Study on the Anti-Inflammatory and Analgesic Effects of Mongolian Medicine Maqianzi-2

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Abstract: This study aims to evaluate the anti-inflammatory and analgesic properties of Mongolian Medicine Maqianzi-2 (MM-2) through the use of a 1% carrageenan-induced rat paw edema model and the hot plate test in mice. Seventy-two Sprague-Dawley rats were randomly assigned to a blank control group, vehicle control group, positive control group, and low, medium, and high dosage MM-2 groups. Following continuous administration, paw volume was measured to assess the inhibition of swelling. Additionally, eighty-four Kunming mice were divided similarly for dosing, with pain thresholds recorded at various time points to analyze analgesic effects. Results from the anti-inflammatory assay demonstrated that the low-dose MM-2 group and the positive control significantly suppressed paw edema at 0.5 and 1 hour post-inflammation induction (*P<0.05*), while the medium-dose group also showed an inhibitory trend, the difference was not statistically significant. In the analgesic test, the low-dose MM-2 group markedly elevated pain thresholds at 1 hour post-administration (*P<0.01*), whereas no significant differences were observed at other time points or in the medium- and high-dose groups. Transdermal application of MM-2 effectively attenuates carrageenan-induced rat paw edema and enhances pain thresholds in mice, manifesting pronounced anti-inflammatory and analgesic activities.

Keywords: Mongolian Medicine Maqianzi-2; Anti-inflammatory Effect; Analgesic Effect

1. Introduction

Mongolian medicinal formulations represent a cornerstone in the prevention and treatment of diseases within traditional Mongolian medicine [1]. Rooted in Mongolian therapeutic principles, these compounds employ carefully balanced combinations of natural herbs, offering distinct advantages in managing inflammatory and painful conditions. Their topical application enables direct delivery to affected areas while minimizing systemic side effects [2], making them a pivotal focus in the modernization of Mongolian medicine research. Previous studies have shown that such formulations achieve their anti-inflammatory and analgesic effects through the synergistic action of multiple components targeting various biological pathways [3,4]. Maqianzi-2 (MM-2) is a topical preparation developed based on Mongolian medical theory [5], with our team having previously optimized its production process. Clinically, MM-2 is used to alleviate inflammation and pain, particularly for the management of PFP [6] and arthritis. However, the fundamental pharmacological mechanisms underlying its anti-inflammatory and analgesic effects have not yet been clearly defined. This study aims to evaluate these effects of MM-2 through well-designed animal experiments, thereby clarifying its pharmacodynamic characteristics, expanding the scientific data supporting its use, and providing a robust experimental foundation for its wider clinical application and future development.

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2. Materials and Experimental Methods

2.1 Reagents and Materials

2.1.1 Experimental Animals

Seventy-two SPF-grade Sprague-Dawley (SD) rats, equally divided by sex, weighing 200±20g, and 84 female SPF-grade Kunming mice, weighing18±2g, were procured from Changsheng Laboratory Animal Co., Ltd., Shenyang, China (Animal License No.: SCXK (Liao) 2020-0001). The animals were housed under standardized SPF conditions at a temperature of 22±2℃ and relative humidity of 50±5%, with ad libitum access to standard rodent feed and sterilized water. Following a seven-day acclimatization period, experiments commenced. All experimental protocols were approved by the Animal Ethics Committee of the Affiliated Hospital of Inner Mongolia Minzu University (Approval No.: 220441237), and all procedures conformed strictly to the ethical guidelines outlined in the *Guidelines for the Ethical Review of Experimental Animals*.

2.1.2 Drugs and Reagents

Fluocinolone acetonide cream (National Medicine Approval Number: H12020838, Tianjin Pacific Pharmaceutical Co., Ltd.); Safflower oil (National Medicine Approval Number: Z20093693, Liang Jiefu Pharmacy); Carrageenan (Product No.: C107615, Aladdin Company); 10% Chloral hydrate solution (Batch No.: 300375666, China National Pharmaceutical Group Chemical Reagent Co.).

2.1.3 Instruments

Plethysmometer (Model: YLS-7C, Jinan Yiyan Technology Development Co, Ltd.); Cold Hot plate analgesia meter (Model: YLS-21A, Jinan Yiyan Technology Development Co, Ltd., temperature control accuracy (55±0.5℃); Electronic analytical balance (Model: BSA124S-CW, Sartorius).

2.2 Experimental Methods

2.2.1 Preparation of MM-2

Ten grams of the powdered herbal mixture were combined with 100 mL of 45% ethanol and subjected to ultrasonic extraction (power: 200 W; frequency: 40 kHz) for 30 minutes. The extract was then filtered, and the filtrate volume was adjusted back to 100 mL with 45% ethanol. Subsequently, 10 g of PVA1788 vehicle material was weighed and mixed with the filtered extract, followed by the addition of 1 mL glycerol and 2 mL Azone. The components were magnetically stirred until a homogeneous gel-like formulation of MM-2 was obtained.

2.2.2 Anti-Inflammatory Activity Assay

Seventy-two SD rats were randomized into six groups of twelve animals each, with equal representation of both sexes per group. The dosing regimen was established based on the FDA-recommended body surface area (BSA) scaling for interspecies dose conversion: the clinical topical human dose of 1.5 g per application for a 70 kg adult translates to approximately 0.021 g/kg. With a rat-to-human conversion coefficient of 6.17, the equivalent dose for rats was calculated to be approximately 0.13 g/kg. Considering preliminary data, three dosage levels were set: low (0.05 mL/cm²), medium (0.1 mL/cm²), and high (0.2 mL/cm²), applied over approximately 2 cm², resulting in doses of about 0.3, 0.6, and 1.2 g/kg, respectively. The groups and administration protocols were as follows:

Blank control group: 0.9% saline applied topically to the abdominal area (~2 cm²), twice daily for 15 consecutive days. Vehicle control group: topical application of the drug-free base matrix solution to the same site, dose, and frequency as the blank control, for 15 days. Positive control group: topical application of fluocinolone acetonide cream at the same site and dose as the blank control, once daily for 5 days. Low-, medium-, and high-dose MM-2 groups: respective concentrations applied topically to the same area, dose, and frequency as the blank control, for 15 days.

Thirty minutes after the final administration, acute inflammation was induced by subcutaneous injection of 0.05 mL of 1% carrageenan suspension into the right hind paw plantar of each rat. Two hours post-induction, the vehicle control group and MM-2 dose groups received an additional application. Paw volume was measured using a plethysmometer at 0.5, 1, 2, 4, and 6 hours following the inflammatory challenge. Baseline paw volume prior to inflammation induction was recorded as (V0), and subsequent volumes as (Vt). The swelling rate was calculated by the formula: Swelling rate (%) = (Vt - V0) / V0 × 100%.

2.2.3 Analgesic Activity Assay

Eighty-four female Kunming mice were randomly divided into six groups (n = 14 per

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group), mirroring the grouping and dosing regimen employed in the anti-inflammatory experiment. The positive control treatment consisted of topical application of safflower oil. The groups and administration protocols were as follows: blank control group, vehicle control group, and low-, medium-, and high-dose MM-2 groups—all receiving topical application at the same dosage, site, and frequency as in the anti-inflammatory assay, administered continuously for 15 days; the positive control group received topical safflower oil applied to the abdominal region (~2 cm²), twice daily for 15 consecutive days.

One day prior to the experiment, baseline pain thresholds were determined using a hot plate analgesia system maintained at (55±0.5℃). The latency period from placement on the hot plate until the exhibition of nocifensive behaviors such as hind paw licking, lifting, or jumping was recorded. Mice exhibiting baseline pain thresholds shorter than 5 seconds or exceeding 30 seconds were excluded to ensure uniform nociceptive sensitivity among experimental subjects. After the final administration, pain thresholds were measured at 0.5, 1, 2, and 4 hours post-treatment. The cut-off time was set at 60 seconds, whereby animals not exhibiting nociceptive responses within this interval were assigned the maximal latency of 60 seconds. The recorded latencies at each time point were utilized to assess the analgesic efficacy of MM-2.

2.3 Statistical Analysis

All data were statistically analyzed using GraphPad Prism 9.5 software. Quantitative data were presented as mean ± standard deviation (mean ± SD). One-way ANOVA was applied for multi-group comparisons, and the LSD t-test was used for subsequent pairwise comparisons. A value of $P<0.05$ was considered statistically significant, and $P<0.01$ was defined as a highly significant difference.

3. Experimental Results

3.1 Effects of MM-2 on Carrageenan-Induced Paw Edema in Rats

Following induction of inflammation with 1% carrageenan, all groups exhibited pronounced swelling of the right hind paw compared to baseline, indicating successful establishment of the acute inflammatory model. The findings demonstrated that both the low-dose MM-2 group and the positive control group significantly attenuated carrageenan-induced paw edema at 0.5 and 1 hour post-inflammation induction, with differences reaching statistical significance compared to the blank control group ($P<0.05$). The medium-dose MM-2 group displayed a modest inhibitory trend at all measured time points (0.5, 1, 2, 4, and 6 hours), although these reductions did not attain statistical significance (see Figure 1).

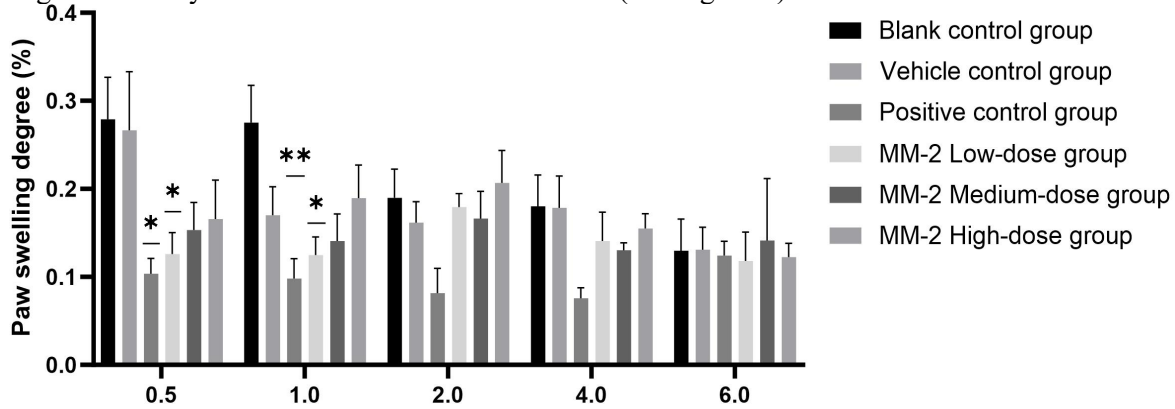

Figure 1. Effects of MM-2 on Carrageenan-Induced Paw Edema in Rats

Note: Compared with the blank control group, *$P<0.05$, **$P<0.01$.

3.2 Effects of MM-2 on Pain Threshold in Mice

Compared to the blank control group, all dosage groups of MM-2 exhibited an upward trend in pain threshold, with the low-dose group demonstrating a significant increase at 1 hour post-administration ($P<0.01$); no statistically significant differences were observed at other time points. These findings suggest that MM-2 possesses a certain analgesic effect, most pronounced at the low dose, albeit overall efficacy was inferior to that of the positive control drug, indicating its potential analgesic properties (see Figure 2).

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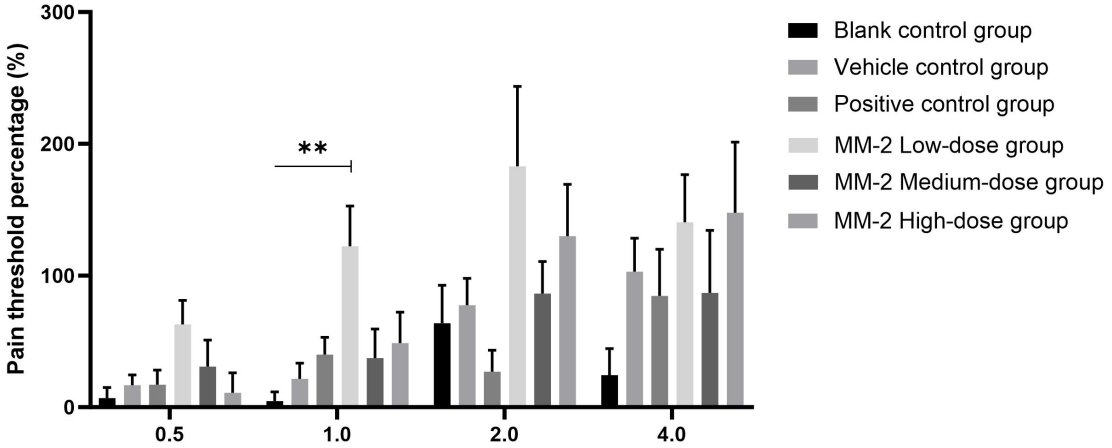

Figure 2. Effects of MM-2 on Pain Threshold in Mice

Note: Compared with the blank control group, $**P<0.01$.

4. Discussion

In Mongolian medicine, peripheral facial paralysis (PFP) is referred to as "Miansa", which, based on its clinical manifestations and syndromic characteristics, is classified under the category of "Heyisa" within the "Baimai" diseases [7-9]. Contemporary medical research indicates that the pathogenesis of PFP is closely associated with inflammatory responses, virus-induced vasospasm of the facial nerve vessels, localized ischemia, and neural edema. The carrageenan-induced rat paw edema model and the hot plate test are classical methodologies for evaluating the anti-inflammatory and analgesic efficacy of topical agents [10,11]. The former simulates the pathophysiological processes of acute inflammation such as vascular dilation and exudative edema, while the latter reflects the drug's inhibitory effect on central nociception. Notably, the inflammatory mediator release pathways involved share commonalities with the inflammatory responses consequent to facial nerve compression in PFP, thereby offering an experimental foundation for the clinical application of MM-2 [12-14]. The present study revealed that the low-dose MM-2 group significantly suppressed carrageenan-induced paw edema in rats ($P<0.05$) and markedly elevated the pain threshold in mice at one hour post-administration ($P<0.01$), indicating its definite anti-inflammatory and analgesic properties [15]. Such effects may effectively alleviate inflammatory edema in PFP patients, mitigate facial asymmetry symptoms such as oral deviation and incomplete eyelid closure, as well as relieve facial and postauricular pain and discomfort. This may indirectly facilitate neural repair, thereby underscoring its potential clinical value [16,17]. MM-2, as a time-honored Mongolian medicinal formula, has been extensively employed in PFP treatment; however, its molecular mechanisms remain elusive, and experimental data are scarce. This study's pharmacodynamic evaluation substantiates its anti-inflammatory and analgesic effects, providing an experimental basis for MM-2's clinical application in PFP and inspiring novel avenues for subsequent research.

The absence of significant pharmacological effects in the medium- and high-dose groups of MM-2 might be attributable to factors such as impaired transdermal absorption or altered local metabolism at higher drug concentrations, warranting further mechanistic investigation. Moreover, the differing administration schedules between the positive control drug and MM-2 were deliberately designed in accordance with clinical medication habits and formulation characteristics, aiming to objectively assess their respective therapeutic advantages rather than direct comparison. Future research should be centered on the isolation and identification of active constituents, validation of target pathways, and formulation optimization to enhance efficacy and elucidate molecular mechanisms, thereby furnishing more precise theoretical guidance for the rational clinical use of MM-2.

5. Conclusion

Transdermal administration of MM-2 exhibits notable anti-inflammatory and analgesic activities, providing robust pharmacological evidence and experimental support for its

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therapeutic application in PFP. This study also lays a foundational framework for future mechanistic investigations and clinical utilization.

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