

Assessment of celecoxib pharmacodynamics in pancreatic cancer

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Abstract

Cyclooxygenase-2 (COX-2) inhibitors are being developed as chemopreventive and anticancer agents. This study aimed to determine the biological effect of the COX-2 inhibitor celecoxib in pancreatic cancer as an early step to the further development of the agent in this disease. Eight patients scheduled for resection of an infiltrating adenocarcinoma of the pancreas were randomized to receive celecoxib at a dose of 400 mg twice daily or placebo for 5 to 15 days before the surgery. In addition, carcinomas from nine additional patients were xenografted in nude mice, expanded, and treated with vehicle or celecoxib for 28 days. Celecoxib markedly decreased the intra-tumor levels of prostaglandin E₂ in patient carcinomas and in the heterotransplanted xenografts. However, this effect did not result in inhibition of cell proliferation or microvessel density (as assessed by Ki67 and CD31 staining). In addition, a panel of markers, including *bcl-2*, *COX-1*, *COX-2*, and *VEGF*, did not change with treatment in a significant manner. Furthermore, there was no evidence of antitumor effects in the xenografted carcinomas. In

summary, celecoxib efficiently inhibited the synthesis of prostaglandin E₂ both in pancreatic cancer surgical specimens and in xenografted carcinomas but did not exert evident antitumor, antiproliferative, or antiangiogenic effect as a single agent. The direct pancreatic cancer xenograft model proved to be a valuable tool for drug evaluation and biological studies and showed similar results to those observed in resected pancreatic cancer specimens. [Mol Cancer Ther 2006;5(12):3240–7]

Introduction

Pancreatic cancer remains a devastating disease, as shown by the equivalence between incidence and mortality rates (1). At the time of diagnosis, 80% of patients have unresectable locally advanced or advanced, and >80% of surgically resected carcinomas will recur in the first 2 years after surgical resection (2). Only two agents are formally approved for this patient population: gemcitabine and erlotinib. New treatment options are needed. In addition, it is increasingly being recognized that a subset of patients have a strong familial predisposition to developing pancreatic cancer (3, 4), opening the possibility of chemoprevention strategies.

One potential target for pancreatic cancer treatment and chemoprevention is cyclooxygenase-2 (COX-2). COX-2 is the inducible isoenzyme of COX, a key enzyme in the conversion of arachidonic acid to prostaglandins and other eicosanoids. The expression of COX-2 is induced by a number of cytokines and growth factors, and COX-2 is overexpressed in neoplastic diseases (5). COX-2 is highly expressed in a number of human cancers and cancer cell lines, including colon (6–10), gastric (11, 12), esophageal (13), biliary (14), squamous carcinoma of the head and neck (15), cervical (16, 17), non-small cell lung (18–20), breast (21–23), prostate (24–29), and pancreatic cancer and its precursors (30, 31). This overexpression in carcinomas has been associated with a more aggressive behavior and a worse prognosis (6, 17, 18, 20). There are a large number of selective COX-2 inhibitors in development. One of them, celecoxib (Celebrex; Pfizer, Inc., New York, NY), has shown to decrease the size and number of colonic (32) and duodenal polyps (33) in patients with familial hereditary polyposis and has gained Food and Drug Administration approval for the treatment of patients with familial adenomatous polyposis to prevent the occurrence of colon cancer. These studies employed a dose (400 mg twice daily) that doubles the standard anti-inflammatory dosing schedule (200 mg twice daily). Laboratory studies with pancreatic cancer indicate that nonsteroidal anti-inflammatory drugs may inhibit cancer growth, but epidemiologic data to support this finding are limited and contradictory. Whereas a prospective study in 28,283

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postmenopausal women documented a reduction in the incidence of pancreatic cancer associated with aspirin use (34), other results with longer sample size and follow-up have evidenced inconclusive (35) or even a deleterious (36) effect.

The purpose of this study was to determine the biological effects of celecoxib in pancreatic cancer to gather rationale for pharmacologic approaches to COX-2 modulation in the prevention and/or treatment of this uniformly lethal disease. The objectives of the clinical trial were (a) to determine the biological effect of celecoxib by comparing the differences in intra-tumor (i.t.) prostaglandin E₂ (PGE₂) levels between patients treated with placebo and celecoxib and (b) to determine the variation in key pathway-related genes induced by celecoxib treatment. The objectives of the follow-up preclinical part of the project were (a) to determine the activity of celecoxib in primary pancreatic cancer xenografts, (b) to determine the biological effect in terms of target inhibition by measuring i.t. PGE₂ levels, and (c) to characterize the variation in key pathway-related genes induced by celecoxib treatment.

Materials and Methods

Patient Eligibility

Patients were required to be 18 years of age or older with suspected pancreatic cancer that was scheduled for surgical resection at our institution; Eastern Cooperative Oncology Group performance status of ≤ 2 ; and adequate bone marrow, hepatic, and renal function (leucocytes $\geq 3,000$ per μL , absolute neutrophil count $\geq 1,500$ per μL , platelet count $\geq 100,000$ per μL , normal bilirubin level, aspartate aminotransferase or alanine aminotransferase levels $< 2.5 \times$ the upper limit of normal, creatinine within normal limits or creatinine clearance ≥ 60 mL/min/1.73 m² for patients with creatinine levels above institutional normal limits). Patients could not have received any prior chemotherapy or radiation therapy for pancreatic cancer and could not be receiving nor have received nonsteroidal anti-inflammatory or anticoagulants drugs in the last 28 days. The scientific review board of our institution granted protocol approval. Patients were required to provide written informed consent before enrollment into the study.

Treatment Plan and Patient Monitoring

Celecoxib and identical-appearing placebo were supplied by the National Cancer Comprehensive Network (Jenkin-town, PA). Treatment consisted of celecoxib/placebo given every 12 hours for 5 to 15 days before surgery, at a total daily dose of celecoxib of 800 mg. Given the exploratory nature of this clinical trial, the occurrence of any grade ≥ 1 toxicity thought to be related to the study drug prompted discontinuation of therapy and removal from the trial. Patients were followed twice weekly by telephone interview until surgery. Toxic events were recorded on a continuous basis and followed until they were resolved to baseline or grade < 1 . Immediately before study entry, patients had a complete clinical history and physical examination, vital sign assessment, complete blood counts,

and serum biochemistry tests (albumin, alkaline phosphatase, total bilirubin, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, lactate dehydrogenase, phosphorus, potassium, total protein, aspartate aminotransferase, alanine aminotransferase, sodium, magnesium, and amylase).

In vivo Growth Inhibition Studies

Resected pancreatic adenocarcinomas are routinely implanted in nude mice at the Johns Hopkins Medical Institutions as a method to obtain enriched populations of neoplastic cells under an Institutional Review Board-approved protocol from residual pancreatic cancer tumors (37). Briefly, tumor specimens from Whipple resection specimens are divided into 2- to 3-mm³ pieces in antibiotic-containing RPMI. Pieces of nonnecrotic tissue are selected and immersed in Matrigel. Under anesthesia with isoflurane, tumors are implanted into 5- to 6-week-old female athymic (*nu/nu*) mice purchased from Harlan Laboratories (Washington, DC). This research protocol was approved by the Johns Hopkins University Animal Use and Care Committee, and animals were maintained in accordance to guidelines of the American Association of Laboratory Animal Care. Tumors are propagated to subsequent cohorts of mice until sufficient number is available for drug testing. Tumors were allowed to grow until reaching 200 mm³, at which time mice were randomized in the following two groups of treatment, with 10 evaluable tumors in each group: (a) control and (b) 20 mg/kg celecoxib. Treatment was given daily by oral gavage for 28 days. Mice were monitored daily for signs of toxicity and weighed thrice per week. Tumor size were evaluated twice per week by caliper measurements using the following formula: tumor volume = (length \times width²) / 2 as previously reported (38). Relative tumor growth inhibition was calculated by relative tumor growth of treated mice divided by relative tumor growth of control mice (T/C). A tumor growth inhibition of $\geq 50\%$ (as documented by a T/C of $\leq 50\%$) was considered significant. Experiments were terminated on day 28.

Biological Studies: Blinding

The blinding of the treatment allocation of the clinical samples was broken at the completion of biological analyses. The pathology and laboratory teams were blinded for treatment allocation and efficacy of the xenograft samples.

PGE₂ Extraction

PGE₂ was extracted, purified, and measured from fresh-frozen tumor following manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). In brief, the frozen tumor tissue was minced on dry ice first and then transferred it to a new Eppendorf tube to homogenize in tissue lysis buffer. PGE₂ was extracted with acetone and purified with C18 reverse-phase columns. The raw data were standardized to tissue weight.

Real-time PCR

The expression of a relevant set of genes was determined in fresh-frozen tissues from (a) the surgical resection in placebo or celecoxib treated samples and (b) from F3

generation xenografts untreated and treated with celecoxib. Genes included *COX-1*, *COX-2*, *bcl-2*, and *vascular endothelial growth factor (VEGF)*. RNA was extracted by a proprietary method (Response Genetics, Inc., Los Angeles, CA) and evaluated by standard real-time PCR technique. Four tumors per group were analyzed.

Immunohistochemical Analysis

Five-micrometer sections were used for Ki67 staining that was done following the manufacturer's instructions (08-0156; Invitrogen, Carlsbad, CA) and scored as percent staining nuclei. Three tumors per treatment group were analyzed. For statistical analyses, an index of intensity \times percentage was calculated. CD31 staining (550274; BD PharMingen, San Diego, CA) was done on zinc-fixed tissue (51-7538KZ; BD PharMingen) and scored quantitatively.

Statistical Analysis

The comparisons between means and proportions obtained from the biological studies were done using Student's *t* test and χ^2 method, respectively. ANOVA was used when three or more variables were compared.

Results

Clinical Trial

Between December 2003 and December 2004, 102 patients with potentially resectable pancreatic cancer were evaluated for participation in the clinical trial JHH-J0265, and 66 did not meet eligibility criteria. The main reasons were concomitant or recent nonsteroidal anti-inflammatory drugs intake ($n = 25$), elevated liver function tests ($n = 18$), and insufficient time window before the surgery ($n = 9$). Thirty-six patients preliminarily met eligibility criteria. Of these, 25 patients were not interested (20 due to safety concerns, 3 to logistic concerns, and 2 did not disclose reason), and 11 signed informed consent. Of these, two developed elevated liver function tests, one withdrew consent, and eight were randomized. Of these eight, one was unresectable at the time of surgery; thus, samples were available from seven subjects. The patients included five males and three females, and they received an average of 7 days of drug (range, 5–12). Regarding tolerability, one patient developed grade 1 stomach upset that was thought to be possibly related and that subsided after initiating proton pump inhibitors. One patient developed a grade 3 transaminitis after surgery that was thought to be related to a mechanical compression of the liver by the robotic retractor during surgery. It resolved without specific treatment and was felt to be unrelated to the study drug. No other incidences were documented.

After eight patients had been accrued and randomized, several celecoxib-based clinical trials were put on hold by the Food and Drug Administration due to cardiac toxicity concerns. Because the currently reported clinical trial was not a therapeutic trial, and despite the short administration of study drug, the principal investigator, the sponsor, and the Food and Drug Administration agreed to terminate the trial. After discussions with the sponsor, it was felt that the primary objectives of the study could be further investi-

gated in the available direct pancreatic cancer xenograft model (39).

Efficacy of Celecoxib in the Xenograft Model

Nine surgically resected pancreatic cancers were xenografted into multiple mice, and 20 xenografts from each of these primary cancers were treated with celecoxib ($n = 10$) or vehicle ($n = 10$) for 28 days (Fig. 1A). None of the treated xenografts showed a meaningful growth arrest (defined as a T/C $< 50\%$). Cases 215 and 410 showed T/Cs of 62% and 63%, respectively, that were statistically different from their respective control groups. However, this single-agent level of activity is not felt to be clinically relevant.

Pharmacodynamic Effect: PGE₂ in Tumors

Of the six patient samples that were assessable, PGE₂ levels were 18.7, 14.7, and 4.9 pg/mg of dry tissue in the three patients that received placebo and 4.6, 4.2, and 2.9 pg/mg of dry tissue in the three patients that received a short course of celecoxib ($P = 0.10$; Fig. 1B). Of the nine groups of xenografts, six (cases 215, 410, 286, 159, 163, and 281) had a decrease in tumor PGE₂ levels ranging from 50% to 100% (Fig. 1B; all $P < 0.05$), and in three (cases 253, 194, and 265), no significant change was documented (Fig. 1C). The tumors where a decrease in PGE₂ was seen after celecoxib had more baseline PGE₂ than tumors where no change was observed (20.7 versus 4.3 pg/mg of dry tissue, respectively, $P < 0.05$). The comparison of the average T/C of these two groups did not show significant differences (72% versus 82%, $P = 0.19$).

Gene Expression

The range of expression of the markers assessed in the untreated groups was similar in the human pancreatectomy and in the heterotransplanted pancreatic cancer samples, as can be seen in Fig. 2. In particular, the expression of *COX-2* and *VEGF* was remarkably parallel, with *bcl-2* and *COX-1* showing a higher expression in the primary carcinomas (that was significant for *COX-1*). Interestingly, one of the patients participating in the clinical trial (and that was allocated to placebo) had her carcinoma xenografted, and this xenograft was used in the xenograft portion of this study. Her *COX-2* and *VEGFR* mRNA levels in the frozen pancreatectomy and xenograft samples were in the same range of high expression for both markers (37.09 and 20.23 for *COX-2* and 38.55 and 43.87 for *VEGF* in the initial and xenograft samples, respectively).

There were no differences in *bcl-2*, *COX-1*, *COX-2*, or *VEGFR* mRNA expression between the samples from patients receiving celecoxib or placebo. In the xenografted tumors, the average baseline levels of *bcl-2*, *COX-1*, *COX-2*, or *VEGFR* varied within a range of 22-, 23-, 89-, and 26-fold between cases, respectively (Table 1). One case (case 281) did not express *COX-1*. The changes within cases when comparing the control and treated tumors were of great magnitude, but these changes were only significant for *COX-2* in two cases: in cases 281 and 410, *COX-2* mRNA significantly decreased in the treatment group (20.2 versus 12.8, $P = 0.046$ and 42.9 versus 19.1, $P = 0.08$, respectively; see Tables 1 and 2). In the rest of the cases, there were no statistically significant differences when the nine group

readouts were averaged, and when a comparison of control versus treated with celecoxib was done. No solid trends were apparent in any of the given four genes. There were no statistically significant differences in the baseline levels of those four markers when grouping the cases according to the presence ($n = 6$) or absence ($n = 3$) of PGE₂ inhibition with celecoxib. There were no patterns when separately analyzing the cases where some degree of tumor growth inhibition had been documented (cases 215 and 410).

Immunohistochemistry

No differences were seen in paraffin slides immunolabeled for the proliferation index Ki67 between the placebo and celecoxib pancreatectomy specimens (Fig. 3A). We did not observe any vascular changes in the Whipple specimens. CD31 staining did not reveal any capillary ingrowth in the areas involved by infiltrating carcinoma beyond the expected supply to the surrounding pancreatic and associated fibroadipose tissue. Both Ki67 and CD31 were also calculated for tumors from the three groups with pharmacodynamic effect that had the more noticeable growth inhibitory effect (cases 215, 410, and 286) and from

two groups where no pharmacodynamic or antitumor effect was seen (cases 194 and 265). As depicted in Fig. 3B and C, only in case 215 was a significant (50%) decrease in microvessel density compared with control were observed in any of the assessed groups. There were no differences between the baseline Ki67 and CD31 levels of the groups with and without pharmacodynamic effect after treatment.

Discussion

In this study we assessed whether celecoxib inhibited COX-2 activation and COX-2-regulated pathways in pancreatic cancer. The long-term goal was to document the ability of celecoxib to inhibit COX-2 in preparation for future prevention and treatment studies. Although the role of aspirin in pancreas cancer is unresolved, the available data suggest that COX-2 inhibition may have a therapeutic potential in several malignancies, including pancreatic cancer. The approach was a presurgical, placebo-controlled clinical trial in patients with pancreatic cancer scheduled

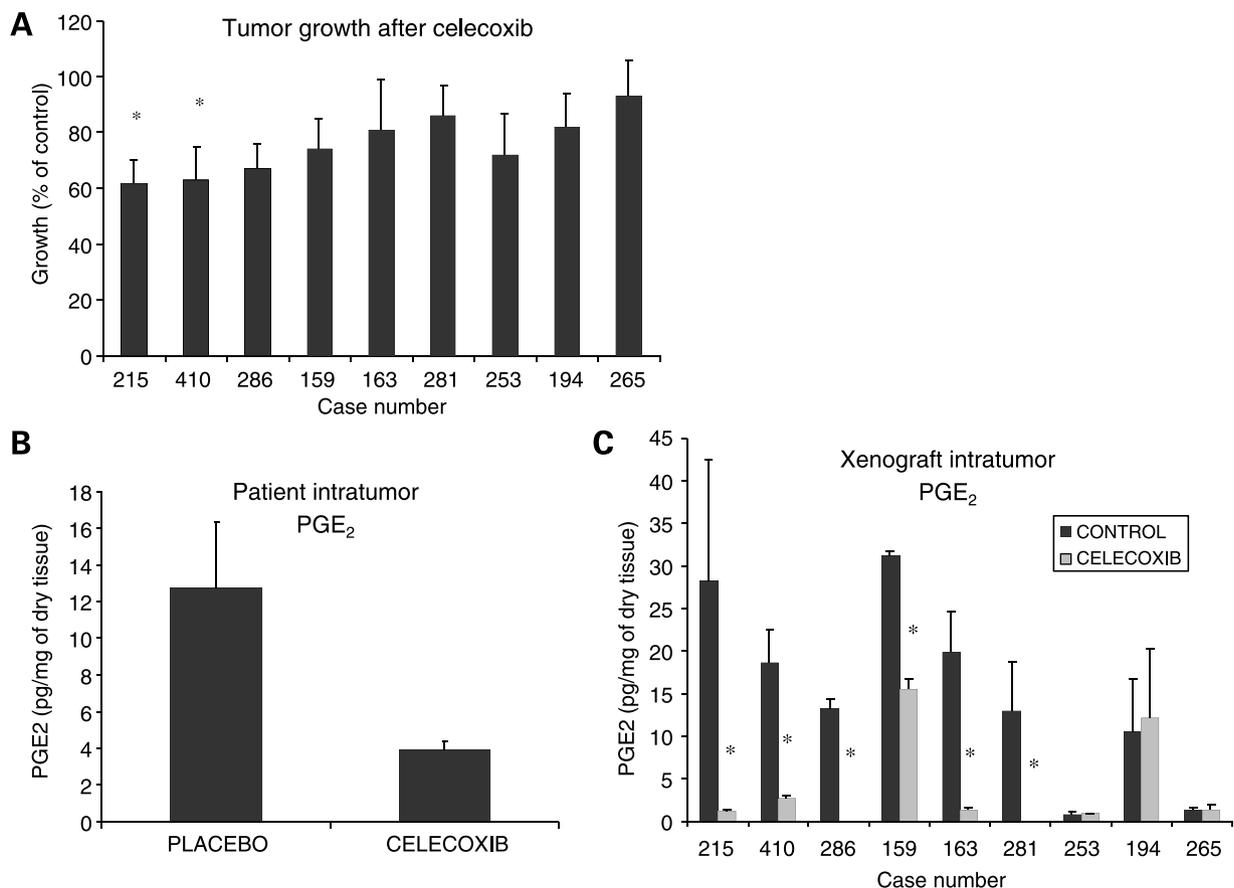


Figure 1. **A**, tumor growth after celecoxib treatment for 28 d. T/C is presented, where each value has been normalized to the control (100%). Each case comprised 20 evaluable tumors that were randomized to receive either 20 mg/kg celecoxib or vehicle orally daily for 28 d for a total of 180 mice used. *Columns*, mean ($n = 10$ tumors per group); *bars*, SE. **B**, the samples of patients allocated to placebo had more PGE₂ than those that received celecoxib, although the difference was not statistically significant ($P = 0.10$). **C**, of the nine groups of xenografts, six (cases 215, 410, 286, 159, 163, and 281) had a decrease in tumor PGE₂ levels ranging from 50% to 100%, and in three (cases 253, 194, and 265), no significant change was documented. *, $P \leq 0.05$, compared with control (Student's t test).

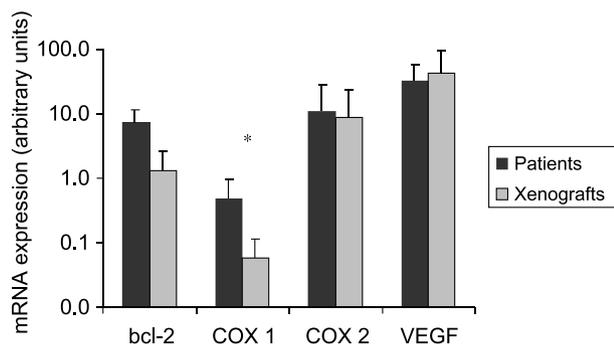


Figure 2. Comparison of the range of expression of the markers assessed in the untreated groups of the human pancreatotomy (Whipple) and in the heterotransplanted pancreatic cancer samples, respectively. *Bcl-2* and *COX-1* showed a higher expression in the Whipple samples (significant for *COX-1*), and the expression of *COX-2* and *VEGF* was similar. (Y-axis in logarithmic scale, with arbitrary expression units shown).

for surgical resection. Due to an early closure of the clinical trial because of safety concerns, the investigations were finalized using a novel direct pancreatic cancer xenograft model in which patient tumors obtained at the time of surgical resection are implanted in nude mice, expanded in nude mice, and treated with drugs (39). Celecoxib significantly decreased PGE_2 both in the pancreatotomy samples and in xenografted tumors treated with the COX-2 inhibitor. However, a decrease in proliferation was only observed in a fraction of the analyzed xenograft groups, and no relevant changes in angiogenesis or tumor growth were observed.

A first conclusion is that the preoperative approach seemed to work as a model to explore the biology of drugs and as a tool for target validation in pancreatic cancers. Furthermore, the addition of the novel xenograft platform gave equivalent results, suggesting that it is representative of the biology of pancreatic cancer as seen by the preservation of features such as gene expression. There were variations between the patient and xenografted

specimens in the genetic expression of one of the four genes compared. This may represent a technical artifact, or *COX-1* expression may be down-regulated in the murine environment.

Limited information on factors that are relevant to the efficacy of a drug at the time clinical trials are initiated is one of the underlying factors for the low yield rate in anticancer drug development. This problem is particularly evident in pancreatic cancer. Before entering clinical development, agents are usually tested against high-passage commercially obtained cell lines and xenografts established from these cell lines. It is unclear how representative these models are of the biology of pancreatic cancer. Although the true relevance of the described xenograft model is currently undergoing prospective evaluation in the context of a controlled clinical trial, it has several features that are intuitively appealing. It has shown to be feasible, with a high engraftment rate, but more importantly for new drug development, it was stable over time and passages, both genetically and from the perspective of drug sensitivity (39). The possibility of testing several drugs simultaneously allows for back-to-back comparisons, and the availability of large amounts of tissue permits profound biological analyses as well as the development of novel techniques for seriated pharmacodynamic assessment. One future approach may be to test drugs in the xenograft platform first and only translate a drug to the clinic if it shows either activity or biological effect in the preclinical model.

In the presented experiments, celecoxib clearly showed pharmacodynamic effects, some degree of antiproliferative effect, and modest inhibition of tumor progression but did not meet our criteria for meaningful antitumor effects. In other settings, such as analgesia models, there is a good correlation between pharmacodynamic effect of celecoxib, as assessed by PGE_2 inhibition, and clinical effect (40). Indeed, in the currently presented data, there was profound PGE_2 down-regulation in both cases (cases 215 and 410) where some growth inhibition was seen, and this

Table 1. Average levels of *bcl-2*, *COX-1*, *COX-2*, or *VEGF* in the untreated and treated groups

Case no.	<i>bcl-2</i>		<i>COX-1</i>		<i>COX-2</i>		<i>VEGF</i>	
	Control	Celecoxib	Control	Celecoxib	Control	Celecoxib	Control	Celecoxib
215	0.63	1.01	0.02	0.04	1.57	3.51	15.90	18.93
410	4.50	3.55	0.02	0.03	42.86	19.13	169.00	130.00
286	0.27	1.60	0.06	0.09	1.43	14.61	9.69	8.24
159	1.18	1.58	0.06	0.31	8.30	1.46	7.46	8.45
163	0.90	0.70	0.02	0.02	1.24	1.58	30.65	51.42
281	0.98	1.41	NA	NA	20.23	12.80	43.87	60.68
253	2.06	2.46	0.18	0.06	0.48	0.70	78.13	122.94
194	0.82	0.88	0.09	0.02	3.68	5.73	12.34	10.51
265	0.21	0.41	0.01	0.01	0.59	0.29	6.53	6.96

NOTE: Each cell represents the average of three to four tumors per treatment group (in arbitrary units). Bold indicates a difference that is statistically significant ($P \leq 0.05$).

Abbreviation: NA, not available.

Table 2. Normalized levels of *bcl-2*, *COX-1*, *COX-2*, or *VEGF* in the untreated and treated groups

Case no.	<i>bcl-2</i>		<i>COX-1</i>		<i>COX-2</i>		<i>VEGF</i>	
	Control	Celecoxib	Control	Celecoxib	Control	Celecoxib	Control	Celecoxib
215	100	160	100	221	100	224	100	119
410	100	79	100	131	100	45	100	77
286	100	590	100	145	100	1022	100	85
159	100	134	100	502	100	18	100	113
163	100	77	100	77	100	127	100	168
281	100	144	NA	NA	100	63	100	138
253	100	120	100	35	100	145	100	157
194	100	107	100	23	100	156	100	85
265	100	193	100	118	100	49	100	107

NOTE: Each cell represents the average of three to four tumors per treatment group. Bold indicates a difference that is statistically significant ($P \leq 0.05$). Abbreviation: NA, not available.

pathway inhibitory effect seemed higher in cases with more abundance of PGE_2 . However, an even deeper pharmacodynamic effect was seen in other cases (such as case 163 or 281) that was not associated with any decrease in tumor growth. It may be that pharmacodynamic effects are necessary but not sufficient to induce antitumor effect (39). In addition, there is strong evidence suggesting that the antitumor effect of celecoxib may be mediated by targets other than COX-2, such as the down-regulation of survivin (41) or the generation of reactive oxygen species (42). If this is true, then PGE_2 down-regulation may be

irrelevant for pancreatic cancer growth. In addition, there is data indicating that the potentiation of gemcitabine effects seen in preclinical models of pancreatic cancer is mediated through modulation of other targets, such as nuclear factor- κ B activation, which may contribute to the induction of apoptosis (43). There is evidence in models other than the pancreas that achieving efficacious drug concentrations is a problem with celecoxib and other COX inhibitors (44). To improve the bioavailability and decrease the systemic effects of celecoxib, alternative delivering strategies, such as chitosan-based, biodegradable slow-releasing platforms,

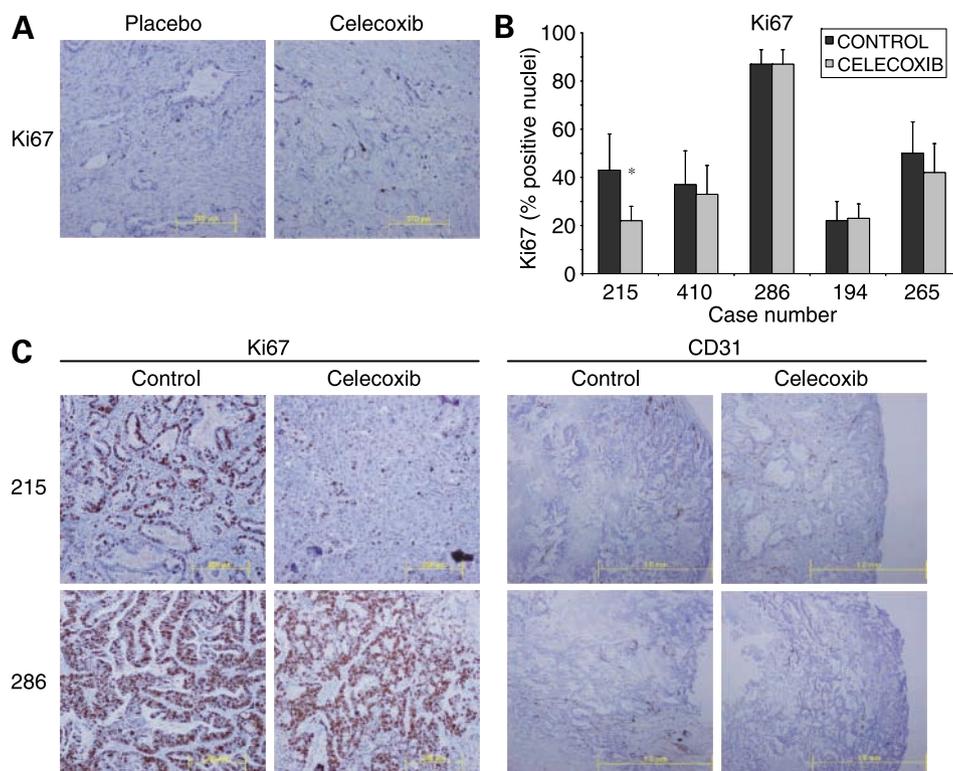


Figure 3. Proliferation index Ki67 was calculated from the reaction specimens (A) and was also calculated in tumors from the three groups with pharmacodynamic effect that had the more noticeable growth inhibitory effect (cases 215, 410, and 286) and from two groups where no pharmacodynamic or antitumor effect was seen (cases 194 and 265) (B). Only in case 215 was a significant (50%) decrease in proliferation documented. No quantitative changes in microvessel density compared with control were observed in any of the assessed groups. *, $P \leq 0.05$, compared with control (Student's *t* test).

have been developed (45). Others have compared oral versus aerosolized celecoxib in lung cancer animal models, showing improved efficacy with lower dose with the local approach (46).

Our interest in assessing the effects of celecoxib in pancreatic cancer was based on the potential applications of the agent in the prevention and treatment of pancreatic cancer. Indeed, laboratory studies indicate that nonsteroidal anti-inflammatory drugs may inhibit pancreatic cancer. However, epidemiologic data to support this finding are limited and contradicting. Anderson et al. conducted a prospective study from 1992 through 1999 among 28,283 postmenopausal women to examine the association between the use of aspirin and other nonsteroidal anti-inflammatory drugs and the incidence of pancreatic cancer (34). Eighty incident cases of pancreatic cancer were identified during 7 years of follow-up; the multivariate-adjusted relative risk of pancreatic cancer associated with any current use of aspirin versus no use was 0.57 (95% confidence interval, 0.36–0.90), and there was a trend of decreasing risk of pancreatic cancer incidence with increasing frequency of aspirin use per week ($P = 0.005$). However, in another report that examined the association between aspirin use and pancreatic cancer mortality among 987,590 subjects, aspirin use was not associated with pancreatic cancer mortality (35). In the Nurses' Health Study, the risk of pancreatic cancer was not associated with regular aspirin use compared with use of fewer than two tablets per week (relative risk, 1.20; 95% confidence interval, 0.87–1.65), but increasing duration of regular aspirin use, compared with nonuse, was associated with a significant increase in risk (36). Similar studies, however, are not available with COX-2 inhibitors, and celecoxib has been shown to decrease the incidence of colorectal cancer in familial syndromes (32). It is well known today that in addition to some genetic syndromes, in which there is an increased risk of pancreatic cancer, there is an increase risk of pancreatic cancer in first-degree relatives of affected patients. In these high-risk populations, there is the unmet need to develop efficacious preventive strategies, including pharmacologic approaches.

There is increasing evidence of the potential value of COX-2 inhibition with a therapeutic intent in cancer patients. Ferrandina et al. showed that a short treatment with celecoxib decreased tumor COX-2 expression and markers of proliferation and neoangiogenesis in cervical cancer, whereas it was ineffective at lowering stromal COX-2 levels, thus suggesting that selective COX-2 inhibitors may be a promising strategy not only for chemopreventive approaches but also for therapeutic approaches in this neoplasm (47). There have been clinical evaluations of celecoxib together with protracted i.v. infusion 5-fluorouracil (48) and gemcitabine plus irinotecan (49) in pancreatic cancer patients, showing adequate tolerance. However, both series are single-arm trials too small to draw any significant conclusions regarding efficacy. Xiong et al. conducted a pharmacologic trial to evaluate whether celecoxib alters the conversion of gemcitabine into its

active metabolite difluoro-dCTP in peripheral blood mononuclear cells of patients with untreated advanced pancreatic cancer (50); no interactions, but significant toxicity, were observed. The currently presented results clearly do not support single-agent studies. However, the fact that pharmacodynamic effects were present and the evidence of combination activity with epidermal growth factor receptor inhibitors in other disease models where epidermal growth factor receptor targeting has a therapeutic role would support conducting combination studies (51).

In our study, celecoxib was able to inhibit PGE₂ synthesis in patients with pancreatic cancer and in xenografted pancreatic tumors. However, this did not translate into a significant level of antiproliferative, antitumor, or antiangiogenic effect. This could be due to pharmacologic obstacles (although there was clear-cut pharmacodynamic effect in the majority of the cases), or it could be related to the lack of relevance of the target in pancreas cancer. Expression analysis rendered a wide range of variation that could be due to individual differences between cases or to assay variability. We were not able to establish clear patterns of variation according to treatment group allocation. Although anecdotal, it is relevant to note the stability of the model in view of the preservation of the level of expression of key genes after xenografting the tumors in mice (39). *VEGF* had been previously described as one of the most stable genes between the initial surgical sample and the third generation of xenografts, and this isolated example of the assessment of the originator and originated tumor 1 year apart seems to also indicate this.

In summary, celecoxib was able to decrease i.t. PGE₂ levels both in pancreatic cancer patients and in xenografted pancreatic cancer tumors. In the xenograft experiments, this effect did not induce a significant antitumor response, and proliferation was decreased in just one of the nine cases tested. The direct pancreatic cancer xenograft model was a valuable tool for drug evaluation and biological studies and showed similar results to those observed in resected pancreatic cancer specimens.

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