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Fast onset of action and the analgesic and sedative efficacy of essential oil from *Rhizoma Chuanxiong* after nasal administration

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In this study, the analgesic and sedative efficacy of Chuanxiong essential oil after nasal administration was compared with that of the commonly used oral administration route. The essential oil significantly reduced nociception only 5 min after nasal administration but not by i.g. administration. The essential oil significantly decreased the sleep latency and prolonged the sleeping time only 5 min after i.n. administration but not by the i.g. route. Taken together, these data demonstrate that the essential oil from *Rhizoma Chuanxiong* had faster onset of action as well as better analgesic and sedative efficacy after i.n. administration than given orally.

1. Introduction

Rhizoma Chuanxiong, known as Chuanxiong in China is a commonly used Chinese medicinal (CM) herb with haemodynamic and analgesic effects and has been widely used for treating cardiovascular diseases and migraine in China for centuries (China Pharmacopoeia). Chuanxiong essential oil (CXEO) extracted from this herb is generally claimed to play the major role in the haemodynamic and analgesic effects of Chuanxiong. It was thought to be composed of many important biologically active components (Xie et al. 2007) and was reported to have properties of vasodilatation (Chan et al. 2007; Cao et al. 2006), antiplatelet aggregation (Song et al. 2004), analgesic (Du et al. 2007), neuroprotection (Peng et al. 2007; Kuang et al. 2006), antithrombotic and antiproliferation (Lu et al. 2006). In particular, the phthalides presented in large quantities in Chuanxiong essential oil (CXEO) have been shown to have many pharmacological activities (Naito et al. 1995; Ko et al. 1997, 2002; Matsumoto et al. 1998; Chong et al. 1999). Chuanxiong essential oil is taken orally in Chinese Medicine practice, but there were reports about the low oral bioavailability of ligustilide, a major ingredient in Chuanxiong essential oil contributing to the therapeutic effects of Chuanxiong. The oral bioavailability of ligustilide in rats, is only 2.6% which is partly due to extensive first-pass metabolism in the liver (Yan et al. 2008). There are also reports about another bioactive phthalide in Chuanxiong, senkyunolide A, which showed that this compound is unstable in the gut and undergoes extensive first-pass metabolism in the liver, leading to a low oral bioavailability (Yan et al. 2007). These reports provided important scientific data to challenge the validity of conventional p.o. administration for Chuanxiong essential oil (Yan et al. 2008). Therefore, other administrative routes such as intranasal administration, to avoid extensive first-pass effects in the gut and the liver may be preferable.

Nasal administration offers many advantages such as avoidance of first-pass metabolism and the fast onset of therapeutic

action (Illum 2000, 2003). Recently, focus has been on the nasal mucosa as an alternate route to achieve higher and faster drug absorption. The nasal mucosa is believed to be more permeable to compounds than the gastrointestinal tract due to lack of gastric enzymatic activity, neutral pH of the nasal mucus and avoidance of dilution by gastrointestinal contents. A number of drugs, including vasopressin, opiates, antihistamines and corticosteroids are effectively administered intranasally (e.g., budesonide, triamcinolone, butorphanol, sumatriptan, mometasone, beclomethasone) (Chrstensen et al. 2007).

In the treatment of neurological diseases, intranasal administration has attracted much attention in the past decades because of its noninvasive route that can offer advantages such as rapid absorption, avoidance of first-pass metabolism, ease of convenience and self-mediation (Illum 2000, 2003). Consequently, the nasal route may be important for drugs that are used for the treatment of central system diseases. As a matter of fact, the 'nasal herbal stuff therapy' has been used in the treatment of headache for a long period in China. For example, the fresh juice squeezed from the herbal leaves or herbal extracts was snuffed up the nostrils and this was sometimes efficacious in the treatment of headache (Liu et al. 2004). It was reported that low molecular weight and lipophilic compounds can be rapidly absorbed into the brain after nasal administration (Illum 2000, 2003). Therefore, following nasal administration, the lipophilic phthalides in Chuanxiong essential oil may have beneficial therapeutic effects at lower dose and have a faster onset of action. In our laboratory, a HPLC-UV method was developed for the determination of ligustilide concentration in rat brain sample after intranasal administration (Guo et al. 2009). The result showed that ligustilide could be detected in rat brain as soon as after 5 min of nasal administration; which showed that ligustilide may have a rapid onset of action. In the present study, the analgesic efficacy of essential oil after nasal administration was tested and compared with conventionally used oral administration in three mice experimental models: acetic acid

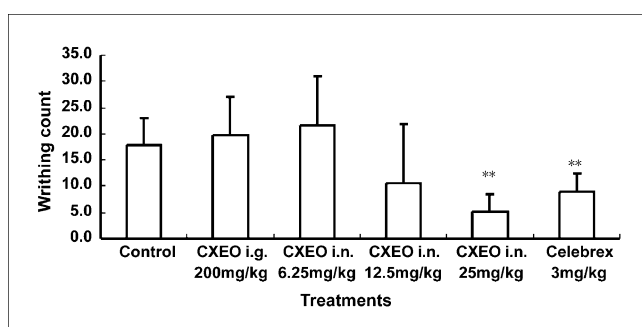


Fig. 1: Effect of CXEO (Chuanxiong essential oil) on acetic acid-induced writhing response in mice following i.n. and i.g. administration. Each mouse was administered with CXEO (6.25, 12.5, 25 mg/kg) or vehicle (3% Tween 80) through intranasal route 5 min before an intraperitoneal injection of 0.6% acetic acid (0.1 ml/10 g bodyweight). For i.g. administration, CXEO (200 mg/kg) or vehicle (3% Tween 80) was administered, and 5 min later, 0.6% acetic acid was injected. The number of writhing was recorded for 10 min after acetic acid injection. Each column represents mean (S.D.) of 15 mice. * $P < 0.05$ and ** $P < 0.01$ versus control

writhing tests, hot plate tests and pentobarbital-induced sleep model.

2. Investigations and results

2.1. Acetic acid-induced writhing response in mice

Fifteen minutes after oral administration of Chuanxiong essential oil, the number of writhing movements at doses of 100 and 200 mg/kg were 16.8 ± 7.3 and 13.7 ± 8.4 respectively, which were significantly lower than that of the control (23.0 ± 8.7). This data showed that Chuanxiong essential oil dose-dependently reduced the number of acetic acid-induced writhing movements in mice at 15 min following i.g. administration.

In order to compare the onset time of Chuanxiong essential oil after oral and nasal administration, the analgesic efficacy was assessed at 5 min following drug administration. Acetic acid was injected 5 min after the administration and the number of acetic acid-induced writhing movements was observed. The essential oil significantly reduced nociception by acetic acid intraperitoneal injection in a dose-dependent manner (6.25, 12.5 and 25 mg/kg) only 5 min after nasal administration (Fig. 1). In contrast, Chuanxiong essential oil had no effect on writhing movements 5 min after oral administration at doses of 200 mg/kg. Therefore, in the acetic acid-induced writhing model, Chuanxiong essential oil had faster onset of action (5 min versus 15 min) and better analgesic efficacy (25 mg/kg versus 100 mg/kg) after i.n. administration in comparison with that by i.g. route.

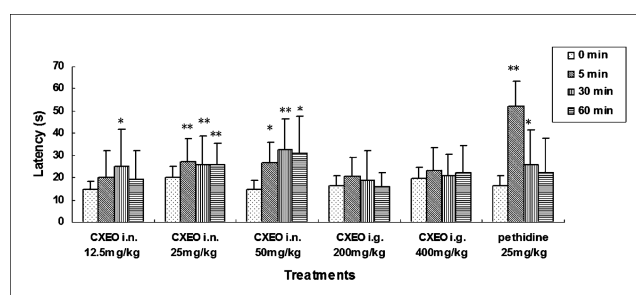


Fig. 2: Effect of CXEO (Chuanxiong essential oil) on hot-plate test in mice following i.n. and i.g. administration. Each mouse was given a single dose of CXEO or vehicle by i.n. (12.5, 25 and 50 mg/kg) or i.g. route (200 and 400 mg/kg) and then the hot-plate latent response time of each animal was recorded at 5, 30, 60 min. Each column represents mean (S.D.) of 15 mice. * $P < 0.05$ and ** $P < 0.01$ versus control

2.2. Hot-plate latent pain response test in mice

Fig. 2 shows that as early as 5 min after nasal administration, Chuanxiong essential oil significantly increased hot-plate latency in a dose-dependent manner (12.5, 25 and 50 mg/kg). And this efficacy lasted for 60 min at doses of 25 and 50 mg/kg. While after i.g. administration, Chuanxiong essential oil had no effect on hot-plate latency at doses of 200 and 400 mg/kg within 60 min.

2.3. Effect of Chuanxiong essential oil on potentiation of pentobarbital-induced sleep in mice

Effects of pre-treatment with Chuanxiong essential oil on the number of mice falling asleep, the sleep latency and duration induced by sodium pentobarbital (respectively, 24 and 40 mg/kg) are shown in Tables 1 and 2. The result showed that at doses of 100 mg/kg, Chuanxiong essential oil could increase the number of mice falling asleep, and also decrease the sleep latency and prolong the sleeping time induced by pentobarbital. Diazepam, the positive control used in this study, also potentiated pentobarbital hypnosis.

3. Discussion

Chuanxiong has been used clinically for treating cardiovascular diseases and migraine in China for centuries. The essential oil extracted from the herb is generally claimed to play the major role in the haemodynamic and analgesic effects. In recent years, several drugs containing Chuanxiong essential oil extract as the primary ingredient have been approved to be used clinically. For example, Quick Acting Heart Saving Pill (Chinese name, Suxiao Jiuxin Wan), a product manufactured for sublingual delivery, has become one of the five best-selling CM-based cardiovascular products in China (Yan et al. 2008; Sun et al. 2002).

Table 1: Effect of CXEO on the onset and duration of sleep in mice induced by sub-hypnotic dosage of pentobarbital

Groups	Dose (mg/kg)	n	Number of falling sleep	Sleep latency (min)	Sleeping time (min)
Normal		10	3	46.2 ± 22.3	5.8 ± 9.4
CXEO i.n.	25 mg/kg	10	4	41.0 ± 24.6	9.0 ± 12.7
CXEO i.n.	50 mg/kg	10	6	29.3 ± 26.4	20.0 ± 19.8
CXEO i.n.	100 mg/kg	10	6	31.7 ± 24.5	24.1 ± 23.3*
CXEO i.g.	100 mg/kg	10	1	55.1 ± 15.5	1.6 ± 5.05
DZP	3 mg/kg	10	9	9.3 ± 2.0	42.3 ± 21.1*

Mice received pentobarbital (24 mg/kg, i.p.) 5 min after the pre-treatment of CXEO (25, 50, 100 mg/kg, i.n.) and diazepam (3 mg/kg, i.g.). If mouse did not lose righting reflex in 1 h after injecting pentobarbital, the sleep latency and the sleeping time were recorded 60 and 0 min, respectively. Sleep latency and time were expressed as the means ± S.D. (n = 10)

* $P < 0.05$,

** $P < 0.01$, significant as compared to the normal group

Table 2: Effect of CXEO on the onset and duration of sleep in mice induced by hypnotic dosage of pentobarbital

Groups	Dose (mg/kg)	n	Number of falling sleep	Sleep latency (min)	Sleeping time (min)
Normal		10	10	11.1 ± 17.6	35.9 ± 23.1
CXEO i.n.	25 mg/kg	10	10	4.0 ± 1.3	54.8 ± 23.2
CXEO i.n.	50 mg/kg	10	10	5.3 ± 1.7	48.2 ± 19.6
CXEO i.n.	100 mg/kg	10	10	4.8 ± 2.5	56.8 ± 13.6*
CXEO i.g.	100 mg/kg	10	10	4.3 ± 1.6	44.3 ± 13.5
DZP	3 mg/kg	10	10	3.2 ± 0.9	81.7 ± 39.4*

Mice received pentobarbital (40 mg/kg, i.p.) 5 min after the pre-treatment of CXEO (25, 50, 100 mg/kg, i.n.) and diazepam (3 mg/kg, i.g.). If mouse did not lose righting reflex in 1 h after injecting pentobarbital, the sleep latency and the sleeping time were recorded 60 and 0 min, respectively. Sleep latency and time were expressed as the means ± S.D. (n = 10)

* $P < 0.05$

** $P < 0.01$, significant as compared to the normal group

Nasal drug delivery offers many advantages over oral administration such as the fast onset of therapeutic action due to the rapid absorption. Consequently, the nasal route may be important for drugs that are used for the treatment of acute central system diseases. In this study, the analgesic efficacy and onset of action of essential oil through nasal route was compared with that via the commonly used oral route. Our results show that the onset of action of essential oil after nasal administration is achieved as early as 5 min after dosing, which demonstrates the fast onset of action of essential oil via nasal route. After nasal administration, Chuanxiong essential oil exhibited potent analgesic efficacy in acetic acid-induced writhing model at doses of 25 mg/kg. Through the i.g. route, Chuanxiong essential oil had analgesic efficacy at doses of 200 mg/kg 15 min after administration, which showed that Chuanxiong essential oil had higher analgesic efficacy after nasal administration than after i.g. administration.

The writhing test and hot-plate test were selected to investigate the peripheral and central analgesic activities of Chuanxiong essential oil, respectively. The results showed that Chuanxiong essential oil exhibited both peripheral and central analgesic efficacy after nasal administration.

In the test of pentobarbital-induced mice sleep, the sedative effect of Chuanxiong essential oil after i.n. administration was represented. It not only prolonged the sleeping time induced by pentobarbital, but also decreased the latency of falling asleep and increased the rate of sleep onset (Tables 1 and 2). In contrast, after oral administration, Chuanxiong essential oil did not exhibit the sedative efficacy at dose of 100 mg/kg.

In summary, based on the result reported in this paper, it is confirmed that Chuanxiong essential oil has faster onset of action as well as more potent efficacy after nasal administration at lower dosage than by i.g. route. This result illustrates that intranasal administration may act as a promising alternative to conventional routes of administration which would improve the therapeutic efficacy and reduce peripheral side effects of essential oil. This report also provides more scientific information for further understanding of the clinical use of the herb.

4. Experimental

4.1. Plant, chemicals and reagents

Ligusticum chuanxiong Hort. (Umbelliferae) was cultivated and harvested in Pengzhou Sichuan, China. Rhizoma Chuanxiong, derived from the rhizome of *Ligusticum chuanxiong* Hort. was identified by the correspondence author. The voucher specimens (No. NJUTCM-20080201) were deposited in Nanjing University of Chinese Medicine.

All reagents used in the experiments were analytical grade and from commercial sources. Distilled water was prepared in EPED Superpure water purification system (Nanjing, China).

4.2. Extraction

Two hundred grams of dry Rhizoma Chuanxiong were transferred into a 5000 ml distillation flask. Two liters of distilled water were added and the

volatile oil distillation apparatus was set according to the Chinese Pharmacopoeia; the mixture was distilled for 10 h. Oil was collected from the condenser, dried over anhydrous sodium sulfate, and the recorded yield of the sample was 0.25%. The concentration of ligustilide in essential oil was determined by HPLC to be 225 mg/ml. As the condition used for the determination of ligustilide, the detector was operated at 322 nm. The mobile phase consisted of A (0.3% aqueous acetic acid, v/v) and B (methanol). Gradient elution was as follows: 30% B increased linearly to 90% B in 20 min, and back to 30% B from 20 to 30 min, then maintained at 30% B for 10 min. The flow rate was 1.0 ml/min.

4.3. Animal experiments

ICR female mice (18–22 g) were obtained from the Experimental Animal Center of Nanjing Medical University (Nanjing). The animals were housed in a temperature-controlled ($22 \pm 1^\circ\text{C}$) animal room on 12 h light/dark cycles with free access to food and water. Animal welfare and experimental procedures were strictly in accordance with the *Guide for the Care and Use of Laboratory Animals* (US National Research Council, 1996) and the related ethics regulations of Nanjing University of Chinese Medicine. Chuanxiong essential oil was formulated in normal saline containing 3% Tween 80 and administered to mice nasal cavity (10 μl /20 g body weight) using a fine tip attached to a micropipette.

4.4. Acetic acid-induced writhing response in mice

The writhing test was carried out as described by Nakamura et al. (1986). The analgesic efficacy was assessed at 5 and 15 min following drug administration. Each mouse weighing 18–22 g was administered with Chuanxiong essential oil (6.25, 12.5, 25 mg/kg) or vehicle (3% Tween 80) through intranasal route 5 min before an intraperitoneal injection of 0.6% acetic acid (0.1 ml/10 g bodyweight). For i.g. administration, Chuanxiong essential oil (200 mg/kg) or vehicle (3% Tween 80) was administered, and 5 or 15 min later, 0.6% acetic acid was injected. The number of writhing movements was recorded for 10 min after acetic acid injection. Mice of the positive control group were given Celebrex® (3 mg/kg) by i.g. route, and 60 min later, 0.6% acetic acid was injected.

4.5. Hot-plate latent pain response test in mice

The hot-plate test was carried out according to the method of Eddy and Leimback (Eddy et al. 1953). Each female mouse weighing 18–22 g was placed on a $55 \pm 0.5^\circ\text{C}$ hot-plate to observe its pain responses (hind-paw-licking or jumping). Before determination each mouse was first habituated to the hot-plate twice. The latent time before the occurrence of the pain response was recorded as an analgesic parameter. Untreated mice with a background latent response time shorter than 10 s or longer than 30 s were excluded from the study. Each mouse received a single dose of Chuanxiong essential oil or vehicle by i.n. (12.5, 25 and 50 mg/kg) or i.g. route (200 and 400 mg/kg) and then the hot-plate latent response time of each animal was recorded at 5, 30, 60 min. Mice of the positive control group received pethidine intraperitoneally (25 mg/kg).

4.6. Potentiation of pentobarbital-induced sleep in mice

In this test, Chuanxiong essential oil or vehicle was administered intranasally (25, 50, 100 mg/kg) or orally (100, 200 mg/kg) 5 min before the test. Following the pentobarbital injection (40 mg/kg pentobarbital was used as hypnotic dosage and 24 mg/kg was used as sub-hypnotic dosage through i.p. injection), each mouse was observed for the sleep onset, with mice that lost righting reflex over 1 min was considered to be asleep. The sleeping latency was recorded from the injection of pentobarbital to the sleep onset and the sleeping time was recorded from the loss of righting reflex to recovery.

4.7. Statistical analysis

Results were expressed as mean \pm S.D. Statistical analyses were performed by Student's t-test. $P < 0.05$ was considered as statistically significant.

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References

- Cao YX, Zhang W, He JY, He LC, Xu CB (2006) Ligustilide induces vasodilatation via inhibiting voltage dependent calcium channel and receptor-mediated Ca^{2+} influx and release. *Vascul Pharmacol* 45: 171–176.
- Chan SS, Cheng TY, Lin G (2007) Relaxation effects of ligustilide and senkyunolide A, two main constituents of *Ligusticum chuanxiong*, in rat isolated aorta. *J Ethnopharmacol* 111: 677–680.
- China Pharmacopoeia, Vol. I [in Chinese]. Beijing, Chemical Industry Press, State Pharmacopoeia Commission of P.R. China, 2005, 28.
- Chong ZZ, Feng YP (1999) dl-3-n-Butylphthalide improves regional cerebral blood flow after experimental subarachnoid hemorrhage in rats. *Acta Pharmacol Sin* 20: 509–512.
- Christensen K, Rogers E, Green GA, Hamilton DA, Mermelstein Fred, Liao Edward, Wright Curtis, Carr Daniel B (2007) Safety and efficacy of intranasal ketamine for acute postoperative pain. *Acute Pain* 9: 183–192.
- Du J, Yu Y, Ke Y, Wang C, Zhu L, Qian ZM (2007) Ligustilide attenuates pain behavior induced by acetic acid or formalin. *J Ethnopharmacol* 112: 211–214.
- Eddy NB, Leimback D (1953) Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J Pharmacol Exper Ther* 107: 385–393.
- Guo JM, Duan JA, Shang EX, Tang YP, Qian DW (2009) Determination of ligustilide in rat brain after nasal administration of essential oil from *Rhizoma Chuanxiong*. *Fitoterapia* 80: 168–172.
- Illum L (2000) Transport of drugs from the nasal cavity to the central nervous system. *Eur J Pharm Sci* 11: 1–18.
- Illum L (2003) Nasal drug delivery-possibilities, problems and solutions. *J Control Release* 87: 187–198.
- Ko WC, Liao CC, Shih CH, Lei CB, Chen CM (2002) Relaxant effects of butylidenephthalide in isolated dog blood vessels. *Planta Med* 68: 1004–1009.
- Ko WC, Sheu JR, Leu YR, Tzeng SH, Chen CM (1997) Stereoselectivity of butylidenephthalide on voltage-dependent calcium channels in guinea-pig isolated ileum. *J Pharm Pharmacol* 49: 1121–1125.
- Kuang X, Yao Y, Du JR, Liu YX, Wang CY, Qian ZM (2006) Neuroprotective role of Z-ligustilide against forebrain ischemic injury in ICR mice. *Brain Res* 1102: 145–153.
- Liu PZ, Wu W, Chen HG (2004) The progress of intranasal administration in the treatment of migraine. *J Liaoning Coll of TCM* 6: 125–126.
- Lu Q, Qiu TQ, Yang H (2006) Ligustilide inhibits vascular smooth muscle cells proliferation. *Eur J Pharmacol* 542: 136–140.
- Matsumoto K, Kohno SI, Ojima K, Tezuka Y, Kadota S, Watanabe H (1998) Effects of methylenechloride-soluble fraction of Japanese Angelica root extract, ligustilide and butylidenephthalide, on pentobarbital sleep in group-housed and socially isolated rats. *Life Sci* 62: 2073–2082.
- Naito T, Kubota K, Shimoda Y, Sato T, Ikeya Y, Okada M, Maruno M (1995) Effects of constituents in a Chinese crude drug *Ligustici Chuanxiong Rhizoma* on vasoconstriction and blood viscosity. *Natural Med* 49: 288–292.
- Nakamura H, Shimoda A, Ishii K, Kadokawa T (1986) Central and peripheral analgesic action of non-acidic non-steroidal anti-inflammatory drugs in mice and rats. *Arch Int Pharmacodyn Ther* 282: 16–25.
- Peng HY, Du JR, Zhang GY, Kuang X, Liu YX, Qian ZM, Wang CY (2007) Neuroprotective effect of Z-ligustilide against permanent focal ischemic damage in rats. *Biol Pharm Bull* 30: 309–312.
- Song ZH, Ji ZN, Lo CK, Dong TT, Zhao KJ, Li OT, Haines CJ, Kung SD, Tsim KW (2004) Chemical and biological assessment of a traditional chinese herbal decoction prepared from *Radix Astragali* and *Radix Angelicae Sinensis*, orthogonal array design to optimize the extraction of chemical constituents. *Planta Med* 70: 1222–1227.
- Sun SR, Huang X, Zhang L (2002) Advances in studies on pharmacokinetics, pharmacodynamics and clinical research of Suxiao Jiuxin Wan. *Chinese Traditional Herbal Drugs* 33: 89–91.
- Xie XQ, Zhan K, Yin RL, Yang LH (2007) Progress of *Chuanxiong Essential Oil*. *Lishizhen Med Materia Medica Res* 18: 1508–1510.
- Yan R, Lin G, Ko NL, Tam YK (2007) Low oral bioavailability and pharmacokinetics of senkyunolide A, a major bioactive component in *Rhizoma Chuanxiong*, in the rat. *Therap Drug Monitor* 29: 49–56.
- Yan R, Ko NL, Li SL, Tam YK, Lin G (2008) Pharmacokinetics and metabolism of ligustilide, a major bioactive component in *Rhizoma Chuanxiong*, in the rat. *Drug Metab Dispos* 36: 400–408.