Kappa-Opioid Receptor-Mediated Antinociceptive Effects of Stereoisomers and Derivatives of (+)-Matrine in Mice

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Abstract: The antinociceptive effects of seven matrine-type lupin alkaloids were examined using the acetic acid-induced abdominal contraction test (the writhing test) and the tail-flick test in mice. (+)-Allomatrine, the C-6 epimer of (+)-matrine, produced the antinociceptive effect at 1/3 potency of (+)-matrine or pentazocine. It was demonstrated that the antinociceptive effects of (+)-allamatrine were mediated through the activation of κ-opioid receptors, while the antinociceptive effect of (+)-matrine was mediated by both μ- and κ-opioid receptors. (-)-Sophoridine, the C-6 epimer of (+)-matrine, (+)-sophoranol, (-)-14β-hydroxyomatrine and (+)-matrine N-oxide, which possess a hydrophilic group, and (-)-sophocarpine and (-)-sophoramine having a double bond(s) did not show significant antinociceptive activity.

Key words: Sophora, Leguminosae, lupin alkaloid, derivatives of (+)-matrine, antinociception, κ-opioid receptor.

Abbreviations:
β-FNA: β-fumaltrexamine
Nor-BNI: nor-binaltorphimine
NTI: naltrindole

Introduction
The plants of the genus Sophora (subfamily Papilionaceae in the family Leguminosae) range from the temperate to the tropic areas in the world. Some of the Sophora plants are important sources of Chinese drugs, such as Ku-shen (roots of S. flavescens Ait.), Shan-dou-gen (roots of S. tonkinensis Gagnep.), and Ku-dou-zhi (roots of S. alopecuroides L.), which have been used mainly for the treatment of fever, inflammation, edema and pain (1). Phytochemical investigations have revealed that the plants accumulate lupin alkaloids, especially matrine-type alkaloids (2). During the course of our investigation on relationships between the medicinal application and alkaloid components in Chinese drugs, we have recently reported that a typical matrine-type lupin alkaloid (+)-matrine (1) possesses an antinociceptive potency which is identical to that of pentazocine and is produced mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors (3).

This paper describes the evaluation on the antinociceptive effects of two stereoisomers, (+)-allomatrine (2) and (-)-sophoridine (3), two hydroxy derivatives, (+)-sophoranol (6) and (-)-14β-hydroxyomatrine (7), two dehydro derivatives, (-)-sophocarpine (4) and (-)-sophoramine (5) of (+)-matrine (1), and (+)-matrine N-oxide (8) (Chart 1) in mice.

Materials and Methods
General experimental procedures
^1H-NMR spectra were recorded on a JEOL JNM-GSX 270 (1H: 270 MHz). Optical rotations were measured on a JASCO DIP-181 polarimeter. TLC was carried out on silica gel 60 plates (0.25 mm). Analytical HPLC was performed on a LiChrosorb SI 60 (Merck, 5 μm, i.d. 4 × 250 mm) column, using a UV detector.

Plant material
The seeds of S. alopecuroides L. were collected in Ning-Xia province of China in August 1993 and identified by Prof. Jia-Shi Li, Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine. A voucher specimen (No. 1022) is deposited in the Herbarium of Beijing University of Traditional Chinese Medicine.

Isolation of lupin alkaloids
The dry seeds of S. alopecuroides (1 kg) were extracted with 75% MeOH three times at room temperature. The aqueous concentrate was acidified with 10% HCl to pH 3 and the resulting precipitate was filtered off. The filtrate was ex-
trated three times with Et₂O. The aqueous layer was made strongly alkaline with K₂CO₃ and extracted with CH₂Cl₂ three times. The CH₂Cl₂ extracts were combined, dried over K₂CO₃ and concentrated in vacuo to give the crude base (49 g, 5/5 plant material). The crude alkaloid mixture (15 g) was chromatographed on silica gel (Wakogel C300, Wako, 450 g) with a solvent gradient of CH₂Cl₂-MeOH-25% NH₄OH (500 : 5 : 1) to yield (-)-14β-Hydroxymatrine (1) (500 mg, 2.0 mmol) with a solvent gradient of CH₂Cl₂-MeOH (95 : 5 : 0.5). The elution volume of each fraction was 250 ml. The fractions 2 (1.1 g) and 3 (0.3 g) were combined and separated by silica gel column chromatography (Merck, 100 g) column chromatography with CH₂Cl₂ : MeOH : H₂O : 25% NH₄OH (500 : 5 : 1 : 0.5) to give four fractions (500 ml elution volume each). The 2nd (1 g) and 3rd (0.4 g) fractions were combined and then separated by Al₂O₃ (aluminium oxide 90, Merck, 100 g) column chromatography with CH₂Cl₂ : Me₂CO : CH₃OH (34 : 3 : 3), the elution volume of each fraction was 100 ml. (-)-Sophoridine [3, 520 mg, [α]₂D: −60° (c 0.3, EtOH)] was obtained from the fractions 3-6. (-)-Sophaocarpine [4, 2.0 mg, [α]₂D: −29° (c 0.5, EtOH)] was isolated from the crude base (15 g) obtained from seeds (540 g) of S. vicilfolia Hance by the similar manner to that reported previously (5). (+)-Matrine (1), (+)-Matrinine N-oxide (8) were isolated from the roots of S. tonkinensis Gagnep (750 g) according to the methods that have been reported previously (4). Alkaloids obtained were identified by direct comparison with authentic samples (TLC, co-HPLC, ¹H-NMR (6) and [α]D).

Preparation of (+)-allomatrine (2) by isomerization of 1 (7)

(+)-Matrine (1) (500 mg, 2.0 mmol) was dissolved in 25 ml of water, and then PtO₂ (60 mg, 0.26 mmol) was added. The mixture was heated at 95-98°C under vigorous stirring in 1 atm of H₂ for 12 h. The catalyst was removed by filtration and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (50 g) with 7% MeOH in ether; 25% NH₄OH (400 : 2) and recrystallized from benzene to give 2 [452 mg, [α]₂D: + 38° (c 0.5, H₂O)] as a white amorphous solid and was identified with authentic sample (TLC, ¹H-NMR (6) and [α]D).

Synthesis of (−)-14β-hydroxymatrine (7) (4)

A 1.6 M solution of n-BuLi in hexane (0.6 ml, 0.96 mmol) was added to a solution of diisopropylamine (33.6 ml) in THF (3 ml) at 0°C with stirring. After several minutes, a solution of 1 (51 mg, 0.12 mmol) in THF (2 ml) was added to the LDA solution at 0°C. The reaction mixture was stirred for 10 min, and then exposed to dry O₂ gas. An aqueous solution of NaHSO₃ was added, stirred for 10 min, made alkaline with K₂CO₃, and extracted three times with CH₂Cl₂. The organic layers were combined, dried over K₂CO₃ and evaporated in vacuo. The residue was subjected to middle pressure column (Merck, Lobar, Art. 10400, 1 x 25 cm) chromatography with 4% MeOH in ether; H₂O: 25% NH₄OH (500 : 5 : 1.5) to give (−)-14β-hydroxymatrine [16 mg, [α]₂D: −26° (c 0.1, EtOH)] in 18% yield.

Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 30-35 g (6 weeks old) were used. They were kept in groups of ten and had free access to food and water in an animal room that was maintained at 24 ± 1°C with a 12 h light-dark cycle (lights on 8:00 a.m. to 8:00 p.m.).

Acetic acid-induced abdominal contraction assay (Writhing test)

Each mouse was injected i.p. with 0.7% acetic acid in a volume of 10 ml/kg, 30 min after administration of the test drug. After 10 min, the animals were observed for an additional 10 min, during which the abdominal contractions were counted. The number of abdominal contractions in each test period was normalized to the mean number shown by the control group. Percent antinociception was expressed as: 100 × (mean control responses – test responses) / (mean control responses).

Tail-flick assay

The intensity of the thermal stimulus was adjusted so that the animal flicked its tail in 3 - 4 s. A cut-off latency of 15 s was used to prevent injury to the tail. Animals which did not respond within 15 s were removed and assigned a score of 15 s. Percent antinociception was calculated for each animal using the formula: 100 × (post-drug latency – pre-drug latency) / (15 – pre-drug latency).

Drugs

Drugs were dissolved or diluted in saline. µ-Opioid receptor selective antagonist β-FNA (20 mg/kg, s.c.) was injected 24 h before injection of each alkaloid. δ-Opioid receptor selective antagonist NalBNI (20 mg/kg, s.c.) was injected before each alkaloid injection. The other assays were the same as in the section on tail-flick assay. The dose and schedule for each opioid antagonist in this study were determined as described previously (3).

Results

Matrine (1), (+)-sophoranol (6, 5-hydroxymatrine) and (+)-matrine N-oxide (8) were isolated from the roots of S. tonkinensis Gagnep as reported in our previous paper (4). (-)-Sophaocarpine (4) (13,14-didehydromatrine) was isolated from the seeds of S. vicilfolia Hance as reported in our previous paper (5). (-)-Sophoridine (3), which is the C-5 epimer of 1, and (-)-sophaocarpine (5, 11,12,13,14-tetradehydomatrine) were isolated from the seeds of S. olpeculoides. (+)-Allomatrine (2), which is the C-6 epimer of 1, and (-)-14β-Hydroxymatrine (7) was prepared by isomerization (7) and oxidation (4) of 1 in yields of 90 % and 18 %, respectively.

The antinociceptive activities of alkaloids 2-8 were evaluated using the acetic acid-induced abdominal contraction test in mice, in which the test samples were administered subcutaneously (Fig.1). Pentazocine and (+)-matrine (1) were also tested for comparative purpose. The stereoisomers of 1, (+)-allomatrine (2) and (-)-sophoridine (3), and the hydroxyl
derivative, (+)-sophoranol (6) showed dose-dependent antinociception. The antinociceptive potencies of alkaloids 2, 3 and 6 were approximately 3-, 3- and 10-fold less than that of 1, respectively. Figure 2a shows the time courses of the antinociception produced by alkaloids 2 and 3 in the tail-flick assay. The antinociceptive potency reached its peak 30 minutes after administration and then gradually decreased. Figure 2b shows the dose-response lines for the antinociception. (+)-Matrine (1, 10–100 mg/kg, s.c.) and (+)-allomatrine (2, 30–180 mg/kg, s.c.) produced dose-dependent antinociception. The antinociceptive potency of 2 was less than that of 1 and the potency ratio (95% confidence interval) of 1 versus 2 was 2.9 (1.6–5.1). (-)-Sophoridine (3) had no significant effect on the tail-flick latency at doses of 10 and 30 mg/kg, s.c. Furthermore, 3, at doses of higher than 100 mg/kg, s.c., exhibited toxic effects, such as wild running, convolution and death.

The effects of β-FNA, NTI and Nor-BNI, a selective μ-, δ- and κ-opioid receptor antagonist, respectively, on the antinociceptive activity of (+)-allomatrine (2) in the tail-flick assay are summarized in Figure 3. When Nor-BNI was administered 3 h before the administration of 2, the antinociceptive activity of 2 was markedly antagonized. However, either β-FNA or NTI had no significant effect on the antinociception of 2.

**Discussion**

Introduction of a hydrophilic substituent such as a hydroxy (6 and 7) and an N-oxide group (8) into (+)-matrine (1) negatively influenced the antinociceptive activities of 1. The presence of a double bond(s) (4 and 5) in 1 also markedly decreased the activities. One of the stereoisomers of 1, (+)-allomatrine (2) revealed significant antinociceptive effects. The antinociceptive potency of 2 was reduced to about 1/3 relative to that of 1 but selectivity for κ-opioid receptors...
increased in comparison of that of 1. Another stereoisomer of 1, (-)-sophoridine (3), did not show significant effects for antinociception in the tail-flick test, though 3 exhibited the activity identical to that of 2 in the acetic acid-induced abdominal contraction test. Thus, it appears that stereoisomerism and lipophilicity influence the antinociceptive potency of matrine-type alkaloids, especially the stereoisomerism seemed to be related to the selectivity for the \( \kappa \)-opioid receptors.

(+)-Matrine (1) is a main alkaloid of the Sophora plants described above which have been used as the Chinese drugs, and 2 is a minor one in the plants (4, 5, 8). Thus, the active principle for analgesic activity of the Chinese drugs, Ku-shen, Shan-du-gen, etc. might be considered to be (+)-matrine (1).

On the other hand, there are requirements for development of selective agonists for the \( \kappa \) or \( \delta \)-opioid receptors, because they have been suggested to produce good analgesic activity without the side-effects associated with the use of \( \mu \)-receptor agonists, such as (-)-morphine and its derivatives (9-11). The selective \( \kappa \)-opioid receptor agonists found up to the present are structurally divided into two classes. One is a group which possesses the framework of (-)-morphine, such as ketocyclazocine, KT-95 (12), and TRK-820 (13), the other is a group which has the N-C-C-N (sp\(^2\)) pharmacophore sequence (11), such as U-50, 488H, ICI 199441, and PD 117302 (Chart 2). But it was reported that the latter group had a different type of side effects (14). The framework of (+)-matrine (1) and (+)-allomatrine (2) is evidently different from those of the above two groups and hence it is of interest as a new type of selective antinociceptive agents mediated by \( \kappa \)-opioid receptors. Modifications of the parent structure are currently under investigation to improve both potency and selectivity and to get detailed information on structure-activity relationships.

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References


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