Anti-arthritic effects of microneedling with bee venom gel

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Abstract  Objective: To combine with transdermal drug delivery using microneedle to simulate the bee venom therapy to evaluate the permeation of bee venom gel.

Methods: In this study, the sodium urate and LPS were used on rats and mice to construct the model. Bee venom gel—microneedle combination effect on the model is to determine the role of microneedle gel permeation by observing inflammation factors.

Results: Compared with the model group, the bee venom gel—microneedle combination group can reduce the level of serum nitric oxide of the acute gouty inflammation model caused by sodium urate, and on LPS induced mouse model of acute inflammation effect and the micro.

Conclusions: Bee venom can significantly suppress the occurrence of gouty arthritis inflammation in rats and mice LPS inflammatory reaction. Choose the 750 μm microneedle with 10N force on skin about 3 minutes, bee venom can play the optimal role, and the anti-inflammatory effect is obvious. Microneedles can promote the percutaneous absorption of the active macromolecules bee venom gel.

Introduction

Arthritis is an inflammatory disease that affects the joints and surrounding tissues. The chronic pain associated with arthritis affects the quality of life of sufferers Pharmaceutical agents, such as nonsteroidal anti-inflammatory drugs, may be used to relieve inflammation and other symptoms, but their long-term use can lead to side-effects, such as diabetes and hypertension.1

Bee venom (BV) has been used in traditional Chinese medicine (TCM) to relieve pain and treat inflammatory
diseases such as rheumatoid arthritis. BV has anti-inflammatory, analgesic, and immune system-enhancing effects. Bee venom is a fragrant light-yellow liquid secreted by stimulated worker bees. It is comprised of melittin, phospholipase A2, histamine, hyaluronidase, catecholamine, and serotonin. Melittin is the main medicinal ingredient, and accounts for 50% of the dry weight of BV. Melittin has strong anti-inflammatory and analgesic effects, and can be combined into natural or artificial membranes. Melittin has hormone-like effects, but no hormone-like side effects. It can activate release of adenocorticotrophic hormone, reduce capillary permeability, and inhibit the synthesis of prostaglandin E2 and neutrophils. It has been reported that melittin also can disrupt the nuclear factor-kappa B (NF-κB) pathway, inhibit the c-Jun N-terminal kinase pathway and hamper the activity of NF-κB and signal transducer and activator of transcription. The analgesic intensity of melittin is 40% that of morphine and its analgesic duration is longer. BV does not affect the digestive tract like salicylic acid-based drugs and it does not inhibit the immune system like corticosteroids.

In Chinese medicine, bee sting therapy is applied to acupuncture to treat arthritis. This form of acupuncture promotes the flow of qi and blood, dredges the channels, and expels pathogens, while simultaneously enabling BV to elicit its anti-inflammatory and analgesic effects. However, live bee stings can result in pain, itching, or an allergic reaction.

Transdermal drug delivery (TDD) offers several advantages over systemic administration, such as oral and intra-vonous. While the early TDD approach, the transdermal patch, is advantageous, only small-molecule drugs can be absorbed because of the barrier action of the skin’s stratum corneum. The recent development of microneedles is an attempt to circumvent the stratum corneum barrier. Microneedles can be used to enhance TDD. Gel is a solid jelly-like material, it made by extract and matrix. Gels have good biocompatibility, can extend the time for which the effect occurs, are associated with good patient compliance, and are suitable for polypeptide drugs. The microneedle can breakdown the skin and make the therapeutic dose gel into the skin.

In the present study, a bee venom in gel form was prepared. Melittin is not stable in water and the main factor affecting its stability is oxidation. We used the stability of melittin as an index to screen additives for gel preparation. We created two models in experimental animal models: acute gouty inflammation induced by sodium uratein rats and acute inflammation induced by lipopolysaccharide (LPS) in mice. We then tested microneedling with bee venom gel (BVG) in these two models to verify the anti-inflammatory effect.

Materials and methods

Animals

Animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals published by the Beijing University of Chinese Medicine (Beijing, China).

Male Kunming mice (18–22 g) and Sprague–Dawley rats (SCXX 2006-0009) were purchased from Vital River Laboratories (Beijing, China) and randomly allocated into 8 groups of 8 animals each. Eight mice were housed in a cage at ambient temperature and pressure.

Screening of bee venom gel materials and selection of antioxidants

First, an appropriate gel matrix was chosen from CMC-Na, Methylcellulose, carbomer 934, Sodium alga alca, Hydroxypropyl methylcellulose-E15. Then, 0.2% vitamin C, 0.2% citric acid, 0.2% sodium sulfate, 0.2% thioiuera, 0.2% sodium thiourea, 0.2% glucose, 0.2% mannitol, 0.2% gelatin and 0.1% stabilizer S. After 72 hours, the content and retention rate of melittin was determined to select the suitable antioxidant. The concentration of the antioxidant was determined by comparing the viscosity and calculating the retention rate of melittin in the 60°C heating test.

Bee venom gel preparation

Bee venom (Hangzhou Tianchumiyuan Health Products, Hangzhou, China) with a total protein content of approximately 80% was weighed and mixed with deoxygenated water (Hangzhou Wahaha Group, Hangzhou, China) to prepare 100 μg/mL BV solution. To this was added the antioxidant. The mixture was then dissolved in 10% propylene glycol followed by addition of 0.01% butylparaben. The resultant mixture was added to the matrix to form BVG.

Accelerated stability test

The appearance of the BVG was uniform and transparent, with a pH of 7.53. There was no discoloration, phase separation, or peculiar smell in 6 months. Centrifugation at 2500 rpm for 30 minutes at 25°C did not result in stratification. The BVG was placed in a constant climate chamber (LHS-100CL; Shanghai Scientific Instruments & Materials, Shanghai, China) for 6 months. Melittin content at 0, 1, 2, 3, and 6 months was measured. The appearance, shape and concentration of melittin were observed.

In vitro study of melittin release from BVG

Release of melittin from BVG with and without stabilizer was studied. Using a Franz-type diffusion cell, a microporous membrane (0.8 μm) was placed between the diffusion and receiving chambers. Volume of the receiving chamber was 18 mL with a diffusion area of 3.14 cm². Deaerated water was added to the receiving chamber until the liquid surface was fully in contact with the microporous membrane. The two types of gels were placed on the microporous membrane, followed by magnetic stirring at 300 rpm for 72 hours at 32°C. To draw receptor fluid (1.0 mL) at 0.5, 1, 2, 4, 6 12, 24, 36, 48, 60, and 72 hours simultaneously, the same volume of water was added the chamber. Melittin content was determined by high-performance liquid chromatography.
Different conditions of microneedles in rat skin in vitro

Five groups were created: A, no microneedle; B, 250-μm microneedle with 10-N force on the skin for 3 minutes; C, 750-μm microneedle with 10-N force on the skin for 30 seconds; D, 750-μm microneedle with 5-N force on the skin for 3 minutes; E, 750-μm microneedle with a 10-N force on the skin for 3 minutes.

The animals were applied 1000N DTR microneedles. Rat skin was fixed in 10% formalin and dehydrated in a graded series of alcohol. Skin was embedded in paraffin and stained with hematoxylin and eosin, followed by observation under light microscopy.

Effect of microneedling plus BVG on sodium urate-induced acute gouty arthritis

Modeling and animal grouping
Male SD rats were anesthetized with 10% chloral hydrate. After administration 0.5 hour, 5% sodium urate (0.2 mL) was injected into the joint cavity of the right hind ankle. Rats were divided into eight groups of 8 animals each (Table 1).

Microneedling of rats
Rats were anesthetized with 10% chloral hydrate. Fur on the back of each rat was shaved 12 hours prior to microneedling. Apart from the normal, model, and bee venom I groups, microneedling was performed on the back of each animal using various parameters based on the group the animal belonged to (Table 1). At 24 and 30 hours, the back of each animal was dressed.

Effect of microneedling plus BVG on sodium urate-induced level of nitric oxide in serum

Nitric oxide (NO) is a proinflammatory factor in arthritis that is commonly elevated in serum. Forty-eight hours after modeling, the blood sample was drawn from abdominal aorta. Samples were centrifuged 5000 rpm for 20 minutes at 25°C to prepare serum, which was stored at −20°C. The content of NO was determinate by enzyme mark instrument.

Results

Screening of BVG materials and selection of antioxidants

The appropriate gel matrix was chosen, the matrix is CMC-Na, the appearance gel which used the CMC-Na is colorless, tasteless and translucent. CMC-Na was used as the gel matrix. Therefore, ensuing experiments were conducted for CMC-Na gel. We used thiourea, sodium thiosulfate, glucose, and stabilizer S as antioxidants (Tables 3 and 4). The gel was stable after 72 hours. Apart from thiourea and stabilizer S, gels with the other additives were not stable at 7 days. In the glucose gel, glucose precipitated, and melittin degraded completely. Mannitol, gelatin, and glucose could combine

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Animal groups and test conditions.</th>
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<tbody>
<tr>
<td>Groups</td>
<td>Treatment (mg/kg)</td>
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<tr>
<td>Normal</td>
<td>Normal saline (1.5)</td>
</tr>
<tr>
<td>Model</td>
<td>Normal saline (1.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>Dexamethasone (1.8)</td>
</tr>
<tr>
<td>Bee venom</td>
<td>Bee venom (1.5)</td>
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<tr>
<td>Bee venom I</td>
<td>Bee venom (1.5)</td>
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<tr>
<td>Bee venom II</td>
<td>Bee venom (1.5)</td>
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<td>Bee venom group III</td>
<td>Bee venom (1.5)</td>
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<td>Bee venom group IV</td>
<td>Bee venom (1.5)</td>
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with melittin, form stable hydrogen bonds and a native conformation, which could enhance the stability of the gel, but this stability was temporary. Rupture of the structure resulted in gradual precipitation of the additive. In the gel with thiourea and stabilizer S, melittin was stable and gel color did not change. The gel with sodium thiosulfate turned yellow after 5 days, and was not selected.

The 60°C heating test showed that melittin in the thiourea gel was not stable, and that thiourea caused severe irritation people use the thiourea for a long time, it can be absorbed through the skin and cause influence heart function, palsy the CNS. Compared with 0.05% stabilizer S, 0.1% stabilizer S was more stable. Possibly, the 0.05% stabilizer S could not reach the critical micelle concentration, which affected the stability and thus detection of melittin. For this reason, the 0.1% stabilizer S was chosen as the additive for the BVG.

### Accelerated stability study

Melittin in the gel was stable during the period of storage (6 months) (Fig. 1). The gel was a colorless semi-solid translucent substance. There was no discoloration, phase separation, or peculiar smell.

### In vitro release

The cumulative release rate of melittin was determined using the formula shown below:

![Figure 1](image_url)  
**Figure 1** Accelerated stability test of melittin in bee venom gel over 6 months.
Melittin in a gel without stabilizer S showed rapid release before 6 hours, after which release was more gradual (Fig. 2). After 48 hours, degradation rate of melittin was faster than the release rate, and its concentration decreased gradually. Melittin in the normal gel did not release in the first 2 hours and showed a slow release rate at 2–6 hours. At 6 hours, melittin release was rapid. After 24 hours, degradation rate of melittin was faster than the release rate, and its concentration decreased gradually.

Effects of varying microneedle parameters on rat skin

Varying parameters applied during microneedling resulted in different depths and sizes of channels in rat skin (Fig. 3). Compared with group A, needling in group B failed to break down the cuticle of the skin, forming a superficial channel. In groups C and D, needling lead to break down of the cuticle and the superficial channel. Needling in group E resulted in cuticle breakdown and a deep channel.

Figure 2 Cumulative release rate of melittin from stabilizer S.

\[
Q = \left( \frac{VC_n + \sum_{i=1}^{n-1} V_i C_i}{W} \right) \times 100
\]

V: Volume of receptor fluid;
\(V_i\): Volume of receptor fluid drawn;
W: Melittin content in the gel;
\(C_n\): Concentration of melittin in receptor fluid at N hours.

Figure 3 Skin channels produced by varying microneedle parameters. Note: A, no microneedling; B, 250-\(\mu\)m microneedle with 10-N force on the skin for 3 minutes; C, 750-\(\mu\)m microneedle with 10-N force on the skin for 30 seconds; D, 750-\(\mu\)m microneedle with 5-N force on the skin for 3 minutes; E, 750-\(\mu\)m microneedle with a 10-N force on the skin for 3 minutes.
Effect of microneedling plus BVG on sodium urate-induced serum levels of NO in rats

Compared with the normal and model groups, serum NO levels in the BVG group rats were markedly reduced \((P < .01)\) (Fig. 4), especially in animals that underwent 750-\(\mu\)m microneedling with 10-N force.

Effect of microneedling with BVG on LPS-induced acute inflammation in mice

Compared with the normal saline LPS group and normal saline, serum NO level was obviously reduced in the BVG mice \((** P < .01)\) (Fig. 5). Serum NO levels did not differ substantially in the normal saline LPS group, the group without a microneedling, and the group that underwent 750-\(\mu\)m microneedling with 5-N force for 3 minutes. However, serum NO levels in other groups that underwent microneedling with BVG using varying parameters were reduced, in particular animals in the 750-\(\mu\)m microneedling with 10 N force for 3 minutes.

Discussion

We used the stability of melittin as an indicator to screen a BVG formulation in rats and mice. We prepared a stable BVG and combined it with microneedling technology to study the penetration ability of BVG in the murine models of arthritis.

In the previous study, Melittin is not stability in water, at preparation course, the instability would create waste the melittin and affect the efficacy. By examining the stability of melittin in gels with different additives, we found that melittin in a gel with 0.1% stabilizer was stable for 6 months.

Our results indicate the bee venom and its melittin content could reduce NO levels in MUC- and LPS-induced inflammation. Different penetration enhancements were seen under different microneedling parameters. Based on the skin experiments, melittin could not penetrate the skin without the assistance of the microneedle. Shorter microneedles could not puncture the stratum corneum, thus efficacy was not obvious. Moreover, the smaller force and the shorter application time, the lesser the amount of melittin penetration, such that the therapeutic dose could not be reached. Our results indicated that a 750-\(\mu\)m microneedle with a 10-N force applied for 3 minutes had the greatest anti-inflammatory effect.

Conclusion

Microneedles can promote percutaneous absorption of active macromolecules in bee venom gel. The present study provides an experimental basis for microneedle administration.

Author contributions

MDZ, KXS, and JB conducted the experiments. SYD and YL designed the study and provided the reagents and materials. MDZ undertook all the percutaneous experiment and drafted the manuscript. PYL, LY, BYD, and NT performed the statistical analyses, drafted and critically revised the manuscript. All authors read and approved the manuscript.

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References