Enhancing Radiotherapy With Cyclooxygenase-2 Enzyme Inhibitors: A Rational Advance?

Hak Choy, Luka Milas

Results of preclinical studies suggesting that the efficacy of molecular therapies is enhanced when they are combined with radiation have generated a surge of clinical trials combining these modalities. We reviewed the literature to identify the rationale and experimental foundation supporting the use of cyclooxygenase-2 (COX-2) inhibitors with standard radiotherapy regimens in current clinical trials. Radiation affects the ability of cells to divide and proliferate and induces the expression of genes involved in signaling pathways that promote cell survival or trigger cell death. Future advances in radiotherapy will hinge on understanding mechanisms by which radiation-induced transcription of genes governs cell death and survival, the selective control of this process, and the optimal approaches to combining this knowledge with existing therapeutic modalities. COX-2 is expressed in all stages of cancer, and in several cancers its overexpression is associated with poor prognosis. Evidence from clinical and preclinical studies indicates that COX-2–derived prostaglandins participate in carcinogenesis, inflammation, immune response suppression, apoptosis inhibition, angiogenesis, and tumor cell invasion and metastasis. Clinical trial results have demonstrated that selective inhibition of COX-2 can alter the development and the progression of cancer. In animal models, selective inhibition of COX-2 activity is associated with the enhanced radiation sensitivity of tumors without appreciably increasing the effects of radiation on normal tissue, and preclinical evidence suggests that the principal mechanism of radiation potentiation through selective COX-2 inhibition is the direct increase in cellular radiation sensitivity and the direct inhibition of tumor neovascularization. Results of current early-phase studies of non–small-cell lung, esophageal, cervical, and brain cancers will determine whether therapies that combine COX-2 inhibitors and radiation will enter randomized clinical trials. [J Natl Cancer Inst 2003;95:1440–52]

The rapid expansion of knowledge of the molecular biology and genetics of cancer has resulted in the genesis of recent heralded advances in cancer treatment. The swift clinical acceptance of the first molecular agents that have successfully completed development has validated molecular therapeutics as a new cancer treatment modality and confirmed the potential promise of agents under development. It is already evident, however, that the full benefits of these new therapeutics will be realized only when their use is optimally integrated with other therapeutic modalities—cytotoxic drugs, radiation, hormonal agents, and biologics—and other molecular therapeutics in a rational, multi-targeted approach to cancer treatment.

Radiotherapy in combination with surgery and chemotherapy has improved treatment for patients with localized and locally advanced cancers. For example, radiotherapy may facilitate tumor removal by permitting more complete or less radical surgery and may help ensure the elimination of cancer cells following surgery. In addition, radiotherapy can be combined with certain chemotherapeutic agents to increase the radiation sensitivity of cancers and to eliminate occult tumor cells located beyond the effective radiation field. Combined-modality therapy has produced moderate improvements in the therapeutic outcome for several cancers, including those of the breast and colon, non–small-cell lung and esophageal cancers, and head-and-neck squamous cell carcinoma.

Results of preclinical investigations demonstrating that molecular therapies enhance the effect of radiation have generated a surge in the number of clinical trials designed to evaluate combined-modality therapy. Since the early 1980s it has been known that inhibiting the production of prostaglandins, hormone-like substances that control blood pressure, muscle contractions, and inflammation, potentiates radiation responses in irradiated tissues. However, it has only been within the past decade that the existence of an inducible form of the key enzyme in prostaglandin synthesis, cyclooxygenase 2 (COX-2), has been appreciated. Studies conducted in experimental models have shown that selectively blocking COX-2 activity is associated with the antitumor effects of radiation without enhancing the effects of radiation in normal tissues. These experimental findings are now being tested in clinical trials as a particularly promising approach to combined-modality therapy.

Here we review the English-language literature with respect to studies of the influence of prostaglandins on tissue radiation response, and we propose a potential mechanism by which enhancement of radioreponse in tumors occurs through inhibition of prostaglandin production. Our objective is to present the experimental foundation supporting current clinical trials that combine standard radiotherapy or radiochemotherapy regimens with COX-2 inhibition for the purpose of stimulating interest and participation in these trials. We searched the entire MEDLINE database for articles on prostaglandin responses to radiation published since 1992 and references contained therein. We also searched the MEDLINE database for references to cyclooxygenase and to cyclooxygenase and radiation in the published pro-
proceedings of the annual meetings of the major cancer research organizations for 1998 through 2003.

**CELLULAR EFFECTS OF RADIATION**

When therapeutic gamma radiation and x-rays interact with biologically important molecules, either directly or indirectly through oxygen-containing radicals generated from water, the principal molecular target is DNA and the net effect is on the ability of cells to proliferate. Exposure to radiation also induces expression of genes whose products are involved in internal signaling pathways that either promote cell survival or trigger cell death (1), and it is the balance between expression of factors that promote survival and those that promote cell death that determines the immediate fate of the irradiated cell (2,3). Radiation resistance, particularly resistance to low-dose radiation, is influenced by cellular resistance to the activation of apoptosis-signaling pathways and subsequent commitment to cell death (4,5). Other genes whose expression is activated by radiation are those that encode angiogenic growth factors (i.e., vascular endothelial growth factor [VEGF]) and basic fibroblast growth factor [bFGF] and platelet-derived growth factor [PDGF]), which contribute to both short- and long-term vascular events that are associated with radiation exposure (6–9); those that encode proteins that control cell cycling (i.e., cyclins and p27); and those whose products control the production of growth factors, signaling proteins, and regulators of gene transcription (10). Advances in the precise focus and concentration of radiation to tumor tissue are reaching a practical limit. Future improvements in the therapeutic index for radiotherapy will likely depend on increasing the sensitivity of tumor cells to radiation and reducing the effects of radiation on normal tissues. Advances in radiotherapy will hinge upon our understanding of the mechanisms of radiation-induced transcription of genes governing cell death and survival and the mechanisms that selectively control this process and our ability to translate this knowledge into optimal combinations of radiation and other therapeutic modalities (11).

**PROSTAGLANDINS AND RADIATION**

The indirect effects of ionizing radiation (i.e., those not related to direct effects on DNA) principally involve the effects of oxygen-containing free radicals on membrane lipids and membrane transport, which may lead to radiation-induced edema and cell death even in the absence of nuclear damage (2,9). Ionizing radiation activates cytoplasmic phospholipase A2, triggering the release of arachidonic acid from membrane phospholipids and subsequent production of eicosanoids through the cyclooxygenase and lipoygenase pathways.

Eicosanoids are biologically active lipid molecules (i.e., prostaglandins, thromboxanes, and leukotrienes) that mediate the diverse responses of the affected cell and neighboring cells to radiation (9,12,13). Eicosanoids affect the immune, vascular, and coagulation systems and regulate cell growth and differentiation. Most cells produce a variety of eicosanoids that may have complementary or antagonistic activities. For example, prostaglandin E2 (PGE2) is a potent vasodilator and is immunosuppressive; prostaglandin F2α (PGF2α) is a potent vasoconstrictor, PGD2 is a vasodilator and inhibitor of platelet aggregation, and thromboxane A2 (TXA2) is a platelet aggregator and vasoconstrictor (13).

In many tissues, radiation exposure is associated with an increase in eicosanoid production. Within hours after irradiation, increased levels of prostaglandins and thromboxanes—prostaglandin E1 (PGE1), PGE2, PGF2α, PGD2, TXA2, and TXB2—are detectable in most tissues, and the increased eicosanoid levels may persist for several days or weeks (14–24). Although in vitro findings have been inconsistent, data indicate that the eicosanoids produced in response to radiation, particularly prostaglandins, are radioprotective for normal cells, but only when administered before irradiation. For example, in studies with cultured cells, an analogue of PGE2 given 2 hours before 10 Gy of radiation (137Cs) was associated with an increase in the proportion of surviving normal fibroblasts (25). In animal models, treatment with various eicosanoids 1–2 hours before radiation consistently conferred protection to the normal gastrointestinal tract (26–29), bone marrow (30), and hair follicles (31).

In experimental and clinical studies with prostaglandin synthesis inhibitors such as indomethacin, the therapeutic index of radiotherapy was improved in that the antitumor effects or radiation were enhanced without substantially enhancing its effects on normal tissues. For example, in a mouse model of fibrosarcoma, daily treatment with indomethacin before radiotherapy was associated with improvements in responses to both single- (32) and fractionated-dose (33) radiotherapy. Indomethacin treatment was associated with prolonged delays in tumor growth, an increased rate of cure, and increased time to recurrence with little or no change in the radiosensitivity of normal tissues. The radiation-enhancing effect of indomethacin appears to require a functioning immune system, because mice with an immune system compromised either genetically or by whole-body irradiation showed less radiation enhancement with indomethacin treatment than tumor-bearing mice with intact immune systems. These findings are consistent with and supported by other studies reporting that indomethacin treatment is associated with both the reversal of the immunosuppressive effects of radiation (34–37) and the radioprotection of hematopoietic cells (32,38,39).

Indomethacin and other nonsteroidal anti-inflammatory drugs (NSAIDs) also have direct antitumor effects that have been demonstrated preclinically in numerous systems (41) and clinically in one randomized controlled trial (40). In the clinical study, 135 patients with a variety of metastatic solid tumors were randomly assigned to receive treatment with indomethacin, prednisolone, or placebo. Patients treated with indomethacin survived statistically significantly longer (P<.05) than placebo-treated patients, and pooled observations from patients on anti-inflammatory treatment (indomethacin group plus prednisone group) revealed a significantly prolonged survival compared with placebo-treated patients (P<.03) (Fig. 1) (40). Furuta and colleagues (41) reported that in murine tumor models the tumor growth-inhibiting effects of indomethacin were seen only against prostaglandin-producing tumors, indicating that inhibition of prostaglandin synthesis was likely related to the antitumor activity. In addition, they reported that the direct antitumor activity of indomethacin was not affected by the immunogenicity of the tumor or by host immunocompetence but was associated with the inhibition of the neovascularization that is required for tumor growth (39). Thus, potential mechanisms for the radiation-enhancing effects of NSAIDs include the reversal of prostaglandin-induced immunosuppression and the direct inhibitory effects of NSAIDs on tumor neovascularization independent of any radiation-induced response.
Results of two trials extended these findings to cancer patients receiving radiation therapy. In a randomized study of 160 women undergoing radiotherapy for carcinoma of the cervix, 76 women received radiation and daily treatment with the prostaglandin synthesis inhibitor oxyphenbutazone and 84 women received radiation alone. The women treated with combined-modality therapy had 5- and 10-year survival rates of 70% and 62%, respectively; whereas women treated with radiotherapy alone had rates of 55% and 44%, respectively (42). In a small comparative study (43) of 19 patients with head-and-neck cancer, patients were treated with daily radiation (3 Gy/day up to a total dose of 62 Gy) or indomethacin (25 mg, four times a day) plus radiation. Combination therapy was associated with a delay in the appearance of radiation-induced mucositis (grades 1 to 3). For grade 3 mucositis, the delay favoring the patients treated with combination therapy was statistically significant.

**COX-2 Expression and Tumorigenesis**

Despite evidence that indomethacin and other NSAIDs enhance tumor responses to radiation, clinical studies combining NSAIDs and radiation therapy have been few. One reason for this has been the risk of gastrointestinal toxicity associated with regular NSAID use (44–48). The development of COX enzyme inhibitors with less potential for gastrointestinal toxicity is stimulating the investigation of combined therapy with COX inhibitors and radiation.

Cyclooxygenase, also called prostaglandin H (PGH) synthase and prostaglandin endoperoxide synthase, is the rate-limiting enzyme in the conversion of membrane-derived arachidonic acid to prostaglandin H2 (PGH2). PGH2 is the common precursor that isomerases convert into the various prostaglandins and TXA2 (Fig. 2) (49). Cyclooxygenase exists in two forms, COX-1 and COX-2 (50–53). NSAIDs inhibit the activities of both COX-1 and COX-2, whereas selective COX-2 inhibitors only inhibit COX-2 activity. COX-1 inhibition is believed to cause the adverse effects of NSAIDs on the upper gastrointestinal tract (54–57). Details of the biochemistry and molecular biology of the COX-1 and COX-2 isoforms have been previously reviewed (58–61).

There is considerable evidence that the COX-2 isoform contributes to carcinogenesis and tumor growth. Cellular expression of COX-2 is elevated above normal in the earliest stages of carcinogenesis and through tumor development and invasive tumor growth. COX-2–derived prostaglandins participate in carcinogenesis, inflammation, immune response suppression, apoptosis inhibition, angiogenesis, and tumor cell invasion and metastasis [reviewed in (62)]. The role of COX-2–derived prostaglandins in human carcinogenesis is supported by retrospective and epidemiologic findings suggesting that regular NSAID use reduces the incidence of certain human cancers (particularly those of the breast, colon, and lung) [reviewed in (63)]. Experimental studies in at least one model have shown that COX-2 over-expression by itself is sufficient to induce formation of mammary tumors (64). Specifically, in transgenic mice engineered to express human COX-2 in their mammary glands, low levels of human COX-2 were expressed in the mammary glands of virgin mice but increased substantially during pregnancy and lactation. In virgin transgenic mice and multiparous wild-type (i.e., nontransgenic) mice, mammary tumors were rarely observed. By contrast, multiparous transgenic animals displayed a high incidence of focal mammary hyperplasia and dysplasia, and at least one tumor was observed in mammary tissue from 85% of the multiparous transgenic mice. Additional evidence of a role for COX-2 in the development of cancer was seen with selective COX-2 inhibition in preclinical studies and in clinical trials in patients with familial adenomatous polyposis (FAP). FAP is a heritable condition characterized by the formation of multiple colonic polyps in very young patients and a nearly 100% prob-
ability of a polyp developing into colon cancer. Two mouse models of FAP, both having alterations in the Adenomatous polyposis coli (Apc) gene, which is mutated in FAP patients, have been studied: the Apc\(^{–/–}\) knockout mouse and the multiple intestinal neoplasia (Min) mouse. Studies in these models provided the experimental evidence that COX-2 activity contributes to colon tumorigenesis. Oshima and colleagues evaluated the importance of COX-2 activity in the development of polyps in Apc\(^{–/–}\) mice by creating a double knock-out model. They introduced a knock-out mutation in the COX-2 gene in Apc\(^{–/–}\) knockout mice. These mice, lacking both COX-2 (COX-2\(^{–/–}\)) and Apc gene function, developed 86% fewer intestinal polyps compared with the positive control littermate Apc\(^{–/–}\) mice with intact COX-2 (COX-2\(^{+/+}\)). Interestingly, Apc\(^{–/–}\) mice heterozygous for COX-2 (COX-2\(^{+/–}\)) produced an intermediate amount of COX-2 and generated 66% fewer intestinal polyps than the positive control mice, providing evidence of a quantitative relationship of COX-2 expression and formation of intestinal polyps in this model (65). Jacoby and coworkers (66) examined the effects of selective COX-2 inhibition on tumor development in Min mice, which have an autosomal dominant heterozygous mutation in the Apc gene and produce large numbers of intestinal polyps. Treatment with a selective COX-2 inhibitor, celecoxib, reduced the total tumor load (a reflection of both tumor number and volume) by 85% compared with untreated Min mice (66). The results of these experiments clearly indicated a role for COX-2 in polyp formation and colon cancer carcinogenesis. In the Jacoby et al. study (66), tumor number decreased to the same extent regardless whether the mice were treated with a selective COX-2 inhibitor, celecoxib, or the non-specific NSAID, piroxicam. However, piroxicam administration was associated with ulcers, bleeding, and perforation of the gastrointestinal tract. Results in the murine models were translated in a randomized clinical trial to patients with FAP. Treatment with the selective COX-2 inhibitor celecoxib produced a statistically significant reduction in the number of polyps (28% reduction versus placebo, \(P = .003\)) in 30 FAP patients treated with 400 mg oral celecoxib twice daily for 6 months (67). Side effects were similar in patients receiving placebo or celecoxib (either 100 mg or 400 mg two times a day). This trial established the ability of celecoxib to reduce polyp burden in FAP patients and its use was approved for this indication by the U.S. Food and Drug Administration.

**ROLE OF COX-2 IN CANCER PROGRESSION**

In addition to its role in carcinogenesis, elevated expression of COX-2 is also associated with the progression of established human cancer. Tumor cells and the cellular components of the tumor stroma (i.e., infiltrating macrophages, lymphocytes, fibroblasts, and endothelial cells) produce COX-2, which in turn increases production of various prostaglandins, most commonly PGE\(_2\). Elevated COX-2 expression has been reported in a broad range of human cancers, including at least 80% of cancers of the breast, colon, esophagus, liver, lung, pancreas, prostate, cervix, and head and neck (Table 1) (68–113). Although there is often broad variation in expression among tumors that overexpress COX-2, overexpression is generally associated with a more ma-

<table>
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<td>Colorectal</td>
<td>60–100</td>
<td>Tumor grade and stage; overall survival; extent of angiogenesis.</td>
<td>Soslow et al. (68), Zhang and Sun (69), Eberhart et al. (70), Mastferrera et al. (71), Sheehan et al. (72), Gianchi et al. (73)</td>
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<td></td>
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<td>Overexpression highest in metastases.</td>
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<td>Esophageal</td>
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<td>Tumor grade; overall survival. Highest in recurrent tumor and metastases. Higher in adenocarcinoma than squamous.</td>
<td>Buskens et al. (77), Morris et al. (78), Zimmermann et al. (79), Wilson et al. (80), Ratnasige et al. (81)</td>
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<td>Pancreatic</td>
<td>31–90</td>
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<td>Lung adenocarcinoma</td>
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<td></td>
<td></td>
<td>Overexpression highest in metastases.</td>
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<tr>
<td>Squamous cell lung</td>
<td>20–100</td>
<td>Tumor grade; overall survival in patients with stage I disease.</td>
<td>Wolff et al. (89), Solosov et al. (68), Hida et al. (90), Mastferrera et al. (71)</td>
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<tr>
<td>Small-cell lung</td>
<td>Negligible-weak</td>
<td>None</td>
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<td>Bladder</td>
<td>31–75</td>
<td>Tumor grade; COX-2 expression may correlate with invasive disease.</td>
<td>Shirahama (97), Bosstrom et al. (98), Ristimaki et al. (99), Mohammed et al. (100), Komhoff et al. (101)</td>
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<td>Breast</td>
<td>29–89</td>
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<td>Cervical</td>
<td>28–100</td>
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<td>Head-and-neck</td>
<td>100</td>
<td>None</td>
<td>Chan et al. (112)</td>
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<tr>
<td>Melanoma</td>
<td>68</td>
<td>None</td>
<td>Denkert et al. (113)</td>
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*PIN = prostatic intraepithelial neoplasia; BPH = benign prostatic hyperplasia.*
lignant phenotype. In several cancers, COX-2 overexpression is associated with aggressive tumor behavior, worse prognosis (72,79,80,89–91,114,115), and the development of metastatic disease (72,116).

Selective COX-2 inhibitors exhibit antitumor activity against tumors in several animal models. In mice bearing established human colon tumor xenografts derived from HT-29 cells, celecoxib treatment was associated with the inhibition of tumor growth by up to 67% and a reduction in the development of lung metastases by as much as 91% compared with untreated mice (71). Pathologic examination of the gastrointestinal tracts of the treated mice showed no gross abnormalities. Selective COX-2 inhibitors have also demonstrated antitumor activity against murine mammary cancer (117), canine bladder cancer (118), and human head-and-neck cancer xenografts in mice (119).

COX-2 AND TUMOR ANGIogenesis

An important, and possibly the principal, contribution of COX-2 to tumor growth may be its role in stimulating tumor neovascularization (i.e., angiogenesis). Angiogenesis is essential for expansive tumor growth, because a tumor cannot grow beyond the limits of its blood supply (120,121). Tumor angiogenesis involves the proliferation of endothelial cells in vessels adjacent to the tumor, the subsequent migration of those cells toward the tumor and their formation into tube-like vessels, and finally the maturation of the vessels with the incorporation of a basement membrane and covering of pericytes and smooth muscle cells. These processes are driven by growth factors produced by tumor cells, endothelial cells, fibroblasts, and tumor-infiltrating inflammatory cells. The most potent angiogenic growth factor in tumor angiogenesis is VEGF. VEGF production by tumor cells and the extent of tumor vascularization (measured as microvessel density in the tumor) are good prognostic indicators for response to therapy and survival in many types of cancer (120,121).

COX-2–derived prostaglandins stimulate production of angiogenic growth factors (122–124), and several studies (73,125,126) have reported associations between COX-2 expression in human cancers and levels of VEGF production and tumor microvessel density. Selective inhibition of COX-2 activity in several animal models is associated with decreases in VEGF production in tumors (100,119,126), decreases in new vessel formation (127–130), and increases in tumor cell apoptosis (96,100,127). Direct evidence that COX-2 stimulates angiogenesis was obtained in the rat corneal model: A basic fibroblast growth factor (bFGF)–containing pellet implanted in the rat cornea induces a strong neovascularization response accompanied by corneal thickening and an expanded stroma filled with COX-2–expressing fibroblasts, endothelial cells, and macrophages (131). Although the bFGF-induced neovascularization contained many COX-2–expressing endothelial cells, no such cells were seen in the established limbic vessels in the corneal tissue sections. PGE2 and TXB2 expression was increased in the angiogenic cornea, compared with the normal cornea. Compared with untreated angiogenic corneal tissue, oral celecoxib (30 mg/kg per day) inhibited the angiogenic response of angiogenic corneal tissue by 78.6%, reduced PGE2 production by 78%, reduced TXB2 production by 68%, and induced endothelial cell apoptosis in the corneal microvessels (131).

Much of the COX-2 stimulating tumor angiogenesis appears to be derived from host stromal tissue. The contribution of host-derived COX-2 to tumor growth was investigated in mice genetically deficient in COX-2 production (COX-2−/− mice) by Williams and colleagues (125). They reported that the growth of tumors derived from Lewis lung carcinoma cells was markedly attenuated in COX-2−/− mice compared with COX-2+/+ (i.e., wild-type) mice. Tumors in the COX-2−/− mice had a 30% reduction in vascular density compared with tumors in the wild-type mice, and VEGF production by stromal fibroblasts in the COX-2-deficient mice was reduced by 94% compared with that in the wild-type mice. Wild-type mice treated with the COX-2–selective inhibitor celecoxib also showed reduced tumor growth and 92% reduction in VEGF production by stromal fibroblasts, compared with tumors in untreated wild-type mice, confirming the role of COX-2 in promoting angiogenesis and tumor growth in this model. Additional evidence that stimulation of tumor vascularization is an important, and possibly the principal, contribution of COX-2 to tumor growth has also been seen in other studies (71,123,127,132).

COX-2 EXPRESSION AND RADIOTherapy

Earlier findings that prostaglandin synthesis is increased in tumor cells and cancer tissues following irradiation and that inhibiting prostaglandin synthesis with NSAIDs can potentiate the antitumor effects of irradiation in vivo provided the rationale for conducting studies to investigate the role of COX-2 in tumor responses to radiation and the potential benefit of combining a selective COX-2 inhibitor with radiation. Many in vivo studies have reported the radiation-potentiating effects of selective COX-2 inhibition. For example, treatment of mice with the selective COX-2 inhibitor SC-236 was associated with a dramatic enhancement of the radiation response of two murine fibrosarcomas, NFSa and FSA (133,134), and a human U251 glioma xenograft (135), confirming earlier findings with indomethacin (32). In these studies, Milas and colleagues (133) administered oral SC-236 (6 mg/kg) daily for 10 days to mice bearing NFSa-derived fibrosarcomas beginning after the tumors reached 6 mm in diameter, and tumors were irradiated when they reached 8 mm in diameter. Radiation sensitizing ratio was calculated for tumor growth delay from the tumor growth rates in SC-236–treated and untreated mice and for tumor cure by comparing the radiation doses required to cure 50% of the SC-236–treated and untreated animals. For the NFSa tumors, the enhancement factor was 3.64 for growth delay and 1.77 for tumor cure (Figs. 3 and 4) (133). By contrast, in the same tumor model, the enhancement factors for growth delay and tumor cure for indomethacin were 1.4 and 1.26, respectively, suggesting that the selective COX-2 inhibitor SC-236 was associated with greater radiation potentiation than the prostaglandin synthesis inhibitor indomethacin (32). Treatment with SC-236 before irradiation was also associated with enhanced radiation-induced growth delay of FSA sarcoma and U251 glioma tumors (134,135). In studies with the FSA sarcoma, no substantial increase in radioreponse was seen in normal tissue, suggesting that selective COX-2 inhibition combined with radiotherapy was associated with a true therapeutic gain (134). In both studies with murine fibrosarcomas (133,134), the response was associated with reduced tumor neovascularization in the SC-236–treated tumors, rather than with enhancement of radiation-induced apoptosis.

Investigators at Vanderbilt University (Nashville, TN) studied the selective COX-2 inhibitor NS-398 in nude mice bearing tumors derived from human NCI-H460 lung cancer cells and...
showed that the ability of a COX-2 inhibitor to enhance the effects of radiation on tumors was associated with the ability of the tumors to produce COX-2. NS-398 (at 36 mg/kg) enhanced the growth-inhibiting effects of radiation in NCI-H460 human lung tumors in nude mice by a factor of 2.5, but not those in HCT-116 human colon tumor xenografts that lacked COX-2, compared with untreated tumor-bearing control mice (136).

An association between COX-2 expression and responses to radiation has also been seen in patients with cancer of the cervix or breast. In patients with invasive cervical cancer treated with radiotherapy, 5-year survival was 35% in those with high COX-2-expressing cervical cancers, compared with 75% in those whose tumors produced no or low levels of COX-2 (Fig. 5) (137). In patients with locally advanced breast cancer treated with twice-weekly paclitaxel and radiation, a 30-fold lower expression of COX-2 was seen in the tumors of the 7 of 21 patients who achieved a complete pathologic response than in those patients that did not have a complete response (138). These studies suggest that COX-2 level in the tumor may predict response to radiotherapy and raise questions about how the COX-2 status of the tumor affects tumor radiosensitivity.

**MECHANISMS OF RADIATION POTENTIATION BY COX-2 INHIBITORS**

Mechanisms of radiation potentiation through inhibition of COX-2 activity are difficult to define because of the paucity of such studies and their apparently conflicting results. Contributions to this problem are the potentially antagonistic effects of individual prostaglandins and the possibility that NSAIDs and selective COX-2 inhibitors have distinct activities and thus may have effects independent of COX-2 inhibition (139,140). In addition, most of the in vivo and in vitro studies have been conducted with long-established tumor cell lines or genetically modified cells that may be atypical in their behavior, especially with regard to their resistance to apoptosis induction, a potential determinant of radiosensitivity (1,4,5).
Increased COX-2 Expression Following Irradiation

Exposure to ionizing or ultraviolet radiation increases the in vitro expression of COX-2 and the synthesis of prostaglandins in normal and tumor cells (14–16,20,135,141,142). In tumor cells, these increases may be a protective response to counter the cytotoxic effects of radiation and can be blocked with nonselective as well as selective inhibitors of COX activity, thereby increasing the radiation sensitivity of the cells (137). The apoptosis-inhibiting activity of COX-2-derived prostaglandins has been described in several studies carried out in different experimental systems (135,143). Prostaglandins may contribute to repair of sublethal DNA damage caused directly or indirectly by radiation, and inhibiting this activity may enhance radiosensitivity (135,143). Alternatively, or in addition, COX-2 inhibition of cell cycling, mediated indirectly through effects on regulatory cyclins and cyclin-dependent kinases, may arrest the cells in G2M, the most radiosensitive phase of the cell cycle. This cell cycle arrest has been seen in some model systems (32,143) but not in others (135) and, thus, may vary among different types of cells.

Induction of Apoptosis

Radiation potentiation by COX-2 may involve effects on p53. Cells with irreparably damaged DNA activate p53 and die through the controlled process of apoptosis regulated in part by p53 activity. Han and colleagues (144) investigated potential functional interactions between COX-2 and p53 in several human and murine cell lines. They found that p53-induced apoptosis was enhanced greatly in COX-2 knockout cells, compared with cells expressing wild-type levels of COX-2, suggesting that COX-2 has an inhibitory effect on p53-induced apoptosis. Furthermore, they found that apoptosis induced by DNA damage in p53+/+ normal cells was enhanced in cells treated with the selective COX-2 inhibitor NS-398 compared with untreated cells. These results suggest that COX-2 expression increases in response to genotoxic stresses that induce p53 expression, that COX-2 expression protects cells from apoptosis in response to stresses that induce p53 expression, and that this protection can be reversed by inhibiting COX-2 activity.

Immunostimulation and Inhibition of Angiogenesis

In animal models, selective inhibition of COX-2 activity is associated with enhanced radiation sensitivity of tumor tissue but not of normal (i.e., non-tumor) tissues (134). The potentiation of radiation effects on tumor xenografts appears to require COX-2 expression by the tumor cells themselves (136), although in at least one report, the antitumor effect of selective COX-2 inhibition was independent of the ability of the tumor cells to express COX-2 (71). In the animal models used in this latter study (71), COX-2 was expressed primarily by the angiogenic blood vessels, the preexisting vasculature adjacent to the primary tumor, and the blood vessels invading metastatic lesions rather than by the tumors themselves. In another study (68), COX-2 expression in human pulmonary, colonic, and mammary tumors was examined with the use of antibodies specific for COX-2. In those human tumors, COX-2 was localized to the cytoplasm of the tumor cells and the non-neoplastic epithelial cells adjacent to the tumors, but was not expressed in epithelial cells distal to the tumors. These findings suggest that the tumors themselves, as well as the tumor environment (i.e., stromal components and infiltrating cells), contribute to COX-2 overexpression and thus may be affected by selective COX-2 inhibition.

Inhibition of the angiogenic response in irradiated tumors is one of the potential mechanisms that may play a major role in radiation potentiation through selective COX-2 inhibition (133,134). Radiation affects the tumor vasculature, particularly the proliferating angiogenic endothelial cells, as well as other stromal components and the tumor cells themselves. In addition, COX-2–derived prostaglandins stimulate tumor angiogenesis, and selective COX-2 inhibitors block angiogenic activity (71).

Other mechanisms, such as immunosuppression, may also contribute to radiation potentiation through selective COX-2 inhibition. The immunosuppressive effects of prostaglandins are known (145–148), and increased prostaglandin production by radiation-induced COX-2 may relieve suppressive effects of humoral and cellular immunologic mediators on tumor growth. Conversely, reducing the immunosuppressive effects of prostaglandins might inhibit tumor growth and increase the efficacy of radiotherapy. For example, Stolina and colleagues (149) reported that COX-2 inhibition in a murine model of Lewis lung carcinoma was associated with a marked lymphocytic infiltration of the tumor, which was associated with reduced tumor growth. In this experimental system, COX-2 inhibition was associated with a decrease in the production of the immunosuppressive cytokine interleukin (IL)-10 by antigen-presenting cells and restoration of production of the immunostimulatory cytokine IL-12. Results of studies with indomethacin in the murine fibrosarcoma model also suggested that host immunocompetence is a factor in radioenhancement with indomethacin (33). Although it appears likely that immunologic factors contribute to the antitumor activity of COX-2 inhibition and, possibly, to its radioenhancing effects, this remains an emerging concept. To date, no studies have directly evaluated the extent to which host immunocompetence contributes to radioenhancement.

VEGF as a Mediator of Endothelial Cell Response to Radiation

The endothelium may be important in the radiation resistance of some tumors, and VEGF may be the principal factor mediating the endothelial cell response to radiation. The tumor microvasculature is relatively radioresistant: high doses of radiation are required to substantially reduce tumor blood flow. However, several studies have shown that when low-dose radiation (≤5 Gy) is administered to a tumor, blood flow in the tumor increases within 3–7 days of irradiation (150–153). Radiation increases VEGF expression in the tumor, and this response may mediate or at least contribute to the resistance of endothelial cells to radiation (7,94,154). Gorski and coworkers (7) proposed that increased VEGF production by irradiated tumor cells protects the vascular endothelium from the effects of radiation and that disruption of this paracrine relationship may enhance the radiosensitivity. In their studies, mice bearing transplanted murine Lewis lung tumors or human tumor (squamous cell carcinoma, esophageal adenocarcinoma, glioblastoma) xenografts were treated with a neutralizing antibody to VEGF before tumor irradiation (20–40 Gy). The combination of anti-VEGF antibody and radiation inhibited tumor growth by 78% to 93%, which was more than the inhibition found with either antibody or radiation used alone. In vitro studies with cultures of human umbilical vein endothelial cells showed that the addition of VEGF to the cell cultures decreased radiation-induced cell death, and this...
effect was reversed if the cells were treated with an anti-VEGF antibody before irradiation. In vivo, treating mice with low doses of anti-VEGF antibody immediately before irradiation produced an antitumor effect greater than the additive effect expected from the radiation and antibody. This effect of tumor-derived COX-2 on the tumor endothelium may have clinical significance in the treatment of radioresistant tumors. For example, investigators have observed that whereas certain neoplasms, such as glioblastoma multiforme and melanoma, are radioresistant in vivo, cell lines derived from those neoplasms are radiosensitive in vitro (154,155). Geng and colleagues (154) examined whether the combination of a VEGF inhibitor (either a construct that expressed the soluble VEGF receptor Flk-1 or SU5416) and low-dose radiation would overcome the in vivo radioresistance of VEGF-expressing glioblastoma multiforme and melanoma tumors in animal models. The response of the tumor vasculature to radiation was assessed by direct observation through vascular windows and by measuring Doppler blood flow. Although minimal or no regression of tumor blood vessels was observed following treatment with either a VEGF inhibitor or a sublethal dose of radiation alone, combination therapy using both modalities was associated with a marked increase in the tumor blood vessel destruction. The investigators speculated that VEGF may protect endothelial cells from radiation-induced apoptosis by increasing the expression of the apoptosis inhibitor protein Bel-2. Fig. 6 illustrates a possible mechanism through which COX-2 inhibition may enhance radiotherapy. Other investigators have also reported enhancement of the antitumor effects of radiation when combined with antiangiogenic agents, such as angiostatin (156–157), TNP-470 (158), anti-VEGF antibody (7), and the VEGF receptor tyrosine kinase inhibitors PTK787/ZK222584 (159), SU5416 (160), and SU6668 (160,161) in murine models of lung, breast, and squamous cell cancers and in mice bearing human colorectal, esophageal, and pancreatic cancers, gliomas, and squamous cell cancers.

**CLINICAL TRIALS WITH CLECOXIB AND RADIOTHERAPY**

Clinical and experimental data discussed above show that COX-2 expression correlates with reduced survival in patients with cervical cancer (108) and non–small-cell lung cancer (NSCLC) (114), that radiation treatment is associated with an increase in COX-2 expression in tumors (14), and that inhibiting COX-2 activity in tumors is associated with an improved response to radiation (133–136). Furthermore, experimental evidence suggests that selective COX-2 inhibitors enhance tumor response to radiation without substantially enhancing the radiosensitivity of normal tissues (134). These findings have provided the impetus for clinical trials to evaluate combined-modality therapy, selective COX-2 inhibitors and radiation, in cancer (Table 2).

**NSCLC**

NSCLC presents difficult challenges at every stage of treatment, from therapy for operable disease to management of metastatic cancer. Radiotherapy is assuming an increasing role in the management of NSCLC, and several studies combining celecoxib and radiation have been initiated. The Radiation Therapy Oncology Group (RTOG) (a multi-institutional cooperative organization with foundation headquarters in Philadelphia, PA) is conducting two studies: a phase II study of postoperative adjuvant therapy focusing on the combination of celecoxib (400 mg two times/day) and radiation (50.4 Gy) in patients with completely resected stage I/II NSCLC, and a phase I/II trial of the same treatment combination in patients with locally advanced NSCLC (stages IIB, IIIA, and IIIB) and an intermediate prognosis. In the phase I/II study, patients are treated with fractionated radiation (66 Gy) and receive twice-daily celecoxib for up to 2 years. In both studies, objectives include assessing the tolerability of celecoxib at 400 mg twice daily, evaluating the role of biomarkers as predictors of celecoxib activity, and examining whether celecoxib improves response and survival.

Patients with previously untreated stage III NSCLC are being enrolled in a phase II trial of celecoxib in combination with standard paclitaxel, carboplatin, and radiation therapy at the Vanderbilt Cancer Center (VCC, Nashville, TN). Preliminary results from this study suggest that changes in circulating levels of VEGF may be a marker of the response to celecoxib (162). Another phase II study at VCC combining celecoxib with radiation and taxane chemotherapy in patients with recurrent NSCLC...
was grade 3 pneumonitis, which was seen in two patients. The only toxicity other than the acute radiation-induced toxicity was with any increase in radiation-induced toxicity in normal tissues.

The University of Texas M.D. Anderson Cancer Center (MDACC, Houston, TX) is evaluating radiotherapy and celecoxib (100–400 mg twice daily) in patients with medically inoperable NSCLC, including those with locally advanced disease and poor prognosis patients. A phase I study at the University of Texas M.D. Anderson Cancer Center (MDACC, Houston, TX) is evaluating radiotherapy and celecoxib (100–400 mg twice daily) in patients with medically inoperable NSCLC, including those with locally advanced disease and poor performance status, stage I/II disease and co-morbidities that preclude surgery, and stage IIIA/B disease previously treated with platinum-based induction therapy. Toxicity data for the 27 patients enrolled to date indicate that celecoxib is not associated with any increase in radiation-induced toxicity in normal tissues. The only toxicity other than the acute radiation-induced toxicity was grade 3 pneumonitis, which was seen in two patients 1 month after completion of radiation and celecoxib therapy.

### Other Cancers

Clinical trials combining celecoxib, chemotherapy, and radiotherapy are also being conducted in other cancers in which radiation is a prominent component of standard therapy. A phase I study at MDACC is examining escalating doses of celecoxib (at 0, 400, or 800 mg/day) and radiation (50.4 Gy) combined with fluorouracil and cisplatin in patients with unresectable or recurrent esophageal cancer. The Hoosier Oncology Group (Walther Cancer Institute, Indianapolis, IN) is conducting a phase II study of celecoxib with chemoradiation therapy in patients with potentially resectable esophageal cancer. The Hoosier Oncology Group (Walther Cancer Institute, Indianapolis, IN) is conducting a phase II study of celecoxib with chemoradiation therapy in patients with potentially resectable esophageal cancer. In a preliminary report of this study, it was concluded that the addition of celecoxib to chemotherapy and radiation is well tolerated. Thirty-one patients have enrolled, and 22 patients have undergone surgery; 24 patients remain alive and are receiving celecoxib maintenance therapy (164). The RTOG is evaluating the effects of celecoxib combined with external-beam radiotherapy and brachytherapy concurrent with 5-fluorouracil and cisplatin and radiation (45 Gy) on locoregional and distant control of disease, disease-free survival, and overall survival in patients with locally advanced cancer of the cervix. The New Approaches to Brain Tumor Therapy (NABTT) Central Nervous System (CNS) Consortium (Johns Hopkins University, Baltimore, MD) is conducting a trial of radiation and celecoxib in patients with glioblastoma multiforme. Recently, Pannullo and colleagues (/165) reported encouraging preliminary results of a phase I trial with temozolomide and celecoxib in 18 patients with relapsed or refractory malignant glioma following resection and radiotherapy. The treatment in that trial was well tolerated, with partial response observed in four of 13 patients and disease stabilization in eight of 13 patients.

The results of these early-phase studies in NSCLC and esophageal, cervical, and brain cancer, which are expected over the next few years, will determine whether the current enthusiasm for combining COX-2 inhibitors and radiation is maintained and justifies the initiation of randomized controlled clinical trials.

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NOTES

Editor’s note: Dr. Choy is conducting research sponsored by Pharmacia/Pfizer, manufacturer of celecoxib. He is also a consultant for this company and a member of its speaker’s bureau.

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