Strong Antinociceptive Effect of Incarvillateine, a Novel Monoterpene Alkaloid from *Incarvillea sinensis*

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Incarvillea sinensis is a wild plant distributed in northern China. The dried whole plant has been traditionally used to treat rheumatism and relieve pain as an ancient Chinese crude drug. To investigate its antinociceptive activity, we evaluated several fractions derived from the methanolic extract of *Incarvillea sinensis* in the formalin-induced pain model in mice. Incarvillateine, a novel monoterpene alkaloid, has been found to show significant antinociceptive activity. Here we report the antinociceptive activity of incarvillateine and compare its activity with that of morphine. Additionally, we suggest that its action may be related to influence on the central opioid pathways.

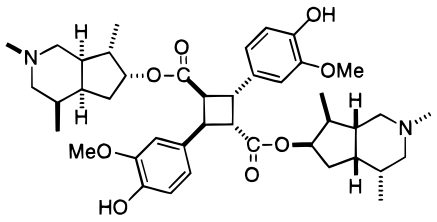
The whole dried plant, *Incarvillea sinensis*, has been used in traditional Chinese medicine as a drug ["Jiaohao (kakko)" or "Cheron"] to treat rheumatism and relieve pain. Although we have previously reported the isolation and structure elucidation of many new alkaloids from *Incarvillea sinensis* Lam. (Bignoniaceae),[1−6] including the monoterpene alkaloid, incarvilline, as well as monoterpene- and C-6−C-3-conjugated alkaloidal derivatives. No studies of the pharmacological properties of this plant have been undertaken.

To verify the analgesic activity of the plant, a suspension of several fractions was administered to mice prior to formalin injection, under the surface of the right hindpaw. Then the time of their pain reaction (paw licking) was measured. These fractions were prepared by extracting the dried whole part of *I. sinensis* (18 kg) with MeOH, followed by reversed-phase column chromatography of the extract.

The formalin-induced licking response has been used as a model for evaluating new analgesics.[7−10] The duration of these nociceptive responses induced by formalin can be divided into two phases. The first phase is from 0 to 10 min after formalin injection, and the second phase is from 10 to 30 min after the injection. These phases have obvious differential properties. The pain of the early phase is evoked by the direct stimulation of the nerve fibers; that of the later phase is due to an inflammatory reaction. Centrally acting drugs such as morphine inhibit both phases equally. On the other hand, peripherally acting drugs, such as aspirin, inhibit only the later phase.

The 60% methanol eluent from Diaion HP-20 chromatography of the extract was injected subcutaneously 10 min prior to 1.0% formalin injection and exhibited significant antinociceptive effect in both phases of the formalin-induced paw-licking response. An active principle was isolated from this fraction by Si gel column chromatography and, by means of nuclear magnetic resonance and mass spectrometry, was identified as incarvillateine, a monoterpene alkaloid derivative. Although we had previously isolated this compound from the plant, its biological effects have not been previously described.[1]

Purified incarvillateine (1) was injected intraperitoneally 10 min prior to 1% formalin injection and was found to produce graded inhibition of both the neurogenic (early phase) and inflammatory (later phase) phases in a dose-related manner (Figure 1). In comparison with antinociceptive effects of different doses of incarvillateine and morphine, the ED_{50} values of incarvillateine were about 1.06 (early phase) and 1.33 (later phase) times lower than those of morphine (Table 1). In addition, the incarvillateine-induced early phase was partially reversed by pretreatment with naloxone (5 mg/kg, sc), although the effect of morphine was completely reversed (Figure 2). These results suggest the possibility that its action may be, in part, related to influence on the central opioid pathways.



incarvillateine (1)

In conclusion, incarvillateine displayed potent antinociceptive action that was partially blocked by naloxone, indicating an interaction with central opioid mechanisms. These results suggest that incarvillateine may be a new type of antinociceptive agent with a different mechanism of action from that of morphine. Further investigation is required to elucidate the exact mechanisms underlying these effects.

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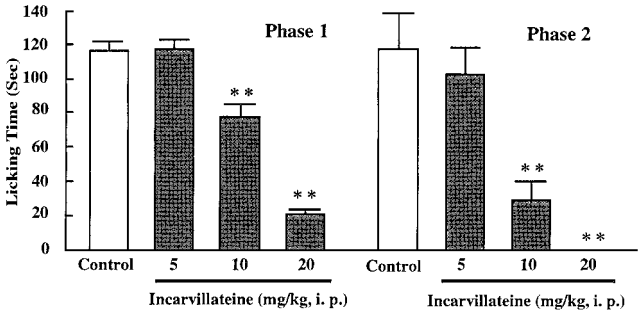

Figure 1. The effects of incarvillateine (**1**) on the early and later phases of formalin-induced pain response in mice. Each value represents mean ± SE ($n = 10$). Significant differences between control and drug treated groups are indicated by **<0.01.

Table 1. Comparison of ED_{50} Values of Morphine and Incarvillateine (**1**) on the Early and Later Phases of Formalin-induced Pain[a]

	ED_{50} (mmol/kg)		relative potency
	morphine	incarvillateine	M/I
early phase	0.0184**	0.0174**	1.06
later phase	0.0104**	0.0078**	1.33

[a] The effects of morphine and incarvillateine on the early and later phases of formalin-induced pain response in mice ($n = 10$). Significant differences between control and drug treated groups are indicated by **<0.01.

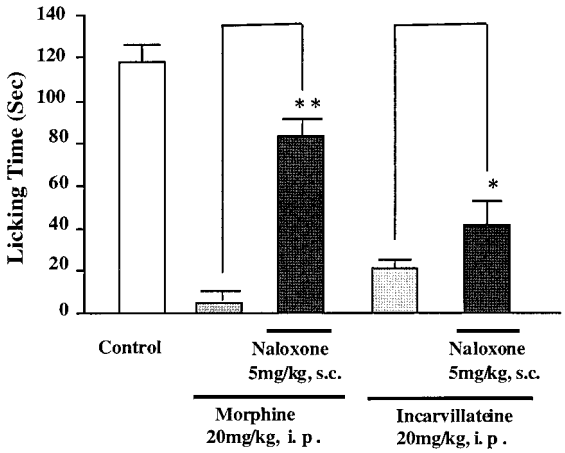

Figure 2. The effects of incarvillateine on the early phases (neurogenic) of formalin-induced pain response in mice. Each value represents mean ± SE ($n = 10$). Significant differences between drug treated groups and naloxone pretreatment groups are indicated by *<0.05, **<0.01.

Experimental Section

Plant Material. The aerial parts of *I. sinensis* were collected at Wuan County, Hebei Province, China, in August 1986, and authenticated by Prof. W.-M. Yan.

Extraction and Isolation. Aerial parts (1.25 kg) were extracted twice with MeOH at room temperature, and the combined extracts were concentrated to a syrup at 60 °C. The syrup was dissolved in H_2O and applied to a reversed-phase column of Diaion HP-20 (Mitsubishi materials), which was then eluted with gradient of H_2O−MeOH, which resulted in seven fractions. Incarvillateine (**1**) was derived from the active fraction (60% MeOH eluate) performed by Si gel chromatography and eluted with cyclohexane−MeOH−Et_2NH (45:1:1).

Recrystallization and X-ray Analysis of Incarvillateine (1). Incarvillateine (**1**) was repeatedly recrystallized from MeOH. The alkaloid crystallized in the triclinic system, space group *P*1. The crystallographic parameters are: a = 6.585(3) Å, b = 9.270(5) Å, c = 17.244(7) Å, α = 82.03(4)°, β = 96.61(3)°, γ = 109.05(4)°, V = 982.7(8) Å^3, D_c = 1.213 g/cm^3, Z = 1.2177; observed reflections were refined to R = 0.08. Because the absolute structure of the monoterpene unit, named incarvilline, was established previously,[5] the absolute configuration of incarvillateine (**1**) was determined by X-ray crystallography.

Formalin Test and Treatments. This method represented a modification of that described by Dubuisson and Dennis.[7] Mice (ddY-male) weighing 25 ± 5 g were used in all experiments. The tested drugs were prepared as suspensions with 0.5% Tween 80−saline. Various fractions (120 mg/kg, sc), incarvillateine (5, 10, 20 mg/kg, ip), and morphine (2.5, 5, 10 mg/kg, ip) were administered 10 min prior to the injection of an inducer (1% formalin−saline, 20 mL). The mice were observed for 30 min, and the time that the mice spent licking the injected right hindpaw was recorded. Because this test has biphasic pain response with two peaks, from 0 to 10 min (first phase) and from 10 to 30 min (second phase), the time spent licking the injected paw was recorded, and the data were expressed as total licking time in the first phase and the second phase. In some experiments, naloxone (5 mg/kg, sc) was administered 10 min before treatment in order to evaluate the potential involvement of morphine receptors.[11−13]

Statistical Analysis. The data are shown in mean ± SE ($n = 10$). The Dunnett's test was employed to determine the significance of differences between reference and experimental samples.

References and Notes

(1) Chi, Y. M.; Yan, W. M.; Chen, D. C.; Noguchi, H.; Iitaka, Y.; Sankawa, U. *Phytochemistry* **1992**, *31*, 2930−2932.
(2) Chi, Y. M.; Yan, W. M.; Li, J. S. *Phytochemistry* **1990**, *29*, 2376−2378.
(3) Chi, Y. M.; Hashimoto, F., Yan, W. M.; Nohara, T. *Phytochemistry* **1995**, *40*, 353−354.
(4) Chi, Y. M.; Hashimoto, F.; Yan, W. M.; Nohara, T. *Phytochemistry* **1995**, *39*, 1485−1487.
(5) Chi, Y. M.; Hashimoto, F.; Yan, W. M.; Nohara, T.; Yamashita, M.; Marubayashi, N. *Chem. Pharm. Bull.* **1997**, *45*, 495−498.
(6) Chi, Y. M.; Hashimoto, F.; Yan, W. M.; Nohara, T. *Phytochemistry* **1997**, *46*, 763−769.
(7) Dubuisson, D.; Dennis, S. G. *Pain* **1977**, *4*, 161−174.
(8) Brown, J. H.; Kissel, J. W.; Lish, P. M. *J. Pharmacol. Exp. Ther.* **1968**, *160*, 231−242.
(9) Hunskaar, S.; Hole, K. *Pain* **1987**, *30*, 103−114.
(10) Shibata, M.; Ohkubo, T.; Takahashi, H.; Inoki, R. *Pain* **1977**, *38*, 347−352.
(11) Jasinski, D. R.; Martin, W. R.; Haertzen, C. A. *J. Pharmacol. Exp. Ther.* **1967**, *157*, 420−426.
(12) Smits, S.; Takemori, A. E. *Br. J. Pharmacol.* **1970**, *39*, 627−638.
(13) Takemori, A. E.; Hayashi, G.; Smits, S. E. *Eur. J. Pharmacol.* **1972**, 20, 85−92.

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