 ISSN: 2362-0412

Invited Oration
Pharma-nature Fusion: Integrated Approaches for Effective Pain Control and Relief

Dr. P.W.D. Wasana

Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna

Abstract:

Pain management poses a significant healthcare challenge, prompting exploration into innovative strategies. Our work embodies a fusion of pharmaceutical and natural approaches to enhance pain relief using curcumin, a natural polyphenolic compound. Through synergistic drug combinations (curcumin-metformin and curcumin-piperine), nanoformulations (curcumin-metformin-coloaded chitosan/alginate nanoparticles), and prodrug development (curcumin diglutaric acid, curcumin diethyl diglutarate, and curcumin diethyl γ-aminobutyrate), we aimed to fortify curcumin's efficacy against diverse pain types. Findings demonstrated enhanced analgesic effects, improved pharmacokinetic profiles, and promising safety profiles, laying the foundation for advanced pain management solutions integrating pharmaceutical and natural elements.

Keywords: *Alginate, Chitosan, Curcumin, Pain, Piperine, Prodrugs, Metformin, Nanoparticles*

Background

Pain is a response of nociceptors to either noxious or non-noxious stimuli, which triggers the central nervous system to experience it. Though pain serves a protective function, the excessive triggering of nociceptors leads to a devasting condition identified as nociceptive pain. If left untreated, acute pain can evolve into a chronic state, emphasizing the significance of effective pain management. Moreover, the widespread prevalence and substantial impact of pain on physical, social, and emotional well-being highlight its critical importance in healthcare (Yong et al., 2022). Current pain management methods, encompassing non-opioid analgesics (nonsteroidal anti-inflammatory drugs, local anesthetics, and steroids), opioids, and adjuvant analgesics (anticonvulsants and antidepressants), face limitations such as reduced efficacy

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and associated side effects. This drives the exploration of alternative strategies in pain management, leading to increased interest in natural bioactive compounds like curcumin (Cur). Its diverse pharmacological activities and superior safety profile have drawn significant attention in this pursuit (Dasuni Wasana et al., 2022).

Curcumin, derived from *Curcuma longa* (turmeric), is a natural polyphenolic compound renowned for its pleiotropic properties, such as anti-inflammatory, antioxidant, and potential anticancer effects. Its extensive historical use in Asian traditional medicine speaks to its safety, considered 'generally recognized as safe' by the US FDA (US FDA, 2011), with minimal short- and long-term toxicity. Studies in animals and humans have highlighted its therapeutic potential in various pain conditions, including nociceptive, inflammatory, and neuropathic pain (Sun et al., 2018). Considering the mechanism of action, curcumin acts through various mechanisms, modulating pain-related neurotransmitters, suppressing immune responses, blocking TRPV1 receptors, and affecting chemokine receptors, effectively providing pain relief. Nonetheless, it faces challenges due to its low stability, poor water solubility, and rapid breakdown in the gastrointestinal tract and liver, despite its evident advantages (Hasriadi et al., 2021). As a result, ongoing research endeavors are actively exploring diverse strategies aimed at boosting curcumin's therapeutic effectiveness.

Our research aims to assess diverse strategies to enhance the bioavailability of curcumin, encompassing approaches such as drug combinations, chemical modifications, and nanoformulations. Evaluations were conducted to explore the synergistic analgesic potential of curcumin when combined with other compounds, such as metformin (Dasuni Wasana et al., 2022), and piperine (Boonrueng et al., 2022). Furthermore, these findings guided the development of curcumin and metformin-loaded chitosan-alginate nanoparticles, enabling the co-delivery of both agents within a single formulation (Wasana et al., 2023). Exploring the chemical modification approach, our investigation involved the conjugation of curcumin with various entities to enhance its physicochemical profile, hindering gastrointestinal degradation and rapid metabolism. Analgesic assessments were conducted with several curcumin prodrugs: curcumin diethyl γ-aminobutyrate (Hasriadi et al., 2022), curcumin diethyl diglutarate (Limcharoen et al., 2021), and curcumin diglutaric acid (Limcharoen et al., 2020). Through these multifaceted

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methodologies, we substantiated the heightened therapeutic efficacy of curcumin across various mouse models of pain.

Aims and Objectives

General Objective

To enhance the therapeutic potential of curcumin in pain management by evaluating strategies that improve its bioavailability and analgesic efficacy.

Specific Objectives

1. To investigate the synergistic analgesic effects of curcumin when combined with other compounds such as metformin and piperine.
2. To develop and characterize curcumin–metformin co-loaded chitosan-alginate nanoformulations for improved co-delivery, sustained release, and enhanced analgesic effects.
3. To assess the physicochemical and analgesic properties of chemically modified curcumin prodrugs aimed at improving its stability, bioavailability, and therapeutic efficacy.
4. To evaluate the central nervous system safety of curcumin in its combined, nanoformulated, and chemically modified (prodrug) forms.

Materials and Methods

Animals

Male ICR mice (4–8 weeks old) were obtained from Nomura Siam International Co., Ltd. (Bangkok, Thailand) and housed (4–5 per cage) under controlled conditions (22 ± 2 °C, 40–60% humidity, 12:12 h light/dark cycle) with free access to food and water at the Laboratory Animal Research Facility, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Mice were acclimatized for at least one week before experiments. Ethical approval was granted by the Institutional Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, before the commencement of the study.

Drugs and Treatments

Curcumin (>95.0 %) and piperine (97%) were purchased from Shaanxi Kanglai Ecology Agriculture Co., Ltd. (Xi'an, China), and Sigma, St. Louis (MO, USA), respoectively. Met was provided by the Siam Bheasach Co. Ltd. (Bangkok, Thailand).

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Statistical Analysis

The data analysis was performed using GraphPad Prism 9.1 (GraphPad Software Inc., La Jolla, CA, USA). All the collected data were summarized with counts percentage for categorical variables. The numerical values were presented as mean ± SD for *in vitro* data and nanoparticle properties and mean ± SEM for animal data (8-10 mice per group). The difference between groups was determined by the One-way analysis of variance (ANOVA) followed by the *post hoc* test. Statistical significance was considered when the *p*-value is < 0.05 (95% confidence level).

1. Curcumin and other drug combination approach for synergistic analgesia

In recent years, research has highlighted the advantages of combining drugs in pain management, showcasing higher efficacy, and reduced adverse effects compared to single-drug approaches. This could be attributed to their capacity to target multiple sites along the pain pathway, thereby minimizing the required doses of each compound in the combination. Based on this understanding, our focus has been on assessing the potential synergistic effects between curcumin and various other compounds possessing analgesic properties.

1.1. Synergistic interaction: curcumin and metformin

This study assessed whether curcumin could synergistically interact with metformin, potentially reducing the required doses of both compounds. Initial in vitro experiments using lipopolysaccharide-induced RAW 264.7 macrophage and BV-2 microglia cells demonstrated that curcumin amplified the anti-inflammatory effects of metformin. The metformin-curcumin combination notably suppressed LPS-induced inflammatory mediators (nitric oxide, TNF-α, and IL-6) more effectively than individual components, indicating potential synergy in peripheral and central pain pathways. This interaction was further explored in a mouse model of formalin-induced pain. Mice received oral doses of curcumin, metformin (3, 10, 30, 100, and 300 mg/kg), or a fixed 1:1 ED_{50} ratio combination, one-hour post-formalin administration, and hind paw licking behavior was observed for 40 minutes. The doses achieving 50% antinociception (ED_{50}) were established at 82.8 ± 17.6 mg/kg for curcumin and 248.9 ± 106.5 mg/kg for metformin using a linear-logarithmic dose-response model. Co-administration of curcumin and metformin at their 1:1 ED_{50} ratio significantly reduced the dose required for a

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50% effect in phase II of the formalin test (39.6 ± 7.1 mg/kg), supported by a combination index value of 0.24 (**Table 1**). Crucially, this synergistic interaction did not induce severe CNS side effects, as evidenced by the absence of motor alterations in rotarod tests, short-term and long-term locomotion changes, and overall well-being evaluated through LABORAS automated home cage monitoring. These findings collectively suggest that curcumin may enhance the anti-inflammatory effects of metformin synergistically without adverse effects on the CNS (Dasuni Wasana et al., 2022).

Table 1. The summary of curcumin interaction with metformin in mouse model of formalin-induced pain-like behaviors.

Combination	**ED_{50} Theoretical (Additive)**	**ED_{50} Experimental**	**CI**	**Interaction**
Curcumin-Metformin	165.8 ± 62.0	39.6 ± 7.1***	0.24	Synergistic

CI = Combination Index; = 1, additive; < 1, synergistic; > 1, antagonistic.
*** represents statistical significance between ED_{50} theoretical and ED_{50} experimental by t-test ($p < 0.001$)

1.2. Synergistic interaction: curcumin and piperine

Similar to the previous study, our group evaluated the synergism between curcumin and piperine in pain attenuation. Curcumin and piperine, prominent bioactive compounds in *Curcuma longa* and *Piper nigrum*, are commonly used together in Southeast Asia. The combination was assessed in animal models for chemical-induced pain, thermal hyperalgesia, and cold allodynia using isobolographic analysis. Curcumin alone demonstrated dose-dependent improvements in pain-like behaviors, with ED_{50} values of 71.4, 34.4, and 31.9 mg/kg in formalin, tail-flick, and cold plate tests. Piperine also exhibited efficacy with ED_{50} values of 18.4, 8.1, and 28.1 mg/kg, respectively. The combination significantly reduced the dose required for 50% antinociception (5.9, 5.2, and 5.5 mg/kg) compared to predicted additive ED_{50} doses (44.9, 21.3, and 30.0 mg/kg) in formalin, tail-flick, and cold plate tests. The combination index values were 0.13, 0.24, and 0.18, indicating strong synergism **(Table 2).** LABORAS automated home-cage analysis revealed reduced locomotive behaviors with piperine (100 mg/kg) alone, but the combination had no impact on spontaneous locomotion. In conclusion, the findings show curcumin and piperine synergistically inhibit pain in mice

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models with no significant CNS side effects, suggesting its application to clinical trials (Boonrueng et al., 2022).

Table 2. The summary of curcumin interaction with piperine in mouse models of pain

Animal Model	ED_{50} Theoretical (Additive)	ED_{50} Experimental	CI	Interaction
Formalin model	44.9 ± 12.5	5.9 ± 2.2***	0.13	Synergistic
Tail-flick	21.3 ± 3.4	5.2 ± 0.6***	0.24	Synergistic
Cold plate	30.0 ± 5.9	5.5 ± 0.7***	0.18	Synergistic

CI = Combination Index; = 1, additive; < 1, synergistic; > 1, antagonistic.
*** represents statistical significance between ED_{50} theoretical and ED_{50} experimental by t-test ($p < 0.001$)

Overall, these results support the possibility of combined use of curcumin with other compounds: metformin and piperine in the treatment of pain with the least amount of medication while taking the easiness of administration, cost of the therapy, and side effect profile of medicines into the account.

2. Curcumin nanoparticle approach for improved therapeutic efficacy against pain.

Nanotechnology plays a pivotal role in multimodal analgesia by providing an exceptional opportunity to finely tune drug loading and co-delivery of therapeutic agents to target sites, thereby enhancing the therapeutic efficacy and safety of analgesic agents. Endogenously, CTS/ALG NPs contribute to enhancing the oral bioavailability of the payload through multiple mechanisms. Firstly, they enhance solubility, safeguarding therapeutic agents from gastrointestinal (GI) degradation, including acid hydrolysis and enzymatic breakdown. Additionally, these nanoparticles improve drug absorption through the GI epithelium, utilizing both transcellular and paracellular transport pathways. Moreover, CTS/ALG promotes mucoadhesion, thereby extending nanoparticle retention in the GI tract, a notable advantage. These natural polymers possess several other advantages, including cost-effectiveness, biocompatibility, biodegradability, and low toxicity. These qualities strongly advocate for the use of CTS/ALG as a nanocarrier for delivering the curcumin-metformin combination orally.

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Co-encapsulation of curcumin (Cur) and metformin (Met) into chitosan/alginate (CTS/ALG) nanoparticles (NPs) at synergistic drug ratios was achieved using response surface methodology (RSM). The optimized Cur-Met-CTS/ALG-NPs were achieved with Pluronic® F-127 2.33% (w/v), Met 5.91 mg, and CTS/ALG mass ratio 0.05:1. The prepared Cur-Met-CTS/ALG-NPs exhibited a particle size of 243 ± 13 nm, a zeta potential of -21.6 ± 0.9 mV, and encapsulations of Cur at 44.2 ± 1.4% and Met at 32.6 ± 0.4%. The loading of Cur and Met was 6.8 ± 0.5% and 19.6 ± 0.9%, respectively, with a Cur/Met mass ratio of 1:2.9.

Polymeric nanoparticles (NPs) are susceptible to instability caused by biochemical attack and swelling. Hence, extensive studies on long-term colloidal stability were conducted to assess their durability during storage. The optimized Cur-Met-CTS/ALG-NPs, stored at 4°C, displayed superior stability compared to those stored at 25°C, where aggregation occurred within two months. Furthermore, their resistance to enzymatic degradation in simulated gastrointestinal (GI) fluids was investigated. The Cur-Met-CTS/ALG-NPs exhibited enhanced stability against enzymatic degradation in these conditions (**Table 4**), highlighting their potential as a dependable carrier for oral Cur and Met delivery.

Table 4. Stability of Met-Cur-CTS/ALG-NPs under simulated GI conditions

GI condition	Size (nm)	Zeta potential (mV)
Original	243 ± 13	-21.6 ± 0.9
Mouth phase	235 ± 24	-14.8 ± 2.5***
Stomach phase	640 ± 34***	-2.8 ± 0.3***
Small intestine phase	256 ± 29	-8.0 ± 0.6***

***$p < 0.001$ compared to the particle size and zeta potential before digestion (student's t-test)

The release pattern of Cur and Met from Cur-Met-CTS/ALG-NPs under various pH conditions (simulating gastric, intestinal, and body fluids) was examined using the dialysis bag method. These NPs demonstrated an initial burst release followed by sustained release across all pH levels. Additionally, the Cur-Met-CTS/ALG-NPs exhibited increased mucoadhesion, supporting their suitability as an oral drug delivery system. Enhanced mucoadhesion prolongs NP retention in the GI tract, elevating local drug concentration and

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subsequent oral bioavailability. Finally, the cellular uptake of Cur in its free and nano forms was assessed using epithelial cells. Caco-2 cells treated with Cur-Met-CTS/ALG-NPs displayed intensified cytoplasmic green fluorescence surrounding the nuclei compared to cells treated with free Cur. This observation suggests a higher cellular internalization of Cur when utilizing NPs, indicating their potential effectiveness.

Furthermore, the efficacy of Cur-Met-CTS/ALG-NPs was evaluated in mice. The animals were treated with ED_{50} dose of a Met-Cur physical mixture (40 mg/kg; 10 mg/kg Cur + 30 mg/kg Met), an equivalent dose of Met-Cur-NPs, blank NPs, or the vehicle. One hour after treatment administration, the mice received an intraplantar injection of formalin (5%, 10 µL), and pain-like behaviors were assessed by measuring hind paw licking responses. Orally administered Cur-Met-CTS/ALG-NPs resulted in significantly greater attenuation of phase II pain-like behaviors (68 ± 3 % inhibition of hind paw licking) compared to the vehilce control group (50 ± 2 %). Similarly, the release of proinflammatory cytokines in paw tissues was significantly reduced in the Cur-Met-CTS/ALG-NP group compared to the group treated with the Met-Cur physical mixture. Additionally, a central nervous system safety pharmacology study in mice revealed no potential CNS side effects associated with Cur-Met-CTS/ALG-NPs as mice treated with the NPs showed comparable locomotive behaviors (climbing, rearing, locomotion, and immobility), distance travelled, and speed to those of the control mice.

In conclusion, these findings lay the groundwork for a nano delivery system for Cur-Met combination therapy against pain, promising improved efficacy and safety. However, future studies are required to evaluate the safety of higher doses or chronic administration to better understand their potential benefits and limitations in clinical applications (Wasana et al., 2023).

3. Chemical modification of curcumin for improved therapeutic efficacy against pain.

A prodrug is an inactive form of the parental drug that undergoes biotransformation into an active form upon administration to the body, aiming to enhance the performance of the parent drug. This approach is employed to improve various properties of the parent drug, including its physicochemical and pharmacokinetic attributes. Recently, several prodrugs exhibiting excellent anti-inflammatory enhancement and superior safety profiles compared to their parent drugs have been reported. Among these are commercially available

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medications like sulindac, parecoxib, nabumetone, and nepafenac (Phumsuay et al., 2020). Therefore, we applied this approach to curcumin, developing several curcumin conjugates. Among them, the therapeutic efficacy of its ester prodrugs: curcumin diglutaric acid (CurDG) and curcumin diethyl diglutarate (CurDDG), and the amino acid prodrug: curcumin diethyl γ-aminobutyrate (CUR-2GE) were evaluated for their effectiveness against pain, comparing them with pure curcumin.

CurDG represents a novel prodrug known for significantly increasing water solubility and promptly converting to curcumin in human plasma. Additionally, the ester prodrug CurDDG demonstrates the capability to bypass curcumin's phase II metabolism through glucuronidation and sulfation pathways. Consequently, our studies aimed to assess the analgesic potential of both prodrugs using a mouse model of chronic constriction-induced neuropathic pain. Mice received various oral doses of curcumin (25, 50, 100, and 200 mg/kg/day) or equimolar doses of CurDG and CurDDG for 14 consecutive days. CurDG and CurDDG, at all doses administered, significantly alleviated CCI-induced thermal hyperalgesia and mechanical allodynia compared to the CCI-control group. Furthermore, both CurDG and CurDDG demonstrated significantly superior efficacy in addressing both mechanical and thermal hypersensitivities compared to curcumin alone. Notably, the effect of CurDDG corresponded with the suppression of TNF-α and IL-6 levels in both the sciatic nerve and spinal cord, relative to their respective control groups. Overall, our findings illustrate the enhanced pharmacological effects of curcumin against neuropathic pain through its ester conjugates, CurDG (Limcharoen et al., 2020) and CurDDG (Limcharoen et al., 2021).

Other than the aforementioned prodrugs, CUR-2GE, a carbamate prodrug of curcumin, was developed to overcome limitations associated with curcumin. We investigated CUR-2GE and curcumin in mouse models induced by carrageenan and lipopolysaccharide (LPS). CUR-2GE outperformed curcumin, significantly improving hyperalgesia and locomotor activity in carrageenan-induced mice. It also suppressed peripheral inflammation indicated by reduced TNF-α and IL-6 levels. In LPS-induced mice, CUR-2GE demonstrated better control over sickness and pain-like behaviors compared to curcumin, significantly reducing proinflammatory cytokines in plasma and spinal cord tissue. Importantly, acute and chronic CUR-2GE administration did not affect motor coordination or locomotive behaviors, suggesting no potential

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CNS side effects. These findings highlight CUR-2GE's enhanced therapeutic efficacy against inflammatory pain (Hasriadi et al., 2022).

These comprehensive studies underscore the potential of prodrugs, particularly CurDG, CurDDG, and CUR-2GE, as promising candidates for improving curcumin's therapeutic efficacy against neuropathic and inflammatory pain conditions, offering enhanced treatment options with potentially fewer side effects.

Limitations

Despite promising outcomes, the studies on curcumin-based pain relief strategies face several limitations. Firstly, the findings are predominantly based on preclinical mouse models, which may not fully translate to human physiology. The bioavailability enhancement techniques, including drug combinations, nanoformulations, and prodrugs, though effective in controlled settings, require validation through human clinical trials to assess real-world applicability, long-term safety, and efficacy. Additionally, the chemical modifications and nanoparticle systems may pose challenges in large-scale manufacturing, stability, and regulatory approval. Moreover, pharmacokinetic interactions between curcumin and co-administered agents like metformin and piperine require deeper exploration to ensure no adverse metabolic effects. These limitations indicate the need for advanced pharmacological and clinical studies to bridge the gap from bench to bedside.

Conclusion

Curcumin has shown therapeutic efficacy against pain by targeting multiple mechanisms in the pain pathway; however, its poor pharmacokinetics and low oral bioavailability limit its clinical application. In response, our study delved into multiple pharmaceutical approaches aimed at enhancing curcumin's therapeutic potential for pain management. Our approaches included a drug combination strategy, a nanotechnology approach employing nanoparticles, and a prodrug approach. These innovative formulations, such as the curcumin-metformin and curcumin-piperine combinations, the Cur-Met-CTS/ALG-NPs nanoparticles, and the prodrugs (CurDG, CurDDG, and Cur-2GE), exhibit the potential for clinical trials. They hold promise as future analgesics with improved efficacy and safety profiles. The diverse strategies explored in this research lay the groundwork for advancing curcumin-based formulations into potential clinical applications for pain management.

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