Combination Chemoprevention of HER2/neu-Induced Breast Cancer Using a Cyclooxygenase-2 Inhibitor and a Retinoid X Receptor–Selective Retinoid

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Abstract  The inducible prostaglandin synthase isoform cyclooxygenase-2 (COX-2) is overexpressed in ∼40% of human breast carcinomas and in precancerous breast lesions, particularly in association with overexpression of human epidermal growth factor receptor 2 (HER2/neu). Experimental breast cancer can be suppressed by pharmacologic inhibition or genetic ablation of Cox-2, suggesting potential clinical utility of COX-2 inhibitors with respect to breast cancer. Importantly, several clinical trials have found reduced colorectal adenoma formation in individuals administered selective COX-2 inhibitors. However, such trials also identified increased cardiovascular risk associated with COX-2 inhibitor use. The goal of this research was to test whether improved chemopreventive efficacy could be achieved by combining submaximal doses of a selective COX-2 inhibitor and a retinoid X receptor–selective retinoid (rexinoid). The rate of HER2/neu-induced mammary tumor formation was substantially delayed by coadministration of the COX-2 inhibitor celecoxib (500 ppm in diet) and the rexinoid LGD1069 (10 mg/kg body weight; oral gavage) to MMTV/neu mice. Median time to tumor formation was increased from 304 to >600 days (P < 0.0001). The combination was substantially more effective than either drug individually. Similarly, potent suppression of aromatase activity was observed in mammary tissues from the combination cohort (44% of control; P < 0.001). Regulation of aromatase expression and activity by COX-derived prostaglandins is well established. Interestingly however, single agent LGD1069 significantly reduced mammary aromatase activity (71% of control; P < 0.001) without modulating eicosanoid levels. Our data show that simultaneous blockade of COX/prostaglandin signaling and retinoid X receptor–dependent transcription confers potent anticancer efficacy, suggesting a novel avenue for clinical evaluation.

The inducible prostaglandin (PG) synthase cyclooxygenase-2 (COX-2) is strongly implicated in breast neoplasia (1, 2). COX-2 is overexpressed in ∼40% of human breast carcinomas and in 60% to 80% of preinvasive ductal carcinoma in situ lesions (1, 2). COX-2 overexpression in breast carcinomas correlates with poor prognosis and with several associated clinical variables, including overexpression of human epidermal growth factor receptor 2 (HER2; also called neu and c-ERBB2; refs. 3–8). Epidemiologic data are broadly consistent with a protumorigenic role for COX enzymes. Several studies have identified reduced breast cancer incidence associated with the use of COX-inhibiting drugs including aspirin, nonsteroidal anti-inflammatory drugs, and selective COX-2 inhibitors (9–17).

Consistent with the human expression data, Cox-2 is also up-regulated in rodent mammary tumors (18–22), and thus rodent breast cancer models have proved useful for analyzing the contribution of Cox-2 to mammary neoplasia. Numerous studies have shown that experimental breast cancer can be suppressed by inhibiting Cox activity using either conventional nonsteroidal antiinflammatory drugs or selective Cox-2 inhibitors (1, 2). Given the observed correlations between COX-2 and HER2/neu overexpression in human breast tumors (3–6), HER2/neu-driven models are of particular interest. Both we and others have shown that HER2/neu-induced tumor formation is significantly delayed

Cancer Prev Res 2008;1(3) August 2008 208 www.aacrjournals.org

©2008 American Association for Cancer Research. doi:10.1158/1940-6207.CAPR-08-0021

Received 02/20/2008; accepted 02/27/2008.

Grant support: NIH grants R03 CA105458 (L.R. Howe), R03 CA119273 (L.R. Howe), and R01 CA078480 (P.H. Brown); a Breast Cancer Alliance Young Investigator Grant (L.R. Howe); the Breast Cancer Research Foundation (P.H. Brown and A.J. Dannenberg); and the Irving Weinstein Foundation, Inc. (L.R. Howe).

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by administration of the selective COX-2 inhibitor celecoxib (20, 23). Furthermore, knocking out Cox-2 significantly decreases HER2/neu-induced mammary tumor formation in mice (24). Conversely, transgenic overexpression of COX-2 is sufficient to induce mammary neoplasia in multiparous mice, providing direct evidence of the in vivo oncogenicity of COX-2 (25). Mechanistically, the antitumor action of Cox-2 inhibition has been ascribed to induction of apoptosis, suppression of proliferation, and modulation of tumor cell invasiveness and immunosurveillance (2). Additionally, genetic manipulations of Cox-2 gene dosage suggest an important role for Cox-2 in regulating both angiogenesis and the activity of the estrogen synthase aromatase in breast tissues (24, 26–28).

Together, the above data sets identify COX-2 as a potential target for inhibiting breast carcinogenesis, stimulating the development of several clinical trials designed to evaluate COX-2 inhibitors in breast cancer patients. In support of this approach, randomized clinical trials have shown that COX-2 inhibitors can reduce the incidence of colorectal adenomas (29, 30). Less propitiously, some studies reported an increased risk of cardiovascular events associated with COX-2 inhibitor (29, 30). Nevertheless, the demonstrated utility of retinoids for preventing cancer, using vitamin A derivatives such as 13-cis-retinoid acid, all-trans retinoic acid, and 9-cis-retinoic acid. However, these trials also identified unacceptable toxicity associated with high-dose regimens of these naturally occurring retinoids. Limiting toxicities include teratogenicity, hepatotoxicity, severe headaches, and mucocutaneous toxicity. More recently, compounds with greater selectivity, and hence decreased toxicity, have been developed through increased understanding of retinoid receptor biology (31, 36). Two distinct classes of retinoid receptors have been identified: retinoic acid receptors and retinoid X receptors (RXR). Whereas both retinoic acid receptors and RXRs are ligand-regulated transcription factors of the steroid/thyroid hormone receptor superfamily, the two classes are distinguished by their differential ligand affinities and by the spectrum of proteins with which they interact. Importantly, RXR-selective retinoids, or rexinoids, have markedly diminished toxicities relative to pan-retinoids and retinoid acid receptor ligands (37), and thus offer considerable promise as anticancer agents. Of particular interest, both LGD1069 (bexarotene, Tar-gretin) and LG100268 have robust chemopreventive efficacy with respect to experimental breast cancer, including HER2/neu-induced tumorigenesis (31, 36, 38–40).

Here we have evaluated the combination of the selective COX-2 inhibitor celecoxib and the RXR-selective retinoid LGD1069 for preventing HER2/neu-induced mammary tumorigenesis. We show that celecoxib and LGD1069 in combination mediate greater-than-additive suppression of mammary tumor formation in HER2/neu transgenic mice. Furthermore, parallel decreases in mammary aromatase activity were observed, suggesting that aromatase modulation may contribute to tumor suppression mediated by the celecoxib/LGD1069 combination.

### Materials and Methods

#### Reagents and chemicals

Celecoxib [Celebrex; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene-sulfonamide] was purchased from LKT Laboratories, Inc. LGD1069 (Targretin; bexarotene) was provided by Ligand Pharmaceuticals.

#### Mouse experimental procedures

FVB/N-Tg(MMTVneu)202Mul/J mice (MMTV/neu) were obtained from The Jackson Laboratory and bred to produce multiple litters. Virgin female offspring were used in three separate studies.

**Study I.** Study I has previously been reported (20), and tumor latency data from that study are provided in Table 4 for comparative purposes only. To briefly recapitulate the study design, females were randomly assigned to one of two groups at 5 wk of age (vehicle or celecoxib), both of which were fed Laboratory Autoclavable Rodent Diet 5010 ad libitum. The diet of mice in the celecoxib group was supplemented with 500 ppm celecoxib.

**Study II.** In study II, females were randomly assigned to one of four groups at 8 wk of age as shown in Table 1 (group 1, vehicle; group 2, celecoxib; group 3, LGD1069; group 4, celecoxib and LGD1069). All mice were fed AIN-76A diet (Research Diets, Inc.) ad libitum. The diet of groups 2 and 4 was supplemented with 500 ppm celecoxib. Animals in groups 1 and 2 were gavaged 5 d/wk with 100 μL sesame oil (Cro-da, Inc.). Animals in groups 3 and 4 were gavaged 5 d/wk with LGD1069 (10 mg/kg body weight) in 100 μL sesame oil. Mild

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet, ad libitum</th>
<th>Gavage, 5 d/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vehicle</td>
<td>AIN-76A</td>
<td>Sesame oil</td>
</tr>
<tr>
<td>2. Celecoxib</td>
<td>AIN-76A containing celecoxib (500 ppm)</td>
<td>Sesame oil</td>
</tr>
<tr>
<td>3. LGD1069</td>
<td>AIN-76A</td>
<td>LGD1069 in sesame oil (10 mg/kg body weight)</td>
</tr>
<tr>
<td>4. Both (celecoxib and LGD1069)</td>
<td>AIN-76A containing celecoxib (500 ppm)</td>
<td>LGD1069 in sesame oil (10 mg/kg body weight)</td>
</tr>
</tbody>
</table>

Table 1. Experimental groups

Aromatase activity assays

Resected mammary glands were homogenized, and mammary aromatase activity was assayed by measuring the release of $[^3H]$H2O from [1-$^3H$]androstenedione as previously described (28).

Statistical analysis

Kaplan-Meier curves of tumor-free survival in control and drug-treated cohorts were compared with a log-rank test (two-tailed; Prism 4.0 for Macintosh, GraphPad Software, Inc.). Poisson regression analysis was used to compare tumor multiplicity in each group based on the assumption that the number of tumors per animal would follow a Poisson distribution. Mean tumor multiplicity was compared in a pairwise manner, and $P$ values were corrected for multiple comparisons using Tukey’s method. Repeated-measure ANOVA was used to compare the mean mouse weights in each experimental group over time. Mammary eicosanoid data, shown as mean ± SD, were compared by one-way ANOVA and further analyzed by Dunnett’s test to effect comparisons between each treatment group and the control group, adjusting for multiple comparisons. Aromatase activity data, shown as mean ± SD, were evaluated for statistical significance with one-way ANOVA followed by pairwise testing using Tukey’s method to adjust for multiple comparisons.

Results and Discussion

Celecoxib and LGD1069 mediate greater-than-additive suppression of mammary tumorigenesis

The primary goal of this research was to compare the chemopreventive efficacy of the COX-2 inhibitor celecoxib and the RXR-selective retinoid LGD1069, alone and in combination, in a breast cancer model. Our ultimate aim was to determine whether greater protection could be achieved by combining drugs from two distinct classes than could be achieved using either drug individually. This combination chemoprevention approach offers the possibility of increased preventive efficacy with minimal associated toxicity through utilization of submaximal doses of individual agents. Thus, drug doses for our study were selected based on the following information. Celecoxib delays tumor formation in MMTV/neu mice when administered in food at either 500 or 900 ppm, whereas 1,500 ppm celecoxib induces cachexia and weight loss in this mouse strain (20, 23). LGD1069 has dose-dependent activity in the MMTV/neu model over the range 10 to 100 mg/kg body weight (40). Therefore, we selected 500 ppm celecoxib and 10 mg/kg LGD1069 as submaximal doses for use in our study.

Virgin female MMTV/neu mice were randomly assigned to one of four experimental groups at 8 weeks of age, as shown in Table 1. Tumor incidence was measured as a function of time in all four groups. The rate of formation of mammary tumors was significantly delayed in the LGD1069 cohort relative to the vehicle group (Fig. 1; Table 2), consistent with the previously reported chemopreventive action of LGD1069 with respect to HER2/neu-induced breast cancer (40). Mammary tumors were detected in 50% of control animals by 304 days of age, versus 420 days of age in LGD1069-treated mice ($T_{50} = 304$ versus 420 days; $P = 0.0085$). Unexpectedly, celecoxib alone failed to delay tumor onset in this study (Fig. 1; Table 2), in contrast to previous reports (20, 23). Failure to achieve comparable circulating celecoxib levels in this study relative to our earlier study would provide a trivial explanation for the lack of effect of celecoxib in the present study. However, serum celecoxib levels in this

dermatitis was observed in a subset of animals in each group, predominantly manifesting as auricular irritation and alopecia on dorsal surfaces in the neck and scapular region. Onset occurred between 7 and 15 mo of age (group 1, n = 1; group 2, n = 2; group 3, n = 5; group 4, n = 6). Mouse weights were measured weekly. No significant difference in animal weight was observed in any treatment group relative to the control group, indicative of the absence of drug-induced cachexia. Thus, all drug regimens were well tolerated with minimal side effects. Expression of the neu transgene was unaltered by any of the treatment regimens (data not shown).

Study III. In study III, females were randomly assigned to one of two groups (vehicle or celecoxib) at 3 wk of age and fed control AIN-76A diet or AIN-76A diet supplemented with 500 ppm celecoxib, respectively.

In all three studies, mice were palpated twice weekly for mammary gland tumors, and the age at appearance of the first tumor was recorded (tumor latency). In studies II and III, animals were sacrificed 2 mo after initial tumor detection, and the number of tumors was recorded (tumor latency). In studies II and III, animals were sacrificed 2 mo after initial tumor detection, and the number of tumors was recorded (tumor latency). In studies II and III, animals were sacrificed 2 mo after initial tumor detection, and the number of tumors was recorded (tumor latency). In studies II and III, animals were sacrificed 2 mo after initial tumor detection, and the number of tumors was recorded (tumor latency). In studies II and III, animals were sacrificed 2 mo after initial tumor detection, and the number of tumors was recorded (tumor latency). In studies II and III, animals were sacrificed 2 mo after initial tumor detection, and the number of tumors was recorded (tumor latency).

Determination of eicosanoid levels

Resected mammary glands were homogenized, and eicosanoids were extracted and assayed by liquid chromatography-tandem mass spectrometry as previously described (41).

Assay of celecoxib levels

Blood was collected from 5.5-mo-old animals by retroorbital bleeding, and plasma levels of celecoxib were assayed by liquid chromatography-mass spectrometry as previously described (20).
experiment were 2.8 ± 0.7 μmol/L (mean ± SD; range, 2.2-3.6 μmol/L), and thus extremely similar to those achieved in our previous study (mean, 2.4 μmol/L; ref. 20). We hypothesize that the variable protective efficacy of celecoxib between studies may be attributable to the use of different diets in each experiment (discussed below).

The combination of celecoxib and LGD1069 was markedly more effective at suppressing tumor formation than either drug alone (Fig. 1; Table 2). Tumor latency was more than doubled in the celecoxib/LGD1069 cohort relative to the untreated group (group 1, T₅₀ = 304 days versus group 4, T₅₀ >600 days; P = 0.0001). Tumor multiplicity was also significantly reduced in the combination group relative to the control cohort (Table 2; P = 0.041). Celecoxib, although ineffective as a single agent in this study, substantially enhanced the delay in tumor onset elicited by LGD1069 (LGD1069 alone, T₅₀ = 420 days versus LGD1069/celecoxib, T₅₀ >600 days; P = 0.0125). Importantly, serum celecoxib levels were not elevated in the combination group relative to the celecoxib cohort, excluding the possibility that increased efficacy reflected elevated circulating celecoxib levels (group 2, 2.8 ± 0.7 μmol/L; group 4, 1.6 ± 0.8 μmol/L; P = 0.12). A similar greater-than-additive antitumor effect of celecoxib and LGD1069 was also observed in the C3(1)-SV40 T antigen breast cancer model (data not shown), in which we have previously demonstrated single-agent activity of LGD1069 (42). Thus, enhanced suppression of mammary tumorigenesis can be achieved by combining the selective COX-2 inhibitor celecoxib and the RXX-selective retinoid LGD1069.

Drug-mediated perturbations of mammary eicosanoid levels

We previously reported a reduction of ~50% in mammary PGE₂ levels in celecoxib-treated mice (20). In the present study, celecoxib administration resulted in a modest 34% decrease in mean mammary PGE₂ levels and a 49% decrease in mean mammary PGD₂ levels (Table 3), although neither of these changes achieved statistical significance. Interestingly, substantially increased levels of leukotriene B₄ and 12-hydroxyeicosatetraenoic acid were observed in mammary tissues from celecoxib-treated animals (Table 3). Mean levels of mammary leukotriene B₄ and 12-hydroxyeicosatetraenoic acid were increased to 337% and 183%, respectively, of those in mammary tissues from vehicle-treated mice (P < 0.001 and P = 0.11, respectively). These data suggest that a metabolic shunt is occurring in mammary tissues from celecoxib-treated animals, with arachidonic acid being metabolized by 5-lipoxygenase to leukotriene A₄ and subsequent byproducts, and by 12-lipoxygenase to 12-hydroxyeicosatetraenoic acid, (rather than by Cox-2).

Although LGD1069 has been shown to down-regulate COX-2 expression both in vitro in human breast cancer cell lines and in vivo in mammary glands from MMTV/neu mice (43, 44), LGD1069 alone was ineffective at modulating mammary PG levels in vivo (Table 3). Unexpectedly, LGD1069 coadministration seemed to suppress the celecoxib-mediated arachidonic acid shunt to 5-lipoxygenase and 12-lipoxygenase. These data show that LGD1069 has complex effects on eicosanoid metabolism, elucidation of which will require further studies.

Mouse diet modulates susceptibility to celecoxib-mediated tumor suppression

The failure of celecoxib to delay tumor formation in MMTV/neu mice in the present study contrasts with previous data from our group and from other investigators (20, 23). Three alterations in experimental design relative to our earlier study were implemented in the present study, any of which could provide a basis for the discrepant observations. First, the age at initiation of drug administration was changed from 3 weeks in our first study (study I) to 8 weeks in the present study (study II). Second, the diet was switched from 5010 mouse chow to AIN-76A, a purified diet. Third, all animals received 100 μL of sesame oil by gavage 5 d/wk. To evaluate the influence of timing of drug initiation on celecoxib efficacy, a third study was done (study III) in which animals were fed AIN-76A, as in study II, and celecoxib administration was initiated at 3 weeks, as in study I. Tumor latency in the vehicle cohort was similar to that observed in study II, and the rate of tumor formation was not delayed by celecoxib administration (Table 4). Comparison of tumor latencies in control cohorts in all three experiments suggests that the alteration in diet could account for the lack of concordance between these studies. Specifically, tumors formed substantially more rapidly in the vehicle cohort in study I than in study II or III (e.g., T₅₀ = 32 weeks in study I versus T₅₀ = 43 weeks in study II; Table 4), suggesting that tumor formation is accelerated in MMTV/neu mice fed 5010 mouse chow compared with those fed AIN-76A.

Table 2. Effect of celecoxib and LGD1069 on tumor latency and multiplicity in MMTV/neu mice

<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>Median time to tumor formation, d</th>
<th>P</th>
<th>Tumor multiplicity (mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>304 (n = 32)</td>
<td>—</td>
<td>1.31 ± 1.00 (n = 32)</td>
<td>—</td>
</tr>
<tr>
<td>Celecoxib (500 ppm)</td>
<td>285 (n = 29)</td>
<td>0.26*</td>
<td>2.03 ± 1.38 (n = 29)</td>
<td>0.13†</td>
</tr>
<tr>
<td>LGD1069 (10 mg/kg)</td>
<td>420 (n = 26)</td>
<td>0.0085*</td>
<td>1.27 ± 0.87 (n = 26)</td>
<td>1.0‡</td>
</tr>
<tr>
<td>Celecoxib and LGD1069 &gt;600 (n = 26)</td>
<td>&lt;0.0001* 0.0125†</td>
<td>0.58 ± 0.78 (n = 24)</td>
<td>0.041, 0.068§</td>
<td></td>
</tr>
</tbody>
</table>

*P value obtained by comparison with vehicle-treated cohort (log-rank test).
†P value obtained by comparison with LGD1069-treated cohort (log-rank test).
‡P value obtained by comparison with vehicle-treated cohort (Poisson regression, corrected for multiple comparisons by Tukey’s method).
§P value obtained by comparison with LGD1069-treated cohort (Poisson regression, corrected for multiple comparisons by Tukey’s method).
diet, presumably due to differences in nutritional constituents. Because tumor latency was similar in the vehicle cohort in studies II and III, but only animals in study II received sesame oil, we infer that sesame oil administration in study II is unlikely to account for the increased tumor latency observed in studies II and III relative to study I.

Comparisons of tumor latencies in vehicle-treated animals in the three studies and of the relative chemopreventive efficacy of celecoxib thus support the following conclusions. First, the rate of mammary tumor formation is accelerated in mice fed 5010 mouse chow relative to those consuming AIN-76A purified diet (study I versus studies II and III). Second, consumption of 5010 mouse chow is associated with susceptibility to celecoxib-mediated chemoprotection against breast cancer (study I versus studies II and III). Third, initiating drug treatment at 3 weeks of age in AIN-76A-fed mice is insufficient to confer susceptibility to celecoxib-mediated protection (study II versus study III).

Because HER2/neu-induced breast neoplasia is suppressed by either pharmacologic inhibition or genetic ablation of the PG synthase Cox-2 (20, 23, 24), increasing mammary PG levels would be predicted to accelerate HER2/neu-driven mammary tumor formation. We speculate that 5010 chow may contain components that increase mammary eicosanoid levels. Consistent with this notion, dietary administration of celecoxib reduced mammary PGE2 by only 34% in animals consuming AIN-76A (Table 3), compared with the 47% reduction we previously reported for 5010-fed animals (20), suggesting that animals consuming 5010 chow may have elevated basal mammary eicosanoid levels.

**Celecoxib and LGD1069 suppress mammary aromatase activity**

Our next goal was to investigate potential mechanisms by which celecoxib and LGD1069 might induce greater-than-additive suppression of HER2/neu-induced mammary tumor formation. Substantial evidence supports a functional relationship between COX/PG signaling and the estrogen synthase aromatase. Correlations have been identified between expression of COX enzymes and the aromatase cytochrome P450 in human breast cancers (45, 46). These correlations are believed to reflect a causal relationship because PG signaling can stimulate transcription of the CYP19 gene encoding aromatase (28, 47–51). Furthermore, aromatase activity is reduced in mammary tissues from Cox-2 knockout mice and, conversely, is increased by transgenic COX-2 overexpression (27, 28).

Therefore, we focused on aromatase modulation as a potential effector of celecoxib/LGD1069-mediated chemoprotection.

Aromatase activity was compared in mammary tissues from all four experimental groups of the celecoxib/LGD1069 combination chemoprevention experiment (study II). Celecoxib alone caused a 17% decrease in mammary aromatase activity relative to that in the vehicle cohort ($P < 0.001$; Fig. 2). The magnitude of the celecoxib effect was substantially less than the ~60% reduction in mammary aromatase activity we had previously observed in Cox-2 knockout mice (28), consistent with the comparatively modest celecoxib-mediated reductions in mammary PGE2 levels in the current experiment (Table 3).

Mammary aromatase activity in LGD1069-treated animals was reduced by 29% ($P < 0.001$; Fig. 2). The rexinoid LG101305 has previously been shown to reduce transcription from the CYP19 promoter in cultured human breast adipose cells (52). Our data provide the first in vivo correlate for these tissue culture data. The mechanism by which rexinoids suppress aromatase transcription has not yet been elucidated, but may be independent of prostanoid metabolism because

### Table 3. Effect of celecoxib and LGD1069 on mammary eicosanoid levels in MMTV/neu mice

<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>PGE2, ng/mg protein ($P = 0.09$)*</th>
<th>PGD2, ng/mg protein ($P = 0.15$)*</th>
<th>LTB4, ng/mg protein ($P &lt; 0.001$)*</th>
<th>12-HETE, ng/mg protein ($P = 0.19$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.29 ± 0.50</td>
<td>2.86 ± 1.55</td>
<td>0.070 ± 0.029</td>
<td>7.85 ± 2.99</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>0.85 ± 0.49 ($P = 0.46$)†</td>
<td>1.45 ± 0.65 ($P = 0.17$)†</td>
<td>0.236 ± 0.082 ($P &lt; 0.001$)†</td>
<td>14.36 ± 7.20 ($P = 0.11$)†</td>
</tr>
<tr>
<td>LGD1069</td>
<td>1.50 ± 0.93 ($P = 0.91$)†</td>
<td>2.63 ± 1.88 ($P = 0.98$)†</td>
<td>0.097 ± 0.049 ($P = 0.78$)†</td>
<td>9.04 ± 5.55 ($P = 0.96$)†</td>
</tr>
<tr>
<td>Celecoxib and LGD1069</td>
<td>0.62 ± 0.37 ($P = 0.18$)†</td>
<td>1.53 ± 0.57 ($P = 0.20$)†</td>
<td>0.102 ± 0.064 ($P = 0.69$)†</td>
<td>9.79 ± 4.31 ($P = 0.86$)†</td>
</tr>
</tbody>
</table>

Abbreviations: PGE2, prostaglandin E2; PGD2, prostaglandin D2; LTB4, leukotriene B4; 12-HETE, 12-hydroxyeicosatetraenoic acid.

* $P$ value obtained by one-way ANOVA analysis across the different treatment groups.

† $P$ value obtained by comparison with vehicle-treated cohort (by Dunnett’s test and correcting for multiple comparisons).
LGD1069 alone did not significantly affect in vivo mammary eicosanoid levels (Table 3).

Strikingly, the combination of celecoxib and LGD1069 potently suppressed mammary aromatase activity, causing a greater-than-additive reduction (56%; P < 0.001, compared with vehicle; Fig. 2). The relative potency of the three drug regimens for depressing mammary aromatase activity was celecoxib/LGD1069 > LGD1069 > celecoxib. Because the drug regimens ranked in the same order of efficacy with respect to tumor suppression, we speculate that inhibition of aromatase expression may contribute to the observed chemopreventive activity. Whereas frank tumors in MMTV/neu mice lack estrogen receptor expression, estrogen receptor is expressed in normal-appearing and hyperplastic mammary tissues (40, 53). Importantly, tumor formation in the MMTV/neu strain is antagonized by the selective estrogen receptor modulators arzoxifene and acolectin, indicating that estrogen signaling contributes to tumorigenesis in this model (39). Thus, we conclude that drug-mediated alterations in mammary aromatase activity may contribute to the observed chemopreventive activity of the celecoxib/LGD1069 combination.

**Conclusions**

The major goal of this study was to compare the chemopreventive efficacy of celecoxib and LGD1069 in combination with that of each of the two drugs individually in a breast cancer model. The MMTV/neu strain was used for this experiment because we had previously demonstrated chemopreventive efficacy of both celecoxib and LGD1069 as single agents in this model (20, 40). We observed a potent, greater-than-additive action of celecoxib and LGD1069 in suppressing mammary tumour formation (Fig. 1). Interestingly, we detected corresponding decreases in mammary aromatase activity (Fig. 2), leading us to conclude that aromatase suppression may be a component of the anticancer action of the celecoxib/LGD1069 combination.

Our data have potential implications for the clinical management of breast cancer. Combination approaches are thought likely to afford greater benefit than can be achieved using single agents, with the additional benefit of minimizing collateral toxicity (31–33). For example, simultaneous targeting of COX-2 and HER-2/neu with celecoxib and anti–HER-2 antibodies has been shown to inhibit experimental colorectal carcinoma growth more effectively than either agent alone (54). This combination approach has been extended to experimental breast cancer. Rexinoid/selective estrogen receptor modulator combinations, for example, have striking synergistic efficacy in rodent breast cancer models (31, 39). Furthermore, clinical trials are ongoing to evaluate celecoxib in combination with aromatase inhibitors as neoadjuvant therapy. LGD1069 is Food and Drug Administration approved for treating refractory advanced stage cutaneous T-cell lymphoma, and is currently being evaluated in numerous clinical studies, including breast cancer-related trials. Additionally, several rexinoids with even greater RXR selectivity, and hence diminished side effects, have been developed, including LG100268 and UAB30 (31, 36, 55). These agents have demonstrated cancer-preventive activity and may be useful candidates for future clinical development. The results of our present study suggest that drug combinations targeting COX/PG signaling and RXR-dependent transcription may have clinical utility for breast cancer prevention.

**Disclosure of Potential Conflicts of Interest**


**Acknowledgments**

We thank Paul Christos for providing preliminary statistical support for this study.

**Table 4.** Diet affects tumor latency and the protective efficacy of celecoxib in MMTV/neu mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mouse diet (gavage)</th>
<th>Age at drug initiation, wk</th>
<th>Tumor latency, wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>Study I‡</td>
<td>5010 (no gavage)</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>Study II†</td>
<td>AIN-76A (sesame oil)</td>
<td>8</td>
<td>43</td>
</tr>
<tr>
<td>Study III†</td>
<td>AIN-76A (no gavage)</td>
<td>3</td>
<td>41</td>
</tr>
</tbody>
</table>

*Data for study I were previously reported by Howe et al. (20).

†P = 0.003, compared with vehicle-treated cohort.

‡P = 0.28, compared with vehicle-treated cohort.

§P = 0.17, compared with vehicle-treated cohort.

References


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