ANTINOCICEPTIVE EFFECTS OF CURCUMIN DIETHYL DISUCCINATE IN ANIMAL MODELS

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ABSTRACT: Curcumin diethyl disuccinate (CurDD) is a succinate prodrug of curcumin. The antinociceptive effects of CurDD was evaluated in hot-plate, tail-flick and acetic acid-induced writhing models in mice. Hot-plate and tail-flick latencies were determined in male ICR mice prior to the administration of 0.9% normal saline solution (10 ml/kg, i.p.), morphine (10 mg/kg, i.p.), 0.5% carboxymethylcellulose (CMC; 10 ml/kg, p.o.) or various doses of CurDD (25, 50, 100 and 200 mg/kg, p.o.) and were subsequently determined at 15, 30, 45, 60, 90, 120 and 240 min. The mean percent maximum possible effect (%MPE) was calculated and used in the determination of the area of analgesia (%MPE-min). All doses of CurDD showed significant analgesic responses in the hot-plate test (p<0.05), while the lowest dose of CurDD (25 mg/kg) gave a significant analgesic response in the tail-flick test (p<0.05). In the acetic acid-induced writhing test, mice were induced with intraperitoneal injection of 0.6% acetic acid 1 hr after the oral administration of 0.5% CMC, indomethacin (10 mg/kg) or various doses of CurDD (25, 50, 100 and 200 mg/kg) and the mean writhing response was determined for 30 min. CurDD 200 mg/kg significantly decreased the mean writhing response compared to vehicle controls (p<0.05). These results demonstrated that CurDD possesses antinociceptive activity in mice and likely produced both central and peripheral analgesic responses.

Keywords: Curcumin diethyl disuccinate, Hot-plate, Tail-flick, Writhing test, Analgesic effect

INTRODUCTION: Pain is the most common reason for patients to seek medical treatment. Almost half of individuals who suffer from pain choose a nonprescription analgesic as their initial choice for pain relief. Nonopioids including acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present e.g., trauma, postoperative, cancer and arthritis pain. Non-opioids are especially effective for certain types of somatic pain e.g., muscle and joint pain, bone/dental pain, and inflammatory pain. Opioids such as morphine, fentanyl, and codeine are used to treat moderate to severe pain that does not respond to nonopioids alone[1)].

Although opioids and NSAIDs are still the mainstay of analgesic therapy, these drugs possess a number of adverse effects. For example, opioids frequently cause respiratory depression, sedation, urinary retention, constipation, nausea and vomiting while adverse effects of NSAIDs produce gastrointestinal problems (e.g., dyspepsia, ulcers, perforation and bleeding), inhibition of platelet aggregation, kidney and liver dysfunction, and hypersensitivity reaction[1)]. Therefore, the investigation of novel analgesic drugs lacking the above adverse effects are being researched as alternatives to NSAIDs and opioids.

Curcumin is an active component commonly found in the rhizome of turmeric (*Curcuma longa* L.). It possesses multifunctional pharmacological applications in a variety of diseases such as inflammation, cardiovascular disease, liver fibrosis and cancer. Current traditional Indian medicine claims the use of turmeric against biliary disorders, anorexia, coryza, cough, diabetic wounds, rheumatism and sinusitis. Chinese traditional medicine uses turmeric in diseases associated with abdominal pain[2-3)]. For analgesic purposes, Sharma *et al.* (2006) investigated the effect of curcumin in diabetic neuropathic pain. They found that chronic treatment with curcumin (15, 30, and 60 mg/kg, p.o.) for 4 weeks significantly attenuated thermal hyperalgesia and the hot-plate latencies[4)]. Tajik *et al.* (2008) studied

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the effect of chronic oral administration of curcumin (10, 20, and 40 mg/kg) once daily for 8 days on the visceral nociception induced by acetic acid in rats. The latency time to the beginning of the first writhe was significantly (p<0.05) increased and the number of writhes was also significantly (p<0.05) decreased by curcumin (20 and 40 mg/kg)[5].

Curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses[2]. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent because it possessed several disadvantages such as chemical instability and low bioavailability[2-3]. As a result, curcumin diethyl disuccinate (CurDD), a prodrug of curcumin, was synthesized to overcome the instability problems of curcumin (Figure 1). Thus, the present study was designed to investigate the antinociceptive effects of CurDD using thermal and visceral pain models in mice in order to provide information that may contribute to better evaluate the potential usefulness of CurDD in the treatment of painful conditions.

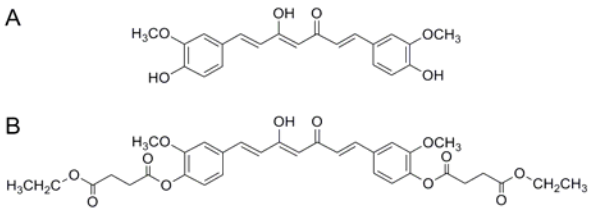


Figure 1 Chemical structures of (A) curcumin and (B) curcumin diethyl disuccinate

MATERIALS AND METHODS:

Drugs and chemicals

Curcumin diethyl disuccinate (CurDD) was synthesized in the Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. The synthesized compound was characterized by IR, MS and NMR and its purity was ca. >99%. CurDD and indomethacin (IND; Sigma Chemical Co., USA) were suspended in 0.5% carboxymethylcellulose solution (CMC; Sigma Chemical Co., USA) before use. Morphine sulphate (MO; Thai FDA) and acetic acid (Merck, Germany) were dissolved in 0.9% sodium chloride solution (NSS). MO (10 mg/kg) and IND (10 mg/kg) were used as standard analgesic drugs. Acetic acid at 0.6% (10 ml/kg) was used as an irritating agent. The control animals were given with an equivalent volume of vehicle via the same route.

Animals

Male ICR mice weighing 18-25 g from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom, Thailand served as experimental subjects in the study. The animals were housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under standard conditions of temperature (25±2°C), 50-60% of humidity and 12 hr/12 hr light/dark cycles. The animals were kept under laboratory conditions for one week prior to the start of the experiments and allowed food and water *ad libitum*. At the end of each experiment, the animals were sacrificed with carbon dioxide asphyxiation. This study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Mouse hot-plate test

Male ICR mice weighing 18-25 g were used (N=10 per group). Analgesic testing was determined using the hot-plate method. The surface of the hot-plate (Harvard Apparatus) measuring 28×28 cm was set at 55±0.5°C and surrounded by a clear Plexiglas wall cylinder, 20 cm in diameter and 30 cm in height to confine the animal to the heated surface during testing. On the day of testing, animals were randomly assigned to one of seven treatment groups and underwent 3 predrug baseline trials on the hot-plate spaced 5-10 min apart. Only those animals which had a pretreatment hot-plate latency time of less than 45 sec were utilized in these studies. Mice were then administered various treatments and retested. Each mouse was placed on the hot-plate from an elevation of 5 cm and the latency to the licking of a hind paw or vigorous jumping up

from the surface of the metal plate was used as the end point and recorded with a stopwatch. If this behavior was not observed within 45 sec the animal was removed from the hot-plate, given a score of 45 sec for its paw-lick latency and returned to its cage. The average of the last two trials served as the baseline predrug paw-lick latency.

Immediately, after the third baseline trial on the hot-plate, the drug administration took place with NSS (10 ml/kg) and MO (10 mg/kg) intraperitoneally (i.p.) or 0.5% CMC (10 ml/kg) and various doses of CurDD (25, 50, 100 and 200 mg/kg) orally (p.o.). All animals were placed on the hot-plate for 7 subsequent trials at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of hot-plate latency was expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

$$\%MPE = \frac{(\text{postdrug latency}) - (\text{predrug latency})}{(\text{cut-off time}) - (\text{predrug latency})} \times 100$$

where the cut-off time was set at 45 sec.

The area of analgesia for the hot-plate assays was derived by computing the area under the corresponding 0-240 min time-course-%MPE curves; areas were calculated using the trapezoidal rule[6].

Mouse tail-flick test

These studies employed the tail-flick assay described by D'Amour and Smith in 1941[7], with minor modifications. Male ICR mice weighing 18-25 g were used (N=10 per group). Mice were placed in individual Plexiglas restrainers with an opening to allow the tail to protrude. Each tail rested in a shallow groove housing a light sensitive sensor. A beam of radiant heat (24 V, high amperage 150-watt light bulb situated 8 cm above the tail) was aimed at the middle of a marked dorsal portion of the distal part of each subject's tail that has been blackened length 1 cm with a black ink marker pen in order to absorb the maximum amount of heat and for uniform heat absorption. The device (Harvard Tail-flick Analgesia meter) automatically recorded the latency between the onset of the light beam stimulus and the response to heat, at which point the light beam was terminated. The maximum duration of each test was set at 4.0 sec to minimize the potential for thermal injury. The stimulus intensity was set so that the baseline tail-flick latencies were approximately 1.0-1.5 sec, and the intensity remained constant throughout the experiment. Animals failing to respond within 1.5 sec were excluded from testing. On the day of testing, all animals were tested for 3 predrug tail-flick baselines conducted at 10-15 min intervals. The average score of the last two trials served as the baseline measure for each subject.

Immediately after the third baseline trial on the tail-flick test, the drugs were administered: vehicle (NSS; 10 ml/kg, i.p.), MO (10 mg/kg, i.p.), or 0.5% CMC (10 ml/kg, p.o.) and various doses of CurDD (25, 50, 100 and 200 mg/kg, p.o.). Tail-flick latencies were recorded at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of the tail-flick latency was expressed as the mean percent maximum possible effect (%MPE) according to the formula as above, with a cut-off time of 4 sec. The area of analgesia for the tail-flick assays was calculated as described above for the hot-plate assays.

Acetic acid-induced writhing test

Male ICR mice weighing 18-25 g were used (N=6 per group). Analgesic testing was determined using the acetic acid-induced writhing method described by Koster *et al.*[8] On the day of testing, animals were randomly assigned to one of seven treatment groups. Mice were then administered 0.5% CMC (10 ml/kg, p.o.), IND (10 mg/kg, p.o.) or various doses of CurDD (25-200 mg/kg, p.o.) 1 hr before administration of 0.6% acetic acid (10 ml/kg, i.p.). Each animal was placed in a transparent observational cage. The number of writhing events (abdominal constriction with hind limb extension) was observed and counted at 5 min intervals for 30 min after the acetic acid administration. Antinociceptive activity was reported as the percentage of inhibition of writhing response compared with the vehicle

control group. The percentage of inhibition of the writhing response was calculated using the following formula:

$$\% \text{ Inhibition of writhing response} = \frac{\text{Wr (control)} - \text{Wr (test)}}{\text{Wr (control)}} \times 100$$

with Wr = mean writhing response.

Analysis of data

The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) followed by a *post-hoc* Fisher LSD test for multiple comparisons. Statistical significance was assessed as $p<0.05$.

RESULTS:

Mouse hot-plate test

Morphine 10 mg/kg significantly ($p<0.01$) increased the hot-plate latency producing an area of analgesia of 10,921.68 ± 2,277.58 %MPE-min compared with that of normal saline solution (NSS) (2,066.08 ± 1,508.68 %MPE-min; Figure 2). All doses of CurDD significantly ($p<0.05$) increased the hot-plate latencies when compared to the vehicle group (Figure 3).

Mouse tail-flick test

Morphine 10 mg/kg significantly ($p<0.01$) increased tail-flick latency producing an area of analgesia of 9,303.42 ± 1,624.33 %MPE-min compared with that of normal saline solution (NSS) (-3,444.30 ± 2,350.11; Figure 4). CurDD 25 mg/kg significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 5).

Acetic acid-induced writhing test

Indomethacin (10 mg/kg) significantly ($p<0.01$) decreased the writhing response by 81.56% producing a mean number if writhes of 5.5 ± 1.54 compared with that of vehicle control (29.83 ± 2.98). CurDD 200 mg/kg significantly ($p<0.05$) decreased the number of writhes induced by acetic acid by 75.76% when compared to 0.5% CMC. Indomethacin produced the greatest degree of analgesia compared to all test groups (Figure 6).

DISCUSSION: These studies have demonstrated the antinociceptive effects of CurDD in various animal models. Antinociceptive activity was assessed utilizing thermal (hot-plate and tail-flick tests) and chemical (writhing test) models.

In order to investigate the antinociceptive properties of CurDD, hot-plate, tail-flick and writhing tests were performed in mice. The standard hot-plate test, a central analgesic activity testing model, measures two behavioral components including paw licking and jumpings which are both considered to be supraspinally integrated responses[9]. This model usually employed MO as a reference drug. MO demonstrated potent analgesic effects in this model indicating the sensitivity of this test. The significant analgesic action of CurDD was observed during the 240 min test. We also investigated the effectiveness of CurDD utilizing the mouse tail-flick technique, another central analgesic activity testing model that is believed to measure spinal reflex. MO administered i.p. produced a significant analgesic response as expected. The significant analgesic effect of CurDD (25 mg/kg) was observed during the 240 min test. The results obtained from both hot-plate and tail-flick tests indicated that CurDD has analgesic activity at both supraspinal and spinal levels.

Additionally, the acetic acid-induced writhing test which is considered a model of visceral inflammation pain was also chosen. This method is commonly used for measuring peripheral analgesic activity. Writhing responses consisted of contraction of the abdomen, twisting and turning of the trunk, and extension of the hind limbs[10]. IND, a non-steroidal anti-inflammatory drug, was used as a reference drug. Oral administration of IND (10 mg/kg) produced a significant analgesic response. CurDD 200 mg/kg demonstrated significant analgesic response, although it was less efficacious compared to IND. Additional studies are required to determine the mechanisms underlying the analgesic properties of CurDD.

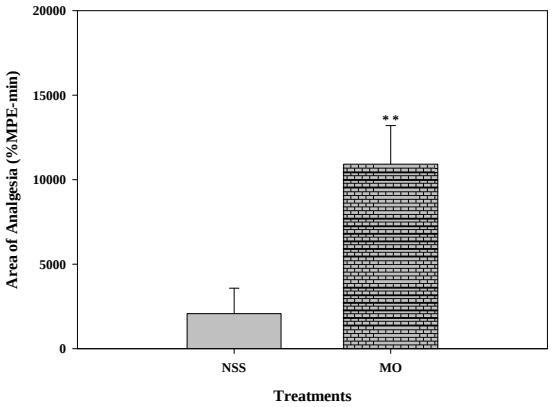


Figure 2 Mouse Hot-Plate Test. Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. $^{**}p<0.01$ significantly different compared to control.

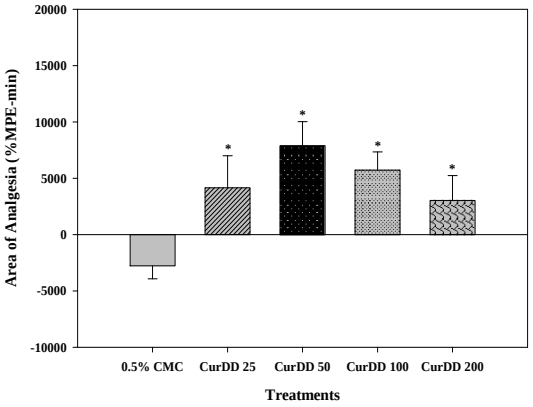


Figure 3 Mouse Hot-Plate Test. Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 0.5% CMC and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. $^{*}p<0.05$ significantly different compared to control.

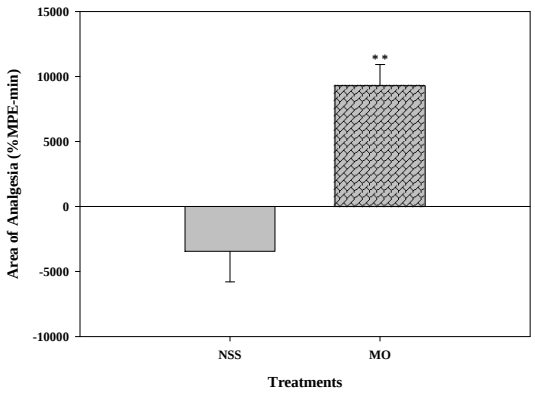


Figure 4 Mouse Tail-Flick Test. Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. $^{**}p<0.01$ significantly different compared to control.

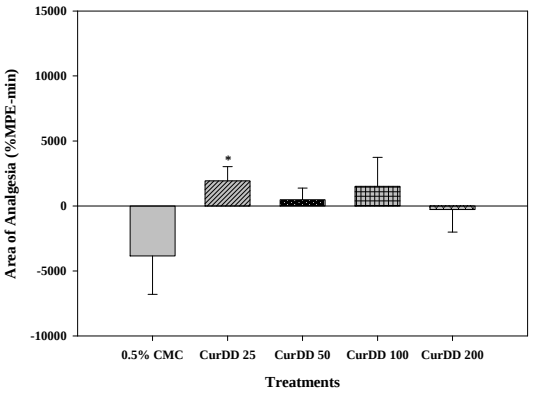


Figure 5 Mouse Tail-Flick Test. Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 0.5% CMC and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=10 for all groups. Values represent the mean±S.E.M. $^{*}p<0.05$ significantly different compared to control.

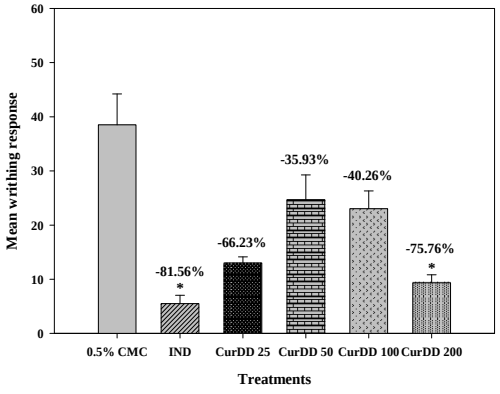


Figure 6 Acetic Acid-induced Writhing Test. Mean writhing response after oral administration of 0.5% CMC, indomethacin (IND; 10 mg/kg) and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=6 for all groups. Values represent the mean±S.E.M. $^{*}p<0.05$ significantly different compared to control. Inhibition is reported as a percentage compared to 0.5% CMC.

CONCLUSION: Curcumin diethyl disuccinate possesses antinociceptive property in both central and peripheral models of nociception in mice. Additional studies are required to better understand the mechanism responsible for the antinociceptive action of this prodrug of curcumin. The knowledge of the analgesic activity of this compound may lead to the discovery of effective and safe drugs in the treatment of different painful conditions.

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