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Journal of Traditional Chinese Medical Sciences (2016) xx, 1–7

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: http://www.elsevier.com/locate/jtcms

Anti-arthritic effects of microneedling with bee venom gel

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Received 14 June 2016; accepted 30 September 2016
Available online ■ ■ ■

KEYWORDS
Arthritis;
Bee venom;
Gel;
Melittin;
Microneedle

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 NO 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 min, bee venom can play the biggest 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

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Peer review under responsibility of Beijing University of Chinese Medicine.
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http://dx.doi.org/10.1016/j.jtcms.2016.09.005

Please cite this article in press as: Zhao M, et al., Anti-arthritic effects of microneedling with bee venom gel, Journal of Traditional Chinese Medical Sciences (2016), http://dx.doi.org/10.1016/j.jtcms.2016.09.005

diseases such as rheumatoid arthritis[2] BV has anti-inflammatory, analgesic, and immune system-enhancing effects.[3]

Bee venom is a fragrant light-yellow liquid secreted by stimulated worker bees.[4] It is comprised of melittin, phospholipase A2, histamine, hyaluronidase, catecholamine, and serotonin.[5] Melittin is the main medicinal ingredient, and accounts for 50% of the dry weight of BV.[6] 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.[7] It can activate release of adrenocorticotropic 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-3.[8–10] 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.[11]

In Chinese medicine, bee sting therapy is applied to acupoints 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.[12–13] However, live bee stings can result in pain, itching, or an allergic reaction.[14]

Transdermal drug delivery (TDD) offers several advantages over systemic administration, such as oral and intravenous.[15] 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.[16] Microneedles can be used to enhance TDD.[17] 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.[18] The mirconeedle can breakdown the skin and make the therapeutic dose gel into the skin.[19]

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. 8 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 acid, Hydroxypropyl methylcellulose-E15. Then, 0.2% vitamin C, 0.2% citric acid, 0.2% sodium sulfite, 0.2% thiourea, 0.2% sodium thiosulfate, 0.2% glucose, 0.2% mannitol, 0.2% gelatin and 0.1% stabilizer S. After 72 h, 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 min 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 cm^2. 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 h at 32°C.To draw receptor fluid (1.0 mL) at 0.5, 1, 2, 4, 6 12, 24, 36, 48, 60, and 72 h simultaneously, the same volume of water was added the chamber. Melittin content was determined by high-performance liquid chromatography.

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