Inhibition of Cyclooxygenase-2 by Celecoxib Reverses Tumor-Induced Wasting

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Received November 17, 2003; accepted November 21, 2003

ABSTRACT

There have been a number of reports suggesting inhibition of prostaglandin production may impact tumor-mediated wasting and levels of associated humoral factors such as hypercalcemia. These reductions were achieved using traditional nonsteroidal anti-inflammatory drugs (NSAIDs), which are often contraindicated in cancer patients. This is especially true during chemotherapeutic regimens due to concerns of bleeding from gastrointestinal and hematopoietic toxicities associated with inhibition of the housekeeping cyclooxygenase enzyme COX-1. Here, we report that celecoxib, one of the new class of selective COX-2 inhibitors, has the potential to reverse tumor-mediated wasting and associated humoral factors such as interleukin (IL)-6 and hypercalcemia in preclinical models of cachexia. Tumor bearing mice in late stage cachexia regained weight within days of the start of celecoxib treatment. Two models were tested. The first was the Colon 26 (Col26) syngeneic murine model that induces high levels of circulating IL-6 and hypercalcemia. The second was the human head and neck 1483 HNSCC xenograft model, which is less inflammatory and produces less prostaglandin than Col26. Despite the observation that no significant impact on tumor growth was observed between vehicle and celecoxib-treated animals over the course of the studies, celecoxib rapidly reversed weight loss in both cachectic models. With the added safety of celecoxib over traditional NSAIDs, these results suggest a possible therapeutic use for celecoxib for treating tumor-mediated wasting.

Cachexia, characterized by a selective loss of lean body mass, is a critical problem in cancer patients, with up to 20% of cancer deaths directly attributable to this syndrome (Fearon and Carter, 1988; Tisdale, 1993, 2002). Patients with even a modest weight loss at time of cancer diagnosis (~5%) show a poorer prognosis and a reduced response to chemotherapy (Dewys et al., 1980). Cachexia differs from anorexia or starvation in that weight is lost from skeletal muscle and fat stores equally, whereas anorexia/starvation targets fat stores and visceral protein for degradation (Fearon, 1992). Furthermore, although anorexia and starvation can be attributed to reduced food intake, weight loss from cachexia cannot. In fact, nutritional supplementation given to cachectic patients often leads to a transitory increase in body fat rather than in lean muscle mass (Evans et al., 1985). This loss of muscle mass leads to a reduction in quality of life and general well being. Numerous circulating factors have been implicated in cachexia, including increased levels of cytokines and eicosanoids (Fearon and Moses, 2002; Tisdale, 2002). A therapeutic approach used by some oncologists to combat cachexia involves reducing production of proinflammatory cytokines (Popiela et al., 1989) by progestogens, such as medroxyprogesterone acetate (megestrol acetate) (Loprinzi et al., 1992). The synthetic progestogens seem to affect appetite and in some cases lead to weight gain. However, they have proven less effective than once hoped because the weight gain, if observed, is often largely due to edema and fat tissue gain (Tisdale, 2002).

There is also increasing evidence that eicosanoids such as prostaglandins have a role in the development of cachexia. Review of the literature showed a study from as early as 1975 demonstrating that indomethacin (a nonsteroidal anti-inflammatory, NSAID) was able to inhibit levels of urinary PGE2 metabolites and this reduction correlated with reduced serum hypercalcemia (Seyberth et al., 1975), a condition often associated with cachexia. Hypercalcemia with cancer is classified as either humoral hypercalcemia of malignancy (caused by circulating hormones) or local osteolytic hypercalcemia (caused by paracrine factors secreted by cancers that act on bone metabolism). Nearly all cases of humoral hypercalcemia of malignancy can be attributed to increases in

ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; IL, interleukin; COX, cyclooxygenase; TXB2, thromboxane B2; SC-560, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-H pyrazole.
parathyroid hormone-related peptide, whereas the mediators of local osteolytic hypercalcemia in bone are heterogeneous and include transforming growth factors α and β, interleukin (IL)-1, IL-6, prostaglandins, and tumor necrosis factor. Hypercalcemia is uncommon at presentation (<1% of patients) but becomes more common as the cancer progresses and is present in 10 to 20% of patients near the time of death (Johnson, 2003).

Prostaglandin release is a component of the signaling cascade in skeletal muscle protein turnover in vitro, which suggest these agents may play a role in pathologies of muscle catabolism, and therefore wasting (for review, see Thompson and Palmer, 1998). Aside from direct effects on muscle biology, prostaglandins can regulate the expression of proinflammatory cytokines as well, such as IL-6 and tumor necrosis factor-α (Rothwell, 1992). NSAIDs have been shown to block the protein catabolic effects of serum from cachectic mice (Smith and Tisdale, 1993) and have been reported to prevent muscle protein breakdown in some tumor-bearing rats, with no observed increase in food intake (Homem-de-Bittencourt Junior et al., 1989), although this observation does not seem to be universal (McCarthy, 1999).

Short-term administration of the NSAID ibuprofen reduced hypermetabolism and acute phase response in colon cancer and pancreatic cancer patients, suggesting an antica- chetic benefit via lowering energy expenditure (McMillan et al., 1995; Preston et al., 1995; Wigmore et al., 1995). Another study by Lundholm et al. found a survival advantage from indomethacin treatment among late stage cancer patients, although cachexia was not specifically addressed (Lundholm et al., 1994). A prospective study comparing maeostag acetate versus meastag acetate/ibuprofen was recently done in gastrointestinal cancer patients (McMillan et al., 1999). Of those evaluable at 12 weeks (38%), there was a decrease in weight (median 2.8 kg) in the megestrol acetate/placebo group compared with an increase (median 2.3 kg) in the megestrol acetate/ibuprofen group (P < 0.001). There was also an improvement in the EuroQol-EQ-5D quality of life scores of the latter group (P < 0.05).

However, chronic use of traditional NSAIDs is not wides- pread in cancer patients due to concerns about inhibition of COX-1 activity and possible adverse effects on mucosal (especially gastrointestinal) and hematological tissues, particularly in the context of chemotherapy. The discovery of the inducible form of cyclooxygenase, COX-2, and subsequent development of selective COX-2 inhibitors raises the possibility of safely reducing tumor-mediated prostaglandin lev- els, which may lead to control of some aspects of cachexia.

COX-2 is induced in many human tumors and is associated with aberrant angiogenesis in a number of pathological settings, especially those involving inflammation. Selective COX-2 inhibitors such as celecoxib have been demonstrated to have potent antitumor (prevention) and growth inhibitory effects in preclinical tumor models (McEntee et al., 1999; Williams et al., 1999; Jacoby et al., 2000; Masferrer, 2001; Leahy et al., 2002; Zweifel et al., 2002). We have observed in numerous models that tumor-bearing animals treated with COX-2 inhibitors retained body weight and overall health compared with vehicle-treated animals. This held true even when treated tumors eventually exceeded the size in which vehicle-treated animals had to be sacrificed. We have conducted specific studies in two tumor models where cachexia is apparent to explore the possible effects of COX-2 inhibition on tumor-induced wasting. It was observed that acute treatment of severely cachectic tumor-bearing animals with the COX-2 inhibitor celecoxib maintained or reduced serum calcium levels and produced a rapid and significant weight gain (reversal of weight loss) compared with tumor-matched vehicle-treated animals. In one model, this also correlated with reduced circulating IL-6 observable as early as 24 h after initiation of treatment. These data suggest that COX-2 derived prostaglandins can mediate cachexia and further suggest a possible benefit for the use of celecoxib in the treat- ment of tumor-induced wasting.

Materials and Methods

Tumor Models. Colon 26 (Col 26) tumor cells were obtained from the NCI. Col 26 is a COX-2/COX-1 positive murine colon adenocarcinoma line maintained in RPMI 1640 medium (In Vitrogen, Carls- bad, CA) supplemented with 10% fetal bovine serum with 2 mM l-glutamine. Col 26 cells (3 × 10⁶) were suspended in 30% matrigel (BD Biosciences, San Jose, CA) in Hank’s balanced salt solution (In Vitrogen) and implanted into the right hind paw of athymic nude mice. Human head and neck squamous cell carcinoma (HNSCC 1483) tumor cells (Zweifel et al., 2002) are COX-2/COX-1 positive and were maintained in DMEM/F-12 medium (In Vitrogen) supplemented with 10% fetal bovine serum, 15 mM HEPES buffer, 2 mM l-glutamine, 2.0 mg/l pyridoxine hydrochloride, and 0.05 mg/ml gentami- cin. HNSCC H&N 1483 cells (1 × 10⁶) were suspended in 30% matrigel (BD Biosciences) in Hanks balanced salt solution (In Vitrogen) and implanted into the right hind paw of athymic nude mice. In both models, body weights and tumor volume were measured twice weekly using a plethysmometer. As described under Results and Discussion, body weights and tumor volume were measured before dosing, at 48 h and at the end of the study (96 h). At the end of the 4 days of dosing, mice were euthanized by CO2 inhalation, tumor tissue was excised, and blood drawn for serum. All animal treatment protocols were reviewed by and were in compliance with Pharmacia’s Institutional Animal Care and Used Committee.

COX Inhibitors. Celecoxib and SC-560 were manufactured by Pharmacia (Peapack, NJ). Indomethacin was purchased from Sigma Aldrich (St. Louis, MO). Drugs were administered by oral gavage b.i.d. in a solution of 0.5% methylcellulose (Sigma-Aldrich) and 0.025% Tween 20 (Sigma-Aldrich). Celecoxib therapy that was in the diet was initiated when tumors reached a mean volume 0.1 ml (Fig. 1) or 0.6 ml (Fig. 2) and maintained for the duration of the experiment (160 and 1600 ppm, equivalent to 25 and 250 mg/kg/day p.o. or 250 mg/kg/day via gavage b.i.d.). Indomethacin was administered via gavage at 2 mg/kg/day b.i.d. SC-560 was administered at 3 mg/kg/ day via gavage b.i.d.

Description of Statistical Methodology. Treatment group means are compared based on a one-way analysis of variance, on the raw data values (parametric analysis), or on the ranks of the raw data values (nonparametric analysis). The α = 0.05 level of signifi- cance is used for the comparison of means. The means comparison method used is least significant differences. This method provides more significant differences than other means comparison proce- dures. The LSD means comparison procedure uses the pooled within group mean square error as the common estimate of the variance for all means comparisons. S.P.E.M. is used throughout.

Tumor Prostaglandin Analyses. Tumors were harvested, fil- leted from skin and bones of the foot pad, and snap-frozen in liquid N₂. Tumor tissue was then pulverized with mortar and pestle and maintained at ~80°C until 100 mg tissue/1 ml of methanol was solubilized to extract the lipid fraction, dried under N₂, and assayed using enzyme-linked immunoassortant assay for PGE₂ and thrombox- ane B₂ levels (Cayman Chemical, Ann Arbor, MI).
**IL-6, Ca²⁺, Blood Glucose, and Serum Thromboxane Analyses.** Blood was clotted at 4°C for 24 h and centrifuged for 5 min at 6000 rpm. Mouse IL-6 was determined using Quantikine Immunoassay (R&D Systems, Minneapolis, MN). Serum calcium was determined with a calcium kit (Sigma-Aldrich). Serum was diluted into enzyme immunoassay buffer, and TXB₂ was measured by enzyme immunoassay (Cayman Chemical). Blood glucose levels were measured from a drop of whole blood placed on an Ascensia Elite blood glucose test strip and analyzed with an Ascensia Elite blood glucose meter (Bayer Corp., Mishawaka, IN).

**Measurement of Food Intake.** Amount of food (pelleted chow) and each animal was weighed on day 0, day 2, and day 4. Animals were housed four per cage. Food intake per gram of body weight was calculated as (gram food eaten/total mouse gram per cage/no. of days). This value was assessed at day 2 and day 4 of the study. Because there were only two cages per treatment group, statistical analysis was not possible.

**Results**

Many studies have implicated COX-2 in tumorigenesis (Oshima et al., 1996; William et al., 1999; Liu et al., 2001). Also, several studies have shown that inhibitors of COX-2 activity result in the prevention of tumor generation and growth (Grubbs et al., 2000; Abou-Issa et al., 2001; Leahy et al., 2002; Zweifel et al., 2002). Interestingly, while assessing the antitumor activities of celecoxib in a number of preclinical tumor models, it was observed that tumor-dependent body weight loss was prevented by celecoxib treatment. For example, celecoxib inhibits the COX-2-positive HNSCC 1483 tumor growth 82–95% when administered in the feed at doses from 160 to 1600 ppm (approximately equivalent to doses of 25–250 mg/kg/day of celecoxib). Figure 1A shows a representative tumor growth curve with 160 ppm celecoxib versus vehicle control. Vehicle-treated animals began to lose body weight when the tumors reached 0.5 ml in volume (day 22), resulting in a rapid deterioration in the animals' health. These animals were sacrificed on day 28 when tumors averaged 1.0 ml in volume (Fig. 1A) and animals had lost 5.1 g from their average net body weight (Fig. 1B).

In contrast, celecoxib-fed mice maintained body weight throughout the treatment period of 64 days (Fig. 1B). In fact, the celecoxib-treated animals did not lose weight even when the tumors reached an average of 0.8 ml in volume at day 64 (Fig. 1, A and B). Similar results with regard to tumor growth control and body weight maintenance were observed in the COX-2-positive Col26 syngeneic model. In this model, tumors reached the volume 1.8 ml by day 18, and animals sacrificed on day 22 had lost 1.0 ml in volume (Fig. 1A) and had lost 7.1 g from their average net body weight (Fig. 1B).
tumor implantation (Fig. 1D). In contrast, celecoxib inhibited tumor growth by 91% at day 18 (Fig. 1C) and so were kept until day 32 when tumors reached volumes of 1.1 ml (Fig. 1C). Celecoxib-treated animals maintained net body weight from the beginning to the end of the study (22 versus 22.7 g; Fig. 1D). Thus, in these two models, celecoxib was clearly able to delay tumor growth and later proved very effective in controlling tumor-induced wasting even in animals carrying a large tumor burden. This suggests that COX-2-derived prostaglandins have a role not only in tumor growth but also may impact systemic wasting.

To directly assess the effects of celecoxib on tumor-mediated wasting observed in HNSCC 1483 and Col26 tumor-bearing animals, studies were designed using late-stage, cachectic animals treated acutely (4 days). Under these conditions, changes in body weight, but not in tumor growth kinetics, were observed. Studies were begun when animals had lost ~15% of their maximal net body weight (Col26-bearing animals, 16.8 g; HNSCC 1483-bearing animals, 24 g). Furthermore, to determine the contribution of COX-1 versus COX-2-derived prostaglandins in wasting we compared the effects of celecoxib to the nonselective COX-1/COX-2 inhibitor indomethacin, and the COX-1-selective inhibitor SC-560 (Smith et al., 1998). Tumor-bearing animals treated with either celecoxib or indomethacin showed a reversal of weight loss and posted net weight gains at the end of 96 h of treatment (Fig. 2). In the HNSCC 1483 tumor model, vehicle-treated animals lost a median of 2.0 ± 0.6 g (8.8%) over the 4 days of the study, whereas celecoxib-treated animals gained 0.6 ± 0.4 g (2.3%), a difference of 2.6 g (Fig. 2A). Similar results were observed with indomethacin with a weight gain of 0.8 ± 0.8 g (3.3%) over the same period (Fig. 2A). To demonstrate that the effects of celecoxib and indomethacin were mediated via COX-2 inhibition and not due to COX-1, the selective COX-1 inhibitor SC-560 was also assessed (Smith et al., 1998). SC-560 at 3 mg/kg/day, a dose that clearly inhibits COX-1 activity (measured as serum TXB2: 7.8 ± 1.2 ng/ml versus 40 ± 12.4 ng/ml for vehicle) did not reverse tumor-induced wasting. In fact, these animals continued to lose 2.6 ± 1.4 g (10.5%) of net body weight during the 4 days of treatment, similar to vehicle animals (Fig. 2A). Based on the efficacy of celecoxib and indomethacin, but not SC-560, the results of this study strongly suggest a role of COX-2-derived prostaglandins in tumor-induced wasting.

In the Col26 model, the study was initiated in animals bearing large tumors averaging 1.8 ml in volume. Although these animals had already lost 15% of their net body weight, a further median loss of 1.4 ± 0.4 g (9.9%) was observed in the vehicle-treated animals during the 4 days of the study. In contrast and similar to the results observed in the HNSCC 1483 model, animals treated with celecoxib or indomethacin gained 1.0 ± 0.3 g (5.7%) and 0.7 ± 0.3 g (5.1%) of net body weight, respectively, over the 96-h dosing schedule (Fig. 2B). Thus, inhibition of COX-2 activity (celecoxib or indomethacin) reversed the tumor-mediated weight loss in two independent models of cachexia, whereas a selective COX-1 inhibitor (SC-560) had no effect on wasting when tested in the HNSCC 1483 tumor model.

A recent report with a model of LPS-induced systemic immunological inflammation has linked COX-2 inhibition with reversal of the observed anorexia (Johnson et al., 2002). We therefore sought to determine whether the positive changes in net weight caused by the COX-2 inhibitors were due to differences in food intake (anorexia) between treatment groups. It was observed, however, that 1483 tumor-bearing animals consumed equal amounts of food per gram of body weight daily over the 4-day period compared with non-tumor-bearing controls (0.20 g), indicating that these animals did not suffer from anorexia. Also, similar food intake per gram of body weight was observed between treatment groups and vehicles over the 4-day study. The average consumption for the HNSCC 1483 model ranged between 0.20 and 0.23 g of food/g body weight/day for all of the groups. The average consumption in the Col26 model ranged between
TABLE 1
Intratumor PG levels are reduced by celecoxib and indomethacin
Both H&N 1483 and Col26 tumors contain high levels of COX-2-derived prostaglandins that are reduced by celecoxib or indomethacin treatment. The COX-1 inhibitor SC-560 did not produce a significant reduction in PGE2 or PGF2α in H&N 1483 tumors.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>H&amp;N 1483</th>
<th>H&amp;N 1483</th>
<th>Col26</th>
<th>Col26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Type</td>
<td>PGE2</td>
<td>PGE-2a</td>
<td>PGE2</td>
<td>TxB2</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------</td>
<td>----------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Vehicle</td>
<td>394.98 ± 29.71</td>
<td>254.84 ± 61.34</td>
<td>1635.25 ± 172.22</td>
<td>111.14 ± 20.87</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>24.71 ± 8.12*</td>
<td>23.99 ± 8.59*</td>
<td>202.9 ± 57.3*</td>
<td>35.81 ± 10.81*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6.73 ± 0.59*</td>
<td>3.77 ± 0.82*</td>
<td>8.94 ± 4.27*</td>
<td>7.65 ± 1.94*</td>
</tr>
<tr>
<td>SC-560</td>
<td>447.52 ± 74.93</td>
<td>220.77 ± 20.36</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.
* Statistically different from vehicle, P < 0.05.

0.13 and 0.17 g/g body weight/day, for all groups. These data indicate that the reversal of weight loss observed after COX-2 inhibition was not solely attributable to increased food intake.

Blood glucose levels were also determined at the end of the studies. No treatment affected blood glucose levels in the Col26 study (116.3 ± 2.5, 112.3 ± 6.4, and 111 ± 7.6 mg/dl for vehicle, celecoxib, and indomethacin-treated groups). Similarly, no treatment had a statistically significant effect on blood glucose levels in the 1483 model (vehicle, 124.8 ± 14.6; indomethacin, 124.7 ± 14.2; celecoxib, 157.1 ± 9.6; SC-560, 111.7 ± 13.2 mg/dl), although celecoxib treatment did slightly elevate blood glucose.

To demonstrate that celecoxib and indomethacin efficiently inhibited the COX-2 enzyme, tumors were excised and levels of major prostaglandins were reduced by enzyme-linked immunoabsorbent assay. As shown in Table 1, intratumoral levels of prostaglandins were reduced by more than 90% by both inhibitors of COX-2 (Table 1 in contrast). SC-560 did not inhibit intratumoral PGE2 or PGF2α levels, indicating that COX-1 does not contribute to overall prostaglandin synthesis in this tumor (Table 1). In the Col26 model, PGE2 and TxB2 are the major prostanoids observed. Again, celecoxib and indomethacin reduced these prostanoids substantially (Table 1).

Hypercalcemia was observed in both tumor models tested, with calcium levels reaching 16 ± 0.9 and 12.2 ± 0.6 mg/dl for the 1483 and Col26 models, respectively. Celecoxib and indomethacin reduced or stabilized circulating calcium levels in both tested models, with the reduction reaching statistical significance with celecoxib in both models and indomethacin in the Col26 model (Table 2). Although hypercalcemia is often observed concurrently with cachexia, its role in the malady is unclear.

Proinflammatory cytokines such as IL-6 have been implicated in numerous models of cachexia, including the Col26 model (Strassmann et al., 1993; Soda et al., 1995a,b), and cachexia has been at least partially inhibited/reversed with a neutralizing IL-6 antibody in this model (Fujita et al., 1996). We observed that both celecoxib and indomethacin stabilized circulating IL-6 levels, whereas vehicle-treated animals went from 189 to 289 pg/ml (P < 0.05) during the 4-day study (Table 2).

Discussion
In conclusion, some current therapies to control cachexia are targeted toward the proinflammatory cytokines. This approach has some activity with appetite stimulation and weight gain; however, the majority of this is in the fat reserves, not the skeletal muscle. Preclinical data (Gelin et al., 1988; Homem-de-Bittencourt et al., 1989), and recently some limited clinical data in cancer patients, have suggested that modification of the eicosanoid factors associated with cachexia [either dietary via eicosapentaenoic acid (Wigmore et al., 2000) or pharmacologically via ibuprofen (Preston et al., 1995; McMillan DC et al., 1999)] can yield gains in muscle mass (for review, see Ross and Fearon, 2002). Here, we have shown that tumor-derived, COX-2-mediated prostaglandins contribute to tumor-induced wasting in two tumor models demonstrating cachexia and that the selective COX-2 inhibitor celecoxib can rapidly reverse this trend. Animals treated with celecoxib or indomethacin seemed more active than vehicle or SC-560-treated cohorts. Further studies are needed to determine the mechanism of this effect, and clinical trials will be necessary to establish this activity in humans.

References


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