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Protective effects of seahorse extracts in a rat castration and testosterone-induced benign prostatic hyperplasia model and mouse oligospermatism model

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ABSTRACT

This study investigated the effects of seahorse (*Hippocampus* spp.) extracts in a rat model of benign prostatic hyperplasia (BPH) and mouse model of oligospermatism. Compared to the sham operated group, castration and testosterone induced BPH, indicated by increased penile erection latency; decreased penis nitric oxide synthase (NOS) activity; reduced serum acid phosphatase (ACP) activity; increased prostate index; and epithelial thickening, increased glandular perimeter, increased proliferating cell nuclear antigen (PCNA) index and upregulation of basic fibroblast growth factor (bFGF) in the prostate. Seahorse extracts significantly ameliorated the histopathological changes associated with BPH, reduced the latency of penile erection and increased penile NOS activity. Administration of seahorse extracts also reversed epididymal sperm viability and motility in mice treated with cyclophosphamide (CP). Seahorse extracts have potential as a candidate marine drug for treating BPH without inducing the side effects of erectile dysfunction (ED) or oligospermatism associated with the BPH drug finasteride.

1. Introduction

Benign prostatic hyperplasia (BPH) is a common disease in the aging male, which is histologically characterized by significant proliferation of the epithelial cells within the prostate gland leading to lower urinary tract symptoms (LUTS) (Gacci et al., 2012). The drug finasteride is used for the treatment of BPH and decreases dihydrotestosterone (DHT) production by inhibiting the 5α-reductase which converts testosterone to DHT, and can induce a statistically significant reduction in the volume of the enlarged prostate in males with BPH (Kaplan et al., 2011). However, the onset of finasteride-related adverse events are frequently reported at the initiation of therapy, including impotence, erectile dysfunction (ED) and ejaculatory dysfunction (EJD), testicular pain and male infertility due to a reduced sperm count (Chiba et al., 2011).

Seahorses (*Hippocampus* spp.), highly unusual marine animals in the teleost group, are recognized for the unique feature of male pregnancy as well as their medicinal value (Koldewey

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and Martin-Smith, 2010). Dried seahorses are used extensively in traditional medicine, particularly traditional Chinese medicine (TCM). *Hippocampus trimaculatus* Leach (three spottedseahorse) and *Hippocampus kuda* Bleeker are highly valued species in both medicinal and aquarium trades, and male and female seahorses have been used to improve sexual function in TCM (Xu et al., 2000).

We initiated aquaculture of *H. trimaculatus* Leach and *H. kuda* Bleeker in 1990, to reduce overexploitation of the wild seahorse populations (Sheng et al., 2006, 2007). Our previous pharmacological studies suggested that seahorse extracts possessed not only anti-thrombotic pharmacological effects, but could also enhance sexual function and tonify the kidney (Xu and Xu, 1997; Xu et al., 2000; Mei et al., 2005).

According to the theory of TCM for BPH, we speculated that seahorse extracts may exert pharmacological effects in BPH by tonifying the kidney and promoting blood circulation. In the present study, the effects of different seahorse extracts (*H. kuda* Bleeker and *H. trimaculatus* Leach) were examined in a castration and testosterone-induced rat model of BPH, and a cyclophosphamide (CP)-induced mouse model of oligospermatism.

2. Materials and methods

2.1. Animals

Ninety adult male Sprague–Dawley rats weighing 260–280 g and eighty adult Chinese KM male mice weighing 18–22 g were used in this study, in accordance with the guidelines approved by the Animal Ethics Committee of Sun Yat-sen University. All animals housed under a 12:12 h light: dark cycle at a room temperature of 25 °C with free access to food and water (SPF, Certificate No SYXK 2004-0020).

2.2. Sample preparation

Dried seahorses (*H. trimaculatus* Leach, HTL; *H. kuda* Bleeker, HKB) were collected from the Research Center of Yi Da Zhou Marine Biology, Guangdong, China. The seahorses were separated by species (HTL and HKB) and sex (female, f; male, m or an equal mixture of females and males, fm) resulting in the mixtures fHTL, mHTL, fmHTL, fHKB, mHKB and fmHKB. One kilogram of dried seahorse samples were separately extracted using acetic ether. Finasteride was obtained from Merck (Hangzhou, China). Testosterone propionate was manufactured by Guangzhou Minxing Pharmacy Co. Cyclophosphamide (CP) was manufactured Hengrui Pharmacy Co. (Jiangsu, China). All other chemicals used in the study were of analytical grade.

2.3. Castration and testosterone-induced rat model of BPH

To exclude the influence of intrinsic testosterone, the rats were anesthetized by intraperitoneal (i.p.) injection of pentobarbital, and castrated after intramuscular administration of penicillin (7.14×10^4 IU/kg body weight). Castration was performed by removing the testicles and epididymal fat through the scrotal sac, according to a previously published method (Coppenolle et al., 2000). One week later, the rats were divided into nine groups ($n = 10$ each) as follows: (1) sham operated plus vehicle-treated control group; (2) BPH model control group, which received testosterone propionate (TP) (0.5 mg/kg body weight, s.c.); (C) finasteride group, which received finasteride (0.45 mg/kg body weight, p.o.) and TP (0.5 mg/kg, s.c.); (D–F) the fHTL, mHTL, mfHTL, fHKB, mHKB and mfHKB groups, which received extracts of fHTL, mHTL, mfHTL, fHKB, mHKB or mfHKB, respectively, (equivalent to 0.9 g dry seahorse/kg body weight, p.o.) by oral administration and TP (0.5 mg/kg, s.c.). The rats were treated once a day for 30 consecutive days.

2.4. Evaluation of erectile function

Erectile function was assessed via cavernous nerve electrostimulation, as described previously (Mills et al., 1992). Intracavernosal pressure was evoked using electro-stimulations of 50 V at 30 Hz for a duration of 0.2 ms, and the latency of erection after electro-stimulation was recorded using electrophysiological apparatus.

2.5. Body and prostate weights

On day 31, the animals were placed under light ether anesthesia after overnight fasting. Blood samples were drawn from the retro-orbital blood vessels and then the rats were euthanized. Total serum ACP activity was analyzed using an acid phosphatase assay kit. Immediately after euthanasia, the whole prostates were removed and weighed. The mean body weight and prostate index (PI; prostate to body weight ratio, mg/100 g) were calculated for each group. The rat penes were excised, homogenized, and centrifuged at 10,000*g* for 30 min. The supernatant was collected and NOS activity was measured using a NOS assay kit (Jianchen Biotech, Co., Nanjin, China).

2.6. Histological studies and immunohistochemical analysis

After prostatic weight measurements, the ventral prostate lobes were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) for routine histological analysis. 10×40 magnification images were obtained from randomly selected sections to measure 2D parameters including the area, perimeter, maximum diameter, and minimum diameter of the ventral prostate lobes and the thickness of the epithelial cell layer using BI-2000 software.

The remainder of each prostate was stored at −70 °C and used for immunohistochemical analysis. Immunohistochemical staining for PCNA and bFGF were performed using the streptavidin-peroxidase (SP) method (Nardone et al., 1999). Cells with brownish-yellow granules in the nuclei were regarded as PCNA-positive, and the PCNA index was calculated as the percentage of PCNA-positive cells from more than 10×40 fields of view for each specimen. The expression of bFGF in the prostate was calculated by converting the number of pixels to a calibrated optical density (OD).