Journal of Steroid Biochemistry & Molecular Biology 145 (2015) 28–37

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

The prevention and treatment effects of tanshinone IIA on oestrogen/androgen-induced benign prostatic hyperplasia in rats

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ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 23 September 2014
Accepted 26 September 2014
Available online 5 October 2014

Keywords:
Benign prostatic hyperplasia
Tanshinone IIA
Androgen receptor
Oestrogen receptor α
Cyclins

ABSTRACT

Benign prostatic hyperplasia (BPH) is one of the major diseases of the urinary system in elderly men. Tanshinone IIA (Tan IIA) is the active ingredient extracted from the traditional Chinese medicine Salvia, and it has effects of anti-oxidation, anti-inflammation, vascular smooth muscle relaxation and tumour growth inhibition. The present study aimed to investigate the therapeutic potential of Tan IIA in the prevention and treatment of BPH. In a rat model of oestradiol/testosterone-induced BPH, Tan IIA inhibited the increase in the thickness of the peri-glandular smooth muscle layer, suppressed the expression of proliferating cell nuclear antigen (PCNA) in both prostate epithelial cells and stromal cells, downregulated the expression of androgen receptor (AR), oestrogen receptor α (ERα), cyclin B1 (CCNB1) and cyclin D1 (CCND1), and effectively prevented the development of the disorder. *In vitro*, Tan IIA inhibited the proliferation of human prostate stromal cell line WPMY-1 and epithelial cell line RWPE-1 in a dose- and time-dependent manner. In WPMY-1 cells, Tan IIA treatment arrested the cell cycle at the G2/M phase and downregulated the expression of CCNB1. However, in RWPE-1 cells, Tan IIA treatment arrested cell cycle at the G0/G1 phase and reduced the expression of CCND1. Tan IIA also reduced the expression of ERα and AR in WPMY-1 and RWPE-1 cells. These results suggest that Tan IIA can inhibit the growth of prostate stromal and epithelial cells both *in vivo* and *in vitro* by a mechanism that may involve arresting the cell cycle and downregulating ERα and AR expression.

1. Introduction

Benign prostatic hyperplasia (BPH) is one of the common urinary system diseases in elderly men [1]. The enlarged prostate volume causes physical compression of the urethra and results in anatomic bladder outlet obstruction (BOO), which seriously impacts the quality of life of elderly men [2]. BPH is characterised by hyper-proliferation of the epithelium and stroma, which coalesce into microscopic and macroscopic nodules in the prostate gland [3]. In BPH, the number of prostate epithelial and stromal cells increases by 9- and 37-fold, respectively, compared with a normal prostate [4].

Hormone imbalance plays an important role in the development of BPH. Normal levels of androgens and oestrogens synergistically maintain the development and growth of the prostate [5]. Androgens promote the differentiation of epithelial cells and the proliferation of mesenchymal cells *via* androgen receptor (AR) [6,7]. The prostate is also a target tissue for oestrogens. As elderly men increase in age, the ratio of androgens to oestrogens decreases in both the serum and the prostate tissue [8], and ERα is highly expressed in the prostate in both epithelial and stromal cells [9,10]. All these changes enhance the effects of oestrogen in the prostate of elderly men. A recent study has shown that an imbalance of the androgen level and enhanced oestrogenic effects were the main cause of BPH [11].

Salvia miltiorrhiza Bunge (Salvia) is a traditional Chinese medicine. The lipid-soluble ingredients of Salvia include tanshione I, tanshinone IIA, tanshinone IIB and cryptotanshinone [12].

Abbreviations: BPH, benign prostatic hyperplasia; Tan IIA, tanshinone IIA; PCNA, proliferating cell nuclear antigen; AR, androgen receptor; ERα, oestrogen receptorα; E2/T, oestradiol benzoate/testosterone propionate; PI, prostatic index; α-SMA, α-smooth muscle actin; SMC, smooth muscle cell; HPRT, hypoxanthine phosphoribosyltransferase 1; CCNB1, cyclin B1; CCND1, cyclin D1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CTL, control.

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http://dx.doi.org/10.1016/j.jsbmb.2014.09.026
0960-0760/

Table 1
Grouping method and drug manipulation (daily dose).

Group	E2/T[e] (in corn oil, s.c.[f]) 10 μg/1000 μg (per rat)	Tan IIA(in corn oil, i.p.[g]) 24 mg/kg (b.w.[i])	Finasteride (in PBS, i.g.[h]) 0.5 mg/kg(b.w.[i])
Sham[a]	0.1 ml blank corn oil	0.18 ml blank corn oil	0.1 ml PBS
Model[b]	0.1 ml E2/T	0.18 ml blank corn oil	0.1 ml PBS
Tan IIA[c]	0.1 ml E2/T	0.18 ml Tan IIA	0.1 ml PBS
Fin[d]	0.1 ml E2/T	0.18 ml blank corn oil	0.1 ml finasteride

[a] Sham = sham-operated group.
[b] Model = oestradiol benzoate/testosterone-induced BPH model group.
[c] Tan IIA = tanshinone IIA group.
[d] Fin = positive control finasteride group.
[e] E2/T = oestradiol benzoate/testosterone propionate.
[f] s.c. = subcutaneous injection.
[g] i.p. = intraperitoneal injection.
[h] i.g. = intragastric administration.
[i] b.w. = body weight.

Tanshinone IIA (C19H18O3, Tan IIA) has effects of anti-oxidation [13], anti-inflammation [14], heart protection [15] and vascular smooth muscle relaxation. Tan IIA also has cytotoxic effects on some tumour cells [16,17]. It can inhibit cell proliferation and induce tumour cell apoptosis in breast cancer [18], nasopharyngeal carcinoma [19], glioma [20], leukaemia [21–23], liver cancer [17,24,25] and pancreatic cancer [26].

Tan IIA has also been shown to impact the proliferation of prostate cancer cells. Tan IIA inhibited cell growth by inducing the endoplasmic reticulum stress response, inactivating the AR signalling pathway and activating the p53 signalling pathway in human prostate cancer cells [27–29]. Tan IIA could also induce prostate cancer cells to undergo mitochondria-dependent apoptosis and inhibit PI3 K/AKT signalling pathway activation [30]. In this study, we investigated a possible inhibitory effect of Tan IIA on BPH through *in vivo* and *in vitro* analyses. Our findings provide an experimental basis for developing new drugs for BPH prevention and treatment.

2. Materials and methods

2.1. Animals and hormonal manipulations

A total number of 40 adult male Wistar rats (250–300 g body weight) were purchased from Weitong-Lihua Experimental Animal Central (Beijing, China). Animal care and experiments were conducted in accordance with the guidelines of the Chinese Council on Animal Care and approved by the NanKai University Animal Care and Use Committee. BPH induction in the rat model was conducted using our previously published method [31–33]. Briefly, 10 rats were randomly separated as a sham-operated group, and 30 rats were castrated and randomly assigned to three experimental groups with 10 rats per group. All rats were maintained in an animal facility under standard laboratory conditions for 3 weeks. The experimental treatments of each group are listed in Table 1. Oestradiol benzoate and testosterone propionate were purchased from Jinyao Amino Acid Manufacturer, Tianjin, China. Tan IIA was purchased from Zhong Xin Medicine Company, Tianjin, China. The dose of Tan IIA was determined by our preliminary experiments (data not shown), which were based on findings of other investigators [28,34]. Finasteride, a positive control for experimental drugs in BPH studies [32,35], was obtained from Hangzhou MSD Pharmaceutical Company Limited, Hangzhou, China. The dose of finasteride was determined according to our previous studies and other reports [32,36]. The drugs were administered once daily for 28 days and weighed weekly during the experiments. The rats were euthanized and weighed 48 h after the last injection. The whole prostates were dissected and weighed for calculating the prostatic index (PI) by the following formula: PI = gross wet weight of prostate/weight of whole animal × 100% [31,37]. After prostate weighing, the dorsal lobe, ventral lobe and anterior lobe of the prostates were isolated and weighed separately. The ventral lobe was fixed in phosphate-buffered formalin and embedded in paraffin for histological and immunohistochemical studies.

2.2. Histological and immunohistochemical studies

Haematoxylin and eosin (H&E) staining and immunohistochemical (IHC) staining were conducted as previously described [31]. Briefly, 5-μm sections were deparaffinised in xylene and rehydrated in a graded series of alcohol. One section was stained with haematoxylin and eosin for histological examination, and other sections were processed for immunohistochemistry using the avidin–biotin–peroxidase complex method. Thus, the endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 10 min, followed by incubation with 10% cow serum for 30 min at room temperature. The sections were incubated with primary antibodies for 2 h at 37 °C. The primary antibodies used for IHC staining are listed in Table 2. A biotinylated secondary antibody was added for 30 min at 37 °C, followed by peroxidase-labelled streptavidin. Then, chromogen 3′,3-diaminobenzidine was added and counterstained with hematoxylin. Secondary antibodies used were biotinylated goat anti-rabbit IgG (1/200; Product No.

Table 2
Characteristics of the primary antibodies.

Primary antibodies	Host species	Supplier	IHC/Immunoblottting	Dilution	Clone/code
a-SMA	Mouse	Sigma–Aldrich, St. Louis, MO, USA	IHC	1/400	A 2547
PCNA	Mouse	Santa Cruz Biotechnology, CA,USA	IHC	1/100	sc-25280
ERα	Rabbit	Santa Cruz Biotechnology,CA, USA	IHC	1/500	sc-542
AR	Rabbit	Santa Cruz Biotechnology, CA,USA	IHC	1/500	sc-816
CyclinB1	Rabbit	Boster, Wu Han, China	Immunoblottting	1/200	BA0766
CyclinD1	Rabbit	Boster, Wu Han, China	Immunoblottting	1/200	BA0770
ERα	Rabbit	Santa Cruz Biotechnology,CA, USA	Immunoblottting	1/1000	sc-542
AR	Rabbit	Santa Cruz Biotechnology,CA, USA	Immunoblottting	1/1000	sc-816
GAPDH	Mouse	Santa Cruz Biotechnology, CA,USA	Immunoblottting	1/1000	sc-166574