Celecoxib and difluoromethylornithine in combination have strong therapeutic activity against UV-induced skin tumors in mice

Susan M. Fischer1,3, Claudio J. Conti1, Jaye Viner2, C. Marcelo Aldaz1 and Ronald A. Lubet2

1The University of Texas M.D. Anderson Cancer Center, Science Park Research Division, PO Box 389, Park Road 1C, Smithville, TX 78957 and 2Division of Cancer Prevention and Control, National Institutes of Health, Bethesda, MD 20892, USA

3To whom correspondence should be addressed
Email: sa83161@odin.mdacc.tmc.edu

The cyclooxygenase-2 (COX-2) inhibitor celecoxib and the ornithine decarboxylase (ODC) inhibitor difluoromethylornithine (DFMO) were each previously shown to prevent skin tumor development when administered throughout the course of UV irradiation. This raised the question of whether maintenance or continued growth of existing tumors required prostaglandins, the product of COX, or polyamines, the product of ODC. To address this question, SKH hairless mice were irradiated 3 times/week with 90 mJ/cm²; this dose was increased 10% weekly to a maximum of 175 mJ/cm². UV was stopped at 27 weeks, at which time there were an average of 5 papillomas/mouse. The mice were then placed in one of four treatment groups: group 1, no treatment; group 2, 0.4% DFMO in the drinking water; group 3, 500 p.p.m. celecoxib in the diet (AIN76); and group 4, both DFMO and celecoxib. The control group continued to produce new tumors in a nearly linear manner such that by week 31 the tumor number had nearly doubled, i.e. ~10 tumors/mouse. The group receiving DFMO showed significant tumor regression, losing an average of 1 tumor/mouse/week, such that 50% of the tumors remained at week 31. The celecoxib group showed a 25% reduction in tumor number. The group receiving the combination of celecoxib and DFMO showed the greatest regression, with an 89% reduction in tumor number compared with the control group. There was also a corresponding reduction in the size of the tumors. To determine whether tumor regression was permanent or required continued treatment, all treatments were stopped at 31 weeks. Over the next 4 weeks, tumors reappeared at the same rate in all treatment groups. It is concluded that the combination of celecoxib and DFMO are potent therapeutic agents for skin cancer, although the benefits are lost with the cessation of treatment.

Introduction

The major etiological factor leading to the development of cutaneous squamous and basal cell carcinoma, and possibly melanoma, is exposure to UV light (1). The direct absorption of UV by DNA leads to the formation of pyrimidine-pyrimidine (6–4) dimers and cyclobutane pyrimidine dimers of DNA bases. Reactive oxygen intermediates generated by UV exposure can also result in DNA adducts and other types of oxidative damage (1). In addition to its DNA-damaging or tumor-initiating activity, UV also has tumor-promoting activity. Exposure to UV elicits inflammation, epidermal hyperplasia and changes in the expression of numerous genes associated with proliferation and differentiation, eicosanoid and cytokine production and growth factor synthesis and responsiveness (1,2).

The identification of several signaling or metabolic pathways that are related to proliferation or inflammation that are also up-regulated after UV exposure offers the opportunity to intervene and block these events with specific agents. Previous UV carcinogenesis studies have shown that agents that inhibit either ornithine decarboxylase (ODC) or cyclooxygenase (COX) are effective chemoprevention agents (3,4). The induction of ODC by chemical tumor promoters also led to the proposal that up-regulation of ODC, with subsequent increases in the levels of the polyamines, is an obligatory step in skin tumor development (5,6). ODC is the first and rate-limiting enzyme in polyamine synthesis and catalyzes the synthesis of putrescine from ornithine. Putrescine is further metabolized to spermidine and spermine by S-adenosylmethylthione decarboxylase. High levels of these polyamines are usually correlated with rapid proliferation, suggesting that ODC should be a critical target for chemoprevention approaches (5). This has been well demonstrated by the finding that inhibitors of ODC activity, notably the analog D,L-α-difluoromethylornithine (DFMO), which is an irreversible inhibitor, significantly reduces skin tumor development in chemical initiation–promotion models (7–9) and in UV carcinogenesis models (3,10). For example, we previously showed that administration of 0.4% DFMO in the drinking water during the course of UV exposure resulted in a 75% reduction in UV-induced skin tumors (3).

Inflammation, denoted by erythema and edema, is a characteristic dermal response to UBV (290–320 nm) exposure, as well as to chemical tumor promoters (11,12). Among the known mediators of inflammation are the arachidonic acid metabolites, collectively referred to as eicosanoids (13). One of the major classes of arachidonic acid metabolites is the prostaglandins, which have been shown to play a role in UV-induced erythema (14). The enzymes responsible for the first step in the synthesis of prostaglandins are the cyclooxygenases, COX-1 and COX-2. Both COX enzymes produce the same prostaglandin intermediates, but the enzymes are differentially regulated (15). In skin, as in most tissues, COX-1 is constitutively expressed, whereas COX-2 is readily induced by irritating and promoting agents, including UV (4,13). The importance of elevated prostaglandins in tumor promotion has been deduced through the use of inhibitors, referred to as non-steroidal anti-inflammatory drugs (NSAIDs), including
agents such as aspirin, indomethacin and piroxicam, which show activity against both COX isoforms (16,17). The problem of gastrointestinal toxicity associated with COX-1 inhibition and the observation that COX-2 is highly expressed at sites of inflammation spurred the development of specific COX-2 inhibitors (18). These inhibitors, most notably celecoxib, have also been shown to have very strong chemopreventive activity in a number of rodent carcinogenesis models, including UV-induced skin tumors (4,19,20). For example, mice fed 500 p.p.m. celecoxib had 89% fewer skin tumors than the control group (4). On the strength of these data, celecoxib is now being tested as a chemopreventive in individuals at increased risk for non-melanoma skin cancer.  

One of the questions that arises from prevention studies in which agents are administered continuously, beginning before tumors are visible, is whether preventive agents would also be effective as therapeutic agents. For at least some preventive targets this would seem likely, based on the observation that the target molecules are constitutively overexpressed in tumors even after removal of the promoting agent. This is true for both ODC and COX-2, which have been shown to be overexpressed in skin tumors (4,6,19,20). Another question that arises is whether the reduction in tumor numbers with chemopreventive administration is permanent or whether a rebound phenomenon would occur after withdrawal of chemopreventive intervention (3). Thus the goals of this study were: (i) to determine whether DFMO or celecoxib or a combination of both has therapeutic activity when administered to animals with pre-existing tumors; (ii) to determine whether any therapeutic activity was permanent or required continued administration of these agents.  

**Materials and methods**  

**Animal treatment**  

Hairless SKH:HR-1-hbr (SKH-1) mice at 3–4 weeks of age were purchased from Charles River Laboratories (Wilmington, MA), and were used at 8 weeks of age. Upon arrival, the mice were housed in climate controlled quarters (22 ± 1 °C at 50% humidity), with a 12/12 h light/dark cycle under yellow fluorescent lights. Animals were allowed free access to water and AIN-76A diet and observed daily during the UV-irradiation protocol. Powdered AIN-76 diet, purchased from Dyets, Inc. (Bethlehem, PA), was stored at 4°C and fresh diet was supplied three times weekly in clean glass jars with stainless steel lids. Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1]-benzene-sulfonamide; SC-58635) was purchased from LKT Laboratories Inc. (St. Paul, MN). The celecoxib-containing diets (500 p.p.m.) were prepared weekly by mixing celecoxib with an electric mixer as previously described (4). DFMO was provided by the National Cancer Institute, Division of Cancer Prevention and Control (lot no. 76494) and used at a dose of 0.4% in the drinking water as previously described (3). Fresh solutions of DFMO or celecoxib diets were supplied to the mice thrice weekly according to the schedule shown in Figure 1.  

**Irradiation**  

For the tumor study, 120 SKH-1 hairless mice were placed on control AIN76 diets 1 week before beginning the UV-irradiation protocol. The UV apparatus consisted of eight Westinghouse FS40 sunlamps, an IL-1400 radiometer and an attached UVB photometer. The spectral irradiance of the UV lamps was 280–400 nm, providing 80% UVB and 20% UVA. The peak intensity of the light source was 297 nm. The fluence at 60 cm from the dorsal surface of the mice was 0.48–0.50 mJ/cm²/s. The mice were placed in individual compartments of a plastic cage on a rotating base to abate any differences in fluence across the UV light bulbs. The cage was covered by a UVB-transparent lid that filtered out the small amount of UVC radiation emitted from these lamps. Mice were exposed to 90 mJ/cm² three times per week; the dose was increased by 10% per week until the dose reached 175 mJ/cm².  

UV treatment was stopped at 27 weeks; this time frame was designated part A (Figure 1). At this time the mice had an average of 5 papillomas/mouse. Thirty mice were assigned to one of the four treatment groups such that each group retained the 5 tumors/mouse average. Treatment was continued for 4 weeks; this time frame is referred to as part B. At the end of 4 weeks, the DFMO and/or celecoxib groups were subdivided into two groups, with one continuing to receive treatment, whereas in the other treatment was removed (part C, Figure 1). After an additional 3 weeks, the experiment was terminated. At the end of part B the control group (untreated) was subdivided into two groups, with one continuing to receive no treatment and the other administered the combination of DFMO and celecoxib. These animals were killed 1 week later in order to assess proliferative or apoptotic changes within the tumors in response to treatment.  

**Tumor analysis**  

Weekly tumor counts were performed following the appearance of the first tumor and continued until termination of the experiment at 34 weeks. The tumor data are expressed both as multiplicity, i.e. mean number of tumors per mouse, and incidence, i.e. percent of mice with tumors. The diameters of all tumors were measured at the end of part B (Figure 1) and assigned to a size category. Tumors were removed, fixed in formalin and processed for histological evaluation of tumor type (papilloma or squamous or spindle cell carcinoma).  

All data from parts B and C were analyzed using SPSS software (SPSS Inc., Chicago, IL) using the analysis of variance with Tukey honestly significant difference mean rank tests and regression methods. In part C an analysis of covariance was also performed using AOCsLOPE software available at www.odin.mdacc.tmc.edu.  

**Proliferation and apoptosis**  

The mice in the part C no treatment and celecoxib + DFMO for 1 week groups were injected i.p. with a filter-sterilized solution of 2.0% 5-bromo-2'-deoxyuridine (BrdU) (Sigma Chemical Co., St. Louis, MO) at 0.1 mg/g body weight in phosphate-buffered saline. One hour later mice were killed and several tumors were excised from the dorsal surface and fixed in 10% neutral buffered formalin. The tissue samples were paraffin embedded and 4 μm sections were cut and immunohistochemically stained for BrdU incorporation using a monoclonal rat anti-BrdU antibody (1:1) (Accurate Chemical and Scientific Corp., Westbury, NY). The slides were visualized with 3,3’-diaminobenzidine (Sigma) using avidin–biotin–horseradish peroxidase linked to an affinity-purified biotin-labeled rabbit anti-rat IgG (Vectorstain Elite ABC Kit; Vector Laboratories). The numbers of BrdU-positive cells per μm length of epidermis were counted on three to five random areas for each section and the mean percentage and standard deviation of each treatment group was determined.  

Tissue sections were also used to assess apoptosis, using the TUNEL assay (ApopTag; Intergen, Purchase, NY) according to the manufacturer’s protocol. Because TUNEL-positive cells were not homogeneous across the tumor, accurate quantitation was not possible.
Results

Tumor generation (part A)
The study was designed to test and compare the efficacy of celecoxib and DFMO, alone and together, as therapeutic agents for UV-induced skin cancer. To accomplish this, tumors were first generated by thrice weekly UV irradiation (part A of the experimental design shown in Figure 1). As shown in Figure 2, after 26 weeks of irradiation there were on average 5.1 tumors/mouse and an incidence of 95%. At this time, UV treatment stopped and the mice were divided into four groups (part B of the experimental design), with each group having ~5 tumors/mouse. This resulted in some differences in incidence among the groups such that the control group had a 100% incidence while the group arbitrarily designated to receive DFMO had an incidence of 89%.

Tumor regression (part B)
For part B of the experiment, mice were placed on the designated drug (Figure 1) and tumor multiplicities and incidences determined weekly. As expected, the control group (Figure 2) continued to produce a significant number ($P < 0.001$) of new tumors in a nearly linear manner, such that by week 31 the tumor number had almost doubled, i.e. there were approximately 10 grossly observable exophytic tumors per mouse. The incidence for this group remained at 100%. The group receiving DFMO showed significant tumor regression starting in the first week of treatment. For the first 3 weeks of treatment this group lost an average of 1 tumor/mouse/week. There was no additional regression in week 4, suggesting that subsets of tumors are refractory or insensitive to DFMO. At this time the tumor multiplicities were significantly different from the control group, as well as from the other treatment groups ($P < 0.001$, two-factor analysis of variance). Over the course of 4 weeks of treatment only 50% of the tumors remained as visible exophytic lesions. In some cases the regression appeared complete, i.e. the skin appeared normal, whereas in other cases the location of the tumor was only evident as a light colored spot.

The group receiving celecoxib showed a 25% reduction in tumor number over the first 2 weeks of treatment. This was followed by an increase such that by the end of the 4 week treatment period the tumor number was the same as at the start of treatment, i.e. little overall change. It should be noted that this number, 4.7 tumors/mouse, is still considerably less ($P < 0.001$) than the control group, which had 9.9 tumors/mouse at 31 weeks. These results suggest that celecoxib is generally more efficacious in preventing the appearance of new tumors than in causing regression of existing tumors. Interestingly, the tumor incidence in this group rose slightly in the first 3 weeks, followed by a drop during the last week. This was due to the complete loss of tumors in some mice, suggesting that celecoxib does have some therapeutic activity.

The group receiving the combination of DFMO and celecoxib showed the greatest activity (significantly different from all other groups, $P < 0.001$) in reducing both tumor multiplicity and incidence. During the first 3 weeks of treatment these mice lost ~1.5 tumors/week. During the fourth week of treatment a slight increase in tumor multiplicity was noted. The same pattern was seen for tumor incidence, which dropped from 93 to 47% over the first 3 weeks. At the end of the 4 week treatment the difference between the untreated control group and the DFMO plus celecoxib group was significant, with an 89% reduction in tumor number and a 48% reduction in tumor incidence, compared with untreated controls.

At week 31, the end of part B, all tumors were measured and assigned to size groups (Table I). In accordance with the tumor multiplicity data, the control group had the most tumors that were >10 mm in diameter, while the DFMO plus celecoxib group had none in this category.

Tumor regrowth (part C)
In order to determine whether the regression of tumors is permanent or requires continuous treatment, each treatment group was divided in half, with one half continuing treatment while the other half was taken off treatment. For the DFMO group, removal of treatment resulted in a significant ($P < 0.001$) increase in tumor multiplicities, with an ~3-fold increase within 3 weeks (Figure 4). The subgroup that continued to receive DFMO also showed a significant ($P < 0.001$) increase of ~2-fold from week 31 to 34. This suggests that the initial responsiveness of the tumors to DFMO (part B, Figure 3) is transient. There was no difference between the subgroups with regard to tumor incidence, which increased only slightly over the 3 week period.

The celecoxib subgroup that continued to receive celecoxib sustained a 44% loss of tumors. The tumor incidence was also reduced by 20%. The subgroup in which celecoxib was discontinued, however, showed a significant ($P < 0.001$)

<table>
<thead>
<tr>
<th>Table I. Distribution of tumors by size</th>
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<tr>
<td>Group</td>
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<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>DFMO</td>
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<td>Celecoxib</td>
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<td>Celecoxib + DFMO</td>
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At week 31 the diameters of all tumors were measured in each experimental group. For each size group data are expressed as the total number of tumors and as a percentage of the total (in parentheses).
increase in tumor multiplicities, from an average of initially 4.6 to 9.7 tumors/mouse in 3 weeks. This indicates that tumor regression is not permanent even though visually or by palpation tumors were not apparent.

The DFMO and celecoxib subgroup that was removed from treatment also showed a significant increase in tumor multiplicity, from 0.7 to 6.2 per mouse over the 3 week period. The rate of increase was similar to the celecoxib only and DFMO only subgroups that were taken off treatment. This suggests that neither drug caused irreversible regression of tumors. The DFMO and celecoxib subgroup that remained on treatment showed little change in tumor multiplicity (0.82 to 0.90), although there was a slight increase in tumor incidence (45 to 56%) over the 3 week period.

The rates of increase in tumor multiplicity for the three subgroups removed from treatment were the same ($P = 0.05$, comparison of regression lines using analysis of covariance), which might be expected in the absence of therapeutic suppression of growth.

At the end of week 34, tumors were removed from all mice and histologically classified (Table II). For all the groups that had been removed from treatment there were more squamous cell carcinomas (SCCs) than in the groups continuing to

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total tumor no.</th>
<th>SCC No.</th>
<th>Percent</th>
</tr>
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<tbody>
<tr>
<td>DFMO → DFMO</td>
<td>26</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>DFMO → no treatment</td>
<td>116</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Celecoxib → celecoxib</td>
<td>54</td>
<td>2</td>
<td>3.7</td>
</tr>
<tr>
<td>Celecoxib → no treatment</td>
<td>85</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td>Celecoxib + DFMO →</td>
<td>8</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>celecoxib + DFMO → no treatment</td>
<td>62</td>
<td>4</td>
<td>6.5</td>
</tr>
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</table>

At week 34 (end of part C) tumors were removed and histologically classified as papilloma or SCC. Total tumor number represents the sum of papillomas plus SCC.
receive treatment. However, because the total number of tumors was much lower in the continuous treatment groups, the percentage of the total tumors that were carcinomas was higher than in two of the three no treatment counterparts. This suggests that the treatments were more effective in causing regression of benign tumors than carcinomas.

In an effort to understand the mechanism by which the combination of celecoxib and DFMO caused tumor regression, measures of proliferation and apoptosis were made. The labeling index for histologically normal skin between tumors was 14.1 ± 1.83% (SEM) for untreated mice and 13.0 ± 1.76% for mice on celecoxib and DFMO for 1 week. While there was a trend toward reduced proliferation in the treatment group it was not statistically significant. Very few TUNEL-positive cells were found in the epidermis of either the treated or untreated groups.

Fig. 5. DFMO- and celecoxib-induced changes in proliferation and apoptosis in skin tumors. Carcinomas from untreated mice (A and C) and mice treated for 1 week with DFMO and celecoxib (B and D) were fixed in formalin and processed for immunohistochemistry, as described in Materials and methods. BrdU labeling was used as an indicator of proliferation (A and B); TUNEL staining was used to evaluate apoptosis (C and D).
BrdU labeling in the tumors was heterogeneous across all tumors, regardless of treatment, making reliable quantitation difficult. While the overall level of labeling appears to be higher in the tumors from untreated mice, some tumors (or some areas) were very heavily labeled while others were necrotic. In the celecoxib plus DFMO treatment group, the areas of necrosis were more prominent but in some areas of some tumors labeling was moderately heavy. It is likely that the tumors in which labeling was low represent ones that would regress with continued treatment, whereas areas or tumors with high labeling represent areas that are refractory to treatment.

The extent of apoptosis after 1 week of combined celecoxib plus DFMO was compared with the no treatment control. As with BrdU labeling, TUNEL staining was heterogeneous. However, there was considerably more TUNEL staining in the tumors from the treatment group than in the no treatment tumors (compare Figure 5C and D). Additionally, tumors from the celecoxib plus DFMO group had large areas of necrotic tissue (data not shown).

Discussion

Genes that are transiently up-regulated by UV (or chemical tumor promoters) are frequently constitutively up-regulated in skin tumors, even in the absence of UV or exogenous promoters (21). This suggests that these genes contribute to or are required for continued tumor growth. This in turn suggests that agents that target these gene products should be good chemopreventive agents as well as exert a cytostatic activity on existing tumors. Because celecoxib, which targets COX-2, and DFMO, which targets ODC, are both excellent inhibitors for their respective enzymes and excellent chemopreventive agents for UV-induced skin cancer (3,4), we hypothesized that they should be effective cytostatic agents when administered to mice with existing tumors. Additionally, because celecoxib and DFMO target different enzymes, and previous data in the colon had shown synergy between NSAIDs and DFMO (22), we hypothesized that a combination treatment should be more effective than the use of single agents.

We recently showed that DFMO and celecoxib each have strong chemopreventive activity against UV-induced skin carcinogenesis (3,4). Additionally, when administration of DFMO was delayed, i.e. using a late intervention rather than prevention protocol, it was effective in both reducing the appearance of new tumors and in causing partial regression of existing tumors. This observation suggested that DFMO might have therapeutic efficacy against skin tumors. This premise was supported by the report that DFMO caused regression of skin papillomas induced by low doses of the carcinogen 7,12-dimethylbenz[a]anthracene in transgenic mice overexpressing ODC (23). A topical DFMO formulation was also reported to be moderately effective in the therapeutic treatment of human actinic keratoses (24). MCF-7 human breast cancer cells grown in athymic nude mice were significantly growth inhibited with DFMO treatment (25). Similar inhibition was seen when a human gastric cancer cell line was transplanted into nude mice (26). This study also provided evidence that DFMO reduces vessel density and is pro-apoptotic (26). Human recurrent gliomas also responded to DFMO treatment, although it was effective in < 50% of the patients (27). DFMO was also reported to cause growth arrest of liver metastasis in a breast cancer patient (28). This and other studies support the conclusion that elevated ODC activity is needed not only for initial tumor development but also for tumor maintenance (29).

As shown in this study, DFMO caused a significant regression or loss of tumors over the first 3 weeks of administration. During the subsequent week no further loss was observed. One interpretation of these data is that the majority of the tumors require ODC activity for tumor survival while a minority of tumors have either circumvented the ODC requirement or are otherwise refractory to DFMO. A similar observation was made in one of our earlier studies in which DFMO was administered shortly after the appearance of tumors (~2 tumors/mouse), causing a significant regression over several weeks, which was followed by a rebound, although never to the level of the control group (3).

Celecoxib, as well as many other NSAIDs, has significant chemopreventive activity in several model systems (4,30-34). In UV carcinogenesis in particular, celecoxib at 500 p.p.m. in the diet was found to reduce tumor multiplicities by nearly 90% (4). Pentland et al. (35) also found that intervening late in the UV carcinogenesis process with celecoxib significantly reduced subsequent tumor multiplicity and size. The basis for this inhibition is believed to be a reduction in prostaglandin synthesis, although prostaglandin-independent mechanisms are also proposed for celecoxib (36). While prostaglandins are synthesized by both COX-1 and COX-2, the induction of COX-2 by UV (or other irritants) is largely responsible for the high levels of prostaglandins in UV-exposed skin (4). Inhibition of both COX-2 and subsequent tumor development suggests that COX-2/high prostaglandin levels are needed for tumor development. In skin tumors, whether elicited by chemical carcinogens or UV exposure, COX-2 is constitutively up-regulated in the absence of UV or phorbol ester and high levels of prostaglandins are produced (4,20). This suggests that prostaglandins are required for tumor maintenance and continued growth. The target cells for the prostaglandins is not clear, however, and may involve multiple cell types, including the tumor cells themselves and/or vascular endothelial cells, as has been suggested for colon cancer (37).

As shown in this study, celecoxib has moderate therapeutic efficacy, at least initially. Approximately 1.5 tumors/mouse were lost over the first 2 weeks of treatment, however, small increases were observed for the subsequent 2 weeks, although not to the extent of the increase in tumor multiplicity seen in the untreated control group. These data suggest that celecoxib has relatively weak therapeutic activity alone while having strong preventive activity, i.e. it had a minor effect on tumor regression but a significant effect in preventing new tumor development. However, the overall lack of an increase in the growth of existing tumors suggests that celecoxib has cytostatic activity.

NSAIDs have been used therapeutically to only a limited extent. In familial adenomatosis polyposis (FAP) patients the non-selective NSAID sulindac was found to cause the nearly complete regression of adenomas (38). A recent study on the efficacy of celecoxib on polyps in FAP patients showed a 31% reduction in the area of the duodenum covered by adenomas (39). This study supports an animal model for FAP in which celecoxib caused regression of adenomas by 50%, both in terms of multiplicity and size (40). Human gastrointestinal cells grown as xenografts in mice also responded with growth inhibition to the COX-2 inhibitor NS-398 and the COX-1/COX-2 inhibitor indomethacin (41). In a recent study
extending these findings to another tissue type, oral adminis-
tration of either celecoxib or a selective COX-1 inhibitor to
mice with established mammary tumors resulted in significant
inhibition of tumor growth and reduced metastatic incidence
(42). Our observation that celecoxib is growth inhibitory to
established skin tumors is the first demonstration that NSAIDs,
and celecoxib in particular, have therapeutic activity in skin
cancer. Collectively, all these studies suggest that NSAIDs
hold promise as therapeutic agents and that further studies
are warranted.

Because combinations of agents, particularly with dissimilar
targets, are often more effective than single agents alone, we
hypothesized that the combination of DFMO and celecoxib
would be additive. As shown here, the ability of this combina-
tion to cause the loss of tumors is significant, with an almost
90% loss or regression of tumors which was sustained over the
4 weeks of the experiment.

Ideally a therapeutic agent that causes tumor loss should do
so completely, i.e. once the tumor has disappeared, the drug
cannot be removed without tumor recurrence. To determine
whether DFMO and/or celecoxib had lasting or curative
effects, the drugs were withdrawn from animals in which
they produced significant tumor loss. Within 1 week tumors
began to appear and increased in number in a linear manner
over the next 4 weeks. The rate of increase was very similar for
all of the treatment groups. These data can be interpreted as
showing that while DFMO and celecoxib have strong tumor
regression activities, they do not in fact cure or completely
remove tumors. However, because the regressed tumors were
not mapped, it is not known whether the emerging tumors
represented regressed tumors or tumors arising de novo. In
either case therapeutic efficacy is dependent on continual
drug exposure. This also raises the question of whether
more extended exposure to the combination of celecoxib
and DFMO for 12–16 weeks might have had a longer term
effect.

The mechanisms involved in DFMO- and celecoxib-induced
tumor regression are not known and are likely to be complex.
Neither drug appears to affect normal skin proliferation nor
function, but both have marked effects on skin tumor prolif-
eration and cell death. For DFMO this differential activity is
likely due to the low proliferation index in normal skin and
very high proliferation in tumors. For celecoxib, no effect
was expected in normal skin because COX-2 is not expressed
(4,13). Both DFMO and celecoxib were shown to have
apoptotic activity in cancer cells (24,33). We observed both
a decrease in proliferation and an increase in the number of
apoptotic cells in tumors from animals receiving the
combination of DFMO and celecoxib. However, further
studies are needed to determine how these drugs induce
tumor cell death.

In summary, the administration of DFMO and/or celecoxib
to mice with skin tumors elicited by UV causes a marked
regression of tumors. The regression is dependent on contin-
ued drug administration however. Overall these studies may
have applicability to the management of human skin cancer
where a reduction in the size of tumors may make them more
amenable to other therapies. Overall, the many reports that
NSAIDs are excellent cancer preventive agents for at least
several human tumor types suggest that NSAIDs, particularly
when used in combination with other types of agents, can be of
significant benefit to reducing the cancer development process
at many levels (43).

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