Effect of Saw Palmetto on the Development and Progression of Prostate Carcinoma in TRAMP Mice

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We established a breeding colony for TRAMP (transgenic adenocarcinoma of the mouse prostate) mice in order to study the effects of saw palmetto berry extract (SPE) on the spontaneous development of prostate cancer. Four week old mice have been assigned to one of two study groups: 1) short-term (8 week) SPE treatment, and 2) long-term (20 week) SPE treatment. There were 4 different cohorts within each study group: WT control diet, TRAMP control diet, TRAMP low dose SPE diet (50 mg/kg) and TRAMP high dose SPE diet (300 mg/kg). The study was statistically empowered at the P=0.05 levels and consisted of ~17 mice/cohort. We found that SPE was well tolerated by mice having no adverse effects on body or organ weights. Although SPE did not affect prostate weight at either the 12 or 24 week time points, high dose SPE treatment resulted in a significantly decreased percentage of differentiated cancer and increased PIN compared to controls. These results are the first to suggest that SPE can delay prostate cancer tumor progression in TRAMP mice. We are currently evaluating the cellular mechanism by which SPE exerts this effect.

prostate cancer, saw palmetto, serenoa repens, preclinical, TRAMP mice, chemoprevention, dietary supplement
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INTRODUCTION

Apart from non-melanoma skin cancer, prostate cancer (CaP) is the most commonly diagnosed cancer among men in the United States today. It is generally accepted that the incidence of death from CaP can be reduced through a three-pronged approach of prevention, early diagnosis, and appropriate treatment. Although dietary factors and pharmaceutical agents have helped reduce the mortality rate of men with CaP, more information is needed on potential chemopreventative agents. One such agent is saw palmetto extract (SPE), an herbal product that is a lipid extract of the fruit of Serona repens. SPE is widely used for treatment of benign prostate hyperplasia, but has never been tested for its potential to prevent CaP. At least two of SPE’s effects are exerted on known risk factors for CaP. SPE inhibits the synthesis of a powerful androgen that drives prostate growth and suppresses the action of a specific protein called insulin-like growth factor-1 (IGF-I) that governs the growth of the particular cell type that becomes malignant. The aim of this Exploration: Hypothesis Development Award is to evaluate the chemopreventative potential on the development and progression of CaP in a unique mouse model (TRAMP) that develops CaP that mimics human disease. TRAMP mice will be treated with phytoestrogen-free control diet (AIN76A) or SPE-formulated diets (50 and 300 mg SPE/kg/day) from 4 weeks of age until they are sacrificed at 12 weeks of age (short term treatment) or 24 weeks of age (long term treatment). The specific objectives are: 1) To determine whether in vivo SPE treatment prevents the formation and progression of CaP; 2) To determine whether in vivo SPE treatment suppresses the activation of the IGF-I signaling pathway; and 3) To determine whether in vivo SPE treatment affects hormone and growth factor concentrations in serum and prostate.

BODY

The following describes the research accomplishments associated with each task outlined in the approved statement of work.

A. Task 1: We established a breeding strategy that utilized breeding harems composed of one wild type C57/Bl6 male and two heterozygous TRAMP females. With eight harems in operation, approximately 20 heterozygous TRAMP males were born each month. Breeding harems were replaced as needed. The PCR protocol for genotyping was established. Our study started with 2 breeding pairs obtained from the National Cancer Institute.

B. Task 2: We have completed the long-term SPE treatments. Long-term treatment cohorts were filled between April 2005 and Sept 2005 (months 6-12). Dissections were completed the end of February 2006. We have frozen tissues, paraffin-embedded slides and plasma from 18 wt controls, 15 TRAMP controls, 13 TRAMP mice treated with 50 mg/kg SPE and 18 TRAMP mice treated with 300 mg/kg SPE. There is variability in the number of mice per cohort because fighting occurred with some animals and therefore animals were removed from the study because of skin lacerations that culminated in infection.

C. Task 3 is integrated into task 5, described in E.

D. Task 4: We have completed the short-term treatment study. The cohorts in this study were initially started after quarantine in December 2004 and necropsies were completed at the end of December 2005. We have frozen tissues, paraffin-embedded slides and plasma from 14 wt controls, 18 TRAMP controls, 18 TRAMP mice treated with 50 mg/kg SPE, and 18 TRAMP mice treated with 300 mg/kg SPE.

E. Tasks 3 & 5: Assess toxicity and effects on urogenital tract (UGT) and dorsolateral prostate (DLP) weights, process tissues, score for pathology, and perform immunohistochemistry and western blots on DLPs from mice on short and long term SPE treatments. Results are enumerated below.
1. **SPE administered in the diet is not toxic.** Changes in body weight and changes in organ weight were used as criteria to evaluate SPE toxicity. Weekly body weights were not affected by SPE treatment (Fig 1a). Organ weights at necropsy at 12 weeks (data not shown) and 24 weeks (Fig 1b) were similar between treatment groups. These results established that the concentrations of SPE used in our study are not toxic in mice.

![Fig 1. Effect of dietary SPE on body weight (a) and organ weight (b). Between 4-24 weeks of age, wild type controls and TRAMP males were fed AIN-76A containing 0 mg/kg SPE (Wt Ct, n=18; Tr Ct, n=15) and TRAMP males fed AIN-76A supplemented with 50 mg/kg or 300 mg/kg SPE (Tr 50, n=13; Tr 300, n=18). Results are mean ± SE of the respective organ weights. One-way ANOVA revealed no significant difference in body or organ weights between treatments.](image)

2. **SPE does not affect relative UGT or DLP weights.** We were unable to detect significant differences in relative UGT or DLP weights with short-term SPE treatment (Fig 2). There is a non-statistically significant trend suggesting that long-term treatment with 50 mg/kg and 300 mg/kg SPE decreases relative UGT and DLP weights in 24-week old TRAMP mice.

![Fig 2. Effects of short-term SPE treatment on relative UGT (a) or DLP (b) weights. Between 4-12 weeks of age, wild type controls and TRAMP males were fed AIN-76A containing 0 mg/kg SPE (Wt Ct, n=15; Tr Ct, n=18) and TRAMP males were fed AIN-76A supplemented with 50 mg/kg or 300 mg/kg SPE (Tr 50, n=18; Tr 300, n=18).](image)
3. **SPE decreases tumor incidence in 24-week old TRAMP mice.** Frank tumors were observed in 4/15 of 24-week old TRAMP mice fed control diet, 1/13 of TRAMP mice treated with 50 mg/kg SPE and 1/18 of TRAMP mice treated with 300 mg/kg SPE (Fig 4).

4. **SPE decreases tumor grade in 24-week old TRAMP mice.** We met and consulted with Dr. Norman Greenberg and followed the procedure described by Kaplan-Lefko et al. (1) for pathological scoring of collected prostates. Short term SPE treatment had no effect on pathological scores (Fig 5a). Long term treatment with 50 mg/kg and 300 mg/kg SPE significantly increased the incidence of PIN lesions and decreased the incidence of differentiated tumors when compared to TRAMP mice treated with control diet (Fig 5b). **The effect of SPE to reduce the incidence of differentiated cancer was statistically significant for the highest dose of SPE and represents the major novel finding of this study.**

5. **SPE decreases cell proliferation.** Immunohistochemical staining for Ki67 in the DLPs of 12-week old TRAMP mice shows a trend towards decreased cell proliferation in mice treated with both doses of SPE compared to control treatment (Fig 6). These results suggest that SPE inhibits epithelial proliferation and is consistent with our previous findings in epithelial cell culture. Analysis of Ki67 immunohistochemistry in 24-week old mice is not yet completed.
Fig 5. Effect of SPE on pathologic cancer grade. The (%) PIN, well-differentiated (WD) or poorly differentiated (PD) tumors from a) 12 week-old TRAMP mice treated as described in the legend to Fig 2 and b) 24 week-old TRAMP mice treated with as described in the legend to Fig 1. **One-way ANOVA followed by the Dunnets test showed that after 24 wk of treatment, Tr300 exhibited significantly higher PIN and significantly lower differentiated cancer compared to Tr Cr, p<0.05.

Fig 6. Effect of SPE on the % of Ki67-positive cells in DLP tissues from 12 week-old TRAMP mice treated as described in the legend to Fig 2.

6. Effects of SPE on apoptosis. Apoptotic nuclei in paraffin-embedded DLP tissue sections from 24-week old TRAMP mice from each cohort are yet to be analyzed by fragment end labeling of DNA (TdT-FragEL™).

7. Effects of SPE on P-AKT. Western blot analysis of phosphorylation/activation of Akt in DLPs from the TRAMP mice will be used to assess whether SPE suppresses the activation of the IGF-I signaling pathway. These studies have not yet been completed.

8. Effect of SPE on prostate DHT concentration. The concentration of DHT/T in the ventral prostate is yet to be determined by RIA.

9. Diet composition. To confirm incorporation of SPE into the diet, the fatty acid content of SPE, control mouse chow and SPE-supplemented mouse chow was determined by previously described methods (2). The diets contained the expected amounts of lauric, capric and myristic acids (data not shown). Surprisingly, we were unable to detect lauric acid in the plasma or prostates of mice treated with control or SPE-supplemented diets.
KEY RESEARCH ACCOMPLISHMENTS

- Established a TRAMP breeding colony
- Filled all treatment groups for the short-term and long-term SPE treatment studies
- Established that short-term treatment with SPE at doses of 50 mg/kg and 300 mg/kg are not toxic.
- Prostate tissue from both studies were histologically processed, stained with H&E and scored pathologically.
- Established that long-term treatment with SPE delays tumor progression in the DLP of TRAMP mice.
- Established that long-term treatment with SPE decreases the number of frank tumors in 24-week old TRAMP mice
- Immunocytochemistry for Ki 67 (indicator of cell proliferation) is established and the effect of short-term SPE treatment has been analyzed.

REPORTABLE OUTCOMES

- A manuscript describing the results of this study is currently being prepared.
- Data from this study was used to apply for a R21 grant award from NIH NCCAM and this grant is currently pending (R21 AT003397-01)

CONCLUSIONS

These data suggest that SPE is a non-toxic dietary supplement that can reduce epithelial proliferation in the dorsolateral prostate of TRAMP mice and lead to a delay in prostate cancer progression. We are currently completing studies that will help identify and understand the mechanism of this effect.

REFERENCES


APPENDIX

List of personnel from the research effort.
1. Charles Roselli, Ph.D; PI
2. Charles Roberts, Ph.D.; Co-I
3. Teri Wadsworth, Ph.D.; Postdoctoral fellow