Effects of motherwort alkaloids on rat ear acne

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Abstract

The aim of this study was to explore the effects of motherwort alkaloids on rat ear acne. The rats that were administered high, medium, and low doses of motherwort alkaloids, tanshinone capsules, a model and a control group. Each group of rats was subjected to gavage once daily for 14 consecutive days. On the first day of testing, the control and model groups were administered an intradermal auricle injection of sterilized saline solution and the remaining groups were administered an intradermal auricle injection of *Staphylococcus epidermidis* in addition to the gavage. The thicknesses of the rats' auricles were measured for five consecutive days following the injections. Anticoagulated blood was used for erythrocyte rheology index measurement. In addition, the entire ear of each rat was removed for morphological examination. Compared to the model group, the group administered motherwort alkaloids exhibited significantly reduced swelling, improved localized auricle proliferation, and reduced blood viscosity. This result suggests motherwort alkaloids are effective in rat ear acne.

Introduction

Acne is a chronic inflammatory skin disease that affects follicles. In clinical settings, acne is most frequently observed in adolescents. Acne can significantly affect a patient's appearance, resulting in loss of self-esteem and even mental illness. Modern medical acne treatments primarily consist of antibiotics, estrogens or anti-androgens, and retinoids, which can cause varying degrees of adverse effects. However, in recent years, researchers have begun to investigate the therapeutic effects of herbal medicine in an attempt to develop safer and more effective treatments for acne.

Motherwort, which can be dried or fresh, consists of the shoot of the Lamiaceae plant *Leonurus heterophyllus* (National Pharmacopoeia Committee, 2010). *L. hetero-*

This paper was presented in the 3rd International Conference on Biomedicine and Pharmaceutics in Zhuhai, China, on December 11-13, 2015.

phyllus can be found throughout China and has several distinct properties. Motherwort has been described as tasting spicy, slightly bitter, and slightly cold. Motherwort affects the body in various ways. It can pass through the pericardium and liver meridian, improve blood flow, regulate menstruation, act as a diuretic, reduce swelling, and promote detoxification. Motherwort primarily consists of alkaloids, diterpenoids, phenylpropanol glycosides, volatile oils, and annular polypeptides (Tian and Miao, 2014).

In clinical settings, motherwort is used to treat irregular menstruation, postpartum pain, cardiovascular diseases, blood-borne diseases, and prostitutes (Liu and Wang, 2012), In addition, recent studies have shown that, due to its anti-inflammatory and antibacterial properties, motherwort can be used as a topical treatment for pruritus, urticarial (Miao et al., 2013), carbuncles, and cosmetological purposes (Wang et al., 2009). In this paper, the effects of motherwort alkaloids on acne

induced by *Staphylococcus epidermidis* in rats were investigated using a rat ear acne model.

Materials and Methods

Animals

Wistar male rats weighing 180-200 g were supplied by the Experimental Animal Center of Hebei Province (Animal permit No.: 812074). Laboratory Certificate of Conformity was SYXK (Henan) 2010-001.

Experimental reagents and drugs

The TALH was provided by the chemical room. Content was 52%; danshentong capsule (Hebei Xinglong Force Pharmaceutical Co., Ltd. Batch No. 20090819). *S. epidermidis* strain No. 12228 was provided by Zhengzhou University Microbiology Laboratory, experimental passages before backup. IL-6 radiation immunoassay kit, Beijing's ear cause Biological Technology Co., Ltd., Batch No. 20091226.

Experimental instrument

Instrument were Bio-optical microscope (Chongqing Optical Instrument Factory, model XSZ-H); Electronic balance (Mettler Toledo Instruments Co. Ltd., model AL204); Automatic blood rheology detector (Chongqing Meiko Instrument Co., Ltd, model XLB201).

Experimental methods

Sixty healthy rats with weights ranging from 180-200 g were randomly divided into six groups by weight. The six groups consisted of three group of rats that were administered high, medium, and low doses (200, 100 and 50 mg/kg, respectively) of motherwort alkaloids; one group of rats administered Tanshinone capsules (360 mg/kg); a model group; and a control group (Miao et al., 2014). All of the groups were administered their corresponding medications via gavage. The model and control groups received gavage as well as the same volume of distilled water. The rats were subjected to gavage once daily for 14 consecutive days (Liu and Wang, 2010). On the first day of administration, the control and model groups were administered a 50-µL intradermal auricle injection of sterilized saline solution and the remaining groups were administered a 50-µL intradermal auricle injection of a solution containing *S. epidermidis* (Liu et al., 2014). The thicknesses of the rats' auricles were measured with a micrometer every 24 hours for five consecutive days following the injections. The auricle swelling rate was defined as:

Thickness of the auricle after the injection - the thickness of the auricle before the injection) / thickness of the auricle before the injection × 100%

One hour after the last gavage, 3 mL of anticoagulated blood was collected from an eye of each rat for erythrocyte rheology index measurements. In addition, the entire ear of each rat was removed, fixed with 10% formalin, embedded with paraffin, sectioned, and stained with HE for observation under a light microscope.

Statistical analysis

With regard to data analysis, SPSS 13.0 statistical package was applied for statistical treatment of data information, while as to measuring data, mean value ± standard deviation was applied.

Results

Impact on auricle swelling rate of rats acne model

Table I shows the comparison of the blank group with the model group. Rats auricle swelling rate in the day 2-5 was significant difference (p<0.01). Injection of *S. epidermidis* acne model was successfully and longer duration. Compared with model group, in the day 2-5, the high and medium dose of TALH group and Danshentong group could significantly inhibited the

Table I						
TALH affect acne *Staphylococcus epidermidis*-induced ear edema model in rats						
Group	n	Auricle swelling rate (%)				
		Day 1	Day 2	Day 3	Day 4	Day 5
Blank group	10	93.8 ± 19.3	70.2 ± 8.6[b]	41.6 ± 16.6[b]	20.1 ± 9.0[b]	7.8 ± 4.4[b]
Model group	10	99.1 ± 20.7	95.5 ± 14.5	85.2 ± 11.8	76.4 ± 14.2	77.2 ± 10.9
Danshentong group	10	98.7 ± 16.4	78.8 ± 9.5[b]	65.6 ± 8.3[b]	31.2 ± 12.5[b]	6.5 ± 5.3[b]
High dose of TALH group	10	92.4 ± 14.9	76.4 ± 1.1[b]	61.3 ± 7.6[b]	35.2 ± 7.5[b]	7.5 ± 7.6[b]
Medium dose of TALH group	10	94.9 ± 11.6	78.0 ± 11.2[b]	65.6 ± 8.6[b]	43.4 ± 11.1[b]	20.0 ± 7.9[b]
Low dose of TALH group	10	96.1 ± 12.3	80.9 ± 5.9[a]	65.8 ± 13.7[b]	44.1 ± 14.7[b]	22.2 ± 12.3[b]

Data are mean ± SD; n=10; Compared with the model group, [a]p<0.05; [b]p<0.01

acne model rats auricle swelling degree (p<0.01). In second day, the low dose of TALH group obviously inhibited the acne model rats auricle swelling degree (p<0.05). In third, fourth, fifth day, the low dose of TALH group obviously inhibited acne model rats auricle swelling degree (p<0.05).

Impact on acne model rats auricle organization form

Blank group rats auricle local skin squamous epithelium, hair follicles, sebaceous glands and subcutaneous tissue were normal (Figure 1A). Model group rats auricle partial skin squamous epithelial hyperplasia of obvious thickening, subcutaneous tissue appeared a lot of inflammatory cells invasion (Figure 1B). Danshentong group rats auricle partial skin squamous epithelial cells did not show proliferous thickening, subcutaneous tissue with a small amount of inflammatory cells inva-

sion (Figure 1C). High dose of TALH group rats auricle partial skin squamous epithelial cells did not show proliferous thickening. Thickening of the subcutaneous tissue and inflammatory cells decreased significantly (Figure 1D). Medium dose of TALH group rats auricle partial skin squamous epithelial cells did not show proliferous thickening. Thickening of the subcutaneous tissue and inflammatory cells were reduced (Figure 1E). Low dose of TALH group rats auricle partial thickened skin squamous cell hyperplasia, thickening of the subcutaneous tissue and reduction of inflammatory cells (Figure 1F).

Table II shows the analysis by Ridit test, compared with the control group. In the model group, local skin acne showed significant pathological lesions (p<0.01), described as the successful model. Compared with the model
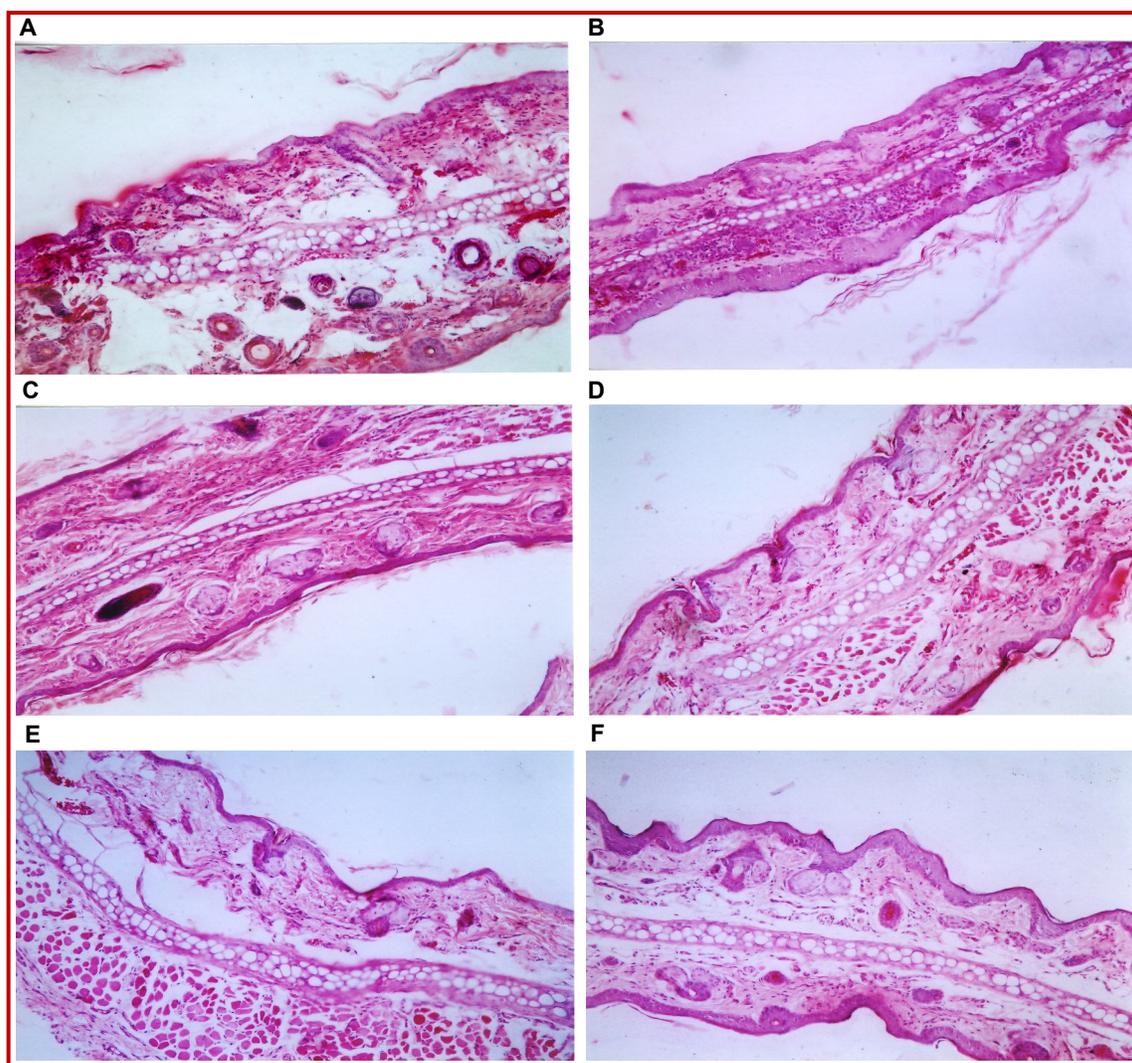

Figure 1: Motherwort total base of acne model rats auricle histologic changes of the organization HE ×100）

Note: A. Blank group; B. Model group; C. Danshentong group; D. High dose of TALH group; E. Medium dose of TALH group; F Low dose of TALH group

Table II						
Effect of TALH on *S. epidermidis*-induced rat model of auricular tissue forms of acne						
Group	n	–	+	++	+++	p
Blank group	10	10	0	0	0	<0.01
Model group	10	0	2	3	5	<0.01
Danshentong group	10	1	8	1	0	<0.01
High dose of TALH group	10	1	7	2	0	<0.01
Medium dose of TALH group	10	0	5	4	1	<0.01
Low dose of TALH group	10	0	3	5	2	

Note: "-": auricle squamous epithelial skin, hair follicles, sebaceous glands and subcutaneous tissue were normal; "+": pinna skin squamous epithelial cells mild hyperplasia, thickening of a small amount of subcutaneous tissue inflammatory cells invasion; "++": pinna thickened skin squamous cell hyperplasia, thickening of the subcutaneous tissue and inflammatory cells invasion; "+++": pinna skin squamous epithelial hyperplasia of obvious thickening, subcutaneous tissue appeared a lot of inflammatory cells invasion

group, high, medium and low dose of TALH group and danshentong group could significantly improve the pathological changes in rats acne topical skin (p<0.01).

Effect of hemorheology on rat acne model

From the Table III, we can see that compared with the blank group, blood viscosity in model group was significantly increased (p<0.01), indicating a successful model. Compared with the model group, high dose of TALH group, medium dose of TALH group and danshentong group the blood viscosity values were significantly lower (p<0.01). In low dose of TALH group, the blood viscosity value of the total base group was significantly decreased (p<0.05).

Discussion

The results of the experiment indicated that motherwort alkaloids could be used to significantly improve the symptoms of rat acne. However, the administration of the tanshinone capsules did not result in any significant changes in acne. The results also indicated

that motherwort alkaloids could inhibit the auricle swelling rate, alleviate ear hyperplasia, and significantly reduce the blood viscosity. Furthermore, the effects of the motherwort alkaloids were positively correlated with the dosage, indicating that the motherwort alkaloids therapeutically affected the rat ear acne induced by *S. epidermidis*. Thus, the results of this study, which suggested that motherwort could be used to clinically treat acne, could be used as a foundation for further studies concerning the therapeutic effects of motherwort on acne.

Acne primarily results from the excessive secretion of sebaceous glands. This excessive secretion of sebum and keratosis as well as the invasion of pathogens, particularly *S. epidermidis*, *S. aureus*, and *S. albus*, block the lumens of hair follicles. Thus, an ideal acne treatment would inhibit the growth of pathogenic bacteria and sebaceous glands, reduce sebum secretion, and alleviate inflammation (Xu et al., 2009). Previous studies have shown that motherwort alkaloids have anti-inflammatory effects that could effectively treat acne (Miao et al., 2013).

Table III				
Effect of TALH on hemorheology in rats of acne				
Group	Dose (g/kg/d)	High shear viscosity of whole blood (mPa.s)	Medium shear viscosity of whole blood (mPa.s)	Low shear viscosity of whole blood (mPa.s)
Blank group		6.9 ± 0.3[b]	6.4 ± 0.4[b]	12.7 ± 0.5[b]
Model group		9.2 ± 0.6	10.0 ± 0.7	19.0 ± 0.7
Danshentong group	0.36	6.0 ± 0.5[b]	6.0 ± 0.8[b]	14.1 ± 0.8[b]
High dose of TALH group	0.2	5.5 ± 0.5[b]	.0 ± 0.5[b]	12.1 ± 0.7[b]
Medium dose of TALH group	0.1	5.6 ± 0.6[b]	6.4 ± 0.7[b]	12.5 ± 1.0[b]
Low dose of TALH group	0.05	6.0 ± 0.6[b]	7.1 ± 0.6[a]	13.0 ± 0.8[a]

Data are mean ± SD; n=10; Compared with the model group, [a]p<0.05, [b]p<0.01

In the experiment, rat auricles, which contain numerous sebaceous glands, were infected with intradermal injections of a solution containing *S. epidermidis* in order to expand their follicular cavities and induce micro acne. Since the excessive secretion of sebaceous glands contributes significantly to acne formation and the main characteristic of acne is the expansion of follicles, the developed model was consistent with the clinical symptoms of acne. In addition, the experimental method used in this study could be easily replicated.

The primary indicators of acne observed in this study included auricle swelling, local histopathological changes, and hemorheology. The auricle swelling rate was one of the most objective indicators of the degree of acne. In addition, the auricle morphology was the primary indicator of proliferation and infection. Whole blood viscosity, an essential parameter of hemorheological studies, can be used as an indicator of thick, sticky, agglomerate, or condensed blood. The shear rate of blood affects its viscosity. Thus, reductions in whole blood viscosity resulting from changes in the shear rate can be used to evaluate erythrocyte rheology. Reductions in blood viscosity at high shear rates can be evaluated using the erythrocyte rigidity index. High erythrocyte rigidity index values are associated with greater degrees of erythrocyte deformability and, thereby, harder erythrocytes. Reductions in blood viscosity at intermediate shear rate values are associated with significant erythrocyte deformation but no obvious aggregation. Reductions in blood viscosity at low shear rate values can be evaluated using the erythrocyte aggregation index. High erythrocyte aggregation index values are associated with higher degrees of aggregation.

Therefore, by calculating reductions in blood viscosity at high, intermediate, and low shear rates, the extent of erythrocyte deformation and aggregation, which is closely related to the occurrence of acne, can be analyzed.

Acknowledgement

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