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Research Article

The therapeutic effects of docosahexaenoic acid on oestrogen/androgen-induced benign prostatic hyperplasia in rats

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ABSTRACT

Benign prostatic hyperplasia (BPH) is one of the major disorders of the urinary system in elderly men. Docosahexaenoic acid (DHA) is the main component of n-3 polyunsaturated fatty acids (n-3 PUFAs) and has nerve protective, anti-inflammatory and tumour-growth inhibitory effects. Here, the therapeutic potential of DHA in treating BPH was investigated. Seal oil effectively prevented the development of prostatic hyperplasia induced by oestradiol/testosterone in a rat model by suppressing the increase of the prostatic index (PI), reducing the thickness of the peri-glandular smooth muscle layer, inhibiting the proliferation of both prostate epithelial and stromal cells, and downregulating the expression of androgen receptor (AR) and oestrogen receptor α (ERα). An in vitro study showed that DHA inhibited the growth of the human prostate stromal cell line WPMY-1 and the epithelial cell line RWPE-1 in a dose- and time-dependent manner. In both cell lines, the DHA arrested the cell cycle in the G2/M phase. In addition, DHA also reduced the expression of ERα and AR in the WPMY-1 and RWPE-1 cells. These results indicate that DHA inhibits the multiplication of prostate stromal and epithelial cells through a mechanism that may involve cell cycle arrest and the downregulation of ERα and AR expression.

Abbreviations: BPH, benign prostatic hyperplasia; DHA, Docosahexaenoic acid; PCNA, proliferating cell nuclear antigen; AR, androgen receptor; ERα, oestrogen receptor α; ERβ, oestrogen receptor β; GPR30, G protein-coupled receptor 30; 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

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Introduction

Benign prostatic hyperplasia (BPH) is one of the major diseases of the urinary system in men over 50 years old [1]. The increase in prostate volume causes the physical compression of the urethra and clinically manifests as lower urinary tract symptoms (LUTS), which seriously impact the quality of life of elderly men [2]. Histopathologically, BPH is characterised by hyperplasia of both glandular epithelial and stromal cell compartments that together result in an expansion of the prostate gland [3,4], and the amounts of epithelial and stromal cells are greatly increased compared with normal tissue [5].

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), the main components of n-3 polyunsaturated fatty acids (n-3 PUFAs) [6], are primarily found in fish oils and seal oils [7,8]. DHA improves memory abilities of nondemented, elderly individuals with memory problems [9]. It is also used to treat coronary heart disease and hypertriglyceridemia, fight inflammation and improve paediatric nonalcoholic fatty liver disease [10–12]. Furthermore, DHA is cytotoxic to some tumour cells and inhibits the proliferation and induces tumour cell apoptosis in breast cancer, lung adenocarcinoma, gastric cancer, hepatocellular carcinoma, colon cancer and melanoma [13–18].

In addition, DHA has also been shown to attenuate growth and induce apoptosis in prostate cancer cells. It upregulates the expression of SDC-1 by activating PPARγ, thus inhibiting activation of the PDK1/Akt/Bad signalling pathway and inducing apoptosis in the human prostate cancer cell lines PC-3 and LNCaP [19,20]. DHA selectively enhanced the sensitivity of human prostate cancer LNCaP cells and Pac MetUT1 cells to oxidative stress by modulating NF-κB [21,22]. DHA can suppress the proliferation of LNCaP cells induced by androgen, reduce the expression of PSA in a dose-dependent manner and increase the expression of c-jun to disrupt the transcriptional activity of AR [23]. Herein, in vivo and in vitro studies demonstrated that DHA is effective in the prevention and treatment of BPH. Furthermore, we also investigated the possible underlying molecular mechanism. These findings provide an experimental basis for finding new drugs that are applicable to BPH research and therapy.

Materials and methods

Animals and hormonal manipulations

A total of 40 adult male Wistar rats, weighing 250–300 g, were obtained from Weitong-Lihua Experimental Animal Central (Beijing, China). Animal care procedures and experiments were conducted following the guidelines of the Chinese Council on Animal Care and were approved by the NanKai University Animal Care and Use Committee. BPH induction in the rat was performed with our previous method [24–26]. Briefly, 10 rats were randomly separated into a sham-operated group, and the other 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 treatment of each group is listed in Table 1. Oestradiol benzoate and testosterone propionate were purchased from Jinyao Amino Acid Manufacturer, Tianjin, China. The dose of seal oil (contains 12% DHA and 18% EPA, Qi Kang Science and Technology Ltd., Tianjin, China) used in the animal experiment was determined using the method of equal effective dose conversion among different animals and based on reports published by other investigators [27–29]. In addition, Finasteride (MSD Pharmaceutical Company Ltd., Hangzhou, China) was used as a positive control for the experimental drugs in the BPH studies [25,30], and the dose of which was determined based on our previous studies and other reports [25,31]. The drugs were administered once daily for 28 days and the weights were recorded weekly. 48 h after the last injection, blood samples were taken under anaesthesia. Then, the rats were euthanized and weighed. The prostates were dissected and weighed to calculate the prostatic index (PI) using the following formula: PI = gross wet weight of prostate/weight of whole animal × 100% [24,32]. Afterwards, the dorsal, ventral and anterior lobes of the prostates were isolated and weighed separately. Part of ventral lobe was fixed in phosphate-buffered formalin and embedded in paraffin for histological and immunohistochemical (IHC) studies. The serum is separated after centrifuging 2500g for 10 min. The levels of DHA in the serum and the remaining prostate ventral lobe of different groups were then detected using 22 C six acid (DHA) ELISA Kit (Jiang Lai Biotechnology Co., Ltd., Shanghai, China) according to manufacturer's instructions.

Histological and IHC studies

Haematoxylin and eosin (H&E) staining and IHC staining were conducted as previously described [24]. Briefly, 5-μm sections were deparaffinised in xylene and rehydrated in a graded series of alcohol. One section was used for staining with H&E for histological examination, and the other sections were processed for immunohistochemistry using the avidin–biotin–peroxidase complex method. Next, 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. The sections were then incubated in biotinylated secondary antibody for 30 min at 37 °C followed by a brief incubation with peroxidase-labelled streptavidin. Then, chromogen 3′,3-diaminobenzidine was added, and the sections were counterstained with haematoxylin. The secondary antibodies used were biotinylated goat anti-rabbit IgG (1/200; Product no. ab64256, Abcam, CA, USA) and biotinylated goat anti-mouse IgG (1/200; Product no. ab64255, Abcam, CA, USA). The peroxidase-labelled streptavidin (Streptavidin-HRP, Product no. BA1088, Boster, Wuhan, China) was used at a dilution of 1/200. For a negative control, the primary antibodies were replaced with non-specific immunoglobulins, a purified mouse IgG antibody (Product no. A7028; Beyotime Inc., Shanghai, China) and a purified rabbit IgG antibody (Product no. A7016; Beyotime Inc., Shanghai, China).

Light microscopy was performed using an Olympus microscope CX-41 (Olympus, Tokyo, Japan) as previously described [25]. Briefly, the thickness of the peri-glandular SMC (SMA-positive) layer was measured with an ocular micrometre (AX0067, Olympus, Tokyo, Japan) in units of 2.5 μm at 400 × magnification. For cell number determination, positive cells in the prostate stromal and epithelial tissues in a unit area of 250 μm × 250 μm at 400 × magnification were counted. The investigator was blinded to the

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