Celecoxib, a Cyclooxygenase 2 Inhibitor as a Potential Chemopreventive to UV-Induced Skin Cancer

A Study in the Hairless Mouse Model

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Objective: To assess the preventive effect of a cyclooxygenase 2 inhibitor, celecoxib (Celebrex; G.D. Searle & Co, Skokie, Ill), in UV-induced skin cancer in hairless mice.

Design: Randomized dose-response study. A total of 75 SKH-HR-1 female hairless mice, aged 2 months, were randomized into control, low-dose (200 mg twice daily human dose equivalent), and high-dose (400 mg twice daily human dose equivalent) celecoxib treatment groups. Animals received 1 J/cm² daily (5 d/wk) total irradiation. The animals were evaluated weekly for appearance of tumors, and the data were analyzed with respect to tumor latency period and tumor multiplicity using statistical software and Wilcoxon rank sum analyses, respectively. Prostaglandin E₂ levels in the blood and epidermis were assessed in each group.

Setting: Veterans Affairs Medical Center, Research and Dermatology Services.

Intervention: Animals received restricted diets containing the Food and Drug Administration–approved human equivalent doses of 200 mg (low dose) and 400 mg (high dose) of celecoxib twice daily. Controls received no drug. Tumors were induced in all animals with an equivalent UV dose.

Main Outcome Measures: Animals were evaluated weekly for the appearance of tumors, and data were analyzed with regard to tumor latency period and tumor multiplicity. Constitutive prostaglandin E₂ levels in blood and epidermis were assessed in each group.

Results: Low doses and high doses of celecoxib significantly lengthened the tumor latency period (P < .03 and P < .003, respectively) and reduced tumor multiplicity (P < .005 and P < .001, respectively) compared with controls. There were no differences in the constitutive levels of blood or epidermal prostaglandin E₂ in the low- or high-dose treated animals compared with controls when analyzed at study termination.

Conclusions: Celecoxib is an effective and safe chemopreventive agent in UV carcinogenesis. The epidemiologic, laboratory, and animal studies of the influence of celecoxib on cancer incidence and its low association with systemic adverse effects have led to a potentially new therapeutic approach for the prevention of skin cancer.

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CYCLOOXYGENASE (COX) 1 and COX-2 enzymes are prostaglandin synthases that catalyze the conversion of arachidonic acid to prostaglandins. Prostaglandin E₂ (PGE₂), in particular, is a proinflammatory and immune-regulating eicosanoid, the cutaneous levels of which increase on UV irradiation. Indeed, the COX-2 gene has been shown to be highly inducible by cytokines, growth factors, and tumor promoters and is overexpressed in many types of human neoplastic tissues, including esophageal, gastric, colon, breast, and lung tissue.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, and piroxicam inhibit the COX-1 and COX-2 isoforms. Recent investigations have indicated that regular NSAID administration reduces the relative risk of death from colorectal cancer by 40% to 50%. Similar trials have shown that regular aspirin and ibuprofen ingestion decreased breast cancer incidence rates by 40% and 50%, respectively. This evidence implies a potential chemotherapeutic role for NSAIDs. Concomitant inhibition of COX-1, however, blocks cytoprotective prostaglandin production by the gastric mucosa, resulting in gastrointestinal bleed-
MATERIALS AND METHODS

ANIMALS AND IRRADIATION

A total of 75 SKH-HR-1 female hairless mice, aged 2 months, were randomized into 3 groups of 25 animals each: control, low-dose celecoxib treatment, and high-dose celecoxib treatment. On randomization, each animal was identified by abdominal tattoo and weighed. Thereafter, body weights were recorded biweekly and mortality records maintained. After a 2-week run-in period of the respective treatments, animals were irradiated with Kodacel 401 filtered FS-40 sunlamps (Westinghouse, Bloomfield, NJ). The Kodacel 401 filters all UV-C radiation, resulting in a radiance spectrum of 290 to 360 nm (approximately 80% in the UV-B region). Animals received 1 J/cm² total irradiation daily (5 d/wk), as determined by an Eppley circular thermopile. This level of irradiation is suberythemic, equivalent to about 0.8 of a minimum erythemal dose. Irradiation continued for 11 weeks when 53 J/cm² had been delivered, at which point irradiation was halted.

DIET AND DRUG TREATMENT

Isocaloric semisynthetic diets were fed to each group of animals for a 2-week run-in period and for the duration of the study. The diet has been described previously23 and is composed of approximately 27% casein, 38% corn starch, 12% corn oil, with the remainder consisting of mineral and vitamin mixtures and nonnutritive filler. Fat content of 12% was chosen because this level is roughly equivalent to consumption of 40% of total calories as fat—a level similar to that consumed by the US population. This level of fat is also known to promote UV carcinogenesis in the mouse.34 From preliminary feeding trials it was determined that animals would completely consume a weight of the diet equivalent to 15 kcal/d. Thus, an equivalent weight in grams of the semisynthetic diet was dispensed daily per animal.

The COX-2 inhibitor celecoxib was administered in the diet. Because the weight of the diet representing the daily energy requirement was shown to be completely consumed, drug intake was more uniform when administered in this manner than by supplying the drug in a diet that would be consumed ad libitum.

The Celebrex capsules used contained 200 mg of celecoxib. Capsules were opened and the contents carefully removed and weighed. Contents of each capsule contained 74% active ingredient; the remainder was nonactive filler. According to the manufacturer's data sheet,35 50 mg of drug per kilogram of body weight in female mice provides a total absorption equivalent to human exposure of 200 mg twice daily. Using the calculated percentage of active ingredient and drug equivalents, we weighed quantities of capsule ingredients and thoroughly mixed them with the semisynthetic diet to provide the human equivalent doses of 200 and 400 mg of celecoxib. Thus, 1.25 mg of drug per mouse and 2.5 mg of drug per mouse was delivered daily to provide the equivalent of 200 mg and 400 mg human twice-daily exposure.

TUMOR EVALUATION AND STATISTICS

Animals were evaluated weekly for the appearance of tumors using a 1-mm-diameter lesion as biological endpoint. Histologically, tumor types were either papillomas or squamous cell carcinomas. Tumor data were analyzed using the SAS Life Table Analysis program (tumor latency, median tumor time)36 and the Wilcoxon rank sum analysis37 for tumor multiplicity (number of tumors per animal). Comparison of body weights among all treatment groups was performed using analysis of variance.

PGE₂ ANALYSIS

Nine animals from each of the 3 groups (control and 2 treatment groups) were killed at week 28. Blood was collected by cardiac puncture into tubes containing ethylenediaminetetraacetic acid and indomethacin. Epidermis was isolated from nonirradiated abdominal skin by blunt dissection after a brief treatment at 55°C.38 Triplicate tissue samples, blood or epidermis, were prepared by pooling tissues from 3 animals each of the 9 from the respective groups.

Tissue PGE₂ levels were determined using the Biotrak prostaglandin E₁ iodine 125 (¹²⁵I) assay system from Amer sham Pharmacia Biotech (Piscataway, NJ). Briefly, whole blood was centrifuged and plasma removed. Epidermal samples were homogenized in buffer containing ethylenediaminetetraacetic acid and indomethacin, centrifuged, and supernatant recovered. Plasma and epidermal homogenates were, thereafter, handled similarly. To 0.5-mL samples, 0.5 mL of water, ethanol (1:4; vol/vol) and 10 µL of acetic acid was added. Samples were centrifuged at 2500g for 2 minutes, and the supernatant loaded onto primed C18 minicolumns. The columns were washed with 1 volume of water and hexane, respectively, and the samples eluted twice with 0.75 µL of ethyl acetate. Samples were dried under nitrogen.

The dried samples were reconstituted with 100 µL of phosphate-buffered gelatin saline (pH 7.0) with 100 µL of methyl oximation reagent added. Samples were incubated at 60°C for 1 hour to allow completion of the methyl oximation reaction. Following oximation, the samples were diluted to a final volume of 500 µL with the phosphate-buffered gelatin saline and assayed. The assay uses the competition between unlabeled methyl-oximated PGE₂ and a fixed quantity of ¹²⁵I-labeled PGE₂ (oximated derivative for a specific antibody raised against oximated PGE₂). A standard curve was generated from which sample values were determined. Significance was tested using a 2-tailed t test.

Basal and squamous cell carcinomas, the 2 most common human skin cancers, are etiologically related to UV exposure.27-29 UV-induced prostaglandin synthesis and up-regulation of COX-2 may be contributing factors to the initiation and promotion of skin carcinogenesis.30 Immunohistochemical studies have revealed COX-2 overexpression in cutaneous squamous cell carcinomas compared with biopsy specimens of skin not
exposed to the sun. In addition, Buckman et al demonstrated, via Western blot analysis, acute UV induction of COX-2 synthesis in human epidermis. The association of COX-2 up-regulation in inflamed and cancerous tissues implies the potential of specific COX-2 inhibitors in chemoprevention. Thus, a controlled study was undertaken to evaluate the influence of celecoxib (Celebrex; G.D. Searle & Co, Skokie, Ill), a selective COX-2 inhibitor, on UV-induced carcinogenesis in the hairless mouse.

RESULTS

The influence of the COX-2 inhibitor celecoxib on UV carcinogenesis is shown in Figure 1 and Figure 2. Tumor incidence plots for low (200-mg equivalent) and high (400-mg equivalent) doses are represented in Figure 1. The effect of celecoxib on tumor multiplicity is shown in Figure 2. Both high and low doses significantly lengthened the tumor latency period (median time of tumor incidence) and reduced tumor multiplicity (number of tumors per animal) compared with controls. Although the high-dose treatment resulted in a longer tumor latency period and lower tumor multiplicity than low-dose treatment, the differences were not statistically significant. The median tumor time for the control, low-dose, and high-dose groups were 18.8, 22.7, and 24 weeks, respectively. The mean±SD number of tumors per animal for these groups were 2.46±2.7, 0.71±1.1, and 0.44±0.8, respectively.

Long-term administration of celecoxib resulted in no statistically significant differences in mortality. Over the course of the 28-week experiment, 1 animal died in the low-dose group at week 20, and 2 animals in the control group died at weeks 24 and 28. No animals died in the high-dose group. A loss in body weight occurred in all groups on transfer of the animals from commercial rodent chow to the semisynthetic diet (week 0 to week 2), after which there was an overall gain (Figure 3). There were no systemic differences in body weights between control and treatment groups. Nor were there differences in constitutive levels of blood or epidermal PGE2 levels. No significant differences were observed in either blood or epidermal constitutive PGE2 levels. Error bars indicate SD.
Recognition of the potential role of COX-catalyzed reactions in carcinogenesis has resulted from convergent evidence, epidemiologic and experimental, that has shown an inverse relationship between regular NSAID intake and the development of colon, breast, esophageal, rectal, and lung cancers. The chemopreventive mechanisms of NSAIDs have been partially elucidated. The NSAIDs are thought to exert their anticarcinogenic effect by inhibiting the biosynthesis of certain products of arachidonic acid metabolism, notably prostaglandins. Accumulating evidence suggests prostaglandins are pathogenically linked to carcinogenesis via their influence on cell proliferation, tumor growth, and immune responsiveness.

Experimental work by DuBois et al demonstrated significantly elevated COX-2 messenger RNA and protein levels in chemically induced colon tumors. Furthermore, Tsuji and DuBois reported that cells that overexpressed the COX-2 gene developed altered adhesion properties and resisted undergoing apoptosis. The adhesion and apoptotic effects were reversible with NSAID administration. In addition, Oshima et al demonstrated a greater than 6-fold reduction of intestinal polyp development in COX-2 null mice compared with COX-2 wild-type mice. Moreover, clinical evidence revealed that the NSAID sulindac suppressed colonic and rectal polyp formation in humans with familial adenomatous polyposis. These studies suggest a pivotal role of COX-2 in colonic carcinogenesis.

Similarly, significant COX-2 gene overexpression in human breast tumors has been reported. Animal studies have illustrated a significant reduction of tumor burden and size that paralleled inhibition of genetic expression of COX-2 with ibuprofen. An inverse relationship between NSAID administration and chemically induced breast carcinogenesis in animals has also been shown. Likewise, prostaglandin up-regulation and COX-2 expression have been pathogenically linked to UV carcinogenesis. Evidence of this association comes from the finding that significantly increased expression of COX-2 occurs in squamous cell carcinomas and actinic keratoses when compared with nonlesional skin. Western blot analysis revealed UV-irradiation induction of COX-2 in human epidermis.

Indeed, it has been shown that COX inhibition by NSAIDs leads to suppression of skin tumorigenesis in animal studies. Bisset et al reported a delay in the appearance of UV-B–induced tumors in hairless mice treated with topical naproxen and ibuprofen. In agreement with these findings, Lowe et al demonstrated suppression of photocarcinogenesis in mice with topical indomethacin. Subsequent studies have shown that orally administered indomethacin reduces tumor incidence and tumor burden in UV-irradiated hairless mice. These studies imply a primary role of COX and prostaglandins as facilitators of cutaneous carcinogenesis in addition to a chemopreventive role of NSAIDs.

As noted previously, the aforementioned NSAIDs are nonspecific in their activity and inhibit the cytoprotective actions of the COX-1 isozyme. Adverse effects of long-term oral NSAID administration are not uncommon and include gastrointestinal bleeding and ulceration and renal toxic effects. Celecoxib, a specific COX-2 inhibitor, exhibits a lower adverse-effect profile than other NSAIDs and could avert many of these problems.

Pentland et al demonstrated a significant difference in tumor burden in UV-irradiated mice treated with celecoxib. In their study, celecoxib was orally administered 6 weeks after UV irradiation was completed and at a time when 90% of the animals exhibited at least 1 tumor. Ten weeks thereafter, the celecoxib-treated mice had only 56% of the tumor burden exhibited by the control group. These results imply an effect on the postinitiation events of UV carcinogenesis and suggest that celecoxib has potential benefit as an intervention therapy to prevent the appearance of subsequent skin cancers arising from clonal expansion of previously initiated cells. Fischer et al have provided evidence that celecoxib acts during UV initiation as well. In UV-irradiated mice fed AIN-76 diets (approximately 12% of total caloric intake as fat) containing 150 or 500 ppm celecoxib, a dose-dependent reduction in tumor yield resulted. Indomethacin at 4 ppm was as effective as 500 ppm celecoxib in reducing this tumor parameter. In this respect, it is interesting to note that these investigators found that neither celecoxib nor indomethacin altered the level of COX-2 expression, although both significantly reduced the levels of UV-increased PGE2 levels.

In the present study, we used a semisynthetic diet that provided approximately 40% of total intake of calories through fat, not unlike that consumed by the general US public. This factor is important because endogenous PGE2 levels are influenced by total fat intake, and level of fat intake is directly related to UV carcinogenic expression. Suberythemic levels (about 0.8 of a mouse minimum erythema) of UV were used. The animals’ diet was restricted to an energy intake adequate to meet their requirements for normal growth and development but that was completely consumed, assuring delivery of the designated drug levels. The specific COX-2 inhibitor celecoxib, based on published 0- to 24-hour absorption data for the mouse, was administered at equivalent levels currently prescribed for humans (ie, 200 and 400 mg twice daily). Celecoxib treatment significantly increased the UV-induced tumor latency period in a dose-dependent manner. Tumor multiplicity was significantly reduced, by about the same magnitude, at both doses. Constitutive levels of PGE2 in blood and nonirradiated abdominal epidermis were unaffected by the drug at experiment termination, some 17 weeks after UV irradiation was administered. There were no obvious deleterious effects of long-term drug treatment with respect to growth rate or mortality.

In toto, celecoxib has been shown to be an effective and safe chemopreventive agent to UV carcinogenesis in the hairless mouse model at doses equivalent to those prescribed in humans. These findings warrant further human investigations to explore this potential. The inherent pharmacologic selectivity of celecoxib on the COX-2 isozyme and limited effect on COX-1 at human therapeutic levels should theoretically result in minimal gastrointestinal and renal cytotoxic effects with long-term administration compared with NSAIDs. The epidemiologic, laboratory, and animal studies of the influence of celecoxib on carcinogenesis and its low association with sys-
temic adverse effects have pointed to a potentially new therapeutic approach for the treatment and prevention of skin cancers.

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