Exploring Antipruritic and Analgesic Effects of Matrine by Allergic Contact Dermatitis Mouse Model through Behavioral Analysis

ABSTRACT

Aim This study investigated the antipruritic and analgesic effects of matrine on allergic contact dermatitis (ACD) through behavioral analyses and cheek fold thickness. To provide theoretical evidences for further development and application of antipruritic and analgesic traditional Chinese medicines (TCM), the contact sensitizer squaric acid dibutylester (SADBE) was used to elicit contact hypersensitivity in the mice as a model of ACD in humans.

Methods C57BL/6 mice were divided into control group, model group, matrine groups (including 80 mg/kg, 40 mg/kg and 20 mg/kg dose groups), and dexamethasone acetate group (positive control). The sensitizer was first applied on the mice abdomen for sensitization and then on the cheek for challenge to elicit ACD. We confirmed the antipruritic and analgesic effects of matrine by comparing the spontaneous scratching and wiping directed to the cheek as well as the changes of cheekfold thickness in the different groups of mice.

Results The scratching of model group was increased significantly when it compared with the control group (P < 0.05). Compared to the model group, the scratching of each matrine group decreased significantly (P < 0.05); The wiping of model group was increased significantly when it compared to control group (P < 0.01). Compared to the model group, the wiping of each matrine group decreased significantly (P < 0.01); The changes of skin fold thickness of model group increased significantly when it compared to control group (P < 0.01). Compared to model group, the changes of skin fold thickness of each matrine group decreased significantly (P < 0.01).

Conclusion We have successfully established an ACD model that provides a behavioral differentiation by itch-like scratching and pain-like wiping in mice. Results proved that matrine can obviously improve the itch and pain sensations of ACD mice, and the effects of matrine has a dose-dependent manner obviously.

KEYWORDS allergic contact dermatitis, antipruritic effect, analgesic effect, behavioral analysis, matrine

INTRODUCTION

Many skin diseases (atopic dermatitis, ACD, psoriasis and xeroderma), liver diseases (cholestatics), renal diseases (uremia) and metabolic diseases (diabetes mellitus) occurred with severe pruritic symptoms. This severe itch was recurrent and hard to cure which damaged patients’ health and had negative influences on life quality. According to statistics, ACD is the most common form of occupationally acquired skin disease and patients with severe itch could not work normally which brought negative effects on social economy.

ACD is a highly prevalent skin inflammatory disease, which is also known as contact hypersensitivity mediated by hapten-specific T-cells and caused by repeated exposure of skin to the hapten such as chemical fiber, cosmetics, compounds, etc. People with ACD usually had severe pruritic and pain symptoms. Due to the complexity of antigen, the peripheral nervous mechanism of ACD was still not clarified thus it was a challenging topic. Moreover, current compounds for ACD have lots of side effects and lack of efficiency.

TCMs like Sophora flavescens radix have specific therapeutic effects on pruritic and allergic symptoms with less side effects which provided a new direction to develop ACD compounds. Matrine and oxymatrine were the effective compounds, etc. People with ACD usually had severe pruritic and pain symptoms. Due to the complexity of antigen, the peripheral nervous mechanism of ACD was still not clarified thus it was a challenging topic. Moreover, current compounds for ACD have lots of side effects and lack of efficiency.

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components of *Sophora flavescentis* radix which showed anti-pruritic and analgesic effects. However, after *Sophora flavescentis* radix was used as medicine or combined with other compounds, most oxymatrine would transform into matrine. Shrabanit found that *Sophora flavescentis* radix extract (mainly matrine) could inhibit H1 receptors and the mRNA expression of decarboxylase which showed anti-allergic effect. The emulsion of *Sophora flavescentis* radix could be applied locally to increase the itch threshold of animals and decrease the swelling degree in the inflammatory skin. Yamaguchi found that the methanol extracts of *Sophora flavescentis* radix could markedly inhibit the pruritic symptom of mice caused by 5-hydroxytryptamine, and it showed dose dependency. Furthermore, matrine has analgesic effect on vincristine-induced neuropathic pain in animal model and many different kinds of neuropathic pains. Therefore, this study chose matrine to explore its antipruritic and analgesic effects by ACD in mice.

This study is to investigate a compound from potential traditional TCMs with less side effects and efficiency for ACD. The contact sensitizer SADBE was used to elicit contact hypersensitivity in the mice as a model of ACD in humans for exploring the antipruritic and analgesic effect of matrine by behavioral analysis and the measurement of cheek skin fold thickness.

**MATERIALS AND METHODS**

**Animals**

C57BL/6SPF mice, 72 males (Animal Experimental Center of Guangdong Province No. 44007200017744) each weighing between 20 and 25 g. All the animals were housed under a 12 h light/dark cycle. Also, the welfare and experimental procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals and related ethical regulations of Jinan University according to the internationally accepted standards. Standard diet and water were always available and consumed *ad libitum*. Mice were divided into six groups for control group, model group, matrine groups (including 80 mg/kg, 40 mg/kg and 20 mg/kg dose), and dexamethasone acetate (positive control, 0.08 mg/kg) group, respectively.

**Materials**

SADBE (Sigma-Aldrich Co., Ltd, Lot: MKBQ1172); Matrine (XI’ an Linhe Biotechnology Co., Ltd, Lot: 20141222); dexamethasone acetate tablets (Tianjin Pharmaceutical Group Xinzheng Co., Ltd, Lot: 140526); normal saline; Acetone; 10% chloral hydrate; 4% paraformaldehyde solution; 10% formaldehyde solution; PBS solution.

**Compound administration and behavioral testing**

On day 0, under anesthesia, the abdomen fur of mice was shaved for preparing sensitizing area. From day 1 to day 3 after shaving, 25 μL of 1% SADBE/acetone (model group, matrine groups and positive control group) or 25 μL acetone (control group) was applied on the sensitizing area once a day. From day 4 to day 7, continue habitation to test chambers was carried out for an hour two times a day, and the cheek fur of mice was shaved on day 6 under anesthesia. After the last habitation on day 7, the skin fold thickness was measured three times on right cheek. From day 8 to day 10, 25 μL of 1% SADBE/acetone (model group, matrine groups and positive control group) or 25 μL acetone (control group) was applied on the right cheek once a day. On day 9 and day 10, before sensitization by SADBE, different dose (80 mg/kg, 40 mg/kg and 20 mg/kg) of matrine, dexamethasone acetate (0.08 mg/kg) and normal saline (0.1 mL/10 g) were given into matrine group, positive control group and model group, respectively, and intragastrically. On day 11, after giving compounds, the mice were transferred into test chambers and the spontaneous behavior of mice was recorded for the next 1.5 hour. After that, the skin fold thickness of mice was measured three times on the right cheek (Fig. 1).

**Behavioral test and data analysis**

During the video recording, the mice were placed in the independent and transparent plastic chamber (9×9×13 cm). There were small pores on the upside edge used to exchange air from the outside. The camera was placed on the right above the chambers. Two mice were videotaped at the same time. On each side of chamber, there was a piece of mirror in order to access 360-degree views of mice’s behavior. The number of bouts of spontaneous scratching with the hindlimb and wiping with the forelimb were counted for 1.5 h. The total behavior was calculated by recording the times of wiping and scratching per minute. When the mice wipe their cheek, usually they quickly move their one forelimb from the backside of cheek to the nose. Compared to scratching, wiping is more quickly and gently with less than 0.5 s of each movement. Mice usually use the medial side of forelimb to wipe cheek with the front claw fisted, and the claw will not touch the cheek. When mice scratch their cheek by hindlimb, one scratching will be counted from mice up lifting hindlimb to put down on the ground or mouth, and multiple scratching action usually occur in one scratching. After...
scratching on cheek, there are often some skin or hair residue on the hind claw. So, mice usually put the hind claw into mouth for cleaning which doesn’t happen in wiping.

As the actions of scratching and wiping are very rapid and not easy to distinguish, we analyze the video by observing four times slow down record repeatedly on Blu-ray disc drive and high-definition television.

**Skin-fold thickness**

The skin-fold thickness in each group was measured three times with a caliper by two laboratory technicians before the first challenge of SADBE on day 7 and after the video recording on day 10.

**Statistical analysis**

All final values were presented as mean ± SEM with the help of statistical analysis which was performed with the independent samples t-test by SPSS 18.0 software. Differences should be considered significant when \( P < 0.05 \).

**RESULTS**

**The effects of matrine on scratching**

As shown in Fig. 2, the scratching bouts of model group were increased significantly when it compared with the control group \( (P < 0.05) \). Compared to the model group, the scratching bouts of each matrine group decreased significantly \( (P < 0.05) \). Results showed that matrine groups can decrease the scratching bouts in SADBE elicited ACD mice with a dose-dependent manner.

**The effects of matrine on wiping**

As shown in Fig. 3, the wiping bouts of model group were increased when it compared with the control group significantly \( (P < 0.01) \). Compared to the model group, the wiping bouts of each matrine group decreased significantly \( (P < 0.01) \). Results showed that matrine groups can decrease the wiping bouts in SADBE elicited ACD mice with a dose-dependent manner.

**The effects of matrine on the changes of cheek skin-fold thickness**

As shown in Fig. 4, the changes of cheek skin-fold thickness of model group increased when it is compared with the control group significantly \( (P < 0.01) \). Compared to model group, the changes of the cheek skin-fold thickness of each matrine group decreased significantly \( (P < 0.01) \). Results showed that matrine groups can decrease the changes of the skin-fold thickness in SADBE elicited ACD mice with a dose-dependent manner.

**DISCUSSION**

SADBE is a common compound to treat pelade, but it will cause ACD with severe itch after repeated usage\(^{11}\). At present, SADBE is one of the antigens to induce ACD and prepare contact hypersensitivity model\(^ {12,13} \). Contact hypersensitivity mainly includes two stages, sensitization...
and challenge. Sensitization usually means the time from the skin firstly exposed to sensitigen to the activation of T cells. Sensitization usually begins with applying sensitizer (antigen) on the abdomen of mice. When the skin firstly contacts the antigen, the antigen will combine with the endogenous protein of skin and form immune complexes. And then the immune complexes will migrate from epidermis to lymphoglandula and send messages to native T lymphocytes. Next, the activated T cells are proliferated and left the lymphoglandula for circulatory system; Challenge stage means the skin is exposed to the same antigen again which usually begins with applying the same sensitizer on ears, cheek or other places. When the skin contacts the same antigen, the antigen will activate the effector cells in dermis and induce inflammation, finally resulting in skin damage, itch and pain. The challenge stage of human usually lasts for 72 hours and for mice it lasts for 24 to 28 hours.

At present, there are some reports about studying the mechanisms of ACD by contact hypersensitivity animal model. But most of them prepared contact hypersensitivity on ears and focused on the inflammation along with ACD. There were few reports on the study of itch and pain sensations of ACD mice. Experiment proved that 1% SADBE could cause pain and itch of mice in the same time, which was consistent with the results of physio-psychological experiment of human. Itch and pain are sorts of subjective sensations, which cannot be expressed by animals but the corresponding behaviors like scratching and wiping can be expressed. By using the cheek model, we counted scratching with the hindlimb as an indicator of itch and wiping with the forelimb as an indicator of pain. The itch and pain sensations can be quantified by using video camera to record the behaviors of mice in a period of time and analyzing the times of scratching and wiping through the replay function.

Pain and itch sensations are common sense in many skin diseases. Itch is regulated by both histamine-dependent and independent pathways. At present, anti-histamine compound is the main method to treat itch symptom in clinic. However, antihistamine has no obvious effect on chronic and refractory itch. It is very significant to find a promising compound to treat histamine-independent itch. Pain is induced by real or potential tissue damages, and it is also a sort of uncomfortable sensation and emotional experience occurs in ACD. In the result so far four study, after the usage of SADBE, scratching increased obviously in the contact hypersensitivity model group ($P < 0.05$), which indicated that mice felt strong itch sensation after the challenge of SADBE. In the meanwhile, wiping also increased significantly in the contact hypersensitivity model group ($P < 0.01$), which indicated that mice felt strong pain sensation after the challenge of SADBE. Both results indicated that contact hypersensitivity model, the model of ACD in humans, was established successfully in this study.

Matrine has been widely used in China to treat several diseases like hepatitis, enteritis, and atopic dermatitis. The injection of matrine has been approved by the State Food and Drug Administration to treat the itch symptom of skin, chronic HBV, eczema and contact dermatitis. Intramuscular or intravenous injection of matrine can treat each type of eczema and dermatitis with 84.4% effective rate. It has been shown to possess various biological properties including anti-inflammation and immunity-regulation activity. Therefore, it can be considered that matrine might have the possibility to be an antipruritic and analgesic compound for ACD. The results of this study showed that each dose of matrine can obviously restrain the scratching of mice in contact hypersensitivity model group with a dose-dependent manner ($P < 0.05$). As scratching on the cheek is a behavioral indicator of itch sensation, therefore, it can be indicated that matrine has antipruritic effect on ACD mice. Itch sensation usually occurs along with pain, and they have many things in common. Our study showed that each dose of matrine also can restrain the wiping of mice in contact hypersensitivity model group with a dose-dependent manner ($P < 0.01$). As wiping on the cheek is a behavioral indicator of pain sensation, therefore, it can be indicated that matrine has analgesic effect on ACD mice. We also found that wiping usually would not appear individually but appearing after scratching and occasionally before scratching. Therefore, we inferred that the itch sensation of mice came out first and the scratching might cause the pain sensation.

As we known, ACD can be considered as the results of representative inflammatory responses in the skin. In ACD mice, the challenged part of cheek skin was swollen and turned red, which means inflammation reaction happened. Therefore, skin-fold thickness could be relevant to the swelling degree and the severity of inflammation in mice. In the result of our study, changes of the cheek skin-fold thickness increased significantly in the contact hypersensitivity model group ($P < 0.01$). Furthermore, each dose of matrine could significantly decrease the changes of the cheek skin-fold thickness with a dose-dependent manner ($P < 0.01$). Results show that contact hypersensitivity model has been established successfully and matrine might have anti-inflammatory effect on ACD mice.

Itch sensation usually occurs with pain. It is confirmed that TRPV1 is an important molecule for the development of thermal hyperalgesia under the inflammatory pain state. Reports showed that selective TRPV1 antagonists could alleviate thermal hyperalgesia in the formalin and the carrageen an models of pain in rats. A new study found that interleukin-31 (IL-31) was increased in pruritic atopic skin and severe pruritic behaviors occurred in IL-31 transgenic mice model with the involvement of TRPV1. Therefore, we could speculate that matrine might improve ACD by regulating the TRPV1 and IL-31 expressions in mice.

In conclusion, we have successfully established an ACD model that provides a behavioral differentiation between itch and pain sensations in the mice by SADBE. We firstly demonstrated that matrine can restrain itch and
pain sensations with a dose-dependent manner, which are quantified as scratching and wiping. Furthermore, we demonstrated that each dose of matrine might have anti-inflammatory effect by decreasing the changes of challenged cheek skin fold thickness in ACD mice. The results of this study showed some hints of antipruritic and analgesic efficiency of matrine in ACD.

**Conflicts of interest and source of funding**

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