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EFFECT OF MOTHERWORT TOTAL ALKALOIDS ON A TESTOSTERONE PROPIONATE-INDUCED ACNE: MOUSE MODEL

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ABSTRACT

We aimed to investigate the effect of motherwort total alkaloids (MTAs) on an acne mouse model induced by injection of testosterone propionate. High, medium and low dose of MTAs was each given to different mouse groups. The content of testosterone (T) in serum was determined. The morphological changes of testes, epididymides and seminal vesicles after each treatment were detected. Compared with the model control group, the mice treated with high, medium and low dose of MTAs had significantly different (P<0.05) content of serum T. Among tested groups, the MTA high dose group showed the most significant difference (P<0.01). The testis, epididymis and seminal vesicle weight of mice in each MTA group was decreased and the reduction was significantly different (P<0.05). The MTA high and medium dose groups had most significant difference (P<0.01). Motherwort herb promoted generation of various spermatogonial cells and increased sperm production in the seminiferous tubules of the testes. The higher the dosage was, the more effective MTAs were. Moreover, MTAs improved the function of epithelial cells in the epididymis tubule. High and low dose of MTAs had better effect. MTAs have a therapeutic effect on the acne mouse model.

Keywords: Motherwort total alkaloids; Acne; Mouse model.

INTRODUCTION

Acne is a common human skin disease, also known as "Whelk". The incidence rate reaches as high as 85% among the young at the age of 12-24 and it is higher in male than female. In recent years, the incidence rate and the onset age of this disease have increased gradually. Motherwort is with the flavor of bitter, pungent and slightly cold, and the systems affected are mainly the liver, pericardium and bladder. The main effective components of motherwort are total alkaloids, including stachydrine, leonurine, etc. Modern pharmacological research shows that motherwort has antibacterial, anti-inflammatory and estrogen-like functions. According to clinical reports, motherwort has been used for the treatment of cardiovascular, peripheral vascular and other disease, having good prospect of modern clinical application (Chen, 2012). In the present study, we investigated the effect of motherwort total alkaloids (MTAs) on an acne mouse model induced by injection of testosterone propionate (TP).

MATERIALS AND METHODS

Experimental reagents and drugs TP was purchased from Tianjin Jinyao Amino Acid Co. Ltd. (batch number, 080427). Serum testosterone (T) was analyzed with a radioimmunoassay kit purchased from Beijing Kemei Leya Biological Technology Co., Ltd. (batch number, 20090125). Danshentong Capsules were obtained from Hebei Xinglong Xili Pharmaceutical Co., Ltd. (batch number, 20080819). MTAs were purchased from Xi'an Garden Biological Preparation Factory (content of 52%; batch number, 20081018).

Experimental instrument A bio-optical microscope was purchased from Chongqing Optical Instrument Factory (model XSZ-H), and an automatic gamma immunity counting meter was from the State-owned 2-6-2 Medical Nuclear Instrument Factory (model FJ2003PS).

Animals SPF grade KM mice (weight, 18～20g) were supplied by Experimental Animal Center of Henan Province (animal permit number, 902076).

Methods: 60 KM male mice (weight, 18～20g) were randomly divided into the blank control (BC), model control (MC), positive control (PC) and MTA high dose (MTA-HD), medium dose (MTA-MD) and low dose (MTA-LD) groups. Animals were drenched with 0.2ml/10g drug solutions. The BC and MC mice were given distilled water, while the PC mice were given Danshentong capsules (0.72g/kg, 16 capsules dissolved in 20ml distilled water). The MTA-HD, MD and LD mice respectively received 400mg/kg, 200mg/kg and 100mg/kg of MTA solutions, which were made by dissolving 800mg, 400mg and 200mg MTAs in 20ml distilled water. Five days after administration, TP (0.5mg/kg) was intramuscularly injected into the MC, PC, MTA-HD, MTA-MD and MTA-LD mice. 30min after the last administration, eyeball blood was collected and the serum was separated. The content of serum T was determined with a serum T RIA kit. FJ2003PS γ counting

instrument was used to calculate the B/B0%. The content of serum T was obtained according to the standard curve (μg/dl). The mice were executed and the testis, epididymis and seminal vesicle were weighted. The tissues were stained with HE for pathological section observation.

Statistical analysis The SPSS 13.0 statistical software for Windows was used for data analysis. The measurement results were expressed as"mean ± standard deviation ($\bar{x} \pm s$)".Comparison between groups was performed by the LSD method.

RESULTS

The content of serum T. After TP injection, the serum T values of animals in each group were detected (Table 1).

Table 1. The effect of motherwort total alkaloids on the serum T levels in mice ($\bar{x} \pm s$).

Group	n	Serum T(ng/dl)
BC	10	64.1 ± 2.9
MC	10	153.8 ± 3.1★★
PC	10	117.5 ± 3.3★★**
MTA-HD	10	116.9 ± 4.2★★**
MTA-MD	10	127.4 ± 4.8★★*
MTA-LD	10	134.0 ± 5.9★★*
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Note: Stars mean comparison with the blank control group (★★ $P<0.01$) and snowflakes indicate comparison with the model control group (* *$P<0.01$ and * $P<0.05$)

As shown in Table1, The serum T content of the MC and MTA mice were significantly different with that of the BC mice ($P<0.01$) (Table1), suggesting a sharp increase of serum T in response to TP administration. The results indicated the model was established successfully. Compared with the MC group, the serum T levels in the PC, MTA-HD, MTA-MD and MTA-LD groups showed significant differences ($P<0.05$). In addition, there was extremely significant difference between the PC and MTA-HD groups ($P<0.01$). Hence, Danshentong capsules and MTAs show a strong effect against the increase of male hormones, indicating an estrogen-like role.

Weight of the testes, epididymides and seminal vesicles After TP injection, the testes, epididymides and seminal vesicles from mice in each group were collected and weighted (Table 2).

As shown in Table2, compared with the BC group, the average weight of each organ in the BC, MC and MTA mice was dramatically increased after TP injection ($P<0.01$). Compared with the MC group, the average weight of testes, epididymides and seminal vesicles in each group was significantly decreased ($P<0.05$). In addition, there was extremely significant difference between the PC and MTA-HD/MD groups ($P<0.01$). Hence, Danshentong capsules and high/medium dosage of MTAs have a strong effect against male hormones

Histopathology observed with a light microscope Histopathology of the testes, epididymides and seminal vesicles in each group was studied and the results were summarized as follows.

Testes: All kinds of spermatogonial cells and sperm in the seminiferous tubules of the testeswere absolutely normal in the BC, PC and MTA-MD mice (Figure 1A, C and E) and basicallynormal in the MTA-LD mcie (Figure1F). They were significantly reduced in the MG mcie (Figure 1B), while obviously increased in the MTA-HD mice (Figure 1D).

Table 2. The effect of motherwort total alkaloids on organ weight in mice ($\bar{x} \pm s$,n=10)

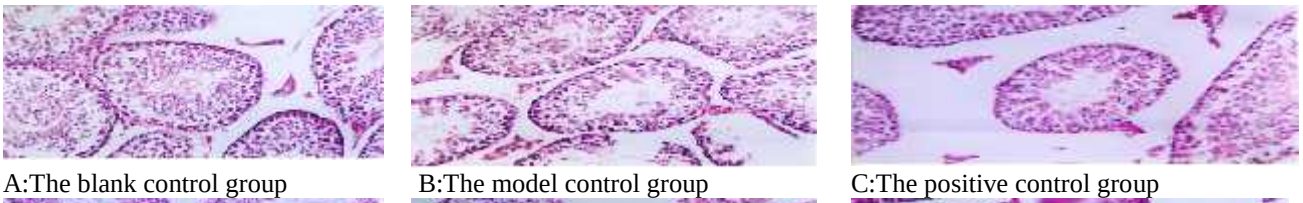
Group	Testis（mg）	epididymis（mg）	Seminal vesicle（mg）
BC	150.9 ± 5.5	26.2 ± 3.7	41.2 ± 5.5
MC	280.5 ± 5.6★★	42.0 ± 4.9★★	88.4 ± 5.6★★
PC	220.1 ± 5.1★★**	36.4 ± 5.3★★*	64.0 ± 4.7★★*
MTA-HD	216.9 ± 4.7★★**	35.0 ± 5.4★★**	60.0 ± 4.5★★**
MTA-MD	229.9 ± 4.5★★**	37.5 ± 3.1★★**	69.0 ± 3.5★★**
MTA-LD	253.6 ± 6.7★★*	42.1 ± 3.2★★*	76.4 ± 6.8★★

Note: Stars mean comparison with the blank control group (★★$P<0.01$) and snowflakes indicate comparison with the model control group (* *$P<0.01$ and * $P<0.05$).

Epididymides: The epididymal epithelial cells (EECs) of the BC mice were rich of cytoplasm, but there were no sperm cells inside the epididymal tubule (ET) (Figure2A). In the MC animals, a small part of EECs

were atrophic, having reduced cytoplasm and shrunk nuclei. In addition, no sperm was observed inside the ET (Figure2B). As to the PC mice, their EECs were full of cytoplasm and the nuclei were large and transparent. The ET contained a huge amount of sperm cells (Figure2C). The MTA-HD and MTA-LD animals showed the same histopathology as the PC mice (Figure2D and F). However, the EECs in the MTA-MD mice had less cytoplasm and karyopyknosis appeared. Moreover, the intraluminal sperm number was obviously reduced (Figure2E).

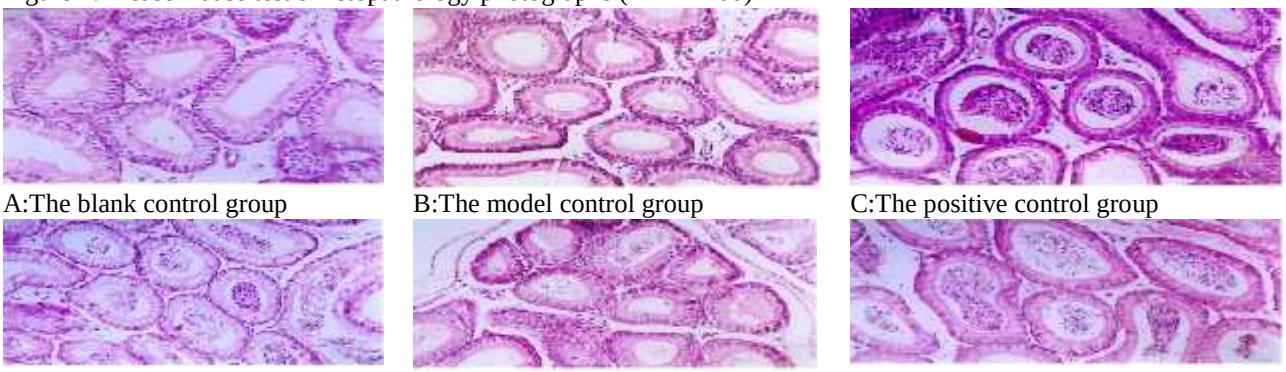
Seminal vesicles: In the BC and MC animals, the seminal vesicle epithelial cells (SVECs) consisted of a lining of columnar cells and intraluminal bleeding was observed (Figure3 A and B). However, obvious hyperplasia of SVECs was detected in the PC mice, and intraluminal bleeding also occurred (Figure3C). The SVECs of MTA-HD animals were significantly shrunk and the number was reduced. Intraluminal bleeding was observed (Figure3D). In contrast, the SVECs of MTA-MD mice had columnar cells, but no intraluminal bleeding was detected (Figure3E). In the MTA-LD mice, their SVECs were significantly shrunk and the number was reduced. Intraluminal leakage was observed (Figure3F).



A:The blank control group B:The model control group C:The positive control group

D:The MTA-HD group E:The MTA-MD group F:The MTA-LD group

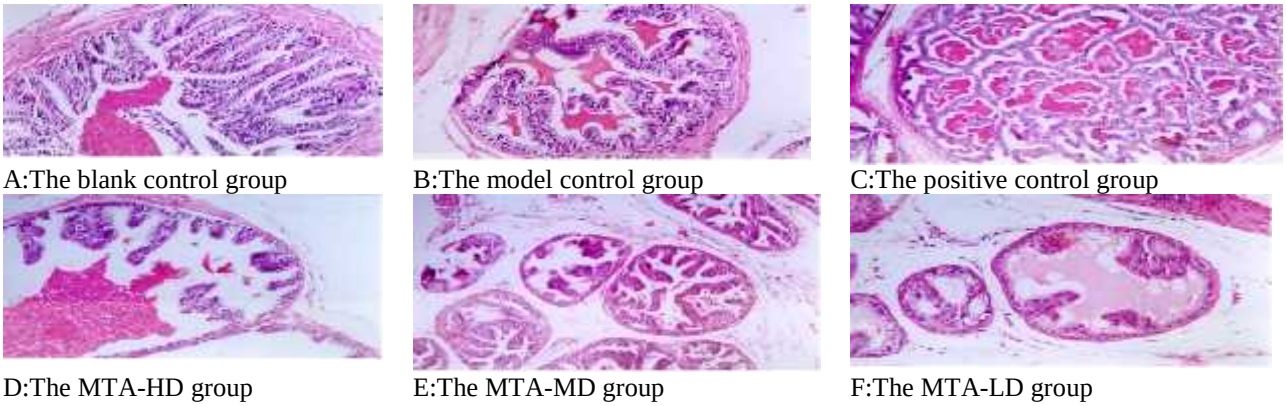
Figure 1: PleaseMouse testis histopathology photographs (HE ×100)



A:The blank control group B:The model control group C:The positive control group

D:The MTA-HD group E:The MTA-MD group F:The MTA-LD group

Figure 2: Mouse epididymis histopathology photographs(HE ×100)



A:The blank control group B:The model control group C:The positive control group

D:The MTA-HD group E:The MTA-MD group F:The MTA-LD group

Figure3: Mouse seminal vesicle histopathology photographs (HE ×100)

DISCUSSION

In order to evaluate the effect of MTAs on the acne mouse model induced by TP injection, we measured the serum T content, weighted related organs including the testis, epididymis and seminal vesicle, and observed the pathological changes of these organs in response to various treatments. The content change of serum T is a critical factor to determine whether the model is established successfully. Testosterone is synthesized in the testis and its secretion changes the pathological properties of the epididymis and seminal vesicle.

Testosterone is reduced to dihydrotestosterone (DHT) by an enzyme. DHT binds to hair follicle receptors to stimulate keratinization of hair follicle sebaceous glands, leading to horny thickening, keratinocyte adhesion and consequently locking the tubule. The inhibited sebum production and discharge are the main causes of acne (Ji *et al*, 2012). The changes of testosterone content directly reflect the anti-androgen effect of a drug, and the pathological changes of testes, epididymdes and seminal vesicles give a more comprehensive reflection of the drug's effect.

The results showed that the serum T levels in the MTA-HD, MD and LD mice were significantly different with that in the MC mice, and the difference was dramatically significant for the MTA-HD group. Hence, MTAs showed a strong effect against the increase of male hormones, confirming the estrogen-like function. Compared with the MC group, the testis, epididymis and seminal vesicle weight of MTA-HD and MTA-MD mice was significantly different, indicating a strong effect of high or medium MTA dosage against male hormones. Motherwort herb promoted generation of all kinds of spermatogonial cells and sperm cells in the seminiferous tubules of the testes. The effect was increased along with the enhanced dosage. In addition, MTAs improved EEC functions and the high and low doses showed better effects.

In traditional Chinese medicine, acne is commonly due to poor diet, pathogenic heat in the lungs and intestines and deficiency of kidney-Yin (Huang *et al*,2014). The pathogenic heat and damp heat is an important cause and basis of acne generation and development. Clearing heat from the appropriate channel or organ is the key of acne treatment (Sun *et al*, 2014). Motherwort is good for relieving inflammation and swelling. It is diuretic and can clear heat. Hence, it is widely used for acne treatment in traditional Chinese medicine.

Acne is a common human skin disease, usually occurring in adolescence. It is mainly due to chronic hair follicle and associated sebaceous gland inflammatory caused by many factors like microorganism infection and excessive sex hormone secretion (Niu *et al*, 2012). Acne is not only a physical disease; it also affects patients in the social, psychological, emotional and other aspects. Acne patients are prone to depression, anxiety and anger, so acne is a physical as well as psychological disease that cannot be underestimated. The findings in this study provide evidence for clinical application of motherwort in acne treatment. It also provides new insights into developing methods for the prevention and cure of acne.

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