

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

Thomas W. Davis, Ben S. Zweifel, Janet M. O'Neal, Deborah M. Heuvelman, Ann L. Abegg, Todd O. Hendrich, and Jaime L. Masferrer

Oncology, PTC Therapeutics, South Plainfield, New Jersey (T.W.D.); and Oncology Discovery Research, Pfizer Corporation, St. Louis, Missouri (B.S.Z., J.M.O., D.M.H., A.L.A., T.O.H.)

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

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.
DOI: 10.1124/jpet.103.063099.

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 anticachectic 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 megestrol acetate versus megestrol 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 widespread 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 levels, 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 treatment 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 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum with 2 mM L-glutamine. Col 26 cells (3×10^6) were suspended in 30% matrigel (BD Biosciences, San Jose, CA) in Hank's balanced salt solution (Invitrogen) and implanted into the right hind paw of BALB/c male 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 (Invitrogen) 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 gentamicin. HNSCC H&N 1483 cells (1×10^6) were suspended in 30% matrigel (BD Biosciences) in Hanks balanced salt solution (Invitrogen) and implanted into the right hind paw of athymic male 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 $\alpha = 0.05$ level of significance 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 procedures. 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.E.M. is used throughout.

Tumor Prostaglandin Analyses. Tumors were harvested, filleted 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 immunosorbent assay for PGE₂ and thromboxane B₂ levels (Cayman Chemical, Ann Arbor, MI).

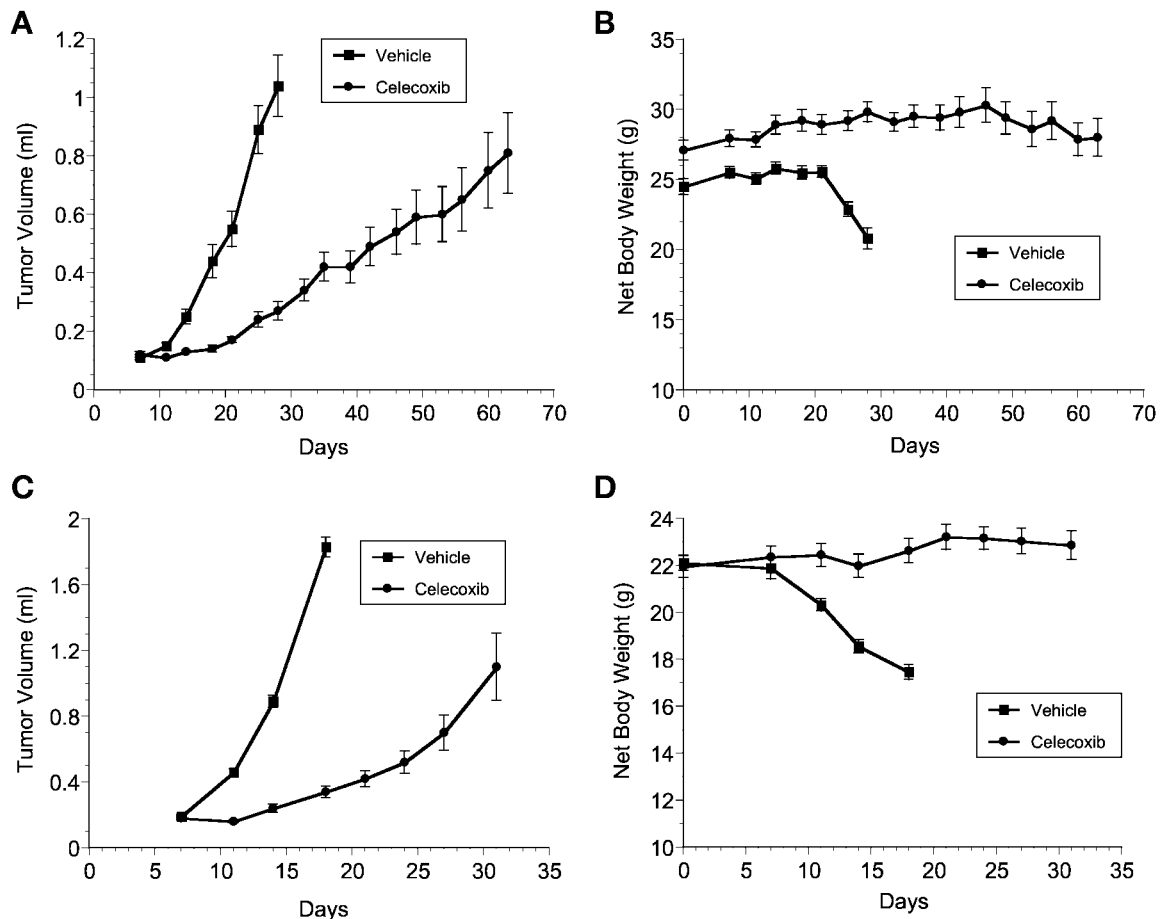


Fig. 1. Tumor growth kinetics and body weights of the HNSCC 1483 and Col26 tumor models. A, celecoxib (■, 160 parts per million in the feed) delays tumor growth of HNSCC 1483 tumors compared with vehicle-treated animals; ●, tumor cells were implanted in the right hind footpad of nude mice and treatment was begun when tumors reached 0.1 ml in volume. B, net body weights during HNSCC 1483 tumor growth, note celecoxib-treated animals did not lose weight as vehicle-treated animals. C, celecoxib delays Col26 tumor growth in BALB/c mice (■, 250 mg/kg/day celecoxib in meal; ●, vehicle-treated). Tumors were established as for HNSCC 1483. D, celecoxib prevents tumor-induced body weight loss. Dosing began on day 7 when tumors averaged 0.1 ml in volume (HNSCC 1483 model) or 0.2 ml (Col26 model), $n = 8$ animals/group.

IL-6, Ca^{2+} , Blood Glucose, and Serum Thromboxane Analyses. Blood was clotted at 4°C for 24 h and centrifuge 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 TXB_2 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 (Fig. 1C). These vehicle-treated animals averaged 17.2 g in net body weight (subtracting tumor), a 4.8-g loss of average net body weight since

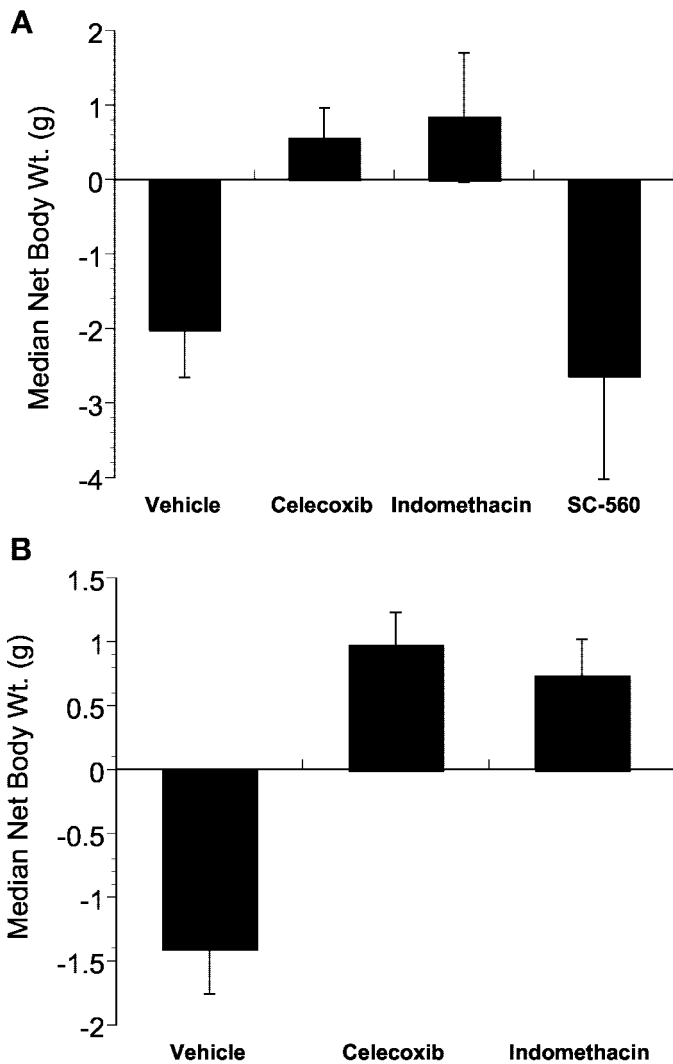


Fig. 2. Effects of COX inhibitors on cachexia. Tumors were allowed to grow until tumor-induced cachexia had resulted in ~15% of body weight loss before therapeutic treatment was started (A, HNSCC 1483 tumor growth; B, Col26) with celecoxib (250 mg/kg/day, ●), indomethacin (2 mg/kg/day, ▲), or SC-560 (3 mg/kg/day, ◆) (HNSCC 1483 tumors, a) or celecoxib ● or indomethacin ▲ (Col26, C). Tumor growth was not different between any treatment group over the 4-day study. Inhibitors of COX-2 reversed tumor-induced weight loss. Celecoxib- and indomethacin-treated animals gained weight over the course of the study, whereas vehicle-treated animals continued to lose body weight (A and B). The COX-1 inhibitor SC-560 did not effect wasting of HNSCC 1483-bearing animals (A). Shown are data from experiments representative of at least three repeated experiments with between six and eight animals per treatment group.

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 2.6g (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 TXB_2 : 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 PGF₂-α in H&N 1483 tumors.

| Tumor Type Treatment Group | H&N 1483 PGE ₂ | H&N 1483 PGF ₂ -α | Col26 PGE ₂ | Col26 TxB ₂ |
|-------------------------------|------------------------------|---------------------------------|---------------------------|---------------------------|
| | <i>pg/g</i> | | | |
| Vehicle | 394.98 ± 29.71 | 254.84 ± 61.34 | 1635.25 ± 172.22 | 111.14 ± 20.87 |
| Celecoxib | 24.71 ± 8.12* | 23.99 ± 8.59* | 202.9 ± 57.3* | 35.81 ± 10.81* |
| Indomethacin | 6.73 ± 0.59* | 3.77 ± 0.82* | 8.04 ± 4.27* | 7.65 ± 1.24* |
| SC-560 | 447.52 ± 74.93 | 220.77 ± 20.36 | ND | ND |

ND, not determined.

* Statistically different from vehicle, $P < 0.05$.

0.13 and 0.17g/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 determined by enzyme-linked immunosorbent 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 PGF₂-α 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

TABLE 2

COX-2 inhibition reduces circulating IL-6 and calcium levels

Circulating levels of calcium and IL-6 were elevated in animals bearing Col26 tumors concurrent with wasting. Celecoxib or indomethacin treatment moderated these levels. Only elevated calcium was observed in animals bearing H&N 1483 tumors. Celecoxib-treated animals had significantly lower levels of circulating calcium than vehicle controls.

| Treatment | Col26 IL-6 | Col26 Ca ⁺ | H&N 1483 Ca ⁺ |
|--------------|----------------|--------------------------|-----------------------------|
| | <i>pg/ml</i> | | <i>mg/dl</i> |
| Normal | 110.5 ± 7.0 | 9.1 ± 0.2 | 10.5 ± 0.1 |
| Vehicle | 289.1 ± 53.5 | 12.2 ± 0.6 | 16.0 ± 0.8 |
| Celecoxib | 182.5 ± 15.8* | 10.3 ± 0.3* | 13.8 ± 0.5* |
| Indomethacin | 144.33 ± 16.5* | 9.5 ± 0.3* | 14.5 ± 0.6 |
| SC-560 | nt | nt | 14.6 ± 0.3 |

nt, not tested.

* Statistically different from vehicle, $P < 0.05$.

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

- Abou-Issa HM, Alshafie GA, Seibert K, Koki AT, Masferrer JL, and Harris RE (2001) Dose-response effects of the COX-2 inhibitor, celecoxib, on the chemoprevention of mammary carcinogenesis. *Anticancer Res* 21:3425–3432.
- Dewys WD, Begg C, Lavin PT, Band PR, Bennett JM, Bertino JR, Cohen MH, Douglass HO Jr, Engstrom PF, Ezdinli EZ, et al. (1980) Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group. *Am J Med* 69:491–497.
- Evans WK, Makuch R, Clamon GH, Feld R, Weiner RS, Moran E, Blum R, Shepherd FA, Jeejeebhoy KN, et al. (1985) Limited impact of total parenteral nutrition on nutritional status during treatment for small cell lung cancer. *Cancer Res* 45:3347–3353.
- Fearon KC (1992) The mechanisms and treatment of weight loss in cancer. *Proc Nutr Soc* 51:251–265.
- Fearon KC and Carter DC (1988) Cancer cachexia. *Ann Surg* 208:1–5.
- Fearon KC and Moses AG (2002) Cancer cachexia. *Int J Cardiol* 85:73–81.
- Fujita J, Tsujinaka T, Yano M, Ebisui C, Saito H, Katsume A, Akamatsu K, Ohsugi Y, Shiozaki H, and Monden M (1996) Anti-interleukin-6 receptor antibody pre-

- vents muscle atrophy in colon-26 adenocarcinoma-bearing mice with modulation of lysosomal and ATP-ubiquitin-dependent proteolytic pathways. *Int J Cancer* **68**: 637–643.
- Gelin J, Moldawer LL, Lonnroth C, deMan P, Svanborg-Eden C, Lowry SF, and Lundholm KG (1988) Appearance of hybridoma growth factor/interleukin-6 in the serum of mice bearing a methylcholanthrene-induced sarcoma. *Biochem Biophys Res Commun* **157**:575–579.
- Grubbs CJ, Lubet RA, Koki AT, Leahy KM, Masferrer JL, Steele VE, Kelloff GJ, Hill DL, and Seibert K (2000) Celecoxib inhibits *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. *Cancer Res* **60**:5599–5602.
- Homem-de-Bittencourt Junior PI, Pontieri V, Curi R, and Lopes OU (1989) Effects of aspirin-like drugs on Walker 256 tumor growth and cachexia in rats. *Braz J Med Biol Res* **22**:1039–1042.
- Jacoby RF, Seibert K, Cole CE, Kelloff G, and Lubet RA (2000) The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res* **60**:5040–5044.
- Johnson B (2003) Paraneoplastic syndromes, in *Harrison's Principles of Internal Medicine* (Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL and Jameson JL eds) McGraw-Hill Companies, New York.
- Johnson PM, Vogt SK, Burney MW, and Muglia LJ (2002) COX-2 inhibition attenuates anorexia during systemic inflammation without impairing cytokine production. *Am J Physiol Endocrinol Metab* **282**:E650–E656.
- Leahy KM, Ornberg RL, Wang Y, Zweifel BS, Koki AT, and Masferrer JL (2002) Cyclooxygenase-2 inhibition by celecoxib reduces proliferation and induces apoptosis in angiogenic endothelial cells in vivo. *Cancer Res* **62**:625–631.
- Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, and Hla T (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J Biol Chem* **276**:18563–18569.
- Loprinzi CL, Johnson PA, and Jensen M (1992) Megestrol acetate for anorexia and cachexia. *Oncology* **49** (Suppl 2):46–49.
- Lundholm K, Gelin J, Hyltander A, Lonnroth C, Sandstrom R, Svaninger G, Korner U, Gulich M, Karrefors I, Norli B, et al. (1994) Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. *Cancer Res* **54**:5602–5606.
- Masferrer J (2001) Approach to angiogenesis inhibition based on cyclooxygenase-2. *Cancer J* **7** (Suppl 3):S144–S150.
- McCarthy DO (1999) Inhibitors of prostaglandin synthesis do not improve food intake or body weight of tumor-bearing rats. *Res Nurs Health* **22**:380–387.
- McEntee MF, Chiu CH, and Whelan J (1999) Relationship of β -catenin and Bcl-2 expression to sulindac-induced regression of intestinal tumors in Min mice. *Carcinogenesis* **20**:635–640.
- McMillan DC, Leen E, Smith J, Sturgeon C, Preston T, Cooke TG, and McArdle CS (1995) Effect of extended ibuprofen administration on the acute phase protein response in colorectal cancer patients. *Eur J Surg Oncol* **21**:531–534.
- McMillan DC, Wigmore SJ, Fearon KC, O'Gorman P, Wright CE, and McArdle CS (1999) A prospective randomized study of megestrol acetate and ibuprofen in gastrointestinal cancer patients with weight loss. *Br J Cancer* **79**:495–500.
- Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, and Taketo MM (1996) Suppression of intestinal polyposis in *Apc* δ 716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* **87**:803–809.
- Popiela T, Lucchi R, and Giongo F (1989) Methylprednisolone as palliative therapy for female terminal cancer patients. The Methylprednisolone Female Preterminal Cancer Study Group. *Eur J Cancer Clin Oncol* **25**:1823–1829.
- Preston T, Fearon KC, McMillan DC, Winstanley FP, Slater C, Shenkin A, and Carter DC (1995) Effect of ibuprofen on the acute-phase response and protein metabolism in patients with cancer and weight loss. *Br J Surg* **82**:229–234.
- Ross JA and Fearon KC (2002) Eicosanoid-dependent cancer cachexia and wasting. *Curr Opin Clin Nutr Metab Care* **5**:241–248.
- Rothwell NJ (1992) Eicosanoids, thermogenesis and thermoregulation. *Prostaglandins Leukot Essent Fatty Acids* **46**:1–7.
- Seyberth HW, Segre GV, Sweetmen BJ, Potts JT, and Oates JA (1975) Prostaglandins as mediators of hypercalcemia associated with certain types of cancer. *N Engl J Med* **293**:1278–1285.
- Smith CJ, Zhang Y, Kobolt CM, Muhammad J, Zweifel BS, Shaffer A, Talley JJ, Masferrer J, Seibert K, and Isakson P (1998) Pharmacological analysis of COX-1 in inflammation. *Proc Natl Acad Sci USA* **95**:13313–13318.
- Smith KL and Tisdale MJ (1993) Mechanism of muscle protein degradation in cancer cachexia. *Br J Cancer* **68**:314–318.
- Soda K, Kawakami M, Kashii A, and Miyata M (1995a) Manifestations of cancer cachexia induced by colon 26 adenocarcinoma are not fully ascribable to interleukin-6. *Int J Cancer* **62**:332–336.
- Soda K, Kawakami M, Takagi S, Kashii A, and Miyata M (1995b) Splenectomy before tumor inoculation prolongs the survival time of cachectic mice. *Cancer Immunol Immunother* **41**:203–209.
- Strassmann G, Fong M, Windsor S, and Neta R (1993) The role of interleukin-6 in lipopolysaccharide-induced weight loss, hypoglycemia and fibrinogen production, in vivo. *Cytokine* **5**:285–290.
- Thompson MG and Palmer RM (1998) Signalling pathways regulating protein turnover in skeletal muscle. *Cell Signal* **10**:1–11.
- Tisdale MJ (1993) Cancer cachexia. *Anticancer Drugs* **4**:115–125.
- Tisdale MJ (2002) Cachexia in cancer patients. *Nat Rev Cancer* **2**:862–871.
- Wigmore SJ, Barber MD, Ross JA, Tisdale MJ, and Fearon KC (2000) Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr Cancer* **36**:177–184.
- Wigmore SJ, Falconer JS, Plester CE, Ross JA, Maingay JP, Carter DC, and Fearon KC (1995) Ibuprofen reduces energy expenditure and acute-phase protein production compared with placebo in pancreatic cancer patients. *Br J Cancer* **72**:185–188.
- Williams CS, Mann M, and DuBois RN (1999) The role of cyclooxygenases in inflammation, cancer and development. *Oncogene* **18**:7908–7916.
- Zweifel BS, Davis TW, Ornberg RL, and Masferrer JL (2002) Direct evidence for a role of cyclooxygenase 2-derived prostaglandin E₂ in human head and neck xenograft tumors. *Cancer Res* **62**:6706–6711.

Address correspondence to: Dr. Thomas W. Davis, PTC Therapeutics, 100 Corporate Ct., South Plainfield, NJ 07080. E-mail: tdavis@ptcbio.com
