Effects of Chloramphenicol Prednisone Liniment on Anti-inflammatory and Anti-pruritic Responses and Skin Barrier Function in an Acute Eczema Mouse Model

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Abstract [**Objectives**] To observe the effects of chloramphenicol prednisone liniment on anti-inflammatory and anti-pruritic responses and skin barrier function in an acute eczema mouse model and explore its potential underlying mechanism. [**Methods**] Twenty-four female SPF-grade ICR mice were randomly and equally assigned to three groups: the blank control group, the acute eczema group, and the chloramphenicol prednisone liniment group according to the random number table method, with 8 mice per group. Except for the blank control group, the acute eczema model was established by applying 2,4-dinitrochlorobenzene (DNCB) to the right dorsal area. On day 10 (d10), 0.1 mL of normal saline was administered to the modeling site in both the blank control group and the acute eczema group, whereas chloramphenicol prednisone liniment was applied to the positive drug group. Medication was applied twice daily in all three groups for a total duration of 14 d. Sixty minutes following the final administration of the drug, the development of eczema in mice was visually assessed, and the severity of skin lesions was scored. Trans-epidermal water loss (TEWL) was measured using a multifunctional skin tester. Experiments inducing and alleviating pruritus were performed to compare the frequency of mice licking their bodies, the latency period before pruritus onset, and the duration of pruritus episodes. Levels of histamine and substance P (SP) in the lesion tissues were quantified using enzyme-linked immunosorbent assay (ELISA). [**Results**] Compared to the acute eczema group, the chloramphenicol prednisone liniment group exhibited a prolonged latency period of pruritus, an increased inhibition rate, and a shortened duration of pruritus. Additionally, there was a significant reduction in the frequency of mice licking their bodies, as well as in six eczema severity indicators: redness and swelling, scratch marks, papules, blisters, exudation or erosion at the lesion site, and the degree of skin swelling. Furthermore, levels of TEWL, histamine, and SP were also significantly decreased ($P<0.05$). [**Conclusions**] Chloramphenicol prednisone liniment exhibits anti-inflammatory and anti-pruritic properties. Its mechanism of action may involve the inhibition of mast cell activation within the lesion tissues of eczema model mice, thereby reducing the release of histamine and other active substances. This process alleviates inflammatory damage associated with eczema and contributes to the restoration of skin barrier function.

Key words Chloramphenicol prednisone liniment, Acute eczema model, Anti-inflammatory and anti-pruritic, Mast cells, Histamine, Skin barrier function

1 Introduction

Acute eczema is a prevalent form of delayed-type allergic inflammatory skin disease affecting the epidermis and superficial dermis in clinical practice[1]. Acute eczema may manifest at any age. In its initial stage, it is characterized by punctate erythema, often accompanied by burning sensations and pruritus. Subsequently, scattered or confluent papules and small vesicles develop on the erythematous areas. Mechanical irritation, such as scratching or rubbing, can result in erosion and exudation. Due to its recurrent nature and the challenges associated with achieving complete remission, acute eczema significantly impairs patients' quality of life[2]. Hypersensitive inflammatory responses are currently recognized as the underlying cause[1,3]. Consequently, the primary treatment approaches involve inhibiting allergic reactions through anti-inflammatory and anti-allergic therapies. Additionally, chloramphenicol prednisone liniment has demonstrated efficacy in the treatment of acute eczema[4]. Currently, there is a paucity of research concerning the treatment of acute eczema. In this study, an acute eczema model was established by applying 2,4-dinitrochlorobenzene (DNCB) to the right dorsal area of mice. The effects of chloramphenicol prednisone liniment on anti-inflammatory and anti-pruritic responses, as well as on skin barrier function, were evaluated in this acute eczema mouse model. Additionally, the potential underlying mechanisms were investigated to provide an experimental foundation and method for clinical treatment and drug development.

2 Materials and methods

2.1 Animals and grouping ICR mice of specific pathogen-free (SPF) grade, weighing between 20.5 and 22.5 g and aged 25 to 28 d, were utilized. Each mouse was assigned an identification number using a random number table and subsequently randomized and evenly distributed into three groups: the blank control group, the acute eczema group, and the chloramphenicol prednisone liniment group, with 8 mice per group ($n=8$). The experimental procedures conformed to the principles of 3R (Replacement, Reduction, and Refinement). Environmental conditions were controlled, maintaining a relative humidity of 70% - 80% and a room temperature of 22 - 24 ℃. The mice had *ad libitum* access to drinking water and were provided with standard laboratory feed. A 12 h light/dark cycle was maintained throughout the study.

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2.2 Pharmaceuticals and instruments The pharmaceuticals and instruments utilized in this study included the following: chloramphenicol prednisone liniment (manufactured by the Preparation Room of Shiyan Taihe Hospital, batch No.: H2017CM16); dexamethasone sodium phosphate injection (manufactured by Chenxin Pharmaceutical Co., Ltd., batch No.: 2112072212); DNCB (manufactured by Hefei Tianjian Chemical Co., Ltd., batch No.: 97-00-7); 4-aminopyridine (4-AP) (manufactured by Alpha Company, batch No.: 200706); and the tissue P substance (SP) histamine ELISA kit (procured from Shanghai Genetimes Biotechnology Co., Ltd.).

2.3 Methods

2.3.1 Acute eczema modeling in mice. On day 1 (d1), with the exception of 8 mice in the blank control group, a total of 16 mice in the acute eczema group and the chloramphenicol prednisone liniment group (positive drug group) were initially sensitized with 30 μL of 5% DNCB applied to the abdominal skin. The hair on the right dorsal area of the mice was removed prior to application. On day 3, 50 μL of 0.2% DNCB was applied for secondary stimulation. This application was repeated 3 times at intervals of 3 d. On day 9 (d9), the modeling criteria were established based on the presence of symptoms including redness, swelling, scratches, papules, blisters, exudation, or erosion on the right dorsal skin[1,5]. The blank control group received only normal saline applied to the corresponding area at the same time points.

2.3.2 Treatment. On day 10 (d10), the positive drug group received applications of chloramphenicol prednisone liniment on the right dorsal area. The blank control group and the acute eczema group were treated with 1 mL of distilled water applied to the same area. Applications were administered twice daily for a total of 14 d.

2.3.3 Detection indicators and methods. The skin condition of each group was observed and compared 60 min after the final drug administration. At this time point, hair was removed from the right dorsal area of the mice in both the acute eczema group and the chloramphenicol prednisone liniment group, followed by a subcutaneous injection of 4-AP at a dose of 1 mg/kg. The blank control group received a subcutaneous injection of normal saline at the same volume. Subsequently, the duration, latency, and frequency of licking behaviors were recorded over a 10-min period. Licking behavior was defined as the repeated turning of the head to lick the skin on the right dorsal area, with continuous licking interrupted only by brief pauses counted as a single licking event. The inhibition rate of licking behavior was then calculated[6]. Inhibition rate (%) = [(Number of licking behaviors in the acute eczema group − Number of licking behaviors in the positive drug group or the chloramphenicol prednisone liniment group)/Number of licking behaviors in the acute eczema group] × 100%. TEWL was measured 60 min following the final application using a multifunctional skin tester[1]. To assess the degree of skin swelling, mice were euthanized and excised immediately after the TEWL measurement. Skin samples from both the eczema-affected area and the corresponding healthy area were collected using a 6 mm biopsy punch. The degree of skin swelling was calculated as the difference in weight between the eczema-affected skin and the healthy skin (Skin swelling degree = Weight of eczema skin − Weight of healthy skin). The six-item scoring system for eczema severity was based on six symptoms: redness and swelling, scratching, papules, blisters, exudation, and erosion. Each symptom was graded on a four-point scale for statistical analysis: none (0 point), mild (1 point), moderate (2 points), and severe (3 points). A higher total score indicated greater severity of eczema[5,7]. Approximately 0.5 g of tissue was excised and stored at −80 ℃. Following thawing of the frozen mouse tissues, a 10% tissue homogenate was prepared and subsequently centrifuged. The concentrations of histamine and substance P (SP) were determined in accordance with the instructions provided by the ELISA kit[1].

2.4 Statistical processing The experimental data were analyzed using SPSS 26.0 and were presented as $(\bar{x} \pm s)$. A multifactor analysis of variance was initially conducted to assess mean differences among multiple groups. For pairwise comparisons between groups exhibiting normal distribution, the *t*-test was employed, whereas the corrected *LSD-t* test was applied for pairwise comparisons between groups that did not meet the normal distribution. A *P*-value of less than 0.05 was considered indicative of statistical significance.

3 Results and analysis

3.1 Experiments results of inducing and alleviating pruritus Pronounced licking responses were observed in the acute eczema group. Statistically significant differences were identified in pruritus latency, pruritus duration, frequency of licking behavior, and inhibition rate in mice when compared to the blank control group ($P < 0.05$). Relative to the acute eczema group, the chloramphenicol prednisone liniment group exhibited prolonged latency, reduced duration, decreased frequency of licking behaviors, and an increased inhibition rate ($P < 0.05$), with all differences reaching statistical significance (Table 1).

Table 1 Pruritus latency, pruritus duration, frequency of licking behavior, and inhibition rate of mice across experimental groups ($n = 8$, $\bar{x} \pm s$)

Group	Latency//sec	Duration//sec	Frequency of licking behavior//times	Inhibition rate//%
Blank control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100.00
Acute eczema	44.61 ± 4.27[a]	140.95 ± 5.61[a]	47.17 ± 2.92[a]	0.00[a]
Chloramphenicol prednisone liniment	65.29 ± 4.31[b]	113.17 ± 4.40[b]	21.27 ± 1.93[b]	54.90[b]

NOTE Compared to the blank control group, [a]$P < 0.05$; compared to the acute eczema group, [b]$P < 0.05$; the same below.

3.2 Assessment of eczema severity The scores for six indicators of eczema severity, namely redness and swelling, scratch marks, papules, blisters, exudation, and erosion, in the acute eczema group were significantly higher than those in the blank control group ($P < 0.05$). Furthermore, compared to the acute eczema group, the scores for these six indicators in the chloramphenicol prednisone liniment group were significantly lower ($P < 0.05$), indicating a statistically significant difference (Table 2).

Table 2 Scores of six indicators of eczema severity ($n = 8$, $\bar{x} \pm s$)

Group	Redness and swelling//point	Scratch marks//point	Papules//point	Blisters// point	Exudation//point	Erosion//point
Blank control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Acute eczema	2.61 ± 0.04[a]	1.87 ± 0.31[a]	2.10 ± 0.27[a]	1.06 ± 0.18[a]	1.47 ± 0.22[a]	1.20 ± 0.09[a]
Chloramphenicol prednisone liniment	0.74 ± 0.05[b]	0.78 ± 0.05[b]	0.87 ± 0.04[bc]	0.70 ± 0.03[b]	0.62 ± 0.07[bc]	0.63 ± 0.08[b]

3.3 Test of inducing and alleviating pruritus and detection of histamine and SP levels Compared to the blank control group, the acute eczema group exhibited significant increases in TEWL, skin swelling degree, histamine, and SP levels ($P < 0.05$). Conversely, treatment with chloramphenicol prednisone liniment resulted in significant reductions in TEWL, skin swelling degree, histamine, and SP levels ($P < 0.05$) (Table 3).

Table 3 TEWL, skin swelling degree, histamine, and SP levels across experimental groups ($n = 8$, $\bar{x} \pm s$)

Group	TEWL//sec	Skin swelling degree//mg	Histamine//mg	SP//ng/L
Blank control	2.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	102.81 ± 9.04
Acute eczema	9.24 ± 1.02[a]	40.91 ± 2.69[a]	30.54 ± 0.91[a]	219.35 ± 10.62[a]
Chloramphenicol prednisone liniment	3.56 ± 0.44[b]	20.72 ± 1.79[b]	19.57 ± 0.55[b]	141.68 ± 9.47[b]

4 Discussion

Acute eczema, a common inflammatory skin disease, typically manifests with characteristic symptoms during childhood and is marked by recurrent episodes and challenges in achieving complete remission[8]. Chloramphenicol, a component of chloramphenicol prednisone liniment, is a broad-spectrum antibiotic. This study demonstrates that chloramphenicol exhibits bactericidal and anti-inflammatory properties, which can reduce or prevent the exacerbation of inflammatory responses following skin infections. When combined with the glucocorticoid prednisone for topical application, the formulation produces a synergistic effect, enhancing anti-inflammatory, bactericidal, and anti-pruritic activities. In the pruritus and anti-pruritus experiments, chloramphenicol prednisone liniment significantly reduced the duration of the licking response induced by subcutaneous injection of 4-AP, prolonged the latency period, decreased the frequency of licking behavior, and markedly increased the inhibition rate of pruritus. These findings suggest that chloramphenicol prednisone liniment possesses notable anti-inflammatory and anti-pruritic properties. Analysis indicates that 4-AP, functioning as a potassium channel blocker, activates calcium channels through the inhibition of potassium channels. This activation promotes the contraction of vascular smooth muscle and subsequently facilitates mast cell degranulation, leading to the release of histamine. The resulting allergic reaction manifests as skin pruritus, which is evidenced by the licking behavior observed in mice[9]. Glucocorticoids present in chloramphenicol prednisone liniment play a crucial role in stabilizing lysosome and inhibiting mast cell degranulation, thereby reducing the release of histamine and other inflammatory mediators[10]. SP is involved in regulating mast cell degranulation and promoting mast cell activation, thus contributing to the inflammatory response observed in eczema[11]. Mast cells, as key effectors in classic allergic reactions, can be activated by various antigens, leading to the release of bioactive substances through degranulation that trigger allergic responses. Furthermore, mast cell degranulation and the subsequent release of inflammatory mediators contribute to type I allergic immune damage, primarily manifesting on the surfaces of the skin or mucous membranes, and represent a direct causative factor in the development of skin lesions[12]. Histamine and SP levels were significantly reduced in the chloramphenicol prednisone liniment group, indicating that this treatment can inhibit mast cell activation, prevent mast cell degranulation, and suppress the release of inflammatory mediators, thereby mitigating inflammatory skin damage in acute eczema. TEWL serves as a critical parameter for assessing the integrity of the skin barrier function. Elevated TEWL values correspond to increased water loss from the stratum corneum, indicating a more pronounced impairment of the skin barrier[1,13]. The application of chloramphenicol prednisone liniment has been shown to significantly reduce TEWL and skin edema, suggesting its efficacy in minimizing water loss from the stratum corneum and facilitating the repair of the compromised skin barrier.

In conclusion, chloramphenicol prednisone liniment may contribute to the alleviation of inflammatory damage symptoms associated with eczema and the restoration of skin barrier function. This effect is potentially mediated through the inhibition of SP expression in lesion tissues and the suppression of mast cell activation and degranulation, thereby reducing the release of histamine and other active substances in the lesion tissues of eczema model mice.

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(To page 79)

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(From page 68)

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(From page 74)

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