Celecoxib Inhibits Meningioma Tumor Growth in a Mouse Xenograft Model

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BACKGROUND. Treatments for recurrent meningiomas are limited. We previously demonstrated universal expression of COX-2 in meningiomas and dose-dependent growth inhibition in vitro with celecoxib, a COX-2 inhibitor. We therefore tested the effects of celecoxib on meningioma growth in a mouse xenograft model.

METHODS. Meningioma cell lines (IOMM-Lee, CH157-MN, WHO grade I primary cultured tumor) were transplanted into flanks of nude mice fed mouse chow with celecoxib at varying concentrations (0, 500, 1000, 1500 ppm) ad libitum. Tumors were measured biweekly and processed for MIB-1, Factor VIII, COX-2, and VEGF, and assayed with transferase-mediated dUTP-biotin nick-end labeling (TUNEL).

RESULTS. Celecoxib reduced growth of mean tumor volume by 66% (P < .05), 25% (P > .05), and 65% (P < .05) compared with untreated controls in IOMM-Lee, CH157-MN, and benign tumors, respectively. IOMM-Lee tumors removed from celecoxib treatment regained a growth rate similar to the control. Blood vessel density decreased and apoptotic cells increased in treated flank tumors. Diminished COX-2 expression and VEGF were observed in treated IOMM-Lee tumors. Mean plasma celecoxib levels were 845, 1540, and 2869 ng/mL, for low-, medium-, and high-dose celecoxib, respectively.

CONCLUSIONS. Celecoxib inhibits meningioma growth in vivo at plasma levels achievable in humans. Celecoxib-treated tumors were less vascular with increased apoptosis. IOMM-Lee tumors treated with celecoxib showed decreased COX-2 and VEGF expression. COX-2 inhibitors may have a role in the treatment of recurrent meningiomas. Cancer 2007;109:588–97. © 2006 American Cancer Society.

KEYWORDS: meningioma, cyclooxygenase-2, COX-2, celecoxib, in vivo.

Meninigonomas account for approximately 20% of all primary intracranial tumors, making them the second most common symptomatic adult central nervous system tumor. Meningioma progression or recurrence may occur because of aggressive biology or unresectable location.1–3 Recurrence rates for benign meningiomas 5 years after complete removal are only 2% to 3%, whereas recurrence rates for atypical and anaplastic meningiomas are 38% to 50% and 33% to 78%, respectively.4,5 Treatment options for recurrent meningiomas are limited.6,7 Radiation therapy is limited by side effects and tumor size, and systemic therapies have largely been disappointing.7–9 Therefore, exploration of new treatment options is necessary.

The results of several head trauma epidemiologic studies have suggested the possibility that the inflammatory process is involved in meningioma pathogenesis.10 Cyclooxygenase-2 (COX-2) is an inducible inflammatory response enzyme that is up-regulated in animal head trauma models.11 Cyclooxygenase is the rate-limiting enzyme...
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MATERIALS AND METHODS

Meningioma Specimens
A meningioma surgical specimen was obtained under an Institutional Review Board-approved protocol and processed for cell culture. The tumor was graded as a WHO grade I meningioma, which, for the purposes of this article, we will describe as a primary cultured “benign meningioma.” This tumor specimen was grown as a monolayer culture as described previously. Briefly, the specimen was taken immediately from the operating room, digested in collagenase, and placed in Dulbecco Modified Eagle Medium (DMEM; Sigma, St. Louis, MO) supplemented with 10% fetal calf serum, L-glutamine (2 μM), penicillin (50 IU/mL), and streptomycin (50 mg/mL). Cultured cells were maintained at 37°C in 7.5% CO2.

The human meningioma immortal cell lines IOMM-Lee and CH157-MN were also grown in DMEM supplemented with 10% fetal calf serum, L-glutamine (2 μM), penicillin (50 IU/mL), and streptomycin (50 mg/mL) at 37°C in 7.5% CO2. The IOMM-Lee and CH157-MN cell lines were kind gifts from Dr. Ian McCutcheon (University of Texas, M. D. Anderson Cancer Center, Houston, TX) and Dr. Yancey Gillespie (University of Alabama School of Medicine, Birmingham, Alabama), respectively.

In Vitro Assays for COX-2 Function
To determine the effects of celecoxib treatment on the COX-2 enzyme, we analyzed IOMM-Lee cells grown in cell culture for COX-2 activity as well as cellular levels of prostaglandin E2 (PGE2). IOMM-Lee cells were plated in T-175 flasks and allowed to grow to 75% confluence. Flasks were treated with vehicle control (0.1% dimethyl sulfoxide [DMSO]) or 1.00 mM celecoxib, as previously described. Cells were harvested after 24 hours of treatment and protein was isolated. A total of 4 samples were processed for each treatment group.

Briefly, cells were rinsed with ice-cold Dulbecco phosphate-buffered saline (PBS), scraped from the culture dish, pelleted at 1000g for 10 minutes at 4°C, resuspended in 250 μL of ice-cold buffer (0.1 M Tris-HCl, pH 7.8, containing 1 mM ethylene diamine tetraacetic acid [EDTA]), and sonicated for 10 seconds 3 times. Cellular debris was pelleted by centrifugation at 10,000g at 4°C for 15 minutes. The supernatant (nuclear and cytoplasmic) was removed and stored at −80°C until use. Samples were processed in triplicate for COX-2 activity (COX Activity Assay, Cayman Chemical, Ann Arbor, MI) and PGE2 enzyme immunoassay (Prostaglandin E2 EIA Kit-Monoclonal, Cayman Chemical) per the manufacturer’s protocol.

Meningioma Mouse Xenograft Flank Model
All animal experiments were approved by the Animal Care and Use Committee of the University of Utah. Subcutaneous meningioma flank tumors were implanted as described previously. Briefly, cells were grown to approximately 80% confluence in T-175 flasks. All steps were carried out on ice. The cells were rinsed with PBS, trypsinized, counted using a bright-line hemocytometer, pelleted at 1000 rpm for 5 minutes at 4°C, and resuspended in media (IOMM-Lee and CH157-MN cell lines) or Matrigel (BD Biosciences, San Diego, CA; primary meningioma cell line). Desired cell counts were 500,000 cells per mouse flank for the immortal cell lines and 5,000,000 per mouse flank for the primary meningioma cell line. A total volume of 0.10–0.15 mL was injected subcutaneously into the flanks of immunodeficient mice (CD1, Charles River Laboratories, Wilmington, MA) using a tuberculin syringe and 25 G needle. Three-week-old mice were used for the dose-response experiments, whereas 3-month-old mice were used for the prophylactic treatment experiments, as described below.

First, we performed dose-response experiments using 3-week-old mice and the IOMM-Lee cell line (control, n = 20; low-dose celecoxib, n = 10; medium-dose celecoxib, n = 10; high-dose celecoxib, n = 15). Second, we repeated the high-celecoxib dosing regime with 3-week-old mice and both the CH157-MN cell line (control, n = 10; high-dose celecoxib, n = 5) and the benign cell line (control, n = 5; high-dose celecoxib, n = 5). Mice were sacrificed with a lethal intraperitoneal injection of pentobarbital. Flank tumors were excised, cut into blocks, and placed in 10% formalin.
for paraffin blocks or snap-frozen in liquid nitrogen. Cardiac puncture was performed after euthanasia to obtain blood for celecoxib serum analysis.

**Treatment With Celecoxib**

Celecoxib (G.D. Searle, Chicago, IL) was included in mouse chow (Rodent Diet 8656, Harlan Teklad, Madison, WI) at 500 (low celecoxib), 1000 (medium), and 1500 ppm (high). Tumors were allowed to grow for 10–14 days before the initiation of treatment to allow the Matrigel or medium to resorb completely. The start of tumor measurements was correlated with the initiation of the treatments. Control mice were fed regular mouse chow ad libitum. Treated mice were fed celecoxib mouse chow ad libitum for prophylactic treatment groups were fed low-, medium-, or high-dose celecoxib for 6 weeks before subcutaneous flank injections and until sacrifice or the end of treatment.

**Analysis of Celecoxib Serum Levels**

After the sacrifice of the mice, whole blood was aspirated via cardiac puncture, placed into plasma separator tubes, and spun at 1000 rcf for 5 minutes. The isolated plasma was placed into cryogenic tubes and stored at −80°C. Serum samples were shipped on dry ice to National Medical Services (Willow Grove, PA) for celecoxib serum analysis by high-performance liquid spectroscopy.

**Immunohistochemistry**

Immunohistochemistry analysis on formalin-fixed, paraffin-embedded meningioma mouse xenograft tumors was accomplished for MIB-1, Factor VIII, VEGF, and COX-2. Slides were cut at 4 μm, then melted at 55–60°C for 30 minutes, deparaffinized in xylene for 5 minutes, and rehydrated in graded alcohols (100%, 70%, 55–60°C for 30 minutes, deparaffinized in xylene for 5 minutes, and rehydrated in graded alcohols (100% ×2, 95% ×2, 70% ×1) for 1 minute each. The following steps were performed on the Ventana ES (Ventana Medical Systems, Tucson, AZ) at 40°C. Heat-induced epitope retrieval was accomplished by applying citrate buffer (pH 6.0) in a microwave oven for 15 minutes at half power and allowing it to cool for 15 minutes for tissue stained for Factor VIII, VEGF, and COX-2. For the MIB-1 immunohistochemical staining, heat-induced epitope retrieval was accomplished by applying citrate buffer (pH 6.0) in an electric pressure cooker for 3 minutes and allowing it to cool for 27 minutes. The primary antibodies (Ki-67, 1:100, mouse monoclonal Ab, Clone MIB-1, Dako Cytomation, Carpinteria, CA; Factor VIII, 1:1600, rabbit polyclonal Ab, Dako Cytomation; COX-2, 1:200, rabbit polyclonal, Lab Vision, Fremont, CA; or VEGF; 1:200, mouse monoclonal, Dako Cytomation) were applied for 32 minutes followed by the appropriate secondary antibody (mouse primary antibody: Mouse Fab, 1:200, Mouse IgG, Dako Cytomation; rabbit primary antibody: goat antimouse/antirabbit, 1:300, Rabbit Fab, Dako Cytomation) for 8 minutes. Detection was done using the IView DAB detection kit (Ventana) and the counterstain was done with hematoxylin (Ventana) for 4 minutes. Slides were then dehydrated through graded alcohols (70% ×1, 95% ×2, 100% ×2) for 30 seconds each, dipped in 4 changes of xylene, and covered with a coverslip. Positive controls consisted of pancreas tumor, normal tonsil, colon cancer, and normal colon for MIB-1, Factor VIII, COX-2, and VEGF, respectively. Negative controls were accomplished by running the above positive control tissue without the primary antibody.

**Apoptosis Assay**

The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique was used to label the fragmented DNA of apoptotic cells. Formalin-fixed, paraffin-embedded mouse meningioma xenograft tumors were processed as recommended by the manufacturer (TUNEL Assay, Promega, Madison, WI), staining the fragmented DNA of apoptotic cells brown.

**Immunohistochemical and Apoptosis Analysis**

Analysis of MIB-1 staining was performed by taking 6 random pictures per slide at ×400 (×10 ocular ×40 objective) using an Olympus Microfire camera. The images were analyzed using the Image-Pro Plus 5.0 graphic analysis program (Media Cybernetics, Silver Spring, MD). Results were reported as number of MIB-1 cells positive per ×40 high-powered field (hpf) and represent the mean (±SD) of 6 random fields. Three flank tumors were analyzed per treatment group, 1 slide per tumor.

Microvascular density analysis was achieved by scoring Factor VIII-stained slides, counting the number blood vessels positive in 3 × 200 fields (×10 ocular ×20 objective). Microscope fields were chosen based on the 3 fields with the maximal number of blood vessels stained (ie, Factor VIII “hot-spots”). Three flank tumors were analyzed per treatment group, 1 slide per tumor.

Immunohistochemical analysis for COX-2 and VEGF was accomplished by scoring 6 random fields per slide at ×200 (×10 ocular ×20 objective) for the percentage of cells stained and staining intensity. Areas of tumor necrosis and edge artifact were avoided. The percentage of cells stained was estimated to be 0%, 25%, 50%, 75%, or 100%. The intensity score was measured using a numerical scale (0 = no expression,
1+ = weak expression, 2+ = moderate expression, 3+ = strong expression.) A weight index was calculated [WI = % positive staining (≥0) × intensity score]. Three flank tumors were analyzed per treatment group, 1 slide per tumor.

TUNEL staining for apoptosis was analyzed by counting the number of positively stained cells in 6 random fields per slide at ×400 (×10 ocular × ×40 objective), avoiding areas of necrosis. Three flank tumors were analyzed per treatment group, 1 slide per tumor.

**Statistical Analysis**

In vitro data and immunohistochemical staining data (MIB-1, Factor 8, and TUNEL) were analyzed with the Student t-test (unpaired, 2-tailed t-test with confidence intervals set to 95%), whereas tumor growth curves and weighted immunohistochemical staining data (COX-2 and VEGF) were analyzed with 1-way analysis of variance (1-way ANOVA, Tukey post-test with confidence intervals set to 95%) to compare control and treated groups, with statistical significance set at P < .05. Survival curves were analyzed with the χ² test, with statistical significance set at a P < .05. Statistics were analyzed using the GraphPad Prism 4.0 (San Diego, CA) statistical program.

**RESULTS**

**Celecoxib Inhibits COX-2 Activity and Decreases PGE₂ Concentrations In Vitro**

The IOMM-Lee cell line was grown in culture, treated with 1.0 mM celecoxib for 24 hours, and processed for COX-2 function to include the COX-2 activity assay and PGE₂ metabolite levels. Celecoxib completely inhibited mean (±SD) COX-2 activity in vitro with an overall reduction of 100% (2.9 ± 1.2 U/mL vs −0.1 ± 0.5 U/mL; t-test P = .004) (Fig. 1). Correspondingly, cellular PGE₂ levels fell by 51% (50.9 ± 21.5 ng/mL vs 24.9 ± 10.3 ng/mL; t-test P = .003) (Fig. 1).

**Meningioma Flank Tumor Characteristics**

The IOMM-Lee immortal cell line was originally obtained from a 55-year-old man with an interosseous meningioma, WHO grade 3, in 1990. The CH157-MN immortal cell line was originally obtained from a 55-year-old woman. The WHO grade I tumor was a convexity, transitional meningioma, obtained from a 55-year-old man. All three meningiomas underwent Giemsa-band karyotype showing chromosomal abnormalities consistent with meningiomas (eg, loss of chromosomes 1 and 22, as well as addition of chromosomes 1, 9q, 12q, 18, 20) (data not shown). Histologically, flank tumors derived from IOMM-Lee and CH157-MN cell lines exhibited sheeting cytoarchitecture composed of large pleomorphic tumor cells. The benign cell line showed a cytoarchitecture vaguely resembling the original operative transitional meningioma subtype. Macroscopically, all three of these tumors exhibited a necrotic core with a peripheral ring of live tumor. Immunohistochemically, the IOMM-Lee, CH157-MN, and benign meningioma flank tumors were vimentin-positive, epithelial membrane antigen-positive, and glial fibrillary acid protein-negative (data not shown). Transmission electron microscopy of the IOMM-Lee and CH157-MN flank tumors exhibited ultrastructural features consistent with meningiomas (eg, intercellular junctions; data not shown). These features (ie, karyotypic abnormalities, immunohistochemistry, and ultrastructural features) are typical of meningiomas.

**Effect of Celecoxib Treatment on Meningioma Growth In Vivo**

The results of dose-response experiments comparing the growth rate of IOMM-Lee tumors treated with low-, medium-, and high-dose celecoxib showed a trend toward a dose-dependent growth inhibition response (Fig. 2A). No statistically significant difference in tumor growth inhibition was found between the control and low- and medium-dose celecoxib treatment groups (ANOVA, P > .05), whereas the high-dose group showed statistically significant growth inhibition by Day 43 (ANOVA, P < .01). Doubling time for the IOMM-Lee tumors in the control and high-dose treatment groups was approximately 2 and 4 weeks, respectively. High-dose celecoxib treatment reduced the mean (±SD) tumor volume over control by 66% at 6 weeks (2821 ± 1907 mm³ vs 948 ± 728 mm³, ANOVA, P < .01).
On the basis of the results from the IOMM-Lee dose-response experiments, we repeated the high-dose celecoxib treatment regime in vivo with the CH157-MN line and a benign meningioma mouse xenograft. The CH157-MN tumors in the control and high-dose celecoxib treatment groups doubled in approximately 6 and 7 days, respectively (Fig. 2B). Treatment reduced mean tumor volume by 25% by 6 weeks (3196 ± 257 mm³ vs 2405 ± 1944 mm³, ANOVA, \( P > .05 \)). The benign meningioma in the control and high-dose treatment groups doubled in approximately 2 and 5 weeks, respectively (Fig. 2C). Treatment reduced mean tumor volume by 65% by 5 weeks (5588 ± 3405 mm³ vs 1966 ± 1685 mm³, ANOVA, \( P < .05 \)). Statistically significant differences in tumor growth inhibition were noted between control and high-dose celecoxib treatment groups for the IOMM-Lee and benign meningioma flank tumors.

The IOMM-Lee, CH157-MN, and benign groups each showed 100% tumor induction (55/55, 15/15, and 10/10, respectively). The IOMM-Lee control group had one tumor that regressed in size (5%, 1/20).

Survival curves showed no statistically significant difference in the death rate between control and treated groups (\( \chi^2 \) test, \( P > .05 \), data not shown). Three of 20 control IOMM-Lee mice died, whereas none (0/10) in the low-dose group, none (0/10) in the medium-dose group, and two (2/15) mice in the high-dose celecoxib group died by Day 43. No animals died in the CH157-MN tumor groups. One animal in the control benign meningioma flank tumor group died, and none (0/5) of the high-dose celecoxib group died by Day 35.
Celecoxib Plasma Levels

Celecoxib serum levels obtained on sacrificed animals fed low-, medium-, and high-dose celecoxib mouse chow ad libitum for a minimum of 35 days (i.e., drug levels reflect steady state) showed mean celecoxib plasma values (±SD) of 845 (±267) ng/mL (n = 6), 1540 (±493) ng/mL (n = 5), and 2869 (±828) ng/mL (n = 3), respectively (Fig. 3).

**Immunohistochemical Staining and TUNEL Results**

Immunohistochemistry was performed to analyze proliferative rates (MIB-1), microvascular density (Factor VIII), and COX-2 and VEGF expression in flank tumors treated with celecoxib. Immunohistochemical staining for the MIB-1 cell proliferative marker showed a slight increase in the mean number of cells positive in the IOMM-Lee flank tumors treated with high-dose celecoxib by 6% (211 ± 75 vs 223 ± 64, P = .627) and a significant increase of 14% (190 ± 24 vs 217 ± 23, P = .005) in the CH157-MN tumors (Fig. 4A). Conversely, the benign flank tumors treated with high-dose celecoxib showed a significant decrease in the mean number of MIB-1-positive cells by 21% (233 ± 60 vs 184 ± 61, t-test, P = .024, Fig. 4A,B).

Factor VIII microvascular density analysis showed a decrease in microvascular density of 22% (t-test, P = .278), 77% (t-test, P = .009), and 37% (t-test, P = .015) in the IOMM-Lee, CH157-MN, and benign flank tumors treated with high-dose celecoxib compared with the controls. Mean microvascular densities (±SD) per ×20 hpf for the IOMM-Lee, CH157-MN, and benign flank tumors for control vs high-dose celecoxib treatment were 4.9 (±2.8) vs 3.8 (±0.8), 5.2 (±1.2) vs 1.2 (±0.4), and 5.1 (±1.4) vs 3.2 (±1.5), respectively (Fig. 4B).

The TUNEL apoptosis stain showed that treated groups had 36% (t-test, P = .516), 152% (t-test, P = .002), and 288% (t-test, P = .022) more cells undergoing apoptosis for IOMM-Lee, CH157-MN, and benign flank tumors treated with high-dose celecoxib than did the control groups. Mean TUNEL positive cells (±SD) per ×40 hpf for the IOMM-Lee, CH157-MN, and benign flank tumors for control vs high-dose celecoxib treatment were 1.4 (±1.8) vs 1.9 (±2.0), 1.3 (±1.5) vs 3.2 (±1.9), and 0.8 (±0.9) vs 3.1 (±3.4), respectively (Fig. 4C).

Immunohistochemical staining for COX-2 and VEGF accomplished on IOMM-Lee xenograft tumors showed decreased staining scores with increasing celecoxib dose (Fig. 5A-C). Statistical significance was achieved for both COX-2 and VEGF at high-celecoxib dosing (ANOVA, P < .001).

**DISCUSSION**

The COX-2 enzyme is ubiquitously expressed in meningioma cells and is also present in endothelial cells, macrophages, and stromal tissues. Up-regulation of COX-2 contributes to tumor growth in cancer models by increasing angiogenesis and cellular pro-
liferation, as well as decreasing apoptosis.\textsuperscript{14,22–25} Celecoxib, a selective COX-2 inhibitor, was chosen because of its FDA approval and it has been shown to decrease cancer growth in vitro and in vivo by both COX-2-dependent and COX-2-independent mechanisms, making it difficult to ascertain whether the drug effect is due to direct COX-2 inhibition.\textsuperscript{26,27} Regardless of the mechanism, we have demonstrated that celecoxib decreases meningioma growth in vivo with evidence of decreased microvascular density, increased apoptosis, and decreased COX-2 and VEGF expression. Celecoxib mouse serum levels for the low- and medium-dose regimes were well within reported ranges, whereas the high-dose regime, although achievable, exceeds the recommended dose that would normally be used clinically in humans.

First, we showed that celecoxib eliminates COX-2 activity in vitro, corresponding to a 51\% reduction in PGE\textsubscript{2} levels. Our previous in vitro work showed growth inhibition and apoptosis at similar celecoxib doses; however, it was unclear whether these findings were because of direct-COX-2 inhibition and subsequent reduction in prostaglandins or via other mechanisms.\textsuperscript{15} These results fit with the extensive in vitro data on numerous cell lines showing growth inhibition by selective COX-2 inhibitors.\textsuperscript{15,23,28} Specifically, another study on other brain tumors with the COX-2 inhibitor NS-398 showed inhibition of cell proliferation and migration of the glioma cell lines U-87MG and U-251MG.\textsuperscript{15,29}

We demonstrated a statistically significant decrease in meningioma mouse flank tumor sizes with high-dose celecoxib treatment in 2 of 3 cell lines, with a final mean tumor volume reduction between 25\% and 66\%. A trend toward a dose-dependent response was seen in 1 cell line grown in mice flanks, but it did not reach statistical significance for the low- or medium-dose celecoxib groups. Prophylactically treating mice with high-dose celecoxib for 6 weeks before induction of IOMM-Lee xenograft tumors resulted in decreased tumor induction rates compared with control, low-, and medium-dose celecoxib groups. Prophylactically treating mice with high-dose celecoxib for 6 weeks before induction of IOMM-Lee xenograft tumors resulted in decreased tumor induction rates compared with control, low-, and medium-dose celecoxib groups. Prophylactically treating mice with high-dose celecoxib for 6 weeks before induction of IOMM-Lee xenograft tumors resulted in decreased tumor induction rates compared with control, low-, and medium-dose celecoxib groups. Prophylactically treating mice with high-dose celecoxib for 6 weeks before induction of IOMM-Lee xenograft tumors resulted in decreased tumor induction rates compared with control, low-, and medium-dose celecoxib groups.

![FIGURE 5](image.png)

\textbf{FIGURE 5.} (A) Representative pictures of COX-2 and VEGF immunohistochemical staining of IOMM-Lee xenograft tumors fed low-, medium-, and high-dose celecoxib (500, 1000, and 1500 ppm, respectively) mouse chow ad libitum for 43 days. Magnification is $\times 200$. Decreased COX-2 and VEGF staining intensity was noted as celecoxib dose increased. Arrows identify internal positive controls; both COX-2 and VEGF stain vascular endothelium. (B,C) Weighted index of (B) COX-2 and (C) VEGF staining showing a decrease in score with increasing celecoxib dose. Statistical significance achieved for both COX-2 and VEGF (ANOVA, $^{*}P < .001$).
Studies consistently show that selective COX-2 inhibitors reduce angiogenesis, probably via direct inhibition of the COX-2 enzyme. COX-2 has been shown to regulate angiogenesis primarily through PGE₂, which is a key regulator in stimulating VEGF expression. Furthermore, several in vitro studies show that COX-2 inhibitors down-regulate COX-2 expression. We verify these findings by showing that celecoxib reduced microvascular density in meningioma flank tumors by 23% to 78%. Furthermore, we provide evidence that COX-2 inhibition is diminished in celecoxib-treated meningioma flank tumors. Finally, we observe that VEGF expression is diminished, suggesting that celecoxib ultimately inhibits microvascular proliferation by inhibiting VEGF stimulation. Liu et al. showed that selective COX-2 inhibition suppressed tumor growth through a down-regulation of a VEGF-mediated tumor angiogenesis. Taken together, these results indicate that selective COX-2 inhibitors probably mediate their antiangiogenic effects via direct inhibition of the COX-2 enzyme (ie, COX-2-dependent effect). Although we are unclear whether the antiangiogenic effects in this study are due to direct COX-2 inhibition, we provide evidence to support that this is the case.

Selective COX-2 inhibition has been shown to promote apoptosis in numerous cell lines in vivo and in vitro. In this study, we have shown that celecoxib increases the number of cells undergoing programmed cell death by 36% to 288%. Although the specific antiapoptotic mechanism(s) remain unclear, proposed mechanisms include modulation of the BAX-to-bcl-2 ratio and activation of caspase pathways. Previous in vitro work accomplished in our laboratory has shown no significant change in BAX or bcl-2 expression by Western blot analysis. Thus, it appears that modulation of the BAX-to-bcl-2 ratio is not a factor in driving meningioma apoptosis in vitro, although we have not specifically studied this in vivo.

Celecoxib treatment of flank meningiomas resulted in slight increases in MIB-1 staining of the immortal meningioma cell lines (IOMM-Lee and CH157-MN). Conversely, flank tumors induced by the benign cell line showed a decrease in the number of cells undergoing cell division. This corresponds with the mixed results reported by others. For example, the COX-2 inhibitor NS-398 inhibits cell proliferation and migration of the glioma cell lines U-87MG and U-251MG in vitro, whereas others have shown no change in cell proliferation with selective COX-2 inhibition. Interestingly, because the MIB-1 antibody recognizes the Ki-67 nuclear antigen during G1, S, G2, and M phases of the cell cycle, treatment with drugs that halt cells in G1 or the G1-S transition (beyond G0 but before S phase) can cause a paradoxical increase in labeling while still arresting cell growth. This may explain our findings of increased Ki-67 labeling in the immortal cell lines flank tumors.

The recommended dose for celecoxib in the treatment of rheumatoid arthritis, osteoarthritis, and familial adenomatous polyposis ranges from 200 to 800 mg a day, and for clinical trials doses up to 800 mg a day have been reported. The plasma half-life of celecoxib is 13 hours, with steady state reached at 5 days. Peak plasma levels of approximately 800 ng/mL (approximately 0.8 μg/mL) have been observed after single 200-mg doses in healthy, fasting subjects. Peak plasma levels of 1234 μg/L (approximately 1.2 μg/mL) and 1160 μg/L (approximately 1.2 μg/mL) have been observed an average of 3 hours after 300-mg single dose (mean) and at steady state, respectively, in 11 pediatric patients ages 6 to 15 being treated for solid tumors. Frank et al. analyzed 35 adults taking celecoxib 400 mg daily and reported a plasma concentration steady state of 600.61 ng/mL (approximately 0.6 μg/mL) at 6 weeks. Our in vivo celecoxib inclusion diets ranged from 500 to 1500 ppm with mean plasma levels of 845 ng/mL (approximately 0.85 μg/mL), 1540 ng/mL (approximately 1.4 μg/mL), and 2869 μg/mL (approximately 2.9 μg/mL) for the low-, medium-, and high-dose celecoxib doses, respectively. Therefore, our low- and medium-dose celecoxib diets yield plasma levels within reported human ranges. Plasma levels of the high-dose celecoxib diet, although achievable in humans, would require ingestion of roughly 3 g of celecoxib daily, which is not realistic (Pfizer). Unlike other central nervous system tumors (eg, astrocytomas), meningiomas derive their blood supply primarily from extracranial blood vessels, thus they are located outside the blood-brain barrier. Therefore, intratumor drug concentrations from systemic therapies should mimic serum levels to a greater extent.

Celecoxib significantly inhibits meningioma growth in vivo at high plasma levels in this model. Overall, celecoxib-treated tumors were less vascular and had increased apoptosis than the control. IOMM-Lee tumors treated with celecoxib showed decreased COX-2 and VEGF expression. Interestingly, the tumor lines used in this study were highly aggressive, with areas of necrosis noted on histologic analysis. These findings are consistent with anaplastic meningiomas, perhaps making these findings more applicable to higher-grade tumors. COX-2 inhibitors may have a role in the treatment of recurrent meningiomas.


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