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Original Research Article

HuChou San ameliorates symptoms of bromhidrosis via down-regulation of expressions of apolipoprotein D and androgen receptor genes

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Abstract

Purpose: *To investigate the effect of HuChou San on bromhidrosis, and the mechanism of action involved.*
Methods: *Patients with bromhidrosis (n = 49) were recruited over a year and 5 months period for this study and were randomly assigned to five groups: saponin group, Halite violaceous (HV) group, Rhizoma typhonii (RT) group, Impatiens balsam (IB) group, and HuChou San group. A sixth group comprised of healthy individuals (n = 9) served as control. The patients were treated with saponins, HV, RT, IB, or HuChou San once a day for 2 months. Fresh skin tissue from bilateral axilla was surgically collected from the patients or control, and used for assay of the expression of apolipoprotein D (ApoD) and androgen receptor (AR) mRNAs with real-time quantitative polymerase chain reaction (qRT-PCR).*
Results: *Treatment of bromhidrosis patients with saponins, HV, RT, IB or HuChou San powder led to significant reductions in the levels of expression of ApoD and AR mRNAs, when compared with the control group ($p < 0.05$). The expressions of these genes were significantly reduced in HuChou San-treated group, relative to the other treatment groups ($p < 0.05$).*
Conclusion: *The results of this study show that HuChou San ameliorates symptoms of bromhidrosis via down-regulation of expressions of ApoD and AR genes.*

Keywords: *HuChou San, Bromhidrosis, Saponins, Apolipoprotein D, Androgen receptor.*

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INTRODUCTION

Axillary bromhidrosis refers to the offensive body odor that results from the interactions between apocrine gland secretions and bacteria in the armpit [1,2]. Topical treatment, laser therapy, ultrasonic and/or liposuction curettage, and surgical intervention are currently being used to treat this condition. Multiple surgical procedures such as minimally invasive surgery have been developed to completely remove apocrine and eccrine sweat glands [3,4]. These surgeries are relatively safe and produce positive long-term outcomes. However, they are not without side effects such as varied degrees of malodor recurrence [5,6].

Studies have shown that primary hyperhidrosis is

promoted by a genetic factor, and its estimated prevalence is put at 0.6 to 16.7 % in the general population [7], and approximately 14.76 % in specific patient groups [8]. Primary plantar hyperhidrosis (PPH) is characterized by over-activation of plantar eccrine sweat glands. This condition occurs during childhood or puberty, and its pathogenesis remains unknown. More than half of the cases of PPH are associated with primary palmar hyperhidrosis [9,10]. The treatment of PPH is aimed at reducing perspiration in body areas prone to hypersecretion of sweat. Nonsurgical approach in the management of PPH comprises the use of anxiolytics and anticholinergics, application of astringent solutions or creams, iontophoresis, subdermal injection of botulinum toxin, or destruction of axillary sweat glands with microwave technology [11,12]. Saponins, HV, RT, and IB are herbs commonly used in Traditional Chinese Medicine (TCM) to treat axillary bromhidrosis. These herbs are also used in the treatment of other diseases. Studies have shown that they are effective against bromhidrosis.

The expressions of ApoD and AR mRNAs have been reported to be upregulated in patients with bromhidrosis. The present study investigated the effect of "*HuChou San*" on bromhidrosis, and the mechanism involved.

EXPERIMENTAL

Materials

Total RNA extraction and QuantiNova Reverse Transcription kits were purchased from Qiagen (Belgium). NanoDrop 2000 spectrophotometer was product of Isogen Life Science (Belgium), and optical 96-well plates were obtained from Applied Biosystems (Belgium). The SYBR Green qRT-PCR probe was product of Invitrogen (Belgium). qBase software was obtained from Biogazelle (Belgium).

Preparation of *"HuChou San"*

HuChou San powder used in this study consisted of equal proportions of crude saponins, HV, RT, and IB.

Patients

Patients with bromhidrosis (n = 49) were recruited over 17-month period for this study, and were randomly assigned to five groups: saponin group, *HV* group, *RT* group, *IB* group, and *"HuChou San"* group. The inclusion criteria were: (1) patients aged 16 to 40 years (mean age = 28.00 ± 5.10 years); (2) patients who had no history of axillary bromhidrosis surgery; and (3) patients who signed written informed consent. Patients in the five observation groups comprised 27 males and 13 females. A sixth group comprised healthy individuals (n = 9) who served as control.

Treatment regimen

The patients' armpits were first treated with copper and zinc sulfate solution for 10 min, and then dipped in *"HuChou San"* powder, which was gently applied to the affected areas, once a day for 2 months. Fresh skin tissue from bilateral axilla was surgically collected from the patients or control and refrigerated at - 70 ^{o}C.

qRT-PCR

Trizol RNA extraction reagent was used to extract total RNA from cell suspension obtained from the trypsinization of skin tissues, while cDNA synthesis was performed using QuantiNova Reverse Transcription kit. The quality and concentrations of extracted mRNAs were assessed spectrophotometrically at 260/280 nm. Light Cycler 1536 RT-PCR detection system was used for the estimation of the mRNA expressions. Variation in the cDNA content was normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The PCR reaction mixture (20 µL) consisted of 6.4 µL of dH_2O, 1.6 µL of gene-specific primer (10 µM), 2 µL of synthesized cDNA and 10 µL of SYBR Premix Ex Taq™ II. The amplification reaction conditions were: 95 ℃ for 3 min, 95 ℃ for 10 sec, 60 ℃ (annealing temperature of ApoD, AR and GAPDH) for 30 sec, and 40 cycles of 65 - 95 ℃ for 5 sec. The procedure was performed in triplicate. The relative expression levels of mRNA was calculated using the $2^{-\Delta\Delta CT}$ method. The primer sequences used are shown in Table 1.

Table 1: Gene sequences used for qRT-PCR

Gene	Sequence
ApoD	Forward: 5'-TAAACATCAGAGACCTGAAG-3' Reverse: 5'-AGAATCAGCCGATTTGAGAT-3';
AR	Forward: 5'-CCCCAGGCACCCAGAGGC-3' Reverse: 5'-GAGAACCATCCTCACCCTGCT-3'
GAPDH	Forward: 5'-CTCAGACACCATGGGGAAGGTGA-3' Reverse: 5'-ATGATCTTGAGGCTGTTGTCATA-3'

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using SPSS (23.0). Groups were compared using Student *t*-test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of the different treatments on the levels of expression of ApoD and AR mRNAs

Treatment of bromhidrosis patients with saponins, HV, RT, IB or *HuChou San* powder led to significant reductions in the levels of expression of ApoD and AR mRNAs**,** when compared with the control group ($p < 0.05$). The expressions of these genes were significantly reduced in *HuChou San*-treated group, relative to the other treatment groups ($p < 0.05$). The inhibitory effect of the different treatments on the level of expression of ApoD mRNA was in the order: HuChou San > saponins > IB > RT > HV. Similarly, the inhibitory effect of the different treatments on the expression level of AR mRNA was in the order: HuChou San > RT > HV > IB> saponins. These results are shown in Figure 1.

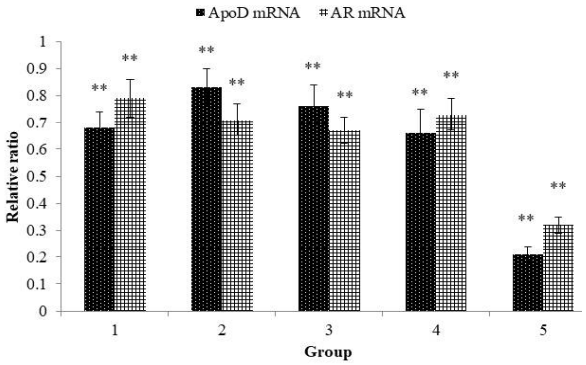

Figure 1: Levels of expression of ApoD and AR mRNAs after treating patients with *HuChou San* powder for 2 months; ** $p < 0.05$, when compared with control group

DISCUSSION

Human apolipoprotein D (apoD) is a 169-amino acid glycoprotein member of the lipocalin family. In this protein family, membership is not based on sequence homology, but on structural homology [13]. The crystal structure of an apoD monomer reveals a typical lipocalin fold with eight strands of antiparallel β-barrel flanked by an α-helix [14]. Its biological functions are associated with its capacity to bind several small hydrophobic molecules [15]. In mice, ApoD expression is limited to the central nervous system (CNS). However, in humans, ApoD is expressed in the CNS, adrenal glands, kidneys, pancreas, placenta, spleen, lungs, ovaries and testes. The expression of ApoD has been studied mainly in the CNS because it is usually overexpressed (up to 500-fold) during neurodegenerative stress [16,17]. Some of the functions of ApoD are mediated via binding to arachidonic acid (ARA), a polyunsaturated omega-6 fatty acid [18]. Evidence in support of the roles of ApoD outside the CNS are beginning to emerge. The androgen receptor (AR) gene consists of eight exons. Exon 1 encodes the N-terminal domain, exons 2 and 3 encode the DNA binding domain (DBD), and exons 4 to 8 encode the hinge region and the ligand binding domain. Androgens such as testosterone and dihydrotestosterone (DHT) regulate male sexual development, and determine the expression of male phenotype. The effects of these hormones are mediated via AR.

Saponins are plant secondary metabolites found in various plant species [19]. They are surface-active because they comprise one or more hydrophilic sugar moieties covalently attached to hydrophobic steroid or triterpene backbone [20]. *Halite violaceous* (HV), RT, and IB are widely used in TCM. Studies have shown that the expressions of ApoD and AR genes are upregulated in patients with bromhidrosis [21].

In this study, treatment of bromhidrosis patients with saponins, HV, RT, IB or *HuChou San* powder led to significant reductions in the levels of expression of ApoD and AR mRNAs**,** when compared with the control group. The expressions of these genes were significantly reduced in *HuChou San*-treated group, relative to the other treatment groups. These results suggest that *HuChou San* may be effective against bromhidrosis.

CONCLUSION

The results of this study show that *HuChou San* ameliorates the symptoms of bromhidrosis via down-regulation of the expressions of ApoD and AR genes.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of authors

This work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Bin Chen designed the study, and revised the manuscript. Chen Wang performed the statistical analysis and wrote the manuscript. Both Bin Chen and Zhen Wang equally contribute to this work.

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