

Chemoprevention of Breast Cancer in Rats by Celecoxib, a Cyclooxygenase 2 Inhibitor¹

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been observed to reduce the relative risk of breast cancer. This prompted our investigation of the chemopreventive potential of celecoxib, a specific cyclooxygenase 2 blocker, against mammary carcinogenesis induced by 7,12-dimethylbenz(a)anthracene in female Sprague Dawley rats. Treatment with celecoxib was examined and compared to treatment with the general NSAID, ibuprofen, and to a control group receiving only dimethylbenz(a)anthracene. Dietary administration of celecoxib (1500 ppm) produced striking reductions in the incidence, multiplicity, and volume of breast tumors relative to the control group (68%, 86%, and 81%, respectively; $P < 0.001$). Ibuprofen also produced significant effects, but of lesser magnitude (40%, 52%, and 57%, respectively; $P < 0.001$). These results help confirm the chemopreventive activity of NSAIDs against breast cancer and provide the first evidence that a cyclooxygenase 2 blocking agent, celecoxib, possesses strong chemopreventive activity against mammary carcinogenesis.

Introduction

Breast cancer is the most commonly diagnosed malignancy, and despite intensive cancer control efforts, it remains the second leading cause of cancer deaths among American women (1). It was therefore of extreme interest when recent epidemiological studies suggested the presence of an inverse association between regular intake of NSAIDs³ and the relative risk of breast cancer (2–4). Animal studies have also demonstrated the effects of NSAIDs against mammary carcinogenesis (5, 6), and in our laboratories, the common over-the-counter compound ibuprofen produced highly significant reductions in tumor size and tumor burden associated with inhibition of the genetic expression of COX isoforms (7–9).

Anti-inflammatory effects of the NSAIDs stem from blockade of the prostaglandin cascade by inhibition of its rate-limiting enzyme, COX (10). Two primary genes are responsible for the genetic control of COX: (a) a constitutive gene (COX-1); and (b) an inducible isoform (COX-2; Ref. 11). Recent molecular studies of breast tumors indicate that COX-2 is inappropriately induced and that both COX-2 and COX-1 are up-regulated in malignant cells (12). Of further importance is the observation by Zhao *et al.* (13) that the chief prostaglandin, PGE₂, effectively and specifically induces the promoter II region of the cytochrome P-450 aromatase gene (CYP-19). This paracrine effect of PGE₂ therefore potentiates local biosynthesis of estrogen and provides a critical link between the prostaglandin cascade and deregulation of estrogen biosynthesis in mammary car-

cinogenesis. *In vivo* evidence supportive of this effect has recently been reported by Brueggemeier *et al.* (14), who observed a significant positive correlation between the genetic expression of COX and CYP-19 in human breast cancer.

Celecoxib (SC-58635) is a new NSAID that specifically inhibits COX-2. It has significant anti-inflammatory and analgesic properties but lesser toxicity than other NSAIDs such as aspirin and ibuprofen, which inhibit both COX-1 and COX-2 (15). Because of our previous studies suggesting that NSAID inhibition of COX reduces the risk of breast cancer, we conducted a preclinical efficacy study to evaluate the chemopreventive effects of a specific COX-2 blockade by this compound against mammary carcinogenesis. For purposes of comparison, we included treatment with the general NSAID, ibuprofen, which has nonspecific activity against COX-1 and COX-2 isozymes but relatively low COX-2 inhibition compared to celecoxib. The investigation was designed to determine the chemopreventive effects of celecoxib against DMBA-induced mammary carcinogenesis in female Sprague Dawley rats.

Materials and Methods

Reagents and Chemicals. Celecoxib (SC-58635; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene-sulfonamide) was supplied by Searle Research and Development (St. Louis, MO). Ibuprofen oral suspension was purchased as Motrin (100 mg/5 ml; McNeil) oral suspension. DMBA and all other reagents with the highest purity were purchased from Sigma Chemical Co. (St. Louis, MO).

Dietary and Tumor Induction Protocols. Female 50-day-old Sprague Dawley rats (Harlan Industries, Indianapolis, IN) were randomly assigned to one of three treatment groups (40 rats/group). The control group received powdered Teklad 22/5 rodent diet (W):8640, the celecoxib group received standard diet supplemented with 1500 mg/kg celecoxib (1500 ppm), and the ibuprofen group received standard diet supplemented with 1500 mg/kg ibuprofen (1500 ppm). After 7 days, each animal was intubated with a single intragastric dose of 15 mg of DMBA in 1.0 ml of sesame oil. The control and experimental diets were then continued for 105 days, and then the experiment was terminated. Food consumption and weight gain were measured weekly throughout the experiment, in addition to monitoring general health status for signs and symptoms of toxicity. Beginning at 28 days after DMBA intubation, the animals were palpated twice weekly to detect the presence and location of mammary tumors. The time of appearance of the first tumor (latency period) and the relative size and location of every tumor were recorded. We also calculated the number of rats with tumors (incidence) and the number of tumors/rat (tumor burden) on a weekly basis and at the end of the study. At the termination of the experiment, each tumor diameter was measured by a micrometer caliper, and the tumor volume was calculated using the formula $V = 4/3 \pi r^3$ where r is half the average diameter. All animals were sacrificed using CO₂ euthanasia. Necropsy included gross examination of all internal organs including the stomach, kidneys, and liver. All tumors plus the stomach and both kidneys of each animal were resected and fixed in 10% buffered formalin. Samples were embedded in paraffin blocks and processed for histological evaluation by routine procedures with H&E staining. Serum samples taken from each animal at the end of the experiment were tested for levels of celecoxib and ibuprofen using high-performance liquid chromatography.

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³ The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; DMBA, dimethylbenz(a)anthracene; PGE₂, prostaglandin E₂.

Statistical Analysis. Descriptive statistics on body weights, tumor latency, tumor incidence, tumor burden, and tumor volumes were examined and compared among the control and experimental treatment groups. The statistical significance of comparisons between the three treatment groups were obtained using χ^2 tests, Fisher's exact test, ANOVA, and multiple mean comparison procedures (16).

Results

General Observations. Average body weights of animals in the three treatment groups were similar throughout the experiment (Table 1). Administration of celecoxib or ibuprofen did not produce any gross or histological changes in the liver, kidneys, stomach, or intestinal tract.

Histopathology of Mammary Tumors. At the completion of the experiment, 127 palpable mammary tumors were excised from control animals, 61 palpable mammary tumors were excised from animals receiving ibuprofen, and 18 palpable mammary tumors were excised from animals receiving celecoxib. Histopathological evaluation revealed that all tumors from the control and ibuprofen groups were adenocarcinomas. Of the 18 tumors excised from animals receiving celecoxib, 15 were adenocarcinomas, and 3 were nonmalignant fibroadenomas.

Mammary Tumor Data. The chemopreventive effects of celecoxib and ibuprofen on mammary tumor development are shown in Figs. 1 and 2 and summarized in Table 2. The specific COX-2 blocker celecoxib produced striking reductions ($P < 0.001$) in the incidence of mammary cancer (68%), tumor burden (86%), and tumor volume (81%) compared to those seen in the control group. In the celecoxib group, only 13 of 40 animals (32%) developed malignant tumors, 3 animals developed fibroadenomas, and the tumors were relatively small (mean volume, 0.45 cm^3). In contrast, 100% of control animals developed malignant tumors, the majority of animals (95%) had multiple tumors, and tumor size was much greater (1.5 cm^3). The weaker COX-2 blocker ibuprofen also produced statistically significant ($P < 0.001$) reductions in cancer risk, tumor burden, and size (40%, 52%, and 57%, respectively), but its effects were of lesser magnitude than those of celecoxib ($P < 0.01$). The administration of NSAIDs also prolonged the latency period of tumor induction. In animals receiving the control diet only, median detection (>50% of tumors) occurred at 58 days after DMBA induction compared with 95 and 86 days in the celecoxib and ibuprofen treatment groups, respectively. In summary, these results reflect strong suppression of mammary carcinogenesis (68% inhibition of breast cancer incidence; $P < 0.001$) by the specific COX-2 blocker celecoxib and intermediate suppression (40% inhibition; $P < 0.01$) by the general NSAID ibuprofen.

Pharmacological Data. Serum levels of celecoxib ranged from 2.3–9.7 $\mu\text{g/ml}$ (mean, 5.1 $\mu\text{g/ml}$). The mean drug level was slightly higher in animals without tumors versus animals with tumors (5.8 versus 4.9 $\mu\text{g/ml}$, respectively; $P < 0.10$). Serum levels of ibuprofen ranged from 4–12 $\mu\text{g/ml}$ (mean, 8.0 $\mu\text{g/ml}$).

Table 1 Effects of celecoxib and ibuprofen diets on body weights of Sprague Dawley rats

Treatment group ^a	Initial weight (g) ^{b,c}	Final weight (g) ^{b,c}
Control	144.1 \pm 1.2	258.2 \pm 3.2
Ibuprofen	148.1 \pm 2.6	251.4 \pm 2.2
Celecoxib	147.6 \pm 1.3	253.5 \pm 2.2

^a Forty rats/group were fed powdered Teklad 22/5 rodent diet (W):8640 supplemented in the experimental diets with 1500 ppm of either ibuprofen or celecoxib.

^b Mean weight \pm SE.

^c Differences among treatment means were not statistically significant (initial weights, $P < 0.24$; final weights, $P < 0.18$).

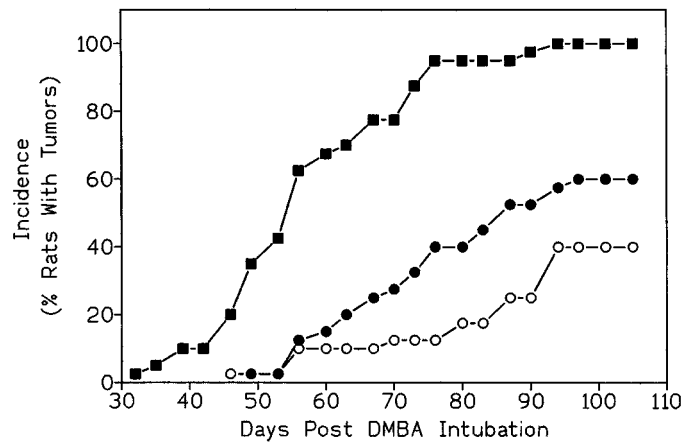


Fig. 1. Effects of celecoxib and ibuprofen on breast cancer incidence. The control group (■) received Teklad powdered chow diet, the celecoxib group (○) received chow plus 1500 mg/kg celecoxib, and the ibuprofen group (●) received chow plus 1500 mg/kg ibuprofen.

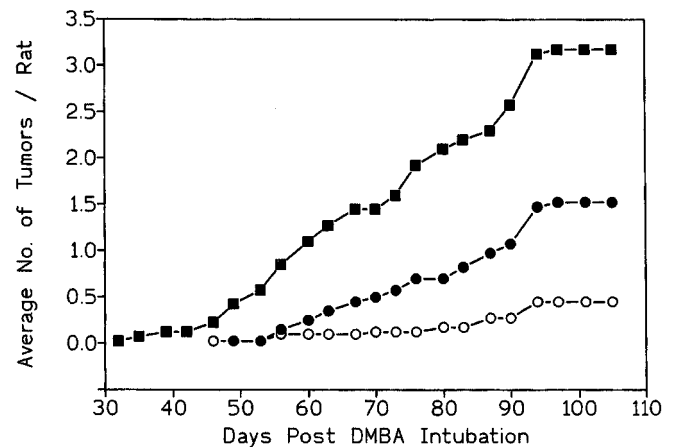


Fig. 2. Effects of celecoxib and ibuprofen on tumor burden. The control group (■) received Teklad powdered chow diet, the celecoxib group (○) received chow plus 1500 mg/kg celecoxib, and the ibuprofen group (●) received chow plus 1500 mg/kg ibuprofen.

Discussion

The major aim of this investigation was to evaluate the chemopreventive effects of the specific COX-2 blocker celecoxib against the development of chemically induced breast cancer. Our results are the first to show dramatic suppression of mammary carcinogenesis in this model by COX-2 blockade. The observed chemopreventive effects of celecoxib exceeded those of the more general NSAID ibuprofen as well as other agents that have shown significant antitumor effects in this animal model, *e.g.*, the retinoic acid 4-HPR and the glucuronidase inhibitor glucarate (17). It is also noteworthy that administration of celecoxib (or ibuprofen) at 1500 ppm did not produce any toxic side effects such as weight loss, gastrointestinal ulceration, or bleeding. These results support earlier epidemiological findings suggesting that NSAIDs may have chemopreventive value against breast cancer and underscore the need for intensive investigation of specific COX-2 blockade *vis à vis* celecoxib as a potentially effective approach to the chemoprevention of this disease. It is also important to note that antitumor effects of celecoxib have been observed against other types of malignancies, the most notable of which is colon cancer (18, 19). The possibility therefore exists that celecoxib may have value as a general chemopreventive agent against a spectrum of malignancies.

The exact mechanism of action by which COX-2 blockade inhibits

Table 2 Effects of celecoxib and ibuprofen on the incidence, growth, and development of DMBA-induced rat mammary tumors

Treatment group ^a	Latency (days)	Incidence (%)		Tumor burden ^d	Tumor volume (cc) ^e
		Cancer ^b	All tumors ^c		
Control	58	100	100	3.2 ± 0.2	1.5 ± 0.4
Ibuprofen	86 ^f	60 (40) ^f	60 (40) ^f	1.5 ± 0.3 (52) ^f	0.6 ± 0.2 (57) ^f
Celecoxib	95 ^f	32 (68) ^f	40 (60) ^f	0.4 ± 0.1 (86) ^f	0.3 ± 0.1 (81) ^f

^a Forty rats/treatment group were fed the control diet supplemented in the experimental diets with 1500 ppm of either ibuprofen or celecoxib.

^b Cancer incidence is the frequency of animals that developed breast cancer.

^c The incidence of all tumors includes three animals in the celecoxib treatment group that developed fibroadenomas.

^d Mean number of tumors/animal ± SE.

^e Mean tumor volume ± SE.

^f Statistical significance relative to the control group at $P < 0.001$. Reductions in the incidence rates, tumor burden, and tumor volume for the experimental diets relative to the control diet are given in parentheses.

mammary carcinogenesis remains to be clarified. Our working hypothesis is that mammary carcinogenesis is triggered by inappropriate induction and up-regulation of COX-2 due to high intake of omega-6 fatty acids in the diet (20). By this hypothesis, conversion of the normally silent COX-2 gene to a heightened state of constitutive activity in the mammary epithelium results in excess production of PGE₂ and potentiates local estrogen biosynthesis by aromatase. The "aberrant turning on of COX-2" could therefore result in at least three major forces that drive the process of mammary carcinogenesis: (a) mutagenesis by creation of free radical molecules involved in sustained prostaglandin biosynthesis; (b) angiogenesis by stimulation of vascular endothelial growth factor by PGE₂; and (c) mitogenesis without natural apoptosis due to estrogen production from aromatase. Cyclooxygenase activity may also be linked to the metabolic activation and metabolism of DMBA and other polycyclic aromatic hydrocarbons through the cytochrome P-450 system (21). Thus, the sustained presence of the COX-2 blocking agent celecoxib could modulate critical steps in the initiation and promotion of mammary carcinogenesis.

In conclusion, administration of celecoxib, a specific COX-2 inhibitor, suppressed the incidence, multiplicity, and size of malignant breast tumors induced by DMBA in female Sprague Dawley rats. The degree of inhibition was more pronounced with celecoxib than with a general NSAID (ibuprofen) as well as other chemopreventive agents evaluated using similar protocols. These results suggest that celecoxib may be an effective chemoprevention agent against human breast cancer.

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