JTCM 中医杂志 Journal of Traditional Chinese Medicine

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J Tradit Chin Med 2016 December 15; 36(6): 743-748
ISSN 0255-2922

EXPERIMENTAL STUDY

Effect of Wuziyanzong pill on levels of sex hormones, and expressions of nuclear- associated antigen Ki-67 and androgen receptor in testes of young rats

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Supported by Natural Science Foundation-funded Project: the Mechanism and Function of Erythropoietin Regulating Blood-Testis Barrier of Rat in Essence of Kidney Dominating Reproduction (No. 81273610)

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Accepted: Augest 21, 2015

Abstract

OBJECTIVE: To evaluate the effects of Wuziyanzong pill on the levels of serum follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T) and the expressions of nuclear-associated antigen Ki67 (Ki67), androgen receptor (AR) in testes of young rats.

METHODS: Sixteen 20-day-old Wistar male rats were randomly divided into control and treatment group (n = 8). Rats in treatment group were administered Wuziyanzong pill by gavage; rats in control group administered the same volume of saline. After 10 days of treatment, the rats were killed, and then serum and testes were taken. The levels of FSH, LH and T were measured by radioimmunoassay (RIA). The histology of seminiferous tubule was observed by hematoxylin-eosin (HE) staining. The expression of Ki67 was detected by immunohistochemical assay (IHC). The mRNA level of Ki67, AR and CK-18 was detected with quantitative real-time polymerase chain reaction (Q-RT-PCR), the protein level of AR and CK-18 were tested by Western blot.

RESULTS: Compared with control group, T level in treatment group increased significantly ($P < 0.05$). HE staining showed that both leydig cells and germ cells increased in treatment group. Expressions of Ki67 and AR became higher after treatment. There were no changes in CK-18 expression.

CONCLUSION: Wuziyanzong pill can up-regulate AR level to promote germ cell proliferation and differentiation in young male rats.

Key words: Wuziyanzong Wan; Follicle stimulating hormone; Ki-67 antigen; Receptors, androgen; Testosterone; Leydig cells; Germ cells

INTRODUCTION

According to the theories of Traditional Chinese Medicine (TCM), kidney is the key organ to store essence and regulate reproductive functions. Wuziyanzong pill (WYP) is an important TCM prescription for the treatment of male infertility. WYP can improve androgen level as well as increase sperm density, activity and decrease deformity rate by up-regulate hypothalamus-pituitary-testis axis.[1-5] The prescription's effect on different male infertility is obvious. Nevertheless, previous studies all focused on infertility patients or experimental animals. Thus the influence of WYP on extraordinary reproductive function under illness conditions has

been discussed before.[6-8] Whether WYP can promote normal young male rat mature or not is uncertain. In addition, most previous studies were limited to the overview of sperm function, such as the number and range of sperm activity. But the research of WYP discussing reproductive function through the function of sertoli cells and germ cell has not been discovered.

Previous studies confirmed that the function of sertoli cells in seminiferous tubule is a necessary factor while adjusting germ cell's function. Normal sertoli cells could support spermatogenesis in every stage and control the differentiation, proliferation and number of germ cell.[9] Nuclear-associated antigen Ki67 (Ki67) is the marker of cell proliferation.[10] Androgen Receptor (AR), which is expressed in sertoli cells, is the most important molecular when androgen is taking effect on germ process.[11-12] Our previous studies indicated that cytokeratin-18 (CK-18), which is a part of cell skeleton,[13] could only express in immature sertoli cell and be used as a marker to examine mature level of sertoli cell. This article aimed to investigate whether WYP could facilitate reproductive function of young male rats or not by observing the changes in follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T), the changes in Ki67, AR and CK-18 as well as the changes in leydig cells and germ cells.

MATERIALS AND METHODS

Animals

Sixteen 20-day-old male Wistar rats weighing (50-60) g, were purchased from the Laboratory Animal Center of Beijing Vital River, Certificate of quality No. SCXK (jing) 2012-0001. The study was approved by the experimental animal ethics committee of Beijing University of Chinese Medicine.

Drugs and reagents

According to the 2005 edition of Pharmacopoeia of the People's Republic of China, WYP were composed of Gouqizi (*Fructus Lycii*), Tusizi (*Semen Cuscutae*), Fupenzi (*Fructus Rubi Chingii*), Cheqianzi (*Semen Plantaginis*), and Wuweizi (*Fructus Schisandrae Chinensis*) on the proportion of 8∶8∶4∶2∶1, then decocted and concentratedto set aside. Anti-Ki67 antibody, Anti-Androgen Receptor antibody, Anti-Cytokeratin 18 antibody, β-actin antibody (Abcam incorporation, Cambridge, USA). spin or vacuum [Total RNA Isolation System (Z3100)], Reverse Transcription System (A3500) (Promega corporation, Beijing, China); Power SYBR Green PCR Master Mix (Applied Biosystems, Shanghai, China). Realtime-RNA primer was synthesized Shanghai Sangon Biotech Co., Ltd. The sequence is shown in Table 1.

Instruments

Microtome (Leica Microsystems, Shanghai, China, CM1510), Inverted Fluorescence Microscope (Nikon Corporation, Shanghai, China, TS100), Fluorescent Quantitative PCR Instrument (Applied Biosystems, Shanghai, China, 7500).

Grouping and treatment

Sixteen 20-day-old male Wistar rats were randomly divided into 2 groups: control group and WYP treatment group (n = 8). The rats in the WYP treatment group were given intra gastrically medicated with WYP at 8.5 g/kg, the decoction were diluted to 1.7 g/mL with saline. The control group was given intragastrically the same volume of saline, once daily for 10 days.

Blood collection and tissue preparation

After anaesthesia with an intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g body weight), blood samples were collected from the aorta abdominal is of each animal, then blood samples were centrifuged for 10 min at 1000 × g, and the serum was separated and stored at − 20 ℃ for hormone assays. One testis was fixed in Modified Davidson's Fluid for 48 h, then embedded in paraffin, and sectioned at 5 μm thickness. The contra lateral testis was frozen in liquid nitrogen and stored at − 80 ℃ for the reverse transcription-polymerase chain reaction (RT-PCR) and western blot assay.

Hormone assays

The serum level of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) was tested by radioimmunoassay (RIA).

Hematoxylin-eosin (HE) staining

Testis sections were deparaffinized, and rehydrated in a graded ethanol series. Then the sections were subsequently stained in hematoxylin and eosin, dehydrated and mounted. For five visions each section was taken to evaluate the changes of seminiferous tubule, including the changes of morphology and number of germ cells.

Immunohistochemistry

The sections of testis were deparaffinized and rehydrat-

Table 1 Oligonucleotide primers used for quantitative real-time PCR

Gene	Sense primer	Antisense primer
β-actin	ACCGTGAAAAGATGACCCAGAT	CCAGAGGCATACAGGGACAA
Ki67	ACCTACCTTCAACGCTCTCTGA	TCCGCTTACTTCTGGACAATCT
AR	AAAGGGTTGGAAGGTGAGAGTC	GCGAGCGGAAAGTTGTAGTAGT
CK-18	CAGAAGAACCGTGAGGAACTG	GGCAGACTTGGTGGTGACTA