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ORIGINAL ARTICLE

Effects of *Panax ginseng* on the nerve growth factor expression in testosterone induced benign prostatic hyperplasia



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KEYWORDS

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Abstract The prostatic hyperplasia in benign prostatic hyperplasia (BPH) leads to obstructive micturition symptoms. Previous studies showed that pontine micturition center (PMC), ventrolateral periaqueductal gray (vlPAG), and medial preopticonucleus (MPA) regions in the brain have been known to regulate the urinary bladder function. The present study shows the influences of *Panax ginseng* on nerve growth factor (NGF) expressions in PMC, vlPAG, and MPA regions in the brain. Wistar rats were used for the present study. The rats split into four groups; 4 groups ($n = 6$) in control group, BPH-induced group, BPH-induced and *P. ginseng*-treated group, and BPH-induced and finasteride-treated group. BPH in rats was induced by testosterone and the animals were evaluated for NGF expression in PMC, vlPAG, and MPA regions in the brain. The NGF expression was identified using immunohistochemistry (IHC). The NGF expression by IHC showed spots with dark brown color. In our results, NGF expressions in PMC, vlPAG, and MPA regions in the brainstem of the BPH-induced group showed increase than the control animal. These increased NGF expressions in three regions were decreased using treatment with *P. ginseng* (200 mg/kg). These results suggest that *P. ginseng* has therapeutic effects on the symptoms of BPH and is associated

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with the regulation of NGF expression in the brain. In conclusion, the administration of *P. ginseng* helps nerve growth factor activation.

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1. Introduction

The most common cause of obstruction in old man was observed in benign prostatic hyperplasia (BPH). BPH shows a prostatic enlargement (Foo, 2010; Nickel, 2006). Previous study reported that neurological factors contributed to smooth muscle dysfunction as risk factor in the development of symptoms of LUTS (Skolarikos et al., 2004).

The regulation of micturition links between various regions in the brainstem and extensive tracts (Fowler et al., 2008). Many neurons in the brainstem regions are involved in the regulation of the micturition (Rickey et al., 2008). The PMC plays an important role in controlling urinary bladder function (Fowler et al., 2008; Yoshimura et al., 2014). Stimulation of neurons in the PMC region contributed to bladder contractions, relaxation of the bladder neck, and external urethral sphincter (Chancellor and Yoshimura, 2004), which results in micturition. And periaqueductal gray (PAG) and medial preoptic nucleus (MPA) reported the relationship with PMC (Blok and Holstege, 1994). PAG and MPA regions of the hypothalamus in the brain associated with the control of urinary bladder function. The connection between PAG and PMC also is contributed to micturition reflex (DasGupta et al., 2007; Blok and Holstege, 1994; Rickey et al., 2008). And the ventrolateral PAG (vlPAG) plays an important role in micturition reflex (Blok and Holstege, 1994; Matsumoto et al., 2004). It was reported that electrical or chemical stimulation in the LUT showed changes in neuronal activity (Bon et al., 1996; Blok, 2002).

Several studies reported that nerve growth factor (NGF) was related to the control of micturition (Chung et al., 2008; Cho et al., 2014). And Stimulation of the bladder increased expressions of NGF in the PAG, PMC, and spinal cord (Dinis et al., 2004; Kavia et al., 2005). NGF also is an important modulator of voiding. It is released in the spinal cord or the urinary bladder (Seki et al., 2004). The NGF has been used to assess neuronal activity (Lee et al., 2003).

Previous study showed that the *Panax ginseng* CAMAYER (*P. ginseng*) protected development of prostate in the rats (Kim et al., 2014, 2015a,b; Park et al., 2017a,b). However, PMC, vlPAG, and MPA regions in the brain have not been investigated. The present study explores the influences of *P. ginseng* on NGF expression in the brain.

2. Material and methods

2.1. Reagents and chemicals

The reagents and chemicals used in this study were procured from the Sigma chemicals (USA). All the reagents and chemical used were of analytical grade.

2.2. Preparation of the *P. ginseng*

P. ginseng was kindly gifted from the Department of Medicinal Crop Research (Eumsung-gun, Chungbuk, Korea) (Kim et al., 2014, 2015a,b).

2.3. Animals

7 week male Wistar rats with an average body weight of 250 ± 10 g were purchased from Central Lab Animal Inc, Korea for the present study. The in vivo and other experiments were performed according the standard methodology of the Korean National Health Institute of Health Animal Facility and it was authenticated and confirmed by the ethical committee of Kyung Hee University.

2.4. Induction of BPH and treatments

Firstly, the rats were operated for orchietomy, except the control group. After orchietomy, they were randomly assigned to the following groups: (A) control group; (B) BPH induced testosterone group; (C) BPH-induced and *P. ginseng*-treated group, 200 mg/kg, administered orally; (D) BPH-induced and finasteride-treated group as the positive treated control. The materials were supplemented to the animal every day up to four weeks. After 4 weeks, all rats in the four groups were sacrificed and fresh prostate was selected for the micromorphological studies.

2.5. Immunohistochemistry (IHC)

The brain of rats were cut into 35 µm thick paraffin-sections using microtome. The sections were attached on slide. Reagents such as peroxides quenching with 3% H₂O₂, primary NGF antibody, biotinylated secondary antibodies (1: 1000), phosphate buffer saline, streptavidin-HRP and diaminobenzidine tetrahydrochloride (DAB) were used. The images were noted at 100×.

2.6. Statistical analyses

Standard error and mean were calculated for all the experiments. ANOVA and Tukey test were applied to evaluate significant differences between the groups. All statistical analyses were done using the SPSS for Windows (Micro Soft, USA).

3. Results

3.1. Effect of *Panax ginseng* on prostatic hyperplasia

Photomicrographs of NGF-positive cells in MPA, vlPAG, and PMC regions are shown in Figs. 1–3. The NGF expression by

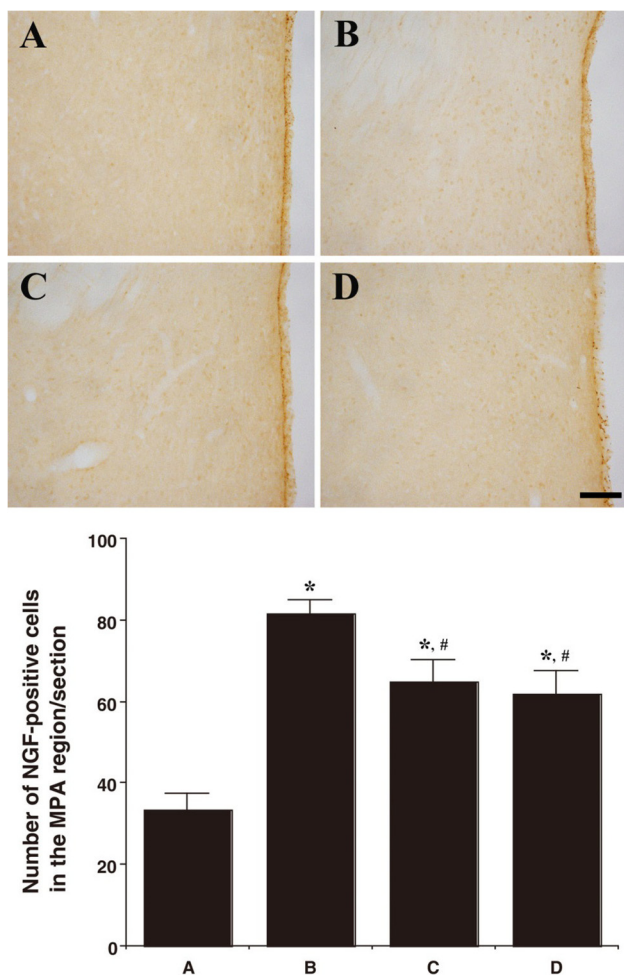


Figure 1 NGF expressions in MPA region of the neuronal voiding centers. The sections were stained for NGF immunoreactivity (brown). The scale bar represents 150 μ m. (A) Control group (B) BPH-induced group (C) BPH-induced and *P. ginseng*-treated group (D) BPH-induced and finasteride-treated group. Upper: Photomicrographs of NGF-positive cells in the neuronal voiding centers. Lower: Number of NGF-positive cells in each group. *Represents $P < 0.05$ compared to the control group. #Represents $P < 0.05$ compared to the BPH-induced group.

IHC showed spots with dark brown color. In the MPA region, the number of NGF-positive cells was 33.66 ± 4.03 /section in the control group, 81.75 ± 3.28 /section in the BPH-induced group, 65.00 ± 5.34 /section in the BPH-induced and *P. ginseng*-treated group, and 68.50 ± 4.78 /section in the BPH-induced and finasteride-treated group (Fig. 1). In the vIPAG region, the number of NGF-positive cells was 41.66 ± 4.67 /section in the control group, 104.25 ± 4.75 /section in the BPH-induced group, 77.91 ± 5.13 /section in the BPH-induced and *P. ginseng*-treated group, and 81.00 ± 5.78 /section in the BPH-induced and finasteride-treated group (Fig. 2). In the PMC region, the number of NGF-positive cells was 35.33 ± 3.69 /section in the control group, 77.75 ± 4.87 /section in the BPH-induced group, 58.58 ± 4.24 /section in the BPH-induced and *P. ginseng*-treated group, and 60.66 ± 3.93 /section in the BPH-induced and finasteride-treated group (Fig. 3). The present results revealed

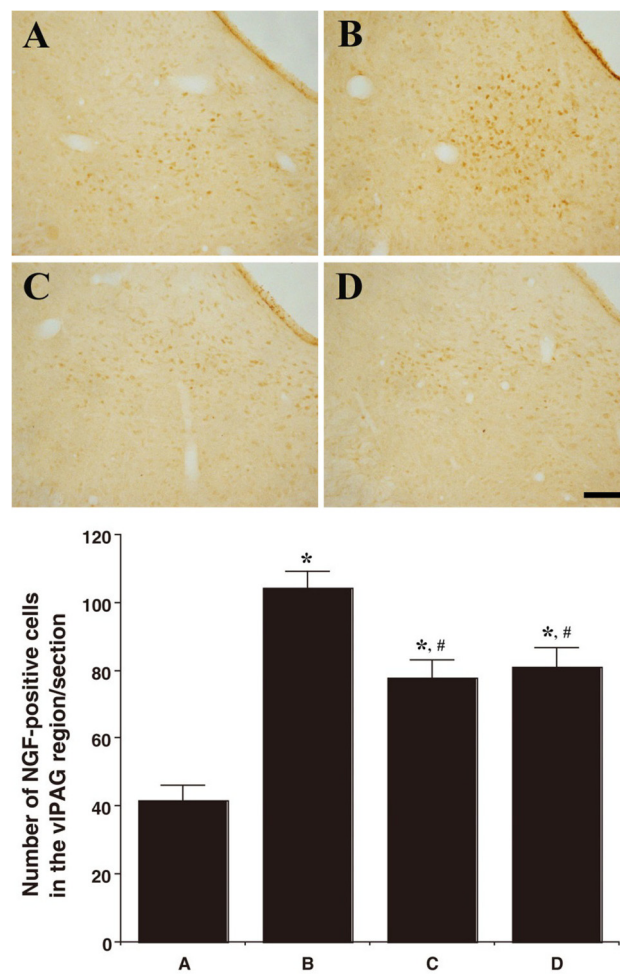


Figure 2 NGF expressions in vIPAG region of the neuronal voiding centers. The sections were stained for NGF immunoreactivity (brown). The scale bar represents 150 μ m. (A) Control group (B) BPH-induced group (C) BPH-induced and *P. ginseng*-treated group (D) BPH-induced and finasteride-treated group. Upper: Photomicrographs of NGF-positive cells in the neuronal voiding centers. Lower: Number of NGF-positive cells in each group. *Represents $P < 0.05$ compared to the control group. #Represents $P < 0.05$ compared to the BPH-induced group.

that NGF expressions were increased in testosterone induced BPH in Wistar rats ($P < 0.05$). The increased NGF expression in MPA, vIPAG, and PMC regions showed that *P. ginseng* treatment significantly decreased ($P < 0.05$).

4. Discussion

In recent study, we reported the influence of *P. ginseng* on testosterone (Kim et al., 2014). The study revealed that *P. ginseng* treatment significantly decreased prostate size and prostate weight compared to those of the normal group. Additionally, *P. ginseng* decreased the expression of EGFR and BCL2 in prostate tissue (Kim et al., 2014). The results suggested that *P. ginseng* may have inhibited alpha-1-adrenergic receptor and suppressed BPH development in the studied rats. However, previous study did not investigate whether many

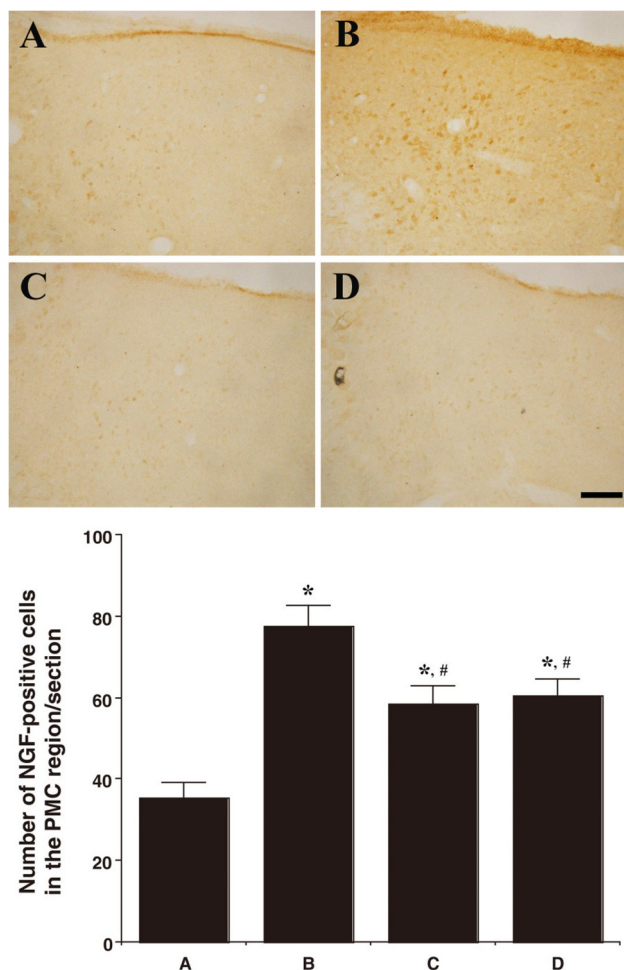


Figure 3 NGF expressions in PMC region of the neuronal voiding centers. The sections were stained for NGF immunoreactivity (brown). The scale bar represents 150 μ m. (A) Control group (B) BPH-induced group (C) BPH-induced and *P. ginseng*-treated group (D) BPH-induced and finasteride-treated group. Upper: Photomicrographs of NGF-positive cells in the neuronal voiding centers. Lower: Number of NGF-positive cells in each group. *Represents $P < 0.05$ compared to the control group. #Represents $P < 0.05$ compared to the BPH-induced group.

neuron regions involved in the control of the bladder, such as PMC, MPA, and vIPAG, were related to *P. ginseng* treatment. The present study evaluated the effects of *P. ginseng* on NGF including PMC, MPA, and vIPAG.

The results of the present study showed that NGF expression in PMC, MPA, and vIPAG regions of brainstem were increased in the BPH group compared to those of the normal group. The increased NGF expression in PMC, MPA, and vIPAG significantly showed decrease in *P. ginseng* treatment group. Overactive bladder (OAB), known as OAB syndrome, is characterized by involuntary bladder contraction (Reynolds et al., 2016). The OAB can lead to the development of BPH. Previous study showed that the incidence of OAB associated with BPH is around 40–75% (Blaivas et al., 2009; Abrams et al., 2002). The storage sub-classification category of LUTS is the same as the OAB (Singh et al., 2015). Several

studies suggested that micturition involves process connection between urinary tract and neurons of brainstem. The NGF has been known as an important modulator of urinary bladder (the storage and voiding of urine). NGF is observed by urothelial tissue and smooth muscle cells. It is one of neurotrophins. It is reported that NGF was involved in the neuro plasticity pathways (Ochodnický et al., 2012). The level of NGF elevated in condition with LUT. Recently, Ergin et al. suggested the NGF level in urine could be used for diagnosis and assessment of dysfunctional voiding, as biomarkers (Ergin et al., 2016). The central NGF production in the injured spinal cord could regulate micturition (Ochodnický et al., 2012).

5. Conclusion

In summary, we confirmed that *P. ginseng* has inhibited effect to develop prostate hyperplasia and prostate weight. Among neuron regions involved in the control of the bladder, PMC, MPA, and vIPAG regions showed that NGF expressions were increased in BPH. And *P. ginseng* significantly decreased the increased NGF expressions in three regions. Thus, these results suggested that *P. ginseng* could be used as an effective treatment to inhibit BPH. The inhibited effect of *P. ginseng* may be associated with modulation NGF expressions in PMC, MPA, and vIPAG regions of the brain.

Author disclosure statement

No competing financial interests exist.

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