Phase II, Randomized, Placebo-Controlled Trial of Neoadjuvant Celecoxib in Men With Clinically Localized Prostate Cancer: Evaluation of Drug-Specific Biomarkers

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A B S T R A C T

Purpose

Cyclooxygenase-2 (COX-2) is a potential pharmacologic target for the prevention of various malignancies, including prostate cancer. We conducted a randomized, double-blind trial to examine the effect of celecoxib on drug-specific biomarkers from prostate tissue obtained at prostatectomy.

Patients and Methods

Patients with localized prostate cancer and Gleason sum ≥ 7 , prostate-specific antigen (PSA) ≥ 15 ng/mL, clinical stage T2b or greater, or any combination with greater than 45% risk of capsular penetration were randomly assigned to celecoxib 400 mg by mouth twice daily or placebo for 4 to 6 weeks before prostatectomy. The primary end point was the difference in prostatic prostaglandin levels between the two groups. Secondary end points were differences in COX-1 and -2 expressions; oxidized DNA bases; and markers of proliferation, apoptosis and angiogenesis. Tissue celecoxib concentrations also were measured. Tertiary end points were drug safety and compliance.

Results

Seventy-three patients consented, and 64 were randomly assigned and included in the intention-to-treat analysis. There were no treatment differences in any of the primary or secondary outcomes. Multivariable regression revealed that tumor tissue had significantly lower COX-2 expression than benign prostatic tissue ($P = .01$) and significantly higher levels of the proliferation marker Ki-67 ($P < .0001$). Celecoxib was measurable in prostate tissue of patients on treatment, demonstrating that celecoxib reached its target. Celecoxib was safe and resulted in only grade 1 toxicities.

Conclusion

Treatment with 4 to 6 weeks of celecoxib had no effect on intermediate biomarkers of prostate carcinogenesis, despite the achievement of measurable tissue levels. We caution against using celecoxib 400 mg twice daily as a preventive agent for prostate cancer in additional studies.

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INTRODUCTION

Prostate cancer is the most common malignancy in men worldwide. In the United States in 2008, there were approximately 186,300 new diagnoses of prostate cancer and 28,700 prostate cancer deaths, which represented 25% of new cancer occurrences and 10% of cancer deaths in men.¹ African Americans and men with a positive family history are at even higher risk of acquiring the disease. Development of effective prevention strategies to diminish the threat of prostate cancer is critical to reducing the burden of this disease on individuals and societies. However,

progress in this field has been slow, as chemoprevention trials that use clinical end points are costly and require long follow-up. Use of intermediate biologic end points in such trials, although clearly investigational, may provide early clues on efficacy and may shorten study duration.

Development of prostate cancer is thought to occur through a multistep process, by which normal prostatic epithelium progresses through proliferative inflammatory atrophy, to prostatic intraepithelial neoplasia, and finally to adenocarcinoma.² Although the precise sequence of genetic changes in this process is unknown, a key early event

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is the methylation and inactivation of the glutathione S-transferase- π gene, which is involved in detoxification of oxidants produced by chronic inflammation.³ In addition, it is known that prolonged oxidative DNA damage promotes progression from proliferative inflammatory atrophy and prostatic intraepithelial neoplasia to prostate adenocarcinoma⁴ and that, when glutathione S-transferase- π is downregulated, nonsteroidal anti-inflammatory drugs may halt oxidative stress.^{5,6}

Cyclooxygenase (COX) enzymes (ie, COX-1 and COX-2) catalyze the conversion of arachidonic acid to prostaglandins, which can in turn inhibit apoptosis, stimulate cell proliferation and migration, suppress immune function, and promote angiogenesis.⁷ Use of celecoxib, a selective COX-2 inhibitor, for cancer prevention is not a new concept. In a randomized trial of patients with familial adenomatous polyposis, celecoxib reduced the size and number of colonic polyps.⁸ Celecoxib also protected against formation of sporadic colorectal adenomas.⁹ Case-control studies also have suggested that this agent decreases the risk of cancers of the breast¹⁰ and lung.¹¹ There is abundant preclinical, clinical, and epidemiologic data to support the potential use of celecoxib in prostate cancer chemoprevention.^{12,13} Many, but not all, studies have found COX-2 overexpression in human prostate cancers and prostate cancer cell lines.¹⁴⁻¹⁶ Moreover, selective COX-2 inhibitors suppressed growth of human prostate cancer cells in vitro and in vivo by inducing apoptosis and by inhibiting cell proliferation and angiogenesis.¹⁷⁻²⁰ Finally, prospective cohort studies have shown that use of nonselective COX-2 inhibitors is associated with reduced risk of prostate cancer.²¹⁻²³

This study aimed to investigate the effect of 4 to 6 weeks of neoadjuvant celecoxib on drug-specific biomarker modulation in normal and malignant prostate tissue obtained at prostatectomy from men with clinically localized prostate cancer. The biomarkers (ie, prostaglandins, COX-1 and -2, oxidized DNA bases, proliferation markers, apoptotic markers, and angiogenic markers) were chosen for their biologic rationale as well as for their relevance to the mechanism of action of celecoxib. We explored the feasibility of analyzing these intermediate biomarkers of carcinogenesis, and we hoped to provide the impetus for larger-scale chemoprevention trials that use celecoxib in men at high-risk for prostate cancer.

PATIENTS AND METHODS

Patients

Participants were recruited from the urology clinic of the Johns Hopkins Hospital (Baltimore, MD). Participants were required to have histologically confirmed, localized prostate adenocarcinoma with at least one of the following high-risk features: Gleason sum ≥ 7 , baseline serum prostate-specific antigen (PSA) ≥ 15 ng/mL, clinical stage T2b or greater, two or more positive cores on biopsy, or any combination of these factors that predicted a greater than 45% chance of capsular penetration.²⁴ Patients must have decided on radical prostatectomy for the treatment of their cancers. Other eligibility criteria included Eastern Cooperative Oncology Group performance status less than 2 and adequate bone marrow, renal, and hepatic functions. Exclusion criteria included any major surgery, radiotherapy, hormonal therapy, or chemotherapy received in the last 28 days; evidence of metastatic disease; chronic use of full-dose aspirin (ie, 325 mg) or other anti-inflammatory drugs, including corticosteroids; prior allergies to sulfa or salicylates; history of bleeding disorders or chronic use of anticoagulants; or any active infectious illness, including HIV. The protocol was approved by the institutional review board. The study was supported by National Cancer Institute (NCI) Grant No. N01-CN-95000-46, and Pfizer.

Study Design

This was a single-center, phase II, placebo-controlled, double-blinded, randomized trial that used intermediate biologic end points.²⁵ Eligible participants provided written informed consent and had their surgeries scheduled before enrollment. By using permuted-block randomization, participants were assigned to either celecoxib 400 mg by mouth twice daily or matching placebo (both provided by Pfizer, New York, NY) for 4 to 6 weeks before prostatectomy. Surgery was never delayed to meet this treatment requirement. Patients were instructed to take the blinded study drug as prescribed to within 8 hours of the surgery. Treatment was stopped for any grade 3 or greater toxicity, but all randomly assigned patients were included in the intention-to-treat analysis. Unblinding did not occur until after study termination.

At baseline, participants had a history and physical examination, complete blood count, routine serum chemistries, coagulation studies, and serum PSA measurement. Clinic visits, routine blood sampling, and safety evaluations were conducted every 2 weeks until the day of surgery. At the final preoperative evaluation, serum PSA was redrawn. The prostate gland was harvested at prostatectomy and was used for investigating the primary and secondary outcomes; some analyses were performed on fresh prostate tissue, and others were performed on fixed tissue. Four weeks postoperatively, patients underwent telephone interviews to assess late treatment complications.

Outcomes

The primary objective was to compare tissue prostaglandin levels (namely prostaglandins D₂, E₂, and F_{2 α} , thromboxane B₂, and 6-keto-prostaglandin F_{1 α}) in resected prostate glands from patients receiving celecoxib and placebo. Prostaglandin levels were measured from fresh prostate tissue, both with and without addition of arachidonic acid, by using gas chromatography-mass spectrometry.²⁶ Assays were performed on paired tissue specimens from each participant (as each specimen contained varying amounts of carcinoma), and the two measurements were averaged. All prostaglandin levels were normalized to the total amount of protein in the sample.

Secondary end points included quantification of tissue COX-1 and COX-2 mRNA expression and measurement of tissue markers of oxidative DNA damage (ie, 8-hydroxy-2-deoxyguanosine [8-OHdG]), cell proliferation (ie, Ki-67), apoptosis (ie, p27^{kip1} and p21^{waf1}), and angiogenesis (ie, factor VIII). COX-1/-2 mRNA levels were determined by performing quantitative reverse transcriptase polymerase chain reaction (RT-PCR) on fresh prostate tissue.²⁷ Levels of 8-OHdG were measured from fresh tissue by using liquid chromatography-mass spectrometry.²⁸ Immunohistochemical staining on fixed tissue samples was performed to assay for Ki-67,²⁹ p27^{kip1},³⁰ p21^{waf1},³¹ and factor VIII.³² All of these assays were conducted in duplicate, and results were averaged. Additional secondary outcomes included analysis of prostate celecoxib levels in fixed tissue by using liquid chromatography-mass spectrometry³³ and serum PSA measurements.

Tertiary outcome measures were drug safety and patient compliance. Safety monitoring included serial physical examinations, adverse event reporting, and routine laboratory evaluations. Adverse events were graded according to the NCI Common Toxicity Criteria version 2.0 and were reported to the medical monitor of the NCI and the pharmaceutical sponsor. Compliance was measured by using self-recorded drug diaries and by counting tablets in returned pill containers. Dosing and scheduling modifications were not permitted.

Patient Samples

Prostatectomy specimens were sectioned fresh; areas of probable tumor and probable normal tissue were obtained by punch biopsy; and tissue samples were subjected to immediate frozen-section analysis. For each patient, we aimed to separately obtain both tumor and normal samples identified on frozen section. In several instances, although we tried to identify tumor-free samples, we often found tumor present within normal specimens. Therefore, for each sample, we used the frozen sections to estimate the percentage of epithelial tissue that consisted of tumor, and this was factored into the multivariable analysis. The fraction of tissue containing epithelium versus stroma was not recorded, although this was generally high (50% to 80% in each instance) and usually was higher in tumor tissue than in benign tissue.

Quantification of Immunohistochemical Staining

We first prepared tissue microarrays (TMAs) by selecting the highest-grade/largest tumor per patient, which we sampled with four-fold redundancy. For each immunohistochemical stain (ie, Ki-67, p27^{kip1}, p21^{waf1}, and factor VIII), TMA slides were scanned with the BLISS virtual slide scanner (Bacus Laboratories, Lombard, IL), and TMA cores first were assigned a diagnosis by the study pathologist and then were subjected to semi-automated image analysis with the BLISS virtual slide scanner. For each biomarker, we divided the area of brown staining by the area of epithelial cells on the TMA core and obtained a normalized ratio.³⁴ The area of epithelium was obtained on each TMA core by staining with cytokeratin-8 and by using automated image analysis.³⁴ Cores with both tumor and normal tissues were excluded if they contained greater than 10% of the other component.

Statistical Analyses

The sample size of 60 patients (30 in each arm) was chosen to provide 85% power to detect a shift in biomarker levels of 0.8 standard deviations by using a two-sided type I error of 5%. Up to 10 additional patients were allowed to enroll, to account for participants who might withdraw from the study.

Means with 95% CIs were used to summarize primary and secondary outcomes by treatment group. Differences in biomarker levels between groups were assessed for statistical significance by using univariable generalized estimating equation (GEE)³⁵ analysis. Multivariable regression analysis with GEE analysis was employed to assess biomarker outcomes as a function of celecoxib treatment and other factors, such as the amount of tumor in the specimen, Gleason score, baseline PSA, age, ethnicity, and presence of arachidonic acid. A compound, symmetric covariance structure was assumed for these regression models, because there was no reason to believe that paired biomarker measurements would have different correlations. Treatment-group comparisons were visualized with box plots of the raw data on a logarithmic scale. Statistical analyses were performed by using SAS (version 8.0; SAS Institute, Cary, NC) and R (version 2.1; National Cancer Institute, Bethesda, MD). No statistical tests were performed on the tertiary outcomes. All *P* values were two sided.

RESULTS

Patient Characteristics

Between April 2002 and January 2005, seventy-three men consented to enroll on the study, but nine withdrew consent before ran-

Fig 1. CONSORT diagram.

dom assignment; thus, 64 participants remained who were included in the intention-to-treat analysis (Fig 1). Thirty-two men were randomly assigned to celecoxib, and 32 were randomly assigned to placebo. Baseline characteristics were balanced in the treatment groups (Table 1). Overall median age was 59 years (range, 46 to 70 years), and overall median PSA was 7.1 ng/mL (range, 0.3 to 25.1 ng/mL). Two patients (each arm, *n* = 1) never took the study drug, although they did have prostate tissue collected for biomarker analysis, and two patients (each arm, *n* = 1) took the study drug but never had prostate tissue harvested.

Biomarker Modulation

Prostatic tissue concentrations of celecoxib were measured in 18 men receiving celecoxib and in 12 men receiving placebo. Concentrations were significantly higher in men receiving celecoxib (mean, 0.16 μ mol/L; 95% CI, 0.04 to 0.27 μ mol/L) than in men receiving placebo (mean, 0.003 μ mol/L; 95% CI, 0.001 to 0.005 μ mol/L; *P* < .0001; Fig 2).

Celecoxib treatment did not influence any of the primary or secondary study outcomes (Table 2, Fig 3), although an association between celecoxib and lower levels of p21^{waf1} approached statistical significance. Prostaglandin figures (Figs 3A to 3E) show only data with

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Treatment Group	
	Celecoxib (n = 32)	Placebo (n = 32)
Age, years		
Mean	58	59
Range	46-68	48-70
SD	6	7
Median	59	60
Ethnicity		
White		
No.	30	28
%	94	88
Black		
No.	2	4
%	6	12
Gleason sum at diagnosis		
6		
No.	18	20
%	56	63
7		
No.	13	9
%	41	28
8-9		
No.	1	3
%	3	9
No. of positive cores		
Mean	4	4
Range	1-10	1-10
SD	2	2
Median	3	3
Baseline PSA, ng/mL		
Mean	8.5	8.9
Range	0.3-24.0	1.2-25.1
SD	5.7	5.7
Median	7.5	6.9

Abbreviations: SD, standard deviation; PSA, prostate-specific antigen.

Fig 2. Tissue levels of celecoxib achieved in the prostate: differences between patients treated with celecoxib and placebo. Tissue celecoxib concentrations are in the log scale. Results are shown as box-and-whisker plots: the height of the box is the interquartile range (IQR), the horizontal line inside the box is the median, and the whiskers extend beyond the upper and lower quartiles by $1.5 \times$ IQR. Values lying outside of this range are considered outliers. Open circles denote that outlier values (solid circles) extend beyond the upper and lower quartiles by more than $1.5 \times$ IQR.

the addition of arachidonic acid. During the 4- to 6-week treatment period, serum PSA decreased, on average, by 0.89 ng/mL (95% CI, 0.10 to 1.89 ng/mL) in the celecoxib group and by 1.18 ng/mL (95% CI, 0.47 to 1.91 ng/mL) in the placebo group ($P = .63$).

After analysis was adjusted for age, ethnicity, tumor percentage, Gleason score, baseline PSA, (and presence of arachidonic acid for prostaglandin analyses), celecoxib was not associated with significant alterations in any of the examined biomarkers. A trend toward lower p21^{waf1} levels with celecoxib treatment was seen ($P = .05$). Representative examples of multivariable GEE regressions are listed in Table 3. Tissue samples with a higher percentage of tumor had lower levels of prostaglandins E₂ ($P = .0002$) and 6kF_{1α} ($P = .0001$), decreased COX-1 and COX-2 expression ($P = .003$ and $P = .01$, respectively), fewer oxidized DNA bases ($P < .0001$), and greater Ki-67 staining ($P < .0001$). Patients with Gleason score ≥ 7 had higher expression of COX-1 ($P = .03$) and COX-2 ($P = .01$) than patients with Gleason scores of 6. White participants had higher levels of prostaglandins E₂ ($P = .04$) and F_{2α} ($P = .03$) and of COX-1 expression ($P = .03$) and had lower levels of p27^{kip1} ($P = .02$) than black participants. Age and baseline PSA were not significantly associated with any biomarker outcomes.

Safety and Compliance

Adverse events for both treatment groups are listed in Table 4. Overall, the study drug was well tolerated. There were no deaths. Elevated transaminase levels, in a patient receiving placebo, represented the only grade 3 toxicity. Other adverse events were all grades 1 to 2 toxicities. Two patients receiving celecoxib developed tachycardia compared with no patients receiving placebo. There were no cardiovascular events or bleeding complications in either group; gastrointestinal toxicities were similar.

Table 2. Biomarker Modulation by Celecoxib in Prostate Cancer Tissue

Biomarker	Treatment Group		P*
	Celecoxib (n = 31)	Placebo (n = 31)	
PGD ₂ , ng/mg of protein†			
Mean	0.75	0.75	.76
95% CI	0.63 to 0.86	0.65 to 0.85	
PGE ₂ , ng/mg of protein†			
Mean	0.97	0.79	.24
95% CI	0.81 to 1.13	0.67 to 0.90	
PGF _{2α} , ng/mg of protein†			
Mean	0.82	0.76	.25
95% CI	0.70 to 0.94	0.61 to 0.91	
TXB ₂ , ng/mg of protein†			
Mean	0.27	0.19	.08
95% CI	0.22 to 0.31	0.17 to 0.21	
6kPGF _{1α} , ng/mg of protein†			
Mean	2.26	1.88	.27
95% CI	1.75 to 2.78	1.44 to 2.31	
COX-1 mRNA, fg/μg of RNA†			
Mean	22.74	14.26	.21
95% CI	13.58 to 31.81	8.26 to 20.27	
COX-2 mRNA, fg/μg of RNA†			
Mean	21.27	13.12	.26
95% CI	13.60 to 28.95	11.00 to 15.24	
8-OHdG, fmol/nmol of 2-dG†			
Mean	49.21	51.43	.33
95% CI	29.23 to 69.18	8.18 to 94.68	
Ki-67, % positive staining‡			
Mean	1.52	1.68	.57
95% CI	1.01 to 2.03	1.20 to 2.17	
p27 ^{kip1} , % positive staining‡			
Mean	16.57	16.10	.29
95% CI	14.28 to 18.86	13.40 to 18.80	
p21 ^{waf1} , % positive staining‡			
Mean	1.08	1.18	.05
95% CI	0.72 to 1.44	0.92 to 1.44	
Factor VIII, % positive staining‡			
Mean	1.53	1.39	.62
95% CI	1.28 to 1.78	1.21 to 1.58	

Abbreviations: PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; PGF_{2α}, prostaglandin F_{2α}; TXB₂, thromboxane B₂; 6kPGF_{1α}, 6-keto prostaglandin F_{1α}; COX, cyclooxygenase; 8-OHdG, 8-hydroxy-2-deoxyguanosine.

*Calculated by using univariable, generalized estimating equation analysis.

†Analysis performed on fresh tissue.

‡Analysis performed on fixed tissue.

Of 62 patients taking the study drug, 52 (84%) took the drug appropriately for the duration of the study (celecoxib arm, n = 28; placebo arm, n = 24). Four patients (each arm, n = 2) took inadequate doses of the drug, whereas six patients (celecoxib arm, n = 1; placebo arm, n = 5) stopped the drug early because of toxicities. An exploratory analysis after exclusion of these 10 patients yielded results similar to the intention-to-treat analysis.

DISCUSSION

This randomized, placebo-controlled trial investigated the effects of short-term, neoadjuvant celecoxib on intermediate biomarkers of prostate carcinogenesis. In our population of men with localized disease, 4 to 6 weeks of celecoxib did not significantly alter prostatic levels

Fig 3. The effect of celecoxib compared with placebo on drug-specific biomarkers collected from (A-H) fresh and (I-L) fixed tissues. Biomarker levels are in the log scale. Only prostaglandin (PG) analyses performed with arachidonic acid are shown. Celecoxib did not significantly alter (A-E) tissue PG levels; (F-G) cyclooxygenase (COX) enzyme expression; (H) levels of oxidized DNA bases; or (I) markers of proliferation, (J-K) apoptosis, or (L) angiogenesis. A trend toward statistical significance was seen with the apoptotic marker p21^{waf1}. TXB₂, thromboxane B₂; 6kPGF_{1α}, 6-keto PG F_{1α}; 8-OHdG, 8-hydroxy-2-deoxyguanosine; 2-dG, 2-deoxyguanosine.

of various prostaglandins, COX-1/2 mRNA, oxidized DNA bases, Ki-67, p27^{kip1}, p21^{waf1}, or factor VIII, despite achieving measurable tissue concentrations. Moreover, effects on serum PSA were not significantly different between treatment arms.

Because of its unique methodology, this study provided additional important results and aided our understanding of the role of COX enzymes in prostate cancer progression. Multivariable regression analysis showed that COX-1 and -2 expressions were significantly lower in tumor samples than in benign prostate tissue, in contrast to many previous observations.^{14,15} Tumor tissue also contained lower levels of prostaglandin E₂, 6-keto-prostaglandin F_{1α}, and oxidized DNA bases and contained higher levels of Ki-67. In addition, a Gleason score ≥ 7 was associated with increased COX-1 and -2 expression. We do not believe this last factor affected interpretation of our results, because the treatment arms were balanced with respect to Gleason grade. Finally, white patients had higher levels of prostaglandins E₂ and F_{1α}, COX-1 expression, and oxidized DNA bases and had lower levels of p27^{kip1} than blacks.

Our observations mirror those of a similar randomized trial that evaluated the effect of 4 weeks of neoadjuvant celecoxib on several biologic end points.³⁶ In that single-blinded study involving 45 men with localized prostate cancer (mean Gleason sum, 6.4; mean pretreat-

ment PSA, 8.2 ng/mL), celecoxib therapy resulted in lower levels of prostatic Ki-67 ($P = .04$) but did not modulate COX-2 expression or markers of angiogenesis, hypoxia, and apoptosis. Effects on serum PSA were not determined.

Other trials have shown potentially more promising results. In a randomized, placebo-controlled trial of celecoxib given for 6 months to 78 men with PSA-recurrent prostate cancer,³⁷ mean PSA velocity increased by 3% with placebo and decreased by 3% with celecoxib ($P = .02$). In a similar, nonrandomized, single-arm study of long-term celecoxib in men with PSA-recurrent disease,³⁸ 48% of 40 patients had a stable or declining PSA at 3 months. Celecoxib is also being studied currently in combination with radiation therapy as definitive treatment for localized prostate cancer³⁹; these results are pending. In addition, a large multi-arm, randomized trial (STAMPEDE)⁴⁰ currently is recruiting men with advanced/metastatic prostate cancer to evaluate the role of hormone therapy plus celecoxib compared with other treatment arms, including hormone therapy used alone. Finally, celecoxib has been examined in two studies as an adjunct to chemotherapy in patients with castration-resistant prostate cancer (CRPC). In a phase II trial in which twice-monthly docetaxel was combined with daily celecoxib in 48 men with CRPC,⁴¹ PSA responses (ie, $> 50\%$ PSA decrease) were 46%, and mean time to PSA progression

Table 3. Multivariable GEE Regression Estimates for PGE₂, COX-2 mRNA, and Ki-67

Parameter	Multivariable Analysis		
	Coefficient*	95% CI	P
PGE₂			
Intercept	−0.45	−1.59 to 0.68	—
Celecoxib†	0.10	−0.18 to 0.38	.49
Tumor‡§	−0.15	−0.21 to −0.09	< .01
Gleason†	0.30	0.00 to 0.60	.06
PSA‡	0.01	−0.01 to 0.04	.26
Age‡	−0.01	−0.03 to 0.01	.30
White ethnicity†	0.41	0.14 to 0.68	.04
Arachidonic acid†	0.31	0.21 to 0.41	< .01
COX-2 mRNA			
Intercept	2.66	1.23 to 4.09	—
Celecoxib†	0.12	−0.21 to 0.45	.47
Tumor‡§	−0.14	−0.23 to −0.05	.01
Gleason†	0.46	0.12 to 0.79	.01
PSA‡	0.02	0.00 to 0.05	.12
Age‡	−0.01	−0.04 to 0.01	.32
White ethnicity†	0.30	−0.17 to 0.78	.24
Ki-67			
Intercept	−6.08	−8.08 to −4.08	—
Celecoxib†	−0.10	−0.47 to 0.27	.59
Tumor‡§	1.01	0.79 to 1.23	< .01
Gleason†	0.04	−0.34 to 0.42	.83
PSA‡	−0.03	−0.07 to 0.01	.14
Age‡	0.02	−0.01 to 0.05	.26
White†	−0.23	−0.64 to 0.17	.30

Abbreviations: GEE, generalized estimating equations; PGE₂, prostaglandin E₂; PSA, prostate-specific antigen; COX, cyclooxygenase.

*The intercept coefficient is the estimated mean log (PGE₂, COX-2, or Ki-67) for a patient with baseline values for all effects. Treatment (ie, celecoxib) and covariate coefficients (ie, tumor, Gleason, PSA, age, white ethnicity) are the estimated changes in the intercept attributed to one unit change in the predictor.

†Analyzed as categorical variables.

‡Analyzed as continuous variables.

§Scale: 0% to 10% tumor, 11% to 50% tumor, 51% to 89% tumor, 90% to 100% tumor.

||Gleason sum ≥ 7.

was 9.3 months. In a separate, phase II study of 48 men with CRPC,⁴² weekly docetaxel and estramustine given together with daily celecoxib produced PSA responses in 58% of men, and the median time to PSA progression was 8.7 months. Although none of these studies are definitive, these data raise the possibility that celecoxib may alter prostate cancer progression by COX-independent mechanisms, a notion that is supported by preclinical observations.^{33,43}

This study had several limitations. Plasma celecoxib levels were not measured; hence, pharmacokinetic analyses could not be performed. We did not evaluate the long-term effects of celecoxib on serum PSA or examine other clinical end points after surgery. The duration of celecoxib treatment may have been too short to result in significant changes in our primary and secondary outcomes.

An additional concern is whether or not celecoxib reached adequate prostatic tissue levels to be able to influence the selected biomarkers. The mean tissue celecoxib concentration in patients receiving celecoxib was 0.16 μmol/L; this is approximately 50 times lower than maximal plasma concentrations, according to prior pharmacokinetic studies.⁴⁴ This raises the issue of a potential blood-prostate barrier,^{45,46}

Table 4. No. of Adverse Events

Adverse event	No. of Events by Treatment Group			
	Celecoxib		Placebo	
	Grades 1-2	Grade 3	Grades 1-2	Grade 3
Fatigue	2	0	1	0
Dyspepsia	1	0	2	0
Tachycardia	2	0	0	0
Headache	0	0	2	0
Diarrhea	0	0	2	0
Nausea	1	0	0	0
ALT/AST elevation	0	0	0	1
Dry mouth	1	0	0	0
Rash	1	0	0	0
Lightheadedness	1	0	0	0
Insomnia	1	0	0	0
Eye irritation	0	0	1	0

capable of inhibiting adequate celecoxib concentrations from reaching the prostate. It also is possible that celecoxib entered the prostate but was unable to penetrate across the luminal side of the glandular epithelium,⁴⁷ where many of the activated inflammatory cells that express COX-2 reside. In contrast, the tissue celecoxib levels achieved here appear adequate for at least partial COX-2 inhibition.⁴⁸ Therefore, it seems unlikely that the lack of effect of celecoxib on our study end points was caused mainly by insufficient tissue celecoxib levels.

Recently, there has been concern about the cardiovascular sequelae of COX-2 inhibitors.⁴⁹ In a trial of colorectal adenoma prevention, a composite cardiovascular end point (ie, cardiovascular death, myocardial infarction, stroke, or heart failure) was 2.9 times higher among 679 patients receiving celecoxib than among 1,356 patients receiving placebo.⁵⁰ However, a recent, retrospective analysis of celecoxib in 67 men with metastatic CRPC reported four cardiovascular events (ie, myocardial infarction or stroke) in men receiving celecoxib compared with three events in men receiving placebo.⁵¹ The potential benefits of celecoxib in patients with prostate cancer should be weighed against the possible increase of cardiovascular toxicities linked to this agent. Notably, there were no cardiovascular events observed in our trial.

In summary, 4 to 6 weeks of neoadjuvant celecoxib (400 mg by mouth twice daily) in patients with localized prostate cancer did not significantly influence any of a number of COX-related biologic markers of prostate cancer progression, despite reaching prostatic tissues. We would caution, therefore, against additional studies to evaluate celecoxib at this dose for the chemoprevention of prostate cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure

Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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