury.

Acetic-acid-induced writhing test[9,11]

The acetic-acid-induced writhing tests were performed with Kunming mice. The mice were divided into five groups to receive p.o. saline, bucinnazine hydrochloride (23 mg/kg) or senkyunolide I (8 mg/kg, 16 mg/kg or 32 mg/kg). Acetic acid (0.9%) was injected intraperitoneally at a dose of 10 ml/kg, 240 min after drug administration. The number of abdominal writhing responses was immediately recorded for a period of 15 min. The typical abdominal writhing response manifested as extension of the hind limb, contraction of the abdomen and raising of the croup.

Nitroglycerin-induced headaches in rats

Sprague–Dawley rats were chosen for the nitroglycerin-induced migraine model, and ergotamine caffeine (tablets) was the reference drug. According to the method reported by Read *et al*.,[12] nitroglycerin was injected subcutaneously for a period of 30 min before treatment to establish the animal model of migraine. Rats were divided into five groups to receive p.o. saline, ergotamine caffeine (1.23 mg/kg) or senkyunolide I (72 mg/kg, 36 mg/kg, 18 mg/kg). Four hours later, 10 rats in each group were anaesthetized with pentobarbital sodium (1%) given intraperitoneally at the dose of 40 mg/kg. Subsequently, the animals were immobilized dorsally, the abdominal cavity was surgically exposed and the blood was collected from the aorta abdominalis. The brain tissue was excised, rinsed with cold saline, immediately frozen in liquid nitrogen and then stored at −80°C until analysed for neurotransmitter concentrations.[13]

Assessment of monoamine neurotransmitter levels[14]

Blood samples were centrifuged at 4000 rpm for 10 min, and plasma samples were harvested. Each plasma sample (100 μl) was mixed with 10 μl of 12 M perchloric acid, vortexed and centrifuged at 5000 rpm for 10 min. The supernatant (20 μl) was then analysed by high-performance liquid chromatography (HPLC).

The frozen brain tissues were homogenized in 500 μl of 0.4 M perchloric acid and centrifuged at 10 000 rpm at 4°C for 15 min. The supernatants were centrifuged at 10 000 rpm at 4°C again for 10 min, and 20 μl of the supernatant was subjected to HPLC analysis.

Table 1 Effect of senkyunolide I on heat-stimulated pain thresholds of mice

Groups	Dose (mg/kg)	0 min (s)	20 min (s)	120 min (s)	180 min (s)	240 min (s)	360 min (s)
Control	–	19.7 ± 3.3	20.8 ± 5.8	23.7 ± 2.7	21.2 ± 6.6	23.3 ± 4.9	21.3 ± 6.0
Bucinnazine hydrochloride	23	20.3 ± 4.0	24.7 ± 6.0	33.7 ± 5.1*	29.7 ± 5.7	33.0 ± 7.6*	21.5 ± 6.8
Senkyunolide I	32	21.2 ± 5.1	28.0 ± 7.0*	33.5 ± 10.0	32.0 ± 7.3*	35.5 ± 7.3*	25.3 ± 5.2
Senkyunolide I	16	20.1 ± 4.1	19.5 ± 2.1	34.3 ± 8.0	30.3 ± 12.2	29.2 ± 11.8	28.5 ± 6.9*
Senkyunolide I	8	19.1 ± 2.2	17.1 ± 1.9	19.7 ± 4.8	22.3 ± 4.8	21.2 ± 3.5	17.0 ± 4.1

Values are mean ± S.D. ($n = 6$). Full-size table. *$P < 0.05$ vs control.

The contents of 5-HT, 5-HIAA, 5-HTP, DA and NE were measured using HPLC with fluorescence detection on an LC-20AB HPLC system (Shimadzu Corporation, Kyoto, Japan) with a Kromasil C_{18} analytical column (4.6 × 250 mm, 5 μm, Eka, Bohus, Sweden). A 20 μl volume of sample was injected. The mobile phase, 90% of 0.1 M acetate buffer (pH 5.1) and 10% methanol, was pumped through the system at a flow rate of 1.0 ml/min, and the column temperature was kept at 25°C. The fluorescence detector was set at 290 nm for excitation and 330 nm for emission. Data acquisition was performed using an LC-Solution workstation (Shimadzu).

Nitric oxide detection

Blood samples were centrifuged at 4000 rpm for 10 min, and plasma samples were harvested. Brain tissues were homogenized on ice in 10 volumes of saline and centrifuged at 3000 rpm at 4°C for 15 min. Using assay kits and following the manufacturer's instructions, proteins and NO in the plasma samples and supernatants of the processed brain tissues were measured and expressed as μmol/l and μmol g/prot, respectively.

Statistical analysis

All statistical procedures were performed by using the SPSS statistical package (version 13.0, SPSS Inc., Chicago, IL, USA). All data analyses were performed by one-way analysis of variance for multiple comparisons. $P < 0.05$ was considered statistically significant, and data are presented as mean ± SD.

Results

Effect of senkyunolide I on pain thresholds

The results of the hot-plate test for pain threshold are shown in Table 1. Senkyunolide I at a dose of 32 mg/kg raised the pain threshold significantly compared to the control group at 20 min and remained high until 240 min post treatment. The 16 mg/kg dose also tended to raise the pain threshold, although not significantly until 360 min post treatment.

Additionally, we found that, compared with the control group, a dose of 32 mg/kg senkyunolide I significantly reduced the number of writhing responses induced in mice by acetic acid (Table 2). These results suggest that senkyunolide I effectively enhances tolerance to pain in mice.

Table 2 Effect of senkyunolide I on acetic-acid-induced writhing responses in mice

Groups	Dose (mg/kg)	Number of writhing responses (15 min)
Control	–	30.0 ± 7.9
Bucinnazine hydrochloride	23	16.8 ± 4.2*
Senkyunolide I	32	18.8 ± 4.4*
Senkyunolide I	16	30.5 ± 7.7
Senkyunolide I	8	33.7 ± 10.4

Values are mean ± S.D. ($n = 6$). *$P < 0.05$ vs control.

Effect of senkyunolide I on levels of plasma monoamines and turnover rates in migraine model rats

As shown in Table 3, the levels of 5-HT, 5-HTP and 5-HIAA in the plasma of the nitroglycerin-induced migraine model rats were lower, although not significantly so, than those in the untreated control group. The administration of senkyunolide I to the migraine model rats at 72 and 18 mg/kg returned the plasma 5-HT levels to near those of the untreated control mice, which was significantly different to the levels in the model rats not given analgesics. Meanwhile, senkyunolide I at 36 mg/kg significantly increased plasma 5-HTP levels compared to the migraine model rats.

The metabolic turnover rate of 5-HTP (5-HT/5-HTP) in the plasma of the migraine model rats was lower, although not significantly so, than that of the control group. However, the turnover rate of 5-HT (5-HIAA/5-HT) was significantly ($P < 0.05$) higher than that of the control group. The administration of senkyunolide I at all three doses tested returned levels of plasma 5-HTP and 5-HT turnover rates close to those of the controls.

Effect of senkyunolide I on brain monoamines and their turnover rates

The levels of monoamine neurotransmitters and their metabolites in brain tissue are shown in Table 4. The levels of 5-HT and NE in the brain tissue of the migraine model rats were higher than those in the control group. The DA, 5-HTP and 5-HIAA levels in the brain tissue of the migraine model rats were lower than those of the normal rats. Administration of senkyunolide I at all three doses generally returned levels of brain tissue 5-HT, NE, DA, 5-HTP and 5-HIAA close to those of the controls. However, despite the trends in these observations, there were no statistically significant differences among the groups.

The metabolic turnover rates of the monoamines in the brain tissue are also given in Table 4. The metabolic turnover rates of DA (NE/DA) in the brain tissue of the migraine model rats were lower than that of the control group. The turnover rates of 5-HTP (5-HT/5-HTP) and 5-HT (5-HIAA/5-HT)

Table 3 Effect of senkyunolide I on the levels of plasma monoamine neurotransmitters and the turnover rates in migraine model rats

Groups	Dose (mg/kg)	Monoamine neurotransmitters (ng/ml)			Monoamine turnover rates	
		5-HT	5-HTP	5-HIAA	5-HT/5-HTP	5-HIAA/5-HT
Control	–	45.6 ± 17.5	20.0 ± 3.48	19.3 ± 1.65	2.418 ± 1.272	0.479 ± 0.177
Model	–	23.2 ± 16.2	16.8 ± 2.99	18.6 ± 6.09	1.174 ± 0.714	0.855 ± 0.448***
Ergotamine and caffeine tablets	1.23	55.6 ± 40.4	19.7 ± 6.27	23.1 ± 7.02	2.963 ± 2.044	0.527 ± 0.335
Senkyunolide I	72	57.3 ± 41.5*	20.8 ± 2.37	19.1 ± 7.24	2.719 ± 1.812	0.535 ± 0.336**
Senkyunolide I	36	46.0 ± 38.3	21.8 ± 2.52*	17.4 ± 2.36	2.042 ± 1.519	0.323 ± 0.206
Senkyunolide I	18	60.5 ± 56.0*	20.8 ± 3.10	14.6 ± 2.30	2.213 ± 1.305	0.530 ± 0.221**

Values are mean ± S.D. ($n = 10$). *$P < 0.05$ vs model; **$P < 0.01$ vs model; ***$P < 0.05$ vs control.

Table 4 Effect of senkyunolide I on the levels of brain tissue monoamine neurotransmitters and the turnover rates in migraine model rats

Groups	Dose (mg/kg)	Monoamine neurotransmitters (ng/g)					Monoamine turnover rates		
		5-HT	5-HTP	5-HIAA	NE	DA	5-HT/5-HTP	5-HIAA/5-HT	NE/DA
Control	–	147.0 ± 41.0	602.6 ± 135.7	240.0 ± 75.2	426.3 ± 175.9	1564.3 ± 592.6	0.268 ± 0.129	1.642 ± 0.301	0.296 ± 0.114
Model	–	241.2 ± 123.4	562.4 ± 129.2	197.0 ± 73.2	452.2 ± 131.4	1230.9 ± 975.6	0.425 ± 0.174*	0.889 ± 0.255**	0.504 ± 0.329
Ergotamine and caffeine tablets	1.23	180.8 ± 67.7	604.5 ± 114.6	185.3 ± 85.2	421.4 ± 128.0	1507.7 ± 651.6	0.319 ± 0.162	1.047 ± 0.464	0.330 ± 0.171
Senkyunolide I	72	184.9 ± 94.4	625.6 ± 70.7	183.1 ± 85.6	382.4 ± 83.84	1505.6 ± 693.7	0.309 ± 0.184	1.078 ± 0.338	0.314 ± 0.171
Senkyunolide I	36	162.6 ± 69.3	584.7 ± 128.0	147.6 ± 86.0	407.2 ± 68.65	1347.1 ± 935.6	0.307 ± 0.166	0.881 ± 0.357	0.371 ± 0.231
Senkyunolide I	18	179.9 ± 42.1	660.7 ± 246.0	219.6 ± 32.8	445.4 ± 100.6	1417.9 ± 754.6	0.294 ± 0.095	1.234 ± 0.397	0.339 ± 0.216

Values are mean ± S.D. ($n = 10$). *$P < 0.05$, **$P < 0.01$ vs control.

Table 5 Effect of senkyunolide I on nitric oxide levels in plasma and brain tissue of rats exposed to nitroglycerin

Groups	Dose (mg/kg)	NO	
		Plasma (μmol/l)	Brain tissue (μmol g/prot)
Control	–	23.98 ± 9.45	1086 ± 0.68
Model	–	137.49 ± 14.21**	2.95 ± 0.22*
Senkyunolide I	72	93.51 ± 49.12***	2.27 ± 0.47
Senkyunolide I	36	117.94 ± 19.67	1.84 ± 0.75
Senkyunolide I	18	135.17 ± 32.26	2.29 ± 1.04

Values are mean ± S.D. ($n = 6$). *$P < 0.05$, **$P < 0.01$ vs control;***$P < 0.01$ vs model.

were significantly higher than those of the control group. Senkyunolide I administered at the three doses generally returned brain tissue NE, 5-HTP and 5-HT turnover rates close to those of the controls.

Effect of senkyunolide I on plasma and brain nitric oxide

As shown in Table 5, the levels of NO in the plasma and brain tissues of the migraine model rats were significantly higher than those of the control group. Senkyunolide I at the dose of 72 mg/kg decreased the levels of NO in plasma and brain tissue.

Discussion

The hot-plate assay is a simple and popular method for detecting central and peripheral acute thermal pain in mice. Additionally, observing acetic-acid-induced writhing is a standard test for visceral pain, which is sensitive to opiate as well as non-opiate analgesics. The results of both these tests in this study showed that senkyunolide I possesses antinociceptive activity in mice. This activity was dose-dependent, with the peak effect occurring at about 240 min after administration.

There is now much evidence to suggest that 5-HT may play a crucial role in migraines.[15] During the prodromal phase of a migraine attack, the level of 5-HT rises and causes vasoconstriction. In the attack stage, 5-HT is metabolized to 5-HIAA and excreted in the urine. The lowered concentration of 5-HT in blood cannot sustain the contraction of blood vessels, resulting in hemangiectasis. The 5-HT reduction also leads to a decrease in the pain threshold within the thalamencephalon. While migraines are regularly caused by these two factors,[16] DA is another stimulant of the migraine process, and its receptors are also involved in the regulation of blood flow in the brain. DA levels are reduced during a migraine attack, leading to cerebrovascular dilation and migraine pain.[17]

A reduction was observed in the levels of three forms of related monoamine neurotransmitters, 5-HTP (the precursor of 5-HT), 5-HT and 5-HIAA (the metabolite of 5-HT), in the plasma following nitroglycerin administration to induce migraine. The turnover rate[18] of 5-HTP (5-HT/5-HTP) was reduced, while that of 5-HT (5-HIAA/5-HT) rose. The change in the monoamine turnover rates caused the reduction in the overall level of 5-HT. Increases (tendentially or significantly) were observed in the levels of all three monoamine neurotransmitters following senkyunolide I administration, and the turnover rates of 5-HTP and 5-HT were reversed (tendentially or significantly). This phenomenon was also observed in the levels of the five monoamine neurotransmitters (5-HTP, 5-HT, 5-HIAA, DA and NE) and their turnover rates (5-HT/5-HTP, 5-HIAA/5-HT, NE/DA) following nitroglycerin or senkyunolide I administration. This suggests that senkyunolide I affects the levels of monoamine neurotransmitters in the plasma and brain tissue of the migraine model rats by adjusting their turnover rates to some extent.

NO is important for the regulation of cerebral and extracerebral cranial blood flow and arterial diameters. It is also involved in nociceptive processing. Glyceryl trinitrate , a prodrug for NO, increases the level of NO in plasma and brain tissue of Sprague–Dawley rats and causes migraines.[19] Our study demonstrates that senkyunolide I decreases (tendentially or significantly) the levels of NO in the plasma and brain tissue of migraine model rats. Similar results were observed with senkyunolide A, an analogue of senkyunolide I, which can decrease the NO content and nitric oxide synthase (NOS) activity in the global cerebral ischemia–reperfusion model in rats.[20]

In preliminary experiments, we studied the time-effect relationship of senkyunolide I on the levels of 5-HT and found that it reached a peak value at 240 min, therefore 240 min was chosen as the time point for testing pain thresholds after drug administration. Moreover, the effects of senkyunolide I on the pain threshold of mice were tested in the first set of experiments at 8 mg/kg, 16 mg/kg and 32 mg/kg, doses chosen according to the results of the preliminary experiments. The most effective dose was apparently 32 mg/kg.

The pain threshold can be influenced to some extent by monoamine neurotransmitters. In order to test a wider range of potentially effective dosages in the rat model, in subsequent experiments we chose doses of 18 mg/kg, 36 mg/kg and 72 mg/kg to evaluate the antimigraine activity of senkyunolide I *in vivo*. The levels of monoamine neurotransmitters can be influenced by emotion, food and medicine, among other factors. In addition, the stimulation given by the experimenter may change the neurotransmitter levels of the laboratory animals. These factors can lead to large differences between individual animals. The monoamine neurotransmitters in biological samples also tend to be unstable and, based on our experience, it was found that the level of 5-HT in plasma samples decreased by 10% within 10 h at room temperature. These reasons may have contributed to the large deviations in Tables 3 and 4, which to some extent caused only trends to be observed, but without significant differences between some groups.

Conclusions

The present study suggests that senkyunolide I may be a key effective component of the traditional *L. chuanxiong* medicine used to treat migraines. The mechanism of its effects on relieving pain in migraine model rats may be through adjustment of the levels of monoamine neurotransmitters and their turnover rates in blood and brain tissue, and decreasing NO levels in plasma and the brain. Senkyunolide I therefore has considerable therapeutic potential and is worthy of clinical development as a potential agent for the treatment of migraines.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The work was supported by the Shanghai Municipal Education Committee (J50302 and 09YZ117) and the National Sci-Tech Major Special Item (2009ZX09502-009).

References

1. Peng C *et al*. Pharmacodynamic action and mechanism of volatile oil from *Rhizoma Ligustici Chuanxiong* Hort. on treating headache. *Phytomedicine* 2009; 16: 25–34 [PubMed: 19121572].
2. Cui F *et al*. Factors affecting stability of z-ligustilide in the volatile oil of *Radix angelicae sinensis* and *Ligusticum chuanxiong* and its stability prediction. *Drug Dev Ind Pharm* 2006; 32: 747–755 [PubMed: 16885129].
3. Zhou CX *et al*. Studies on the stability of ligustilide with solvent effect. *Yao Xue Xue Bao* 2001; 36: 793–795 (in Chinese) [PubMed: 12579984].
4. Yuan Y *et al*. Studies on the in vivo transmigration of chuanxiong bioactive parts for the treatment of migraine. *Chin Pharma J* 2010; 45: 694–697 (in Chinese) [DOI: 1001-2494(2010) 09-0694-04].
5. Li SL *et al*. Post-Harvest Alteration of the Main Chemical Ingredients in *Ligusticum chuanxiong* Hort. (Rhizoma Chuanxiong). *Chem Pharm Bull* 2007; 55: 140–144 [PubMed: 17202719].
6. Yan R *et al*. Pharmacokinetics and metabolism of ligustilide, a major bioactive component in Rhizoma chuanxiong, in the rat. *Drug Metab Dispos* 2008; 36: 400–408 [PubMed: 18039808].
7. Zhu Q *et al*. Effects of ferulic acid, senkyunolide H and senkyunolide I on erythrocytes. Lishizhen medicine and materia medica. *Research* 2003; 14: 738–739 (in Chinese) [DOI: 1008-0805(2003)12-0738-02].
8. Takashi N *et al*. Two phthalides from ligusticum chuanxiong. *Phytochemistry* 1996; 41: 233–236 [DOI: 10.1016/0031-9422(95)00524-2].
9. Liu JW. *Methodology of Pharmacology Experiment-New Technic and Method*, 2nd edn. Beijing: Chemical Industry Press, 2007: 363–366.
10. Menéndez L *et al*. Unilateral hot plate test: a simple and sensitive method for detecting central and peripheral hyperalgesia in mice. *J Neurosci Methods* 2002; 113: 91–97 [PubMed: 11741726].
11. Tajik H *et al*. The Effect of Curcumin (active substance of Turmeric) on the acetic acid-induced visceral nociception in rats. *Pak J Biol Sci* 2008; 11: 312–314 [PubMed: 18817212].

12. Read SJ *et al*. Effects of sumatriptan on nitric oxide and superoxide balance during glyceryl trinitrate infusion in the rat. Implications for antimigraine mechanisms. *Brain Res* 1999; 847: 1–8 [PubMed: 10564729].
13. HZ L. *Pharmaceutical Analysis in Biological Sample*, 1st edn. Beijing: People's Medical Publishing House, 2008: 10–16.
14. Lu YX *et al*. The content determination of the five transmitters in rat brain by RP-HPLC method with fluorescence detector. *Pharm J Chin PLA* 2003; 19: 262–263.
15. Lance JW. 5-Hydroxytryptamine and its role in migraine. *Eur Neurol* 1991; 31: 279–281 [PubMed: 1884718].
16. Stephen D. Serotonin (5-HT) and migraine. *Headache* 1994; 34: 408–417 [DOI: 10.1111/j.1526-4610.1994.hed3407408.x].
17. Peroutka SJ. Dopamine and migraine. *Neurology* 1997; 49: 650–656 [PubMed: 9305317].
18. Kabuki Y *et al*. Different locomotor activities and monoamine levels in the brains of Djungarian Hamsters (D. sungorus) and Roborovskii Hamsters (D. roborovskii). *Exp Anim* 2008; 57: 447–452 [PubMed: 18946181].
19. Olesen J *et al*. Nitric oxide is a key molecule in migraine and other vascular headaches. *Trends Pharmacol Sci* 1994; 15: 149–153 [PubMed: 7538702].
20. Zhang J *et al*. NBPA: a cerebral ischaemic protective agent. *Clin Cxp Pharmacol Physiol* 1999; 26: 845–846 [PubMed: 10549420].