Anti-Inflammatory Effects of Graviola Stem Bark Extracts on the Testosterone-induced Benign Prostatic Hyperplasia Model in Rats

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a common age-related disease. However, pathophysiology for the development of BPH is not clearly known yet. Inflammation plays a critical factor for BPH. Graviola as a traditional medicine has anti-inflammatory effect. This study aims to investigate the potential ameliorative effects of graviola extracts against the development of BPH by inflammatory cytokines and growth factors secreted from mast cell activation. BPH was subcutaneously induced by testosterone propionate (TP; 5 mg/kg) once per day for 4 weeks. Graviola stem bark (GV) extracts (70, 150, 300 mg/kg) or finasteride (1 mg/kg) as a positive control were administrated by gavage feeding once per day concomitantly with TP. Prostatic index (PI) was calculated by ratio prostatic tissue weight to body weight, epithelial thickness was determined by hematoxylin and eosin staining, target molecules for BPH by enzyme-linked immunosorbent assay, inflammatory cytokines, growth factors and migration molecules by reserve transcriptase-polymerase chain reaction, expression of androgen receptor (AR), 5α-reductase, COX-2, inflammatory cytokines (interleukin [IL]-6, IL-8), growth factors (insulin-like growth factor, epidermal growth factor), TGF-β, CCL2 and CCR2, protein expression of AR and TGF-β, and quantification of mast cell population in prostatic tissues of BPH, compared to TP group which increased all the responses in BPH versus the negative control group. Finasteride reduced all the responses similar to or less than those in GV extracts. The data suggest that GV extracts may attenuate the development of BPH caused via inflammation and hypertrophy through inhibiting the inflammatory cytokines, growth factors and COX-2 secreted/synthetized from mast cell activation, which are infiltrated into stromal area of prostatic tissues, and that some single components in GV extracts may offer a potential treatment for BPH.

Keywords: Benign prostatic hyperplasia; Inflammation; Hypertrophy; Mast cells
INTRODUCTION

Benign prostatic hyperplasia (BPH) belongs to the most frequent diseases in aging men. BPH is demonstrable in 30%–40% of men in the decade of life, and its prevalence increases almost linearly to 70%–80% in those older than 80 years.\(^1\) BPH is characterized by a benign enlargement of prostatic tissues, and this kind of benign proliferation induces the lower urinary tract symptom.\(^1,2\)

In the development of BPH, the dominant role of androgen and androgen receptor (AR) system is well defined.\(^1\) AR, which is widely expressed in the epithelium and stroma of prostatic tissues in BPH, is activated by the potent dihydrotestosterone (DHT).\(^4,5\) Synthesis of DHT is controlled by 5α-reductase (5α-R) enzyme.\(^4,6\) However, the pathophysiology of BPH is still incomplete. Many reports for BPH focus on the expression of growth factors and inflammatory cytokines, showing the important role of acute and chronic inflammation in the development of BPH.\(^3,7,8\) It has been recently reported that chronic inflammation plays a role in the stroma or epithelial proliferation in BPH.\(^5\) Elevated levels of inflammatory cells including T- and B-lymphocytes and macrophages were detected to the interstitial area and surrounding epithelial glands of human BPH tissues.\(^9\) Infiltration of these inflammatory cells into prostatic tissues produces proinflammatory cytokines (interleukin [IL]-6, IL-8)\(^12\) and growth factors.\(^13\) Thus, prostatic inflammation is associated with a significantly increased risk of acute urinary retention in the BPH.

Mast cells, which are known as major immune cells in innate immunity,\(^14\) are involved in tissue remodeling,\(^15\) angiogenesis,\(^16\) and immunomodulation in a variety of human tumors\(^17\) including prostatic cancer\(^28,39\) by releasing and synthesized potent mediators, proteases, cytokines (IL-4, -5, -6, -8, etc.) and growth factors (nerve growth factor, stem cell factors etc.), etc.\(^16\) Mast cells infiltrated into prostatic tissues promote BPH development by inflammatory cytokines.\(^20,21\) Thus, there have been many reports concerning the role of mast cells in human BPH, although the exact role of mast cells is still controversial.

Graviola, which is known as soursop and \textit{Annona muricata L.}, is a small deciduous tropical evergreen fruit tree, and is widely grown and distributed in tropical region around the world.\(^22\) Over 121 phytochemical ingredients including alkaloids, phenols, flavonoids, carotenoids and acetogenins have been reported in graviola extracts prepared from different plant parts.\(^22\) It has been reported that the specific bioactive constituents may be used as the traditional medicines of the various diseases, such as, arthritis,\(^23\) hypertension,\(^24\) mamaria,\(^25\) antidiabetics and anti-oxidant,\(^26,28\) anti-inflammation,\(^28,29\) and anticancer,\(^22,30-32\) etc. Additionally, graviola may have a few side effects because it is a natural plant used as the traditional medicines. However, there are no reports how graviola components influence the development of BPH.

The 5α-R inhibitors, finasteride (Fina) and dutasteride, are frequently and effectively used for the treatment of androgenic alopecia and BPH by reducing DHT levels.\(^5,33\) However, both agents have been shown to produce an increase in the incidence of sexual dysfunction, mainly impotence and ejaculation disorders.\(^33\) Repeated Fina administration induces depression-like behavior in male rats.\(^18\)

We hypothesized that graviola stem bark (GV) extracts, which have little side effects as a natural material, may alleviate the inflammation and hypertrophy in BPH caused through...
inflammatory cytokines and growth factors secreted from activation of inflammatory cells like mast cells which are infiltrated into prostatic tissues of BPH. We observed that GV extracts ameliorated inflammation and hypertrophy in the prostatic tissues of BPH model in rats. Thus, the data suggest that GV extracts may be developed as a promising therapeutic agent in order to protect the BPH.

METHODS

Drugs and chemicals
GV extracts and Platycodon grandiflorus (Jacq.) A. DC. (PG) extracts were provided by Hyunsung Vital Co., Ltd. (Seoul, Korea). In brief, the dried GV or PG roots (50 g) was added in 1,000 mL distilled water, and extracted at 90°C for 24 hours, and then filtered using filter paper. The extracts were evaporated by Liquefied extractor (Hyunsung Vital Co. Ltd.) to yield a powder of GV (extraction yield, 9.6%) or PG (extraction yield, 11.5%) extracts.

Graviola contains a variety of ingredients from different plant parts, such as fruits, seeds, roots, leaves and stem bark. We chose GV because it showed superior effect on expression of AR in prostatic tissues of BPH model in rats prepared in preliminary experiments. Testosterone propionate (TP; Tokyo Chemical Industry, Tokyo, Japan), corn oil and Fina (Sigma-Aldrich, St. Louis, MO, USA) was purchased.

Animals
Adult male Wistar rats weighing 300–350 g (10 weeks old) were purchased from DaehanBio Link Co. (Yeumsung, Korea). All animals (rats) were housed at an air-conditioned temperature (23°C–25°C), relative humidity (56%–60%) and a 12 hours light/dark cycle. They were provided access to food and water ad libitum, and maintained in specific-pathogen-free conditions at the Laboratory Animal Research Center of Hyunsung Vital Co., Ltd. The mice were kept in accordance with the guidelines of the Ministry of Food and Drug Safety in Korea. All animal experiments were approved by the Laboratory Animal Research Center of Hyunsung Vital Co., Ltd., which had been approved by the Ministry of Food & Drug Safety (Approval number, Hyunsung-2015-3).

Castration
After maintained 1 week, animals were castrated to remove any internal testosterone influence. In the rats anesthetized with CO₂ and ether, both sides of the scrotum were incised to expose the testicles, the epididymis and the spermatic cord. Castration was performed referring to the OECD Hershberger assay method provided by the Institute of National Toxicology Laboratory. One week after castration, rats were used for experiments.³⁵

Experimental design
The castrated rats were randomly divided into 7 groups (8 mice/group): negative control (NC) or TP, rats injected with phosphate-buffered saline (PBS) and TP, respectively; Fina, TP-injected rats administrated by gavage feeding with Fina (1 mg/kg); GV (70, 150, 300 mg/kg), TP-injected rats administrated by gavage feeding with each GV extracts; PG, TP-injected rats administrated by gavage feeding with PG extracts (300 mg/kg).

Rats were subcutaneously injected with TP (5 mg/kg/100 μL) once per day for 4 weeks.³⁵ GV extracts or PG extracts were administrated by gavage feeding once per day for 4 weeks.
concomitant with TP. NC group was subcutaneously injected with corn oil for vehicle (solvent for TP, 100 μL), and then administrated by gavage feeding with PBS. TP was administrated by gavage feeding with PBS after TP injection.

The viability for PC-3 cells (derived from patient with metastatic site of prostate) (ATCC CRL-1435) treated with various concentrations of GV or PG extracts (10, 70, 150, 300, 500 μg/mL) obtained the results more than 99% (data not shown). The optimal concentrations and time of GV extracts was determined in the expression of AR, transforming growth factor (TGF)-β and COX-2 in the PC-3 cell line (data not shown). The concentration of GV extracts in vivo was determined through the cell viability and experiments of cell line (PC-3) related to BPH. That is, the optimal concentration of GV extracts (in vitro) was first determined targeting the expression of AR, TGF-β and COX-2 in the TP-stimulated PC-3 cells (data not shown). On base of the optimal concentration and time of GV yielded by experiment in vitro, the optimal concentration of GV (in vivo) was finally determined from twice pilot studies in BPH rat model using the deduced concentration (approximately 1,000 times of cell concentration). The dose of PG used only the highest concentration of GV extracts. After 4 weeks, rats were weighed, blood was collected by cardiac puncture, sacrificed, and then prostate was isolated.

**Determination of prostatic index (PI)**

The isolated prostatic tissues were weighed using microbalance. PI (mg/g) was calculated by the ratio prostate weight (mg) to body weight (g) of the prostate of rats. That is, the PI was calculated using an equation, PI (mg/g) = prostate weight (mg)/body weight (g), and the histogram of PI showed mean ± standard error of the mean (SEM; n = 8).

**Biochemical analysis**

The amounts of DHT, prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) (CUSABIO BIOTECH, Wuhan, China) were determined in sera obtained from blood samples of BPH by enzyme-linked immunosorbent assay (ELISA) kit, respectively. To obtain homogenized samples from prostatic tissues, the prostatic tissues (50 mg) were placed in a RIPA lysis buffer (500 μL) with prostatic inhibitors (Thermo Fisher Scientific, Sunnyvale, CA, USA) and allowed to swell on ice for 30 minutes. Then, it was homogenized using Polytron homogenizer (Qiagen, Hilden Germany), and then centrifugated. The amounts of cyclooxygenase-2 (COX-2) (MyBioSource, San Diego, CA, USA) in the supernatants obtained from centrifugation were determined by ELISA kit. The assays were conducted following the manufacturer’s instructions. Briefly, standard diluents (200 μL), serum obtained from each sample (200 μL), and 200 μL of reaction mixture were added in pre-coated 96-well plates; the plates were then incubated on a plate shaker at 4°C for overnight. The optical density was measured spectrophotometrically at a wavelength of 450 nm. Standard curves were made using serial dilutions of a standard sample, and then the activity (n = 8) was calculated according to the manufacturer’s instructions. The lowest detection limits for DHT, PSA, PAP or COX-2 was 10 pg/mL, 0.195 pg/mL, 0.938 ng/mL, 1 ng/mL, respectively.

**Histological examination**

Ventral prostatic tissues were fixed with 4% paraformaldehyde (Sigma-Aldrich) and embedded in paraffin block (Leica, Buffalo, IL, USA). Prostatic tissues cut into 4 μm thickness and were stained with hematoxylin and eosin (H&E) (Sigma-Aldrich) for epithelial thickness, and with toluidine blue solution (Merck, Darmstadt, Germany) for mast cells in the prostate tissues. Prostatic epithelial thickness was quantified at 5 sites of random areas under a microscope (5 sites/each slide × 8 rats = 40 sites), and then the mean ± SEM for 40
sites were shown in the histogram. The number of mast cells was quantified at 5 sites of 200 × 200 μm area under a microscope (5 sites/each slide × 8 rats = 40 sites), and the mean ± SEM for 40 areas is shown in the histogram.

**Reserve transcriptase-polymerase chain reaction (RT-PCR)**

Total cellular RNA was isolated from prostatic tissues (50 mg/500 μL) using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed in a final volume of 50 μL using an amfiRivert one-step RT-PCR kit (GenDEPOT, Barker, TX, USA) in an automated thermal cycler (Bio-Rad, Laboratories, Hercules, CA, USA). The PCR assays were performed for 35 cycles. Each cycle consisted of the following steps: denaturation at 94°C for 30 seconds, annealing at 50°C–65°C for 45 seconds, and extension at 72°C for 3 minutes. The results were expressed as a ratio to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The PCR products were analyzed using 1% agarose gel and visualized under UV light after staining with StaySafe nucleic acid gel stain (Real Biotech Corporation, Banqiao, Taiwan). Numbers below band images (#) show the mean values (n = 4) obtained from the ratio of each band density of each group versus those of the control and GAPDH from 4 independent experiments.

The primer sequences used were as follows: AR sense, 5-CAA AGG GTT GGA AGG TGA GA-3; AR anti-sense, 5-GAG CGA GGC GAA AGT TGT AG-3; COX-2 sense, 5-ATG CTC TTC CGA GCT GTG CTG-3; COX-2 anti-sense, 5-TTA CAG CTC AGT TGA AGG-3; COX-1 sense, 5-ATG AGT CGA AGG AGT CTC TCG-3; COX-1 anti-sense, 5-TCA GAG CTC AGT GGA AGG-3; IL-6 sense, 5-GCC AGA GTC ATT CAG AGC AA-3; IL-6 anti-sense, 5-TAA ACC TTT CCC CAA AT-3; IL-8 sense, 5-ATT GCA TCT GGC AAC CCT AC-3; IL-8 anti-sense, 5-TGA TCT GTG AAG TGG GGG TAG GA-3; α-R sense, 5-GCA AAG TTT CTG TGG AGG A-3; α-R anti-sense, 5-AGG CAA CTG GAA TAA CAA GAG A-3; insulin-like growth factor (IGF)-1 sense, 5-GGC ATT GTG GAT GAG TGT TG-3; IGF-1 anti-sense, 5-CCA TAG GGG CTG GGA CTT-3; epidermal growth factor (EGF) sense, 5-CCC GTG TTC TTC TGC GTT CC-3; EGF anti-sense, 5-TGT AAC CGT GGC TTC CTT CT-3; TGF-β sense, 5-GCA ACA TGT GGA ACT CTA C-3C; TGF-β anti-sense, 5-ATG CTC TTC TGC TGG AGC TG-3; GAPDH sense, 5-AAC CAT TGG GGC ATT GTG GAA GG-3; GAPDH anti-sense, 5-ACA CAT TGG GGG TAG GAA CA-3.

**Immunohistochemical analysis**

Prostatic tissues were fixed with 4% paraformaldehyde and embedded in paraffin block. Prostatic tissues cut into 3 μm thickness and deparaffinized with xylene (Duksan Pure Chemicals, Seoul, Korea), and then hydrated using an ethanol (each percentage from 100% to 70%); (Duksan Pure Chemicals). The tissue sections were treated with 3% hydrogen peroxide (Sigma-Aldrich) in PBS for 5 minutes to remove endogenous peroxidase activity and permeabilized using triton X-100 (Sigma-Aldrich). The sections were blocked with 5% bovine serum albumin (Sigma-Aldrich) with 1.5% fetal bovine serum (Invitrogen) in PBS, and then incubated overnight at 4°C with primary antibodies (1:50) against AR rabbit monoclonal antibody (Abcam, Cambridge, MA, USA). After that, the slides were incubated with hors eradish peroxidase -conjugated goat anti-rat antibody (biotinylated secondary antibody) for 2 hours using aavidin-biotin complex method, and then washed with PBST, followed by staining with diaminobenzidine (Sigma-Aldrich). The slides were counterstained with hematoxylin and finally mounted using aqueous mounting medium, and then examined under a light microscopy (Axiophot, Carl Zeiss, Oberkochen, Germany). The number of AR or TGF-β was quantified at 5 sites of 200 × 200 μm area under a microscope (5 sites/each slide × 8 rats = 40 sites), and the mean ± SEM for 40 areas is shown in the histogram.

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**RESULTS**

**Effects of GV extracts on the PI**

The volume of prostate is increased in the BPH rat model\(^3\) and BPH patients.\(^{39}\) Therefore, increment of prostate weight can be a marker of TP-induced BPH model (TP group) in rats and human. Therefore, we examined whether GV extracts have any influence on PI in the TP group. TP group showed a significant increase of PI, compared to the NC group. GV extracts reduced the PI in the dose-dependent manner, compared to TP group (Fig. 1). Fina group reduced the PI in the BPH. The highest concentration (300 mg/kg) of GV extracts showed the reduced PI more than or similar to that in Fina group, which was used as therapeutic positive control. Thus, hereafter, we did not describe any more about the data for all Fina groups due to similar to or less effects than those in GV extracts group.

PG extracts, which is a potential natural source of prebiotics and intestinal immunomodulator,\(^{40}\) was used as a natural positive control instead of GV extracts as a natural material. The PG did not affect the PI values. Hereafter, we did not describe any more about the data for all PG groups due to no responses in PG treatment.

**Effects of GV extracts on the level of DHT, PSA and PAP or COX-2**

We investigated the level of DHT, which is controlled by\(\alpha\)-R, and increases the size of prostate,\(^{4,6}\) and PSA and PAP, which are produced in the epithelial cells of prostatic tissues in BPH and increased in sera.\(^{41}\) TP group showed the increased amounts of DHT, PSA and PAP in...
the sera of BPH versus NC group. GV extracts significantly decreased amounts of DHT, PSA and PAP in the sera of BPH, compared to the increased TP group (Fig. 2A-C).

COX-2 enzyme was associated with the development of BPH. We checked amounts of COX-2 in the supernatants obtained from homogenizing of ventral prostatic tissues. TP group increased amounts of COX-2 in the prostatic tissues (Fig. 2D). GV extracts showed the decreased amounts of COX-2 in the dose-dependent manner, compared to that in TP group.

**Effects of GV extracts on the epithelial thickness of prostate**
Prostatic epithelial hyperplasia contributes to the development of BPH via interaction with stromal cells. TP group also induced intraluminal papillary folds and irregular shape of multi-layer epithelium. TP group induced the narrow of lacuna and the epithelial hyperplasia in BPH versus NC group (Fig. 3). GV extracts improved the epithelial hyperplasia and the narrowed lacuna in dose-dependent manner, compared to that in TP group.

**Effects of GV extracts on the expression of AR, inflammatory molecules and growth factors**
It has been reported that AR, 5α-R, inflammatory molecules and growth factors play a critical role in the inflammation and hypertrophy of prostatic tissues in BPH. Thus, we examined whether GV extracts blocked the expression level of several factors. GV extracts
Effects of Graviola Extracts on BPH Rat Model

Fig. 3. Effects of GV extracts on the epithelial thickness of prostate in the benign prostatic hyperplasia model in rats. Bar in box or magnification indicates 200 µm and × 200, respectively. Prostatic epithelial thickness is quantified, and then histogram shows the mean ± standard error of the mean (n = 8). (A) Epithelial thickness by hematoxylin and eosin staining. (B) Quantification of epithelial thickness.

GV = graviola stem bark, NC = negative control, TP = testosterone propionate, Fina = finasteride, PG = Platycodon grandiflorus.

***P < 0.001 versus the NC; **P < 0.01, +++P < 0.001 versus the TP.

reduced targets molecules, such as AR and 5α-R, related in the hypertrophy of BPH, compared to TP group increased in the BPH model (Fig. 4). GV extracts also inhibited the expression of inflammatory cytokines (IL-6 and IL-8), and growth factors (IGF and EGF), in the dose-dependent manner versus those in the increased TP group.

Expression of COX-2 was increased in the prostatic tissues of BPH versus NC group (Fig 4A). GV extracts inhibited the expression of COX-2, in the dose-dependent manner, compared to that in TP group increased by TP induction. However, COX-1 expression (constitutive enzyme) was not affected in all groups checked as well as in GV extracts group (Fig. 4A).

GV extracts reduced the expression of TGF-β, which was known as a tissue remodeling molecule in the development of BPH, in the dose-dependent manner (Fig. 4B), compared to TP group which was increased versus NC group.

Fig. 4. Effects of GV extracts on the expression of AR, inflammatory molecules and growth factors in the prostate tissues of BPH model in rats. Molecules related to BPH using reserve transcriptase-polymerase chain reaction (n = 4).

GV = graviola stem bark, NC = negative control, TP = testosterone propionate, Fina = finasteride, PG = Platycodon grandiflorus, AR = androgen receptor, COX = cyclooxygenase, IL = interleukin, 5α-R = 5α-reductase, IGF = insulin-like growth factor, EGF = epidermal growth factor, TGF-β = transforming growth factor beta, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, BPH = benign prostatic hyperplasia.

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Effects of GV extracts on the expression of AR and TGF-β1

To investigate the level of AR and TGF-β1 related to remodeling of prostatic tissues, we observed that TP group increased the protein expression of AR and TGF-β1 (brown dots) in the prostatic tissues in BPH using immunohistochemistry staining (Fig. 5). GV extracts attenuated the protein expression of AR and TGF-β1 in dose-dependent manner versus that in TP group.

Effects of GV extracts on the recruitment of mast cells

We confirmed that GV extracts reduced inflammation and hypertrophy caused in the prostate of BPH by inhibiting inflammatory cytokines and growth factors. Thus, it may imply that inflammatory cells like mast cells may be infiltrated and activated in the inflammatory sites of prostatic tissues. Population of mast cells in the TP group was increased in the stromal region of prostatic tissues versus NC group using toluidine blue staining (Fig. 6A and B). GV extracts inhibited the population of mast cells infiltrated into the stromal region of prostatic tissues in dose-dependent manner versus that in TP group.

We examined the expression of CCR2/CCL2, which are related to the migration of mast cells, in prostatic tissues of BPH. TP group showed the increased expression of CCL2 and CCR2 versus NC group (Fig. 6C). GV extracts decreased the expression of these migration molecules in dose-dependent manner, compared to those in the increased TP group.
data imply that GV extracts may reduce the inflammation and the hypertrophy via inhibiting the infiltration of mast cells into the stromal region of prostatic tissues.

**DISCUSSION**

We demonstrated that natural material GV extracts may ameliorate inflammation and hypertrophy in the development of BPH caused via inflammatory cytokines, growth factors and CoX-2 secreted/synthetized from mast cells, which are infiltrated in the stromal areas of prostatic tissues in BPH.

There have been many reports that the specific bioactive components contained in graviola are used as traditional medicines for lots of diseases, and graviola shows potent efficacy in the various disease models in animals. However, effects of GV extracts on the inflammation and hypertrophy in BPH are not reported yet, although it has anti-inflammatory effects on various diseases related to inflammation.

PI, which is increased in BPH, can be a marker of TP-induced BPH model and human. These reports agree with our data for the enhancement of PI in BPH model in rats (Fig. 1). Our data shows that the BPH is properly formed in this experimental model. Thus, the data suggest that GV extracts may reduce the size of the prostate in BPH model.
Hormonal regulation of BPH is dependent on androgen. AR is essential for normal prostate development. It has been reported that androgen/AR signaling may play important role in promoting the proliferation of epithelial and stroma cells in the periurethral area of the transitional zone, leading to development of BPH with urinary obstruction. However, testosterone appears to function as a prohormone in that the most active form of androgen in prostate is DHT, although it is the primary plasma androgen. The DHT is produced and regulated by 5α-R, which plays an important role in the BPH pathogenesis. Thus, the data suggest that GV extracts may reduce expression of AR and 5α-R related to the proliferation of epithelial and stromal cells of BPH (Fig. 4 and Fig. 5A).

PSA is one of the organ specific marker produced by prostatic tissues. It is correlated with prostate size. PAP is produced by the lysosomes of epithelial cells in prostate, and is elevated in BPH more than prostate cancer. They are diagnostic biomarkers used in BPH. In the present study, we found the enhancement of DHT, PSA and PAP in the sera in BPH (Fig. 2A-C), which were in agreement with results obtained by other laboratories. We also found that GV extracts reduced the levels of them. Therefore, the data suggest that GV extracts may alleviate production of diagnostic biomarkers (DHT, PSA, PAP) in BPH.

In prostate epithelial and stromal cells, inflammation has linked of BPH progression and acute urinary retention, and it is associated with the development of hyperplasia. Thus, increased inflammation is closely associated with the severity of BPH. Infiltration of immune and inflammatory cells, such as, T- and B-lymphocytes, macrophages, and mast cells, is increased in inflammatory sites of a prostate with BPH. The infiltrated cells secrete a variety of inflammatory cytokines and growth factors. Inflammatory cytokine like IL-6, which is secreted in the stimulated-epithelial cells, induces infiltration of mast cells and stromal cell proliferation. EGF is also expressed in the prostatic tissues of BPH. Expression of IGF is increased in stromal compartment of the human BPH tissues, and IGF binding protein potentiate stromal remodeling by TGF-β. The IGF is expressed in mast cells, which are associated with the development of BPH.

Thus, we focused on mast cells in this study. Mast cells are immune cells in innate immunity, and release and secrete a variety of mediators, inflammatory cytokines and growth factors. These molecules are involved in a variety of diseases including BPH. Mast cells are infiltrated/activated into inflammatory sites of stromal area in prostatic tissues by BPH epithelial cells stimulated with some materials like Trichomonas. The activated mast cells may induce the proliferation of stromal cells via inflammatory cytokines (IL-6, IL-8) and growth factors (EGF, IGF). Thus, the data suggest that mast cells may induce inflammation and hypertrophy through interaction of epithelial cells and stromal cells.

The enhancement of the cytokines secreted from various cells, such as epithelial and stromal cells, particularly mast cells, induces cell survival, growth, differentiation and COX-2 expression. The COX-2 is expressed in human prostatic tissues, and it causes a reduction in prostatic cell death. Moreover, chronic inflammation in BPH is characterized by excessive expression of COX-2. Thus, the data imply that the COX-2 overexpressed in mast cells is also associated with the development of BPH (Fig. 2D and Fig. 4A). We demonstrated that GV extracts reduced the PI, expression of AR, 5α-R, inflammatory cytokines (IL-6, IL-8), growth factors (IGF, EGF), COX-2, and tissue remodeling cytokine TGF-β related to the development of BPH. Thus, the data suggest that GV extracts may alleviate inflammation and hypertrophy in the prostatic tissues of BPH through inhibiting COX-2 as well as inflammatory...
cytokines and growth factors secreted/synthesized from mast cells which are infiltrated and activated into stromal areas by epithelial cells stimulated in prostatic tissues of BPH. However, COX-2 is known as enzyme to produce a variety of inflammatory substances, such as, prostaglandins, leukotrienes (LTs), etc. It has shown that treatment of non-steroidal anti-inflammatory drugs inhibits BPH-1 cell line growth. Therefore, it needs further to investigate for relationship between substances (prostaglandins, LTs) and BPH development, and to examine whether GV extracts affect production of various substances in BPH.

It has been reported that T and B lymphocytes, and macrophages are also infiltrated into inflamed prostatic tissue sites of BPH, and these infiltrated cells induce inflammation and hyperplasia of prostatic tissues via nuclear factor-κB activity and AR activity. Therefore, it needs further to examine whether GV extracts have any influence on infiltration of T- and B-lymphocytes and macrophages into the prostatic tissues of BPH.

In conclusion, our data suggest that mast cells may be recruited in stromal area of prostatic tissues by unknown substances or circumstance, and activated by the inflamed epithelial cells, and then the activated mast cells may secrete inflammatory cytokines, growth factors and COX-2, followed as inflammation and hypertrophy related to the pathogenesis of BPH development. This report is our first study to suggest that GV extracts may alleviate inflammation and hypertrophy in prostatic tissues of BPH. Thus, some single or complex components contained in GV extracts may be developed as a potential therapeutic strategy for treating BPH caused by cytokines, growth factors and COX-2 secreted/synthesized by mast cell activation in patients with BPH.

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