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A Double Blind, Randomized, Neoadjuvant Study of the Tissue effects of POMx Pills in Men with Prostate Cancer Prior to Radical Prostatectomy

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Abstract

Pomegranates slow prostate cancer xenograft growth and prolong PSA doubling times in single-arm human studies. Pomegranates' effects on human prostate tissue are understudied. We hypothesized orally administered pomegranate extract (POMx; PomWonderful, Los Angeles, CA) would lower tissue 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidative stress biomarker. 70 men were randomized to 2 tablets POMx or placebo daily up to 4 weeks prior to radical prostatectomy. Tissue was analyzed for intra-prostatic Urolithin A, a pomegranate metabolite, benign and malignant 8-OHdG, and cancer pS6 kinase, NFκB, and Ki67. Primary end-point was differences in 8-OHdG powered to detect 30% reduction. POMx was associated with 16% lower benign tissue 8-OHdG (p=0.095), which was not statistically significant. POMx was well-tolerated with no treatment-related withdrawals. There were no differences in baseline clinicopathological features between arms. Urolithin A was detected in 21/33 patient in the POMx group vs. 12/35 in the placebo group (p=0.031). Cancer pS6 kinase, NFκB, Ki67, and serum PSA changes were similar between arms. POMx prior to surgery results in pomegranate metabolite accumulation in prostate tissues. Our primary end-point in this modest-sized short-term trial was negative. Future larger longer studies are needed to more definitely test whether POMx reduces prostate oxidative

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stress as well as further animal testing to better understand the multiple mechanisms through which POMx may alter prostate cancer biology.

INTRODUCTION

Given the significant morbidity associated with standard prostate cancer (PC) treatments and the lack of FDA-approved agents for PC prevention, there is growing interest in alternative and complementary approaches for PC prevention and treatment.(1) Pomegranate juice and its polyphenol antioxidants have been extensively studied pre-clinically for their *in vivo* and *in vitro* molecular effects, and clinically for their impact on serum PSA kinetics.(2)

Both *in vitro* and animal studies show pomegranate extract and pomegranate juice can inhibit PC growth.(3-8) In a single-arm human trial of men with a rising PSA after primary therapy, pomegranate juice was associated with statistically significant longer PSA doubling times (PSADT) versus pre-study PSADT (i.e. more slowly rising PSA).(9) This finding was further supported by a non-blinded randomized phase II trial of men with a rising PSA after primary treatment randomized to 1 or 2 tablets of daily Pomegranate-X (POMx, Pom Wonderful, Los Angeles, CA), a pill containing concentrated pomegranate extracts.(10) The study found 76-82% of men in both arms had longer on-study PSADT values than pre-study PSADT, though there were no differences in on-study PSADT between arms. Given the lack of placebo control, the lack of a dose-response, the fact a prior placebo-controlled trial found 73% of men on placebo on a similar study had longer on-study PSADT than pre-study,(11) and the lack of prostate tissue to confirm biological effects makes interpreting these data challenging.

Thus, we undertook a randomized, placebo-controlled study of POMx daily for up to 4 weeks prior to radical prostatectomy. The goal was to obtain prostate tissue to objectively measure whether pomegranate extracts were systemically absorbed resulting in Urolithin A, the predominant pomegranate metabolite, being accumulated in the prostate, and to assess what molecular effects, if any, this had on both benign and malignant prostate tissue biology. Our primary outcome was the difference between arms in prostate 8-hydroxydeoxyguanosine (8OHdG) levels. 8OHdG is formed as the result of oxidative damage to the DNA base 2‘-deoxyguanosine(dG) and is a major product of DNA oxidation. We choose 8OHdG levels as our primary outcome because oxidative damage is a key pathway in PC development and progression(12) and pomegranates have been shown to affect oxidative stress,(13) suggesting that altering oxidative stress may be a key pathway through which pomegranates impact PC biology. Moreover, 8OHdG is considered to be a sensitive, stable and integral marker of oxidative damage in cellular DNA, and is considered stable for immunohistochemistry in formalin-fixed, paraffin embedded sections and antibodies have been widely used to evaluate oxidative DNA damage in animal and human tissues.(14)

PATIENTS AND METHODS

Patients

Participants were recruited from the urology clinics at Duke University and Johns Hopkins University between February 2009 to January 2011. Participants were required to have a histologic diagnosis of prostate adenocarcinoma and to be scheduled to undergo radical prostatectomy at least 2 weeks from study entry. The diagnostic needle biopsy was required to have at least 2 cores with cancer to increase the likelihood of having PC tissue for analysis. Subjects were required to stop all commercially available pomegranate products, nutritional supplements and herbal therapies (i.e. lycopene, vitamin E, selenium, genistein,

or saw palmetto) for at least 2 weeks prior to starting the intervention. Subjects were ineligible if they were currently on a 5-alpha reductase inhibitor, anti-androgens, or LHRH agonists, or had received a bilateral orchiectomy. The study was approved by the institutional review board at each participating institution.

Study Design

This was a Phase II, randomized double-blind trial designed to study intermediate biologic end-points in serum and tissue specimens to determine the bioavailability and the effects on prostate inflammation, apoptosis, and proliferation of the study treatment. This trial was registered with clinicaltrial.gov (#NCT00719030). All subjects underwent informed consent prior to study entry. Randomization (1:1) was by a permuted random block design. Study duration was up to 4 weeks, though a window of treatment that included additional days of treatment was permitted to accommodate standard surgical scheduling. All subjects consumed a study prescribed pill twice daily generally starting on the day of randomization until the day of surgery (last tablet the evening before surgery) but was timed to ensure up to 4 weeks on therapy (minimum 2 weeks). For subjects on the POMx arm, this was two 2 POMx tablets taken orally once daily (POM Wonderful, Los Angeles, California) and for those on placebo, it was a matching placebo pill with the same schedule of administration (Paramount Farming, Los Angeles, California). Compliance was recorded as a percent of scheduled intakes of study product consumed. Non-compliance was defined as consumption of <80% of the scheduled intakes. Subjects in both groups were asked not to consume commercially available pomegranates and to make no additional changes to their diets during the study period.

At baseline and at the conclusion of up to four weeks of study treatment, all subjects had a physical examination and blood drawn for PSA, and whole blood for serum and plasma. Following surgical removal and before fixation, a 1,000 mg biopsy of fresh prostate tissue was isolated. This sample was obtained from any prostate tissue, regardless of tumor involvement. The remainder of the prostate was fixed in formalin and embedded in paraffin per standard processing procedures at each institution. All fresh frozen tissues and slides cut from representative formalin-fixed paraffin embedded (FFPE) blocks were shipped to UCLA for analyses.

POMx

POMx (provided by Pom Wonderful, Los Angeles, CA) is a pomegranate (Punica granatum L., Wonderful variety) fruit polyphenol extract. POMx was developed for use as a nutritional supplement and has Generally Recognized as Safe (GRAS) status. Each capsule contains 1000mg of POMx powder which includes up to 600 mg of polyphenol from extract, which delivers pomegranate polyphenols in an amount equivalent to about 8 oz of pomegranate juice. POMx powder is produced in a two-step process: (1) extraction of polyphenols from pomegranate fruit, and (2) purification of the extract to produce a highly concentrated polyphenol powder. Extraction is performed during the fruit harvest using pressed pomegranate skins and arils with the seed completely removed. Product specifications have been established, and batch analyses data confirm the product is consistent in quality and free of microbial or chemical contaminants. The extract has been well characterized and contains the same compounds found in pomegranate juice, differing only in having lower anthocyanidins and significantly higher proportional content of pomegranate polyphenols, primarily punicalagin and isomers, but the levels in food or supplement products are limited to the amount found in 8 oz of 100% juice.

Outcomes

The primary objective was to compare mean differences between arms in prostatic 8-hydroxydeoxyguanosine (8OhdG) levels, a measure of oxidative damage, in the radical prostatectomy specimen. Secondary outcomes included between arm differences in tissue biomarkers of PC inflammation, development and progression (NF-κB expression, pS6 kinase), proliferation (Ki-67), measurement of the pomegranate metabolite, Urolithin A, within the prostate, treatment related toxicity, and serum PSA. Urolithin A was measured from frozen tumor tissue without performing frozen section histological analysis preventing us from knowing whether the tissues were malignant or benign and creating only one value of urolithin A for analyses.

Serum analysis

Serum was assayed for PSA using the standard CLIA-certified laboratories at each center as part of standard of care.

Tissue biomarker analysis

Four μm-thick tissue sections were cut prior to staining. They were first heated to 56°C for 20 minutes, followed by deparaffinization in xylene. The sections were then rehydrated in graded alcohols and endogenous peroxidase was quenched with 3% hydrogen peroxide in methanol at room temperature. The sections were then placed in a 95°C solution of 0.01 M sodium citrate buffer (pH 7.0) for antigen retrieval. Protein blocking was accomplished through application of 5% normal horse serum for 30 minutes. Endogenous biotin was then blocked with sequential application of avidin D, then biotin (A/B blocking system). The sections were then incubated for 1 hour with various primary antibodies at room temperatures. Primary anti-8-OHdG was monoclonal antibody purchased from (JaICA, Japan), anti-Ki67 monoclonal antibody purchased from (DAKO, CA), and anti-NFkB was a polyclonal antibody purchased from (Abcam, MA). For anti-8-OHdG and anti-Ki67, 1:50 dilution was used and for anti-NFkB, 1:300 dilutions were used. After washing, biotinylated horse anti-mouse IgG was applied for 30 min at room temperature. Next, the ABC complex was applied for 25 min and diaminobenzidine DAB (DAKO, CA) was used as the chromagen. TBST buffer at pH 7.4 was used for all intermediate wash steps and a moist humidity chamber was used for prolonged incubations. The sections were counterstained with Harris' Hematoxylin, followed by dehydration and mounting. A negative control section was prepared exactly in the same manner except omitting the primary antibody. Immunohistochemical stained slides were examined independently by a single trained genitourinary pathologist blinded to treatment (JYR). The staining intensities (graded from 0 to 3) and percentage of staining for each staining grade were recorded separately.

Tissue Urolithin analysis

Reagents—All solvents and chemical reagents were HPLC grade from Fisher Scientific Co. (Tustin, CA). Urolithin A was synthesized and characterized at UCLA Center for Human Nutrition. The reference mixture of urolithin A glucuronide was enriched from human urine and characterized at UCLA Center for Human Nutrition.

LCMS sample preparation—Prostate samples (~500 mg) were thawed and homogenized with 1.5 mL of MeOH-HCl-H_2O (79.9:0.1:20.0, v:v:v) solution using a grinder (Kontes*Duall*Tissue Grinder Capacity: 5 mL Size: 22 Plastic Coating). The mixture was centrifuged at 18407 rcf for 5 minutes in a 2 mL micro centrifuge tube. The pellet was further extracted with 1.5 mL of the same methanol solution and centrifuge at 18407 rcf for 5 minutes. Both supernatants were pooled and evaporated to dryness with a Speed-Vac. The dry residue was dissolved with 500 μL of methanol in an ultrasound water bath for 5

minutes and centrifuged at 18407 rcf for 5 minutes. The resulting supernatant was evaporated to dryness. Finally, this dry residue was reconstituted with 200 μL of MeOH: H_2O (1:1) solution and centrifuge at 18407 rcf for 5 minutes. The supernatant was the LCMS sample solution.

LC/MS analyses—The LC/MS system consisted of an LCQ Advance Finnigan system (Thermo Finnigan, San Jose, CA), equipped with a Survey HPLC system consisting of an autosampler/injector, quaternary pump, column heater, and diode array detector with Xcalibur version 1.2 software (Finnigan Corp). A Zorbax SB-C18 5 μm 2.1 × 150 mm column (Agilent) was employed for the separation with a gradient elution condition by increasing the percentage of acetonitrile (with 1% acetic acid) in water (with 1% acetic acid) from 5% to 99% in 50 minutes at a flow rate of 0.19 mL/minute. The MS condition for the detection of urolithin A glucuronide were as follows: electron spray ionization in negative modes; scan range, 150-500 amu; scan rate 1 scan/second; cone voltage 17 eV. Identification of urolithin A glucuronides was obtained by matching the molecular ions ($M-H^+$) obtained by electrospray ionization/MS and MS/MS with the expected theoretical molecular weights from literature data as of urolithin A glucuronide, M-H m/z 403, MS/MS (M-H m/z 227)(1, 2). Subjects with an undetectable Urolithin A value were assigned a value of 0 for statistical analyses.

Statistical Analysis

A sample size of 70 (35 per group) was estimated to provide the t-test with 80% power to detect a corresponding effect difference of 0.35 between groups with a 2-sided alpha of 0.05. This power calculation was based on results from prior interventions.(15, 16). Group size was estimated using statistical power software (Epicenter Software, Pasadena, CA). No interim analyses were planned or conducted. Continuous variables were reported as means (± standard deviation [SD]) and medians (Inter Quartile Range [IQR]). Normal distribution was tested with the Kolmogorov- Smirnov test. Based on whether the continuous data were normally distributed or not, we quantified associations with either Student's t-test or the Mann Whitney U test, respectively. *P*-values <0.05 were considered significant with the Bonferroni correction applied to correct for multiple comparisons. Based on normal distribution either Spearman's or Pearson's correlations were performed to test the association of the tumor markers with Urolithin A. Pearson's Chi-square and Fisher's exact tests were used for comparison of categorical variables.

RESULTS

Baseline Characteristics

Though 70 subjects signed consent forms, one withdrew prior to randomization. Of the remaining 69 men, 33 were randomized to POMx and 36 to placebo. The baseline characteristics of these 69 subjects who completed the study are shown in Table 1. The groups were well balanced in terms of the baseline demographics, biopsy Gleason sum, and PSA. Most patients were white and >90% of men in both arms had biopsy Gleason sums of 7 or less. All patients had an ECOG score of 0.

Treatment Duration, Compliance, and Side Effects

Mean number of days from screening to date of prostatectomy was 37 ± 19 days in the POMx group and 33 ± 11 days in the placebo group (2-sided T Test for comparison of means, p-value=0.280). With the exception of one subject for whom a protocol deviation was approved for 75% compliance, all other subjects in both groups were compliant with the dietary intervention in that all men consumed >80% of the prescribed pills.

There were no serious adverse events in either group. No patient withdrew due to adverse events. Eight subjects (6 POMx, 2 Placebo) reported an adverse event, all of which were grade I. Six of the eight (4 POMx, 2 Placebo) were GI related (nausea, diarhhea) and judged possibly related to study agent.

Pathological Analyses

There were no differences between groups in any pathological end-points (table 2). Most men in both arms had Gleason 7 and organ-confined, margin-negative disease. Seminal vesicle invasion and lymph node positivity was rare.

Primary Outcome

The primary outcome was between arm differences in prostatic 8OHdG. This was assessed both in benign prostate tissue and PC tissue. In benign tissue, 8OHdG levels were 16% lower in the POMx treated arm, though this failed to reach statistical significance (p=0.095, table 2). Though 8OHdG expression in the benign tissue was significantly correlated with levels in cancer tissue (r=0.441, p=0.001), the overall expression was much lower in cancer tissue than in benign tissue (table 2). In cancer tissue, post-treatment 8OHdG levels were 23% lower in the POMx treated arm, though this difference did not reach statistical significance (p=0.372).

Secondary Outcomes

Urothithin A, a pomegranate metabolite, was detected significantly more frequently in men in the POMx arm (21/33=64%) than in the placebo arm (12/35=36%; p=0.022). Moreover, when examined as a continuous variable, Urolithin A levels were significantly higher in the POMx group compared to placebo (1.12 vs. 0.49 ng/gm; p=0.007). There were no differences between arms in PSA prior to surgery (p=0.739) nor in the ratio of baseline to pre-surgery PSA (p=0.443). There were no between arm differences in expression of pS6, nf-kb, or Ki67 within PC tissue (table 3).

Exploratory Outcomes

Among men from both arms combined, Urolithin A levels were inversely correlated with 8OHdG expression (i.e. more Urolithin A associated with lower oxidative damage as measured by 8OHdG) in both benign (r=−0.115, p=0.369) and cancer tissue (r=−0.299, p=0.017), though this only reached statistical significance in the cancer tissue.

DISCUSSION

Though pomegranate extract and juice and POMx pills have shown promise in pre-clinical and limited clinical studies, there are limited data on the bioavailability and distribution of orally consumed pomegranate and its *in vivo* cellular and molecular effects within prostate tissue.(3-10) In a randomized phase II trial of two daily tablets of POMx vs. placebo, we found that urolithin A, a pomegranate metabolite, was significantly more likely to be present and at higher levels in men assigned to POMx. This is the first strong human evidence that orally administered pomegranate routinely reaches and accumulates in the prostate. However, in this modest sized short-term study, POMx treatment did not significantly lower 8OHdG levels, a measure of oxidative damage and our primary outcome. Higher urolithin A levels, a key pomegranate metabolite, were correlated with less 8OHdG providing some evidence to the hypothesis that pomegranates do in fact lower 8OHdG. As such, the current findings support further formal hypothesis testing of POMx for reducing oxidative damage as well as further animal testing to better understand the multiple mechanisms through which POMx may alter prostate cancer biology.

Pomegranates have been touted to have numerous health benefits.(17) In regards to PC, several pre-clinical reports have shown that pomegranates, whether by extract or concentrated juice, can slow PC growth *in vitro* and in animal models.(3-8) Unfortunately, human studies of pomegranate for PC are limited. To date, only three studies have been published.(9, 10, 18) In two of them, men with a rising PSA after primary therapy were treated with pomegranate juice or POMx.(9, 10) Though neither study included a placebo control, both studies found men who consumed pomegranates had longer PSADT values than prior to study enrollment, suggesting that pomegranate consumption may slow human prostate growth. However, placebo controlled trials of men with rising PSA after primary therapy have also shown that the majority of men treated with placebo have longer on-study than pre-study PSADT (11) and thus whether the longer PSADT truly reflected any anti-PC activity of pomegranate is unclear. A randomized, placebo controlled study of pomegranate liquid extract in men with rising PSA after primary therapy is nearing completion.

Pomegranates have been shown to contain more than 100 different phytochemicals, including the bioactive family of ellagitannins.(19) A number of studies have examined the oral bioavailability of pomegranate juice polyphenols(20, 21) determined by plasma bioavailability of ellagic acid and urinary accumulation of urolithin-A glucuronide, a urinary metabolite of ellagic acid. Pomegranate ellagitannins are not absorbed intact into the blood stream but are instead absorbed after being hydrolyzed to ellagic acid in the intestine. Ellagitannins are also further metabolized into urolithins by gut flora, which are subsequently conjugated in the liver and finally excreted in the urine. To date, only one human study examined the effect of pomegranate consumption on prostate tissue.(18) In this study, 19 men prior to surgery for either PC or benign prostatic hyperplasia (BPH) were given pomegranate juice for 3 days prior to surgery and were compared to 14 subjects given walnuts for 3 days prior to surgery and to 30 untreated controls. Pomegranate juice was derived from fresh pomegranates using a laboratory pilot press and patients consumed 200ml per day. Urolithin A was detected in only 2 of the 19 men given pomegranate juice compared to zero of the controls suggesting either limited accumulation of pomegranate metabolites in the prostate or lack of sensitivity in their detection. Though other tissue analyses were limited, the authors did note no significant differences in CDKN1a, Ki67, or c-Myc expression among men treated with either pomegranate juice or walnuts vs. the untreated controls. Unfortunately, this study had numerous limitations including a mixed group of men undergoing surgery not just for PC but also for BPH, small numbers (only 14 men treated with pomegranate), and the use of pomegranate for only a limited duration of 3 days. As such, it is difficult to draw firm conclusions from this one study. In contrast to this prior study, we found that treatment for up to four weeks of a known pomegranate extract (POMx) resulted in significantly increased Urolithin A levels in the prostate. As such, we conclude that treatment with POMx can result in detectable tissue levels of a major pomegranate metabolite. However, it should be noted that the overall levels of urolithin A were low consistent with the known poor uptake of ellagtannins and ellagic acid in blood. Moreover, 12 subjects in the POMx arm had undetectable levels. Whether this reflects different pomegranate metabolism among different subjects, poor compliance (though pill counting showed >75% compliance among all but one man), low overall POMx exposure, or insufficient exposure time is unknown. However, these findings suggest that future studies testing higher doses and for longer duration of POMx may be warranted.

A large body of literature has linked inflammation and the reactive oxygen species (ROS) generated secondary to inflammation to prostate carcinogenesis.(22) Inflammation in the microenvironment of the PC cell may stimulate the multistep process of carcinogenesis by upregulating the production of pro-inflammatory cytokines and their signaling pathways. Evidence supports the concept that proliferative inflammatory atrophy of benign prostate epithelium may be a precursor to prostatic intraepithelial neoplasia and PC.(23)

Inflammation can result in persistent oxidative stress in cancer cells and the ROS may lend cancer cells a survival advantage.(24, 25) Mild levels of oxidative stress stimulate cancer cell proliferation(24) and increase mutation rates through DNA damage and/or epigenetic changes.(26) Furthermore, low levels of antioxidant enzymes and defective DNA repair of oxidative DNA damage in malignant prostatic tissue relative to benign prostate epithelium implicate oxidative DNA damage in prostate carcinogenesis.(23) (27)

Oxidative stress represents an imbalance between the production and quenching of reactive oxygen species, with accumulation of intracellular free radicals that can damage all components of the cell. Oxidative damage to the DNA base 2‘-deoxyguanosine(dG) produces 8-hydroxy-2‘-deoxyguanosine (8-OHdG), a major product of DNA oxidation. The concentration of 8-OHdG within a cell has been proposed as a measurement of oxidant stress and oxidative DNA damage, and when it is incorporated into DNA, 8-OHdG has demonstrated a mutagenic potential, leading to a point mutation via an A to T substitution. 8-OHdG levels have been correlated with the incidence of several cancers. (28) We hypothesized that patients with PC would exhibit a large amount of oxidized DNA adducts as a result of GSTP1 gene inactivation and the chronic oxidant stresses to which they are exposed. We also hypothesized that the number of DNA adducts can be diminished by treatment of patients with agents containing anti-oxidant polyphenols such as POMx. Though the amount of oxidized DNA adducts, such as 8-OHdG, present in the prostate of patients with PC has not been established, a key mechanism through which pomegranates are thought to affect PC growth is via reducing oxidative damage.(9, 13) Indeed, when PC cells are grown in serum from men given pomegranate juice (Wonderful variety) it results in less oxidative state and reduced oxidation of serum lipids vs. cells treated with serum prior to pomegranate intake.(9) In the current study, we indeed found that POMx treatment did lower 8OHdG levels in both benign (16% lower) and cancerous tissue (23%), though this did not reach statistical significance in either analysis. Moreover, higher Urolithin A levels were correlated with lower 8OHdG levels further supporting the notion that POMx may lower 8OHdG. Unfortunately, the effect size of POMx on 8OHdG levels and a clinically significant threshold in change in 8OHdG levels were unknown prior to the study, and thus we estimated our power calculations and sample size upon prior studies of different agents(15) and different tumor types(16) assuming the extent of effect previously reported would be similar compared to the proposed research. Thus, while the current study failed to meet its primary end-point, it does provide better effect estimates for powering a larger study going forward. Moreover, it does suggest that such an approach may be warranted.

We then examined other key PC biomarkers including Ki67 (proliferation), pS6 (a marker of mTOR activity), and Nf-kB (a measure of inflammation). However, we found no effect on POMx on these relevant biomarkers. Of note, prior murine studies did show that pomegranate can affect some of these markers.(4, 6, 7) As such, whether these negative data reflect insufficient dose or duration of POMx therapy, or some other cause is unknown. However, there are multiple putative mechanisms through POMx may affect PC.(2) As such, further studies are needed to more comprehensively investigate potential targets which are altered in response to POMx therapy.

What we did confirm was the relative safety of short-term POMx therapy. No patient had any adverse event at a grade II or higher. Consistent with the known side-effect profile of POMx, we did have some mild GI effects.(10) Thus, while continued efforts to determine the efficacy of POMx for PC are needed, we can conclude based upon this study and prior clinical trials of pomegranate juice and POMx in men with PC that it appears pomegranates are unlikely to be harmful.(9, 10)

This study is not without limitations. First, our primary end-point is an intermediate surrogate biomarker end-point. The clinical relevance of 8OHdG levels is unclear. Thus, we used 8OHdG as a means to test if POMx had "on-target" effects which would support future placebo-controlled randomized studies aimed at more clinically relevant end-points. Second, the number of men included was modest limiting our statistical power to detect important changes. Third, the duration of POMx therapy was short and the dose was modest. As such, further studies are needed to test whether longer duration or higher doses have greater effects are warranted. This is particularly true in that we did find higher Urolithin A levels were correlated with lower 8OHdG levels suggesting higher doses may have greater effects within the prostate. However, this analysis was limited by our inability to separate the benign from the malignant tissue when examined the Urolithin A levels. Moreover, it is possible that urolithin A levels were influenced by dietary sources other than POMx tablets as indeed some men in the control arm had detectable urolithin A levels. Future studies may consider measuring urine urolithin A and other pomegranate metabolites as further controls assessing systemic absorption. Finally, we only examined a small number of secondary end-points. It is hoped that future analyses of these samples including full gene expression analyses should yield valuable information regarding the effects of POMx on the prostate.

SUMMARY

A small randomized placebo-controlled phase II trial of 4 weeks of dietary intervention with POMx prior to radical prostatectomy did not significantly lower 8OHdG levels. However, the fact that urolithin A, an active pomegranate metabolite was capable of absorption and accumulation in prostate tissues and higher Urolithin A levels correlated with lower 8OHdG levels does provide some evidence to support the underlying hypothesis that pomegranates may modulate 8OHdG levels and suggests a role for pomegranate juice in protection against oxidative DNA damage. Further and larger studies with longer duration are needed to formally test whether pomegranates can alter 8OHdG levels and the clinical relevance of this as well as further animal testing to better understand the multiple mechanisms through which POMx may alter prostate cancer biology.

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REFERENCES

1. Bishop FL, Rea A, Lewith H, et al. Complementary medicine use by men with prostate cancer: a systematic review of prevalence studies. Prostate Cancer Prostatic Dis. 2011; 14:1–13. [PubMed: 20956994]
2. Bell C, Hawthorne S. Ellagic acid, pomegranate and prostate cancer -- a mini review. The Journal of pharmacy and pharmacology. 2008; 60:139–44. [PubMed: 18237460]
3. Seeram NP, Aronson WJ, Zhang Y, et al. Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland. J Agric Food Chem. 2007; 55:7732–7. [PubMed: 17722872]
4. Koyama S, Cobb LJ, Mehta HH, et al. Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society. 2010; 20:55–62. [PubMed: 19853487]
5. Sartippour MR, Seeram NP, Rao JY, et al. Ellagitannin-rich pomegranate extract inhibits angiogenesis in prostate cancer in vitro and in vivo. Int J Oncol. 2008; 32:475–80. [PubMed: 18202771]

6. Rettig MB, Heber D, An J, et al. Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-kappaB-dependent mechanism. Mol Cancer Ther. 2008; 7:2662–71. [PubMed: 18790748]
7. Adhami VM, Siddiqui IA, Syed DN, Lall RK, Mukhtar H. Oral infusion of pomegranate fruit extract inhibits prostate carcinogenesis in the TRAMP model. Carcinogenesis. 2012; 33:644–51. [PubMed: 22198212]
8. Wang L, Alcon A, Yuan H, Ho J, Li QJ, Martins-Green M. Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effect on prostate cancer cells. Integrative biology : quantitative biosciences from nano to macro. 2011; 3:742–54. [PubMed: 21594291]
9. Pantuck AJ, Leppert JT, Zomorodian N, et al. Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. Clin Cancer Res. 2006; 12:4018–26. [PubMed: 16818701]
10. Paller CJ, Ye X, Wozniak PJ, et al. A randomized phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer. Prostate Cancer Prostatic Dis. 2012
11. Smith MR, Manola J, Kaufman DS, et al. Rosiglitazone versus placebo for men with prostate carcinoma and a rising serum prostate-specific antigen level after radical prostatectomy and/or radiation therapy. Cancer. 2004; 101:1569–74. [PubMed: 15468186]
12. Gupta-Elera G, Garrett AR, Robison RA, O'Neill KL. The role of oxidative stress in prostate cancer. Eur J Cancer Prev. 2012; 21:155–62. [PubMed: 21857523]
13. Aviram M, Dornfeld L, Rosenblat M, et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr. 2000; 71:1062–76. [PubMed: 10799367]
14. Seki S, Kitada T, Yamada T, et al. Immunohistochemical detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in human chronic cholecystitis. Histopathology. 2002; 40:531–5. [PubMed: 12047764]
15. Chen L, Stacewicz-Sapuntzakis M, Duncan C, et al. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. Journal of the National Cancer Institute. 2001; 93:1872–9. [PubMed: 11752012]
16. Luo H, Tang L, Tang M, et al. Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. Carcinogenesis. 2006; 27:262–8. [PubMed: 15930028]
17. Johanningsmeier SD, Harris GK. Pomegranate as a functional food and nutraceutical source. Annual review of food science and technology. 2011; 2:181–201.
18. Gonzalez-Sarrias A, Gimenez-Bastida JA, Garcia-Conesa MT, et al. Occurrence of urolithins, gut microbiota ellagic acid metabolites and proliferation markers expression response in the human prostate gland upon consumption of walnuts and pomegranate juice. Mol Nutr Food Res. 2010; 54:311–22. [PubMed: 19885850]
19. Heber, D. Pomegranate Ellagitannins. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed.. CRC Press; Boca Raton, FL: 2011.
20. Seeram NP, Lee R, Heber D. Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (Punica granatum L.) juice. Clin Chim Acta. 2004; 348:63–8. [PubMed: 15369737]
21. Seeram NP, Zhang Y, McKeever R, et al. Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects. J Med Food. 2008; 11:390–4. [PubMed: 18598186]
22. Malins DC, Johnson PM, Barker EA, Polissar NL, Wheeler TM, Anderson KM. Cancer-related changes in prostate DNA as men age and early identification of metastasis in primary prostate tumors. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:5401–6. [PubMed: 12702759]
23. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007; 7:256–69. [PubMed: 17384581]

24. Kondo S, Toyokuni S, Iwasa Y, et al. Persistent oxidative stress in human colorectal carcinoma, but not in adenoma. Free radical biology & medicine. 1999; 27:401–10. [PubMed: 10468215]

25. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. European journal of cancer. 1996; 32A:30–8. [PubMed: 8695238]

26. Wainfan E, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. Cancer research. 1992; 52:2071s–7s. [PubMed: 1544143]

27. Trzeciak AR, Nyaga SG, Jaruga P, Lohani A, Dizdaroglu M, Evans MK. Cellular repair of oxidatively induced DNA base lesions is defective in prostate cancer cell lines, PC-3 and DU-145. Carcinogenesis. 2004; 25:1359–70. [PubMed: 15044326]

28. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009; 27:120–39. [PubMed: 19412858]

Translational relevance

The effect of pomegranates on human prostate tissue is unclear. In a randomized double blind study of men with prostate cancer undergoing radical prostatectomy, we found that up to 4 weeks of supplementation with the pomegranate extract, POMx, was associated with no significant reductions in 8-hydroxy-2-deoxyguanosine, a measure of oxidative stress. Given the presumed importance of oxidative stress in prostate cancer development and progression, future larger longer studies are needed to more definitely test whether POMx reduces prostate oxidative stress as well as further animal testing to better understand the multiple mechanisms through which POMx may alter prostate cancer biology.

Table 1

Baseline Characteristics

	POMx		Placebo			
Feature	**No.**	**%**	**No.**	**%**	**p-value**	**Test**
Race						
White	27	81.8	31	86.1		
Black	5	15.2	5	13.9	.563	Chi-Square
Native American	1	3.0	0	0.0		
Height, cm (mean ± SD)	179.62	±8.733	181.25	12.001	.529	T Test
Weight, kg (mean ± SD	92.12	±17.070	94.18	15.836	.610	T Test
Age, y (mean ± SD)	60.03	±7.935	57.09	6.254	.096	T Test
Biopsy Gleason sum						
6	13	39.4	20	55.6		
7	18	54.5	14	38.9	.363	Chi-Square
8	2	6.1	1	2.8		
9	0	0.0	1	2.8		
ECOG						No Test performed
ECOG 0	33	100.0	36	100.0	NA	
PSA, ng/ml (Mean) ± SD	6.89	± 3.884	6.83	± 4.274	.878	Ranksum Test

Table 2

Pathological Prostatectomy Features

	POMx		Pacebo			
Feature	**No.**	**%**	**No.**	**%**	**p-value**	**Test**
Surgical Procedure						
Open	31	93.9	36	100.0		
Laparoscopic	0	0.0	0	0.0	.325	Chi-Square
Robotic	1	3.0	0	0.0		
unknown	1	3.0	0	0.0		
T stage						
pT1	0	0.0	1	2.8		
pT2	22	60.7	23	63.9	.626	Chi-Square
pT3	11	33.3	12	33.3		
Gleason at Surgery						
6	12	36.4	13	36.1		
7	18	54.5	21	58.3	.161	Chi-Square
8	3	9.1	0	0.0		
9	0	0.0	2	5.6		
Surgical Margins						
Negative	20	60.6	22	61.1		
Positive	13	39.4	14	38.9	1.000	Fisher's Exact
Seminal Vesicle Involvement						
No	30	90.9	34	94.4		
Yes	3	9.1	2	5.6	.572	Chi-Square
N stage						
pN0	26	78.8	25	69.4		
pN1	0	0.0	1	2.8	.491	Chi-Square
pNx	7	21.2	10	27.8		

Table 3

Tissue Analyses

	Therapy	N	Mean % positive	Std. Deviation	p-value
8-OHdG Normal Cells	Placebo	33	74.70	31.50	.095
	POM	30	62.67	36.48	
8-OHdG Tumor Cells	Placebo	33	33.52	38.45	.372
	POM	30	25.90	33.76	
pS6 Tumor Cells	Placebo	32	39.53	26.50	.245
	POM	29	46.10	24.85	
NFkB Tumor Cells	Placebo	33	44.85	37.88	.887
	POM	27	44.44	35.47	
Ki-67 Tumor Cells	Placebo	33	.76	.90	.164
	POM	30	.60	.89	