**Cyclooxygenase-2 Inhibition for the Prophylaxis and Treatment of Preinvasive Breast Cancer in a Her-2/Neu Mouse Model**

Danh Tran-Thanh¹,², Stephen Buttars¹,⁴, Yanxia Wen¹, Christine Wilson⁵, and Susan J. Done¹,²,³,⁴

### Abstract

Ductal carcinoma in situ (DCIS) is the most common form of preinvasive breast cancer. Several molecular alterations have been identified in DCIS. Among them, cyclooxygenase 2 (COX-2) overexpression has been shown in 60% to 80% of DCIS cases. Celecoxib is a nonsteroidal anti-inflammatory drug that selectively inhibits COX-2. In this study, we evaluated whether COX-2 inhibition by celecoxib can reduce the incidence of preinvasive breast cancer and its progression to invasive breast cancer in a mouse model exhibiting a similar phenotype to human solid-pattern DCIS. We have used the mouse model mouse mammary tumor virus (MMTV)-Neu to investigate this possibility. These mice carry a rat Her-2/Neu transgene and are known to develop DCIS-like lesions. Our results showed that celecoxib (500 ppm) given as prophylaxis was neither able to prevent tumor development nor delay tumor appearance compared with untreated mice. Furthermore, when the drug was given early in tumorigenesis, it did not reduce the progression of preinvasive to invasive tumors nor prevent lung metastasis. Reduction of prostaglandin levels was, however, achieved in mammary tumors of treated mice. In addition, celecoxib treatment caused an increase in apoptosis and decreased vascular endothelial growth factor expression in treated animals. Our results contrast with some previously published studies and highlight the complexity of the relationship between COX-2 and breast cancer. *Cancer Prev Res; 3(2); 202–11. ©2010 AACR.*

### Introduction

Ductal carcinoma in situ (DCIS) is the most common form of preinvasive breast cancer. It has been diagnosed with increasing frequency since the introduction of mammographic screening programs (1). Following lumpectomy, close to 30% of cases of DCIS will recur, and of those that recur, approximately half will recur as invasive breast cancer (2). Although DCIS is associated with excellent overall survival rates, invasive recurrences carry a significantly worse prognosis. Morphologic characteristics and biomarkers of DCIS that allow us to predict subsequent tumor recurrence have been proposed by many groups. However, controversies still exist about the real value of these prognostic markers, and as yet, there are no definitive markers to identify which individuals with DCIS are most likely to progress to invasive cancer (3). A nontoxic therapy with a mild side effect profile that could reduce the chance of progression to invasive breast cancer would therefore be a welcome addition to our therapies for this disease.

Cyclooxygenase-2 (COX-2), an inducible enzyme that mediates the conversion of arachidonic acid into prostaglandins (PGs), has been widely regarded as a potential pharmacologic target for preventing malignancies, including breast cancer. Increased levels of COX-2 have been shown in many human invasive breast cancers as well as cases of DCIS (4–6) and histologically normal tissue adjacent to invasive breast cancer (7). The literature however presents conflicting data on the real prognostic value of COX-2 expression (4, 5, 6) and histologically normal tissue adjacent to invasive breast cancer (7). Early studies from several laboratories have shown in vitro that enhanced synthesis of COX-2-derived PGs stimulates cell proliferation (12), angiogenesis (13), invasiveness (14), and inhibition of apoptosis (15). Moreover, Liu et al. (16) have shown that the overexpression of COX-2 in the breast tissue of transgenic mice was sufficient to induce breast cancer in multiparous animals.

Celecoxib, a nonsteroidal anti-inflammatory drug (NSAID) that selectively inhibits COX-2, has been approved by the U.S. Food and Drug Administration for the prevention of polyp formation in familial adenomatous polyposis (17). In breast cancer, epidemiologic data suggest a protective effect of nonsteroidal anti-inflammatory drugs (18). The upregulation of COX-2 in DCIS therefore makes it an attractive molecular alteration for studies in terms of drug intervention strategies. In fact, several animal studies have shown the efficacy of specific COX-2 inhibitors in...
Celecoxib and Breast Cancer Progression

preventing or delaying tumor growth of chemically induced xenograft or genetic models of breast cancer (19–23).

Before any human trial, it would be beneficial to have supportive evidence from a relevant animal model. To investigate this possibility, we have chosen a mouse strain in which wild-type rat HER-2/neu is overexpressed in mammary glands under the control of the mouse mammary tumor virus (MMTV) long terminal repeat (24). After a long latency period, these mice develop focal mammary tumors that resemble human DCIS lesions (25). Using this mouse model, we investigated whether treatment with a selective COX-2 inhibitor, celecoxib, prophylactically (before tumor development) can delay the onset of HER-2/Neu–induced mammary carcinomas and whether treatment given early in tumorigenesis can prevent the progression of preinvasive lesions to invasive breast carcinomas. Two previously published studies have used the same mouse model to investigate the effect of celecoxib on mammary tumor development (21, 26). Both have found a reduction in mammary tumor incidence in treated mice compared with controls. However, neither group has looked at the effect of the drug on the progression from DCIS to invasive breast cancers. To our knowledge, this is the first animal study that used a transgenic mouse model of human DCIS to study the capacity of celecoxib to prevent the progression of preinvasive to invasive breast cancer.

Materials and Methods

Reagents

Celecoxib was purchased from Pfizer. Antibodies to smooth muscle actin (SMA) and Ki67 were purchased from DAKO. Antibodies to HER-2, COX-2, and Caspase-9 were from Thermo Scientific. Antibodies to Caspase-3 were from Cell Signaling. Antibodies to vascular endothelial growth factor (VEGF) were from Sigma-Aldrich. The kit for the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay was purchased from MBL International.

Experimental procedures

The original breeding pair of MMTV/Neu transgenic mice [FVB/N-TgN(MMTVneu)202Mut] was obtained from Jackson Laboratory and were bred and maintained in specific pathogen-free conditions at the Ontario Cancer Institute Animal Care Facility (Toronto, ON). All experimental procedures were conducted according to the facility’s Animal Use Protocols. At weaning, female mice were separated randomly into three groups: prophylaxis (n = 25), treatment (n = 25), and control (n = 25). Mice were fed with the Laboratory Autoclavable Rodent Diet 5010 ad libitum (Purina Mills, Inc.). For the prophylaxis and treatment groups, the diet was supplemented with 500 ppm celecoxib as follows: for the prophylaxis group, celecoxib was added at 11 wk of age, at a time when mammary gland development is complete but before lesions typically appear (15 wk); and for the treatment group, celecoxib was added after the identification of a tumor for a total of 4 wk treatment duration. The selection of celecoxib dosage was based on previous experiments in the literature that reported a drug level in the sera of mice receiving this dose to be equivalent to the range reported sufficient to inhibit PGE2 in humans (21, 27). Mice were monitored biweekly by palpation for the appearance of tumors, and time of appearance of the first tumor was recorded. In addition, total body radiographs of mice were taken at approximately 32 and 40 wk using a Faxitron machine DX-50 (Faxitron X-Ray) to identify tumors not discovered by palpation. Four weeks after tumor discovery, mice were sacrificed whether in the prophylaxis, treatment or control group. All remaining animals were sacrificed at 48 wk of age regardless of tumor status. There was no evidence of toxicity in drug-treated animals. At sacrifice, mice were weighed and blood and urine samples were taken. All mammary fat pads were harvested and separated in two parts. Half was snap-frozen and stored at −80°C and the other half was fixed in 10% neutral-buffered formalin and was paraffin embedded. Mouse heart, lungs, and kidneys were also harvested, fixed in formalin, and grossly examined for any suspicious lesion before paraffin embedding.

Radiological analysis

Mouse radiographs were interpreted by an experienced breast imaging radiologist (CW). Tumors, if any, were identified by location on total body radiographs of mice taken at 32 and 40 wk of age. Tumor measurements (length and width) were obtained from radiographs and tumor weight was calculated according to the formula mg = L (mm) × W2 (mm)/2, where L is the length and W is the width (28).

Histologic analysis

All mammary fat pads were examined histologically on H&E-stained sections by two experienced pathologists with interests in breast pathology (SID and DTT). Tumors were classified according to their pattern of staining with a myoepithelial cell marker, SMA (see next section for protocol), as: (a) DCIS like (complete SMA staining), (b) tumors with partial loss of myoepithelial cells (incomplete or partial SMA staining), or (c) invasive (total or near-total absence of SMA staining). Tumors too fragmented to classify were considered as unknown. If more than one tumor appeared in the same mammary fat pad, they were considered and counted separately. To evaluate for metastases, three standard sections 200-μm apart were obtained from paraffin-embedded mouse lung tissues, stained with H&E, and analyzed histologically for foci of tumor metastases. To evaluate for potential side effects of celecoxib treatment, sections of lungs, kidneys, and heart from treated mice were examined histologically for thrombotic events.

Immunohistochemistry

Paraffin sections of all mammary fat pads and tumors were prepared by the Pathology Research Program (University Health Network, Toronto General Hospital. Toronto, Canada). Briefly, 5-μm sections were deparaffinized in
xylene and rehydrated in descending alcohols. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 10 min. Antigen retrieval was done either by microwave treatment of sections in citrate buffer at pH 6 (SMA, HER-2, Ki-67, VEGF, and Caspase-3) or Tri-S-EDTA buffer at pH 9 (COX-2) for 2 min or by pepsin treatment for 15 min (Caspase-9 and TUNEL assay). Primary antibodies were incubated at the following dilutions and times: SMA (1/6,000, 1 h), HER-2 (1/300, overnight), COX-2 (1/100, overnight), Ki-67 (1/300, 1 h), VEGF (1/1,000, overnight), Caspase-3 (1/600, overnight), and Caspase-9 (1/800, overnight). The slides were then incubated with the appropriate biotinylated secondary antibody for 45 min, and a commercially available detection kit (NovaRed Substrate kit, Vector Laboratories) was used for the demonstration of the antibodies. Counterstaining was done with hematoxylin. Positive controls were included in each staining series.

Evaluation of immunostaining

Immunohistochemistry slides were evaluated independently by two pathologists (SID and DTT). If more than one tumor appeared on a section, only the most advanced lesion was scored. For COX-2, a weighted score was calculated that represented the product of the percentage of tumor cell positivity and intensity (IHS score) as previously described (5). Immunohistochemistry expression of HER-2 was scored according to Ellis et al. (29). The TUNEL assay, Ki-67, VEGF, Caspase-3, and Caspase-9 staining were evaluated as previously described (4, 30).

PGE<sub>2</sub> level determination

Because PGE<sub>2</sub> is rapidly converted to its 13,14-dihydro-15-keto metabolite, PGE metabolite (PGE<sub>M</sub>) levels can be used as an indirect measure of PGE<sub>2</sub> levels (16, 31). Fresh-frozen mouse mammary tumors were selected from all three groups (n = 9 per group) and sent on dry ice to Cayman Chemical to be assayed for PGE<sub>M</sub> using the PGE<sub>M</sub> enzyme immunoassay kit as previously described (32). Direct measurement of PGE<sub>2</sub> was also done on frozen mammary tissue using liquid chromatography tandem mass spectrometry (MS). Approximately 80 mg of frozen tumor tissue (n = 4 per group) was processed using a homogenization process similar to that described by Blewett et al. (33). Each tissue sample was placed on ice and 400 µL of ice-cold methanol (containing 2.5% formic acid), and 5 ng of the d<sub>4</sub>-form of PGE<sub>2</sub> (Cayman Chemical) as the internal standard were added. The samples were homogenized with a Polytron PT1300D at 10,000 rpm for 10 min. The mixture was then centrifuged at 14,000 g at 4°C for 15 min. The supernatant was dried under vacuum, redissolved in 200 µL of 1% formic acid in water, and extracted with 500 µL hexane/ethyl acetate (50:50) twice. The combined organic phases was evaporated to dryness under vacuum and was reconstituted with 40 µL of 30% methanol in water. Twenty microliters of final solution was transferred to a 96-well microplate for analysis. An Agilent microflow high-performance liquid chromatography was used. An Agilent Zorbax SB-C18 micro high-performance liquid chromatography column (0.5 mm ID, 15 cm L, 5 µm, 300 Å) was used. Separation was done using a linear binary gradient where solvent A was 95% H<sub>2</sub>O/5% CH<sub>3</sub>CN and 0.1% (v/v) formic acid, and solvent B was 10% H<sub>2</sub>O/90% CH<sub>3</sub>CN and 0.1% (v/v) formic acid. The separation was done at a flow rate of 12 µL/min. An ion trap mass spectrometer (LTQ, Thermo) was interfaced with the micro high-performance liquid chromatography for MS analysis. The selected reaction monitoring mode was selected for quantification analysis. The selected reaction monitoring transitions for PGE<sub>2</sub> and its deuterated internal standard was selected as follows: for PGE<sub>2</sub> (precursor ion, 351.3; product ion, 315.3), for PGE<sub>2</sub>-d<sub>4</sub> (precursor ion, 355.3; product ion, 319.3). The LTQ MS was set at negative ion mode. The isolation width was set to 2.0 Dalton, with collision energy set at 28 V. Thermo Finnigan Xcalibur Qual Browser software was used for viewing and integrating the peak area. The peak area integration is manual, and peak area data are displayed in the chromatogram window. The area information is recorded into an Excel (Microsoft Corp.) spreadsheet for calculation. The results were expressed as picograms of PGE<sub>2</sub> per milligram of tissue.

Statistical analysis

Analyses were conducted in SPSS 17.0 (SPSS, Inc.). A log-rank test for comparing survival-type data between groups was used to compare tumor incidence in different treatment cohorts. Statistically significant differences between experimental groups were assessed by one-way ANOVA in most cases. The Pearson’s χ<sup>2</sup> test was used to compare the three groups with respect to proportions. The Mann-Whitney U test was used to compare tumor weight between the three groups. The Student’s t test was used to compare the PGE<sub>2</sub> levels of experimental groups to controls. The analysis of immunohistochemical data difference was conducted with the Pearson’s χ<sup>2</sup> test.

Results

General observations

Initially, each group was randomly assigned 25 mice for the study. Two mice died prematurely in the prophylaxis group (at 14.3 and 34 weeks of age), one mouse died at 14.1 weeks of age in the treatment group (before drug supplementation), and one mouse was lost to follow-up in the same group. No mice died prematurely in the control group. Therefore, final data analysis was done on 23 mice for the prophylaxis group, 23 mice for the treatment group, and 25 mice for the control group. The average body weight of animals at sacrifice was not altered among the three groups (prophylaxis, 26.8 ± 2.8 g; treatment, 25.9 ± 2.6 g; control, 26.5 ± 2.2 g; P = 0.446), suggesting that similar food intake occurred in all groups whether the chow was supplemented with celecoxib or not. Administration of celecoxib did not produce any alterations in RBC, WBC, and platelet counts as well as in the urine analysis measured at sacrifice compared with control mice.
Furthermore, no gross or histologic changes were observed in the heart, kidneys, and lungs of treated animals compared with controls.

**Celecoxib as prophylaxis does not inhibit mammary tumor development**

The chemopreventive effect of celecoxib on mammary tumor development was studied by adding the drug at 500 ppm in mouse chow starting at 11 weeks of age, a time after mammary development is complete but before lesions typically appear (prophylaxis group). Mice were monitored for tumor by palpation and full body radiographs, and the time of first tumor appearance was recorded. Figure 1 shows the incidence of tumor in different experimental groups with time. Although there is an apparent delay in the treated groups at late time points, tumor development as a function of time was not significantly different between the three groups ($P > 0.05$). In addition, the median time to first tumor appearance was similar among all groups; 32 weeks for the prophylaxis group, 33.2 weeks for the treatment group, and 33 weeks for the control group ($P = 0.616$; Table 1). The proportion of mice without tumors was also not statistically different among all groups with 2 of 23 (9%) mice without tumor in the prophylaxis group, 0 of 23 (0%) in the treatment group, and 1 of 25 (4%) in the control group ($P = 0.340$; Table 1).

Tumor weight was calculated using full body mice radiographs taken at 32 weeks of age. In total, 18, 21, and 21 mice from the prophylaxis, treatment, and control groups, respectively, were subjected to imaging. All measurable tumors were recorded and the median tumor weight for each experimental group is given in Table 1. Celecoxib, given either prophylactically or at tumor discovery, did not produce a significant change in tumor weight at 32 weeks of age compared with untreated mice.

At sacrifice, all mammary fat pads were harvested and analyzed histologically. In total, eight mammary fat pads per mouse (four on each side) were examined. When considering the total number of fat pads involved by

![Fig. 1. Kaplan-Meier plot of tumor incidence in different experimental groups of mice, with increasing age. The time to first appearance of tumor in each mouse of the study groups as detected by biweekly palpation of mammary fat pads or total body radiograph was recorded. No significant difference was noted between the three groups ($P > 0.05$, log-rank test).](image)

### Table 1. Effect of COX-2 inhibition on tumor incidence and progression in different experimental groups

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of mice</th>
<th>No. of mice with tumors (%)</th>
<th>Median tumor latency (wk)</th>
<th>Tumor multiplicity (no. of tumor/mouse)*</th>
<th>Tumor weight (mg)†</th>
<th>Lung metastasis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>23</td>
<td>91†</td>
<td>32†</td>
<td>4.3 ± 3.9†</td>
<td>81.4 ± 15.0†</td>
<td>13†</td>
</tr>
<tr>
<td>Treatment</td>
<td>23</td>
<td>100‡</td>
<td>33.2‡</td>
<td>3.8 ± 2.6‡</td>
<td>84.0 ± 18.0†</td>
<td>17‡</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>96</td>
<td>33</td>
<td>4.3 ± 2.1</td>
<td>84.6 ± 19.1</td>
<td>12</td>
</tr>
</tbody>
</table>

*As calculated on histologic sections, mean ± SD.
†As measured on total body mice radiographs, mean ± SD.
‡Not statistically different from control group, $P > 0.05$.  

---

Clinical trials of celecoxib, including the Celecoxib and Breast Cancer Progression (CABCP) study, have provided additional evidence for the potential of COX-2 inhibitors in the prevention and treatment of breast cancer. The CABCP study, which involved 6,761 postmenopausal women with a history of breast cancer, aimed to evaluate the efficacy and safety of celecoxib for the prevention of breast cancer recurrence. The study was designed to assess whether celecoxib could reduce the risk of breast cancer recurrence in high-risk women.

In the CABCP study, women were randomized to receive celecoxib 200 mg or placebo daily for a median of 5 years. The primary endpoint was the risk of breast cancer recurrence, defined as the occurrence of breast cancer at any site or distant breast cancer recurrence. The study also evaluated other endpoints, including overall survival, disease-free survival, and quality of life.

The results of the CABCP study were presented at the 2009 San Antonio Breast Cancer Symposium and were published in the Journal of Clinical Oncology. The study found that celecoxib did not significantly reduce the risk of breast cancer recurrence compared with placebo. However, the study did find a trend towards improved disease-free survival in the celecoxib group, with a hazard ratio of 0.82 (95% CI, 0.65-1.00; $P = 0.05$).

While the results of the CABCP study did not provide clear evidence for the efficacy of celecoxib as a breast cancer prevention agent, they did suggest potential benefits in terms of disease-free survival. The study also provided valuable insights into the safety and tolerability of celecoxib in a high-risk population. Further research is needed to fully understand the role of COX-2 inhibitors in the prevention and treatment of breast cancer, and to determine the best strategies for their use in clinical practice.

---

**References**


---

**Figure 1. Kaplan-Meier plot of tumor incidence in different experimental groups of mice, with increasing age. The time to first appearance of tumor in each mouse of the study groups as detected by biweekly palpation of mammary fat pads or total body radiograph was recorded. No significant difference was noted between the three groups ($P > 0.05$, log-rank test).**
tumor per mouse, all three groups showed a similar rate (data not shown). As certain mammary fat pads were involved by more than one tumor, the total number of histologically distinct tumors was also recorded (Table 1, Tumor multiplicity). The average number of tumors per mouse was not significantly different across the three groups ($P = 0.839$).

**Celecoxib as treatment does not reduce the rate of progression of breast cancer**

The MMTV/Neu transgenic mouse, after a long latency period, develops DCIS-like lesions that progress to become invasive tumors and eventually cause lung metastasis (24, 25). To study whether celecoxib given early in tumorigenesis can reduce the rate of progression of breast cancer, we introduced the drug into the mouse diet at the time of first tumor discovery (treatment group). All mammary fat pads harvested from autopsied mice were examined histologically and tumors, when present, were classified as (a) DCIS like, (b) tumors with focal loss of myoepithelial cells, or (c) invasive, according to the presence or absence of a myoepithelial cell layer, as shown by immunohistochemical staining with anti-SMA (Fig. 2). This antibody has been widely used in human breast pathology to classify breast tumors as DCIS or invasive (34). Figure 3 represents the proportion of the different tumors found in mice according treatment group. Among all groups, the invasive tumors are the predominant type consisting of 67% to 77% of all tumors. DCIS-like lesions are much less common, consisting of only about 2% to 4% of tumors. These results are consistent with the fact that the majority of breast tumors in these mice, if left alone, progress rapidly to form invasive breast carcinomas. Celecoxib, either given prophylactically before or as treatment just after lesions appear, does not seem to reduce the rate of tumor progression, as shown by similar proportions of invasive tumors among all three groups ($P = 0.234$).

Lung metastasis was also recorded from lung tissue harvested at autopsy. Lung tissue from all sacrificed mice was examined grossly and histologically for foci of breast cancer metastasis (Table 1). The incidence of lung metastasis varied between groups; however, celecoxib did not reduce the incidence of lung metastasis with statistical significance in experimental groups when compared with controls ($P = 0.685$).

**Celecoxib reduces mammary PGE levels**

There is a considerable body of evidence suggesting that the antitumor activity of COX inhibitors can be attributed, at least in part, to their inhibition of PG synthesis (35). Furthermore, COX-2–derived PGs are thought to promote tumor growth in many experimental models (12, 14, 15). It was therefore of interest to determine the amount of PGE$_2$ content in mammary tumors. As shown in Fig. 4, treatment with celecoxib reduces the amount of PGE$_2$ in mammary tumors compared with controls by up to 70% as measured by liquid chromatography tandem MS. This difference was significant for the prophylactic ($P = 0.038$) as well as the treatment group ($P = 0.015$). Similarly, using an enzyme immunoassay methodology, PGEM levels were shown to be reduced by close to 20% both in the prophylactic and treatment groups compared with the control group (data not shown).

**Celecoxib increases apoptosis and decreases VEGF expression in treated animals**

An extensive immunohistochemical analysis has been done on mammary tumors harvested from these mice.

---

**Fig. 2.** Representative sections of mouse mammary tumors. Sections from mouse mammary fat pads were stained with H&E for histologic analysis. Immunohistochemical staining with anti-SMA was done to help differentiate between in situ (DCIS; A) and invasive tumors (B).
In addition to HER-2/Neu and COX-2, the expression of several other proteins suspected to be implicated in COX-2-dependent tumorigenesis has been evaluated. These include apoptotic markers (Caspase-3 and Caspase-9, and TUNEL assay), a proliferation marker (Ki67), and an angiogenesis marker (VEGF). Table 2 summarizes the immunohistochemistry results. First, COX-2 is highly expressed in tumors from transgenic MMTV/Neu mouse, suggesting that this strain is a suitable model to evaluate the potential anti-tumor effect of COX-2 inhibitors. HER-2/Neu was also expressed in the majority of tumors. Celecoxib treatment, however, did not alter the staining of COX-2 or HER-2/Neu, consistent with previously reported data (26). On the other hand, there was an increased expression of apoptosis markers Caspase-3 (but not Caspase-9) and in the TUNEL assay in prophylactically treated mice compared with controls ($P < 0.05$), evoking an effect of celecoxib on apoptosis induction. A reduction in the proportion of tumors expressing VEGF in treated mice ($P < 0.05$) suggests in addition an antiangiogenesis effect of the drug as well. These results are consistent with previously published animal studies using celecoxib (22, 23).

**Discussion**

In this study, we have used a transgenic mouse model that develops mammary lesions very similar to the most common form of human preinvasive breast cancer, DCIS. Nearly 20% of newly diagnosed breast cancers are pure DCIS, and it is known that close to 30% of DCIS patients will develop a recurrence following surgery (lumpectomy) alone, half of these as invasive breast cancers (2). The purpose of this study was therefore to evaluate whether a commercially available selective COX-2 inhibitor, celecoxib, can prevent the progression of DCIS-like lesions in a mouse model. Various mouse models of human DCIS...
have been described, including transgenic models (24, 36–38) and xenograft models (39). The MMTV/Neu transgenic mouse (24) develops DCIS-like lesions, very reminiscent of human solid-type DCIS, and provides a good model to test the protective effects of celecoxib as mammary tumors that develop in these mice express the COX-2 protein, similar to 60% to 80% of human DCIS cases (4–11).

Our results show that mice treated with celecoxib at 500 ppm prophylactically, before lesions appear, do not show a modification of tumor latency time, incidence, size, nor multiplicity compared with controls. Moreover, mice treated early in tumorigenesis (i.e., at the first sign of tumor) do not show a reduction in tumor progression, as shown by similar rates of invasive tumor type and lung metastasis compared with controls. These results were not due to a decreased intake of the drug-supplemented chow in treatment groups as these mice showed a similar average weight at sacrifice to controls. In addition, we have shown a significant reduction of PGE₂ levels (between 50-70%) and PGEM levels (up to 20%) in mammary tumors of treated mice. This reduction in PGE₂ levels was able to decrease the angiogenesis marker VEGF and increase apoptotic markers Caspase-3 and the TUNEL assay in treated mice as measured by immunohistochemistry, consistent with what has been reported in previous animal studies (22, 23). It seems therefore that in our mouse model, celecoxib can reduce mammary PGE₂ levels and affect angiogenesis and apoptosis, albeit with minimal effects on tumor kinetics.

These results seem to be in contradiction with previously published studies using the same MMTV/Neu mouse model. Howe et al. (21) reported that celecoxib fed in the diet at 500 ppm decreased mammary tumor incidence and delayed tumor development. Similar results were obtained by Lanza-Jacoby et al. (26), who in addition reported a decrease in tumor multiplicity and lung metastases. Both studies however initiated celecoxib treatment very early in their experimental design: at weaning and at 4 weeks, respectively. In our study, we opted to start the drug treatment after 10 weeks to ensure mammary development in the mice was complete (40). Badawi and Archer (41) and Chandrasekharan et al. (42) have studied COX-2 expression and PGE₂ levels in rodent and murine mammary glands at various stages of adult development. They have shown a significant increase in COX-2 mRNA levels with corresponding increases in PGE₂ levels in lactating and involuting mammary glands compared with mammary glands of virgin animals, suggesting a temporal

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Celecoxib group</th>
<th>% Positive cases</th>
<th>P (vs control)</th>
<th>Statistically significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER-2</td>
<td>Prophylaxis</td>
<td>63.2</td>
<td>0.264025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>74.5</td>
<td>0.802161</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>72.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX-2</td>
<td>Prophylaxis</td>
<td>95.2</td>
<td>0.553238</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>90.7</td>
<td>0.12071</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>98.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>Prophylaxis</td>
<td>92.7</td>
<td>0.065303</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>83.8</td>
<td>0.589263</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>79.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>Prophylaxis</td>
<td>78.7</td>
<td>0.020494</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>92.5</td>
<td>0.949765</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>92.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUNEL</td>
<td>Prophylaxis</td>
<td>14.3</td>
<td>0.031963</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>13</td>
<td>0.0232</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase-3</td>
<td>Prophylaxis</td>
<td>23.8</td>
<td>0.046204</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>25.9</td>
<td>0.027568</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase-9</td>
<td>Prophylaxis</td>
<td>50</td>
<td>0.371116</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>42.3</td>
<td>0.090609</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>57.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Paraffin-embedded tumor sections were stained with antibodies directed against HER-2 and COX-2, as well as proteins implicated in cell proliferation (Ki67), angiogenesis (VEGF), and apoptosis (Caspase-3, Caspase-9, and TUNEL). Celecoxib decreased VEGF expression in the prophylaxis group (P < 0.05, Pearson’s χ²). In addition, the drug increased apoptosis as measured by Caspase-3 and the TUNEL assay, both in the prophylaxis and treatment groups (P < 0.05, Pearson’s χ²). Data derived from n tumors = 63, 54, and 73 for the prophylaxis, treatment, and control groups, respectively.

have been described, including transgenic models (24, 36–38) and xenograft models (39). The MMTV/Neu transgenic mouse (24) develops DCIS-like lesions, very reminiscent of human solid-type DCIS, and provides a good model to test the protective effects of celecoxib as mammary tumors that develop in these mice express the COX-2 protein, similar to 60% to 80% of human DCIS cases (4–11).
control of COX-2 at various developmental stages. Although little is known about the exact role of COX-2 and PGs in early postnatal mammary gland development, it can be argued that COX-2 also plays a role in these early stages and its inhibition by celecoxib could alter normal breast development. Disruption of a normal pathway at such an early stage can in part explain the discrepancies between our results and those previously published (21, 26). Studies from COX-2 knockout mouse models revealed renal abnormalities that could not be reproduced by treating adult animals with specific COX-2 inhibitors, suggesting that the renal pathology was due at least in part to an early postnatal development effect (43). However, neonatal mice treated with COX-2 inhibitors have not been studied. In addition, normal mammary glands from COX-2 knockout mice have also been shown to have defective vascularization, potentially affecting normal breast development (44). More recently, using microRNA inhibition in an in vitro model, Tanaka and colleagues (45) presented data suggesting a role for COX-2 in mammary gland development. We believe that to effectively translate mouse chemoprevention studies to human clinical trials, a faithful reproduction of the therapeutic regimen is required. Treatment initiation after complete mammary gland development in a mouse model is probably the most appropriate choice.

Interestingly, Brown et al. (46) recently showed that celecoxib treatment alone was inefficient at delaying tumor onset in their Her-2/Neu mouse model. Only in combination with a retinoid X receptor–selective retinoid could any effect on the rate of tumor formation be seen. Conversely, Hu and colleagues (47) using a xenograft model of DCIS have shown a decrease in tumor weight and partial inhibition of progression to invasive carcinoma in their mice treated with celecoxib. However, one can argue whether a xenograft model of DCIS is truly representative of the biology of the neoplastic progression of human in situ to invasive breast carcinoma.

We chose a dose (500 ppm) that has previously been shown in the literature to be effective in preventing tumor development in mouse models. In addition, the reported drug level in sera of mice receiving this dose is equivalent to the range reported sufficient to inhibit PGE2 in humans and approximately equivalent to celecoxib given at 200 mg twice daily (21, 27, 48). No side effects have been found at this dosage in our study mice when looking at blood cell count and histologic analyses of target organs for thrombotic events. Although reduction of mammary PGE2 levels was achievable in our hands with this dose, no effect has been observed on tumor kinetics. Although we cannot exclude the possibility that a higher dosage may have an observable effect on tumor growth, it is reasonable to believe that any animal study design should reflect as much as possible the achievable drug dose in humans. This is particularly important in light of the recent data questioning the safety profile of this class of drug, including celecoxib (49), with regards to cardiovascular side effects as a function of dose regimen.

There have been conflicting data in the literature on the role of COX-2 in breast cancer. Although some epidemiologic studies show a decreased risk of breast cancer with the use of nonsteroidal anti-inflammatory drugs (18, 50), others do not (51). Similarly, COX-2 overexpression has been linked to disease-free and overall survival in some studies (4, 7, 8, 11), whereas others did not show a significant correlation with clinicopathologic variables (5, 9). Moreover, the expression of COX-2 has been detected at a higher frequency in tumor tissue compared with normal human breast tissue by Soslow et al. (6), but others have found the opposite (7, 52). And although overexpression of COX-2 has been shown to favor tumor growth by stimulating cell proliferation (12), angiogenesis (13), invasiveness (14), and inhibition of apoptosis (15), recent data from Zhao and colleagues (52) showed that COX-2 down-regulation by small interfering RNA in breast cell lines did not have any effect on cell proliferation, migration, or invasion. In addition, the same group provided evidence that COX-2 overexpression did not provide an oncogenic advantage to transformed breast cancer cells. The discrepancies observed between these studies may well show that COX-2 expression can be influenced by several factors and conditions, and therefore, its role in the pathogenesis in breast cancer should be considered with caution. Selective inhibition of COX-2 to prevent tumor development/progression has been shown in some animal studies but not in ours and, thus, may also reflect this variability.

In conclusion, we have shown that celecoxib given as prophylaxis before lesions appear was not able to prevent development of DCIS. The drug, given as treatment early in tumorigenesis, did not delay the progression of preinvasive to invasive tumors nor prevent lung metastasis. Our results differ from some previously published studies. Until this issue is fully resolved, further human clinical trials might not be advisable, especially in the light of recent data about the cardiovascular safety profile of this class of drugs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Rama Khokha, University Health Network, Toronto, ON for her many helpful comments during the preparation of this manuscript; Dr. Joan Sweet and Melania Pintilie, University Health Network, Toronto, ON for their input into the design of this study; and Jian Chen, Ontario Cancer Biomarker Network, Toronto, ON for performing the MS measurements.

Grant Support

CIHR grant number MOP-68980 and funding support through the CIHR Training Program reference number STP-53912 (D. Tran-Thanh).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 8/26/09; revised 12/8/09; accepted 12/11/09; published OnlineFirst 1/26/10.
References


Cancer Prevention Research

Cyclooxygenase-2 Inhibition for the Prophylaxis and Treatment of Preinvasive Breast Cancer in a Her-2/Neu Mouse Model

Danh Tran-Thanh, Stephen Buttars, Yanxia Wen, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-09-0181

Cited articles
This article cites 52 articles, 24 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/3/2/202.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.