Lack of Effect of Celecoxib on Prostaglandin E2 Concentrations in Nipple Aspirate Fluid from Women at Increased Risk of Breast Cancer

Edward R. Sauter,† Lisa Schlatter,† John Hewett,† Debra Koivunen,† and John T. Flynn‡

†Department of Surgery, University of Missouri-Columbia, Columbia, Missouri and ‡Thomas Jefferson University, Philadelphia, Pennsylvania

Abstract

Background: Cyclooxygenase enzymes (COX-1, COX-2, and COX-3) convert arachidonic acid to prostaglandins, prostacyclins, thromboxanes, and other hydroxy fatty acids. Among these, prostaglandin E2 (PGE2) has tumor growth-promoting activity. The COX-2 isozyme is the primary enzyme involved in PGE2 production in cancerous tissue.

Objective/Hypothesis: We administered the COX-2 inhibitor celecoxib (200 mg b.i.d.) to women at increased breast cancer risk. Our hypothesis was that PGE2 would be secreted in breast nipple aspirate fluid (NAF), that levels in NAF would be higher than in corresponding plasma, and that celecoxib would decrease PGE2 levels in NAF (reflecting a decreased breast tissue eicosanoid production) and plasma.

Specific Aim: To determine if PGE2 concentrations in NAF and plasma decrease after a 2-week course of celecoxib and then return to baseline 2 weeks after stopping the medication (washout).

Introduction

Women at increased breast cancer risk include those with a strong family history of breast cancer, those with a history of ductal carcinoma in situ (DCIS) or invasive breast cancer (IBC), and those with precancerous changes in their breasts. Eligibility for enrollment in the first National Surgical Adjuvant Breast and Bowel Project breast cancer prevention trial required that a participant have a >1.66% projected 5-year probability of developing IBC (1). The only accepted treatment for these women is tamoxifen, with its attendant risks of pulmonary embolism and uterine cancer. An effective treatment with fewer side effects is desirable.

Three mammalian isoenzymes encoded by two different genes, cyclooxygenase (COX)-1, COX-2, and COX-3, may be present in breast tumors to catalyze the conversion of arachidonic acid to prostaglandins, prostacyclins, or thromboxanes. Prostaglandin E2 (PGE2) is produced from arachidonic acid by either COX-1 or COX-2. PGE2 has tumor and cell growth-promoting activity and is the major prostaglandin found in colorectal cancer (2). Nonsteroidal anti-inflammatory drugs, including aspirin, indomethacin, and ibuprofen, inhibit both COX-1 and COX-2 but not COX-3. Extensive studies suggest that COX-1, which is constitutively expressed, is important in a variety of normal body functions, including maintenance of gastrointestinal mucosa and platelet function (3). Inhibition of COX-1 leads to several side effects, including gastrointestinal ulcers and renal toxicity (3). Recent efforts have therefore focused on pharmacologic agents such as celecoxib, a clinically available medication that selectively inhibits COX-2. The attractiveness of COX-2 inhibitors is that they have a favorable toxicity profile and have potential to prevent and/or treat a wide variety of malignancies. For example, celecoxib, a selective COX-2 inhibitor, has been administered to millions of people without significant side effects.

Whereas COX-1 expression is constitutive, COX-2 can be induced by cytokines, mitogens, growth factors, and tumor promoters (4). Regarding COX activity in malignancies, women with breast cancer tissue PGE2 levels >15 ng/g seem to have a significantly worse survival than those with levels ≤15 ng/g (5). Malignant breast...
tumors produce more PGE2 than benign breast tumors or normal breast tissue (6). COX-2 is up-regulated in a variety of tumors including breast cancers (6). Whereas COX-1 localizes to human breast stroma adjacent to but not within the tumor (7), COX-2 localizes to human breast cancer cells (6). Thus, COX-2 seems to be the significant isoform of the COX system with regard to breast cancer.

Preclinical studies suggest that celecoxib is effective both in preventing and in treating breast cancer. 7,12-Dimethylbenz(a)anthracene–formed rodent mammary tumors shrank after the administration of celecoxib (8). Dietary administration of celecoxib prior to the development of 7,12-dimethylbenz(a)anthracene-formed rat mammary tumors had a significant chemopreventive effect (8), reducing the incidence of cancer by 68%, the multiplicity by 86%, and the volume of tumors by 81%. The inhibition was dose dependent. Nonsteroidal anti-inflammatory drugs had a lesser chemopreventive effect. In a HER-2/neu transgenic model of mammary cancer, celecoxib significantly reduced mammary tumor incidence and PGE2 concentrations (9).

There is good evidence that modulating prostaglandin production through the administration of COX inhibitors is effective in decreasing both colon polyp and colon cancer formation (10). A series of recent publications provide strong support for the hypothesis that an inhibitor of COX-2 would also be effective in both the prevention and the treatment of breast cancer (8, 9). The currently available, generally accepted treatments for women at high breast cancer risk are limited to tamoxifen versus observation. Although effective, tamoxifen presents the potential risks of pulmonary embolism and uterine cancer, resulting in many eligible women electing not to take the medication.

The half-life of celecoxib in humans is 11.2 hours, with steady-state concentrations reached in 5 days (11). The drug is eliminated predominantly by hepatic metabolism. Based on studies to date, dose adjustment in the elderly is not generally necessary unless the subject weighs <50 kg. The medication does not seem to affect platelet aggregation, prothrombin time, partial thromboplastin time, or bleeding time. Only 0.04% of subjects (2 of 5,285) treated for 1 to 6 months with celecoxib and the plasma fraction was decanted and stored at −80°C until use.

Materials and Methods

Subjects. Women were enrolled in an institutional review board–approved protocol that included eligibility criteria to minimize risk. Subjects had to be ≥18 years old and at increased breast cancer risk based on the subject having either a Gail model risk of developing IBC in a 5-year period of >1.66% or previously treated DCIS or IBC. For women with a history of DCIS or IBC, they must have been finished with their treatment and free of disease. NAF was only collected from the breast contralateral to the one with cancer. For all subjects, NAF was collected from the same breast for each of the three visits.

All NAF samples with ≥1 μL were adequate for obtaining a result. Baseline NAF and blood collection were prior to the ingestion of celecoxib. Celecoxib was taken for 14 days. NAF and blood collection on completion of celecoxib was within 11 hours of ingestion of the last pill for all subjects. Washout NAF and blood samples were collected 14 days after stopping celecoxib. Thus, subjects were asked to provide three NAF and three plasma samples (baseline, after celecoxib, and after washout).

Pregnant and lactating women were not eligible. Women could not have been currently on nonsteroidal anti-inflammatory drugs, a COX-2 inhibitor, warfarin, or have taken such a medication within the 2 weeks prior to enrollment. Subjects could not have a significant history of peptic ulcer disease, upper gastrointestinal bleed, and asthma or be allergic to sulfonamides or nonsteroidal anti-inflammatory drugs. A complete blood count, serum electrolytes, and liver panel performed within 2 months prior to enrollment had to be within normal limits. Subjects were recruited from the breast evaluation clinics at the University of Missouri-Columbia.

Specimen Collection. NAF samples were collected into capillary tubes and stored at −80°C until use. Blood (8 mL) was also collected from the subject in a green top tube, the blood was spun for 10 minutes at 1,600 rpm, and the plasma fraction was decanted and stored at −80°C until use.

Biomarker Analysis. The biomarker chosen for analysis was PGE2 due to its established link to cancer growth. NAF and plasma samples were analyzed initially by RIA, and later by chemiluminescence immunoassay (CLIA), for their PGE2 content. We switched from RIA to CLIA because the majority of plasma samples had levels of PGE2 below the limit of detection by RIA.

PGE2 Analysis

RIA. NAF and plasma samples were diluted in 0.01 mol/L Tris buffer (pH 7.3) containing 0.1% gelatin, incubated with a PGE2 polyclonal antibody for 24 hours at 4°C, and terminated by adding dextran-coated charcoal (13). The samples were then centrifuged, the supernatant was dissolved in Scintiverse liquid scintillation cocktail (Fisher Scientific, Pittsburgh, PA), and tritium activity was determined with a “Taurus” four-well liquid scintillation spectrometer (Micromedic, Huntsville, AL).
Samples were run in duplicate. The lower limit of detection was 10 pg of eicosanoid per 0.1 mL sample.

Chemiluminescence Immunoassay. NAF and serum samples were analyzed according to the manufacturer’s instructions (Assay Designs, Ann Arbor, MI). The kit uses a monoclonal antibody to PGE2 to competitively bind the PGE2 in the standard or sample. Briefly, samples were diluted in 100 μL assay buffer supplied by the manufacturer, pipetted into appropriate wells, incubated for 2 hours at room temperature, and washed; substrate solution was added followed by 1-hour incubation; and absorbance was measured at 530 nm.

For both RIA and CLIA NAF and plasma analyses, a standard curve was prepared using serial dilutions of PGE2. A linear regression equation was created fromstandards of known PGE2 concentration and from PGE2 concentrations of unknown samples fit to the standard curve regression equation, corrected for aliquot volume and expressed as nanograms of PGE2 per milliliter of original sample. Specifically, for RIA, the dilutions were 1,250, 625, 312, 156, 78, 39, 20, and 10 pg/mL PGE2. For CLIA, the dilutions were 5,000, 1,000, 200, 40, 8, and 1.6 pg/mL PGE2. For the RIA analyses, the percentage binding versus the known concentration of PGE2 was plotted. For CLIA, relative light units versus known PGE2 concentration was plotted. Once the standard curve was produced, the percentage binding (RIA) or relative light units (CLIA) of the unknown samples were compared with the standard curve, and the number of picograms of PGE2 in each unknown tube was calculated. The standard curve, NAF, and plasma samples were run in duplicate and the average of the two values was reported. The goodness of fit (R^2) of NAF was 0.991 for RIA analysis and 0.997 for CLIA. The goodness of fit was similar for the plasma samples.

Statistical Analysis. Mean and median values of continuous variables were computed for the various groups of subjects. Due to the potential non-normality of the data, ranking procedures were used for all analyses with continuous variables. Data were analyzed using all NAF PGE2 results, including those below the level of detection, which were considered as zero. To compare eicosanoid expression between pretreatment and post-treatment groups, the Wilcoxon signed rank test was used. The Wilcoxon rank sum test was used to compare groups with different diagnoses.

**Results**

**Subjects.** Median age of the subjects was 51 years, with a range of 23 to 68 years (Table 1). Ninety-four percent (16 of 17) were Caucasian and 1 was American Indian. Forty-seven percent (8 of 17) were premenopausal. Thirteen women were enrolled based on their Gail risk using family history and/or age data and 4 because of the findings on breast biopsy.

All subjects provided NAF at all time points. For one subject at the washout time point, only a single tube was collected and the volume of NAF (0.5 μL) was insufficient to provide a reliable PGE2 result. All NAF samples of ≥1 μL were sufficient to obtain a PGE2 result. Blood was not available at three time points for RIA PGE2 analyses and at one time point for CLIA analyses.

**PGE2 Concentrations in Plasma Using RIA as a Measurement Technique.** PGE2 levels in plasma analyzed by RIA were not detectable in the first 3 subjects (Table 2). PGE2 was analyzed in an additional 9 subjects. PGE2 concentrations in plasma of the remaining 9 patients were detectable in 9 of 24 samples. Overall, using RIA as a measurement technique, PGE2 was detectable in plasma in 9 of 33 samples from 12 women.

**PGE2 Concentrations in Plasma Using CLIA as a Measurement Technique.** To increase the sensitivity of detection of PGE2 in plasma, CLIA was employed (Table 2). Plasma samples from the same 12 subjects were analyzed. PGE2 was detectable in all 35 samples. In an additional 5 subjects, plasma samples were tested by CLIA and PGE2 was detectable in all samples. In all, PGE2 was detectable in 50 of 50 plasma samples using the CLIA method.

These data were compared with the results of the plasma concentrations in those subjects whose plasma PGE2 concentrations were measured by RIA (Table 2). PGE2 was detectable in all (50 of 50) plasma samples using CLIA compared with 9 of 33 (27%) samples by RIA. The mean PGE2 values for the before celecoxib (baseline), after celecoxib, and after washout periods were 24, 27, and 100 pg/mL of plasma as measured by RIA and 380, 260, and 430 pg/mL, respectively, when measured by CLIA. Values as measured by CLIA were significantly higher than by RIA for the baseline (P = 0.005), after celecoxib (P = 0.002), and after washout (P = 0.01) periods. Similar directional changes were observed between groups by both techniques.

**PGE2 Concentrations in NAF.** We evaluated NAF samples collected from 12 subjects for PGE2 concentrations as measured by RIA (Table 2). Mean PGE2 concentrations in NAF were 16.4, 13.6, and 15.8 ng/mL of sample for the before celecoxib, after celecoxib, and after washout time points. Median PGE2 concentrations were 6.2, 6.6, and 11.9 ng/mL of sample. Using the CLIA technique in the same 12 subjects as well as an additional 5 subjects, mean PGE2 concentrations in NAF were 23.0, 38.9, and 25.5 ng/mL of sample for the three sample groups, respectively. Median PGE2 concentrations by CLIA were 14.8, 21.9, and 22.9 ng/mL of sample before celecoxib, after celecoxib, and after washout, respectively. When comparing the NAF PGE2 concentrations as measured by RIA and CLIA, there was a relatively constant proportionality between the two techniques.

**Table 1. Demographics**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>17</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>Median 51, Range 23-68</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Family history 13, Atypia 1, History of DCIS 3</td>
</tr>
<tr>
<td>Comorbid medications</td>
<td>Aspirin, nonsteroidal medications 0, Vitamins 5, Alternative therapies 4*</td>
</tr>
</tbody>
</table>

*Four subjects took a soy preparation, each ≤1 serving every other day.
Table 2. Comparison of PGE2 concentrations (ng/mL) in NAF or plasma based on method of PGE2 detection

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIA</td>
<td>35</td>
<td>15.24</td>
<td>8.85</td>
<td>0.50-4.57</td>
</tr>
<tr>
<td>CLIA</td>
<td>30</td>
<td>29.20</td>
<td>22.32</td>
<td>0.11-268.38</td>
</tr>
<tr>
<td><strong>Before celecoxib</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIA</td>
<td>12</td>
<td>16.39</td>
<td>6.16</td>
<td>0.01-4.57</td>
</tr>
<tr>
<td>CLIA</td>
<td>17</td>
<td>23.00</td>
<td>14.87</td>
<td>0.07-72.29</td>
</tr>
<tr>
<td><strong>After celecoxib</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIA</td>
<td>12</td>
<td>13.57</td>
<td>6.59</td>
<td>0.01-4.43</td>
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<tr>
<td>CLIA</td>
<td>16</td>
<td>38.88</td>
<td>21.85</td>
<td>0.02-268.38</td>
</tr>
<tr>
<td><strong>After washout</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIA</td>
<td>11</td>
<td>15.81</td>
<td>11.94</td>
<td>0.10-4.57</td>
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<tr>
<td>CLIA</td>
<td>16</td>
<td>25.50</td>
<td>22.90</td>
<td>0.04-80.59</td>
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</table>

NOTE: There were 12 subjects who provided up to three samples (baseline, after treatment, and after washout). Each sample of NAF and plasma for RIA and an additional 5 subjects who provided three samples of both NAF and plasma for a total of 17 subjects providing NAF and plasma for CLIA. NAF was collected at all time points, and a PGE2 value was obtained at all time points except for one washout sample, which contained only 0.5 μL NAF. Plasma was not available at three time points. The median RIA results were 42%, 30%, and 52% of the corresponding group mean as measured by CLIA for the before celecoxib, after celecoxib, and after washout groups.

**PGE2 Is Concentrated in NAF as Compared with Corresponding Plasma.** PGE2 concentrations in NAF as measured by RIA were detectable in all samples. Unfortunately, quantitative comparison of NAF and plasma PGE2 concentrations by RIA was not possible using the RIA technique, because PGE2 was detectable in only a small percentage of the plasma samples. The remaining plasma samples were below the limit of detection of the assay, which was 0.1 ng/mL of original sample. Comparisons therefore are based only on the CLIA data wherein we could measure both NAF and plasma PGE2 concentrations concurrently. Mean (median) concentrations of PGE2 in NAF were 61 (55), 150 (87), and 29 (92) times higher than in corresponding plasma as measured by CLIA before celecoxib, after celecoxib, and after washout (Table 2). Overall, mean and median NAF PGE2 concentrations (n = 50) were 51- and 56-fold higher than matched plasma concentrations. Thus, there is a significant enrichment of NAF in its eicosanoid concentration as compared with plasma.

**PGE2 Concentrations in NAF and Plasma Are Not Altered by Celecoxib Administration.** We hypothesized that celecoxib would decrease PGE2 concentrations below baseline values in both NAF and plasma. By both RIA and CLIA analyses, NAF PGE2 concentrations were not significantly altered by 2 weeks of celecoxib treatment. Similarly, PGE2 concentrations were not significantly different between the after celecoxib and after washout time periods, nor were the baseline versus the after washout PGE2 time periods significantly different (Table 2). Although mean and median concentrations of PGE2 seemed to increase in NAF and to decrease in plasma after the administration of celecoxib, the differences were not significant.

**PGE2 Expression Is Similar in Women with a History of Breast Cancer as in Those at Risk for the Disease.** We compared the concentration of PGE2 in both NAF and plasma before versus after celecoxib versus after washout in women with different disease backgrounds (Table 3). Levels of PGE2 in neither NAF nor plasma were significantly different based on enrollment criteria (family history or age versus a prior history of atypical ductal hyperplasia or DCIS), whether considering concentrations before celecoxib, after celecoxib, or after washout. PGE2 concentrations seemed to increase in NAF after celecoxib administration both in women with a family history of breast cancer and in those with a history of atypical ductal hyperplasia or DCIS. Plasma concentrations of PGE2 seemed to be stable in women with a family history of breast cancer but decreased following celecoxib treatment in those with a history of atypical ductal hyperplasia or DCIS. None of these apparent differences were significant. Thus, the pattern of eicosanoid change during treatment with the COX-2 inhibitor celecoxib was not significantly influenced by the criterion used for enrollment.

**Discussion**

Despite significant efforts to decrease the burden of breast cancer, it remains a leading cause of death among U.S. women. Women at increased breast cancer risk include those with a strong family history of breast cancer, those with a history of DCIS or IBC, and those with precancerous changes in their breasts. Multi-institutional trials have shown that tamoxifen is effective in significantly decreasing the risk of breast cancer in these women. Unfortunately, tamoxifen is not free from side effects, some of which, including pulmonary embolism and uterine cancer, can be quite serious. For this reason, although tamoxifen is beneficial in decreasing the risk of breast cancer in these women, many (perhaps most) choose not to take it because of the risk of side effects. A safer alternative therefore is needed.

One possible alternative approach to the prevention of cancer involves manipulation of the arachidonic acid system. Arachidonic acid is the precursor molecule for prostaglandins, prostacyclins, thromboxanes, and several other eicosanoids that are involved in the development and progression of breast cancer.
other hydroxy fatty acids (collectively termed eicosanoids). These biologically active molecules can be synthesized by at least three separate COX enzymes termed COX-1, COX-2, and COX-3. In a double-blind, placebo-controlled trial, celecoxib inhibited colorectal polyp formation in patients with familial adenomatous polyposis (14). Subjects receiving 400 mg b.i.d. celecoxib for 6 months had a 28% reduction ($P = 0.003$) and those receiving 100 mg b.i.d. had a 11.9% reduction (a nonsignificant difference) in mean colorectal polyp number as compared with placebo. Another double-blind, placebo-controlled trial was conducted to determine if celecoxib inhibited duodenal polyp formation in patients with familial adenomatous polyposis (15). Subjects with clinically significant disease who received 400 mg b.i.d. celecoxib had a 23% reduction in involved areas compared with placebo ($P = 0.049$), whereas subjects who received 100 mg b.i.d. had a nonsignificant reduction. For both studies, adverse events incidence was similar among the groups. Based on these findings, the Food and Drug Administration approved celecoxib for the prevention of polyp formation in patients with familial adenomatous polyposis.

In the present study, we carried out an initial set of experiments to investigate whether COX-2 inhibitors are of value in the prophylactic treatment of women at increased risk for breast cancer. Although this study does not directly approach the question of whether celecoxib is an effective breast cancer–preventing drug, we lay the groundwork for evaluating that possibility. This study was designed to determine whether eicosanoids are present within NAF, whether the eicosanoid concentrations could be routinely measured in NAF and plasma, and whether the administration of the COX-2 inhibitor celecoxib could affect prostaglandin production within this fluid under normal, nonstressed conditions.

The design of the current experiments required concurrent analysis of eicosanoids in both plasma and NAF to determine whether eicosanoids are concentrated in NAF relative to plasma. Because plasma concentrations of eicosanoids are normally kept very low by extremely efficient metabolic and clearance mechanisms in the lungs, liver, and kidneys, basal circulating concentrations in humans are in the sub–nanogram per milliliter range. We employed both RIA and CLIA to measure the eicosanoid concentrations. Although both techniques possessed the sensitivity to measure eicosanoid concentrations in NAF, only the CLIA technique possessed the sensitivity to routinely measure PGE$_2$ concentrations in plasma.

The plasma concentrations of PGE$_2$ were relatively low in this patient population, whether measured by RIA or CLIA. All of the values fell within the reference range of circulating basal concentrations of PGE$_2$ in nonstressed patient populations. Both RIA and CLIA techniques provided similar qualitative results. Similar directional changes were observed between groups by both techniques. It is likely that the PGE$_2$ levels in plasma as analyzed by RIA were not uniformly detectable because some of the values were near the limit of detection of the RIA assay of 0.1 ng/mL. The CLIA technique provided greater sensitivity of detection and higher quantitative values for PGE$_2$. Mean plasma concentrations of PGE$_2$ of 0.35 ng/mL have been reported previously (16), similar to our findings of PGE$_2$ levels in plasma by CLIA of 0.36 ng/mL.

The RIA value was consistently 50% to 70% below the corresponding CLIA generated values. There was a trend toward higher PGE$_2$ concentrations in NAF as measured by CLIA after 2 weeks of celecoxib treatment ($P = 0.098$). This was not seen by RIA ($P = 0.52$). A trend was also observed using CLIA analysis toward higher plasma PGE$_2$ concentrations comparing samples collected after celecoxib to those collected after washout ($P = 0.10$). Whether these trends would become significant with a larger sample size is unknown.

As mentioned above, celecoxib is effective in preventing colon and duodenal polyps, which are precursors to cancer, presumably through its ability to inhibit COX-2 and therefore prostaglandins. COX-2 is termed the “inducible” form of COX as compared with COX-1, which is presumed to be a constitutive “housekeeping” enzyme. Thus, it is hypothesized that events in precancerous or early cancerous cells initiate increased transcription of COX-2 and the resulting eicosanoids play some causative or supporting role in the evolution of the cancerous state. Our hypothesis was that at least a portion of the eicosanoids present in the NAF of women with a predisposition to breast cancer would be the product of COX-2 and that the administration of celecoxib would decrease that concentration further as a possible prophylactic measure. That hypothesis was not borne out by the data. Instead of decreasing NAF PGE$_2$ concentrations, treatment with celecoxib 200 mg b.i.d. showed a trend toward increased PGE$_2$ concentrations. These results may be explained in several ways. First, the concentration of celecoxib used (200 mg b.i.d.) may not have been high enough to enter the breast tissue and exert full effect. A concentration of 100 mg b.i.d. in the quoted colon and duodenal polyp studies did not show significant results. There are ongoing studies evaluating the effect of celecoxib in breast cancer tissue between the time of diagnostic breast biopsy and definitive breast surgery, in combination with aromatase inhibitors as neoadjuvant therapy, in combination with chemotherapy, or with an aromatase inhibitor in patients with advanced or metastatic breast cancer. Additionally, there are biomarker modulation trials of celecoxib in women at increased breast cancer risk and in those with breast cancer. In general, treatment studies are investigating celecoxib 400 mg b.i.d., whereas prevention studies are investigating 200 mg b.i.d. Second, the time from ingestion of the last celecoxib pill to the time of NAF and blood collection may have influenced our ability to detect an effect, especially given the short half-life of celecoxib of 11.2 hours. However, we were able to collect all NAF and plasma samples within 11 hours of ingesting the last pill, so we do not feel that the time from the last celecoxib dose to specimen collection had a major impact on PGE$_2$ levels. Third, COX-2 activity may be minimal in the breast tissue of women predisposed to new or recurrent breast cancer unless cancer is present. If true, then monitoring eicosanoid concentrations in NAF would be uneventful until the onset of disease. Additional studies are needed to compare eicosanoids in the NAF of women without breast cancer with eicosanoids in the NAF of women with breast cancer to
determine whether PGE2 concentrations in NAF change through the course of the disease. If PGE2 increases with the onset of breast cancer, celecoxib treatment might still be an effective preventative therapy, but the measurement of NAF eicosanoids would not be of clinical value.

COX-2 expression has been evaluated in preclinical models and clinical breast specimens. In a 7,12-dimethylbenz(a)anthracene rat model of breast cancer, celecoxib was found to dramatically reduce the incidence, multiplicity, and volume of breast tumors relative to control (17). There was a dose response, with higher doses leading to fewer tumors per rat and smaller average tumor volumes (18). Celecoxib also was found to protect against HER-2/neu-induced breast cancer (19). COX-2 expression is up-regulated in a subset of cases of DCIS and IBC (7, 20-23). About 40% of primary breast carcinomas are COX-2 positive (24) and overexpression is associated with lymph node metastasis, poor differentiation, and large tumor size. Although COX-2 expression seems to be a good marker of breast cancer in tissue, the lack of reliable commercial immunoassays for bodily fluids at the time that the study was conducted limited our ability to measure this marker in NAF and plasma.

The observation that PGE2 concentrations in NAF do not decrease, and that there is a trend for them to increase following COX-2 inhibition is of note. It is possible that COX-2 is active in breast tissue in compartments where the product of COX-2 activity (PGE2) cannot gain access to NAF. If the synthesis of these COX-2-mediated eicosanoids is inhibited by celecoxib, then there may be compensatory production of eicosanoids by COX-1 activity. This may be part of a homeostatic balance of eicosanoid concentrations within the tissue. If this suggestion is true, then the concept of specific COX-2 inhibition as a universal therapeutic regimen in preventing cancer may not be appropriate for all forms of cancer. The effectiveness of COX-2-specific inhibitory therapy may only apply to those tissues where eicosanoid homeostasis is not well maintained and there is no compensatory eicosanoid synthesis. Another possible mechanism for the increased PGE2 concentrations in NAF during celecoxib treatment may relate to the volume of NAF produced within the breast. The sampling technique for NAF produced different volumes of fluid and the eicosanoid concentrations are expressed as nanograms per milliliter of original sample. If COX-2 inhibition affects fluid balance across the ductal epithelial cell membrane, then the absolute volume of NAF fluid within the breast ducts may change. Thus, if celecoxib reduces the total volume of fluid within the sampled system (e.g., decreases), then the concentration of the eicosanoids may change (e.g., increase) even when the actual content may be decreased. Whether eicosanoid content versus volume in the system changes in the correct directions to explain our observations is unknown.

In summary, PGE2 is measurable and is concentrated in NAF ~80-fold as compared with plasma. Plasma concentrations of PGE2 in women with a predisposition to breast cancer are within the normally accepted basal range (<1 ng/mL). Celecoxib 200 mg b.i.d. did not significantly alter NAF or plasma concentrations of PGE2. Additional studies are required to determine whether monitoring NAF eicosanoid concentrations will be of value in assessing the development and progression of breast cancer or in monitoring the effect of candidate chemopreventive agents such as celecoxib.

References


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