Effects of a High-Selenium Yeast Supplement on Celecoxib Plasma Levels: A Randomized Phase II Trial

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Abstract

A combination of celecoxib and selenium was used in a randomized double-blind Phase II trial as a preliminary study to a multicenter Phase III colorectal cancer chemoprevention trial using these two agents together. The purpose of this trial was to determine whether high-selenium baker’s yeast ([Saccharomyces cerevisiae] 200 μg once daily) in combination with celecoxib (400 mg once daily) altered the steady-state plasma concentration of celecoxib or produced clinically significant toxicities. Seventy-three healthy subjects (ages 40–75 years) were recruited to the 6-week study from the general local population and were randomized to either the celecoxib plus selenized baker’s yeast group or the celecoxib plus placebo group after a 2-week run in period of celecoxib only. Blood samples were taken at baseline (to document that there was no evidence of celecoxib intake), after the 2-week run-in period on celecoxib to verify steady-state blood levels of this agent, and at end of study (4 weeks postrandomization). Toxicities were monitored at 2 weeks after initiation of celecoxib, at 4 weeks after initiation, and at the end of the study. Blood level concentrations of celecoxib did not differ between the two groups as determined by high-performance liquid chromatography analysis nor were there significant differences in blood chemistry values between the two groups. Subjects’ self-report of general physical toxicities was uncommon and limited to National Cancer Institute toxicity grade 2 or less; however, 2 female participants (3%) were removed from the study medications because of grade 2 edema and significant weight gain after 2 and 2.5 weeks of celecoxib administration. In conclusion, high-selenium yeast and celecoxib can be taken at the described doses with minimum short-term negative effects. In future Phase III chemoprevention trials of celecoxib, weight gain should be carefully monitored, and participants should be made aware of this potential side effect before study entry.

Introduction

Colorectal cancer remains the second leading cause of cancer mortality in the United States (1). Risk factors include a combination of environmental (nutrition and physical activity) and genetic variables. Surveillance, early detection, and prevention are key to the reduction of incidence and mortality from this disease (2). Although current colorectal screening techniques such as fecal occult blood testing and screening colonoscopy are known to reduce mortality from this disease, patient compliance with screening recommendations remains low (3–5). For those patients who comply, the removal of precursor lesions (adenomas) appears to reduce the incidence of subsequent colorectal cancer (6, 7). However, colonoscopic polypectomy has an associated miss-rate (8), and the polyp recurrence rate is often high (9). Thus, there is considerable need to develop dietary and chemopreventive intervention strategies that can be applied to large populations to reduce both adenoma recurrence and colorectal cancer risk.

Over the last decade, efforts toward elucidating the biochemical mechanisms of colorectal carcinogenesis have resulted in great advances in our understanding of the carcinogenic process (10). In addition, insights from epidemiological studies and experimental animal data have made targeted chemopreventive measures more promising (11). Recently, Phase III chemoprevention trials have identified both calcium carbonate and low-dose aspirin as having activity in reducing recurrence rates of colorectal adenomas (12, 13).

Evidence from multiple lines of research consistently supports the hypothesis that nonsteroidal anti-inflammatory drugs (NSAIDs) can prevent colorectal cancers. One mechanism by which NSAIDs inhibit carcinogenesis is through the interruption of cyclooxygenase (COX) activity that metabolizes arachidonic acid to a variety of eicosanoids, including prostaglandins. Prostaglandin synthesis is catalyzed by two isozymes, COX-1 and COX-2. COX-2 contributes to colon tumorigenesis through numerous mechanisms, including inhibition of apoptosis, increased angiogenesis, increased invasiveness, modulation of inflammation/immunosuppression, and conversion of procarcino gens to carcinogens (14, 15). However, the major disadvantages of most NSAIDs such as aspirin and ibuprofen are gastrointestinal and renal toxicity primarily because of COX-1 inhibition. The development of selective COX-2 inhibitors is believed to largely circumvent these toxicities. Celecoxib, a selective COX-2 inhibitor, has been shown to effectively protect against colon cancer in carcinogen-treated animals (16); the degree of carcinogenic inhibition is significantly greater than with other nonselective COX inhibitors (17). In human trials, celecoxib has been shown to cause regression of colorectal adenomas in patients with familial adenomatous polyposis (18) and is Food and Drug Administration approved for reducing the...
number of adenomatous colorectal polyps in this patient population.

In human clinical trials, selenium, in the form of selenized baker’s yeast, has been shown in a secondary analysis of a Phase III study to reduce incidence and mortality from colon cancer (19), and experiments in laboratory animals have demonstrated that various selenium compounds inhibit colorectal tumorigenesis (17, 20, 21).

The mechanisms of action of selenium are not completely known but likely occur at several stages in the multistep carcinogenesis process. It has been suggested that selenium metabolites affect apoptosis and redox-regulated proteins, including transcription factors (22). In animal models, selenium compounds have been shown to inhibit COX-2 expression (21). The specific mechanism of action may depend in part on the individual’s baseline nutritional status of this essential micro-nutrient, and at higher levels of supplementation, selenium may affect carcinogen metabolism, immune function, cell cycle regulation, and apoptosis (23).

Recently, we began a multicenter, Phase III double-blind, randomized, factorial trial to determine the efficacy of celecoxib and selenium supplementation in the form of selenized baker’s yeast (SelenoExcell) separately and combined in reducing colorectal adenoma recurrence in patients with sporadic colorectal adenomas. As a preliminary short-term safety study to the Phase III trial, the purpose of this clinical investigation was to determine whether short-term selenized baker’s yeast (Saccharomyces cerevisiae) in combination with celecoxib had any effect on the steady-state plasma concentration of celecoxib or produced unexpected, clinically significant toxicities over that observed with celecoxib alone.

Materials and Methods

Participants. Male and female participants were recruited from the Tucson and Phoenix metropolitan areas. Participants were eligible if they were between the ages of 40 and 75 and had no severe metabolic or life-threatening acute or chronic illness. Exclusion criteria included known allergy to sulfonamides or NSAIDs. Individuals were ineligible if they used more than one aspirin (325 mg) daily, other NSAID drugs, high-potency vitamins, or individual vitamin or mineral supplements containing selenium. Females were ineligible if they were pregnant or nursing.

We attempted to achieve a balance of gender and minority participants in accordance with NIH policy. This study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants before study entry.

Investigational Agents. Celecoxib was generously supplied by Pharmacia (Peapack, NJ) as oral capsules of Celebrex containing 200 mg of active ingredient. Celecoxib is chemically designated as 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide. The inactive ingredients include croscarmellose sodium, edible inks, gelatin, lactose monohydrate, magnesium stearate, povidone, sodium lauryl sulfate, and titanium dioxide.

SelenoExcell high selenium yeast was formulated and supplied by Cypress Systems, Inc. (Fresno, CA). SelenoExcell is produced from the introduction of selenium salt during ac-cultivation of Saccharomyces cerevisiae without selenium. S. cerevisiae grade microbial requirements. The matched placebo, also formulated by Cypress Systems, Inc., contained yeast (S. cerevisiae) without selenium.

Study Design. Initial eligibility of participants was assessed via telephone. At the initial clinic visit, participants completed a health history questionnaire, and a baseline blood sample was collected to verify general health status. Fasting blood was collected in lavender top vacutainers for complete blood count with differential platelet count and in trace-element free EDTA vacutainers to complete the chemistry panel (biochemical profile) and serum lipids (cholesterol, triglycerides, high density lipids, and low density lipids). Chemistry panel and serum lipid determination were performed by Sonora Quest Diagnostics.

Within 1 week from initial blood draw, participants completed a baseline symptom form and were treated with 400 mg of celecoxib once daily for 2 weeks. This dose of celecoxib was chosen because of its safety record in long-term follow up by Pharmacia (data on file; Pfizer, New York, NY). Study participants were instructed to self-administer the celecoxib dose in the evening. Because of its long elimination half-life, celecoxib dosing for 5 days is required to achieve steady-state blood levels. A morning fasting blood sample was collected at the end of the 5-week run-in period to determine the baseline steady-state celecoxib level. On average, the blood sample was collected ~13 h since the last celecoxib dose. Celecoxib plasma concentrations were determined at the Arizona Cancer Center by one of us (H-H. S. C.). Subjects were then randomized and treated with either (a) 400 mg of celecoxib once daily and selenium matched placebo or (b) 400 mg of celecoxib once daily and 200 μg of selenized baker’s yeast once daily for 30 days. At 2 weeks after randomization, a phone visit occurred to discuss toxicities. At study end, 30 days after randomization, a morning fasting blood sample was collected. This sample was collected at ~13 h since the last celecoxib dose. Plasma celecoxib concentrations were measured, and self-reported adverse effects information was collected.

The blood markers evaluated included sodium, potassium, chloride, glucose, creatinine, blood urea nitrogen, blood urea nitrogen/creatinine ratio, calcium, total protein, albumin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, total cholesterol, high-density lipoprotein, low-density lipoprotein, WBCs, RBCs, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, RBC distribution width, platelet count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Toxicity Monitoring. Before entry into the trial, all participants completed an in depth baseline symptoms questionnaire to document the frequency of common health related complaints (i.e., headaches, fatigue, aching joints and muscles, nausea, abdominal pain, diarrhea, constipation, dyspepsia, and so forth). Participants kept a study calendar during the study to record both their capsule intake and the advent of any changes or new symptoms. Participants were instructed to contact the study clinic with any unusual health problems. Severities of the symptoms were graded following the National Cancer Institute’s Common Toxicity Criteria. All severity was based on changes from baseline levels.

Celecoxib Assay. Celecoxib concentrations in plasma were determined using a reversed phase high-performance liquid chromatography procedure developed and validated by one of us (H-H. S. C.). Briefly, the plasma samples were mixed with the internal standard (SC-236, a chemical analogue of celecoxib) and extracted with the BakerBond Octadecyl SPE car-
triglycerides. The extracted samples were injected onto a Nova-Pak® C₈ column. The mobile phase consisted of acetonitrile, tetrahydrofuran, and sodium acetate buffer (20 mM, pH 5.0) in the ratio of 30:8:62. The flow rate of the mobile phase was 1.5 ml/min. The column eluent was monitored with an UV detector at a wavelength of 215 nm. Linear calibration curves were established over a concentration range of 40–4000 ng/mL. The inter- and intraday variation for the assay was <12% at 40 ng/mL and <5% at higher concentrations. For each analytical run, a standard curve was prepared in plasma, analyzed along with the authentic samples, and used to determine the celecoxib concentrations. In addition, quality control samples were prepared and analyzed along with the authentic samples. The whole batch of samples were reprocessed and reanalyzed if the variation of the quality control samples was >15% of the expected values.

Statistical Analysis. The primary goal of this study was to determine whether selenium-enriched yeast impacts the toxicity profile of celecoxib when given in combination. The sample size of 35 subjects/group was originally selected to guarantee that at least 30 subjects/group would complete the treatment period (assuming a dropout rate no greater than 15%). This sample size allowed us to rule out a toxicity rate (grade 3 or 4) of ≥10%. Additionally, assuming a toxicity rate of ≤5% in the celecoxib alone group, we would be able to detect an increase to a toxicity rate of 35% or higher in the combination group with power >80%.

Demographic characteristics of the celecoxib alone versus celecoxib plus selenium-treated groups were compared using a two-sample independent t test for continuous variables and Fisher’s exact test for discrete variables. The change in blood markers with celecoxib supplementation (before the potential addition of selenium) was tested using a paired t test. Because of the many blood markers measured (n = 30), the P-values were adjusted using the Bonferroni method; only those P-values of ≤0.0017 were considered statistically significant (0.05/30 = 0.0017). Analysis of covariance was used to test for the effect of selenium supplementation on the changes in the blood chemistry markers and the steady-state celecoxib concentration. This analytic approach allowed adjustment for the value measured at the end of the run-in period and therefore adjusted for potential regression to the mean. Differences in the proportion of study drug-related toxicities in the selenium supplemented versus nonselenium-supplemented groups were tested using Fisher’s exact test.

Results

Demographic characteristics of the participants are shown in Table 1. Thirty-five participants were randomized to the celecoxib alone arm, and 38 participants were randomized to the celecoxib plus selenium arm. The participants were predominantly female (60 versus 71%, respectively, P = 0.157), with mean age of 54 and 57 years, respectively (P = 0.337). Participants were predominantly white (79 versus 92%, P = 0.325), with a small number of Hispanics, Native Americans, and other races.

Table 2 documents the changes in blood chemistry markers after the 2 weeks of celecoxib treatment (before the potential addition of selenium). The values shown are the mean change (from baseline) with the associated SD. After adjustment for multiple comparisons, only blood urea nitrogen and blood urea nitrogen/creatinine ratio showed a statistically significant increase (P < 0.001) when adjusted for multiple comparisons.

Analysis of the changes in the blood chemistry markers with selenium supplementation (versus the value measured at the end of the 2-week run-in period) did not suggest a statistically significant difference between those who were and were not supplemented with selenium; the only significant unadjusted P-values at the 0.05 level were creatinine (P = 0.021), blood urea nitrogen/creatinine ratio (P = 0.036), and calcium (P = 0.045; Table 3). After adjustment for multiple comparisons, these differences were not statistically significant.

Table 4 documents the most severe study drug-related postrandomization toxicities with and without selenium supplementation. Four participants in this study experienced local or generalized edema that was probably drug related. Two of these...
4 participants had study drugs discontinued at 2 and 2.5 weeks because of grade 2 edema with 5–10-lb. weight gain; however, there was no significant difference between the two intervention groups ($P = 0.74$). The number of grade 1 toxicities was small, with no significant differences between the selenium supplemented versus nonsupplemented groups; the smallest unadjusted $P$ was for dyspepsia ($P = 0.238$).

Finally, the change in the celecoxib steady-state plasma concentration between the 2- and 6-week blood samples in those participants treated with celecoxib plus selenium versus celecoxib alone was assessed. The mean plasma concentration of celecoxib in the celecoxib plus selenium group at 2 weeks was 640.73 ng/mL and at 6 weeks was 699.13 ng/mL. In the celecoxib only group, the mean plasma concentration at 2 weeks was 568.30 ng/mL and at 6 weeks was 600.61 ng/mL. Although the mean plasma concentration change was greater for the celecoxib plus selenium group (58.4 versus 32.3 ng/mL), the standard deviation of the change was quite large (314.7 versus 363.5 ng/mL), suggesting substantial variability. As a result, there was no statistically significant interparticipant difference in the steady-state celecoxib concentrations with the addition of selenium ($P = 0.76$).

### Discussion

An important strategy for developing a chemopreventive regimen is the evaluation of combinations of agents that will potentially maximize their efficacy while minimizing toxicity (24, 25). Novel combinations of pharmacological agents and nutritional supplements might achieve that goal, and celecoxib plus selenium appears promising in the chemoprevention of colorectal carcinogenesis.

In this clinical trial, 200 μg/day selenized baker’s yeast taken concomitantly with 400 mg/day celecoxib did not affect the steady-state blood concentration of celecoxib. The change from baseline serum levels observed in blood urea nitrogen and blood urea nitrogen/creatinine ratio, although significant, remained in the normal clinical range. In general, this drug combination was well tolerated; however, grade 2 edema with 5–10-lb. weight gain was observed in 2 (2.7%) participants, necessitating their discontinuation of study medications. Mild to moderate edema formation has been reported to occur with celecoxib usage (26) as with other NSAIDs (27) but has not been reported for selenium use. As a preliminary model for our recently initiated Phase III trial, the results presented here suggest that celecoxib and selenized baker’s yeast can be combined safely in a chemopreventive regimen; however, as with all celecoxib trials, it is important to inform the study participants of this potential side effect and carefully monitor for weight gain.

We acknowledge that it may require several months of selenized baker’s yeast to achieve steady-state selenium concentrations (28, 29) and that the ultimate evaluation of its effects on steady-state concentration of celecoxib and celecoxib-associated clinical toxicities would require a much longer intervention duration. This is being accomplished with in-depth plasma selenium and celecoxib steady-state and clinical toxicity monitoring for the first year of our ongoing Phase III trial of these drugs to prevent colorectal adenoma recurrence.

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### References


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