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Comparative Anti-Inflammatory Effect of Aceclofenac-Saccharin in Mice Granuloma Model

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Abstract: Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase (COX) and suppresses prostaglandin synthesis. Its clinical utility, however, is limited by poor aqueous solubility and low bioavailability. This study evaluates the anti-inflammatory activity of a multicomponent crystal (MC) of aceclofenac with saccharin using a carrageenan-induced granuloma pouch model in mice. Male mice were divided into three groups (n = 3 per group): control, aceclofenac, and aceclofenac-saccharin multicomponent crystal, administered intraperitoneally. Inflammatory response was assessed via exudate volume and TNF-α levels. Both aceclofenac and MC significantly reduced exudate volume and TNF-α compared to the control ($p < 0.05$), with the MC group showing the greatest reduction. Although not statistically different from aceclofenac in TNF-α suppression, the MC demonstrated superior performance overall. The enhanced efficacy may be attributed to improved solubility and drug delivery. These outcomes support co-crystallization as a promising approach to optimize NSAID therapy.

Keywords: Aceclofenac-Saccharin, Anti-inflammatory; Exudate volume; Granuloma pouch; TNF-α;
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1. INTRODUCTION

Aceclofenac, a phenylacetic acid-derived non-steroidal anti-inflammatory drug (NSAID), exerts its therapeutic effects primarily through cyclooxygenase (COX) inhibition, thereby suppressing prostaglandin synthesis and reducing inflammation (Lolason et al., 2021). Several preclinical studies have demonstrated its ability to reduce both edema and pro-inflammatory cytokines, particularly TNF-α, in various in vivo models. Aceclofenac has also been shown to reduce cortical TNF-α and IL-1β expression following immune activation in neuroinflammatory conditions. However, long-term administration of aceclofenac is associated with gastrointestinal, renal, and cardiovascular adverse effects (Taskiran et al., 2023). In addition, aceclofenac is classified as a Biopharmaceutics Classification System (BCS) Class II compound due to its low aqueous solubility and high permeability, which limits its dissolution and oral bioavailability, often necessitating higher doses to achieve therapeutic levels (Koirala et al., 2021). Poor aqueous solubility may lead to prolonged gastrointestinal residence and dose escalation, thereby increasing the risk of dose-dependent side effects, especially common in NSAID therapy (Savjani et al., 2012; El-Badry et al., 2015).

To address this limitation, recent studies have explored the formation of multicomponent crystals to enhance the physicochemical properties of aceclofenac. A co-crystal of aceclofenac with saccharin, a Generally Recognized As Safe (GRAS) artificial sweetener and hydrogen bond donor, has demonstrated approximately a 2.5-fold increase in solubility and a significantly improved dissolution rate (~92% drug release within 60 minutes compared to ~35% for pure aceclofenac) (Usman et al., 2025). Despite this promising enhancement in solubility, the pharmacodynamic properties of aceclofenac-saccharin co-crystals have yet to be validated in vivo, particularly in the context of inflammation.

In this study, the anti-inflammatory efficacy of the aceclofenac-saccharin multicomponent crystal (MC) is evaluated using the carrageenan-induced granuloma pouch model in mice. This model is widely accepted for studying subacute inflammation due to its reproducibility and capacity to simulate persistent inflammatory states. Carrageenan, a sulfated polysaccharide, induces inflammation through a biphasic mechanism involving histamine and serotonin in the early phase, followed by prostaglandins and pro-inflammatory cytokines in the late phase. Among these, TNF α plays a central role in amplifying the inflammatory cascade by promoting immune cell recruitment, vascular permeability, and the acute-phase response (Fehrenbacher & McCarson., 2021). Therefore, TNF α is selected as a key biomarker in this study alongside exudate volume to comprehensively assess both molecular and physiological components of inflammation.

By integrating improvements in solubility with in vivo pharmacological testing, this study aims to provide the first preclinical evidence supporting the therapeutic potential of aceclofenac-saccharin co-crystals as an effective alternative for inflammation management.

2. METHODS

2.1 Experimental Animals

Healthy male mice (Mus musculus, 6 weeks old, body weight 20-24 g). Animals were housed in standard cages under controlled conditions (22 ± 2 °C, 55–60% humidity, 12-hour light/dark cycle) with ad libitum access to food and water. Prior to the experiment, animals were acclimatized for 5 days. The study was approved by the Animal Ethics Committee of Faculty of Pharmacy Universitas Andalas, with ethical clearance number: 105/UN16.10.D.KEPK-FF/2024, mice were randomly divided into three groups (n = 3 per group).

2.2 Granuloma Pouch Induction

The subcutaneous granuloma pouch model was adapted with slight modifications from the method described by Fehrenbacher and McCarson (2021). Dorsal fur was shaved (~3 cm diameter), and after 24 hours, 5 mL of sterile air was injected subcutaneously to form an air pouch. Simultaneously, 0.1 mL of 2% carrageenan solution was injected into the pouch to initiate inflammation. After 24 hours, residual air was aspirated using a 5 mL syringe to collapse the pouch, followed by reinjection of 0.5 mL of 2% carrageenan solution into the same site to reinforce the inflammatory response.

2.3 Treatment Groups and Drug Administration

The animals were randomly assigned to three experimental groups (n = 3 per group). Group I (negative control) received carrageenan along with an intraperitoneal injection of 0.5% sodium carboxymethylcellulose (Na-CMC) vehicle. Group II (positive control) received carrageenan, followed by aceclofenac administered intraperitoneally at a dose of 2.6 mg/20g body weight. Group III (test group) received carrageenan followed by the aceclofenac-saccharin multicomponent crystal (MC), which was administered intraperitoneally at an equivalent dose of 2.6 mg per 20 g of body weight, with an administration volume of 1 mL per 20 g of body weight. All test substances were prepared as suspensions in 0.5% Na-CMC.

2.4 Exudate Collection and TNF-α Quantification

Three hours after the final carrageenan injection, mice were euthanized using CO_2 inhalation. Exudate fluid was aspirated from the granuloma pouch using a sterile syringe and measured to assess inflammatory edema. TNF-α levels in the exudate were determined using a sandwich ELISA kit (Elabscience®). Absorbance was measured at 450 nm using a microplate reader. All samples were analyzed in triplicate, and results were reported as mean ± SD in ng/L.

2.5 Statistical analysis

Experimental values were expressed as Mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) after confirming homogeneity of variances using Levene's test. Significant differences between groups were further evaluated using Duncan's multiple range test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The anti-inflammatory activity of aceclofenac and its multicomponent crystal (MC) with saccharin was evaluated using a carrageenan-induced granuloma pouch model in mice. Two key parameters were assessed: exudate volume, an indicator of inflammatory edema, and TNF-α concentration, a marker of pro-inflammatory cytokine activity.

3.1 Exudate Volume

One-way ANOVA revealed a highly significant difference in exudate volume among treatment groups ($F = 375.50$, $p < 0.001$). Levene's test confirmed homogeneity of variances ($p = 0.548$), indicating the assumption for ANOVA was met. The negative control group exhibited the highest mean exudate volume (0.93 ± 0.01 mL), followed by the aceclofenac group (0.83 ± 0.01 mL), while the MC group demonstrated the lowest value (0.62 ± 0.02 mL). Post hoc analysis using Duncan's multiple range test showed that all groups differed significantly from each other ($p < 0.05$).

The outcome reinforced that both aceclofenac and its multicomponent crystal significantly reduced inflammation-induced fluid accumulation in the pouch. The greater reduction observed in the MC group indicates enhanced anti-inflammatory efficacy, which may be attributed to improved solubility and dispersion of aceclofenac when formulated as a multicomponent crystal. This observation aligns with previous studies on NSAIDs in air pouch models, where a reduction in exudate volume reflects diminished leukocyte infiltration and plasma extravasation (Martin et al., 1994).

3.2 TNF-α Concentration

TNF-α levels in exudate fluid also showed a significant treatment effect ($F = 10.691$, $p = 0.011$), with no variance heterogeneity detected ($p = 0.499$). The carrageenan control group had the highest TNF-α concentration (55.63 ± 10.32 ng/L), while the aceclofenac and MC groups showed markedly lower levels, at 29.70 ± 6.70 ng/L and 27.48 ± 7.00 ng/L, respectively. Although the difference between aceclofenac and MC was not statistically significant ($p > 0.05$), both were significantly lower than the control, confirming effective cytokine suppression.

TNF-α is a central pro-inflammatory cytokine involved in the recruitment of immune cells and amplification of the inflammatory response. Its reduction indicates that both treatments modulate the inflammatory cascade at the molecular level, likely through COX inhibition and suppression of NF-κB signaling, which are known mechanisms of aceclofenac (Grover et al., 2022).

3.3 Role of Multicomponent Crystal with Saccharin

Interestingly, the MC formulation exhibited greater reductions in both physical (exudate) and biochemical (TNF-α) inflammatory markers. This could be explained by the physicochemical advantages offered by the multicomponent system. Pharmaceutical co-crystals, including those with saccharin, have been shown to enhance the solubility, permeability, and bioavailability of poorly soluble drugs (Guo et al., 2021; Chun et al., 2013). Saccharin, although lacking intrinsic anti-inflammatory activity, acts as an effective co-former that improves drug release profiles and facilitates better delivery of the active pharmaceutical ingredient (API) to the site of inflammation. Similar results have been reported with other NSAID-saccharin co-crystals, such as indomethacin-saccharin, which demonstrated improved dissolution rates and systemic exposure (Connor et al., 2019).

These results support the hypothesis that the multicomponent crystal of aceclofenac with saccharin provides a synergistic advantage in reducing inflammatory responses, not merely due to the pharmacological activity of aceclofenac but also because of enhanced delivery properties conferred by the co-crystal structure

4. CONCLUSION

Findings from this study indicate that the multicomponent crystal (MC) of aceclofenac with saccharin offers enhanced anti-inflammatory activity compared to the parent drug. This was reflected by greater reductions in both exudate volume and TNF-α levels in the carrageenan-induced granuloma pouch model in mice. Although the difference in cytokine suppression between the MC and pure aceclofenac was not statistically significant, the MC consistently showed more favorable outcomes. These improvements are likely due to enhanced solubility and drug release characteristics conferred by the co-crystal structure. Overall, the data support the potential of co-crystallization as an effective approach to optimize the pharmacological performance of poorly soluble NSAIDs such as aceclofenac

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