Inflammatory response and meningioma tumorigenesis and the effect of cyclooxygenase-2 inhibitors

**BRIAN T. RAGEL, M.D.,** 1,2 **RANDY L. JENSEN, M.D., PH.D.,** 1,2 **AND WILLIAM T. COULDWELL, M.D., PH.D.,** 1,2

1 Department of Neurosurgery, University of Utah; and 2 Huntsman Cancer Institute, Salt Lake City, Utah

✓ In this article the authors discuss the rationale and research supporting the hypothesis that meningioma tumorigenesis may, in part, be driven by overexpression of cyclooxygenase-2 (Cox-2) and that treatment with celecoxib, a selective Cox-2 inhibitor, may hold therapeutic promise. Because therapies for recurrent or aggressive meningiomas (atypical or malignant subtypes) such as chemotherapy and radiotherapy generally offer little therapeutic benefit, interest in targeting Cox-2 has grown. This rate-limiting enzyme of prostaglandin synthesis can be inhibited with nonsteroidal antiinflammatory drugs (NSAIDs) such as ibuprofen and celecoxib. Treatment with NSAIDs has been shown to curb the tumorigenic properties of prostaglandins in several cancer models via both Cox-2-dependent and -independent mechanisms. In addition, celecoxib is well tolerated in humans, making its use as a chronic therapy for meningiomas attractive. (DOI: 10.3171/FOC-07/10/E7)

**KEY WORDS** • Cox-2 • inflammatory response • meningioma • nonsteroidal anti-inflammatory drug • tumorigenesis •

---

**Cyclooxygenase-2 Expression Promotes Meningioma Formation**

Clinical evidence supports the concept that meningioma formation may be associated with previous head trauma.32–34,45 In vivo and in vitro studies show Cox-2 up-regulation after head trauma, and previous work has shown that meningiomas stain extensively for Cox-2,19,21,36 which is the rate-limiting enzyme in the synthesis of prostaglandins from arachidonic acid.31 Prostaglandins have angiogenic, cell-proliferative, and antiapoptotic properties and are upregulated in a multitude of cancers (for example, breast, prostate, and lung).43 These lines of evidence suggest that meningioma formation may occur in the setting of chronic inflammation triggered by trauma.2

Of the two isoforms of cyclooxygenase, referred to as Cox-1 and Cox-2, Cox-1 is constitutively expressed in most tissues and primarily performs general “housekeeping” functions (for example, cytoprotection of the stomach and platelet aggregation). In contrast, Cox-2 is induced by migratory cells (for example, macrophages, monocytes, and microglia) responding to proinflammatory stimuli and mediates acute and chronic inflammatory states.11,27 The expression of Cox-2 is ordinarily low at baseline but surges in the context of inflammation or neoplasia.6

The head trauma–meningioma relationship may be explained by the development of neoplastic changes in meningeal tissue caused by the inflammatory state of healing and the associated release of prostaglandins and other growth factors (Fig. 1).19,21,32–34 Several studies have shown an increased incidence of meningiomas in patients with

---

**Abbreviations used in this paper:** Cox = cyclooxygenase; FDA = Food and Drug Administration; NSAIDs = nonsteroidal antiinflammatory drugs; VEGF = vascular endothelial growth factor.
previous history of head trauma, with elevated odds ratios ranging between 1.2 and 6.4 and latency periods between 14 and 24 years. In these patients, it is possible that neoplastic changes in meningeal tissue caused by healing, inflammation, and the release of growth factors may act as inciting factors in tumorigenesis.

Nonsteroidal Anti-Inflammatory Drugs and Cancer

The anti-inflammatory action of NSAIDs results from their ability to inhibit Cox enzyme activity, resulting in decreased synthesis of the proinflammatory prostaglandins. The NSAIDs decrease Cox activity through nonselective binding, selective binding, or noncyclooxygenase Cox-2 mechanisms. Among the nonselective inhibitors, aspirin irreversibly inactivates both Cox-1 and -2, whereas ibuprofen and flurbiprofen are reversible inhibitors of Cox-1 and -2 (although they act via different mechanisms; the former competes with arachidonic acid, whereas the latter forms a salt bridge). Among the selective Cox-2 inhibitors, celecoxib (Celebrex) and rofecoxib (Vioxx) are both irreversible inhibitors of Cox-2. Finally, R-flurbiprofen inhibits NF-κB and AP1 activation of Cox-2 transcription. Selective Cox-2 inhibitors are used in cancer treatment studies because of their low side-effect profile and because they have been approved by the US FDA.

Clinically, selective Cox-2 inhibition produces antiinflammatory and analgesic effects without the side effects of gastric ulcers and platelet dysfunction. These characteristics, along with the following findings, suggest that NSAIDs may be used in the prevention of cancer and as adjuncts for treatment:

1) that Cox inhibitors promote antitumor effects in vitro; 2) that Cox inhibitors reduce the size and number of tumors in cancer animal models; 3) that Cox inhibitors reduce the incidence of colon cancer; and 4) that Cox inhibitors cause precancerous lesions (for example, the aberrant crypt foci of colorectal cancer) to regress in cohorts of patients at risk for genetic and sporadic cancers.

Interest in inhibiting Cox-2 with celecoxib in meningiomas specifically stems from the literature showing Cox-2 expression in meningioma tumor samples and from our ability to inhibit this enzyme with NSAIDs. Celecoxib is appealing because it is US FDA approved for the treatment of rheumatoid arthritis, osteoarthritis, and familial adenomatous polyposis, and it is being tracked by the National Cancer Institute in more than 30 Phase I, II, and III cancer trials in the treatment of colon, prostate, liver, lung, breast, and glioblastoma multiforme (physician’s data query at http://www.nci.nih.gov/clinicaltrials/).

Research

Cyclooxygenase-2 Inhibition in Meningiomas

Cyclooxygenase-2 is extensively expressed in meningiomas, with immunohistochemical and Western blot evidence showing cytoplasmic and nuclear localization (Fig. 2). Normal dura and dura adjacent to meningoma tu-
mors, on the other hand, do not stain for Cox-2 (Fig. 2). Because of the selective expression of Cox-2 in meningioma tissue, its previously reported effects on other cancer cells,\textsuperscript{15,46} and FDA approval of celecoxib, this selective Cox-2 inhibitor was chosen for use in growth inhibition studies in meningiomas. Meningiomas treated both in vitro and in vivo with celecoxib showed decreased growth.\textsuperscript{35,36}

In in vitro studies, celecoxib showed a dose-dependent inhibition of meningioma cell growth in a malignant cell line (IOMM-Lee) and in six benign cell lines.\textsuperscript{36} In the IOMM-Lee cell line, this inhibition was associated with abolition of the Cox-2 enzymatic activity and a 51% reduction in prostaglandin E2 (PGE2) levels.\textsuperscript{36} These findings of growth inhibition in vitro coincide with other findings in the literature in which numerous cell lines, including those derived from brain tumors (for example, glioma cell lines U-87MG and U-251MG), show growth inhibition by selective Cox-2 inhibitors.\textsuperscript{14,17,36,42}

After the successful demonstration of inhibition of cell growth in meningioma cell lines in vitro, a mouse meningioma flank tumor model was used to show a statistically significant decrease in tumor size with high-dose celecoxib treatment in two of three cell lines, with a mean tumor volume reduction between 25 and 66% (Fig. 3).\textsuperscript{35} Interestingly, prophylactic treatment of mice with high-dose celecoxib for 6 weeks before tumor xenografting with a malignant meningioma cell line also resulted in a decreased tumor induction rate compared with the control, low-, and medium-dose groups (80% compared with 100%).\textsuperscript{35} Removal of celecoxib resulted in the return of tumor growth to baseline tumor growth rates, suggesting a growth-suppressive effect (Fig. 3). These findings agree with the results reported in other in vivo solid tumor studies (for example, colorectal, prostate, lung, squamous cell carcinoma, and breast) showing decreased tumor size with celecoxib treatment.\textsuperscript{8,12,18,20,38,39,47}

Analysis of tumors grown in mice and treated with high-dose celecoxib showed decreased microvascular density (by 23 to 78%) and diminished Cox-2 and VEGF staining (Fig. 4), as well as increased apoptosis.\textsuperscript{35} Diminished Cox-2 and VEGF staining implies that celecoxib ultimately inhibits microvascular proliferation by inhibiting VEGF. This is supported by numerous studies showing that selective Cox-2 inhibition reduces blood vessel density, probably through direct inhibition of Cox-2 and downregulation of VEGF-mediated angiogenesis.\textsuperscript{7,11,23,30,42,45} These results indicate that the decreased tumor vascularity shown in celecoxib-treated meningiomas is probably a result of direct inhibition of the Cox-2 enzyme with diminished VEGF expression (that is, the Cox-2-dependent effect).\textsuperscript{35} Furthermore, celecoxib increased the number of apoptotic cells in meningioma flank tumors by 36 to 288%.\textsuperscript{35} These findings are supported by other studies with selective Cox-2 inhibitors, showing increased apoptosis in multiple cell lines in vivo and in vitro.\textsuperscript{5,9,16,45,46}

In the reported studies, celecoxib doses ranged from 500

---

**Fig. 2.** Photomicrographs showing expression of Cox-2 in human colon cancer (A) and human meningiomas (B–F). Positive immunoreactivity appears as reddish-brown staining. A: Positive control. Strong Cox-2 immunoreactivity seen in the cytoplasm of colonic carcinoma cells (solid arrow). Note that vascular endothelium stains positively for Cox-2, as described previously in Maihöfner et al. (open arrow). B: Negative control. No staining was noted when slides were incubated with serum only. C: Normal dura mater showing staining of vascular endothelium (open arrow), as well as meningioma abutting normal dura (solid arrows). D–F: Strong Cox-2 immunoreactivity noted diffusely throughout cytoplasm of meningioma (solid arrows). Note that monocyte or macrophage within blood vessel stains positive as described previously (open arrow in D). Original magnification ×10. Modified from Ragel, 2005.
to 1500 ppm, which yielded mean plasma levels of 845 ng/ml (~0.85 μg/ml), 1540 ng/ml (~1.4 μg/ml), and 2869 μg/ml (~2.9 μg/ml) for the low-, medium-, and high-dose celecoxib doses, respectively. The peak plasma levels in various human studies of celecoxib have been reported from 0.6 μg/ml to approximately 1.2 μg/ml at recommended dosages between 200 and 800 mg a day (for the treatment of rheumatoid arthritis, osteoarthritis, and familial adenomatous polyposis). In clinical cancer trials this dose is as high as 800 mg a day. Thus, the plasma levels achieved from the low- and medium-dose celecoxib diets were within reported human ranges (Fig. 3). Although the mice on the high-dose diet achieved a plasma concentration achievable in humans, it would require ingestion of roughly 3 g of celecoxib daily (Pfizer, personal communication). Thus, although the findings in this study indicate

FIG. 3. Graphs showing that celecoxib (cele) inhibits the growth of meningioma xenograft tumors. A: The IOMM-Lee flank tumors show a dose-dependent growth inhibition with increasing doses of celecoxib. Control mice were fed regular mouse chow. Treated mice were fed low-, medium-, or high-dose celecoxib diets (500, 1000, and 1500 ppm, respectively). No statistically significant difference was found between the control and low-dose (p > 0.05, analysis of variance [ANOVA]) or medium-dose (p > 0.05, ANOVA) celecoxib treatment groups, whereas a statistically significant difference between the control and high-dose celecoxib groups was noted by Day 43 (p < 0.05, ANOVA). Error bars represent 20, 10, 10, and 15 mice for the control, low- and medium-, and high-celecoxib groups, respectively. B: Benign meningioma from operative specimen treated with control or high-dose celecoxib. A statistical difference was noted between groups by Day 43 (p < 0.05, ANOVA). Error bars represent five mice. C: Effects of prophylactically treating mice with low-, medium-, and high-dose celecoxib for 6 weeks before induction of IOMM-Lee xenograft tumors. A dose-dependent tumor growth inhibition is exhibited between control and pretreatment groups (p < 0.05, ANOVA). Error bars represent five mice. The high-dose celecoxib group was changed to regular mouse chow 31 days after IOMM-Lee flank injection (arrow) and divided into groups of animals without palpable tumors (two animals, –TU) and animals with flank tumors (three animals, +TU). The animals without palpable tumors remained tumor free, whereas in animals with tumors, the tumors started to grow at a rate similar to that of control tumors earlier in the study. Error bars after arrow represent at least two mice. D: Celecoxib serum levels in animals fed low-, medium-, and high-dose celecoxib (500, 1000, and 1500 ppm, respectively) mouse chow ad libitum for a minimum of 35 days (drug levels reflect steady state). Mean celecoxib plasma values ± standard deviation for low-, medium-, and high-dose celecoxib are 845 ± 267, 1540 ± 493, and 2869 ± 828 ng/ml, respectively. *p < 0.05, ANOVA (statistically significant). Modified with permission from Ragel et al., 2007.
that celecoxib will be beneficial in the treatment of meningiomas, alternative ways of exploiting its effects should be investigated. Future studies will be used to examine celecoxib as a radiation sensitizer and in combination with other systemic therapies (for example, hydroxyurea).

**Conclusions**

Recurrent meningiomas, particularly those classified as aggressive, currently lack many successful treatment options; however, studies suggest that Cox-2 may offer a therapeutic target that can be inhibited by NSAIDs. Treatment with NSAIDs has been shown to curb the facilitation of tumor properties of Cox-2 in other cancers via several mechanisms. Thus, investigations of Cox-2 inhibitors may reveal benefit for the treatment of recurrent meningiomas. Studies have shown that celecoxib significantly inhibits meningioma growth in vivo at high plasma levels in a meningioma mouse model. The meningioma cell lines used in these studies showed aggressive growth with areas of necrosis on histological analysis, findings consistent with higher-grade meningiomas. Therefore, these Cox-2 inhibition results may be more applicable to higher-grade tumors and demonstrate that Cox-2 inhibition may play a role in the treatment of recurrent meningiomas.

**Acknowledgments**

We thank Kristin Kraus for her excellent editorial assistance and Kelly Johnson for designing the figures.

**References**

44. Ware ML, Larson DA, Sneed PK, Wara WW, McDermott MW: Surgical resection and permanent brachytherapy for recurrent atypical and malignant meningioma. Neurosurgery 54:55–64, 2004

B. T. Ragel, R. L. Jensen, and W. T. Coldwell

Manuscript submitted July 12, 2007. Accepted August 1, 2007.

This work was supported by a grant from the American Association of Neurological Surgeons Neurosurgery Research and Education Foundation to Brian Ragel.

Address correspondence to: William T. Coldwell, M.D., Ph.D., Department of Neurosurgery, University of Utah, 175 North Medical Drive East, Salt Lake City, Utah 84132. email: william.coldwell@hsc.utah.edu.