The Combination of Serenoa Repens, Selenium and Lycopene is More Effective Than Serenoa Repens Alone to Prevent Hormone Dependent Prostatic Growth

Domenica Altavilla, Alessandra Bitto, Francesca Polito, Natasha Irrera, Herbert Marini, Salvatore Arena, Vincenzo Favilla, Francesco Squadrito, Giuseppe Morgia* and Letteria Minutoli

From the Section of Pharmacology, Department of Clinical and Experimental Medicine and Pharmacology (DA, AB, NI, FS, LM), Section of Physiology and Human Nutrition, Department of Biochemical, Physiological and Nutritional Sciences (FP, HM) and Department of Urology (SA), University of Messina, Messina and Department of Urology, Polyclinic Hospital, University of Catania (VF, GM), Catania, Italy

Abbreviations and Acronyms

BPH = benign prostatic hyperplasia
DHT = dihydrotestosterone
EGF = epidermal growth factor
Ly = lycopene
PCR = polymerase chain reaction
SeR = Serenoa repens
VEGF = vascular endothelial growth factor

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* Correspondence and requests for reprints: Department of Urology, School of Medicine Policlinico Hospital, University of Catania, Catania, Italy (telephone: +39 095351816; FAX: +39 0953782373; e-mail: gmorgia@policlinico.unict.it).

Purpose: Serenoa repens is frequently combined with other natural compounds, such as the carotenoid lycopene and the essential trace element Se, to increase its therapeutic activity in benign prostatic hyperplasia. We noted that the lycopene-Se-Serenoa repens combination has greater, enhanced anti-inflammatory activity, which might be of particular interest for benign prostatic hyperplasia treatment. Testosterone administration in rats is a suitable tool for investigating hormone dependent benign prostatic hyperplasia. We performed a comparison experiment between Serenoa repens and the lycopene-Se-Serenoa repens combination on prostate growth induced in rats by testosterone administration.

Materials and Methods: Rats were treated daily with testosterone propionate (3 mg/kg subcutaneously) or its vehicle for 14 days. Testosterone administered animals were randomized to receive vehicle, Serenoa repens (25 mg/kg subcutaneously) or the combination of lycopene (3 mg/kg subcutaneously), Se (3 mg/kg subcutaneously) and Serenoa repens for 14 days. The rats were sacrificed and the prostate was removed for analysis.

Results: Lycopene-Se-Serenoa repens was more effective than Serenoa repens alone for decreasing prostate weight and hyperplasia, augmenting pro-apoptotic Bax and caspase-9, and blunting anti-apoptotic Bcl-2 mRNA. Lycopene-Se-Serenoa repens also markedly decreased epidermal growth factor and vascular endothelial growth factor expression.

Conclusions: The data indicate that the lycopene-Se-Serenoa repens combination is superior to Serenoa repens alone for decreasing hormone dependent prostatic growth.

Key Words: prostate, prostatic hyperplasia, Serenoa, lycopene, selenium

Benign prostatic hyperplasia and prostate cancer are major health concerns that are likely to have an increasing impact in line with the gradual aging of the population.[1] BPH is characterized by smooth muscle and epithelial proliferation, primarily in the prostatic transition zone, which can cause various problems for patients, of which the most common is lower urinary tract symptoms.[1] Prostate cancer mostly originates in the prostate peripheral zone but 1/4 arises in the transition zone, where most BPH originates. Abnormal prostate growth is thought to involve the disruption of DHT supported homeostasis between cell proliferation and cell death so

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that proliferative processes predominate and apoptotic processes are inhibited.[2,3] The key role of DHT in BPH prompted the development of 5α-reductase inhibitors as treatment for BPH and potentially for preventing prostate cancer.[2] Several large trials have also shown the efficacy of α-receptor blocking medications when used alone or in combination with 5α-reductase inhibitors for BPH.[4] However, these therapeutic strategies are not free from side effects on sexuality and blood pressure regulation.[5]

Despite the enormous burden of BPH on public health the pathogenesis of BPH is incompletely understood. Age related systemic/local hormonal and vascular changes appear to represent the predominant mechanism. However, emerging evidence suggests that inflammation may have a key role in BPH development and progression.[6] Inflammation may contribute to tissue injury and cytokines produced by inflammatory cells may drive local growth factor production and angiogenesis.[7] As a consequence, an inflammatory cascade was also suggested to have a role in prostate cancer.

Phytotherapeutic supplements, mainly based on saw palmetto derived SeR, are numerous and used frequently. However, data supporting efficacy are limited, making treatment recommendations difficult.[8]

We recently noted in a bladder outlet obstruction experimental model that a combination of SeR, Se and Ly was more effective than SeR alone to decrease the prostate inflammatory response, growth factor expression and histological features.[9] Others have used the SeR and/or Ly-Se-SeR trimix in multiple animal and human studies.[10,11] Recently in a chronic prostatitis/chronic pelvic pain syndrome, category IIIa, controlled multicenter study the combined use of these natural components showed some efficacy.[10] Ly is known to have antigrowth and death apoptotic inducing ability in prostate cancer cell lines.[12–14] Specifically Ly decreased PC3 cell proliferation,[12] presumably by decreasing VEGF, and it has also been used in BPH models.[14]

Se has multiple bio-organic forms (selenomethionine, Se-methylselenocysteine, methylseleninic acid, etc) and there is debate on what form is best.[15] Se treatment is purported to have antiprostate cancer cell activity alone[16] or in conjunction with survivin gene silencing.[17] However, a meta-analysis of randomized, controlled trials in humans revealed no positive effect of Se on prostate cancer prevention.[18]

Overall we still lack confirmatory data on the Ly-Se-SeR association effect on prostate growth. In this regard testosterone administration in rats is a suitable tool with which to investigate BPH. Thus, we performed a comparison experiment between SeR alone and the Ly-Se-SeR association on testosterone induced prostate growth in rats.

MATERIALS AND METHODS

Animals

All procedures complied with the standards for the care and use of animal subjects, as stated in the National Research Council Guide for the Care and Use of Laboratory Animals. The protocol was reviewed and approved by the University of Messina ethics committee. A total of 28, 3-month-old male Sprague-Dawley® rats weighing 220 to 250 gm were maintained in plastic cages under standard environmental conditions with free access to water and food at the Department of Clinical and Experimental Medicine and Pharmacology animal facility, University of Messina, Messina, Italy. After 1 week of stabilization the rats were randomly assigned to 4 groups, including 7 sham-BPH rats administered vehicle (100 μl corn oil subcutaneously), 7 BPH rats that received testosterone propionate (3 mg/kg subcutaneously diluted in corn oil in a volume of 100 μl), 7 BPH plus SeR rats injected with testosterone propionate and SeR (25 mg/kg subcutaneously in corn oil) and 7 BPH plus Ly-Se-SeR rats administered testosterone and Ly-Se-SeR (1, 3 and 25 mg/kg, respectively, subcutaneously) for 14 days. At the end of the experiment the rats were sacrificed under ether anesthesia. The prostate was immediately removed and used for further analysis. Testosterone propionate (Sigma-Aldrich®), and its dose and administration route were chosen accordingly to previously published reports.[19]

Prostate Weight

At study completion the prostates were removed and weighed. The percent of growth inhibition was calculated using the equation, $100 - [(\text{TG} - \text{sham group})/(\text{BPH} - \text{sham group}) \times 100]$, where TG represents the values of the treated groups.

Histology

Prostates were routinely processed and embedded in paraffin. Sections (5 μm) were cut and stained with hematoxylin and eosin. Acinar structure pathological findings were defined as villamentous or villose projections—uniform epithelial infoldings into the lumen with a connective tissue fine core along the longitudinal axis and an epithelial cell lining, papillary projections—expanded epithelial infoldings with a varying number of ramifications with a cauliflower-like pattern, cribriform structures—result of the fusion of contralateral papillary projections, mimicking a glands in glands pattern and hyperplastic nodules—focal increase in nuclei number, resulting in a multilayered conglomerate.

Real-Time PCR

Total RNA was extracted from the rat prostate and isolated using TRIzol® reagent according to the manufacturer protocol. RNA (5 μg) from each sample was reverse transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems™) according to manufacturer procedures. cDNA (5 ng) from each sample was amplified by real-time reverse transcriptase-PCR with

2 × TaqMan® universal PCR Mastermix, 20 × target primer and probe. cDNA was used to quantify the amount of Bax, Bcl-2, caspase-9, vascular VEGF and EGF with β-actin as the housekeeping gene. Each sample was analyzed in duplicate using SDS 7300 (Applied Biosystems). The results of the target genes are shown as the n-fold difference relative to normal controls (relative expression).

Statistical Analysis

Groups were compared using the Kruskal-Wallis 1-way test. Differences between individual treatment groups were compared using Dunn's test with $p < 0.05$ considered significant. Statistical analysis was done with Prism®, version 5.0 for Windows®.

Drugs

We used Se as sodium selenate, Ly (90% or greater from tomato) (Sigma-Aldrich) and SeR alcoholic extract.

RESULTS

The mean ± SD weight of prostates from sham operated animals was 0.75 ± 0.03 gm. As expected, prostate weight increased after 14 days of testosterone treatment (see table). Concomitant administration of SeR significantly decreased prostate weight and growth but Ly-Se-SeR more effectively inhibited prostate enlargement and growth by 83% in treated animals (see table).

Histology revealed complete derangement of prostate tissue and marked hyperplasia in BPH vehicle treated vs sham treated rats (see Appendix, and fig. 1, *A* and *B*). SeR decreased the altered histological pattern and the exaggerated hyperplasia (see Appendix and fig. 1, *C*). However, Ly-Se-SeR more effectively prevented prostate hyperplasia (see Appendix and fig. 1, *D*). No cribiform pattern was noted.

Prostates from sham operated rats had detectable Bax, Bcl-2 and caspase-9 levels (fig. 2). BPH rats showed a slight decrease in Bcl-2 mRNA with a parallel induction in the message for the pro-apoptotic proteins Bax and caspase-9 (fig. 2). SeR administration decreased anti-apoptotic Bcl-2 gene expression and increased pro-apoptotic Bax and caspase-9 mRNA in BPH rats (fig. 2). Ly-Se-SeR caused a greater pro-apoptosis enhancing effect in the prostate of BPH rats (fig. 2).

Treatment effects on prostate growth in rats that received testosterone

Group	Mean ± SD (gm) Prostate Wt	% Growth Inhibition
Sham treatment	0.75 ± 0.03	—
BPH	1.53 ± 0.05	—
BPH + SeR ($p < 0.005$ vs BPH)	1.05 ± 0.07	62
BPH + Ly-Se-SeR	0.98 ± 0.04 ($p < 0.001$ vs BPH)	83 ($p < 0.001$ vs BPH and < 0.05 vs BPH + SeR)

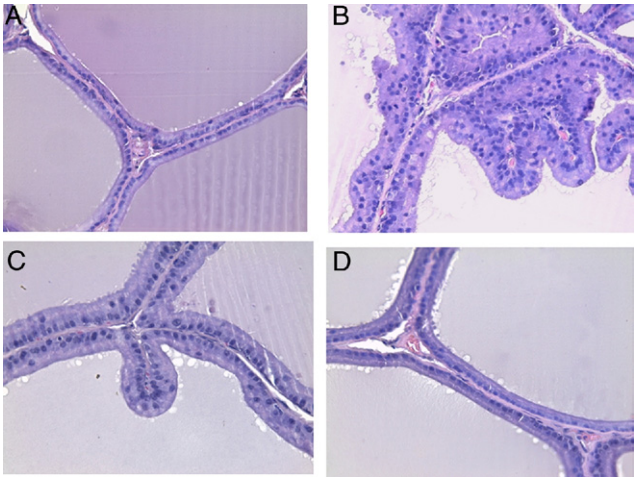

Figure 1. *A*, sham treated prostate has regular acini with cuboidal and low cylindrical epithelium, round nuclei with regular alignment, fine stroma and continuous basal layer. *B*, BPH prostate has irregular acinar shape with villose projection into lumen, foci of piled hyperplastic nodule with loss of epithelial polarity, high cylindrical epithelium, round and irregularly aligned nuclei, moderate stroma and intact basal layer surrounding acini. *C*, BPH prostate treated with SeR has cylindrical/cuboidal epithelial cells, some villose projection, fine stroma and intact basal layer around acini. *D*, BPH prostate treated with Ly-Se-SeR has histological features resembling normal acinar structure. Reduced from ×20.

Prostates harvested from sham treated animals also showed detectable mRNA levels of the growth factors EGF and VEGF (fig. 3). EGF and VEGF mRNA was significantly increased in BPH rats (fig. 3). SeR treatment significantly blunted the expression of both growth factors. The Ly-Se-SeR association caused a further decrease in EGF and VEGF expression (fig. 3).

DISCUSSION

Prostate enlargement induced by testosterone has been used to assess the effects of potential treatments for BPH since it reproduces adequately but not fully the major features of human BPH, including functional and histological changes. This supports the theory that testosterone produces prostate hyperplasia. Our results show prominent prostate growth after testosterone administration and a consequent increase in prostate weight with the typical histological features of BPH. Combined treatment with Ly-Se-SeR was more effective than SeR treatment alone in preventing BPH and it inhibited growth by 83%, suggesting that Se and Ly at pharmacological doses further increase SeR efficacy for BPH. We specifically administered a triple combination of these compounds in rats according to the dose used in humans[10] and it was generally safe and well tolerated.

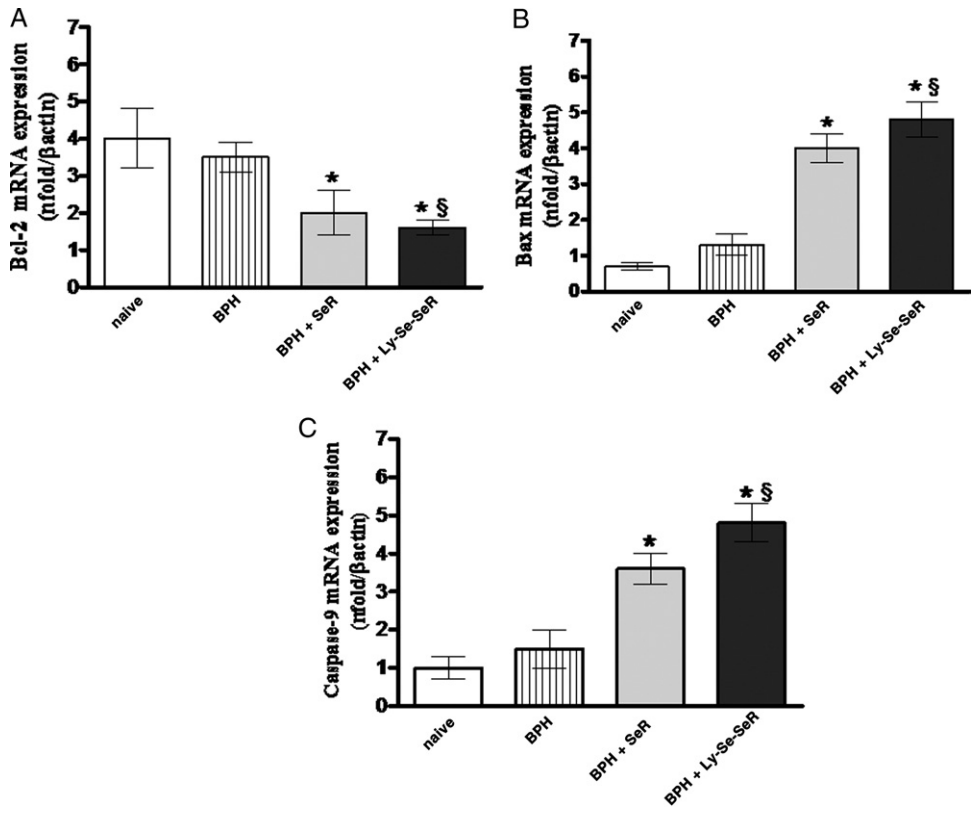

Figure 2. mRNA expression in prostate specimens of sham and testosterone treated rats. *A*, Bcl-2. *B*, Bax. *C*, caspase-9. Asterisk indicates p <0.01 vs BPH. Curley indicates p <0.05 vs BPH plus SeR.

Prostate growth inhibition by Ly-Se-SeR is likely stimulated via a caspase dependent signal through caspase-9 and an independent mechanism involving the pro-apoptotic Bax and the anti-apoptotic Bcl-2 genes. To date 3 major apoptotic signaling pathways have been described, including the mitochondrial pathway, the endoplasmic reticulum pathway and the death receptor pathway.[20] Bcl-2 and Bax are key in apoptotic events. Bcl-2 is an upstream effector molecule in the apoptotic pathway that was identified as a potent suppressor of apoptosis.[21] Most cancers, including prostate cancer, generally over express Bcl-2,[22,23] thereby escaping apoptosis and undermining therapy. Bcl-2 forms a heterodimer with the apoptotic protein Bax, neutralizing its apoptotic effects. The ratio of these proteins determines whether a cell will commit to the apoptosis pathway.

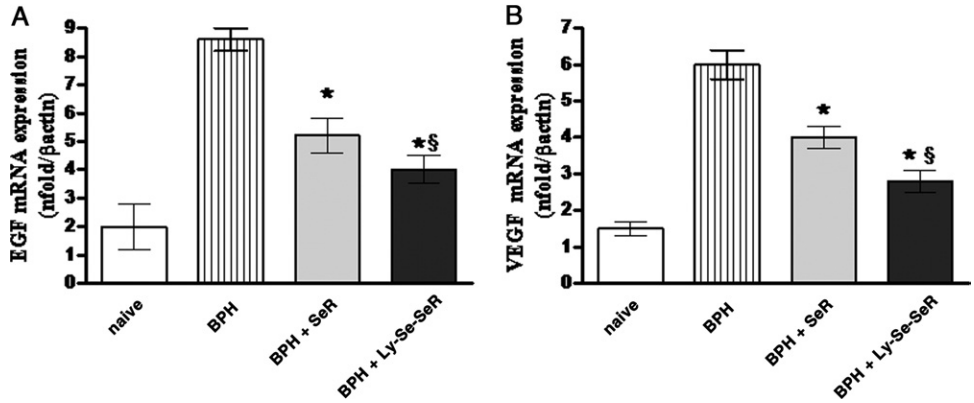

Figure 3. mRNA expression in prostate specimens of sham and testosterone treated rats. *A*, EGF. *B*, VEGF. Asterisk indicates p <0.01 vs BPH. Curley indicates p <0.05 vs BPH plus SeR.

We report that Ly-Se-SeR was more efficient than SeR alone in significantly down-regulating Bcl-2 protein and up-regulating Bax during hormone dependent, testosterone induced BPH. The endoplasmic reticulum, caspase driven cascades can be activated by multiple pathways, including the mitochondrial pathway, which relies on cytochrome c release and, thus, activates the initiator caspase-9.[24] In our study SeR and particularly the Ly-Se-SeR combination induced caspase-9 over expression in prostate tissue, further stimulating the apoptotic machinery triggered by the Bax/Bcl-2 imbalance.

Also, Bcl-2 staining was noted to be intensified in the area of chronic inflammatory infiltrate in radical prostatectomy specimens.[25] This potential concomitant decrease in inflammation, which parallels the increased apoptotic activity in BPH tissue, is supported by our previous findings in the bladder obstruction model, in which we observed a significant decrease in inflammatory infiltrate and tumor necrosis factor-α, an important BPH inflammatory marker.[9]

Inflammatory cells release cytokines as well as growth factors to modulate the immune response but they also promote epithelial and stromal prostatic cell growth. Growth factors have a significant role in the regulation and growth of normal, hyperplastic and malignant prostatic epithelium. Prostatic cells alone secrete inflammatory mediators and stimulate their own growth. After the circle has started it appears that feedback controls can be overwhelmed and prostate volume progressively increases.

SeR decreases in vivo the androgenic support to prostatic cell growth and induces a 50% decrease in DHT and EGF in BPH tissue in treated patients.[26] In agreement with these findings we noted that during testosterone induced growth there was over expression of the growth factor EGF, which was prevented by treatment with SeR and to a greater extent by combined Ly-Se-SeR. EGF activates intracellular signaling cascades to initiate activation of the downstream pathways that lead to cell proliferation, migration, adhesion, anti-apoptosis, angiogenesis and metastasis.[27] Moreover, EGF and its receptor EGFR are frequently over expressed in prostate cancer, which is associated with a more aggressive clinical outcome.[28]

Among other growth factors involved in BPH and cancer development a primary role is played by VEGF, which stimulates neovascularization. VEGF has been observed in BPH stromal cells[29] as well as in prostate cancer epithelial cells,[29] where it has a significant role in tumor growth, inducing angiogenesis.

Recently VEGF was noted to be a possible therapeutic target to reduce prostate growth.[30] Accordingly in our experimental model we observed a sustained anti-VEGF effect of SeR, particularly when combined with Se and Ly. This indicates an antiproliferative effect independent of hormone receptor status, which consequently might be useful for different pathological conditions.

CONCLUSIONS

The Ly-Se-SeR association was more effective than SeR alone for inducing the apoptosis that was likely to occur via caspase-9 and Bax activation, and consequent inhibition of the Bcl-2 pathway in testosterone induced BPH. Ly-Se-SeR also more efficiently inhibited prostate enlargement by inhibiting growth factor expression, suggesting a possible alternative therapy for hormone dependent prostatic growth.

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APPENDIX

Histopathological Features

	Sham Treatment	BPH	BPH + SeR	BPH + Ly-Se-SeR
Acinar irregularity	Present	Absent	Present	Present
Stroma	Slight	Variable	Slight	Slight
Cell:				
Shape	Cuboidal	Cuboidal/cylindrical	Cuboidal/cylindrical	Cuboidal
Polarity	Present	Present/absent	Present	Present
Nuclear shape	Round	Round/ovoid	Round/ovoid	Round
Mitosis	Absent	Isolated	Absent	Absent
Acinar villi	Absent	Focally present	Focally present	Absent
Piling up	Absent	Moderate	Absent	Absent
Budding out	Absent	Slight	Absent	Absent
Basal membrane	Intact	Intact/focal interruption	Intact	Intact

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