ฤทธิ์ระงับปวดและฤทธิ์ต้านอักเสบของเคอร์คิวมินไดเอทิลไดซัคซิเนตในสัตว์ทดลอง

นางสาวจุติรัตน์ วงศ์ศรีสกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยา ภาควิชาเภสัชวิทยาและสรีรวิทยา
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF CURCUMIN DIETHYL DISUCCINATE IN ANIMALS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Pharmacology

Department of Pharmacology and Physiology

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2010

Thesis Title	ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF CURCUMIN DIETHYL DISUCCINATE IN ANIMALS
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Field of Study	Pharmacology
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จุติรัตน์ วงศ์ศรีสกุล : ฤทธิ์ระงับปวดและฤทธิ์ต้านอักเสบของเคอร์คิวมินไดเอทิลไดซัคซิเนตในสัตว์ทดลอง. (ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF CURCUMIN DIETHYL DISUCCINATE IN ANIMALS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ร.ท. หญิง ดร. ภัสราภา โตวิวัฒน์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. พรชัย โรจน์สิทธิศักดิ์, 127 หน้า.

เคอร์คิวมินไดเอทิลไดซัคซิเนตเป็นโปรดรักส์ของเคอร์คิวมิน การทดลองครั้งนี้เริ่มต้นศึกษาฤทธิ์ระงับปวดของเคอร์คิวมินไดเอทิลไดซัคซิเนตในขนาดต่างๆ กันด้วยวิธี hot-plate ในหนูเมาส์เพศผู้ จับเวลาที่หนูเมาส์สามารถทนอยู่บนแผ่นร้อนได้ (hot-plate latencies) ก่อนให้น้ำเกลือ ทางช่องท้อง มอร์ฟีน (10 มก./กก.) ทางช่องท้อง คาร์บอกซีเมทิลเซลลูโลส (0.5%) โดยการป้อน หรือเคอร์คิวมินไดเอทิลไดซัคซิเนตขนาด 25-200 มก./กก. โดยการป้อน และจับเวลาที่หนูสามารถทนอยู่บนแผ่นร้อนได้ ที่เวลา 15, 30, 45, 60, 90, 120 และ 240 นาที หลังให้สารทดสอบ โดยให้หนูเมาส์อยู่บนแผ่นความร้อนไม่เกิน 45 วินาทีในการทดสอบแต่ละครั้ง (cut-off time) คำนวณเปอร์เซ็นต์สูงสุดที่หนูเมาส์สามารถทนต่อความร้อนได้ (%MPE) แล้วนำมาคำนวณหาพื้นที่ใต้กราฟระหว่าง %MPE และเวลา (area of analgesia) พบว่า เคอร์คิวมินไดเอทิลไดซัคซิเนตทุกขนาดที่ใช้ในการทดสอบยกเว้นขนาด 200 มก./กก. มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ ฤทธิ์ระงับปวดของเคอร์คิวมินไดเอทิลไดซัคซิเนตที่ขนาด 50 มก./กก. ถูกยับยั้งได้ด้วยนาลอกโซน แสดงว่ากลไกการออกฤทธิ์ระงับปวดของเคอร์คิวมินไดเอทิลไดซัคซิเนตเกี่ยวข้องกับตัวรับ opioid ในการทดลองด้วยวิธี tail-flick ทำการจับเวลาที่หนูเมาส์ทนต่อความร้อนได้โดยไม่กระดกหางหนี (tail-flick latencies) ก่อนให้น้ำเกลือ มอร์ฟีน คาร์บอกซีเมทิลเซลลูโลส (0.5%) หรือเคอร์คิวมินไดเอทิลไดซัคซิเนตขนาด 25-200 มก./กก. และทำการทดสอบหลังจากได้รับสารอีก 7 ครั้ง ในช่วงเวลา 4 ชม. โดยหางหนูจะได้รับความร้อนจากการส่องไฟแต่ละครั้งไม่เกิน 4 วินาที (cut-off time) พบว่าเคอร์คิวมินไดเอทิลไดซัคซิเนตขนาด 25 มก./กก. มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ ส่วนการทดสอบฤทธิ์ระงับปวดโดยเหนี่ยวนำให้หนูเมาส์เกิดความเจ็บปวดจนเกิดอาการบิดงอลำตัว (writhing) ด้วยกรดอะซิติก ทำการฉีดกรดอะซิติก 0.6% ขนาด 10 มล./กก. เข้าทางช่องท้องของหนูเมาส์ที่เวลา 1 ชม. หลังจากป้อนน้ำเกลือ อินโดเมทาซิน ขนาด 10 มก./กก. คาร์บอกซีเมทิลเซลลูโลส (0.5%) หรือเคอร์คิวมินไดเอทิลไดซัคซิเนตขนาด 25-200 มก./กก. แล้วนับจำนวนครั้งที่หนูเกิดการบิดงอของลำตัวเป็นเวลา 30 นาที พบว่าเคอร์คิวมินไดเอทิลไดซัคซิเนตทุกขนาด สามารถลดการบิดงอลำตัวของหนูเมาส์ได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม ($p<0.05$)

ในการทดสอบฤทธิ์ต้านการอักเสบของเคอร์คิวมินไดเอทิลไดซัคซิเนตในหนูแรทด้วยการเหนี่ยวนำให้อุ้งเท้าหนูบวมด้วยคาราจีแนน พบว่าเคอร์คิวมินไดเอทิลไดซัคซิเนตขนาด 10-80 มก./กก. สามารถลดการบวมของอุ้งเท้าหนูได้อย่างมีนัยสำคัญทางสถิติในระยะที่ 2 ของการบวม แสดงถึงการยับยั้งพรอสตาแกลนดิน จากผลการทดลองทั้งหมดแสดงว่า เคอร์คิวมินไดเอทิลไดซัคซิเนตมีฤทธิ์ระงับปวดทั้งในระดับประสาทส่วนกลางและระดับประสาทส่วนปลาย และกลไกการออกฤทธิ์ระงับปวดมีความเกี่ยวข้องกับวิถีของ opioid ส่วนกลไกการต้านการอักเสบของเคอร์คิวมินไดเอทิลไดซัคซิเนตเกิดจากการยับยั้งการสร้างพรอสตาแกลนดินและการยับยั้งผลของพรอสตาแกลนดิน อี 1

ภาควิชา เภสัชวิทยาและสรีรวิทยา	ลายมือชื่อนิสิต .....
สาขาวิชา เภสัชวิทยา	ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก .....
ปีการศึกษา 2553	ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5176560133 : MAJOR PHARMACOLOGY

KEYWORDS : CURCUMIN DIETHYL DISUCCINATE / ANTINOCICEPTION / ANTI-INFLAMMATORY / HOT-PLATE TEST / TAIL-FLICK TEST / ACETIC ACID-INDUCED WRITHING / CARRAGEENAN-INDUCED PAW EDEMA / HISTAMINE-INDUCED PAW EDEMA / SEROTONIN-INDUCED PAW EDEMA / PGE_1-INDUCED PAW EDEMA

JUTIRAT WONGSRISAKUL: ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF CURCUMIN DIETHYL DISUCCINATE IN ANIMALS. THESIS ADVISOR: ASST. PROF. FLG. OFF. PASARAPA TOWIWAT, Ph.D., THESIS CO-ADVISOR: ASST. PROF. PORNCHAI ROJSITTHISAK, Ph.D., 127 pp.

Curcumin diethyl disuccinate (CurDD) is a succinate prodrug of curcumin. We initially determined the antinociceptive property of a range of CurDD doses in the mouse hot-plate test. Hot-plate latencies (cut-off 45 sec) were determined in male ICR mice prior to the administration of 0.9% normal saline solution (NSS; 10 ml/kg, i.p.), morphine (MO; 10 mg/kg, i.p.), 0.5% carboxymethylcellulose (CMC; 10 ml/kg, p.o.) or various doses of CurDD (25-200 mg/kg, p.o.). Hot-plate latencies were subsequently determined at 15, 30, 45, 60, 90, 120 and 240 min. The percent maximum possible effect (%MPE) was calculated and used in the determination of the area of analgesia (%MPE-min). All doses of CurDD tested except 200 mg/kg produced a significant analgesic response. CurDD 50 mg/kg produced analgesic response that was naloxone-sensitive suggesting opioid-mediated mechanism. In the mouse tail-flick test, tail-flick latencies (cut-off 4 sec) were determined prior to the administration of NSS, MO, 0.5% CMC or various doses of CurDD (25-200 mg/kg, p.o.) and were subsequently determined at 7 intervals over a 4 hr period. CurDD 25 mg/kg produced significant analgesic response. In the acetic acid-induced writhing in mice, the animals were induced with i.p. injection of 0.6% acetic acid (10 ml/kg) 1 hr after the administration of NSS, indomethacin (IND; 10 mg/kg, p.o.), 0.5% CMC or various doses of CurDD (25-200 mg/kg, p.o.) and the mean writhing response was determined for 30 min. All doses of CurDD significantly ($p<0.05$) decreased the mean writhing response compared to vehicle controls.

Studies then determined the anti-inflammatory property of orally administered CurDD (10-80 mg/kg) using carrageenan-induced paw edema model in rats. CurDD 10, 40, and 80 mg/kg significantly reduced paw volume during the second phase of edema suggesting inhibition of prostaglandins. Taken together these results demonstrated that CurDD produced both central and peripheral analgesic activities and mechanism of analgesic action seems to be involved with the opioid pathway. The mechanism of anti-inflammatory effect of CurDD involves in the reduction of prostaglandin synthesis and inhibition of prostaglandin E_1 effects.

Department : Pharmacology and Physiology Student's Signature .....

Field of Study : Pharmacology Advisor's Signature .....

Academic Year : 2010 Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

The author wishes to express her sincere gratitude and appreciation to her advisor, Assistant Professor Flight Officer Dr. Pasarapa Towiwat for her intensive advice, guidance, suggestions and encouragement throughout her study. Thanks are also extended to the committee members.

The author wishes to thank Assistant Professor Dr. Pornchai Rojsitthisak, her thesis co-advisor for his valuable advice and providing curcumin diethyl disuccinate for this study.

The author wishes to thank Mr. Wisut Wichitnithad for his help with the synthesis of curcumin diethyl disuccinate.

The author would like to thank all staff members in the Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University for their help and support.

The author also would like to thank her lab partners especially Anusara Jongchanapong, Mala Insaro and, her friends for their help, advice and encouragement throughout her thesis study.

The author would like to express her gratitude to the Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing research funds. Financial support from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) is also acknowledged.

Finally, the author would like to thank her parents and her elder sister for everything in her life.

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LIST OF ABBREVIATIONS

^{3}H	=	tritium
α	=	alpha
β	=	beta
κ	=	kappa
δ	=	delta
μ	=	mu
µg/ml	=	microgarm per milliliter
µl	=	microlitter
µM	=	micromolar
/	=	per
%	=	percent
%MPE	=	percentage of the maximum possible effect
^{o}C	=	degree of Celsius
AD	=	Alzheimer's disease
AIDS	=	acquired immune deficiency syndrome
ASA	=	acetylsalicylic acid
AUC	=	area under the curve (area of analgesia)
bw/day	=	body weight per day
cm	=	centimeter
COX-2	=	cyclooxygenase-2
CurDD	=	curcumin diethyl disuccinate
CVDs	=	cerebrovascular diseases
CYPRO	=	cyproheptadine
DAC	=	diacetyl curcumin
e.g.	=	exempli gratia
ELISA	=	enzyme-linked immunosorbent assays
et al.	=	et alii (and other)

g	=	gram
g/day	=	gram per day
g/kg	=	gram per kilogram
g/mol	=	gram per mole
sec	=	second
HPLC	=	high pressure liquid chromatography
hr	=	hour
IASP	=	International Association for the Study of Pain
i.e.	=	id est
i.p.	=	intraperitoneal
i.v.	=	intravenous
IκB	=	I-kappa B
IL	=	interleukin
IND	=	indomethacin
iNOS	=	inducible nitric oxide synthase
LPS	=	Lipopolysaccharide
m	=	meter
MCP	=	monocyte chemoattractant protein
mg/kg	=	milligram per kilogram
mmol/kg	=	millimole per kilogram
min	=	minute
ml	=	milliliter
ml/kg	=	milliliter per kilogram
ml/rat	=	milliliter per rat
MO	=	morphine sulphate
N	=	sample size
NaC	=	sodium curcuminate
NAL	=	naloxone
NCI	=	National Cancer Institute

NF-κB	=	nuclear factor kappa B
ng/ml	=	nanogram per milliliter
nm	=	nanometer
NO	=	nitric oxide
NOS	=	nitric oxide synthase
NSAIDs	=	nonsteroidal anti-inflammatory drugs
NSS	=	normal saline
PB	=	phenylbutazone
PGE_1	=	prostaglandin E_1
PRO	=	promethazine
p.o.	=	per os
TEC	=	triethyl curcumin
THC	=	tetrahydro curcumin
TNF-α	=	tumor necrosis factor-alpha
V	=	volt
w/w	=	weight by weight

CHAPTER I

INTRODUCTION

Background and Rationale

Pain is a very frequent symptom of disease and is the most common reason for patients to seek medical treatment. Pain is a sensory experience that can be evoked by many different kinds of stimuli including a burn, a fracture, colic, a tooth extraction which the generating causes are very different. Pain accompanies inflammation and tissue repair, and when it goes away it tells us that the process has been completed, but pain can also last well beyond the point of tissue repair or appear without tissue damage or be felt by stimuli that do not normally evoke pain. (Cervero and Jensen, 2006).

Inflammation is an important protective mechanism that on occasion becomes deregulated and leads to a chronic inflammatory state. It can occur in all tissues and organs in the body in response to a variety of stimuli which may be mechanical, chemical, or infective and initiates both structural and functional repair of damaged tissues (Lombardino, 1985; Sigal and Ron, 1994). The process of inflammation is necessary in healing of wounds but if inflammation becomes excessive or uncontrolled that may begin to see delayed healing or chronic inflammatory diseases include rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, inflammatory bowel disease, and artherosclerosis (Sherwood and Toliver-Kinsky, 2004).

Nonopioids (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, arthritis pain) and are especially effective for certain types of somatic pain (e.g., muscle and joint pain, bone/dental pain, inflammatory pain, postoperative pain). Opioids such as morphine, fentanyl, and codeine, are used to treat moderate to severe pain that does not respond to nonopioids alone (Berry et al., 2006).

Although opioids and NSAIDs are still the mainstays 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 effect of NSAIDs produce gastrointestinal problems (e.g., dyspepsia, ulcers, perforation and bleeding), inhibition of platelet aggregation, kidney and liver dysfunction, and hypersensitivity reactions (Berry et al., 2006). Therefore, the investigation of novel analgesic and anti-inflammatory drugs lacking the above adverse effects are being researched as alternatives to NSAIDs and opioids.

Curcumin (diferuloylmethane), a polyphenol, 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, hepatic disorders, rheumatism and sinusitis. Chinese traditional medicine uses turmeric in diseases associated with abdominal pains. Curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses. However, 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, lack of water solubility, poor bioavailability and fast metabolism (Mukhopadhyay et al., 1982; Sharma et al., 2005; Anand et al., 2008; Goel et al., 2008; Liang et al., 2009).

As a result, curcumin diethyl disuccinate (CurDD), a prodrug of curcumin, was synthesized to overcome the instability problems of curcumin. Thus, the present study was designed to investigate the antinociceptive and anti-inflammatory effects of CurDD using various animal models in order to provide the potential usefulness of CurDD in the treatment of painful conditions and inflammatory diseases.

Purpose of the study

To evaluate the antinociceptive and anti-inflammatory effects of curcumin diethyl disuccinate in comparison with the reference drugs. In addition, the possible mechanisms involved were also investigated.

Hypothesis

Curcumin diethyl disuccinate has antinociceptive and anti-inflammatory effects in various animal models.

Research design

Experimental Research

Expected benefit and application

The knowledge obtained from the studies of antinociceptive and anti-inflammatory effects of curcumin diethyl disuccinate could lead to the development of a new analgesic and/or anti-inflammatory agent.

Key words

Curcumin diethyl disuccinate

Antinociception

Anti-inflammatory

Hot-plate test

Tail-flick test

Acetic acid-induced writhing

Carrageenan-induced paw edema

Histamine-induced paw edema

Serotonin-induced paw edema

PGE_1-induced paw edema

CHAPTER II

LITERATURE REVIEWS

PAIN

Pain can be defined as an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage. Pain is subjective, and the patient's report of pain should always be taken seriously (Roach, 2006). In 1968, McCaffery defined pain as "whatever the experiencing person says it is, existing whenever s/he says it does". This definition emphasizes that pain is a subjective experience with no objective measures. It also stresses that the patient, not clinician, is the authority on the pain and that his or her self-report is the most reliable indicator of pain. In 1979, the International Association for the Study of Pain (IASP) introduced the most widely used definition of pain. The IASP defined pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage''. This definition emphasizes that pain is a complex experience that includes multiple dimensions (Berry et al., 2006).

Pain that is classified on the basis of its presumed underlying pathophysiology is broadly categorized as nociceptive or neuropathic pain.

Nociceptive pain

Nociceptive pain is caused by the ongoing activation of Aδ and C-nociceptors in response to a noxious stimulus (e.g., injury, disease, inflammation). Pain arising from visceral organs (such as the large intestine or pancreas) is called visceral pain, whereas that arising from tissues such as skin, muscle, joint capsules, and bone is called somatic pain. In contrast to neuropathic pain, the nervous system associated with nociceptive pain is functioning properly. Generally, there is a close correspondence between pain perception and stimulus intensity, and the pain is indicative of real or potential tissue damage. While somatic pain most often presents as throbbing and well localized, visceral pain can manifest as pain feeling as if it is coming from other

structures (referred pain) or as a well-localized phenomenon. (Baumann, 2005; Berry et al., 2006).

Neuropathic pain

Neuropathic pain is distinctly different from nociceptive pain. It is pain sustained by abnormal processing of sensory input by the peripheral or CNS. Lesions of the peripheral or central nervous pathways for pain typically result in a loss or impairment of pain sensation. Paradoxically, damage or dysfunction of these pathways can produce pain. For example, damage to peripheral nerves, as occurs in diabetic neuropathy, or to primary afferents, as in herpes zoster, can result in pain that is referred to the body region innervated by the damaged nerves. Though rare, pain may also be produced by damage to the central nervous system, particularly the spinothalamic pathway or thalamus. Such neuropathic pains are often severe and are notoriously intractable to standard treatments for pain.

Clinically, patients present with spontaneous pain transmission (often described as burning, tingling, prickling, jabbing, spasm, squeezing, shock-like, or shooting), exaggerated painful response to normally noxious stimuli (hyperalgesia), or painful response to normally nonnoxious stimuli (allodynia). This change over time may help to explain why this type of pain often manifests long after the actual nerve-related injury (Baumann, 2005; Berry et al., 2006; Kasper et al., 2004).

Classification of pain

Although pain classes are not diagnoses, categorizing pain helps guide treatment. Multiple systems for classifying pain exist. These include multidimensional classification systems, such as the IASP Classification of Chronic Pain, and a variety of systems based on a single dimension of the pain experience. Of the latter systems, those based on pain duration (i.e., acute vs. chronic pain) and underlying pathophysiology (i.e., nociceptive vs. neuropathic pain) are used most often (Berry et al., 2006).

Acute Pain

Acute pain was once defined simply in terms of duration. It is now viewed as a "complex, unpleasant experience with emotional and cognitive, as well as sensory, features that occur in response to tissue trauma". Acute pain is usually nociceptive, but may be neuropathic. Intensity of acute pain is from mild to severe and duration less than 3 to 6 months. Common sources of acute pain include trauma, surgery, labor, medical procedures, and acute disease states. The best way of managing acute pain is to diagnose and treat the cause, though this is often clear, e.g. following any kind of trauma. Temporary relief with analgesics is valuable while healing and recovery proceed (Berry et al., 2006; Greene and Harris, 2008; Roach, 2006).

Chronic Pain

Chronic pain was once defined as pain that extends 3 or 6 months beyond onset or beyond the expected period of healing. It does not signify a danger that requires immediate avoidance and a patient may not interpret such pain as indicating serious disease. Further, adaptation by the autonomic nervous system over time may lead to the absence of objective physical signs. However, there is often progressive physical deterioration, with sleep disturbance and weight loss. In severe cases, patients undergo serious affective and behavioural changes, e.g. major depression.

Chronic pain may be nociceptive, neuropathic, or both and caused by injury (e.g., trauma, surgery), malignant conditions, or a variety of chronic non-life-threatening conditions (e.g., arthritis, fibromyalgia, neuropathy). In some cases, chronic pain exists *de novo* with no apparent cause. Although injury often initiates chronic pain, factors pathogenetically and physically remote from its cause may perpetuate it. Environmental and affective factors also can exacerbate and perpetuate chronic pain, leading to disability and maladaptive behavior (Berry et al., 2006; Greene and Harris, 2008).

Cancer Pain

Pain associated with potentially life-threatening conditions such as cancer is often called “malignant pain” or “cancer pain.” However, there is movement toward the use of new terms such as “pain associated with cancer”. Cancer pain includes pain caused by the disease itself (e.g., tumor invasion of tissue, compression or infiltration of nerves or blood vessels, organ obstruction, infection, inflammation) and/or painful diagnostic procedures or treatments (e.g., biopsy, postoperative pain, toxicities from chemotherapy or radiation treatment).

There are several reasons why some experts feel that cancer pain merits a discrete category. First, its acute and chronic components and multiple etiologies make it difficult to classify based on duration or pathology alone. Second, cancer pain differs from chronic noncancer pain (CNCP) in some significant ways (e.g., time frame, levels of pathology, treatment strategies). However, there is little evidence to support a distinction between these pain types based on underlying neural processes. Therefore, many pain experts categorize cancer pain as acute or chronic pain (Berry et al., 2006).

Chronic noncancer Pain

A subtype of chronic pain is CNCP, which refers to persistent pain not associated with cancer. In contrast to patients with chronic cancer pain, patients with CNCP often report pain levels that only weakly correspond to identifiable levels of tissue pathology and/or respond poorly to standard treatments. As CNCP may last for many years, some consider use of the traditional term for such pain, “chronic nonmalignant pain,” inappropriate. Thus, there is movement toward use of alternate terms such as “chronic noncancer pain” and “chronic noncancer-related pain.”

Causes of CNCP include acute injury that has proceeded to chronic pain (e.g., whiplash) and various chronic conditions (e.g., osteoarthritis, low back pain, myofascial pain, peripheral neuropathy etc.). In some cases, there is no discernable cause, and the pain is considered the disease. CNCP can affect virtually any body system or region, and pain severity ranges from mild to excruciating. Some types of CNCP have well-defined characteristics and patterns, whereas others do not.

Neuropathic and myofascial CNCP can be particularly hard to diagnose, as they may occur in the absence of a known injury or disease process.

Because of its chronicity and impact on daily activities, patients with CNCP may experience vocational, interpersonal, and/or psychological problems. If the symptoms of CNCP consume the attention of and incapacitate the patient, he or she may suffer from a psychosocial disorder known as “chronic pain syndrome” (CPS). The pain experienced by these patients is real, and not all patients with CNCP develop this syndrome. Appropriate management of both CNCP and CPS requires an interdisciplinary approach that addresses the complex interaction of physical, psychological, and social factors that contribute to the ongoing pain (Berry et al., 2006).

Nociceptors

The relatively unspecialized nerve cell endings that initiate the sensation of pain are called nociceptors (*noci* is derived from the Latin *nocere*, “to hurt”). Like other cutaneous and subcutaneous receptors, they transduce a variety of stimuli into receptor potentials, which in turn trigger afferent action potentials. Moreover, nociceptors, like other somatic sensory receptors, arise from cell bodies in dorsal root ganglia (or in the trigeminal ganglion) that send one axonal process to the periphery and the other into the spinal cord or brainstem (Fitzpatrick, 2004).

Three types of nerve fibers are involved in pain transmission. The A-beta (Aβ) fibers are large, myelinated and fast-conducting (30-100 m/s). They have a low stimulation threshold and respond to light touch. The A-delta (Aδ) fibers are small, lightly myelinated and slower-conducting (5-15 m/s). They respond to pressure, heat, chemicals and cooling, and give rise to the sensation of sharp pain, producing reflex withdrawal and other prompt action. The C fibers are small and unmyelinated and therefore slow-conducting (0.5-2 m/s); they respond to all types of noxious stimuli and transmit more prolonged, dull pain signals. The last two of these types of fibers usually require high-intensity stimuli to trigger a response (Figure 1; Greene and Harris, 2008).

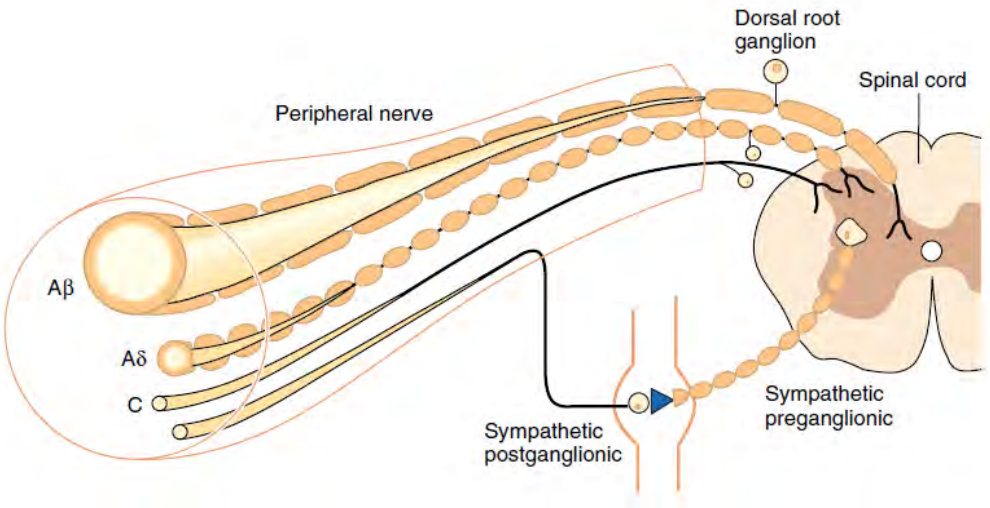

Figure 1 Primary afferents with cell bodies in the dorsal root ganglion, include those with large-diameter myelinated (Aβ), small-diameter myelinated (Aδ), and unmyelinated (C) axons (Kasper et al., 2004).

In general, two categories of pain perception have been described: a sharp **first pain** and a more delayed, diffuse, and longer-lasting sensation that is generally called **second pain**. Stimulation of the large, rapidly conducting Aβ axons in peripheral nerves does not elicit the sensation of pain. When the stimulus intensity is raised to a level that activates a subset of Aδ fibers, however, a tingling sensation or, if the stimulation is intense enough, a feeling of sharp pain is reported, Aδ fibers are responsible for first pain. If the stimulus intensity is increased still further, so that the small-diameter, slowly conducting C fiber axons are brought into play, then a duller, longer-lasting sensation of pain is experienced, C fibers are responsible for the duller, longer-lasting second pain (Fitzpatrick, 2004).

Physiologic pathways

Nociception refers to the process by which information about tissue damage is conveyed to the central nervous system (CNS). Exactly how this information is ultimately perceived as painful is unclear. In addition, there can be pain without nociception (e.g., phantom limb pain) and nociception without pain. But classic descriptions of pain typically include four processes:

Stimulation (Transduction)

The first step leading to the sensation of pain is stimulation of free nerve endings known as nociceptors. These receptors are found in both somatic and visceral structures, distinguish between noxious and innocuous stimuli, and are activated and sensitized by mechanical, thermal, and chemical impulses. The underlying mechanism of these noxious stimuli (which in and of themselves may sensitize/stimulate the receptor) may be the release of bradykinins, K^+, prostaglandins, histamine, leukotrienes, serotonin, and substance P (among others) that sensitize and/or activate the nociceptors. Receptor activation leads to action potentials that are transmitted along afferent nerve fibers to the spinal cord.

Substance P is a polypeptide that facilitates nociception. It is synthesized in the dorsal root ganglia and can be found in the nerve endings. When tissue injury occurs, antidromic (backward) stimulation of peripheral nerves releases substance P into the area of injury. This sensitizes the nerve endings and increases vascular permeability causing edema. Tissue injury also results in norepinephrine release from sympathetic nerve endings; that also sensitizes or depolarizes the nerve endings. The tissue damage itself can facilitate production and release of various chemical compounds including prostaglandins, bradykinin, histamine, and serotonin. These proinflammatory mediators further sensitize the nociceptors and cause local tissue reactions including edema and vasodilation. Bradykinin can increase the production of prostaglandins and serotonin can increase the release of substance P from the nerve endings. When nociception occurs in response to injury. This chain of events perpetuates and accentuates the response (Baumann, 2005; Hare et al., 2004).

Transmission

Nociceptive transmission takes place in Aδ and C-afferent nerve fibers. Stimulation of large-diameter, myelinated Aδ fibers evokes sharp, well-localized pain, whereas stimulation of unmyelinated, small-diameter C fibers produces dull, aching, and poorly localized pain. These afferent, nociceptive pain fibers synapse in various layers (laminae) of the spinal cord's dorsal horn, releasing a variety of neurotransmitters,

including glutamate, substance P, and calcitonin gene–related peptide. The complex array of events that influence pain can be explained in part by the interactions between neuroreceptors and neurotransmitters that take place in this synapse. For example, by stimulating large sensory myelinated fibers (e.g., Aβ) that mutually connect in the dorsal horn with pain fibers, both noxious and nonnoxious stimuli can have an inhibitory effect on pain transmission. Functionally, the importance of the interplay between these different fibers and various neurotransmitters and neuroreceptors is evident in the analgesic response produced by topical irritants or transcutaneous electrical nerve stimulation. These pain-initiated processes reach the brain through a complex array of at least five ascending spinal cord pathways, which include the spinothalamic tract (Figure 2), spinoreticular tract, spinomesencephalic tract, dorsal column postsynaptic spinomedullary pathway, and propriospinal multisynaptic ascending systems. Information other than pain is also carried along these pathways. Thus pain is influenced by many factors supplemental to nociception. It is postulated that the thalamus acts as a relay station as these pathways ascend and passes the impulses to central structures where pain can be processed further (Baumann, 2005).

Pain transmission may be blocked if opioid receptors have already been occupied by endorphins at the spinal level. If successful in passing through the gating mechanisms, and several are probably involved in the total pathway, the pain impulse is transmitted via the reticular activating system of the pons and midbrain to the thalamus. From there, they are directed to the appropriate part of the cerebral cortex where the impulses are perceived as pain. The limbic system, which is anatomically close to these areas, is thought to be responsible for the emotional component of pain. Transmission of the pain impulse may be modified in the CNS by the presence of 5-HT and other chemical mediators (Green and Harris, 2008).

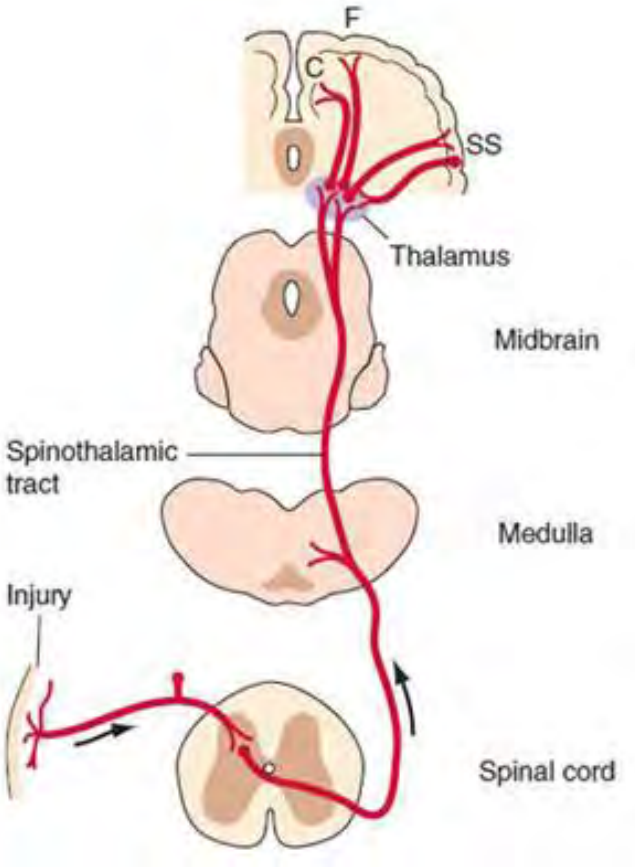

Figure 2 Transmission system for nociceptive messages. Noxious stimuli activate the sensitive peripheral ending of the primary afferent nociceptor by the process of transduction. The message is then transmitted over the peripheral nerve to the spinal cord, where it synapses with cells of origin of the major ascending pain pathway, the spinothalamic tract. The message is relayed in the thalamus to the anterior cingulated (C), frontal insular (F), and somatosensory cortex (SS) (Kasper et al., 2004).

Perception

At this point in transmission, pain is thought to become a conscious experience that takes place in higher cortical structures. The brain may accommodate only a limited number of pain signals; thus cognitive and behavioral functions can modify pain. Relaxation, distraction, meditation, and guided mental imagery may decrease pain by limiting the number of processed pain signals (Baumann, 2005).

Modulation

Inhibition of nociceptive impulses. The body modulates pain through a number of complex processes and occurs at multiple levels (peripheral, spinal, supraspinal). One, known as the endogenous opiate system, consists of neurotransmitters (e.g., enkephalins, dynorphins, and β-endorphins) and receptors (e.g., mu, delta, and kappa) that are found throughout the central nervous system (CNS). Like exogenous opioids, endogenous opioids bind to opioid receptor sites and modulate the transmission of pain impulses. Other receptor types also can influence this system. Activation of N-methyl-D-aspartate (NMDA) receptors, found in the dorsal horn, can decrease the mu receptors' responsiveness to opiates. The CNS also contains a highly organized descending system for control of pain transmission. This system can inhibit synaptic pain transmission at the dorsal horn and originates in the brain. Important neurotransmitters here include endogenous opioids, serotonin, norepinephrine, γ-aminobutyric acid (GABA), and neurotensin. These substances bind to receptors on primary afferent and/or dorsal horn neurons and inhibit nociceptive transmission. Such endogenous modulation may contribute to the wide variations in pain perception observed among patients with similar injuries (Baumann, 2005; Berry, 2006).

The gate control theory

Various theories have tried to integrate the anatomical pain pathways and the psychological and neurological components that contribute to the perception of pain. The generally accepted model is the 'gate control theory', illustrated diagrammatically in Figure 3.

The gate control theory of pain, proposed in 1965 by Melzack and Wall and later updated, is an attempt to describe the mechanism of pain transmission. The dorsal horn of the spinal cord contains a gate mechanism that alters the transmission of painful sensations from peripheral nerve fiber to the thalamus and cortex of the brain. The thalamus and the cortex is where painful sensations are recognized as pain. The transmission flows through the gate mechanism. The gate is closed by large diameter, low-threshold afferent fibers and is opened by small diameter, high-threshold afferent

fibers. In addition to these two sets of fibers, the gate is also influenced by descending control inhibition from the brain to close the gate. When the patient experiences slower-acting painful stimuli, the large-diameter fibers are stimulated. This causes the gate to close, stopping transmission of the painful stimuli. Nonpharmacological pain relief treatments, such as a massage, are based on the gate control theory to ease the patient's pain (Hare et al, 2004; Kamienski and Keogh, 2006).

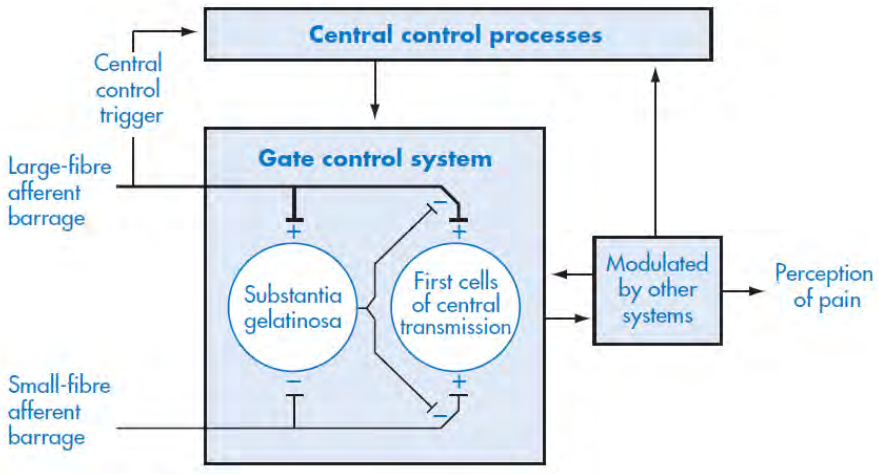

Figure 3 Gate control theory of the transmission of pain impulses. +, excitation; -, inhibition. (Green and Harris, 2008).

Neurotransmitters involved in pain

Opioid receptors and endogenous opioids

The important discoveries of stereospecific opioid receptors (of which several subtypes are known) and endogenous opioids further increased our understanding of the biochemical mechanisms involved in pain transmission and perception. Several families of endogenous opioids have been identified including the endorphins, enkephalins and dynorphins. Each family is derived from a distinct precursor polypeptide and has a characteristic anatomical distribution (Green and Harris, 2008).

Other transmitters and mediators

Physical or chemical insult can stimulate nociceptors. Inflammation, ischemia or other pain inducing stimuli cause the release of noxious chemicals (e.g. bradykinin, histamine and 5-HT) in injured tissues. Prostaglandins (PGs), although not directly producing pain, appear to sensitize nociceptors to various chemical and pressure stimuli. This explains why NSAIDs, which block PG synthesis, are effective analgesics in some situations (Green and Harris, 2008).

Substance P (neurokinin-1), a polypeptide probably released by the small-diameter C-fibers, is believed to be involved in pain transmission in the dorsal horns of the spinal cord. It is probably not the actual transmitter, but initiates a series of events leading to the recruitment of pro-inflammatory agents. The latter release mediators, e.g. PGs, LTs, 5-HT and histamine, which stimulate the nerve endings and cause sensitization. Sensitization involves a lowering of the trigger threshold, producing hyperalgesia. Excitatory amino acid transmitters, e.g. glutamate and aspartate, may also be involved (Green and Harris, 2008).

PHARMACOLOGICAL MANAGEMENT OF PAIN

Analgesia is a state in which no pain is felt despite the presence of normally painful stimuli. Drugs that alleviate pain without major impairment of other sensory modalities are termed analgesics, include nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, narcotics, antidepressants, anticonvulsants, and others. Acetaminophen and NSAIDs include aspirin, ibuprofen, naproxen, and ketoprofen are available as over-the-counter and prescription medications, and are frequently the initial pharmacological treatment for pain. These drugs can also be used as adjuncts to the other drug therapies, which might require a doctor's prescription (Longe, 2002; Wecker et al., 2010).

Nonopioid analgesics (nonopioids): acetaminophen and NSAIDs

Mechanism of action and effects

The primary mechanism of action of NSAIDs is inhibition of the enzyme cyclooxygenase (COX), resulting in blockade of prostaglandin synthesis (Figure 4). Acetaminophen, another nonopioid, appears to act mostly via a central mechanism. All nonopioids have anti-inflammatory, antipyretic, and analgesic effects, but the anti-inflammatory effect of acetaminophen is essentially negligible. The analgesic effect of NSAIDs is prompt (minutes to hours), whereas the anti-inflammatory effect may take longer (1-2 weeks or longer). This latter effect can indirectly relieve some pain by reducing tissue swelling.

The relatively recent discovery that COX has at least two isoforms, COX-1 and COX-2, has advanced NSAID pharmacology. COX-1 is constitutively expressed in most normal tissues, but plays an especially important role in the gastrointestinal (GI) tract, kidneys, and platelets; COX-1 primarily produces prostaglandins with beneficial effects (e.g., regulation of blood flow to the gastric mucosa and kidneys). In contrast, COX-2 is normally not present but may be induced in response to inflammatory stimuli; COX-2 primarily produces prostaglandins that activate and sensitize nociceptors. Nonselective NSAIDs inhibit COX-1 and COX-2, which contributes to both their

therapeutic actions and side effects. Agents that selectively inhibit COX-2 were introduced to minimize the risk of GI side effects without compromising analgesic efficacy. The “coxibs” affect COX-2 both centrally and peripherally. However, an increased risk of myocardial infarction, stroke, and death has been linked to selective COX-2 inhibitors, and this increased risk of cardiovascular side effects appears to be a class effect of NSAIDs, including nonselective agents. Rofecoxib and valdecoxib were voluntarily withdrawn from the market in 2004 and 2005, respectively, because of these cardiovascular safety concerns. Celecoxib is still available because its benefits appear to outweigh its potential risks in certain patients (Berry et al., 2006).

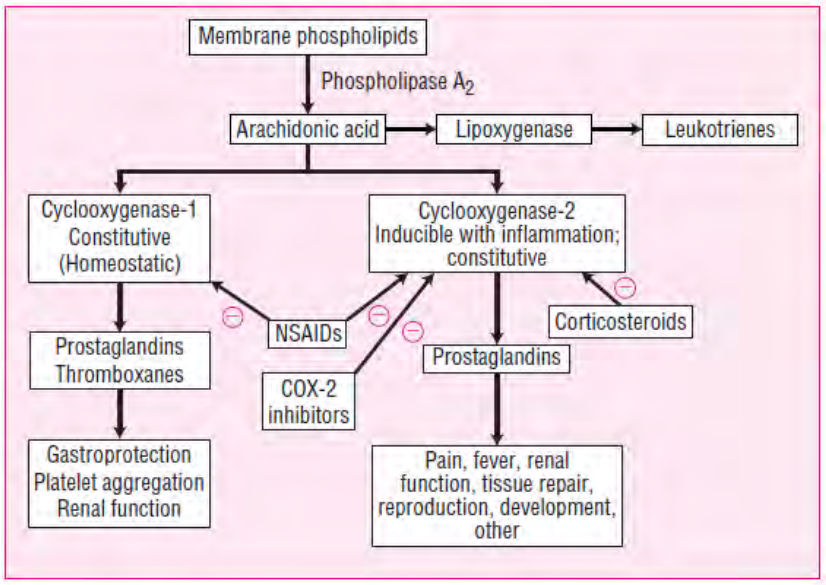

Figure 4 Blockade of prostaglandin synthesis through inhibition of cyclooxygenase (both COX-1 and COX-2 enzymes) is believed to be the principal mechanism by which NSAIDs relieve pain and inflammation (O’Connell and Seaton, 2005).

Indications and uses

Nonopioids relieve a variety of types of acute and chronic pain (e.g., trauma, postoperative, cancer, arthritis pain) and are especially effective for certain types of somatic pain (e.g., muscle and joint pain, bone/dental pain, inflammatory pain, postoperative pain). Acetaminophen and NSAIDs, alone, often relieve mild pain, and some NSAIDs relieve certain types of moderate pain. Even for moderate or severe pain

that does require an opioid, nonopioids are often added to the regimen for their opioid-sparing effect (i.e., they lower the dose of opioid required). Since nonopioids and opioids relieve pain via different mechanisms, combination therapy offers the potential for improved relief with fewer side effects. Nonopioids do not produce tolerance, physical dependence, or addiction. Choice of NSAID is influenced by factors including medication tolerance, dosing frequency, and cost (Berry et al., 2006).

Side effects

Inhibition of COX-1 causes some of the side effects of nonselective NSAIDs. Adverse effects of nonselective NSAIDs as a class include GI problems (e.g., dyspepsia, ulcers, perforation, bleeding, liver dysfunction), bleeding (i.e., "antiplatelet effect"), kidney dysfunction, hypersensitivity reactions (e.g., Skin rash, erythema, irritation, skin eruptions, exfoliative dermatitis, Stevens-Johnson syndrome, ecchymosis, and purpura), and CNS effects such as dizziness, insomnia, confusion, hearing loss, tinnitus, and even headache.

Acetaminophen or a selective COX-2 inhibitor may be an appropriate treatment alternative to nonselective NSAIDs in some patients. Acetaminophen does not damage the gastric mucosa or inhibit platelet aggregation and provides pain relief comparable to that of aspirin. However, acetaminophen has negligible anti-inflammatory activity. In addition, acute or chronic overdose with acetaminophen may cause liver or kidney toxicity, so acetaminophen should be used with caution in patients with certain conditions (e.g., malnutrition, chronic alcoholism, liver disease). Accidental overdosage also may occur in patients taking over-the-counter combination pain relievers containing acetaminophen (Berry et al., 2006; Roach, 2006).

Opioid analgesics (opioids)

Opioid analgesics are the narcotic analgesics obtained from the opium plant. The narcotics obtained from raw opium (also called the opiates, opioids, or opiate narcotics) include morphine, codeine, hydrochlorides of opium alkaloids, and camphorated tincture of opium. Morphine, when extracted from raw opium and treated chemically, yields the semisynthetic narcotics hydromorphone, oxymorphone,

oxycodone, and heroin. Heroin is an illegal narcotic and is not used in medicine. Synthetic narcotics are those man-made analgesics with properties and actions similar to the natural opioids. Examples of synthetic narcotic analgesics are methadone, levorphanol, remifentanil, and meperidine (Roach, 2006).

Mechanism of action and effects

Narcotic analgesics are classified as agonists, partial agonists, and mixed agonists-antagonists. The agonist binds to a receptor and causes a response. A partial agonist binds to a receptor, but the response is limited (i.e., is not as great as with the agonist). Antagonists bind to a receptor and cause no response. An antagonist can reverse the effects of the agonist. This reversal is possible because the antagonist competes with the agonist for a receptor site. An agonist-antagonist has properties of both the agonist and antagonist. These drugs have some agonist activity at the receptor sites and some antagonist activity at other receptor sites. Classification of the narcotic analgesics is based on their activity at the opioid receptor sites. Although five categories of opioid receptors have been identified, only three of these receptors affect the action of the narcotic analgesics:

- **mu** : Morphine-like supraspinal analgesia, respiratory and physical depression, miosis, reduced GI motility
- **kappa** : Sedation and miosis
- **delta** : Dysphoria, psychotomimetic effects (e.g., hallucinations), respiratory and vasomotor stimulations caused by drugs with antagonist activity

Opioid receptor sites exist in the brain, spinal cord and elsewhere where opioids such as morphine bind to produce analgesia (and other pharmacological effects). The body's natural ligands for these receptors are the endorphin, enkephalin and dynorphin peptides. β-endorphin and longer-chain dynorphins have a more limited distribution in the CNS and may not influence pain processing directly. Rather, they may

have hormonal roles in responses to stress and fluid homeostasis, respectively (Greene and Harris, 2008; Wecker et al., 2010).

Pure agonists (e.g., morphine, pethidine) elicit a maximum response if given in sufficient concentration. The morphine-like opioids are thought to exert their agonist effects primarily at the mu receptor and to a lesser degree at the kappa receptor. The mixed agonist-antagonist drugs (e.g., pentazocine) act on the mu receptors by competing with other substances at the mu receptor (antagonist activity) and are agonists at other receptors. Partial agonists (e.g., buprenorphine) have limited agonist activity at the mu receptor (Greene and Harris, 2008; Roach, 2006).

Narcotic antagonists are antidotes for overdoses of narcotic analgesics. They have a higher affinity to the opiate receptor site than the narcotic analgesic and block the narcotic analgesic from binding to the opiate receptor site. They also reverse the respiratory and CNS depression caused by the narcotics. Naloxone is a narcotic antagonist and can be used to determine if an unconscious patient has used an opioid narcotic drug. If the patient wakes up after naloxone is administered intravenously, the patient is likely to have ingested or injected an opioid narcotic (Kamienski and Keogh, 2006).

Indications and uses

Opioids are used to treat moderate to severe pain that does not respond to nonopioids alone. They are often combined with nonopioids because this permits use of lower doses of the opioid (i.e., dose-sparing effect). Nearly all types of pain respond to opioids; however, nociceptive pain is generally more responsive to opioids than neuropathic pain, which may require higher doses of opioids. Opioids play a major role in the treatment of acute pain (e.g., trauma, postoperative pain), breakthrough pain, cancer pain, and some types of chronic noncancer pain (CNCP). Because responsiveness to opioids varies greatly among individuals, a patient who has failed to respond to an adequate trial of one opioid should try another. Although opioids vary in potency, more potent agents are not necessarily superior. Opioids are also categorized as weak opioids and strong opioids (Berry et al., 2006).

Side effects

Binding of mu agonist opioids to receptors in various body regions (e.g., CNS, GI tract) results in therapeutic effects and side effects. Side effects of mu agonist opioids as a class include sedation, mental clouding or confusion, respiratory depression, nausea, vomiting, constipation, pruritus (itching), and urinary retention. With the exception of constipation, these side effects tend to subside with time. Most opioids should be used with caution in patients with impaired ventilation, bronchial asthma, liver failure, or increased intracranial pressure. Opioid-induced respiratory depression is usually short-lived, antagonized by pain, and most common in the opioid-naive patient (Berry et al., 2006).

Narcotics may be ineffective against some forms of chronic pain, especially since changes in the spinal cord may alter the usual pain signaling pathways. Furthermore, narcotics are usually not recommended for long-term use because the body develops a tolerance to narcotics, reducing their effectiveness over time. In such situations, pain can be managed with antidepressants and anticonvulsants, which are also only available with a doctor's prescription (Longe, 2002).

Although antidepressant drugs were developed to treat depression, it has been discovered that they are also effective in combating chronic headaches, cancer pain, and pain associated with nerve damage. Antidepressants that have been shown to have analgesic (pain reducing) properties include amitriptyline, trazodone, and imipramine. Anticonvulsant drugs share a similar background with antidepressants. Developed to treat epilepsy, anticonvulsants were found to relieve pain as well. Drugs such as phenytoin and carbamazepine are prescribed to treat the pain associated with nerve damage (Longe, 2002).

Other prescription drugs are used to treat specific types of pain or specific pain syndromes. For example, corticosteroids are very effective against pain caused by inflammation and swelling, and sumatriptan was developed to treat migraine headaches (Longe, 2002).

INFLAMMATION

Inflammation is a response to infection, antigen challenge or tissue injury that is designed to eradicate microbes or irritants and to potentiate tissue repair.

Causes of an inflammation can be:

- Microorganisms, such as bacteria, viruses, fungi, or parasites;
- Foreign bodies (foreign protein, e.g., pollen, asbestos or silicon crystals);
- Tissue destruction with formation of tissue debris, for example, through *mechanical damage* such as cuts, stabs, scratches or foreign bodies, *chemical compounds* such as acids or alkalis, *physical influences* such as cold, heat, radiation (UV, X-rays, radioactivity), and endogenous causes such as disintegrating tumor cells, extravascular blood, autoimmune reactions, or crystals of substances precipitated in the body (uric acid, calcium oxalate, calcium phosphate, and cholesterol).

Excessive inflammation may lead to tissue injury and can, if severe, cause physiological decompensation, organ dysfunction and death (Sherwood and Toliver-Kinsky, 2004; Silbernagl and Lang, 2000). The classical signs of inflammation-redness (rubor), swelling (tumor), heat (calor) and pain (dolor) are indicative of the acute phase of the inflammatory response where local release of mediators results in an increase in capillary blood flow, leakage of plasma into the tissue, and pain (Dawson and Willoughby, 1985; Sigal and Ron, 1994; Silbernagl and Lang, 2000; Singh et al., 2008).

Inflammation can be divided into two major categories—acute and chronic—based on timing and pathological features.

Acute inflammation is typically of relatively short duration (hours to days) and is characterized by vasodilation, the exudation of protein-rich fluid (plasma) and a migration of cells (primarily neutrophils) into the site of injury and, in some cases, activation of the coagulation cascade. Sepsis, severe trauma and major surgery all have major acute inflammatory components (Sherwood and Toliver-Kinsky, 2004).

Chronic inflammation is a prolonged duration (weeks to months to years) in which active inflammation, tissue destruction and attempts at tissue repair are occurring simultaneously. Typical histological features of chronic inflammation are infiltration of mononuclear cells and fibrosis. The persistent chronic inflammation increases the development of the degenerative diseases such as rheumatoid arthritis, systemic lupus erythematosus, silicosis, atherosclerosis and inflammatory bowel disease (Sherwood and Toliver-Kinsky, 2004).

Injury causes the release of chemicals that stimulate a vascular response that force fluid and white blood cells to flow to the site of the injury. Stimulated nerve endings signal the brain that there is an injury. These chemicals are

• Histamines: this chemical works to bring more blood and lymph fluid to the site of the invasion.

• Serotonin: promotes the aggregation of thrombocytes; it causes pain, can increase the permeability of peripheral capillaries, and can produce edema.

• Kinins: these are blood plasma proteins that influence smooth muscle contractions, increase blood flow throughout the body, increase the permeability of small capillaries, and stimulate pain receptors.

• Prostaglandins: they work as chemical messengers. They do not move but work right in the cell where they are synthesized. They are synthesized in every cell in the body. These chemicals activate the inflammatory response and produce pain and fever. They are produced in response to the white blood cells that flow to the area of injured tissue (Kamienski and Keogh, 2006; Silbernagl and Lang, 2000).

Vasodilation is a classic feature of acute inflammation and is clinically characterized by redness and warmth at the site of injury. The purpose of the vasodilatory response is to facilitate the local delivery of soluble mediators and inflammatory cells. Inflammation-induced vasodilation is mediated primarily by nitric oxide (NO) and vasodilatory prostaglandins. NO production is mediated by several nitric oxide synthase (NOS) isoforms. Inducible NOS (iNOS) production is induced by inflammatory mediators such as TNF-α and IL-1. NO causes vascular smooth muscle

relaxation via cyclic GMP-mediated mechanism resulting in vasodilation at the site of inflammation. The primary vasodilatory prostaglandins are prostacyclin (PGI_2), PGD_2, PGE_2 and $PGF_{2\alpha}$ (Figure 5). These lipid mediators are produced from arachidonic acid through the action of cyclooxygenase (Sherwood and Toliver-Kinsky, 2004).

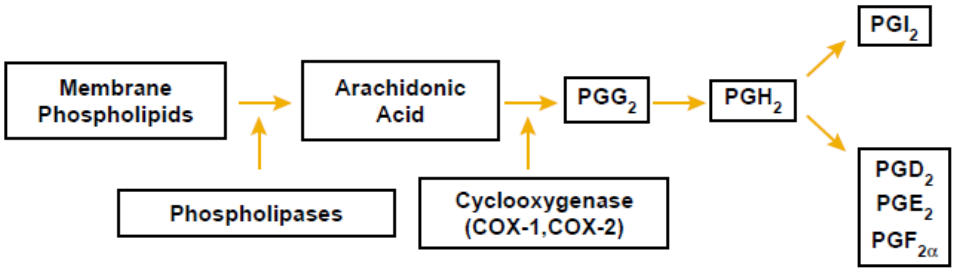

Figure 5 Vasodilatory prostaglandins are produced through the actions of phospholipase and cyclooxygenase. The major vasodilatory prostaglandins are prostacyclin (PGI_2) and the prostaglandins PGD_2, PGE_2 and $PGF_{2\alpha}$ (Sherwood and Toliver-Kinsky, 2004).

Another early sign of inflammation is edema formation. Edema is caused by the transvascular flux of protein-rich fluid from the intravascular compartment into the interstitium as a result of the actions of histamine, bradykinin, leukotrienes, complement components, substance P and platelet-activating factor (PAF). These factors markedly alter the barrier functions of small blood vessels and increase the permeabililty of capillaries venules for both water and protein (Sherwood and Toliver-Kinsky, 2004).

The term eicosanoid is used to represent a large family of endogenous compounds containing oxygenated unsaturated 20-carbon fatty acids and includes the prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs). The name PG was derived from the gland from which these compounds were first isolated. The PGs, TXs, and LTs are synthesized as shown in Figure 6. Most pathways originate with the parent compound arachidonic acid, a major component that is cleaved from membrane phospholipids by phospholipase A_2 and are metabolized by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (Wecker et al., 2010).

Two distinct COXs have been described and have been designated as COX-1 and COX-2. The COX-1 enzyme is involved in “housekeeping” or routine

physiologic functions such as generation of gastroprotective prostaglandins to promote gastric blood flow and bicarbonate generation. COX-1 is expressed constitutively in gastric mucosa, vascular endothelial cells, platelets, and renal collecting tubules, so that COX-1–generated prostaglandins also participate in hemostasis and renal blood flow. In contrast, the COX-2 enzyme is not normally expressed in most body tissues, but is rapidly induced by inflammatory mediators, local injury, and cytokines including interleukins, interferon, and tumor necrosis factor (O'Connell and Seaton, 2005; Wecker et al., 2010).

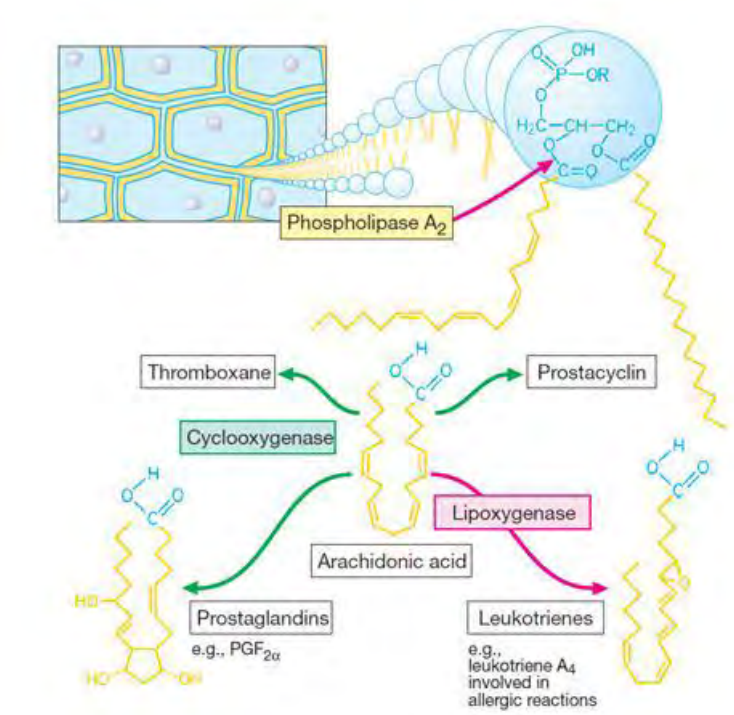

Figure 6 Biosynthesis of the prostaglandins, thromboxanes and leukotrienes by the cyclooxygenase and lipoxygenase pathway (Silbernagl and Lang, 2000).

PHARMACOLOGICAL MANAGEMENT OF INFLAMMATION

Although inflammation is a natural response to injury, this process can be uncomfortable for a patient, especially when there is fever, pain, and swelling. Anti-inflammatory medication can be given to reduce the inflammatory process and bring comfort to the patient. Anti-inflammatory medication stops the production of prostaglandins resulting in a decrease in the inflammatory process (Kamienski and Keogh, 2006).

Glucocorticoids (often Prednisolone) are frequently used to suppress inflammation, allergy and immune response. Anti-inflammatory therapy is used in many diseases (e.g., rheumatoid arthritis, ulcerative colitis, bronchial asthma, severe inflammatory conditions of the eye and skin). Steroids can control inflammation by suppressing or preventing many of the components of the inflammatory process at the injured site, but high doses and prolonged use may cause severe adverse effects such as increased blood pressure, induced to Cushing's syndrome, increased risk of infection, GI problems (e.g., ulceration, bleeding), osteoporosis, pathological fractures, withdrawal syndrome with sudden discontinuation etc (Berry et al., 2006; Kamienski and Keogh, 2006; Neal, 2002).

There are other anti-inflammatory medications that are not chemically the same as corticosteroid medication. These are referred to as nonsteroidal anti-inflammatory drugs (NSAIDs). However, inhibition of prostaglandin synthesis by NSAIDs attenuates rather than abolishes inflammation. Nevertheless, the relatively modest anti-inflammatory actions of the NSAIDs give, to most patients with rheumatoid arthritis, some relief from pain, stiffness and swelling, but they do not alter the course of the disease (Kamienski and Keogh, 2006; Neal, 2002).

Examples of NSAIDs (Berry et al., 2006).

1. Salicylates	Aspirin, Diflunisal, Trolamine salicylate
2. Propionic acid derivatives	Ibuprofen, Naproxen, Ketoprofen, Oxaprozin
3. Indole acetic acids	Indomethacin

4. Benzothiazine derivatives	Piroxicam, Meloxicam
5. Pyrrole acetic acid derivatives	Diclofenac, Ketorolac
6. Selective COX-2 inhibitors	Celecoxib

CURCUMIN

Curcumin is the active ingredient isolated from the rhizome of turmeric (*Curcuma longa L.,* Zingiberaceae) (Mukhopadhyay et al., 1982; Sharma et al., 2005; Jurenka, 2009). Turmeric is a tropical plant cultivated in India, Southeast Asia, China, and other Asian and tropical countries and regions (Goel et al., 2008). Its measures up to 1 m high with a short stem and tufted leaves (Figure 7A), distributed throughout tropical and subtropical regions of the world (Araújo and Leon, 2001; Aggarwal et al., 2004; Jurenka, 2009). For over 4000 years, tumeric has been used in traditional Asian and African medicine to treat a wide variety of ailments because of its wide spectrum of pharmacological activities.

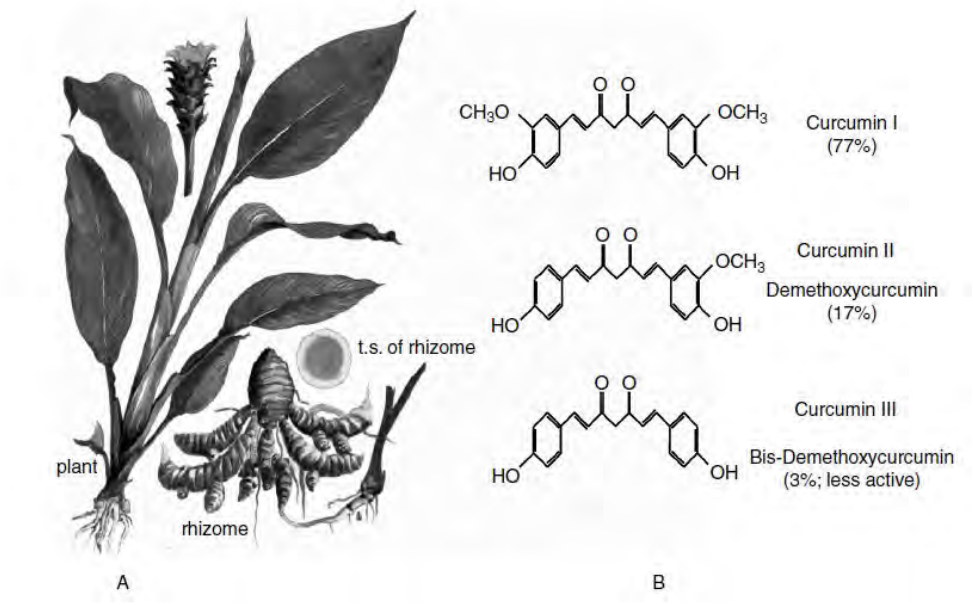

Figure 7 The plant *Curcuma longa* (panel A), from which curcumin is derived, and its structure (panel B) (Aggarwal et al., 2004).

Turmeric is comprised of curcuminoids, volatile oils (tumerone, atlantone, and zingiberone), sugar, proteins, and resins (Jurenka, 2009). Medicinal properties of turmeric have been attributed mainly to the yellow pigment called curcuminiods, phenolic compounds derived from the roots of *Curcuma* spp. (Figure 8) (Sharma et al., 2005). The content of three curcuminoids, curcumin, demethoxycurcumin and bis-

demethoxycurcumin (Figure 7B), were detected in *Curcuma longa* and confirmed that curcumin was rich in *Curcuma longa* (3.9-12.3% in the methanol extract) (Tohda et al., 2006).

In Thailand, *C. longa* can be cultivated in all regions of the country. Variation of total curcuminoids in dried powder of *C. longa* rhizome collected from 13 locations of Thailand have been investigated. The highest total curcuminoids content (8.99±0.83% w/w) was found in the southern samples while the lowest content (4.80±1.83% w/w) was found in the northern samples (Pothitirat and Gritsanapan, 2006).

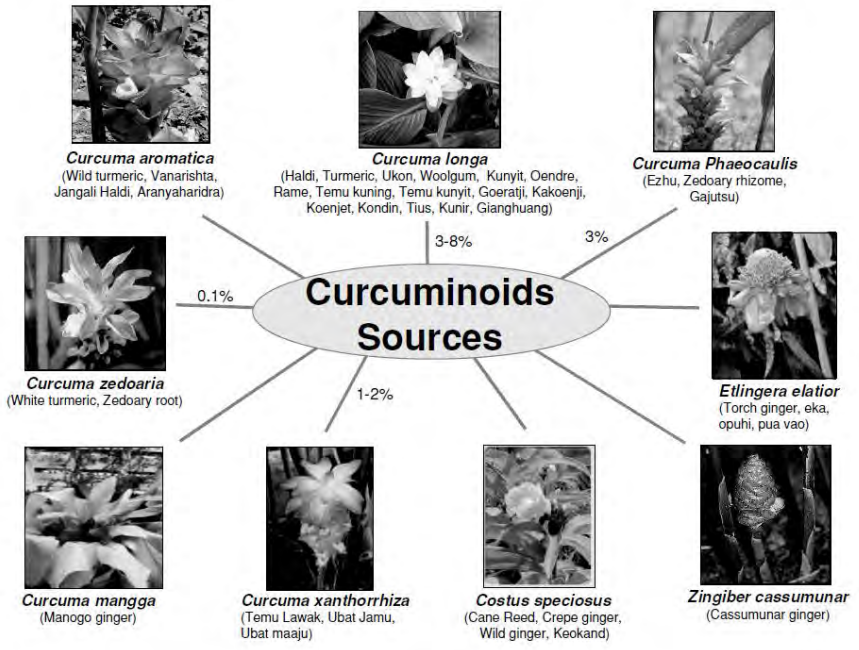

Figure 8 Sources of curcuminoids (Aggarwal et al., 2007)

Curcumin is a low molecular weight polyphenol that gives a yellow color to turmeric and is now recognized as being responsible for most of the therapeutic effects. It is estimated that 2–5% of turmeric is curcumin. Curcumin was first isolated from turmeric in 1815, and the structure was delineated in 1910 as diferuloylmethane and Lampe solved its structure in 1913. Curcumin has a melting point of 183°C, a molecular formula of $C_{21}H_{20}O_6$ with a molecular weight of 368.37 g/mol. The IUPAC name of curcumin is 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 dione which is also known as diferuloylmethane. In chemical terms it is bis-α, β-unsaturated β-diketone,

a linear diarylheptanoid compound, where two oxy-substituted aryl moieties are linked together through a seven carbon chain (Figure 7B). The aryl rings may be substituted by varying numbers of hydroxy or methoxy groups in a symmetrical or asymmetrical fashion to produce analogues of curcumin or curcuminoids. Most currently available preparations of curcumin contain approximately 77% diferuloylmethane, 18% demethoxycurcumin in which one methoxy group is absent, and 5% bis-demethoxycurcumin in which the methoxy group is absent from the aryl rings (Figure 7B). Curcumin is insoluble in water and ether but frequently soluble in dimethylsulfoxide, acetone, ethanol, acetic acid, chloroform and oils. Spectrophotometrically, the maximum absorption (λ_{max}) of curcumin in methanol occurs at 430 nm and in acetone at 415-420 nm. Curcumin is unstable at neutral and basic pH values and is degraded to ferulic acid (4-hydroxy-3-methoxycinnamic acid) and feruloylmethane (4-hydroxy-3 methoxycinnamoylmethane) (Rahman et al., 2006). Although the exact mechanism of degradation is still not fully obvious, the idea that an oxidative mechanism may be involved gains ground from observations that the presence of antioxidants such as ascorbic acid, N-acetyl-L cysteine, or glutathione completely blocks the degradation of curcumin at pH 7.4. However, the stability of curcumin increases in an acidic pH condition. Therefore, curcumin could be stable in the stomach and small intestine because the pH is between 1 and 6, and degradation of curcumin is extremely slow under these conditions (Rahman et al., 2006). When exposed to acidic conditions, the color of curcumin turns from yellow to deep red, the form in which it is used routinely for various religious ceremonies. (Aggarwal et al., 2007; Anand et al., 2008; Epstein et al., 2010; Goel et al., 2008).

Pharmacokinetics and metabolism of curcumin

The absorption, distribution, metabolism, and excretion of curcumin in rodents have been described in at least 10 studies over the past three decades. Collectively, these studies support the notion that curcumin undergoes a rapid and efficient metabolism that severely curtails the availability of parent compound in the biophase. In an early study in rats, a dietary dose (1 g/kg) administered to rats resulted

in about 75% of species related to curcumin being detected in feces, whereas negligible amounts appeared in the urine. In another early article, absorption of oral curcumin was 60%; urinary agent-derived species were characterized as glucuronide and sulfate conjugates. When curcumin bioavailability was investigated using a ^{3}H-radiolabeled agent, the vast majority of the oral dose was excreted in the feces and one-third was excreted unchanged. Intravenous and intraperitoneal administration of curcumin in rats resulted in large quantities of curcumin and metabolites in bile. The metabolites were characterized mainly as glucuronides of tetrahydrocurcumin and hexahydrocurcumin. After intravenous dosing, more than 50% of the dose was excreted in the bile within 5 hr. This finding was interpreted as evidence in support of the hypothesis that curcumin undergoes biotransformation during absorption in the intestinal tract and enterohepatic recirculation.

More recently, curcumin (0.1 g/kg) administered intraperitoneally to the mouse was found to undergo metabolic reduction to dihydrocurcumin and tetrahydrocurcumin, which, in turn, were converted to monoglucuronide conjugates. High pressure liquid chromatography (HPLC) analysis of plasma from rats that had received oral curcumin demonstrated substantial levels of curcumin glucuronide and curcumin sulfate, small quantities of hexahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin glucuronide and negligible amounts of curcumin. In suspensions of isolated human hepatocytes, or in microsomes derived from liver or gut tissues of rats and humans, curcumin was rapidly reduced to metabolites, as shown in figure 9. In a separate study in rats, a high dose of curcumin mixed into the diet (2%, equating to approximately 1.2 g/kg) for 14 days yielded low-nanomolar levels of the parent compound in the plasma, with concentrations in the liver and colon mucosa ranging from 0.1 to 1.8 nmol/g tissue. It is also conceivable that other constituents of the dietary matrix might alter the bioavailability of curcumin. When oral curcumin (2 g/kg) is co-administered to rats with l-piperoylpiperidine (piperine) that induces glucuronyl transferase enzymes, the systemic bioavailability of curcumin might be increased by as much as 154% (Sharma et al., 2007).

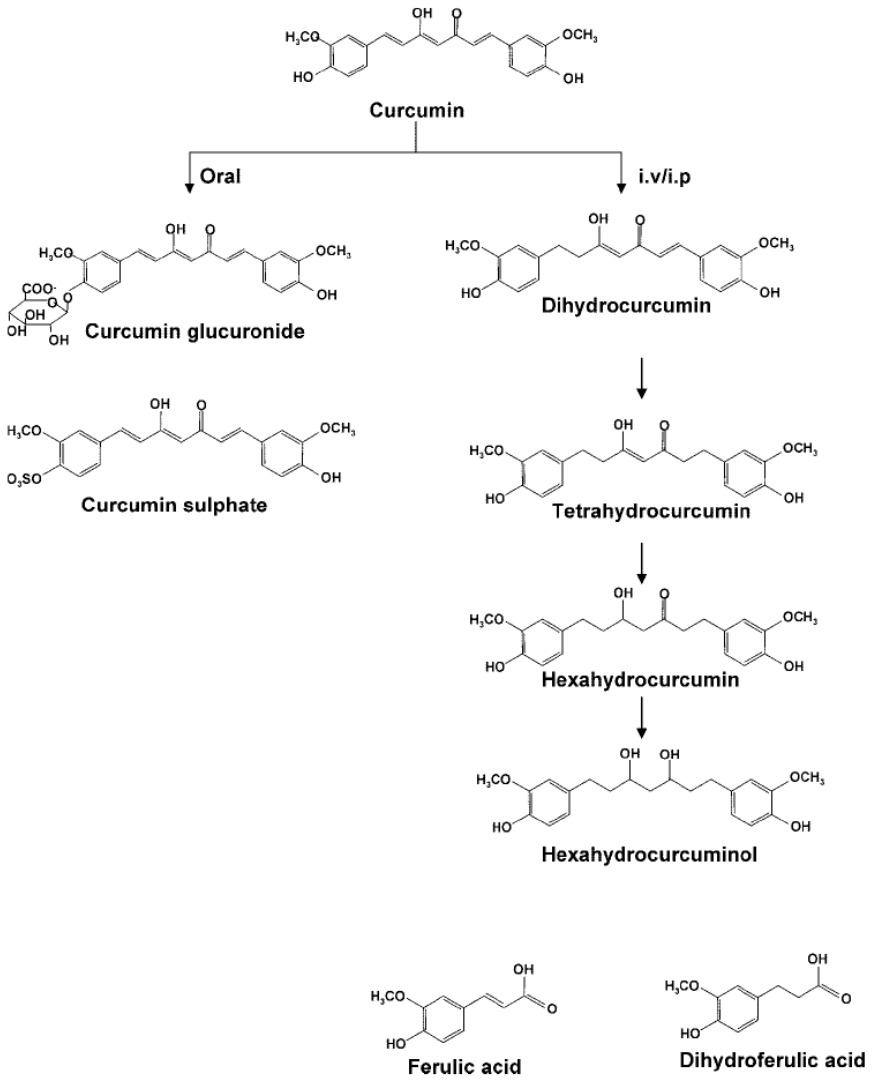

Figure 9 Major metabolites of curcumin detected in rodents and humans (Anand et al., 2007; Sharma et al., 2007).

In comparison to the preclinical work summarized above, comprehensive pharmacokinetic data in humans do not exist. Healthy volunteers who ingested 2 g pure curcumin powder after fasting showed less than 10 ng/ml curcumin in their plasma 1 hr postdose. In a study of oral curcumin, patients with preinvasive malignant or high-risk premalignant conditions of the bladder, skin, cervix, stomach, or oral mucosa received 0.5–8 g curcumin by mouth daily for 3 months. Plasma curcumin concentrations were found to peak 1–2 hr after intake and gradually declined within 12 hours. The 8-g/day dose resulted in a peak serum concentration of 1.75±0.80 μM, indicating curcumin is

poorly absorbed and may have limited systemic bioavilability (Jurenka, 2009). When curcumin in micronized form was administered orally with orange juice at doses of 50–200 mg to 18 healthy volunteers, curcumin was not found in the plasma at or above the limit of quantitation (approximately 0.63 ng/ml). Because of curcumin's rapid plasma clearance and conjugation, its therapeutic usefulness has been somewhat limited, leading researchers to investigate the benefits of complexing curcumin with other substances to increase systemic bioavailability. One substance that has been studied is the alkaloid piperine, a constituent from black pepper and long pepper (*Piper nigrum* and *Piper longum,* respectively). In humans 20 mg piperine given concomitantly with 2 g curcumin increased serum curcumin bioavailability 20-fold (2000%), which was attributed to piperine's inhibition of hepatic glucuronidation and intestinal metabolism (Anand et al., 2007; Jurenka, 2009; Sharma et al., 2007).

Exploratory studies have also been performed in patients undergoing operations for colorectal cancer who consented to have tissues analysed for research purposes. Twelve patients with confirmed colorectal cancer received oral curcumin at 0.45, 1.8, or 3.6 g/day for 7 days prior to surgery. Levels of agent-derived species were determined in blood and colorectal tissue obtained at the time of surgical resection. The mean concentrations of curcumin in normal and malignant colorectal tissue of patients who had ingested 3.6 g curcumin daily were 12.7 and 7.7 nmol/g tissue, respectively. Curcumin sulfate and curcumin glucuronide were also found in the intestinal tissue taken from these patients; trace levels of curcumin were detected in peripheral blood samples. Compatible with the preclinical data presented earlier, these preliminary results in humans suggest that a daily dose of 3.6 g curcumin achieves measurable levels in colorectal tissue with negligible distribution of the parent drug outside of the gut. When 12 patients with liver metastases from colorectal cancer received oral curcumin (0–3.6 g) daily for 7 days prior to hepatic surgery, curcumin was not found in liver tissue resected 6–7 hr after the last dose of curcumin, whereas trace levels of products of its metabolic reduction were detected. Levels of curcumin and glucuronide and sulfate conjugates in the low-nanomolar range were found in blood samples taken 1 hr after the last dose. The results of this pilot study suggest that doses of oral curcumin required to

produce hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans (Sharma et al., 2007).

Although research on curcumin pharmacokinetics in healthy subjects is limited, one study using high doses (10 and 12 g in a single oral dose) in 12 healthy subjects measured serum curcumin as well as its sulfate and glucuronide metabolites at various time points up to 72 hours post-dose. As in previous studies, curcumin was rapidly cleared (only one subject had detectable free curcumin in the serum) and subsequently conjugated in the gastrointestinal tract and liver. Area under the curve (AUC) for curcumin conjugates was surprisingly higher (35.33 ± 3.78 μg/mL) for the 10-g dose than for the 12-g dose (26.57 ± 2.97 μg/mL), perhaps indicating saturation of the transport mechanism in the gut for free curcumin. Maximum serum concentration (C_{max}) for the 10-g dose was 2.30 ± 0.26 μg/mL compared to 1.73 ± 0.19 μg/mL for the 12-g dose (Jurenka, 2009).

To summarize the data from pilot and phase I clinical studies performed with curcumin, it appears that low systemic bioavailability following oral dosing is consistent with the findings in preclinical models presented earlier. Efficient first-pass and some degree of intestinal metabolism of curcumin, particularly glucuronidation and sulfation, might explain its poor systemic availability when administered via the oral route. A daily oral dose of 3.6 g of curcumin results in detectable levels in colorectal tissue, which might be sufficient to exert pharmacological activity, with negligible distribution of the parent drug in hepatic tissue or other tissues beyond the gastrointestinal tract (Sharma et al., 2007).

The pharmacological activities of curcumin have been studied intensively from many country for over 4,000 years. Traditionally, turmeric has been put to use as a foodstuff, cosmetic, and medicine, As a spice, it is used to provide curry with its distinctive yellow color and flavor. It is used as coloring agent in cheese, butter, and other foods. In folk medicine, turmeric and natural curcuminoids have been applied as therapeutic preparations over the centuries in different parts of the world. In Ayurvedic medicine, curcumin is a well-documented treatment for various respiratory conditions (e.g., asthma, bronchial hyperactivity, and allergy) as well as for liver disorders,

anorexia, rheumatism, diabetic wounds, runny nose, cough, and sinusitis (Goel et al., 2008). A poultice of turmeric paste is used to treat common eye infections, and to dress wounds, treat bites, burns, acne and various skin diseases (Hatcher et al., 2008). Moreover, curcumin powder has been claimed to against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Anand et al., 2007; Jayaprakasha et al., 2005). In old Hindu medicine, is extensively used for the treatment of sprains and swellings caused by injury. The traditional medicine in China uses curcumin in diseases, which are associated with abdominal pains (Araújo et al., 2001; Chattopadhyay et al., 2004; Goel et al., 2008).

Throughout the Orient, curcumin has traditionally been used to good therapeutic effect, particularly as an anti-inflammatory, and many of its therapeutic effects have been confirmed by modern scientific research. Such effects include anti-oxidant, anti-inflammatory, anticarcinogenic, anti-platelet aggregation, antiviral, antiparasitic, hepatoprotective, antimutagenic, wound healing, anti-protozoal activity, cardiovascular (i.e., as protection against myocardial infarcton), and antimicrobial (Araújo et al., 2001; Chattopadhyay et al., 2004; Goel et al., 2008; Itokawa et al., 2008; Maheshwari et al., 2006; Srivastava et al., 1995). In various chronic illnesses in which inflammation is known to play a major role, curcumin has been shown to exhibit therapeutic potential. These diseases include Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis, epilepsy, cerebral injury, CVDs, cancer, allergy, asthma, bronchitis, colitis, rheumatoid arthritis, renal ischemia, psoriasis, diabetes, obesity, depression, fatigue, and AIDS (Aggarwal and Harikumar, 2009).

The study concerning anti-inflammatory and antinociceptive effects of curcumin and curcumin derivatives have been investigated in various animal models as followed:

Anti-inflammation of curcumin

Curcumin was isolated from alcoholic extract of powdered rhizomes of *C. longa* and purified by recrystallization. Curcumin analogues, sodium curcuminate (NaC), diacetyl curcumin (DAC), triethyl curcumin (TEC) and tetrahydro curcumin (THC),

were synthesized in laboratories from curcumin. The anti-inflammatory activity of curcumin and curcumin analogues was compared with phenylbutazone (PB) using carrageenan-induced rat paw edema model at 3, 10, 30 and 60 mg/kg administered orally. The rank order of potencies of curcumin analogues and PB were NaC>THC>C>PB>TEC. The curcumin analogues decreased carrageenan-induced paw edema at low doses but at higher doses this effect was partially reversed (Mukhopadhyay et al., 1982).

The synthesis of a series of symmetrical curcumin derivatives, in total 16 curcumin analogues, were determined the anti-inflammatory activity by carrageenan-induced rat paw edema. Rats were given graded doses of the test compounds of 10, 20, 40 and 80 mg/kg body weight administered intraperitoneally. It appeared that the *para* hydroxy groups in curcumin are important for anti-inflammatory activity. This activity is enhanced when, in combination with the *para* hydroxy groups, the *meta* positions are occupied with alkyl groups. Since the methyl derivatives are more active than the corresponding ethyl and *tert-butyl* derivatives, it is suggested that sterical hindrance is involved (Nurfina et al., 1997).

Prophylactic and therapeutic studies on arthritis rats with dietary lipids and spice principles were studied. The combination of curcumin (30 mg/kg bw/day), capsicin (5 mg/kg bw/day) and dietary n-3 polyunsaturated fatty acids (dietary oils 1 ml) were administered on adjuvant induced arthritis in Wistar rats by gavage. These studies indicated that the dietary n-3 polyunsaturated fatty acids, capsaicin, and curcumin can decrease the incidence, delay the onset and reduce the extent of inflammation of adjuvant-induced arthritis in rats (Joe and Lokesh, 1997).

Three series of mono-carbonyl curcumin analogues and curcumin were tested for anti-inflammatory activity *in vitro.* They were measured as the ability of these compounds to inhibit LPS-induced TNF-α and IL-6 expression in mouse J774A.1 using enzyme-linked immunosorbent assays (ELISA). The results indicated that several novel curcumin analogues inhibited LPS-induced TNF-α and IL-6 expression better than curcumin due to the properties and position of the substituent and the space of the linking chain of curcumin analogues (Liang et al., 2008).

Synthetic curcumin (25, 50, 100, 200 and 400 mg/kg, p.o.) was evaluated in a carrageenan-induced rat paw edema test and compared with that of indomethacin (10 mg/kg, p.o.). The results showed that low doses of synthetic 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 inhibitory effect of synthetic curcumin began at 2 hr or later after carrageenan injection suggests that this compound produce anti-inflammatory effects in the second phase of edema, indicating inhibition of prostaglandin synthesis (Buadonpri et al., 2009).

Nine curcumin analogues containing five-carbon linker with mono-carbonyl and curcumin were evaluated their anti-inflammatory properties through inhibiting the LPS-induced TNF-α and IL-6 release in mouse J774A.1 macrophages using enzyme-linked immunosorbent assays (ELISA). The results showed that the 3'-methoxyl group plays an important role in bioactivity and cyclohexanone containing analogues exhibited stronger inflammatory inhibition than other analogues. It is suggested that five-carbon linker analogues containing a cyclohexane ring may be important for the development of anti-inflammatory curcumin drugs (Liang et al., 2009).

Antinociception of curcumin

The antinociceptive effect of curcumin on neuropathic pain in streptozotocin-injected mice was tested in the tail immersion and hot-plate assays. Chronic treatment with curcumin (15, 30 and 60 mg/kg body weight; p.o.) were given in streptozotocin induced diabetic mice for 4 weeks. Curcumin suspension was prepared in 0.5% carboxymethylcellulose solution. The results showed that chronic treatment with curcumin significantly attenuated thermal hyperalgesia and the hot-plate latencies. Curcumin also inhibited tumour necrosis factor-α (TNF-α) and nitric oxide (NO) release in streptozotocin induced diabetic mice in a dose dependent manner. These results indicate an antinociceptive activity of curcumin possibly through its inhibitory action on NO and TNF-α release (Sharma et al., 2006).

The effect of curcumin on the formalin-induced pain was investigated in albino wistar rats. Interaction between curcumin and opioid system using morphine and

naloxone was also examined. Curcumin suspension was prepared in 5% ethanol solution and administered orally at the of 25, 50, 100 and 200 mg/kg body weight 45 min before formalin injection. Curcumin, morphine and naloxone had no effect on the early phase of pain. Late phase of pain was suppressed significant by curcumin (100 and 200 mg/kg) and morphine. Curcumin did not influence the morphine-induced antinociception, but reversed the effect of naloxone on pain indicated that curcumin may produce antinociception by activation of both opioid and non opioid mechanism of pain (Tajik et al., 2007).

The effect of chronic oral administration of curcumin in the presence or absence of morphine and naloxone was investigated on the visceral nociception induced by acetic acid in rats. Curcumin suspension was prepared in 0.15 M NaCl (normal saline) and was administered orally at the doses of 10, 20 and 40 mg/kg body weight once daily for 8 days. Curcumin doses of 20 and 40 mg/kg significantly increased the latency time to the beginning of the first writhe. The number of writhes was significantly decreased by curcumin at the doses of 20 and 40 mg/kg. Curcumin significantly enhanced the effect of morphine on the visceral pain responses but no effect on the action of naloxone. The antinociceptive effect of curcumin is involved in the endogenous opioid analgesic system (Tajik et al., 2008).

Anti-inflammatory mechanisms of curcumin

Curcumin is a highly pleiotropic molecule capable of interacting with numerous molecular targets involved in inflammation. It modulates the inflammatory effect through inhibition of nuclear factor kappa B (NF-κB) activation. NF-κB, a ubiquitous eukaryotic transcription factor, is involved in regulation of inflammation, cellular proliferation, transformation, and tumorigenesis. Curumin is thought to suppress NF-κB activation and proinflammation gene expression by blocking phosphorylation of inhibitory factor I-kappa B kinase (IκB). The anti-inflammatory role of curcumin is also mediated through down-regulating the activity of cyclooxygenase-2 (COX-2), lipoxygenase, and inducible nitric oxide synthetase (iNOS) enzymes; inhibits the production of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α),

interleukin (IL)-1, -2, -6, and -12, monocyte chemoattractant protein (MCP), and migration inhibitory protein. Suppression of NF-κB activation subsequently down-regulates COX-2 and iNOS expression. Curcumin has also been shown to reduce the TNF-α-induced expression of the tissue factor gene in bovine aortic-endothelial cells by repressing activation of both AP-1 and NF-kB. Curcumin also enhances wound-healing in diabetic rats and mice, and in H_2O_2-induced damage in human keratinocytes and fibroblasts. The inhibitory action of curcumin on Janus kinase (JAK)-STAT signaling can contribute to its anti-inflammatory activity in the brain. In both rat primary microglia and murine BV2 microglial cells. These anti-inflammatory effects appear to be due to the suppression of the JAK-STAT inflammatory signaling cascade. In an animal model of inflammation, curcumin also inhibited arachidonic acid metabolism and inflammation in mouse skin epidermis via down-regulation of the cyclooxygenase and lipoxygenase pathways. (Aggarwal et al., 2004; Araujo and Leon, 2001; Chattopadhyay et al., 2004; Jayaprakasha et al., 2005; Jurenka, 2009; Kim et al., 2003; Strimpakos and Sharma, 2008;).

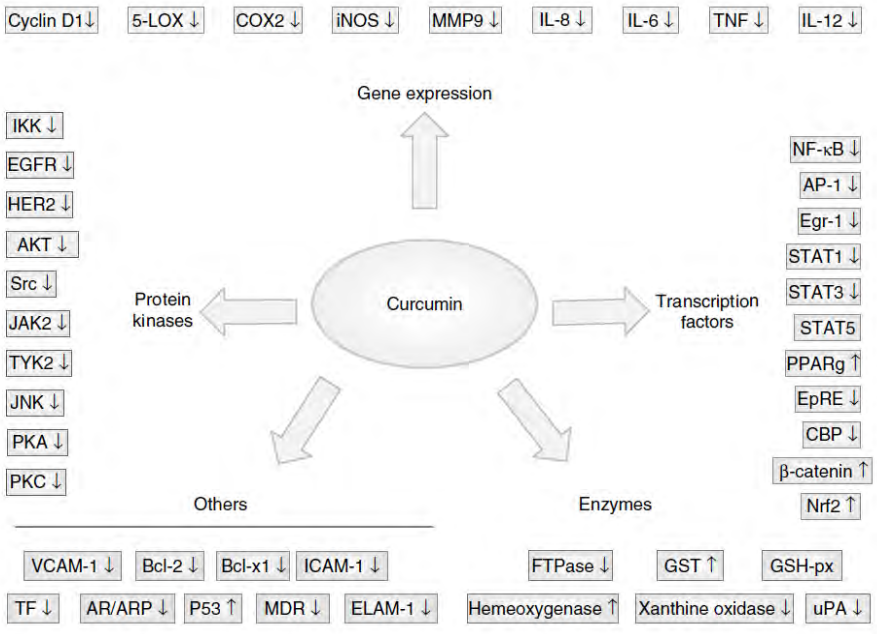

Figure 10 Molecular targets shown to be regulated by curcumin (Aggarwal et al., 2004).

Safety and toxicity

The experience of using curcumin in the diet for many centuries inspires confidence in its safety, but it cannot assume that diet-derived agents are innocuous when administered as pharmaceutical formulations at doses that generally exceed those consumed in the dietary matrix. Anecdotal reports suggest that dietary consumption of turmeric up to 1.5 g per person per day, equating to a probable maximum of 150 mg of curcumin daily, are not associated with adverse effects in humans. Studies of curcumin in animals have confirmed a lack of significant toxicity. In an early investigation, rats treated with up to 3.5 g/kg body weight per day (9.5 mmol/kg body weight) for 90 days displayed only discolored feces, yellow fur, and hematological changes not considered biologically significant. Other investigation, doses up to 5 g/kg were administered orally to Sprague–Dawley rats, resulting in no demonstrable toxicity. Systematic preclinical safety studies orchestrated by the US National Cancer Institute (NCI) did not discover any adverse effects in rats, dogs, or monkeys at doses of up to 3.5 g/kg administered up to 3 months in duration. One early report suggested a potentially ulcerogenic effect of dietary curcumin in the stomach of the albino rat, but this finding has not been replicated in subsequent rodent studies. In more recent preclinical investigations of dietary curcumin, toxicity has not been observed at 2% of the diet in rats (approximately 1.2 g/kg) or at 0.2% of the diet in mice (approximately 300 mg/kg) (Ringman et al., 2005; Sharma et al., 2007).

Although curcumin and turmeric are natural products used in the diet, the doses administered in clinical trials exceed those normally consumed in the diet. This fact underlines the need for systematic safety and toxicity studies. Turmeric is Generally Recognized As Safe (“GRAS”) by the U.S. FDA, and curcumin has been granted an acceptable daily intake (ADI) level of 0.1-3 mg/kg-body weight (BW) by the Joint FAO/WHO Expert Committee on Food Additives, 1996. In terms of dietary use in different countries, according to a study from Nepal, dietary consumption of turmeric up to 1.5 g per person per day, equivalent to 50 mg/day of curcumin, does not appear to be associated with adverse effects in humans. In India, where the average intake of

turmeric can be as high as 2.0-2.5 g per day (corresponding to 60-100 mg of curcumin daily), no toxicities or adverse effects have been reported at the population level. More valuable than such population dietary studies, which are potentially confounded by multiple variables and interactions, are the systematic preclinical studies funded by the Prevention Division of the U.S. National Cancer Institute. These studies did not demonstrate any adverse effects in rats, dogs, or monkeys at doses of curcumin up to 3.5 g/kg-BW administered for up to 90 days. A single report of curcumin-induced gastric ulceration in albino rats was reported in 1980, but this finding has not been replicated in subsequent studies. More recently, no toxicity has been observed in a preclinical study of the administration of 2% dietary curcumin (1.2 g/kg BW) to rats for 14 days or in a study of 0.2% dietary curcumin (300 mg/kg-BW) administered to mice for 14 weeks. Furthermore, a two-generation reproductive toxicity study in Wistar rats found no toxicity, reproductive or otherwise, related to oral curcumin administration (up to 1 g/kg-BW daily) in two successive generations of rats.

Contrary to the lack of toxicity with studies of curcumin, a rarely cited carcinogenicity study of turmeric oleoresin reported: (a) hyperplasia of the mucosal epithelium in the cecum and colon of male and female rats; (b) an increased incidence of hepatocellular adenoma in male and female mice; (c) a significantly increased incidence of thyroid gland follicular cell hyperplasia in female mice; and (d) small but significant increases in sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. Further studies with this preparation of turmeric oleoresin have not been performed (Rahman et al., 2006; Strimpakos and Sharma, 2008).

Similar to the conclusions regarding the safety of curcumin in preclinical models, clinical trials have documented minimal toxicity from administration of curcumin or turmeric, although it has not been clearly stated by the reporters of most of these studies which methodologic criteria have been used to assess potential toxicity. In a study performed in India, administration of 1.2-2.1 g of oral curcumin to patients with rheumatoid arthritis daily for 2-6 weeks did not cause any toxicity. In another study of high-dose oral curcumin by Cheng and colleagues in Taiwan, administration of up to 8 g

daily of curcumin for 3 months to patients with preinvasive malignant or highrisk premalignant conditions had no adverse effects (Cheng et al., 2001). In a phase I clinical trial of oral curcumin in patients with advanced colorectal cancer in which the U.S. National Cancer Institute (NCI) criteria were used to assess potential toxicity, curcumin was well tolerated at all dose levels up to 3.6 g daily for up to 4 months.

Adverse events probably related to curcumin consumption reported by patients in these studies were mainly gastrointestinal (nausea and diarrhea). Diarrhea (U.S. NCI toxicity grades 1 and 2, respectively) was experienced by one patient consuming 0.45 g curcumin daily and by another patient consuming 3.6 g daily, 1 and 4 months into treatment, respectively. A third patient, consuming 0.9 g of curcumin daily, reported nausea (NCI toxicity grade 2), which resolved spontaneously despite continuation of treatment. Two abnormalities were detected in blood tests in this trial, both possibly related to treatment: An increase in serum alkaline phosphatase level was observed in four patients (two were NCI grade 1, and two were grade 2); and three other patients had serum lactate dehydrogenase increases to 1.5 times the upper limit of normal. It is unclear whether these abnormal blood test results were related to the activity of the malignant disease in these patients or to treatment toxicity (Strimpakos and Sharma, 2008)

To date, no studies in either animals or humans have discovered any toxicity associated with the use of curcumin, and it is clear that curcumin is not toxic even at very high doses (Goel et al., 2008).

Curcumin diethyl disuccinate

Curcumin has been the subject of hundreds of published papers over the past three decades, studying its antioxidant, anti-inflammatory, cancer chemopreventive and chemotherapeutic properties (Sharma et al., 2005). Moreover, curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent. The poor aqueous solubility, relatively low bioavailability, and intense staining color of curcumin have been highlighted as major

problems; and consequently search for a ‘‘super curcumin’’ without these problems and with efficacy equal to or better than that of curcumin is ongoing (Anand et al., 2008).

Prodrug approach is one of the strategies searched for ‘‘super curcumin’’. Prodrug can be defined as precursors of active principles that can be used to modify a variety of both pharmaceutical and biological properties including modification of the pharmacokinetics of the drug *in vivo* to improve absorption, distribution, metabolism, and excretion (ADME). This type of strategy has been used in many different ways, including modification of physical-chemical properties to improve delivery, targeting to a specific enzyme or transporter, antibody-directed targeting, or gene-directed targeting. It also may lead to improvement of bioavailability by increased aqueous solubility, increase drug product stability, enhancement of patient acceptance and compliance by minimizing taste and odor problems, elimination of pain on injection, and decrease gastrointestinal irritation. It is usually accepted that a prodrug by itself should not possess any relevant pharmacological activity (Balant, 2003; Barbour and Lipper, 2008).

Succinylation is used to produce prodrug. It is an attachment of a succinic acid derivative to a drug molecule. (Wichitnithad et al., 2011). An example of the succinate prodrug is chloramphenicol for intravenous administration. A carboxylic acid group in the succinate pro-moiety, with a p*K*a value far below physiological pH, will be ionized at pH 7.4 and thereby increase the aqueous solubility of chloramphenicol-succinate ester compared to the parent chloramphenicol. Chloramphenicol itself is an alcohol with a p*K*a value far above pH 7.4, and consequently it is present as neutral species, with lower aqueous solubility, than the corresponding ionized succinate-ester prodrug (Luthman and Steffansen, 2010).

Curcumin diethyl disuccinate (CurDD) is a curcumin prodrug and synthesized by Wichitnithad et al. in 2011 Its chemical name is 4,4'-((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl) bis (2-methyl-4,1-phenylene) diethyl disuccinate (Figure 11). Succinylation of this compound is generated from the ester linkage between phenolic hydroxyl group in a curcumin molecule and succinicyl monoethyl ester. The chemical stability of CurDD in phosphate buffer at pH 7.4 was much more than

curcumin and CurDD was able to release the parent curcumin in human plasma (*in vitro*). CurDD showed anti-colon cancer activity when evaluated using Caco-2 cells with IC_{50} values in the 1.8–9.6 μM range, compared to IC_{50} values of 3.3–4.9 μM for the parent compounds (Wichitnithad et al., 2011).

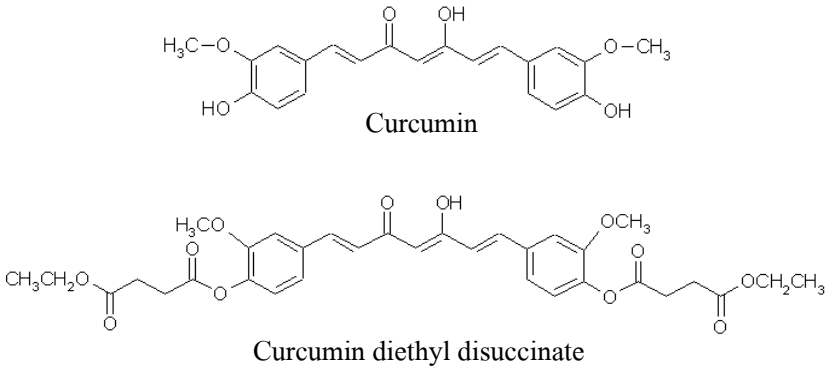

Figure 11 Chemical structures of curcumin and curcumin diethyl disuccinate

ANIMAL MODELS

Nociceptive Activity Testing

Over the years, many tests of nociception and pain in animals have been developed, and the species most frequently used in pain research are rats and mice. The use of animals in this research is necessary for several reasons. Since pain is a complex perceptual experience that can be quantified only indirectly in animal, behavioral measures or nociceptive reflexes in intact animals must be evaluated. Many different methods for testing of pain or nociception in animals have been developed. A few of the most commonly used methods will be described, which may illustrate important aspects of investigations of pain in animals. Special emphasis will be put on methodological problems in the tests (Tjolsen et al., 1994).

Table 1 Ethical guideline for investigations of experimental pain in conscious animals (Tjolsen et al., 1994).

1. Experiments must be reviewed by scientists and lay-persons, and the potential benefit must be made clear.
2. If possible, the investigator should try the pain stimulus on himself.
3. A careful assessment of the animal's deviation from normal behavior should be given.
4. A minimal pain stimulus necessary for the purposes of the experiment should be used.
5. Pain relieving agents or procedures should be used, as long as this will not interfere with the aim of the investigation.
6. The duration must be as short as possible, and the number of animals kept to a minimum.

The committee for Research and Ethical Issues of the International Association for the Study of Pain has published guidelines for investigations of experimental pain in conscious animals (Table 1). Several tests of nociception

measuring acute pain at or close to the pain threshold do not pose problems with respect to these guidelines, while there may be difficulties in applying the present guidelines to chronic pain models. It is not always possible to try the pain stimulus on the experimenter himself, and pain relieving agents most often are prone to interfere with the purpose of the experiments. Adequate evaluations of the intensity and duration of pain necessary to achieve the experimental objective are also difficult. It has been difficult to reach a final agreement about the justification of animal models of chronic pain, and the topic will still have to be thoroughly discussed (Tjolsen et al., 1994).

Hot-Plate Test

The hot-plate test was described in 1944 (Woolfe and MacDonald, 1944), and is one of the most commonly used tests of nociception and analgesia in rodents. Originally the test measured nociceptive responses of mice placed on the hot-plate at temperatures varying from 55 to 70°C. Later the test was modified, and a constant temperature of about 55°C was used. Subsequently most researchers have used this temperature (Tjolsen et al., 1994).

This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid. A plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw-licking and jumping. Both are considered to be supraspinally integrated responses. As far as analgesic substances are concerned, the paw-licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less or if the temperature is increased in a progressive and linear fashion, e.g., from 43 to 52°C at 2.5°C/min. The specificity and sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping, or by lowering the temperature. The behavior is relatively stereotyped in the mouse but is more complex in the rat, which sniffs, licks its forepaws, licks its hind paws, straightens up, stamps its

feet, starts and stops washing itself, among other things. These behaviors have been labeled “chaotic defensive movements” (Bars et al., 2001).

Tail-Flick Test

The tail-flick test using radiant heat is an extremely simplified version of the method used on human subjects by Hardy et al. (1940). The application of thermal radiation to the tail of an animal provokes the withdrawal of the tail by a brief vigorous movement. It is the reaction time of this movement that is recorded (often referred to as “tail-flick latency”). This is achieved by starting a timer at the same time as the application of the heat source. By using a rheostat, the intensity of current through the filament and therefore of radiant heat emission can be controlled in such a way that one can empirically predetermine the time until the withdrawal of the tail. This is usually between 2 and 10 s (most commonly between 2 and 4 s), although it can be much longer. A photoelectric cell stops the timer and switches off the lamp at the moment the tail is withdrawn. A lengthening of the reaction time is interpreted as an analgesic action. It is advisable not to prolong the exposure to radiant heat beyond 10 to 20 s, otherwise the skin may be burned. The advantages of this method are its simplicity and the small interanimal variability in reaction time measurements under a given set of controlled conditions (Bars et al., 2001).

One can demonstrate that the tail-flick is spinal reflex in that, at least in its shorter latency form, it persists after section or cold block of upper parts of the spinal cord. As with all reflexes, it is subject to control by supraspinal structures. It is triggered by C fibers when it is elicited by heat delivered by a CO_2 laser. The tail-flick reflex may not always be purely spinal, notably when the heating slope is slower and there is an increase in the reaction time. Under these conditions, the tail-flick can disappear in the spinal animal. It is possible that the tail-flick is not a purely spinal reflex but is a more complicated one involving higher neural structures (Bars et al., 2001).

From a pharmacological point of view, there is a consensus that this test is truly efficient only for revealing the activity of opioid analgesics (but not of opioid partial agonists). In this context, it is adequate for predicting their analgesic effects in

humans. For morphine itself, it is not difficult to construct dose-response curves for intravenous doses between 1 and 10 mg/kg. As far as opioid partial agonists are concerned, some have been shown to increase the tail-flick reaction time when slow rate of heating are applied. It is probable that this pharmacological observation resulted from the aforementioned fact that supraspinal structures are involved when the test is carried out in this fashion (Bars et al., 2001).

Writhing Test

The widely used test for measuring peripheral analgesic activity is the writhing test in mice. The intraperitoneal injection of an irritant induced a syndrome called “writhing”, which consists of contractions of the abdomen, twisting and turning of the trunk, and extension of the hind limbs. Several compounds have been shown to elicit this syndrome, such as phenylquinone, acetic acid, bradykinin and acetylcholine. In recent years acetic acid and phenylquinone have been used most frequently. Administration of relatively small doses of non-narcotic drugs, especially the nonsteroidal anti-inflammatory drugs, abolishes the writhing syndrome in a dose dependent manner. The test is commonly employed as a screening method because of its simplicity and sensitivity (Tjolsen et al., 1994; Vogel, 2008). The main disadvantage of this method is its lack of specificity, as many drugs without certain analgesic effects in humans can effectively inhibit the writhing response. In addition, there is a large variation in the response between mouse strains; ED_{50} values for acetylsalicylic acid have been found to vary from 50 to 200 mg/kg (Tjolsen et al., 1994).

The mechanism of the syndrome is not known, but many mediators have been proposed. No significant differences were detected in histamine, serotonin, or prostaglandin content of peritoneal fluid from writhing (using phenylquinone) and control mice, while other reports indicate involvement of the prostaglandin system in writhing induced by at least some irritants, the mode of action has been eludicated as an inhibition of cyclooxygenase in the prostaglandin pathway. It has been claimed that different irritants may be considered as models of different pain reaction, e.g., distinguishing between inflammatory and non-inflammatory pain (Tjolsen et al., 1994;

Vogel, 2008). The writhing tests is most widely applied in mice, but it has also been used in a few studies in rats. The test is simple to perform, is sensitive, and is therefore preferred in many laboratories. However, because of the poor specificity, the results of this test should be interpreted with caution (Tjolsen et al., 1994).

Anti-inflammatory Activity Testing

The inflammatory process involves a series of events that can be elicited by numerous stimuli, e.g., infectious agents, ischemia, antigen-antibody interactions chemical, thermal or mechanical injury. The response is accompanied by the clinical signs of erythema, edema, hyperalgesia and pain. Pharmacological methods for testing acute and subacute inflammation include UV-erythema in guinea pigs, vascular permeability, oxazolone-induced ear edema in rats and mice, croton-oil edema in rats and mice, paw edema in rats, pleurisy tests and granuloma pouch technique (Vogel, 2008).

Paw edema

One of the cardinal signs of inflammation is the presence of edema. It is not surprising, then, that edema tests are among the most prominent models used to assess the efficacy of drugs for treating inflammatory disease such as arthritis (Lombardino,1985).

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, sulfate polysaccharides like carrageenin or naphthoylheparamine.

The effect can be measured in several ways. The hind limb can be dissected at the talocrural joint and weighed. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. The increase of paw volume after 3 or 6 hr is

calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema (Vogel, 2008). Maintenance of a constant temperature in the laboratory is important since the amount of paw swelling and the degree of inhibition achieved by NSAIDs decrease with decreasing temperature (Lombardino,1985). The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The differences at the various time intervals give some hints for the duration of the anti-inflammatory effect. A dose-response curve is run for active drugs and ED_{50} values can be determined (Vogel, 2008).

CHAPTER III

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Male ICR mice (weighing 18-25 g) and male Sprague Dawley rats (weighing 100-150 g) from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom served as experimental subjects. They were housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under the standard condition of temperature (25±2°C), 50-60% of humidity, 12 hr/12 hr light/dark cycles and had accessed to the standard pellet diet (Perfect Companion Group Company Limited, Thailand) and tap water *ad libitum*. The animals were allowed to acclimate to the facility for 3-5 days before starting the experiments. At the end of each experiment, the animals were sacrificed with carbon dioxide. The number of animals used in each treatment was typically six to ten per group. The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Appendix A).

PREPARATION OF CURCUMIN DIETHYL DISUCCINATE

Curcumin diethyl disuccinate was obtained from Assistant Professor Dr. Pornchai Rojsitthisak, Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

DRUGS

0.9% Sodium chloride solution (NSS; 10 ml/kg, GHP, Thailand)

0.5% Carboxymethylcellulose (CMC; 10 ml/kg, Sigma Chemical Co., USA)

Morphine sulphate (MO; 10 mg/kg, Thai FDA)

Indomethacin (IND; 10 mg/kg, Sigma Chemical Co., USA),

Naloxone (NAL; 5 mg/kg, Sigma Chemical Co., USA),

0.6% Acetic acid (10 ml/kg, Merck, Germany)

1% λ-Carrageenan solution (0.1 ml/rat, Sigma Chemical Co., USA)

0.1% Serotonin solution (5-HT; 0.1 ml/rat, Sigma Chemical Co., USA)

0.1% Histamine solution (HIS; 0.1 ml/rat, Sigma Chemical Co., USA)

0.0001% Prostaglandin E_1 solution (PGE_1; 0.1 ml/rat, Sigma Chemical Co., USA)

Promethazine (PRO; 30 mg/kg, Sigma Chemical Co., USA)

Cyproheptadine (CYPRO; 10 mg/kg, Sigma Chemical Co., USA)

Curcumin diethyl disuccinate (CurDD; 10-200 mg/kg)

Morphine, indomethacin, naloxone, acetic acid, carrageenan, serotonin, histamine, prostaglandin E_1, promethazine and cyproheptadine were dissolved in 0.9% sodium chloride solution. Curcumin diethyl disuccinate was suspended in 0.5% carboxymethylcellulose. Morphine sulphate and indomethacin were used as the standard analgesic drugs and indomethacin, promethazine and cyproheptadine were used as the standard anti-inflammatory drug. The control animals were given with equivalent volume of vehicle in the same route.

EXPERIMENTAL METHODS

Antinociceptive activity Testing

Hot-plate Analgesic Testing

Analgesic testing was determined using the hot-plate method. The male ICR mice weighing 18-25 g were used (N=10 per group). The surface of the hot-plate (Hot Plate Analgesia meter, Harvard Apparatus, USA) measuring 28×28 cm was set at 55±0.5°C and was 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 pre-drug 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 for its paw-lick latency and returned to its cage (the maximum time allowed for an animal to remain on the surface of the plate during testing was 45 sec). The average of the last two trials served as the baseline pre-drug paw-lick latency.

Immediately, after the third baseline trial on the hot-plate, the drug administration took place with NSS (10 ml/kg) and morphine sulphate (10 mg/kg) intraperitoneal (i.p.) or 0.5% CMC and various doses of curcumin diethyl disuccinate (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:

$$\%MPE = \frac{(\text{post-drug latency}) - (\text{pre-drug latency})}{(\text{cut-off time}) - (\text{pre-drug latency})} \times 100$$

Cut-off time for hot-plate test = 45 sec

Thus, dose- and time response curve was generated. Dose-effect curves for hot-plate assays were derived by computing the area under the corresponding 0-240 min time-course-%MPE curves; area was calculated using the trapezoidal rule (Tallarida and Murray, 1987).

Figure 12 Hot Plate Analgesia Meter

Analysis of the mechanism of antinociceptive action of curcumin diethyl disuccinate

The involvement of the opioid system in the antinociceptive effect of curcumin diethyl disuccinate was investigated using the model of mouse hot-plate test. Animal were pretreated with naloxone (5 mg/kg, i.p.) 10 min before oral administration of curcumin diethyl disuccinate (50 mg/kg).

Tail-flick Analgesic Testing

These studies employed the tail-flick assay described by D'Amour and Smith in 1941, 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 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 (about 4 cm from the tip). The device (Tail Flick Analgesia Meter, Harvard apparatus, USA) automatically recorded (in 0.1 sec) 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 about 1.0-1.5 sec (intensity 3.7 °A). The intensity was not changed for any animals within any given experiment. On the day of testing, all animals were tested for 3 pre-drug tail-flick baselines conducted at 10-15 min intervals. The average score of the last two trials served as the baseline measure for each subjects.

Immediately, after the third baseline trial, the drug administration took place with NSS (10 ml/kg) and morphine sulphate (10 mg/kg) intraperitoneal (i.p.) or 0.5% CMC and various doses of curcumin diethyl disuccinate (25, 50, 100 and 200 mg/kg) orally (p.o.). Tail-flick latencies were recorded at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. The time-course of tail-flick latency was expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

$$\%MPE = \frac{(\text{post-drug latency}) - (\text{pre-drug latency})}{(\text{cut-off time}) - (\text{pre-drug latency})} \times 100$$

Cut-off time for tail-flick test = 4 sec

Thus, dose- and time response curve was generated. Dose-effect curves for the tail-flick assays were derived by computing the area under the corresponding 0-240 min time-course-%MPE curves; area was calculated using the trapezoidal rule (Tallarida and Murray, 1987).

Figure 13 Tail Flick Analgesia Meter

Acetic acid-induced writhing test

Analgesic testing was determined using the acetic acid-induced writhing method described by Koster et al. in 1959. Male ICR mice weighing 18-25 g were used (N=6 per group). On the day of testing, animals were randomly assigned to one of seven treatment groups. Mice were then administered NSS (10 ml/kg), indomethacin (IND; 10 mg/kg), 0.5% CMC (10 ml/kg) or various doses of curcumin diethyl disuccinate (25, 50, 100 and 200 mg/kg) orally (p.o.) 1 hr before intraperitoneal administration of 0.6% acetic acid (10 ml/kg) which used to induce the constriction response.

Each animal was placed in a transparent observational cage. The number of writhes (abdominal constriction) were observed and counted for 30 min after acetic acid administration (Nguemfo et al., 2007). Antinociceptive activity was reported as percentage of inhibition of writhing response compared with the vehicle control group. The percentage of inhibition of writhing response was calculated using the following formula:

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

Wr = mean writhing response

Figure 14 Writhing response

Anti-inflammatory activity Testing

Carrageenan-induced paw edema

The anti-inflammatory activity of curcumin diethyl disuccinate was determined using carrageenan-induced paw edema test in hind paws of rats as described by Winter et al. in 1962. Male Sprague-Dawley rats (N=6 per group), 100-150 g, were fasted overnight before the experiment with free access to water. The animals were treated orally with vehicle control (0.9% NSS or 0.5% CMC; 10 ml/kg), indomethacin (10 mg/kg), or various doses of curcumin diethyl disuccinate (10, 20, 40 and 80 mg/kg). One hour later, the rats were challenged by subcutaneous injection of 0.1 ml of 1% carrageenan solution into the plantar surface of the right hind paw (Amanlou et al., 2005). The rat's paw was marked with black ink at the level of the lateral malleolus. The paw volume was measured at 1 hr prior to the injection of carrageenan

and at 1, 2, 3, 4, 5 and 6 hr after injection using plethysmometer. Edema was expressed as a mean increase in paw volume in relation to control. The percentage of inhibition of edema was calculated using the following formula:

$$\% \text{ Inhibition of edema} = 100 (1- Vt / Vc)$$

Vc = edema volume in control group; Vt = edema volume in tested group

Figure 15 Plethysmometer

Analysis of the mechanism of anti-inflammatory activity of curcumin diethyl disuccinate

The mechanism of anti-inflammatory activity of curcumin diethyl disuccinate was investigated using the model of histamine, serotonin and prostaglandin E_1-induced acute inflammation in rats. Male Sprague-Dawley rats (N=6 per group), 100-150 g, were fasted overnight before the experiment with free access to water. The animals were treated orally with 0.9% NSS (10 ml/kg), antihistamine (promethazine; 30 mg/kg), antiserotonin (cyproheptadine; 10 mg/kg), cyclooxygenase inhibitor (indomethacin; 10 mg/kg) or curcumin diethyl disuccinate (40 mg/kg). One hour later, the rats were challenged by subcutaneous injection of 0.1 ml of 0.1% histamine, 0.1%

serotonin or 0.0001% prostaglandin E_1 solution into the plantar surface of the right hind paw (Kale et al., 2007). The rat's paw was marked with black ink at the level of the lateral malleolus. The paw volume was measured at 1 hr prior to the injection of histamine, serotonin and prostaglandin E_1 and at 1, 2, 3, 4, 5 and 6 hr after injection using plethysmometer. Edema was expressed as a mean increase in paw volume in relation to control. The percentage of inhibition of edema was calculated using the formula as described above.

DATA TREATMENT AND STATISTICAL ANALYSIS

Statistical analyses were performed on the dose-response curves by analysis of variance (ANOVA; SPSS version 15.0 for windows). Post hoc analyses were performed using Fisher's LSD (Hot-plate, Tail-flick, and Writhing tests) and Tukey tests (Carrageenan, Histamine, Serotonin, and PGE_1-induced paw edema tests). The minimum level of statistical significance was set at $p<0.05$.

CHAPTER IV

RESULTS

MOUSE HOT-PLATE TEST

To demonstrate the validity of the hot-plate analgesic testing following drug administration, mice received morphine sulphate (MO; 10 mg/kg) intraperitoneally (i.p.) were tested during the subsequent 240 min period. As expected MO significantly ($p<0.01$) increased the hot-plate latency producing an area of analgesia of 10921.68 ± 2277.58 %MPE-min compared with that of normal saline solution (NSS) (2066.08 ± 1508.68 %MPE-min; Figure 16).

Initial studies utilizing the hot-plate test in mice to examine the efficacy of curcumin diethyl disuccinate in producing analgesia. Mice were administered orally 0.5% carboxymethylcellulose (CMC) or various doses of curcumin diethyl disuccinate (25, 50, 100, 200 mg/kg). All doses of curcumin diethyl disuccinate except 200 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 17). The analgesic peak effects of curcumin diethyl disuccinate (25, 50, 100 and 200 mg/kg) were reached within 15, 240, 240, 15 min after oral administration, respectively. Individual time courses of the responses are shown in Figure 18.

In order to investigate the involvement of opioid receptors in curcumin diethyl disuccinate actions, mice were then administered NSS (10 ml/kg, i.p.), naloxone (NAL; 5 mg/kg, i.p.), a short-acting opioid receptor antagonist, 0.5% CMC (10 ml/kg, p.o.), curcumin diethyl disuccinate (50 mg/kg, p.o.) or the combination of naloxone and curcumin diethyl disuccinate (5/50 mg/kg). Naloxone alone failed to produce significant response when compared to vehicle control. Curcumin diethyl disuccinate 50 mg/kg produced significant ($p<0.05$) response when compared to vehicle control. The inclusion of naloxone with curcumin diethyl disuccinate significantly ($p<0.05$) attenuated the analgesic response due to curcumin diethyl disuccinate indicating that opioid receptors are involved in the analgesic response produced by curcumin diethyl disuccinate (Figure 19). Individual time courses of the responses are shown in Figure 20.

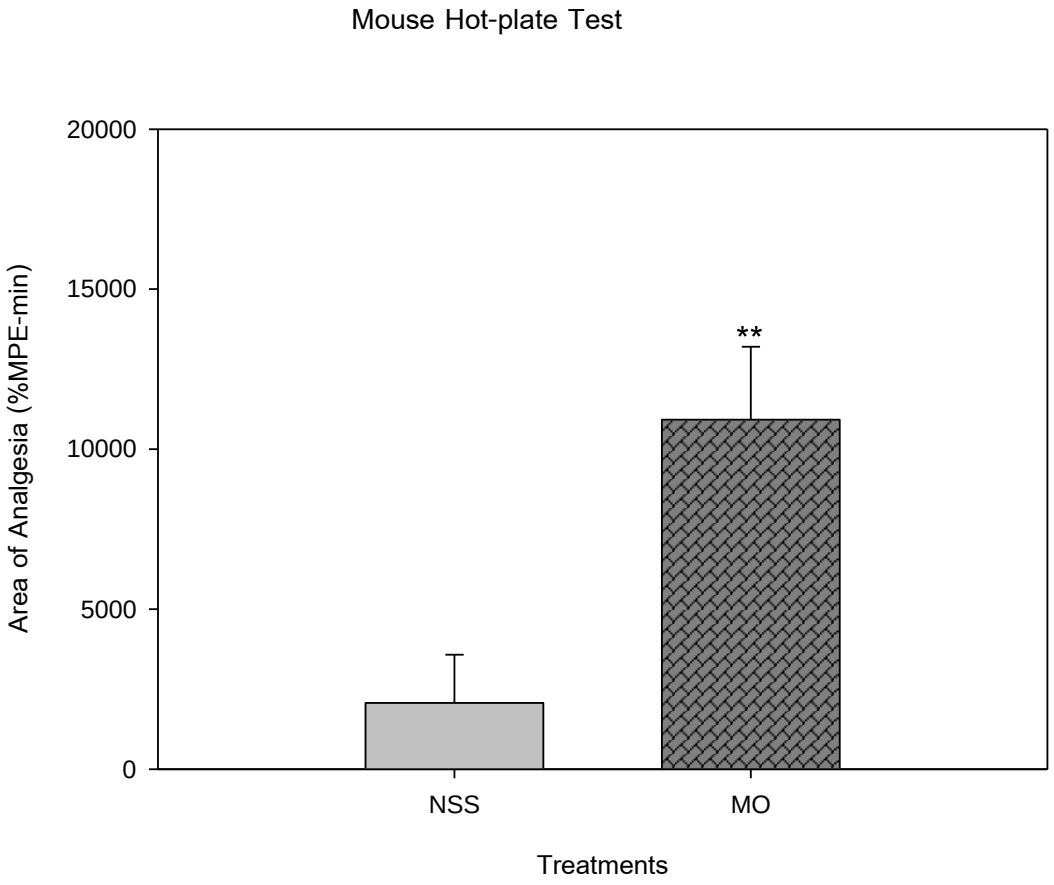

Figure 16 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. ** $p<0.01$ significantly different compared to NSS.

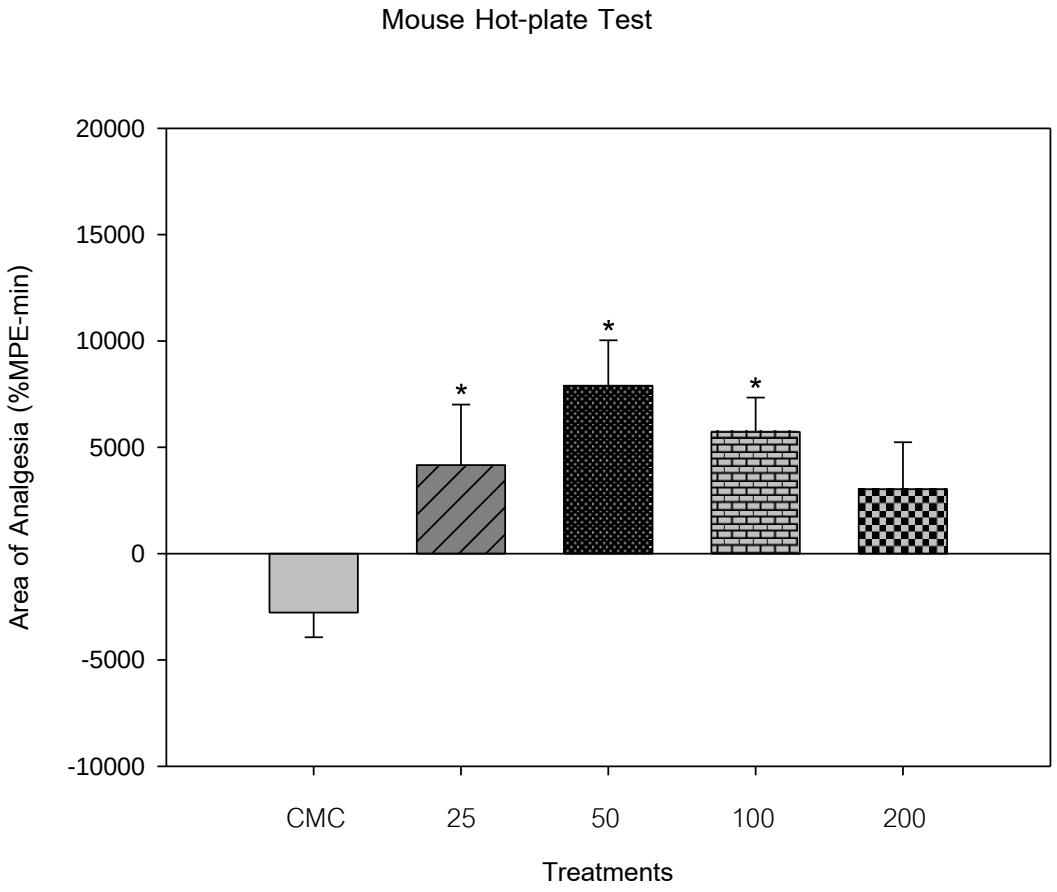

Figure 17 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 0.5% carboxymethylcellulose (CMC; 10 ml/kg) and various doses of curcumin diethyl disuccinate (25-200 mg/kg). N=10 for all groups. *p<0.05 significantly different compared to CMC.

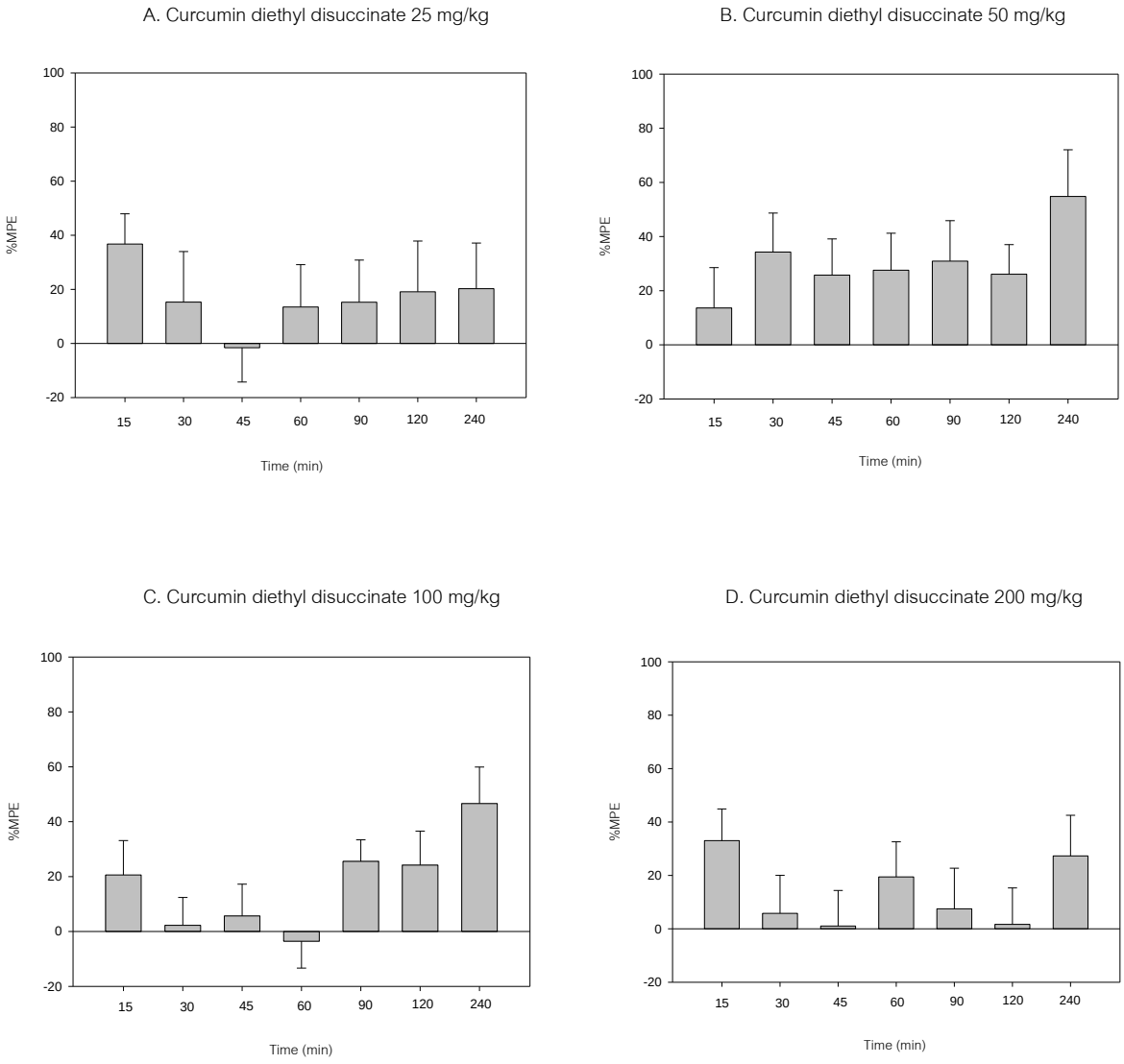

Figure 18 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of curcumin diethyl disuccinate.

A. Curcumin diethyl disuccinate 25 mg/kg, B. Curcumin diethyl disuccinate 50 mg/kg, C. Curcumin diethyl disuccinate 100 mg/kg, D. Curcumin diethyl disuccinate 200 mg/kg. N=10 for all groups.

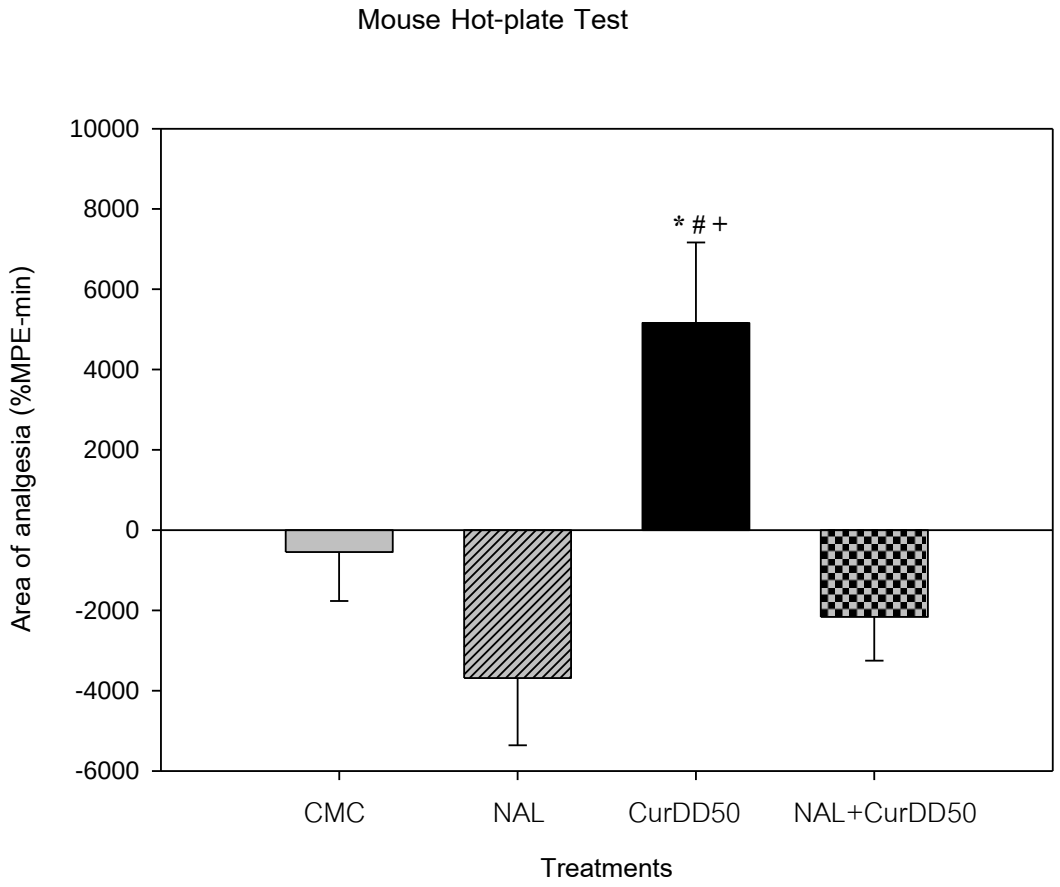

Figure 19 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 0.5% carboxymethylcellulose (CMC; 10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), curcumin diethyl disuccinate (CurDD50; 50 mg/kg, p.o.) and the combination of naloxone and curcumin diethyl disuccinate (5/50 mg/kg). N=10 for all groups. $^{*}p<0.05$ significantly different compared to CMC; $^{\#}p<0.01$ significantly different compared to NAL; $^{+}p<0.01$ significantly different compared to NAL+CurDD50.

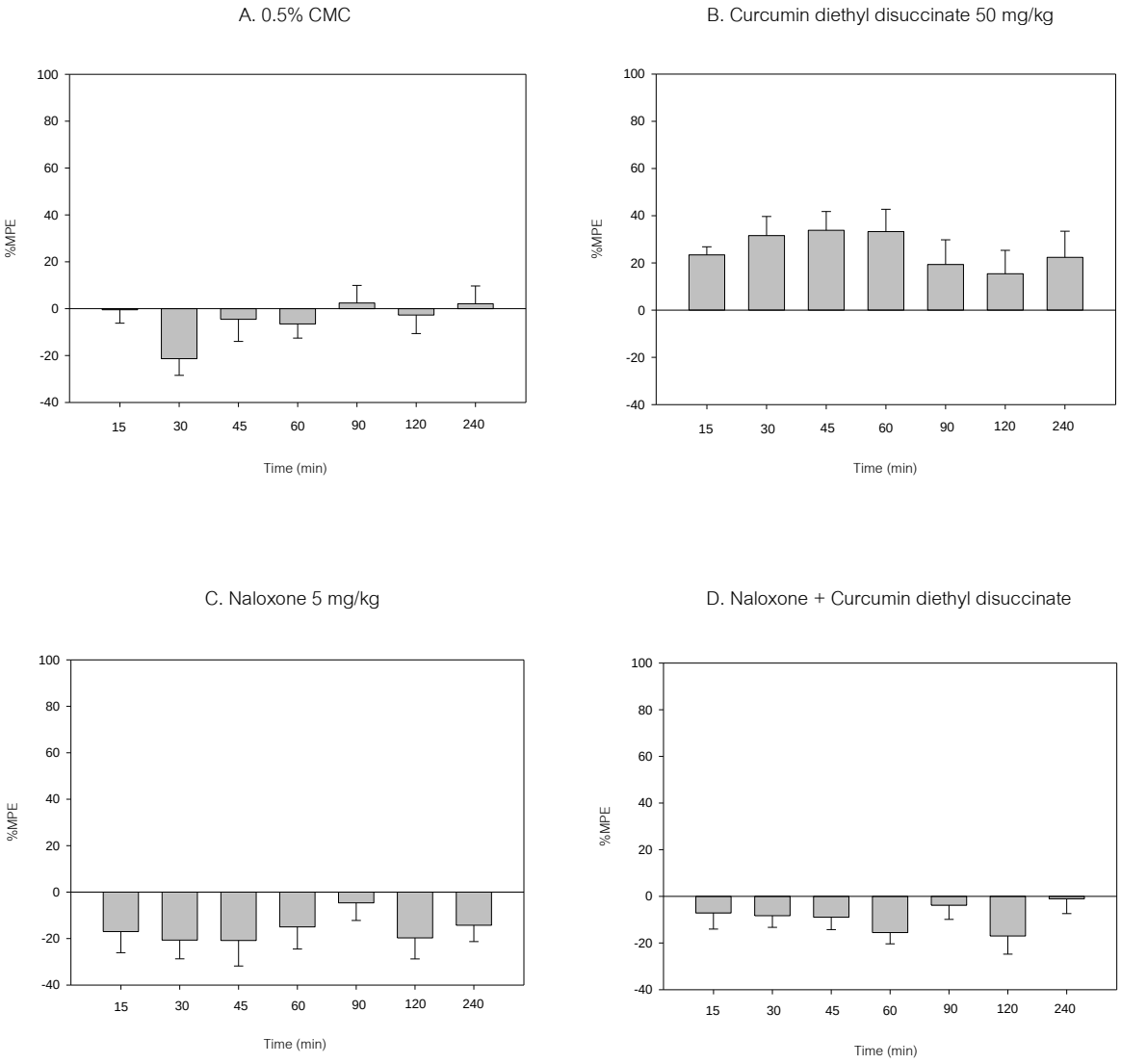

Figure 20 Individual time courses of the response (%MPE versus time (min)) after administration of 0.5% carboxymethylcellulose (CMC, p.o.), curcumin diethyl disuccinate 50 mg/kg (p.o.), naloxone 5 mg/kg (i.p.) and the combination of naloxone and curcumin diethyl disuccinate (5/50 mg/kg).

A. 0.5% CMC, B. Curcumin diethyl disuccinate 50 mg/kg, C. Naloxone 5 mg/kg,

D. Naloxone 5 mg/kg + Curcumin diethyl disuccinate 50 mg/kg. N=10 for all groups.

MOUSE TAIL-FLICK TEST

To demonstrate the validity of the tail-flick analgesic testing following drug administration, mice received morphine sulphate (MO; 10 mg/kg) i.p. and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.01$) increased tail-flick latency producing an area of analgesia of 9303.42 ± 1624.33 %MPE-min compared with that of normal saline solution (NSS) (-3444.30 ± 2350.11 %MPE-min; Figure 21).

Studies then utilized the mouse tail-flick method to examine the efficacy of curcumin diethyl disuccinate in producing analgesia. Mice were orally administered CMC or various doses of curcumin diethyl disuccinate (25, 50, 100, 200 mg/kg). Curcumin diethyl disuccinate at the dose of 25 mg/kg significantly ($p<0.05$) increased the tail-flick latency when compared to the vehicle group (Figure 22). The analgesic peak effects of all doses of curcumin diethyl disuccinate tested were reached within 45, 30, 15, 15 min after oral administration, respectively. Individual time courses of the responses are shown in Figure 23.

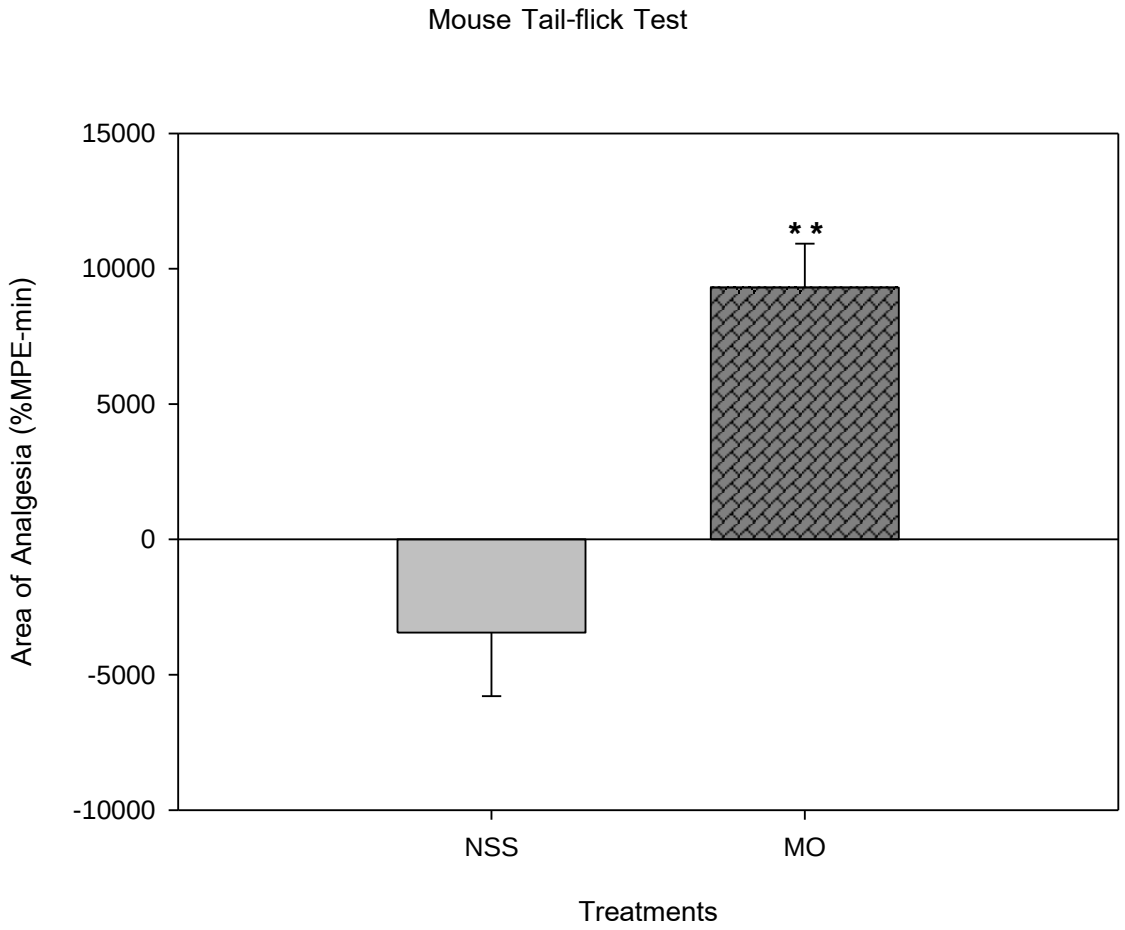

Figure 21 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. ** $p<0.01$ significantly different compared to NSS.

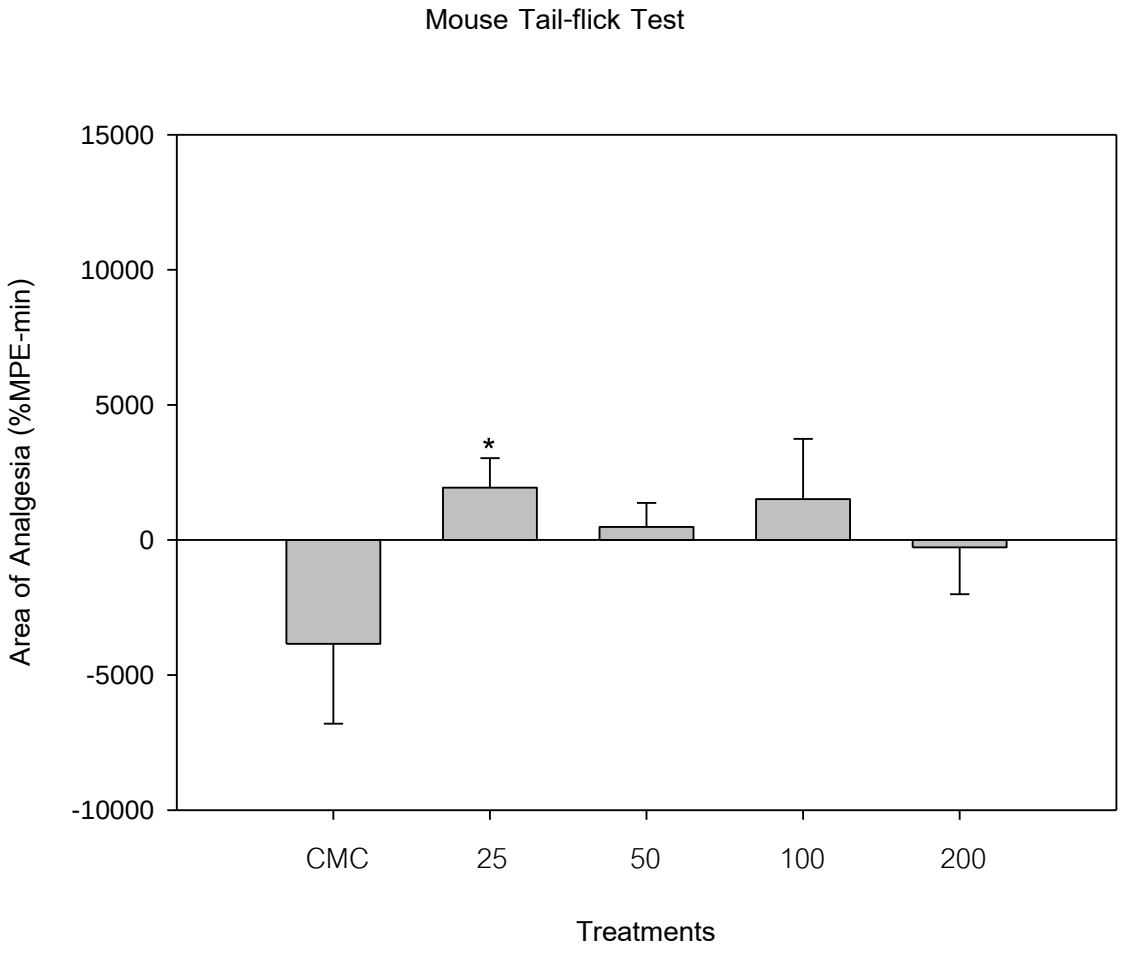

Figure 22 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of curcumin diethyl disuccinate (25-200 mg/kg). N=10 for all groups. *$p<0.05$ significantly different compared to CMC.

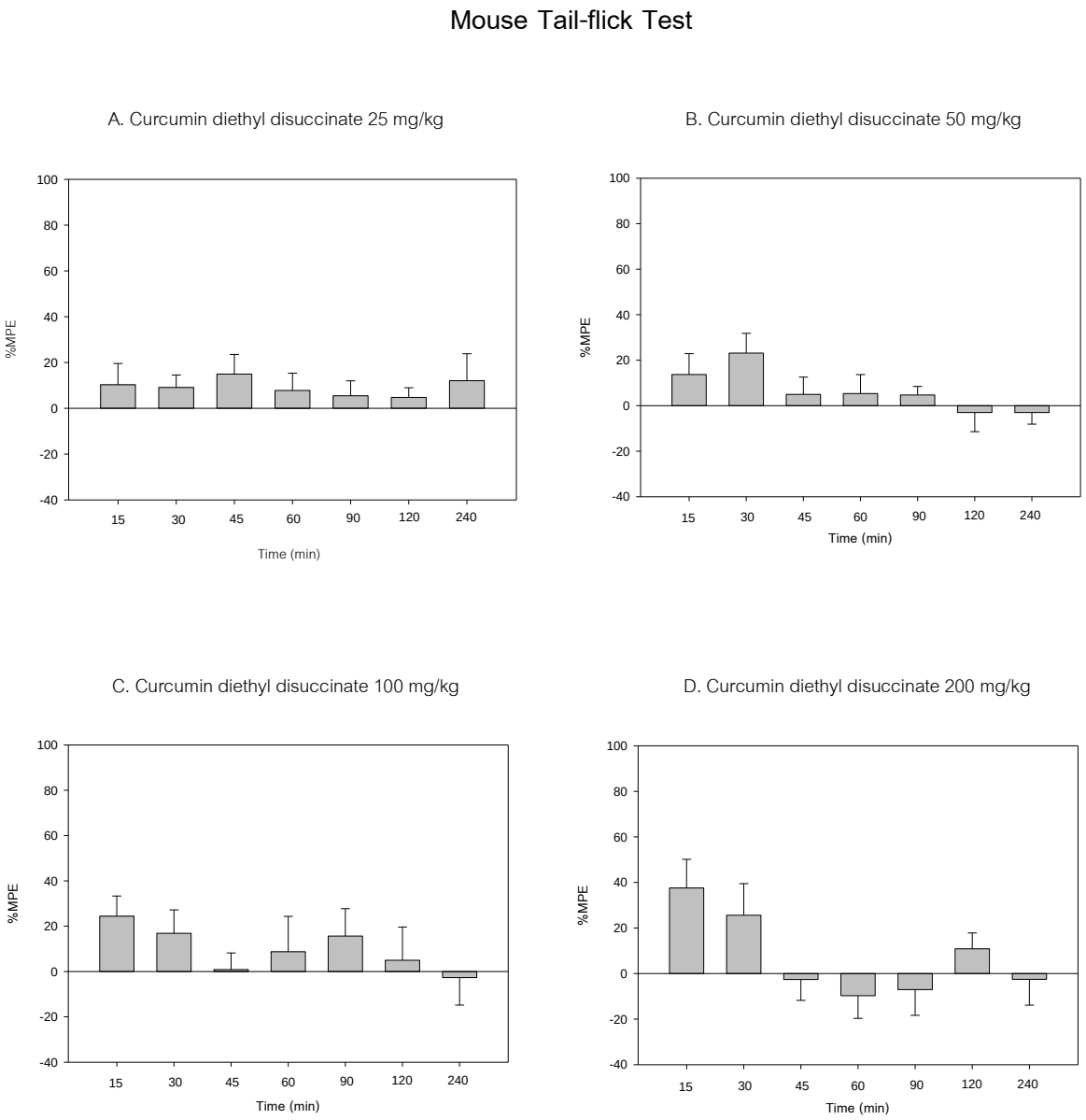

Figure 23 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of curcumin diethyl disuccinate.

A. Curcumin diethyl disuccinate 25 mg/kg, B. Curcumin diethyl disuccinate 50 mg/kg, C. Curcumin diethyl disuccinate 100 mg/kg, D. Curcumin diethyl disuccinate 200 mg/kg. N=10 for all groups.

ACETIC ACID-INDUCED WRITHING IN MICE

To demonstrate the validity of the acetic acid-induced writhing method following drug administration, mice received indomethacin (IND; 10 mg/kg) orally and were tested during the subsequent 30 min period. As expected IND significantly ($p<0.01$) decreased writhing response by 81.56% producing a mean number of writhes of 5.5 ± 1.54 compared with that of NSS (29.83 ± 2.98; Figure 24).

Studies then utilized the acetic acid-induced writhing method to examine the efficacy of curcumin diethyl disuccinate in producing analgesia. Mice were administered orally CMC or various doses of curcumin diethyl disuccinate (25, 50, 100, 200 mg/kg). All doses of curcumin diethyl disuccinate tested significantly ($p<0.05$) decreased the number of writhes induced by acetic acid by 66.23%, 35.93%, 40.26%, and 75.76%, respectively when compared to 0.5% CMC (Figure 25).

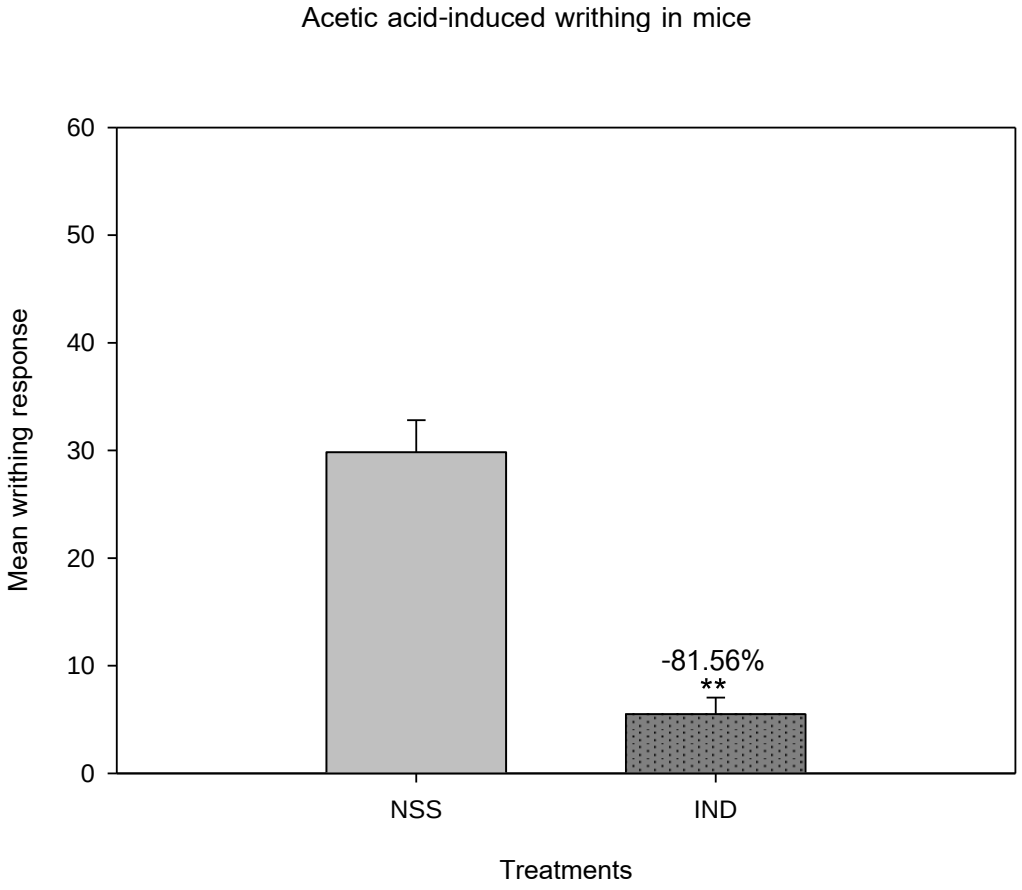

Figure 24 Mean writhing response after oral administration of 0.9% normal saline solution (NSS; 10 ml/kg) and indomethacin (IND; 10 mg/kg). N=6 for all groups. $^{**}p<0.01$ significantly different compared to NSS.

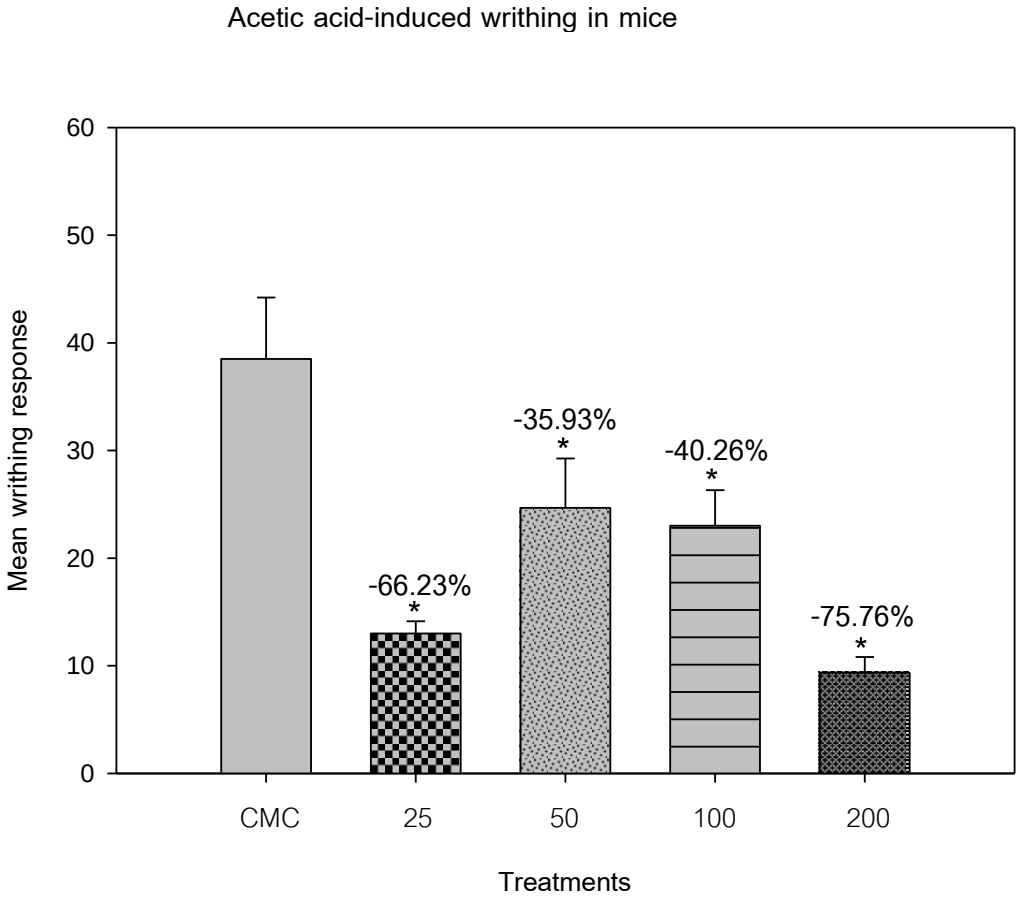

Figure 25 Mean writhing response after oral administration of 0.5% carboxymethylcellulose (CMC; 10 ml/kg) and various doses of curcumin diethyl disuccinate (25-200 mg/kg). N=6 for all groups. *p<0.05 significantly different compared to CMC.

CARRAGEENAN-INDUCED PAW EDEMA

To demonstrate the validity of the carrageenan-induced paw edema test following drug administration, rats received oral administration of indomethacin (IND; 10 mg/kg) one hour before injection of carrageenan into the plantar surface of rat's right hind paw and were tested during the subsequent 6 hr period. As expected IND significantly ($p<0.05$) decreased paw edema at 2, 3, 4, and 5 hr after carrageenan administration compared with that of normal saline solution (NSS; 10 ml/kg), producing a percentage of inhibition of edema of 47.50%, 56.52%, 48.89%, and 44.44%, respectively (Table 2).

Studies then utilized the carrageenan-induced paw edema test to examine the efficacy of CurDD in producing anti-inflammation. Rats were administered orally CMC or various doses of CurDD (10-80 mg/kg) one hour before carrageenan administration. CurDD 10 mg/kg decreased the paw volume significantly ($p<0.05$) at 2 and 4 hr. CurDD 40 mg/kg decreased the paw volume significantly ($p<0.05$) at 2, 3, 4, 5, and 6 hr after carrageenan administration. CurDD 80 mg/kg decreased the paw volume significantly ($p<0.05$) at 4 and 6 hr after carrageenan administration. CurDD 10 mg/kg showed a maximum inhibition of paw edema of 46.81% at 2 hr after carrageenan administration. CurDD 40 mg/kg showed a maximum inhibition of paw edema of 55.77% at 5 hr after carrageenan administration. CurDD 80 mg/kg showed a maximum inhibition of paw edema of 46.94% at 6 hr after carrageenan administration (Table 3). Percentage of inhibition from 1-6 hr after oral administration of 0.5% carboxymethylcellulose (CMC) and various doses of CurDD (10-80 mg/kg) were shown in the Table 3.

Table 2 Change of edema volume (ml) of oral administration of 0.9% normal saline solution (NSS; 10 ml/kg) and indomethacin (IND; 10 mg/kg) from 1-6 hr after carrageenan administration. N=6 for all groups. Inhibition is reported as percentage compared to vehicle control. *p<0.05 significantly different compared to NSS.

Treatments	Paw edema ± S.E.M.(% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
NSS	0.26±0.05	0.40±0.07	0.46±0.05	0.45±0.05	0.36±0.05	0.29±0.05
IND	0.14±0.05 (46.15%)	0.21±0.05* (47.50%)	0.20±0.03* (56.52%)	0.23±0.04* (48.89%)	0.20±0.05* (44.44%)	0.17±0.04 (41.38%)

Table 3 Change of edema volume (ml) of oral administration of 0.5% carboxymethylcellulose (CMC; 10 ml/kg) and various doses of curcumin diethyl disuccinate (10-80 mg/kg) from 1-6 hr after carrageenan administration. N=6 for all groups. Inhibition is reported as percent compared to the vehicle control. *p<0.05 significantly different compared to CMC.

Treatments	Paw edema ± S.E.M.(% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
0.5% CMC	0.34±0.04	0.47±0.04	0.51±0.04	0.57±0.04	0.52±0.06	0.49±0.05
Curcumin DD 10 mg/kg	0.21±0.03 (38.24%)	0.25±0.05* (46.81%)	0.34±0.05 (33.33%)	0.38±0.02* (33.33%)	0.36±0.03 (30.77%)	0.30±0.02 (38.78%)
Curcumin DD 20 mg/kg	0.25±0.03 (26.47%)	0.35±0.03 (25.53%)	0.37±0.05 (27.45%)	0.39±0.05 (31.58%)	0.39±0.04 (25.00%)	0.33±0.06 (32.65%)
Curcumin DD 40 mg/kg	0.22±0.03 (35.29%)	0.27±0.07* (42.55%)	0.27±0.05* (47.06%)	0.30±0.07* (47.37%)	0.23±0.07* (55.77%)	0.22±0.06* (55.10%)
Curcumin DD 80 mg/kg	0.20±0.03 (41.18%)	0.38±0.03 (19.15%)	0.42±0.04 (17.64%)	0.35±0.03* (38.60%)	0.34±0.02 (34.62%)	0.26±0.03* (46.94%)

HISTAMINE, SEROTONIN, PROSTAGLANDIN E_1-INDUCED PAW EDEMA TEST

In order to further investigate the mechanism of CurDD in carrageenan-induced paw edema model. Histamine-induced paw edema, serotonin-induced paw edema and prostaglandin E_1-induced paw edema models were used. Rats were treated orally with NSS, promethazine (PRO; 30 mg/kg), cyproheptadine (CYPRO; 10 mg/kg), indomethacin (IND; 10 mg/kg) or CurDD (40 mg/kg) one hour before injection of various chemical mediators (histamine, serotonin or prostaglandin E_1) into the plantar surface of rat's hind paw and were tested during the subsequent 6 hr period.

PRO, an antihistamine, at the dose of 30 mg/kg decreased paw edema significantly ($p<0.05$) at 1 hr after histamine administration with a maximum inhibition of paw edema of 82.35%. CurDD 40 mg/kg failed to decrease paw volume at all time tested (Table 4).

CYPRO, an antiserotonin, at the dose of 10 mg/kg decreased paw edema significantly ($p<0.05$) at 1, 2, 3, 4, and 6 hr after serotonin administration, CYPRO showed a maximum inhibition of paw edema of 66.67% at 6 hr after serotonin administration. CurDD failed to decrease paw volume at all time tested (Table 5).

IND, a cyclooxygenase inhibitor, at the dose of 10 mg/kg decreased paw edema significantly ($p<0.05$) at 1, 2, and 3 hr after prostaglandin E_1 (PGE_1) administration. IND showed a maximum inhibition of paw edema of 60.00% at 3 hr after PGE_1 administration. CurDD decreased paw volume significantly ($p<0.05$) at 1, 2, 3, and 4 hr and showed a maximum inhibition of paw edema of 80.00% at 4 hr after PGE_1 administration (Table 6).

Table 4 Change of edema volume (ml) of oral administration of 0.9% normal saline solution (NSS; 10 ml/kg), promethazine (PRO; 30 mg/kg) and CurDD 40 mg/kg from 1-6 hr after histamine administration. N=6 for all groups. Inhibition is reported as percentage compared to vehicle control. *p<0.05 significantly different compared to NSS.

Treatments	Paw edema ± S.E.M.(% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
NSS	0.17±0.03	0.12±0.01	0.07±0.03	0.07±0.01	0.02±0.02	0.03±0.01
PRO 30 mg/kg	0.03±0.03* (82.35%)	0.05±0.04 (58.33%)	0.05±0.04 (28.57%)	-0.03±0.02 (142.86%)	0.01±0.04 (50.00%)	-0.01±0.02 (133.33%)
CurDD 40 mg/kg	0.19±0.04 (11.76%)	0.15±0.04 (-25.00%)	0.10±0.02 (-42.86%)	0.03±0.01 (57.14%)	-0.01±0.01 (100.00%)	-0.03±0.02 (200.00%)

Table 5 Change of edema volume (ml) of oral administration of 0.9% normal saline solution (NSS; 10 ml/kg), cyproheptadine (CYPRO; 10 mg/kg) and CurDD 40 mg/kg from 1-6 hr after serotonin administration. N=6 for all groups. Inhibition is reported as percentage compared to the vehicle control. *p<0.05 significantly different compared to NSS.

Treatments	Paw edema ± S.E.M.(% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
NSS	0.82±0.05	0.64±0.05	0.45±0.03	0.40±0.02	0.24±0.02	0.21±0.03
CYPRO 10 mg/kg	0.33±0.09* (59.76%)	0.25±0.09* (59.37%)	0.16±0.07* (64.44%)	0.19±0.06* (52.50%)	0.12±0.05 (50.00%)	0.07±0.04* (66.67%)
CurDD 40 mg/kg	0.90±0.05 (-9.76%)	0.71±0.05 (-10.94%)	0.59±0.06 (-31.11%)	0.42±0.04 (-5.00%)	0.23±0.05 (14.17%)	0.19±0.03 (4.76%)

Table 6 Change of edema volume (ml) of oral administration of 0.9% normal saline solution (NSS; 10 ml/kg), indomethacin (IND; 10 mg/kg) and CurDD 40 mg/kg from 1-6 hr after PGE_1 administration. N=6 for all groups. Inhibition is reported as percentage compared to the vehicle control. *p<0.05 significantly different compared to NSS.

Treatment	Paw edema ± S.E.M.(% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
NSS	0.36±0.04	0.27±0.03	0.20±0.03	0.15±0.03	0.10±0.02	0.05±0.03
IND 10 mg/kg	0.22±0.03* (38.89%)	0.13±0.04* (51.85%)	0.08±0.03* (60.00%)	0.05±0.03 (66.67%)	0.01±0.03 (90.00%)	0.01±0.01 (80.00%)
CurDD 40 mg/kg	0.21±0.04* (41.67%)	0.06±0.04* (77.78%)	0.07±0.04* (65.00%)	0.03±0.02* (80.00%)	0.06±0.03 (40.00%)	0.02±0.03 (60.00%)

CHAPTER V

DISCUSSION AND CONCLUSION

These studies have demonstrated the antinociceptive and anti-inflammatory effects of curcumin diethyl disuccinate (CurDD) in various animal models. Antinociceptive activity was assessed utilizing thermal (hot-plate and tail-flick test) and chemical (acetic acid-induced writhing test) models. Anti-inflammatory effect was assessed in acute inflammation model (carrageenan-induced paw edema). The mechanisms of actions of CurDD in carrageenan-induced paw edema model were further investigated using histamine, serotonin, and prostaglandin E_1-induced paw edema models. Both antinociceptive and anti-inflammatory activities of CurDD, a prodrug of curcumin, are due to the availability of curcumin.

Initial attempts to investigate the analgesic effect of CurDD utilized the standard mouse hot-plate test (Woolfe and MacDonald, 1944), a central analgesic activity testing model which measures two behavioral components including paw licking and jumping. Both are considered to be supraspinally integrated responses. This model usually employs morphine (MO) as a reference drug. MO showed potent analgesic effect on the response in this model indicating the sensitivity of this test (Figure 16). CurDD, a prodrug of curcumin, was administered orally by suspending in 0.5% CMC. The oral administration was chosen in order to imitate the normal human consumption of curcumin. The significant analgesic effect of CurDD (25-100 mg/kg) was observed during 240 min period. CurDD at the dose of 50 mg/kg showed the highest analgesic response (Figure 17). Previous study has shown that curcumin at the dose of 200 mg/kg showed the highest analgesic response in the same model (Buadonpri, 2008). This may be due to better systemic bioavailability of CurDD, a prodrug of curcumin. Considering the doses of CurDD from 25-200 mg/kg, the antinociceptive peak response of lower doses of CurDD was observed earlier than the higher doses of CurDD (Figure 18). Similar results were also observed with the previous study of curcumin (Buadonpri, 2008). This phenomenon may be partly due to the erratic absorption from the gastrointestinal tract and poor systemic bioavailability after oral dosing of curcumin in

rodents (Yang et al., 2007) and the saturation of the transporter mechanism in the gut for free curcumin (Jurenka, 2009). The antinociceptive effect of low doses of CurDD was observed faster (within 15-30 min after administration) as compared to low doses of synthetic curcumin (Buadonpri, 2008). This may be due to systemic bioavailability improvement of CurDD after succinylation of curcumin.

MO acts through μ-opioid receptors and naloxone is a competitive antagonist at the μ-, κ- and σ-receptors with a higher affinity for the μ-receptors (Helm et al., 2008). MO and naloxone are used to explore the involvement of the endogenous opioid analgesic system activated by novel analgesics. Naloxone, a short acting opioid antagonist, was then utilized to investigate the involvement of opioid receptors in the analgesic effects of CurDD. Naloxone attenuated the analgesic response of CurDD in the hot-plate test. The results suggested the involvement of μ opioid receptor in analgesia produced by CurDD (Figure 19). These finding are in accordance with the previous study which showed that curcumin reduced the number of acetic acid-induced writhing in rats and pretreatment with naloxone inhibited the suppressive effect of curcumin (Tajik et al., 2008).

Studies were then undertaken to investigate the effectiveness of CurDD utilizing the mouse tail-flick technique, another central analgesic activity testing model that measures spinal reflex. MO administered i.p. produced significant analgesic response as expected (Figure 21). Only the lowest dose of CurDD (25 mg/kg) showed significant analgesic activity (Figure 22). Previous study of curcumin showed that all doses of curcumin tested (25-400 mg/kg) had no analgesic effect on tail-flick test (Buadonpri, 2008). This could also be due to better systemic bioavailabilty of CurDD. The results obtained from hot-plate and tail-flick tests suggested that CurDD had analgesic activity at both supraspinal and spinal levels.

In order to measure an analgesic effect of CurDD against chemical stimuli, an acetic acid-induced writhing model was chosen. This model is commonly used for measuring peripheral analgesic activity and considered as a model of visceral inflammatory pain. Writhing responses consist of contractions of the abdomen, twisting and turning of the trunk, and extension of the hind limbs (Svender and Hau, 1994).

Acetic acid provoked the biosynthesis or the release of considerable amounts of prostaglandins in the peritoneal fluid and only prevented by various inhibitors of prostaglandin biosynthesis. Various types of prostaglandin biosynthesis inhibitors including nonsteroidal anti-inflammatory agents, non-narcotic analgesics and some monoamine oxidase inhibitors and antioxidants prevented prostaglandin release. Their activity on release paralleled their activity on acetic acid-induced writhing (Deraedt et al., 1980). In the present study, indomethacin (IND), a nonsteroidal anti-inflammatory drug, was used as a reference drug in this model. Oral administration of IND (10 mg/kg) produced significant analgesic response compared to vehicle treated controls (Figure 24). All doses of CurDD (25-200 mg/kg) produced significant peripheral analgesic responses compared to vehicle controls (Figure 25). Oral administration of high doses of curcumin (200 and 400 mg/kg) were shown to produce significant analgesic response in this model (Buadonpri, 2008). Another study also showed that chronic oral administration of curcumin for 8 days at the doses of 20 and 40 mg/kg significantly ($p<0.05$) decreased the number of writhes in acetic acid-induced visceral nociception (Tajik et al., 2008). These results indicated that CurDD had higher analgesic potency than curcumin due to the benefit of prodrug approach.

Carrageenan-induced paw edema was originally described by Winter et al. in 1962. Inflammation induced by carrageenan is acute, nonimmune, well-researched, and highly reproducible. Cardinal signs of inflammation (edema, hyperalgesia, and erythema) develop immediately following subcutaneous carrageenan injection, resulting from action of proinflammatory agents. The inflammatory response is usually quantified by increased in paw size (edema) which is maximal around 5 hr postcarrageenan injection and is modulated by inhibitors of specific molecules within the inflammatory cascade (Morris, 2003). Carrageenan-induced edema is a biphasic response. The first phase (1-2 hr) involves the release of serotonin and histamine, whereas the second phase is attributed to local production of prostaglandins (PGs), especially those of the E series, and the continuity between the two phases is provided by kinins (Vinegar et al., 1969; Morris, 2003; Kale et al., 2007). Histamine, serotonin and PGs are established mediators of acute phase of inflammation causing increase in

vascular permeability and vasodilation (Mahat and Patil, 2007). The precursor of PGs is prostacyclin (PGH_2), derived from arachidonic acid by the action of cyclooxygenase (COX) enzymes. Inhibition of these enzymes is the basis of action of the nonsteroidal anti-inflammatory drugs (NSAIDs), indomethacin is a clinically useful example. Therefore, this model is the standard experimental animal model of acute inflammation and has a vital role in the development of novel drug (Morris, 2003).

In this study, IND (10 mg/kg) significantly reduced paw edema at 2, 3, 4, and 5 hr (second phase) after carrageenan administration (Table 2). The anti-edematous effect of IND at the second phase could be explained by the fact that IND is a cyclooxygenase inhibitor, resulting in the reduction of prostaglandins. This result is consistent with the previous study which showed the strong inhibition of the second phase without affecting the development of the first phase of IND (Vinegar et al., 1969). CurDD doses of 10, 40 and 80 mg/kg showed significant reduction of edema at 2 hr or more, suggesting that CurDD produces an anti-edematous effect at the second phase which involves prostaglandin synthesis. These results are in conformity with previous studies which shown that curcumin exhibits anti-inflammatory effect by inhibition of cyclooxygenase enzyme and hence, reduces PGs (Goel et al., 2001; Kim et al., 2003; Strimpakos and Sharma, 2008). Previous study showed that high dose of curcumin (400 mg/kg) significantly inhibited paw edema starting at 2 hr and the effect was sustained until 5 hr after carrageenan administration (Buadonpri et al., 2009). Low dose of CurDD (40 mg/kg) showed a similar result to curcumin but the anti-edematous effect of CurDD was sustained longer (up to 6 hr). These results also indicated that CurDD had higher anti-inflammatory potency than curcumin.

In order to confirm the action of CurDD during the second phase of inflammation induced by carrageenan. The experiments were carried out using specific blocking agents of histamine, serotonin and PGE_1 (Kale et al., 2007). Histamine is a basic amine related with inflammatory and allergic processes causing, among several effects, both vasodilatation and increase of vascular permeability. Serotonin is present in mast cells and is considerably more potent than histamine in increasing vascular permeability in rats. The main evidence for histamine and serotonin being the mediators

of immediate response in injured tissues comes from the suppression of the inflammatory response by pharmacological histamine and serotonin antagonists (Antonio and Brito, 1998). PRO (antihistamine), CYPRO (antiserotonin) and IND (NSAIDs) significantly inhibited the edematogenic effect of histamine, serotonin and PGE_1, respectively. CurDD significantly inhibited inflammation induced by PGE_1 but could not antagonize the inflammation induced by histamine and serotonin (Table 4-6). Percent inhibition of histamine-induced paw edema values shown at some hours were equal or higher than 100% due to human error in measuring the paw volume of rats. The results from both carrageenan and PGE_1-induced paw edema favored the notion that the anti-inflammatory action of CurDD are not only involved in the reduction of PGs through the inhibition of cyclooxygenase enzyme, but are also involved in the direct blocking of PGE_1 effects. These results provided additional information of curcumin actions.

In conclusion, this present study has demonstrated that CurDD exerts a pronounced antinociceptive activity when assessed with thermal and chemical models of nociception in rodents. CurDD possesses both central and peripheral analgesic activities. The analgesic mechanism is most likely involved with the opioid pathway. In addition, CurDD also has anti-inflammatory effect assessed with carrageenan-induced paw edema model. The anti-inflammatory mechanism of CurDD involves in the reduction of prostaglandin synthesis through the inhibition of cyclooxgenase enzyme and in the inhibition of PGE_1 effects. In addition, CurDD, a prodrug of curcumin seems to be more effective as an analgesic and anti-inflammatory drug than curcumin.

FUTURE RESEARCH

The future research could comprise of several objectives as listed below

(1) To better understand the mechanism of curcumin diethyl disuccinate (CurDD) that involved in producing its analgesic and anti-inflammatory effects.
(2) To better characterize the mechanism of analgesic effects of CurDD using other opioid antagonists.
(3) To investigate the anti-inflammatory effect of CurDD in the standard experimental animal model of chronic inflammation.
(4) To investigate the potential use of CurDD in combination with other analgesics or nonsteroidal anti-inflammatory drugs.
(5) To investigate other routes of administration that might be more appropriate for the use of CurDD and possibly enhance the analgesic or anti-inflammatory effects of CurDD.
(6) To test side effects and toxic effects of CurDD at high doses and long term use.
(7) To investigate the antipyretic effect of various doses of CurDD.

These studies may provide important clues to help understand the mechanism underlying the analgesic and anti-inflammatory effects of CurDD and further support the use of such compound in a clinical setting.

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APPENDICES

Appendix A

Study Protocol Approval by the Institutional Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☐ Original ☐ Renew
Animal Use Protocol No. 10-33-009	**Approval No. 10-33-009**
Protocol Title Antinociceptive and anti-inflammatory activities of curcumin diethyl disuccinate in animals	
Principal Investigator Pasarapa Towiwat, Ph.D.	
Certification of Institutional Animal Care and Use Committee (IACUC) This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.	
Date of Approval April 5, 2010	**Date of Expiration** April 5, 2011
Applicant Faculty/Institution Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Rd., Pathumwan BKK-THAILAND. 10330	
Signature of Chairperson	**Signature of Authorized Official**
Name and Title THONGCHAI SOOKSAWATE, Ph.D. Chairman	**Name and Title** PARKPOOM TENGAMNUAY, Ph.D. Associate Dean (Research and Academic Service)
The official signing above certifies that the information provided on this form is correct. The institution assumes that investigators will take responsibility, and follow university regulations and policies for the care and use of animals. *This approval is subjected to assurance given in the animal use protocol and may be required for future investigations and reviews.*	

Appendix B

Data of mouse hot-plate test

Table 7 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of 0.5% CMC 10 ml/kg and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
CMC 10 ml/kg	18.19±2.51	17.91±1.52	14.79±1.77	14.35±1.95	14.96±1.07	14.83±2.39	16.88±1.24
CurDD 25	29.75±2.54	24.63±4.03	20.12±2.98	23.80±3.54	23.80±3.82	25.75±4.39	26.47±3.58
CurDD 50	23.15±3.42	27.59±3.72	24.96±3.73	25.89±3.93	26.69±4.01	26.10±2.81	33.21±4.28
CurDD 100	23.21±2.84	17.60±2.63	18.76±3.02	16.01±2.27	23.96±2.30	23.61±3.71	28.86±4.23
CurDD 200	27.61±2.92	20.68±3.22	19.48±2.92	23.25±3.72	21.05±3.33	19.35±3.34	25.24±3.87

Table 8 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of 0.5% CMC 10 ml/kg and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
CMC 10 mg/kg	-0.40±8.18	-5.64±11.27	-13.20±2.45	-16.52 ±6.91	-15.93±7.81	-14.26±9.77	-9.36±9.84	-2768.86±1164.99
CurDD 25	36.74±11.18	15.27±18.69	-1.58±12.69	13.49 ±15.63	15.23±15.61	19.10±18.75	20.27±16.86	4165.84±2844.71
CurDD 50	13.61±14.92	34.28±14.39	25.75±13.36	27.57±13.64	30.92±14.94	26.09±10.93	54.79±17.26	7896.79±2130.08
CurDD 100	20.59±12.54	2.23±10.16	5.67±11.56	-3.57±9.76	25.55±7.86	24.19±12.34	24.19±12.34	5723.80±1617.25
CurDD 200	33.02±11.86	5.79±14.24	1.06±13.28	19.45±13.12	7.50±15.19	1.67±13.66	27.30±15.21	3023.47±2216.67

Appendix C

Study of mechanism of analgesic action involving opioid pathways using naloxone in mouse hot-plate test

Table 9 Latency (sec) in mouse hot-plate test from 0-240 min after oral administration of NSS (10 ml/kg; i.p.), naloxone (5 mg/kg; i.p.), 0.5% CMC (10 ml/kg; p.o.), curcumin diethyl disuccinate (CurDD; 50 mg/kg, p.o.) and the combination of naloxone and curcumin diethyl disuccinate (5/50 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
NSS	15.71±1.10	14.67±0.77	14.59±1.38	14.75±1.30	15.21±1.71	14.94±1.79	15.85±1.43
Naloxone 5 mg/kg	17.56±1.46	16.69±1.20	16.88±1.44	18.08±1.51	20.36±1.62	16.55±2.12	17.98±1.49
CMC 10 ml/kg	18.48±1.61	13.39±1.25	17.01±2.58	17.07±1.32	19.11±2.07	18.04±1.88	19.30±1.75
CurDD 50 mg/kg	24.59±0.73	25.76±2.29	26.44±2.19	26.53±2.34	22.49±2.75	21.22±2.80	23.21±3.15
Naloxone + CurDD	15.17±1.65	14.55±1.55	14.31±1.81	12.51±1.58	15.75±1.93	12.37±1.83	16.54±1.94

Table 10 %MPE-Time in mouse hot-plate test from 0-240 min after oral administration of NSS (10 ml/kg; i.p.), naloxone (5 mg/kg; i.p.), 0.5% CMC (10 ml/kg; p.o.), curcumin diethyl disuccinate (CurDD; 50 mg/kg; p.o.) and the combination of naloxone and curcumin diethyl disuccinate (5/50 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia (%MPE-min)
NSS	3.35±2.78	-0.92±4.47	-1.03±5.69	-1.57±8.06	1.64±6.27	-2.04±10.42	3.11±6.43	68.16±1525.49
Naloxone 5 mg/kg	-16.97±9.12	-20.65±8.06	-20.82±10.96	-14.95±9.53	-4.63±7.57	-19.70±9.04	-14.26±7.04	-3685.16±1675.11
CMC 10 ml/kg	-0.48±5.69	-21.32±7.13	-4.54±9.45	-6.52±6.04	2.43±7.49	-2.73±7.89	2.12±7.59	-546.40±1220.64
CurDD 50 mg/kg	23.40±3.44	31.58±8.12	33.83±7.96	33.27±9.46	19.34±10.47	15.43±9.91	22.36±11.10	5159.72±2008.38
Naloxone + CurDD	-7.12±6.90	-8.30±4.96	-8.93±5.32	-15.54±4.80	-3.78±6.09	-16.98±7.77	-1.06±6.32	-2165.17±1087.68

Appendix D

Data of mouse tail-flick test

Table 11 Latency (sec) in mouse tail-flick test from 0-240 min after oral administration of 0.5% CMC 10 ml/kg and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
CMC 10 mg/kg	1.82±0.13	1.75±0.11	1.80±0.19	1.76±0.15	1.82±0.13	1.86±0.16	1.57±0.08
25	1.93±0.17	1.89±0.08	1.99±0.20	1.85±0.16	1.78±0.15	1.76±0.11	1.96±0.25
50	2.10±0.17	2.26±0.19	1.87±0.15	1.85±0.19	1.82±0.12	1.70±0.13	1.64±0.16
100	2.34±0.20	2.06±0.25	1.85±0.11	2.00±0.27	2.19±0.19	2.02±0.22	1.83±0.12
200	2.32±0.28	2.18±0.30	1.90±0.17	1.83±0.15	1.80±0.13	1.89±0.16	1.65±0.23

Table 12 %MPE-Time in mouse tail-flick test from 0-240 min after oral administration of 0.5% CMC 10 ml/kg and various doses of curcumin diethyl disuccinate (CurDD; 25-200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
CMC 10 mg/kg	-12.29±11.18	-18.36±16.03	-10.51±10.29	-17.93 ±15.43	-15.26±15.48	-9.66±12.49	-27.36±15.26	-3845.15±2952.05
CurDD 25	10.27±9.31	9.09±5.45	14.93±8.57	7.77 ±7.57	5.47±6.50	4.75±4.21	12.07±11.78	1933.65±1098.02
CurDD 50	13.71±9.19	23.14±8.67	4.97±7.60	5.36±8.34	4.70±3.77	-3.00±8.40	-2.98±5.12	484.60±883.23
CurDD 100	24.42±8.90	16.89±10.32	0.87±7.32	8.73±15.63	15.65±12.11	5.00±14.63	-2.67±12.13	1512.99±2228.09
CurDD 200	37.58±12.57	25.63±13.85	-2.61±9.18	-9.71±9.96	-7.03±11.31	10.89±6.96	-2.51±11.36	-270.55±1739.81

Appendix E

Data of acetic acid-induced writhing in mice

Table 13 Dose-response and time-course effect of curcumin diethyl disuccinate (CurDD) on acetic acid-induced writhing in mice. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean of no. of writhes						Total
	0-5	6-10	11-15	16-20	21-25	26-30	
CMC 10 ml/kg	1.00±0.63	6.67±1.74	10.33±1.52	7.33±1.38	7.33±0.92	5.83±0.54	38.50±5.71
CurDD 25	0.50±0.34	2.50±0.92	3.50±0.56	2.83±0.48	2.50±0.56	1.17±0.60	13.00±1.13
CurDD 50	0.17±0.17	4.50±2.64	6.67±1.02	6.33±1.20	3.83±1.17	3.17±0.48	24.67±4.59
CurDD 100	0.83±0.65	7.00±1.71	4.50±1.18	4.50±0.85	3.67±0.88	2.50±0.85	23.00±3.32
CurDD 200	0.17±0.17	1.00±1.00	2.83±0.87	3.00±0.86	1.33±0.49	1.00±0.45	9.33±1.48

Appendix F

Data of carrageenan induced-paw edema in rats

Table 14 Effect of 0.5% CMC 10 ml/kg (p.o.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before carrageenan injection		1.22	1.15	1.13	1.13	1.11	1.16		-
1 hr	Paw volume (ml)	1.49	1.36	1.63	1.46	1.52	1.48		-
	Volume of edema (ml)	0.27	0.21	0.50	0.33	0.41	0.32	0.34	
2 hr	Paw volume (ml)	1.65	1.54	1.73	1.47	1.70	1.62		-
	Volume of edema (ml)	0.43	0.39	0.60	0.34	0.59	0.46	0.47	
3 hr	Paw volume (ml)	1.69	1.61	1.77	1.51	1.70	1.63		-
	Volume of edema (ml)	0.47	0.46	0.64	0.38	0.59	0.47	0.50	
4 hr	Paw volume (ml)	1.71	1.48	1.55	1.60	1.82	1.65		-
	Volume of edema (ml)	0.49	0.33	0.42	0.47	0.71	0.49	0.49	
5 hr	Paw volume (ml)	1.62	1.41	1.43	1.55	1.64	1.60		-
	Volume of edema (ml)	0.40	0.26	0.30	0.42	0.53	0.44	0.39	
6 hr	Paw volume (ml)	1.59	1.35	1.37	1.41	1.61	1.58		-
	Volume of edema (ml)	0.37	0.20	0.24	0.28	0.50	0.42	0.34	

Table 15 Effect of indomethacin 10 mg/kg (p.o.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before carrageenan injection		1.26	1.20	1.30	1.16	1.11	1.17		-
1 hr	Paw volume (ml)	1.31	1.26	1.38	1.36	1.49	1.25		46.15
	Volume of edema (ml)	0.05	0.06	0.08	0.20	0.38	0.08	0.14	
2 hr	Paw volume (ml)	1.36	1.45	1.41	1.41	1.48	1.29		47.5
	Volume of edema (ml)	0.10	0.25	0.11	0.25	0.37	0.12	0.20	
3 hr	Paw volume (ml)	1.45	1.43	1.45	1.37	1.40	1.25		56.52
	Volume of edema (ml)	0.19	0.23	0.15	0.21	0.29	0.08	0.19	
4 hr	Paw volume (ml)	1.53	1.40	1.42	1.47	1.46	1.26		48.89
	Volume of edema (ml)	0.27	0.20	0.12	0.31	0.35	0.09	0.22	
5 hr	Paw volume (ml)	1.39	1.40	1.33	1.47	1.44	1.31		44.44
	Volume of edema (ml)	0.13	0.20	0.03	0.31	0.33	0.14	0.19	
6 hr	Paw volume (ml)	1.38	1.43	1.30	1.40	1.36	1.32		41.38
	Volume of edema (ml)	0.12	0.23	0.00	0.24	0.25	0.15	0.17	

Table 16 Effect of curcumin diethyl disuccinate 10 mg/kg (p.o.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before carrageenan injection		1.16	1.24	1.06	1.17	1.23	1.36		-
1 hr	Paw volume (ml)	1.37	1.38	1.21	1.40	1.55	1.56		38.24
	Volume of edema (ml)	0.21	0.14	0.15	0.23	0.32	0.20	0.21	
2 hr	Paw volume (ml)	1.48	1.30	1.37	1.38	1.62	1.56		46.81
	Volume of edema (ml)	0.32	0.06	0.31	0.21	0.39	0.20	0.25	
3 hr	Paw volume (ml)	1.44	1.39	1.37	1.54	1.65	1.83		33.33
	Volume of edema (ml)	0.28	0.15	0.31	0.37	0.42	0.47	0.33	
4 hr	Paw volume (ml)	1.45	1.58	1.41	1.55	1.68	1.78		33.33
	Volume of edema (ml)	0.29	0.34	0.35	0.38	0.45	0.42	0.37	
5 hr	Paw volume (ml)	1.38	1.57	1.42	1.49	1.66	1.80		30.77
	Volume of edema (ml)	0.22	0.33	0.36	0.32	0.43	0.44	0.35	
6 hr	Paw volume (ml)	1.41	1.62	1.33	1.42	1.49	1.70		38.78
	Volume of edema (ml)	0.25	0.38	0.27	0.25	0.26	0.34	0.29	

Table 17 Effect of curcumin diethyl disuccinate 20 mg/kg (p.o.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before carrageenan injection		1.05	1.08	1.13	1.29	1.14	1.21		-
1 hr	Paw volume (ml)	1.31	1.28	1.32	1.67	1.38	1.40		26.47
	Volume of edema (ml)	0.26	0.20	0.19	0.38	0.24	0.19	0.24	
2 hr	Paw volume (ml)	1.29	1.34	1.48	1.69	1.58	1.57		25.53
	Volume of edema (ml)	0.24	0.26	0.35	0.40	0.44	0.36	0.34	
3 hr	Paw volume (ml)	1.30	1.35	1.49	1.70	1.74	1.50		27.45
	Volume of edema (ml)	0.25	0.27	0.36	0.41	0.60	0.29	0.36	
4 hr	Paw volume (ml)	1.38	1.28	1.53	1.72	1.66	1.65		31.58
	Volume of edema (ml)	0.33	0.20	0.40	0.43	0.52	0.44	0.39	
5 hr	Paw volume (ml)	1.32	1.35	1.51	1.75	1.66	1.60		25.00
	Volume of edema (ml)	0.27	0.27	0.38	0.46	0.52	0.39	0.38	
6 hr	Paw volume (ml)	1.25	1.22	1.39	1.79	1.63	1.55		32.65
	Volume of edema (ml)	0.20	0.14	0.26	0.50	0.49	0.34	0.32	

Table 18 Effect of curcumin diethyl disuccinate 40 mg/kg (p.o.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before carrageenan injection		1.34	1.18	1.12	1.21	1.35	1.15		-
1 hr	Paw volume (ml)	1.42	1.32	1.48	1.41	1.66	1.35		35.29
	Volume of edema (ml)	0.08	0.14	0.36	0.20	0.31	0.20	0.22	
2 hr	Paw volume (ml)	1.30	1.40	1.58	1.51	1.73	1.42		42.55
	Volume of edema (ml)	-0.04	0.22	0.46	0.30	0.38	0.27	0.27	
3 hr	Paw volume (ml)	1.37	1.44	1.47	1.48	1.65	1.50		47.06
	Volume of edema (ml)	0.03	0.26	0.35	0.27	0.30	0.35	0.26	
4 hr	Paw volume (ml)	1.30	1.50	1.53	1.49	1.79	1.51		47.37
	Volume of edema (ml)	-0.01	0.32	0.41	0.28	0.44	0.36	0.30	
5 hr	Paw volume (ml)	1.25	1.41	1.45	1.51	1.59	1.48		55.77
	Volume of edema (ml)	-0.09	0.23	0.33	0.30	0.24	0.33	0.22	
6 hr	Paw volume (ml)	1.29	1.39	1.44	1.46	1.65	1.43		55.10
	Volume of edema (ml)	-0.05	0.21	0.32	0.25	0.30	0.28	0.22	

Table 19 Effect of curcumin diethyl disuccinate 80 mg/kg (p.o.) on paw volume in carrageenan-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before carrageenan injection		1.13	1.07	1.12	1.30	1.16	1.28		-
1 hr	Paw volume (ml)	1.25	1.33	1.41	1.49	1.38	1.41		41.18
	Volume of edema (ml)	0.12	0.26	0.29	0.19	0.22	0.13	0.20	
2 hr	Paw volume (ml)	1.44	1.47	1.53	1.58	1.61	1.69		19.15
	Volume of edema (ml)	0.31	0.40	0.41	0.28	0.45	0.41	0.38	
3 hr	Paw volume (ml)	1.45	1.60	1.63	1.64	1.63	1.61		17.65
	Volume of edema (ml)	0.32	0.53	0.51	0.34	0.47	0.33	0.42	
4 hr	Paw volume (ml)	1.52	1.54	1.43	1.53	1.52	1.61		38.60
	Volume of edema (ml)	0.39	0.47	0.31	0.23	0.36	0.33	0.35	
5 hr	Paw volume (ml)	1.40	1.47	1.46	1.67	1.48	1.62		34.62
	Volume of edema (ml)	0.27	0.40	0.34	0.37	0.32	0.34	0.34	
6 hr	Paw volume (ml)	1.33	1.38	1.42	1.56	1.32	1.58		46.94
	Volume of edema (ml)	0.20	0.31	0.30	0.26	0.16	0.30	0.26	

Appendix G

Data of histamine-induced-paw edema in rats

Table 20 Effect of 0.9% NSS on paw volume in histamine-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before histamine injection		1.26	1.39	1.28	1.33	1.45	1.39		-
1 hr	Paw volume (ml)	1.42	1.58	1.43	1.58	1.51	1.57		-
	Volume of edema (ml)	0.16	0.19	0.15	0.23	0.06	0.18	0.16	
2 hr	Paw volume (ml)	1.35	1.51	1.42	1.39	1.56	1.54		-
	Volume of edema (ml)	0.09	0.12	0.14	0.06	0.11	0.15	0.11	
3 hr	Paw volume (ml)	1.31	1.47	1.44	1.30	1.47	1.48		-
	Volume of edema (ml)	0.05	0.08	0.16	-0.03	0.02	0.09	0.06	
4 hr	Paw volume (ml)	1.30	1.49	1.37	1.41	1.48	1.42		-
	Volume of edema (ml)	0.04	0.10	0.09	0.08	0.03	0.03	0.06	
5 hr	Paw volume (ml)	1.27	1.43	1.35	1.29	1.42	1.43		-
	Volume of edema (ml)	0.01	0.04	0.07	-0.04	-0.03	0.04	0.02	
6 hr	Paw volume (ml)	1.27	1.38	1.31	1.36	1.44	1.47		-
	Volume of edema (ml)	0.01	-0.01	0.03	0.03	-0.01	0.08	0.02	

Table 21 Effect of promethazine 30 mg/kg (p.o.) on paw volume in histamine-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before histamine injection		1.44	1.32	1.29	1.59	1.23	1.17		-
1 hr	Paw volume (ml)	1.44	1.29	1.28	1.57	1.29	1.31		82.35
	Volume of edema (ml)	0.00	-0.03	-0.01	-0.02	0.06	0.14	0.02	
2 hr	Paw volume (ml)	1.52	1.31	1.27	1.57	1.23	1.41		58.33
	Volume of edema (ml)	0.08	-0.01	-0.02	-0.02	0.00	0.24	0.05	
3 hr	Paw volume (ml)	1.47	1.32	1.25	1.56	1.32	1.38		28.57
	Volume of edema (ml)	0.03	0.00	-0.04	-0.03	0.09	0.21	0.04	
4 hr	Paw volume (ml)	1.45	1.29	1.22	1.47	1.22	1.19		142.86
	Volume of edema (ml)	0.01	-0.03	-0.07	-0.12	-0.01	0.02	-0.03	
5 hr	Paw volume (ml)	1.52	1.38	1.28	1.40	1.25	1.28		50.00
	Volume of edema (ml)	0.08	0.06	-0.01	-0.19	0.02	0.00	0.01	
6 hr	Paw volume (ml)	1.41	1.36	1.26	1.50	1.24	1.22		133.33
	Volume of edema (ml)	-0.03	0.04	-0.03	-0.09	0.01	0.05	-0.01	

Table 22 Effect of curcumin diethyl disuccinate 40 mg/kg (p.o.) on paw volume in histamine-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before histamine injection		1.31	1.51	1.33	1.47	1.50	1.43		-
1 hr	Paw volume (ml)	1.60	1.81	1.46	1.70	1.58	1.52		-11.76
	Volume of edema (ml)	0.29	0.30	0.13	0.23	0.08	0.09	0.19	
2 hr	Paw volume (ml)	1.41	1.66	1.60	1.73	1.52	1.52		-25.00
	Volume of edema (ml)	0.10	0.15	0.27	0.26	0.02	0.09	0.15	
3 hr	Paw volume (ml)	1.48	1.56	1.42	1.62	1.51	1.53		-42.86
	Volume of edema (ml)	0.17	0.05	0.09	0.15	0.01	0.10	0.10	
4 hr	Paw volume (ml)	1.39	1.53	1.36	1.45	1.53	1.47		57.14
	Volume of edema (ml)	0.08	0.02	0.03	-0.02	0.03	0.04	0.03	
5 hr	Paw volume (ml)	1.30	1.50	1.36	1.46	1.49	1.40		100.00
	Volume of edema (ml)	-0.01	-0.01	0.03	-0.01	-0.01	-0.03	-0.01	
6 hr	Paw volume (ml)	1.32	1.43	1.36	1.42	1.42	1.42		200.00
	Volume of edema (ml)	0.01	-0.08	0.03	-0.05	-0.08	-0.01	-0.03	

Appendix H

Data of serotonin-induced-paw edema in rats

Table 23 Effect of 0.9% NSS on paw volume in serotonin-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before serotonin injection		1.26	1.19	1.46	1.28	1.33	1.35		-
1 hr	Paw volume (ml)	2.11	2.04	2.46	2.08	2.09	1.96		-
	Volume of edema (ml)	0.85	0.85	1.00	0.80	0.76	0.61	0.81	
2 hr	Paw volume (ml)	2.01	1.86	2.22	1.85	1.76	1.96		-
	Volume of edema (ml)	0.75	0.67	0.76	0.57	0.43	0.61	0.63	
3 hr	Paw volume (ml)	1.78	1.55	1.87	1.73	1.86	1.73		-
	Volume of edema (ml)	0.52	0.36	0.41	0.45	0.53	0.38	0.44	
4 hr	Paw volume (ml)	1.67	1.58	1.83	1.59	1.79	1.74		-
	Volume of edema (ml)	0.41	0.39	0.37	0.31	0.46	0.39	0.39	
5 hr	Paw volume (ml)	1.55	1.50	1.67	1.49	1.52	1.54		-
	Volume of edema (ml)	0.29	0.31	0.21	0.21	0.19	0.19	0.23	
6 hr	Paw volume (ml)	1.53	1.48	1.66	1.44	1.45	1.53		-
	Volume of edema (ml)	0.27	0.29	0.20	0.16	0.12	0.18	0.20	

Table 24 Effect of indomethacin 10 mg/kg (p.o.) on paw volume in serotonin-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before serotonin injection		1.35	1.35	1.30	1.48	1.37	1.35		-
1 hr	Paw volume (ml)	2.05	1.58	1.75	1.59	1.52	1.63		59.76
	Volume of edema (ml)	0.70	0.23	0.45	0.11	0.15	0.28	0.32	
2 hr	Paw volume (ml)	1.95	1.53	1.73	1.58	1.37	1.52		59.38
	Volume of edema (ml)	0.60	0.18	0.43	0.10	0.00	0.17	0.25	
3 hr	Paw volume (ml)	1.68	1.56	1.67	1.48	1.34	1.38		64.44
	Volume of edema (ml)	0.33	0.21	0.37	0.00	-0.03	0.03	0.15	
4 hr	Paw volume (ml)	1.69	1.46	1.64	1.64	1.34	1.50		52.50
	Volume of edema (ml)	0.34	0.11	0.34	0.16	-0.03	0.15	0.18	
5 hr	Paw volume (ml)	1.55	1.52	1.58	1.54	1.34	1.34		50.00
	Volume of edema (ml)	0.20	0.17	0.28	0.06	-0.03	-0.01	0.11	
6 hr	Paw volume (ml)	1.58	1.44	1.40	1.45	1.32	1.40		66.67
	Volume of edema (ml)	0.23	0.09	0.10	-0.03	-0.05	0.05	0.07	

Table 25 Effect of curcumin diethyl disuccinate 40 mg/kg (p.o.) on paw volume in serotonin-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before serotonin injection		1.40	1.36	1.35	1.32	1.48	1.37		-
1 hr	Paw volume (ml)	2.42	2.06	2.32	2.32	2.37	2.15		-9.76
	Volume of edema (ml)	1.02	0.70	0.97	1.00	0.89	0.78	0.89	
2 hr	Paw volume (ml)	2.10	1.85	2.10	2.18	2.21	2.06		-10.94
	Volume of edema (ml)	0.70	0.49	0.75	0.86	0.73	0.69	0.70	
3 hr	Paw volume (ml)	2.03	1.75	2.12	1.97	1.95	1.96		-31.11
	Volume of edema (ml)	0.67	0.39	0.77	0.65	0.47	0.59	0.59	
4 hr	Paw volume (ml)	1.71	1.85	1.83	1.84	1.80	1.73		-5.00
	Volume of edema (ml)	0.31	0.49	0.48	0.52	0.32	0.36	0.41	
5 hr	Paw volume (ml)	1.47	1.69	1.71	1.62	1.59	1.57		4.17
	Volume of edema (ml)	0.07	0.33	0.36	0.30	0.11	0.20	0.23	
6 hr	Paw volume (ml)	1.51	1.57	1.55	1.61	1.62	1.57		4.76
	Volume of edema (ml)	0.11	0.21	0.20	0.29	0.14	0.20	0.19	

Appendix I

Data of prostaglandin E_1-induced-paw edema in rats

Table 26 Effect of 0.9% NSS on paw volume in prostaglandin E_1-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before PGE_1 injection		1.31	1.38	1.32	1.53	1.35	1.29		-
1 hr	Paw volume (ml)	1.80	1.76	1.67	1.78	1.59	1.69		-
	Volume of edema (ml)	0.49	0.38	0.35	0.25	0.24	0.40	0.35	
2 hr	Paw volume (ml)	1.51	1.68	1.64	1.69	1.65	1.59		-
	Volume of edema (ml)	0.20	0.30	0.32	0.16	0.30	0.30	0.26	
3 hr	Paw volume (ml)	1.51	1.50	1.52	1.61	1.62	1.57		-
	Volume of edema (ml)	0.20	0.12	0.20	0.08	0.27	0.28	0.19	
4 hr	Paw volume (ml)	1.47	1.51	1.48	1.65	1.40	1.52		-
	Volume of edema (ml)	0.16	0.18	0.16	0.12	0.05	0.23	0.15	
5 hr	Paw volume (ml)	1.41	1.47	1.42	1.55	1.41	1.46		-
	Volume of edema (ml)	0.10	0.09	0.10	0.02	0.06	0.17	0.09	
6 hr	Paw volume (ml)	1.33	1.43	1.39	1.50	1.39	1.42		-
	Volume of edema (ml)	0.02	0.05	0.07	-0.03	0.04	0.13	0.05	

Table 27 Effect of indomethacin 10 mg/kg (p.o.) on paw volume in prostaglandin E_1-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before PGE_1 injection		1.43	1.44	1.65	1.44	1.48	1.32		-
1 hr	Paw volume (ml)	1.68	1.64	1.72	1.65	1.74	1.61		38.89
	Volume of edema (ml)	0.25	0.20	0.07	0.21	0.26	0.29	0.21	
2 hr	Paw volume (ml)	1.54	1.56	1.60	1.59	1.65	1.56		51.85
	Volume of edema (ml)	0.11	0.12	-0.05	0.15	0.17	0.24	0.12	
3 hr	Paw volume (ml)	1.55	1.45	1.64	1.52	1.60	1.45		60.00
	Volume of edema (ml)	0.12	0.01	-0.01	0.08	0.12	0.13	0.08	
4 hr	Paw volume (ml)	1.42	1.54	1.58	1.54	1.55	1.41		66.67
	Volume of edema (ml)	-0.01	0.10	-0.07	0.10	0.07	0.09	0.05	
5 hr	Paw volume (ml)	1.43	1.51	1.52	1.42	1.53	1.39		90.00
	Volume of edema (ml)	0.00	0.07	-0.13	-0.02	0.05	0.07	0.01	
6 hr	Paw volume (ml)	1.44	1.49	1.59	1.44	1.49	1.34		80.00
	Volume of edema (ml)	0.01	0.05	-0.06	0.00	0.01	0.02	0.01	

Table 28 Effect of curcumin diethyl disuccinate 40 mg/kg (p.o.) on paw volume in prostaglandin E_1-induced paw edema in rats.

		NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	average	% Inhibition
Paw volume before PGE_1 injection		1.51	1.29	1.46	1.27	1.58	1.42		-
1 hr	Paw volume (ml)	1.67	1.66	1.57	1.44	1.81	1.59		41.67
	Volume of edema (ml)	0.16	0.37	0.11	0.17	0.23	0.17	0.20	
2 hr	Paw volume (ml)	1.65	1.42	1.56	1.34	1.49	1.43		77.78
	Volume of edema (ml)	0.14	0.13	0.10	0.07	-0.09	0.01	0.06	
3 hr	Paw volume (ml)	1.62	1.43	1.56	1.36	1.48	1.46		65.00
	Volume of edema (ml)	0.11	0.14	0.10	0.09	-0.10	0.04	0.06	
4 hr	Paw volume (ml)	1.60	1.31	1.51	1.35	1.52	1.40		80.00
	Volume of edema (ml)	0.09	0.02	0.05	0.08	-0.06	-0.02	0.03	
5 hr	Paw volume (ml)	1.54	1.38	1.52	1.39	1.52	1.51		40.00
	Volume of edema (ml)	0.03	0.09	0.06	0.12	-0.06	0.09	0.06	
6 hr	Paw volume (ml)	1.50	1.43	1.47	1.30	1.49	1.47		60.00
	Volume of edema (ml)	-0.01	0.14	0.01	0.03	-0.09	0.05	0.02	

VITAE

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