Possible Mechanism Involved in the Antinociceptive Activity of Dimer of Paederosidic Acid and Paederosidic Acid Methyl Ester in Mice

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The roots, leaves, barks, and fruits of *Paederia scandens*, which belongs to the family Rubiaceae, have been traditionally used as folklore medicine to treat inflammation-related disease, toothache, and chest pain in many countries in Asia for thousands of years [1]. Recently, many dimeric iridoid glycosides and iridoid glycosides, such as paederoside and dimer of paederosidic acid and paederoside, have been isolated from *P. scandens* [2]. These compounds possess many activities, for example, anticancer and anti-inflammation activities [2]. Despite that *P. scandens* could be used as analgesic in folklore medicine and there have been a few literatures about the antinociception and the possible mechanisms, the compounds related to the antinociceptive activity in this plant remain unknown.

Since 2006, these compounds contributed to antinociception, which were isolated from *P. scandens*, have been sifting by the method of high throughput screening. Fortunately, these compounds, including paederosidic acid methyl ester and dimer of paederosidic acid and paederosidic acid methyl ester (DPAPAME; Figure 1A), showed significant antinociception in our prelimi-

nary experiments. However, there has been no literature about the antinociception of DPAPAME since it was isolated. Now, the effects of this compound on various stimuli-induced pain were evaluated to demonstrate the antinociception and the underlying mechanism.

Each experimental group included 16 ICR mice (18–22 g). These mice were housed at the room temperature (TM) and took up freely Purina chow and water. Before the experiment, all the experimental animals were fastened for 10 h. These protocols complied with the recommendations of International Association for the study of pain [3].

To evaluate the analgesic activity of DPAPAME, the formalin and hot-plate test were performed. In formalin test, according to the procedure described [4], the experimental mice were injected intraperitoneally with DPAPAME (10, 20, and 40 mg/kg) or the equivalent volume of vehicle, and morphine (10 mg/kg, s.c.) was used as a positive control drug. Thirty min later, all the mice were given 20 μ L of 5% formalin into the right hind paw. The duration of paw licking as an index of painful response was determined at

0–5 min (early phase, neurogenic) and 20–30 min (late phase, inflammatory) after formalin injection. The results showed that DPAPAME (20 and 40 mg/kg) could effectively inhibit the pain response in both phases (Figure. 1B). The character of the formalin model is that it could elucidate pain in its central or peripheral components [5]. Central analgesic drugs could inhibit both phases; while peripheral ones could suppress mainly in the later phase [6]. Therefore, it is considered reasonably that DPAPAME possessed the significant central-acting analgesic property, not peripheral-acting one.

The hot-plate test [7] was performed using an automatic apparatus (model YLS-6B, China), maintained at $54 \pm 0.5^\circ\text{C}$. These mice, which exhibited initial nociceptive threshold between 5 and 30 s, were applied in this experiment. The latency to first sign of hind paw licking or jumping to avoid heat nociception was taken as an index of nociceptive threshold. In this test, pretreatment latencies were determined three times with intervals of 20 min. The groups of mice were pretreated with DPAPAME or vehicle and 30 min later, the measurement started. A morphine-treated (10 mg/kg, s.c. 30 min before the test) animal group was included as positive control. The cutoff time was 60 s in the hot-plate test to minimize skin damages. The results showed that DPAPAME (40 mg/kg) could significantly enhance the threshold of pain response (Figure. 1C). The hot-plate test were predominantly a spinal reflex and were considered to be selective for centrally acting analgesic compounds; while peripheral analgesic was known to be inactive on this kind of painful stimulus [8]. Therefore, this result supported further that DPAPAME could act as a central analgesic drug.

The effect of DPAPAME on spontaneous locomotor activity and exploratory behavior was assessed by the open-field test [9]. The number of rearing responses, the number of areas crossed by all paws, and the total time spent on being immobilized (immobility) were recorded. 30 min before the test, the groups of mice were pretreated with the compound or vehicle. A diazepam-treated (1.0 mg/kg, i.p.) animal group was included as positive control. DPAPAME (10, 20, and 40 mg/kg) did not affect the motor coordination in mice. The mean permanence time of animals and the length of the route in the apparatus, obtained in the DPAPAME-treated groups, were not statistically different from those of vehicle-treated control group over 5-min period. Only diazepam (1.0 mg/kg, i.p.) significantly ($P < 0.01$) affected the mobile performance in comparison with the control group (data were not showed).

To explore further the possible mechanisms, we had examined the effects of naloxone (nonselected opioid receptor antagonist), glibenclamide (blocker of ATP-sensitive K^+ channels), methylene blue (endogenous guanylyl cyclase inhibitor), and yohimbine ($\alpha 2$ -adrenoceptor antagonist) on the antinociceptive activity of DPAPAME in the hot-plate test. Mice were treated with DPAPAME (40 mg/kg, i.p.), the vehicle (10 mL/kg, i.p.) 30 min before the test. Naloxone (1 mg/kg, s.c.), glibenclamide (2 mg/kg, i.p.), methylene blue (10 mg/kg, i.p.), and yohimbine (2 mg/kg, i.p.) were administered 15 min before DPAPAME, diazoxide, or morphine. Moreover, in the combination study, diazoxide (2 mg/kg i.p.), DPAPAME (10 mg/kg i.p.) in combination with diazoxide (1 mg/kg i.p.) were administered 30 min before the hot-plate test, respectively.

Figure 1 (A) The Chemical Structure of dimer of paederosidic acid and paederosidic acid methyl ester (DPAPAME). (B) Effect of DPAPAME and morphine on formalin-induced nociception in mice. The total time spent in licking the injected hind-paw was measured in the early phase (0–5 min, white column) and the late phase (20–30 min, black column). The vehicle (10 mL/kg) or DPAPAME (10, 20 and 40 mg/kg) was administered intraperitoneally and morphine (10 mg/kg) subcutaneously. DPAPAME and morphine was administered 30 min before the test. Each column represented the mean $\pm$ SEM ($n = 16$). * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$ versus vehicle (ANOVA followed by Dunnett's test). (C) Effects of DPAPAME and morphine on thermal-induced anti-nociception in the hot-plate test. The vehicle (10 mL/kg) or DPAPAME (10, 20 and 40 mg/kg) was administered intraperitoneally and morphine (10 mg/kg) subcutaneously. DPAPAME or morphine was administered 30 min before the test respectively, and the time in seconds (s) of first sign of hind paw licking or jump response to avoid heat nociception was recorded. Cut-off time was 60 s. Each column represented the mean $\pm$ SEM ($n = 16$). *** $P < 0.001$ versus vehicle (ANOVA followed by Dunnett's test).

Table 1 Effects of dimer of paederosidic acid and paederosidic acid methyl ester (DPAPAME) administered in combination with drugs on the hot-plate test

Drug	Dose (mg/kg)	Duration on the hot plate (s)
Vehicle	0	15.3 ± 1.22
Naloxone	1	16.6 ± 3.75
Methylene Blue	10	15.7 ± 6.06
Yohimbine	2	16.2 ± 2.65
Glibenclamide	2	15.3 ± 1.66
Morphine	10	50.1 ± 3.38**
Diazoxide	2	35.2 ± 4.36**
Morphine + Naloxone	10 + 1	17.6 ± 2.43 [#]
Diazoxide + Glibenclamide	2 + 2	14.7 ± 1.43 [#]
DPAPAME	40	39.6 ± 5.38**
DPAPAME + Naloxone	40 + 1	43.2 ± 5.39**
DPAPAME + Methylene Blue	40 + 10	38.7 ± 5.28**
DPAPAME + Yohimbine	40 + 2	37.8 ± 6.06*
DPAPAME + Glibenclamide	40 + 2	15.4 ± 1.54 [#]

Mice were treated with DPAPAME, morphine, diazoxide, the vehicle, methylene blue, yohimbine, glibenclamide, or naloxone, respectively, 30 min or 45 min before the hot-plate test. In combination, mice were treated with DPAPAME or the vehicle 15 min after methylene blue, yohimbine, glibenclamide, or naloxone was administered. Thirty min later, the test started. Each group represents the mean ± SEM (n = 16). **P* < 0.01 or ***P* < 0.001 versus vehicle. [#]*P* < 0.01 versus DPAPAME or positive drug (ANOVA followed by Dunnett's test).

When used alone, naloxone, methylene blue, glibenclamide, and yohimbine failed to modify the thermal-induced nociceptive responses in a significant manner. In the naloxone antagonism test, naloxone was able to reverse significantly the antinociceptive activity of morphine (10 mg/kg s.c.), but not sensitive to that of DPAPAME. As we know, the dose of naloxone (1.0 mg/kg, s.c.) was high enough to block opiate receptors [10]. In the present study, naloxone was completely inactive as an antagonist, thus precluding involvement of the central opiate mechanism in the antinociception of DPAPAME.

As we know, ATP-sensitive K⁺ channels played a key role in central and peripheral antinociception. The activity of ATP-sensi-

Table 2 Effect of dimer of paederosidic acid and paederosidic acid methyl ester (DPAPAME) administered in combination with diazoxide on the hot-plate test

Drug	Dose (mg/kg)	Duration on the hot plate (s)
Vehicle	0.	14.8 ± 1.05
Diazoxide	2.	40.2 ± 3.30*
Diazoxide	1	18.2 ± 2.15
DPAPAME	10	20.1 ± 1.70
DPAPAME + Diazoxide	10 + 1	40.6 ± 3.80* [#]

Each group represents the mean ± SEM (n = 16). **P* < 0.001 versus vehicle. [#]*P* < 0.001 versus DPAPAME (ANOVA followed by Dunnett's test).

tive K⁺ channels was regulated not only by intracellular ATP concentration but also by G protein $\beta\gamma$ -subunits through activation of several G protein-coupled receptors, such as α_2 -adrenergic and opioid receptors [11]. In the antagonism test, the yohimbine and methylene blue failed to inactivate the antinociception of DPAPAME. However, glibenclamide at the dose of 2 mg/kg could modify the analgesic activity of DPAPAME and diazoxide. It indicated that DPAPAME might enhance pain threshold by activation directly of ATP-sensitive K⁺ channels, not activating indirectly these channels through G protein-coupled receptors. In addition, DPAPAME (10 mg/kg), in combination with diazoxide (1.0 mg/kg), could exhibit the potential antinociception in the hot-plate test. However, DPAPAME (10 mg/kg) or diazoxide (1 mg/kg) could not show the analgesic activity in itself. The synergic result further confirmed that DPAPAME could enhance the threshold of pain by activating the ATP-sensitive K⁺ channels in the brain and spinal cord level (Tables 1 and 2).

In summary, this study has elucidated that DPAPAME might exhibit significant central analgesic activity by activating ATP-sensitive K⁺ channel in the animal models of chemical and thermal-induced nociception, which merited to be explored further.

Conflict of Interest

The authors declare no conflict of interest.

References

- Zhu W, Pang M, Dong L, et al. Anti-inflammatory and immunomodulatory effects of iridoid glycosides from *Paederia scandens* (LOUR.) MERRILL (Rubiaceae) on uric acid nephropathy rats. *Life Sci* 2012;**91**:369–376.
- Wu ZJ, Wang JH, Fang DM, et al. Analysis of iridoid glucosides from *Paederia scandens* using HPLC-ESI-MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2013;**1**:54–64.
- Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;**16**:109–110.
- Sirceratawong S, Jaiyong K, Soonthornchareonnon N. Evaluation of anti-inflammatory and antinociceptive activity of triphala recipe. *Afr J Tradit Complement Altern Med* 2012;**31**:246–250.
- Neville C, Ostini R. A psychometric evaluation of three pain rating scales for people with moderate to severe dementia. *Pain Manag Nurs* 2013;**19**. doi:pii: S1524-9042 (13)00114-8. 10.1016/j.pmn.2013.08.001.
- Trongsakul S, Panthong A, Kanjanapothi D, et al. The analgesic, antipyretic and anti-inflammatory activity of *Diospyros variegata* Kruz. *J Ethnopharmacol* 2003;**85**:221–225.
- Yu J, Fang Q, Lou GD, et al. Histamine modulation of acute nociception involves regulation of Nav 1.8 in primary afferent neurons in mice. *CNS Neurosci Ther* 2013;**19**:649–658.
- Srinivasan K, Muruganandan S, Lal J, et al. Antinociceptive and antipyretic activities of *Pongamia pinnata* leaves. *Phytother Res* 2003;**17**:259–264.
- Tsuda M, Suzuki T, Misawa M, et al. Involvement of the opioid system in the anxiolytic effect of diazepam in mice. *Eur J Pharmacol* 1996;**307**:7–14.
- Yaksh TL. Pharmacology and mechanisms of opioid analgesic activity. *Acta Anaesthesiol Scand* 1997;**41**:94–111.
- Wada Y, Yamashita T, Imai K, et al. A region of the sulfonylurea receptor critical for a modulation of ATP-sensitive K⁺ channels by G-protein $\beta\gamma$ -subunits. *EMBO J* 2000;**19**:4915–4925.