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CLINICAL INVESTIGATION **Cervix**

A PROSPECTIVE PHASE I–II TRIAL OF THE CYCLOOXYGENASE-2 INHIBITOR CELECOXIB IN PATIENTS WITH CARCINOMA OF THE CERVIX WITH BIOMARKER ASSESSMENT OF THE TUMOR MICROENVIRONMENT

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Purpose: To evaluate the toxicity and effectiveness of celecoxib in combination with definitive chemoradiotherapy (CRT) in women with locally advanced cervical cancer.

Methods and Materials: Thirty-one patients were accrued to a phase I–II trial of celecoxib 400 mg by mouth twice per day for 2 weeks before and during CRT. Tumor oxygenation (HP_5) and interstitial fluid pressure (IFP) were measured before and 2 weeks after celecoxib administration alone. The median follow-up time was 2.7 years (range, 1.1–4.4 years).

Results: The most common acute G3/4 toxicities were hematologic (4/31, 12.9%) and gastrointestinal (5/31, 16.1%) largely attributed to chemotherapy. Late G3/4 toxicity was seen in 4 of 31 patients (13.7% actuarial risk at 2 yr), including fistulas in 3 patients (9.7%). Within the first year of follow-up, 25 of 31 patients (81%) achieved complete response (CR), of whom 20 remained in CR at last follow-up. After 2 weeks of celecoxib administration before CRT, the median IFP decreased slightly (median absolute, –4.6 mm Hg; $p = 0.09$; relative, –21%; $p = 0.07$), whereas HP_5 did not change significantly (absolute increase, 3.6%; $p = 0.51$; median relative increase, 11%; $p = 0.27$). No significant associations were seen between changes in HP_5 or IFP and response to treatment ($p = 0.2$, relative HP_5 change and $p = 0.14$, relative IFP change).

Conclusions: Celecoxib in combination with definitive CRT is associated with acceptable acute toxicity, but higher than expected late complications. Celecoxib is associated with a modest reduction in the angiogenic biomarker IFP, but this does not correspond with tumor response. © 2007 Elsevier Inc.

Celecoxib, Cervical cancer, Chemoradiation, Phase I–II trial.

INTRODUCTION

Carcinoma of the uterine cervix is the second most common cancer of women in the world, and a leading cause of cancer mortality (1). In patients with locally advanced cervical cancer, concurrent radiotherapy (RT) and cisplatin-based chemotherapy (CT) have improved survival rates compared with RT alone and are therefore considered standard management. Despite this improvement, there are still a significant number of patients who do not achieve pelvic control and eventually die of disease (2–5).

Intratumoral hypoxia is an adverse prognostic factor for both local control and survival, and has been associated with increased risk of nodal disease and metastasis in patients with cervical cancer (6, 7). Hypoxia can also cause genetic instability and selection for resistance to apoptosis and has been shown to be the major physiologic stimulus for inducing transcription of vascular endothelial growth factor (VEGF) mediated by hypoxia-inducible factor 1 (HIF-1) (8).

Interstitial fluid pressure (IFP) is elevated in most solid tumors as a result of the abnormal microvasculature, dysfunctional lymphatics (linked with high levels of VEGF-C), and high vasculature permeability. IFP is easily evaluated in human tumors using a needle probe, and has been shown to

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provide unique information about outcome in patients with cervical cancer (9).

Cyclooxygenase-2 (COX-2) is an enzyme required in the conversion of prostaglandins (PGs) from arachidonic acid. Its tumor-promoting activities are mediated via several mechanisms including conversion of procarcinogens to carcinogens, stimulation of tumor cell growth, prevention of apoptosis, promotion of angiogenesis, and immunosuppression (10). COX-2 overexpression has been reported in cervical cancers in association with locally advanced stage, distant metastasis, and poor survival (11–13).

Cervical cancer cell lines treated with celecoxib, a nonsteroidal anti-inflammatory drug (NSAID) that directly inhibits the enzyme COX-2, are more sensitive to radiation-induced apoptosis, and this appears to be the result of an increase in the G2M cell cycle arrest and inhibition of sublethal radiation damage repair (14–16). Treatment with celecoxib decreases expression of COX-2, the proliferation marker Ki67, and the neo-angiogenic marker CD 31 (17). In preclinical models, celecoxib suppresses the growth of corneal capillaries in rats exposed to basic fibroblast growth factors, and this potent angiogenesis inhibition seems to be derived from its capacity to inhibit PG production via COX-2 (18).

Thus, the potential activity of celecoxib to attack hypoxic cells directly, to overcome the adverse characteristics of the tumor microenvironment, and to augment the treatment effect of radiation and concurrent CRT may benefit cervical cancer patients. We therefore report the results of a phase I–II study of the COX-2 inhibitor celecoxib in combination with chemoradiotherapy (CRT) in women with locally advanced cervical cancer.

METHODS AND MATERIALS

Patient eligibility, evaluation, and follow-up

This study included 31 women with locally advanced (FIGO cT1b >4 cm, T2b–4a or any N1, M0), biopsy-proven carcinoma of the cervix, ECOG performance status of 0, 1, or 2, for whom CRT followed by a single insertion of intrauterine brachytherapy (BT), was the planned treatment. Patients with a prior malignancy, active peptic ulcer disease, or a history of ischemic heart disease, stroke, or other significant comorbidity were ineligible, as were patients with prior therapy for cervical cancer or metastatic disease and those who had received NSAIDs within 2 weeks before study enrollment.

Investigation and staging of patients conformed to the policies of the Princess Margaret Hospital Gynecologic Cancer Group. Pre-entry study assessment included physical examination, complete blood count, chest radiograph, magnetic resonance imaging (MRI), and computed tomography of abdomen and pelvis. Positron emission tomography was not part of the staging. An examination under anesthesia (EUA) was performed to determine the stage according to the guidelines established by the Federation Internationale de Gynecologie et d'Obstetrique (FIGO). Tumor oxygenation, reported as the percentage of pO_2 readings <5 mm Hg (hypoxic proportion or HP_5), and IFP were measured at EUA before treatment. The oxygen measurements were performed using the Eppendorf oxygen pressure histograph (Eppendorf-Netheler-Hinz, Hamburg, Germany) and IFP was measured using a wick-in-needle apparatus, as previously described (9, 19). Measurements were obtained at 5 positions symmetrically spaced around the circumference of the tumor to minimize intratumor heterogeneity (19).

After completing treatment, patients were followed at 4–6 weeks and then every 3 months for the first year, every 4 months for the second year, and at 6-monthly intervals thereafter, for a minimum follow-up of 5 years. At each visit, the clinical history was updated and a physical examination, including pelvic examination, was performed. Pelvic MRI was repeated 3–6 months after completing treatment, and primary tumor response was documented using Response Evaluation Criteria in Solid Tumors (20) guidelines. Other laboratory and radiographic tests were obtained as required based on clinical findings. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria, v. 2. The acute effects were those occurring during CRT or within 90 days after its completion. Late toxicity was considered to be those occurring after 90 days from completion of CRT.

Study design

The primary objective of this phase I–II study was to assess the safety and toxicity of this regimen. An interim analysis of acute and late toxicity was planned after 15 patients were enrolled. Secondary objectives were to assess changes in tumor oxygenation and IFP after 2 weeks of celecoxib alone, and to assess tumor response.

Eligible patients were treated with celecoxib (Celebrex™, 4-[5-(4-methylpheny)-3–1H-pyrazol-1-yl] benzenesulfonamide) before and during CRT according to the schema in Fig. 1. Tumor oxygenation and IFP were remeasured after 2 weeks of treatment with celecoxib alone, before the start of CRT. Initially, a celecoxib dose of 400 mg orally twice daily was selected based on extrapolating preclinical data, which suggested that this would provide serum levels sufficient to inhibit angiogenesis (18, 21). This was also the dose used in a double-blind placebo-controlled trial, to reduce the number of adenomatous colorectal polyps in patients with familial adenomatous polyposis (22).

After a planned interim analysis, the celecoxib dose was reduced to 300 mg twice daily because of concerns regarding late toxicity; this dose was chosen with the aim to lower potential toxicity while maintaining high levels of biologic activity. The Princess Margaret Hospital/University Health Network Research Ethics Board approved the trial. Written informed consent was obtained from each patient before study entry.

This study was originally designed to detect a 40% relative decrease in the median HP_5 and IFP with a power of 85% and 89%, respectively, α level 0.025. The 40% relative decrease represents an absolute decrease of 20 units for HP_5 (from 50–30%) and 8 mm Hg for IFP (from 20–12 mm Hg) based on the results of our previous study (6, 7, 9). To accomplish this, 45 patients were required. However, after recognition of the cardiovascular toxicity of COX-2 inhibitors, the study was closed prematurely after accruing 31 patients (23). With this number of patients, the probability of a type II statistical error (falsely rejecting the null hypothesis of no difference in the tumor biomarkers) was 58% for HP_5 and 53% for IFP.

The absolute change in HP_5 or IFP was calculated as the difference between the value after 2 weeks of celecoxib alone and the pretreatment value. The relative change is the absolute change as a percentage of the pretreatment value. The association between

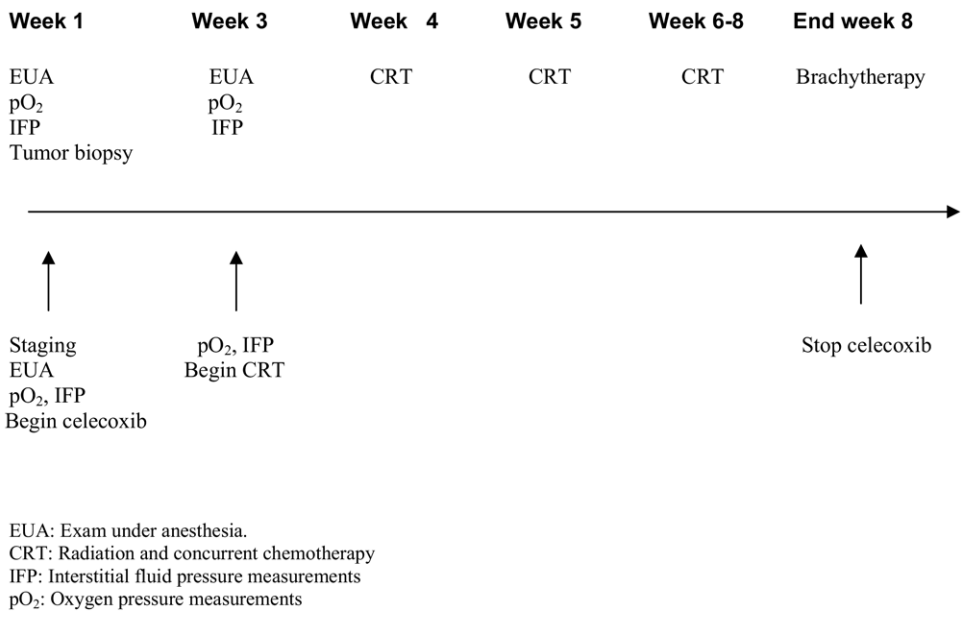

Fig. 1. Study assessments and treatment.

the relative change in HP_5 and IFP and tumor response was evaluated using the Wilcoxon rank-sum test. The survival percentages were calculated using the Kaplan-Meier method.

Standard CRT treatment

As per our institutional protocol, external beam pelvic RT was administered using anterior-posterior fields or a four-field box technique with 18–25 MV photons. The irradiated pelvic volume included the primary tumor as well as the internal, external, and lower common iliac lymph nodes. Typically, a dose of 45–50 Gy (1.8–2 Gy daily), in 25 fractions over 5 weeks, was prescribed at the isocenter, and a 2 half-value layer posterior midline attenuator was used to reduce the rectal dose by 10% to 20%. In addition, external beam para-aortic radiation to between 40 and 45 Gy was administered to 7 patients with involved para-aortic lymph nodes. After external beam radiation, patients were treated with a single intracavitary low-dose rate or pulsed-dose rate BT application using an intrauterine line source without ovoids (24). A dose of 40 Gy (dose rate, 0.5–0.8 Gy/h) was administered 2 cm lateral to the midpoint of the sources. The total RT dose was 85–90 Gy at point A.

Patients received cisplatin, 40 mg/m^2 intravenously with adequate hydration once weekly for 5 weeks, concurrent with external beam RT. Antiemetics and dexamethasone were administered and patients were monitored weekly with clinical examination, complete blood count, creatinine, electrolytes, and magnesium. If a patient developed grades 1 or 2 renal toxicity, the cisplatin dose was reduced to 30 mg/m^2 and 20 mg/m^2, respectively, and celecoxib was stopped. If a patient had other grade 2 acute toxicity, celecoxib was stopped until the toxicity resolved and then was restarted at 50% of the original dose. Celecoxib was permanently discontinued for all other grades 3 or 4 acute events that were felt to be related to the drug, or at any time per patient request.

RESULTS

Patient characteristics

Between January 2001 and April 2004, 31 patients were enrolled in this study. Table 1 shows selected baseline demographics and disease characteristics. The median tumor size was 4.8 cm (range, 2.5–15 cm). FIGO stage was: Ib–IIa (n = 9), IIb–IIIa (13), and IIIb–IVa (9). At presentation, 14 patients had node-negative disease, 14 patients were node-positive (defined as a short-axis diameter of ≥8 mm on CT or MRI), and 3 were equivocal (minimal enlarged lymph nodes classified by CT or MRI as equivocal for metastatic disease). Follow-up of surviving patients ranged from 1.1 year to 4.4 years, with a median follow-up of 2.7 years.

Table 1. Patient and tumor characteristics

	Attribute, n (%)
Tumor stages (FIGO)	
T1b–T2a	9 (29%)
T2b	12 (38.7)
T3a	1 (3.2)
T3b	7 (22.6)
T4a	2 6.5
Histologic diagnosis	
Adenocarcinoma	5 (16.1)
Adenosquamous	4 (12.9)
Squamous cell	22 (71.0)
Tumor grade	
G2	14 (45.2)
G3	12 (38.7)
Not determined	5 (16.1)
Pelvic lymph nodes	
Positive	11 (35.5)
Negative	17 (54.8)
Equivocal	3 (9.7)
Para-aortic lymph nodes	
Positive	7 (22.6)
Negative	23 (74.2)
Equivocal	1 (3.2)
Median age (y)	49 (range, 28–65)

Treatment compliance

Twenty-eight patients (90.3%) completed the planned treatment with CRT and celecoxib. Twenty-one of 31 patients (68%) received celecoxib for the full duration of CRT (56–63 days). Five patients who received celecoxib for between 7 and 35 days abandoned the treatment because of gastrointestinal toxicity. One patient abandoned celecoxib treatment after 15 days because of skin rash. Three patients received celecoxib for >30 days but refused to continue with the treatment for unspecified reasons. One patient entered the study but did not receive celecoxib because of a previously unrecognized cardiac arrhythmia (multifocal premature ventricular complex). One patient did not receive cisplatin because of renal dysfunction. Two patients had cisplatin dose modifications, one because of renal toxicity and the other because of leukopenia.

Brachytherapy was attempted in 2 patients but, as a result of unfavorable anatomy, the procedure was abandoned; one of these patients had a perforation of the lower uterine segment. Conformal external beam RT boost was utilized as an alternative to BT in these 2 patients where an intracavitary applicator could not be inserted. One patient completed her external beam RT but declined to receive either BT or external beam boost because of a skin reaction within the treatment field.

The median treatment duration for the 29 patients who received BT was 41 days (range, 40–57 days).

Toxicity assessment

Eighteen of 31 patients (58%) received celecoxib 400 mg orally twice daily in combination with CRT. An interim analysis of acute and late toxicity was performed for the first 15 patients. Acute toxicity was not found to be significantly elevated, but 2 of 15 patients (13.3%) developed grade 4 rectovaginal fistulas more than a year after completing treatment. As a consequence, the celecoxib dose was reduced to 300 mg orally twice daily, and 13 additional patients were accrued.

Seventeen Grade 3–4 acute toxicity events were seen in 11 of 31 patients (35.5%, Table 2). Hematologic toxicity was reported in 4 of 30 patients (12.9%) and was largely attributable to chemotherapy, with neutropenia reported in 2 patients, anemia in 1 patient, and leukopenia in 4 patients. Acute gastrointestinal toxicity was reported in 5 of 30 patients (16.1%), including nausea and vomiting in 3 patients and diarrhea in 2 patients. Two patients had Grade 3 skin reaction (6.5%) within the RT field. One patient (3.2%) developed a deep vein thrombosis and 1 patient (3.2%) had grade 3 urosepsis.

The major late toxicity for the whole group was Grade 4 rectovaginal fistula, seen in 3 of 31 patients (9.7%). All had a colostomy with good relief of symptoms. One patient had Grade 3 vaginal necrosis (3.2%), which healed after antibiotics and did not require surgery. Two of the 4 patients who developed Grade 3–4 late toxicity received the higher dose of celecoxib, and two the lower dose. All received celecoxib for a total duration of 50 days. Only 1 patient had a preexisting gastrointestinal inflammatory condition (diverticulitis), and 2 patients were treated with extended-field RT to the pelvis and para-aortics followed by BT. In comparison, 5 of 28 patients (18%) without fistula were treated with extended radiation. In all 3 patients with fistulas, extensive investigation, including MRI and surgical exploration at the time of gastrointesinal bypass, failed to demonstrate evidence of recurrent tumor. The actuarial likelihood of grade 3–4 late toxicity at 2 years was 13.7%.

Response and survival

Within the first year of follow-up, 25 of 31 patients (81%) achieved a complete response (CR), of which 20 remained in CR at last follow-up and 5 had a subsequent recurrence (3 in the pelvis, 1 distant, and 1 at both locoregional and distant sites). Two of 31 patients (6.5%) had a partial response (PR), 1 with locoregional and distant progression at the time of last analysis, and the other patient was lost to follow-up. One of 31 patients (3.2%) initially had stable disease, the tumor responded slowly to treatment, and 18 months after finishing treatment she achieved PR. This patient remains in PR at the time of last analysis. Three of 30 patients (9.7%) had progressive disease within the first year and all of them died with local and distant progression.

Disease-free survival (DFS) and overall survival (OS) were 62% and 92% at 3 years, respectively (Figs. 2 and 3). Neither HP_5 nor IFP were associated with DFS in this cohort of patients.

Table 2. Acute toxicity profile (a patient may be counted more than once)

Acute toxicity profile Grades 3–4	Celebrex 400 mg $n = 18$ (%)	Celebrex 300 mg $n = 13$ (%)	Total events $n = 31$ (%)
Hematologic	1 (5.6)	3 (23.1)	4 (12.9)
Gastrointestinal	3 (16.7)	2 (15.4)	5 (16.1)
Deep vein thrombosis	0	1 (7.7)	1 (3.2)
Urosepsis	1 (5.6)	0	1 (3.2)
Skin reaction	1 (5.6)	1 (7.7)	2 (6.5)
Pain	0	2 (15.4)	2 (6.5)
Fatigue	0	1 (7.7)	1 (3.2)
Synocope	0	1 (7.7)	1 (3.2)
Total events (Patients and %)	6 ($n = 6$, 63.3%)	11 ($n = 5$, 38.5%)	17 ($n = 11$, 35.5%)

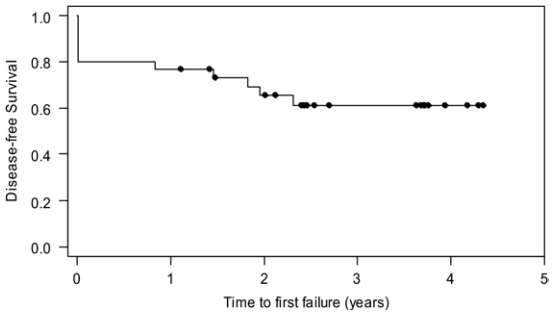

Fig. 2. Disease-free survival.

Microenvironmental biomarker assays

Twenty-one and 19 patients agreed to undergo both the pre- and post-celecoxib HP_5 and IFP measurements, respectively. The median pre-HP_5 and post-HP_5 values were 66.25% (range, 2.3–98.3%) and 68.8% (range, 0–100%), respectively. The median pre-IFP and post-IFP values were 15.6 mm Hg (range, 4.7–39.7 mm Hg) and 14.0 mm Hg (range, –1.15 to 48.1 mm Hg), respectively. The median post-celecoxib HP_5 increased by 3.75% (first and third quartile, –11.1% and 16.3%, respectively; $p = 0.5$), which represents a relative increase of 11% (first and third quartiles, –17% and 43%, respectively; $p = 0.27$), (Fig. 4). IFP decreased slightly (median, –4.6 mm Hg; first and third quartile, –9.3 and 1.1 mm Hg, respectively; $p = 0.09$), with a relative change of –21%, (first and third quartile, –53% and 8%, respectively; $p = 0.07$) Fig. 5).

No association was found between the relative change of HP_5 and IFP and the response to treatment ($p = 0.22$ and $p = 0.14$, respectively). The main caveat of this analysis is the small numbers, especially in the no-CR group ($n = 3$).

DISCUSSION

The results of the present study demonstrate that the addition of celecoxib to CRT is associated with an increased risk of toxicity in patients with cervical cancer. The overall rate of acute toxicity in this series was 33% and was largely hematologic (Grade 3–4, 12.9%) and gastrointestinal (16.1%), and attributable mainly to CRT. The overall rate of acute toxicity reported by Keys *et al.* was in the same range at 35% for CRT vs. 13% for RT alone (4).

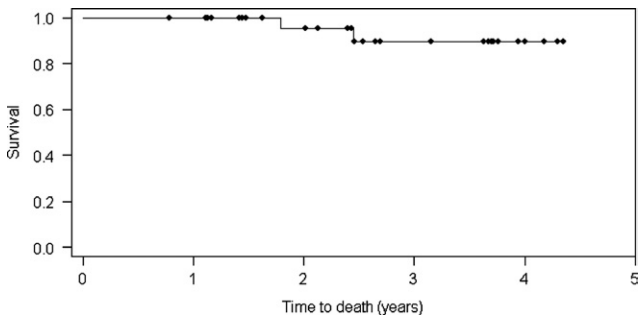

Fig. 3. Overall survival.

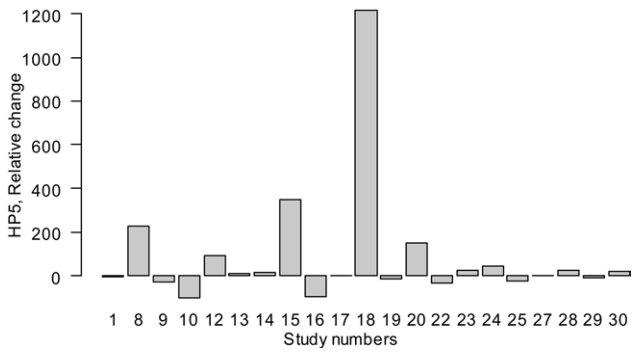

Fig. 4. Relative changes in tumor oxygenation (HP_5).

This contrasts with the phase I–II RTOG 0128 study recently published in abstract, which accrued 81 patients with advanced-stage cervical cancer, treated with celecoxib 400 mg twice daily for 1 year in combination with RT, cisplatin, and 5-fluorouracil (5-FU) chemotherapy. Thirty-five of 75 patients (47%) had Grade 3–4 acute toxicity, which was also mainly hematologic and gastrointestinal. This high rate, perhaps because of the addition of 5-FU to cisplatin, exceeded a toxicity threshold of 35% that was established in advance by the investigators, and the regimen was therefore considered unacceptable for further clinical development (25).

In our trial, 3 of 31 patients (9.7%) had grade 4 rectovaginal fistulas. All of them had advanced disease, 2 had extended field RT, and 1 had pre-existing diverticulitis. In this multifactorial context, it is difficult to ascertain if this late toxicity was related to celecoxib treatment alone. In previous cervical cancer CRT trials, the rate of serious late effects was between 6% and 23.3%, and not different from our actuarial toxicity rate of 13% (26). However, in a recent retrospective paper from our group, the 3-year probability of grade 3–4 late toxicity was only 7.6% and 6.9% for patients treated with RT alone and CRT, respectively (27). Therefore, in terms of our practice, these late toxicities are concerning.

Recognizing the limitations of a phase I–II trial, we were unable to demonstrate efficacy of celecoxib in addition to CRT, either directly or indirectly, by monitoring tumor biomarkers of response in this study. The response rate of

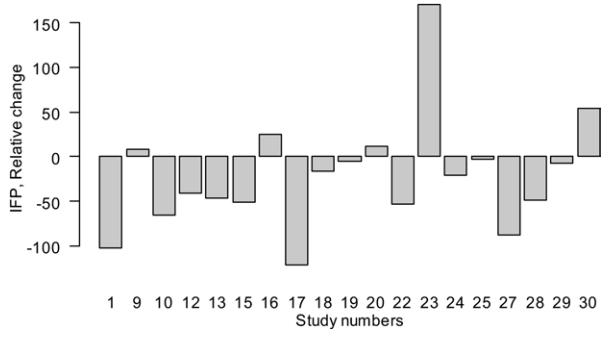

Fig. 5. Relative changes in interstitial fluid pressure (IFP).

81% within the first year of treatment is similar to the experience with CRT alone, considering that most of the regimens in advanced cervical cancer achieve pelvic control rates of 70–75%. The survival rate in this study was also similar to that reported in previous studies of combined CRT (2–4).

This study used HP_5 and IFP as biomarkers of tumor response after celecoxib treatment. This was based on our previous work that showed these parameters to be strong predictors of DFS in cervical cancer patients treated with RT alone. There was no significant change in hypoxia measurements after celecoxib alone, but an important question is whether oxygen measurements 2 weeks apart in the same untreated tumor would yield the same or different results. Cyclic hypoxia has been described in experimental animal models and human tumors (28, 29). This would suggest that sampling of tumor oxygenation at different times could reveal differences due to heterogeneous microregional blood flow fluctuation. There is only a limited amount of information on changes in hypoxia during a course of treatment, especially with noncytotoxic agents. Cooper *et al.* studied pO_2 before and after RT in cervical tumors and found that in most, but not all, tumors hypoxia decreased (30).

IFP is elevated in most solid tumors and has been shown to provide unique information about outcome in patients with cervical cancer. Patients with high IFP are significantly more likely than those with low IFP to recur after RT and die of progressive disease, independent of other prognostic factors (9, 31). Taghian *et al.* (32) showed that paclitaxel decreased IFP and improved oxygenation in breast tumors after neoadjuvant CT. Willet *et al.* (33) demonstrated improvement in several biomarkers, including IFP, in 6 patients with colorectal cancer treated with the anti-VEGF monoclonal antibody bevacizumab. There was a trend toward lower IFP in our study after 2 weeks of celecoxib, but the result did not achieve statistical significance, perhaps because the study was underpowered to detect a small difference in IFP.

The existence of alternative pathways of COX-2 signaling could explain the lack of activity of celecoxib. Recent data suggest that both isoforms, COX-1 and COX-2, contribute to carcinogenesis; this implies that nonselective inhibitors may have advantages over selective COX-2 inhibitors (34–36). In addition, the efficacy of selective COX-2 inhibition for cancer treatment could be compromised by the lack of COX-2 expression in the target lesions. It is clear that COX-2 expression is variable in human malignant tissue and that the biologic and radiation-enhancing effects associated with COX-2 inhibition occur in a COX-2 expression-dependent manner (37, 38). Synchronous coexpression of epidermal growth factor receptor (EGFR) and COX-2 has been reported in 32% of cervical tumors and was related to poor outcome (39). Cross-talk exists between these 2 oncoproteins and it is well recognized that EGFR/RAS/MAPK/PI3K signaling is responsible for the EGFR-mediated induction of COX-2 in squamous cell carcinoma lines (40). Recent studies have demonstrated that COX-2 is overexpressed four-fold in the oral mucosa of active smokers vs. those who have never smoked, and *in vitro* studies have shown that tobacco smoke stimulates EGFR tyrosine kinase (TK) activity, leading to enhanced transcription of COX-2 (41). This provides a new mechanism-based rationale for evaluating whether the combination of a COX-2 inhibitor and an EGFR TK inhibitor might be effective in cervical cancer.

CONCLUSIONS

Although there is biologic rationale for combining COX-2 inhibitors with CRT to treat patients with cervical cancer, our results and those from the RTOG trial suggest that the potential toxicity of COX-2 inhibitors probably offsets any small benefit that might exist and is likely to constrain further investigation of these agents in this clinical context. Therefore, alternate biologic strategies for improving the outcome of patients with cervical cancer should be explored, in combination with evolving techniques for more precise RT delivery. We have chosen instead to evaluate more specific inhibitors of tumor angiogenesis in combination with CRT, aimed at overcoming the unfavorable biologic features of the tumor microenvironment that promote tumor progression and radiation resistance and, ultimately, improving patient survival.

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