SYNTHETIC CURCUMIN INHIBITS CARRAGEENAN-INDUCED PAW EDEMA IN RATS

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ABSTRACT: Curcumin is the main active ingredient in *Curcuma longa* L. (Turmeric) and exhibits potent antioxidative and anti-inflammatory activities. In this study, the anti-inflammatory activity of curcumin was evaluated in a carrageenan-induced rat paw edema test and compared with that of indomethacin. Rats treated with indomethacin (10 mg/kg, p.o.) and curcumin (25, 50, 100, 200 and 400 mg/kg, p.o.) showed a significant reduction in carrageenan-induced paw edema ($p<0.05$). Indomethacin inhibited edema by 46.87% and 65.71% at 2 and 3 h after carrageenan injection, respectively. The inhibitory effect of curcumin began at 2 h or later after carrageenan injection depending upon the administered dose. Low doses of curcumin (25-100 mg/kg) gave significant inhibitory effects of 30.43-34.88%, and higher doses caused significant inhibition at levels of 32.61-58.97%. The reduction of edema by indomethacin and curcumin at 2 h or more after carrageenan injection suggests that both compounds produce anti-inflammatory effects in the second phase of edema, indicating inhibition of prostaglandin synthesis.

Keywords: Anti-inflammation, Paw edema, Carrageenan, Curcumin, *Curcuma longa*, Turmeric

INTRODUCTION: The rhizome of turmeric (*Curcuma longa* L., Zingiberaceae) has been used in indigenous medicine for the treatment of inflammatory disorders since ancient times. Studies of turmeric have revealed numerous pharmacological activities, including antioxidant, anti-inflammatory, antiparasitic, antimutagenic, anticancer, chemoprotective, hepatoprotective, antimicrobial and antiviral properties[1-3]. These activities are attributable to curcuminoid compounds (curcumin, desmethoxycurcumin and bisdesmethoxycurcumin), with curcumin being the main constituent of turmeric (Figure 1).

Inflammation plays a major role in most chronic illnesses, including neurodegenerative, cardiovascular, pulmonary, metabolic, auto-immune and neoplastic diseases. The anti-inflammatory activity of turmeric has been known for centuries, and is now known to be due to the component curcuminoids[4]. These compounds have been reported to regulate a number of inflammatory mediators and enzymes such as transcription factors, cytokines, protein kinases and cyclooxygenases[4,5]. In preclinical studies, curcuminoids have been tested in acute and chronic models of inflammation in rodents[6-10]. Oral administration of curcumin to mycobacterial adjuvant-induced arthritic rats decreases the levels of an inflammatory glycoprotein, GpA-72, with concomitant lowering of paw inflammation[6,7]. Recently, Tohda *et al.* (2006) have investigated

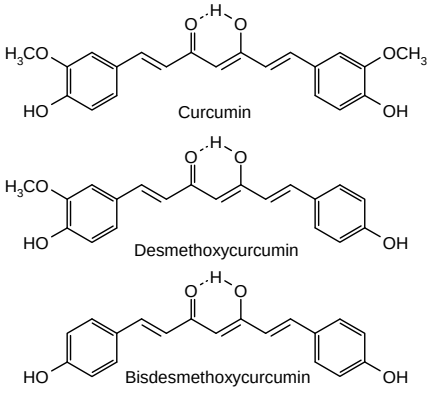

Figure 1 Structures of (A) curcumin, (B) desmethoxycurcumin and (C) bisdesmethoxycurcumin

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the anti-inflammatory activities of methanolic extracts of six *Curcuma* species using Complete Freund's adjuvant-induced arthritic mice. They found that the extract of *Curcuma longa* had no significant inhibitory effects on paw swelling[8].

Prevention of carrageenan-induced paw edema in rats and mice is frequently used for evaluation of anti-inflammatory activity. In 1982, Mukhopadhyay *et al.* studied the anti-inflammatory activities of orally administered curcumin (3-80 mg/kg) using a carrageenan-induced rat paw edema model in comparison with phenylbutazone as a positive control. At 3 h after carrageenan injection, curcumin decreased paw edema at low doses while this effect was reversed at higher doses, indicating both protective and irritant effects[9]. Nurfina *et al.* (1997) also used this animal model to further evaluate the anti-inflammatory activity of curcumin (10-80 mg/kg) after intra-peritoneal administration compared to phenyl-butazone and indomethacin. The paw edema was measured with mercury bath every hour until the forth hour after carrageenan injection, and curcumin showed inhibition of edema only at 80 mg/kg[10]. The aforementioned results demonstrated that the administration dose of curcumin played an important role in its anti-inflammatory response. Further investigation of the anti-inflammatory activity of various doses of curcumin at lower and higher doses is therefore needed.

In this study, the anti-inflammatory activity of curcumin (25-400 mg/kg) in comparison with that of indomethacin was investigated by testing the inhibitory effects of these compounds on paw edema in rats to provide evidence for a potential role of curcumin at various doses in the prevention and treatment of various proinflammatory chronic diseases.

MATERIALS AND METHODS:

Synthesis of Curcumin

Chemicals and Instrumentation

All reagents were obtained from commercial suppliers and used without further purification. Reaction progress was monitored through thin layer chromatography (TLC) on pre-coated glass plates (silica gel 60 F254, 0.25mm thickness) purchased from Merck, Thailand. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova Fourier Transform NMR 500 MHz spectrometer. The NMR spectra were obtained in deuterated chloroform ($CDCl_3$) and referenced to the residual solvent peak; chemical shifts are reported in parts per million, and coupling constants in hertz (Hz). Melting points were determined on a Differential Scanning Calorimeter (DSC823^{e}, Mettler Toledo). Mass spectra were obtained on a Reflex IV Bruker time-of-flight High-Resolution Mass Spectrometer (HRMS).

Procedures

Acetyl acetone (1.03 ml, 10 mmol) followed by tributyl borate (10.8 ml, 40 mmol) were added to a solution of boric anhydride (0.35 g, 5.0 mmol) in ethyl acetate (30.0 ml) at 50°C for 15 min. Vanillin (3.04 g, 20 mmol) was added to the resulting boron complex and stirred at 50°C for 5 min. Butylamine (0.4 mL, mmol) was then added dropwise over 40 min at 50°C and the reaction mixture was refluxed for 4 h, cooled, combined with 1N HCl (30 ml) and stirred for 30 min. The organic layers were separated, extracted three times with ethyl acetate, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by recrystallization from methanol to give curcumin (2.92 g, 81%) as a yellow solid. mp 187-188°C (lit. 184-185°C)[11]; IR (KBr) 3500, 1626, 1601, 1504, 1427, 1261, 1026 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.95 (6H, s), 5.80 (1H, s) 6.48 (2H, d, J = 15.7 Hz), 6.94 (2H, d, J = 8.1 Hz), 7.05 (2H, d, J = 1.8 Hz), 7.13 (1H, dd, J = 8.2, 1.8 Hz), 7.59 (1H, d, J = 15.7 Hz); ^{13}C NMR (125.76 MHz, $CDCl_3$); 183.2, 147.8, 146.7, 140.5, 127.6, 122.8, 121.7, 114.8, 109.6, 101.1, 55.9. HRMS calcd for $C_{21}H_{21}O_6$ [$M+H^+$]: 369.1338; found 369.1335.

Carrageenan-Induced Paw Edema Test

Animals

Male Sprague-Dawley rats (100-150 g) obtained from the National Laboratory Center (Mahidol University, Salaya, Nakornprathom, Thailand) were used in the study. The animals were housed in the animal facility of the Faculty of Pharma-ceutical Sciences, Chulalongkorn University under standard conditions of

temperature (25±2°C) and 12 hr/12 hr light/dark cycles. The animals were kept under laboratory conditions for one week before start of the experiments and allowed food and water *ad libitum*. At the end of each experiment, the animals were sacrificed with carbon dioxide euthanasia. Six animals were used in each treatment group. The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Procedures

The anti-inflammatory activity of curcumin was determined using a carrageenan-induced paw edema test according to the method of Winter *et al.*[12]. Forty-two male Sprague-Dawley rats (100-150 g) were randomly divided into 7 groups and fasted overnight before the experiment with free access to water. Curcumin suspended in 0.5% carboxymethylcellulose (CMC) at doses of 25, 50, 100, 200 and 400 mg/kg was administered orally to rats for one hour before subcutaneous injection of carrageenan (1% in NSS) into the plantar surface of the left hind paw. The control group received an equivalent volume of vehicle (0.5% CMC) and the positive-control group received indomethacin (IND) dissolved in NSS (10 mg/kg). After the carrageenan injection, the paw volumes were measured at 1, 2, 3, 4, 5 and 6 h using a plethysmometer (Model 7150, UGO Basile, Italy). Edema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation: % inhibition of edema = 100 (1-Vt/Vc), where Vc is the edema volume in the control group and Vt is the edema volume in tested group.

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 AND DISCUSSION: Curcumin was synthesized according to Pabon's method[13]. To prevent Knovenagel condensation at the C-3 methylene group, boronic acid was first reacted with acetyl acetone to form the boron complex. Subsequently, less active methyl moieties were reacted further with a large excess of vanillin to ensure that aldol condensation occurred at both ends of the 2, 4-pentanedione. After decomplex-ation using hydrochloric acid, the desired curcumin was obtained in excellent yield as a yellow crystal. The structure of curcumin was confirmed by spectroscopic methods and is consistent with previous reports[14].

Carrageenan-induced paw edema is a suitable experimental animal model for evaluating an anti-edematous effect. Edema developed following injection of carrageenan serves as an index of acute inflammatory changes, was and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 6 hours. Edema induced by carrageenan is believed to be biphasic: the first phase (1 h) involves the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins[15,16].

To demonstrate the validity of the carrageenan-induced paw edema test, rats were administered indomethacin orally as a positive control at a dosage of 10 mg/kg 1 h before carrageenan injection. As expected, indomethacin significantly ($p<0.05$) decreased paw edema at 2 and 3 hr after carrageenan injection compared to saline, with inhibition levels of 46.87% and 65.71%, respectively (Table 1). These results demonstrate that indomethacin, a cyclooxygenase inhibitor, exerts an anti-edematous effect during the second phase of paw edema due to the reduction of prostaglandins, which are second-phase inflam-matory mediators. Our results are also consistent with a previous study showing that indomethacin strongly inhibits the second phase of edema without affecting the first phase[16].

We then utilized the carrageenan-induced paw edema test to examine the anti-inflammatory effect of synthetic curcumin. Rats were administered CMC or various doses of curcumin (25-400 mg/kg) orally 1 h before carrageenan

administration. Curcumin at 25 mg/kg decreased inflammatory effect through cyclooxygenase

Table 1. Changes in edema volume (ml) from 1-6 hr after carrageenan administration following oral administration of normal saline solution (NSS) or indomethacin (IND; 10 mg/kg). N=6 for all groups.

Treatment	Paw edema ± S.E.M. (mL) (% Inhibition[a])					
	1 h	2 h	3 h	4 h	5 h	6 h
NSS	0.09±0.04	0.32±0.03	0.35±0.06	0.33±0.05	0.33±0.03	0.27±0.04
Indomethacin	0.11±0.05 (-22.22%)	0.17±0.04* (46.87%)	0.12±0.04* (65.71%)	0.21±0.07 (36.36%)	0.22±0.04 (33.33%)	0.27±0.08 (0%)

[a] Inhibition is reported as a percentage compared to NSS. * Significantly different compared to NSS (p<0.05).

Table 2. Changes in edema volume (ml) from 1-6 hr after carrageenan administration following oral administration of 0.5% carboxymethylcellulose (CMC) or various doses of curcumin (25-400 mg/kg). N=6 for all groups.

Treatment	Paw edema ± S.E.M. (mL) (% Inhibition[a])					
	1 h	2 h	3 h	4 h	5 h	6 h
0.5% CMC	0.21±0.01	0.39±0.04	0.43±0.04	0.43±0.03	0.46±0.05	0.26±0.05
Curcumin 25 mg/kg	0.17±0.04 (19.05%)	0.26±0.04 (33.33%)	0.33±0.04 (23.25%)	0.28±0.05* (34.88%)	0.33±0.02 (28.26%)	0.20±0.05 (23.08%)
Curcumin 50 mg/kg	0.11±0.04 (47.62%)	0.28±0.07 (28.21%)	0.33±0.06 (23.25%)	0.39±0.05 (9.30%)	0.31±0.04* (32.61%)	0.22±0.04 (15.38%)
Curcumin 100 mg/kg	0.13±0.06 (38.09%)	0.30±0.05 (23.08%)	0.41±0.07 (4.65%)	0.41±0.04 (4.65%)	0.32±0.06* (30.43%)	0.26±0.06 (0%)
Curcumin 200 mg/kg	0.15±0.05 (28.57%)	0.18±0.06* (53.85%)	0.26±0.04* (39.53%)	0.32±0.08 (25.58%)	0.34±0.04 (26.09%)	0.25±0.05 (3.85%)
Curcumin 400 mg/kg	0.10±0.03 (52.38%)	0.16±0.05* (58.97%)	0.27±0.04* (37.21%)	0.31±0.05 (27.91%)	0.31±0.05* (32.61%)	0.26±0.04 (0%)

[a] Inhibition is reported as a percentage compared to CMC. * Significantly different compared to CMC (p<0.05).

the paw volume significantly (p<0.05) at 4 h, while curcumin at 50 and 100 mg/kg significantly decreased the paw volume at 5 h after carrageenan administration compared to vehicle control. Curcumin at 200 mg/kg significantly (p<0.05) decreased the paw volume at 2 and 3 h, and a dose of 400 mg/kg inhibited paw edema significantly at 2, 3 and 5 h after carrageenan administration compared to vehicle control. At 2 h after carrageenan administration, curcumin doses of 200 and 400 mg/kg resulted in inhibition of edema of 53.85% and 58.97%, respectively (Table 2).

All doses of curcumin used in this study showed significant reduction of paw edema at 2 h or more after carrageenan injection, suggesting that curcumin produces an anti-edematous effect during the second phase, similarly to indomethacin. Data from preclinical models suggest that curcumin is able to produce anti-inhibition and consequent reduction of prostaglandins[17-19]. Therefore, our results confirm that the mechanism of the anti-inflammatory effect of curcumin involves reduction of prostaglandins through inhibition of cyclooxygenase. The anti-edematous effect of low-dose curcumin (25-100 mg/kg) had a delayed onset (4-5 h), whereas high-dose curcumin (200 and 400 mg/kg) had a faster onset (2 h). In addition, the efficacy of high-dose curcumin was comparable to that of indomethacin, but with a longer duration of action (Figure 2 and 3). This phenomenon may partly be due to the low systemic bioavailability of curcumin following oral dosing, due to efficient first-pass metabolism and some degree of intestinal metabolism[20]. Although curcumin has low systemic bioavailability after oral dosing, the achievement of efficacious concentrations in the gastrointestinal tract is sufficient for exerting beneficial effects in animals and humans[21].

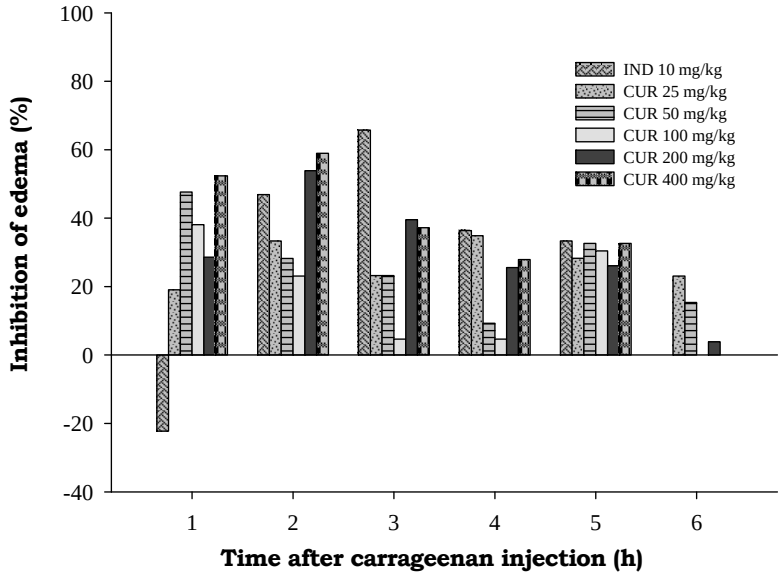

Figure 2 Inhibition of carrageenan-induced rat paw edema by indo-methacin and curcumin measured at 1, 2, 3, 4, 5 and 6 h after carrageenan injection.

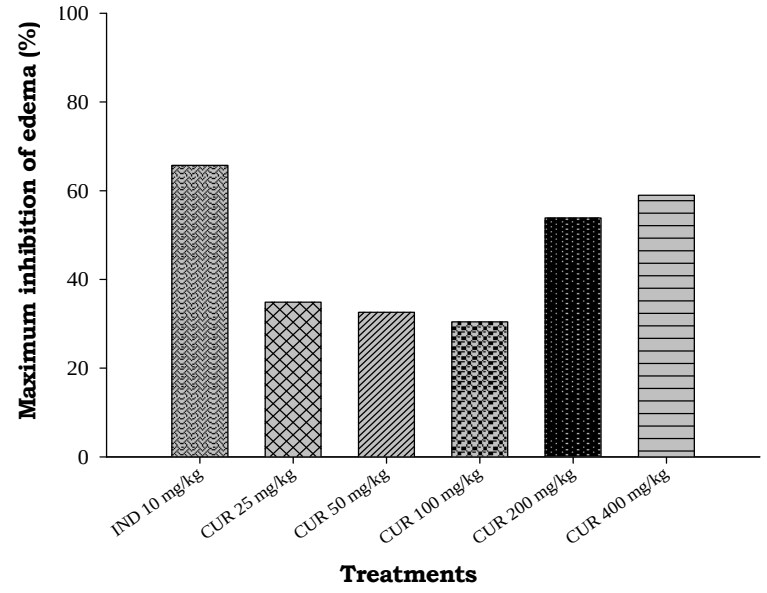

Figure 3 Maximum inhibition of carrageenan-induced rat paw edema by indomethacin and curcumin.

CONCLUSION: In this study, curcumin was demonstrated to inhibit inflammation in the carrageenan-induced rat paw edema model. The onset and duration of action suggest that the anti-inflammatory mechanism of curcumin occurs through inhibition of prostaglandin synthesis via cyclooxygenase pathway. The pharmacological activity combined with the lack of toxicity render curcumin a valuable candidate for further investigation as an agent for treatment of various disorders associated with inflammation.

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