Research Article

Cancer Prevention Research

A Double-Blind, Randomized, Neoadjuvant Study of the Tissue Effects of POMx Pills in Men with Prostate Cancer Before Radical Prostatectomy

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Abstract

Pomegranates slow prostate cancer xenograft growth and prolong prostate-specific antigen (PSA) doubling times in single-arm human studies. Pomegranates' effects on human prostate tissue are understudied. We hypothesized that orally administered pomegranate extract (POMx; Pom Wonderful) would lower tissue 8-hydroxy-2′-deoxyguanosine (8-OHdG), an oxidative stress biomarker. Seventy men were randomized to two tablets, POMx or placebo, daily up to four weeks before radical prostatectomy. Tissue was analyzed for intraprostatic urolithin A, a pomegranate metabolite, benign and malignant 8-OHdG, and cancer pS6 kinase, NF-κB, and Ki67. Primary endpoint was differences in 8-OHdG, and the study was powered to detect 35% 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 of the 33 patients in the POMx group versus 12 of the 35 in the placebo group ($P = 0.031$). Cancer pS6 kinase, NF-κB, Ki67, and serum PSA changes were similar between arms. POMx before surgery results in pomegranate metabolite accumulation in prostate tissues. Our primary endpoint in this modest-sized short-term trial was negative. Future larger longer studies are needed to more definitively 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. *Cancer Prev Res; 6(10); 1120–7. ©2013 AACR.*

Introduction

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

Both *in vitro* and animal studies show that pomegranate extract and pomegranate juice can inhibit prostate cancer 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 prestudy PSADT (i.e., more slowly rising PSA; ref. 9). This finding was further supported by a nonblinded 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), a pill containing concentrated pomegranate extracts (10). The study found that 76% to 82% of men in both arms had longer on-study PSADT values than prestudy 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 prestudy (11), and the lack of prostate tissue to confirm biologic effects make interpreting these data challenging.

Thus, we undertook a randomized, placebo-controlled study of POMx daily for up to 4 weeks before radical

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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 definitively 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.

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-hydroxy-2′-deoxyguanosine (8-OHdG) levels. 8-OHdG is formed as the result of oxidative damage to the DNA base 2′-deoxyguanosine and is a major product of DNA oxidation. We choose 8-OHdG levels as our primary outcome because oxidative damage is a key pathway in prostate cancer 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 prostate cancer biology. Moreover, 8-OHdG 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 (FFPE) 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 (Durham, NC) and Johns Hopkins University (Baltimore, MD) 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 two cores with cancer to increase the likelihood of having prostate cancer 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 before starting the intervention. Subjects were ineligible if they were currently on a 5-α reductase inhibitor, anti-androgens, or luteinizing-hormone releasing hormone (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 endpoints 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 before 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 POMx tablets taken orally once daily (POM Wonderful) and for those on placebo, it was a matching placebo pill with the same schedule of administration (Paramount Farming). Compliance was recorded as a percentage of scheduled intakes of study product consumed. Noncompliance was defined as consumption of less than 80% of the scheduled intakes. Subjects in both the 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 4 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 FFPE blocks were shipped to UCLA (Los Angeles, CA) for analyses.

POMx

POMx (provided by Pom Wonderful) is a pomegranate (Punica granatum L., Wonderful variety) fruit polyphenol extract. POMx was developed to be used as a nutritional supplement and has Generally Recognized as Safe status. Each capsule contains 1,000 mg 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: (i) extraction of polyphenols from pomegranate fruit, and (ii) purification of the extract to produce a highly concentrated polyphenol powder. Extraction is conducted 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 that the product is

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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 the mean differences between arms in prostatic 8-OHdG levels, a measure of oxidative damage, in the radical prostatectomy specimen. Secondary outcomes included between arm differences in tissue biomarkers of prostate cancer inflammation, development and progression (NF-κB expression, pS6 kinase), proliferation (Ki67), 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 conducting frozen section histologic 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 micrometer thick tissue sections were cut before 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 mol/L 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), anti-Ki67 monoclonal antibody purchased from (DAKO), and anti-NF-κB was a polyclonal antibody purchased from (Abcam). For anti-8-OHdG and anti-Ki67, 1:50 dilutions were used and for anti-NF-κB, 1:300 dilutions were used. After washing, biotinylated horse anti-mouse immunoglobulin G was applied for 30 minutes at room temperature. Next, the avidin–biotin complex was applied for 25 minutes, and diaminobenzidine DAB (DAKO) 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 (J.-Y. Rao). 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 high-performance liquid chromatography (HPLC) grade from Fisher Scientific Co. 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.

LC/MS 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 18,407 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 centrifuged at 18,407 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 18,407 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 centrifuged at 18,407 rcf for 5 minutes. The supernatant was the liquid chromatography/mass spectrometry (LC/MS) sample solution.

LC/MS analyses. The LC/MS system consisted of an LCQ Advance Finnigan system (Thermo Finnigan), 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 used 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 conditions for the detection of urolithin A glucuronide were as follows: electron spray ionization in negative modes; scan range, 150 to 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 tandem mass spectrometry (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; refs. 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 two-sided α

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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). No interim analyses were planned or conducted. Continuous variables were reported as mean (±SD) and median (inter quartile range). Normal distribution was tested with the Kolmogorov–Smirnov test. On the basis of whether the continuous data were normally distributed or not, we quantified associations with either Student *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. On the basis of normal distribution, either Spearman or Pearson correlation was conducted to test the association of the tumor markers with Urolithin A. Pearson χ^2 and Fisher exact tests were used for comparison of categorical variables.

Results

Baseline characteristics

Though 70 subjects signed consent forms, one withdrew before 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 more than 90% of men in both arms had biopsy Gleason sums of 7 or less. All patients had an Eastern Cooperative Oncology Group (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 (two-sided *t* test for comparison of means, $P = 0.280$). 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 more than 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 1. Six of the 8 (4 POMx, 2 placebo) were gastrointestinal related (nausea, diarhhea) and judged possibly related to study agent.

Pathologic analyses

There were no differences between groups in any pathologic endpoints (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 8-OHdG. This was assessed both in benign prostate tissue and prostate cancer tissue. In benign tissue, 8-OHdG levels were 16% lower in the POMx-treated arm, though this failed to reach statistical significance ($P = 0.095$; Table 2). Though 8-OHdG expression in the benign tissue was significantly correlated with the 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, posttreatment 8-OHdG 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

Table 1. Baseline characteristics

Feature	POMx N (%)	Placebo N (%)	*P*	Test
Race				
White	27 (81.8)	31 (86.1)		χ^2
Black	5 (15.2)	5 (13.9)	0.563	
Native American	1 (3.0)	0 (0.0)		
Height, cm (mean ± SD)	179.62 (±8.733)	181.25 (12.001)	0.529	*t* test
Weight, kg (mean ± SD)	92.12 (±17.070)	94.18 (15.836)	0.610	*t* test
Age, y (mean ± SD)	60.03 (±7.935)	57.09 (6.254)	0.096	*t* test
Biopsy Gleason sum				
6	13 (39.4)	20 (55.6)		χ^2
7	18 (54.5)	14 (38.9)	0.363	
8	2 (6.1)	1 (2.8)		
9	0 (0.0)	1 (2.8)		
ECOG				
ECOG 0	33 (100.0)	36 (100.0)	NA	No test conducted
PSA, ng/mL (Mean) ± SD	6.89 (±3.884)	6.83 (±4.274)	0.878	Rank-sum test

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Table 2. Pathologic prostatectomy features

Feature	POMx N (%)	Placebo N (%)	P	Test
Surgical procedure				
Open	31 (93.9)	36 (100.0)		χ^2
Laparoscopic	0 (0.0)	0 (0.0)	0.325	
Robotic	1 (3.0)	0 (0.0)		
Unknown	1 (3.0)	0 (0.0)		
T stage				
pT1	0 (0.0)	1 (2.8)		χ^2
pT2	22 (60.7)	23 (63.9)	0.626	
pT3	11 (33.3)	12 (33.3)		
Gleason at surgery				
6	12 (36.4)	13 (36.1)		χ^2
7	18 (54.5)	21 (58.3)	0.161	
8	3 (9.1)	0 (0.0)		
9	0 (0.0)	2 (5.6)		
Surgical margins				
Negative	20 (60.6)	22 (61.1)		Fisher exact
Positive	13 (39.4)	14 (38.9)	1.000	
Seminal vesicle involvement				
No	30 (90.9)	34 (94.4)		χ^2
Yes	3 (9.1)	2 (5.6)	0.572	
N stage				
pN0	26 (78.8)	25 (69.4)		χ^2
pN1	0 (0.0)	1 (2.8)	0.491	
pNx	7 (21.2)	10 (27.8)		

POMx group compared with placebo (1.12 vs. 0.49 ng/gm; $P = 0.007$). There were no differences between arms in PSA before surgery ($P = 0.739$) or in the ratio of baseline to presurgery PSA ($P = 0.443$). There were no between arm differences in expression of pS6, NF-κB, or Ki67 within prostate cancer tissue (Table 3).

Exploratory outcomes

Among men from both arms combined, urolithin A levels were inversely correlated with 8-OHdG expression (i.e., more urolithin A associated with lower oxidative damage as measured by 8-OHdG) 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 preclinical and limited clinical studies, there are limited data on the bioavailability and distribution of orally consumed pomegranate and its *in vivo*

Table 3. Tissue analyses

	Therapy	N	Mean% positive	SD	P
8-OHdG Normal cells	Placebo	33	74.70	31.50	0.095
	POM	30	62.67	36.48	
8-OHdG Tumor cells	Placebo	33	33.52	38.45	0.372
	POM	30	25.90	33.76	
pS6 Tumor cells	Placebo	32	39.53	26.50	0.245
	POM	29	46.10	24.85	
NF-κB Tumor cells	Placebo	33	44.85	37.88	0.887
	POM	27	44.44	35.47	
Ki67 Tumor cells	Placebo	33	0.76	0.90	0.164
	POM	30	0.60	0.89	

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cellular and molecular effects within prostate tissue (3–10). In a randomized phase II trial of two daily tablets of POMx versus 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 8-OHdG levels, a measure of oxidative damage and our primary outcome. Higher urolithin A levels, a key pomegranate metabolite, were correlated with less 8-OHdG providing some evidence to the hypothesis that pomegranates do in fact lower 8-OHdG. 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 prostate cancer, several preclinical reports have shown that pomegranates, whether by extract or concentrated juice, can slow prostate cancer growth *in vitro* and in animal models (3–8). Unfortunately, human studies of pomegranate for prostate cancer 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 before 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 prestudy PSADT (11), and thus whether the longer PSADT truly reflected any anti-prostate cancer 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 before surgery for either prostate cancer or benign prostatic hyperplasia (BPH) were given pomegranate juice for 3 days before surgery and were compared with 14 subjects given walnuts for 3 days before surgery and with 30 untreated controls. Pomegranate juice was derived from fresh pomegranates using a laboratory pilot press and patients consumed 200 mL per day. Urolithin A was detected in only 2 of the 19 men given pomegranate juice compared with 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 versus the untreated controls. Unfortunately, this study had numerous limitations including a mixed group of men undergoing surgery not just for prostate cancer 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 with this prior study, we found that treatment for up to 4 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 prostate cancer cell may stimulate the multistep process of carcinogenesis by upregulating the production of proinflammatory 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 prostate cancer (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 ROS, with accumulation of intracellular free radicals that can damage all components of the cell. Oxidative damage to the DNA base 2′-deoxyguanosine produces8-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

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oxidative DNA damage, and when it is incorporated into DNA, 8-OHdG has shown 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 prostate cancer 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 antioxidant polyphenols such as POMx. Though the amount of oxidized DNA adducts, such as 8-OHdG, present in the prostate of patients with prostate cancer has not been established, a key mechanism through which pomegranates are thought to affect prostate cancer growth is via reducing oxidative damage (9, 13). Indeed, when prostate cancer cells are grown in serum from men given pomegranate juice (Wonderful variety), it results in less oxidative state and reduced oxidation of serum lipids versus cells treated with serum before pomegranate intake (9). In the current study, we indeed found that POMx treatment did lower 8-OHdG 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 8-OHdG levels further supporting the notion that POMx may lower 8-OHdG. Unfortunately, how effectively POMx would alter 8-OHdG levels and a clinically significant threshold in change in 8-OHdG levels were unknown before 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 that the extent of effect previously reported would be similar compared with the proposed research. Thus, although the current study failed to meet its primary endpoint, 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 prostate cancer biomarkers including Ki67 (proliferation), pS6 (a marker of mTOR activity), and NF-κB (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 which POMx may affect prostate cancer (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 grade 2 or higher. Consistent with the known side-effect profile of POMx, we did have some mild gastrointestinal effects (10). Thus, although continued efforts to determine the efficacy of POMx for prostate cancer are needed, we can conclude based upon this study and prior clinical trials of pomegranate juice and POMx in men with prostate cancer that it seems pomegranates are unlikely to be harmful (9, 10).

This study is not without limitations. First, our primary endpoint is an intermediate surrogate biomarker endpoint. The clinical relevance of 8-OHdG levels is unclear. Thus, we used 8-OHdG as a means to test whether POMx had "on-target" effects which would support future placebo-controlled randomized studies aimed at more clinically relevant endpoints. 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. This is particularly true in that we did find higher urolithin A levels were correlated with lower 8-OHdG 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 examining 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 endpoints. It is hoped that future analyses of these samples including full gene expression analyses should yield valuable information about the effects of POMx on the prostate.

Summary

A small randomized placebo-controlled phase II trial of up to 4 weeks of dietary intervention with POMx before radical prostatectomy did not significantly lower 8-OHdG 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 8-OHdG levels does provide some evidence to support the underlying hypothesis that pomegranates may modulate 8-OHdG 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 8-OHdG 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.

Disclosure of Potential Conflicts of Interest

M.A. Carducci is a consultant/advisory board member of POM Wonderful. S. Kerkoutian is a consultant/advisory board member of POM Wonderful. D. Heber has commercial research grant from POM Wonderful. A.J. Pantuck has commercial research grant from POM Wonderful. No potential of conflicts were disclosed by the other authors.

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