Gene modulation by Cox-1 and Cox-2 specific inhibitors in human colorectal carcinoma cancer cells

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Cox-1 and Cox-2 specific inhibitors exert chemo-preventative activity. However, the exact mechanisms for this activity remain unclear. Increasing evidence suggests that non-steroidal anti-inflammatory drugs regulate gene expression, which may be responsible, in part, for this activity. In this study, human colorectal carcinoma HCT-116 cells were treated with the Cox-1 specific inhibitor SC-560 and the Cox-2 specific inhibitor SC-58125 to evaluate their ability to induce apoptosis, inhibit cell proliferation, inhibit growth on soft agar and modulate gene expression. The Cox-1 specific inhibitor, SC-560 significantly induced apoptosis and inhibited the growth of HCT-116 cells on soft agar, an in vitro assay for tumorigenicity. SC-58125 moderately induced apoptosis and inhibited growth on soft agar at higher concentrations than were required for SC-560. Previously, we reported that the potent chemo-preventative drug sulindac sulfide altered the expression of eight genes including several transcription factors that may be linked to this drug’s chemo-preventative activity. HCT-116 cells were treated with various concentrations of SC-560 or SC-58125 and changes in the expression of these eight genes were determined by real-time reverse transcription-polymerase chain reaction. SC-560 modulated mRNA expression of the eight genes studied. In contrast, SC-58125 required ~5-10-fold higher concentrations to achieve similar degrees of gene modulation in six of eight genes. Changes in protein expression by SC-560 also occurred for five of these genes with antibodies available (NAG-1, ATF3, C/EBPβ, MAD2 and MSX1). In conclusion, this is the first report to suggest that like sulindac sulfide, the Cox-1 specific inhibitor SC-560 appears to elicit chemo-preventative activity by altering gene expression, while the chemo-preventative effects of SC-58125 are complex and probably work through these and other mechanisms, such as the inhibition of Cox-2.

Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs), are inhibitors of cyclooxygenase (Cox), which is responsible for the formation of prostaglandins (1). Two distinct forms of Cox exist, the constitutively expressed Cox-1, and the inducible Cox-2. Numerous animal studies (2–7), population-based studies (8,9) and in vitro studies (10–13) with human colorectal carcinoma cells provide evidence that NSAIDs have chemo-preventative activity directed against colorectal cancer as illustrated in several recent reviews (14–18). Colorectal cancer is the third most common cancer in the USA. In humans, the pro-drug sulindac, which is converted to sulindac sulfide, is used in a chemo-preventative manner to suppress the development of adenomatous polyps in patients with familial adenomatous polyposis (FAP) (19,20). Sulindac also causes a rapid regression of pre-existing tumors in APCmin mice (20), however sulindac sulfone, which lacks NSAID activity does not inhibit tumor formation in vivo suggesting the inhibition of Cox is involved (4,5). Many studies on the chemo-preventative activity of Cox inhibitors used classical NSAIDs that inhibit both Cox-1 and Cox-2. However, classical Cox inhibitors such as sulindac sulfide and indomethacin are more potent inhibitors of Cox-1 than Cox-2.

Recently, a concerted effort has been focused on the Cox-2 specific inhibitors since Cox-2 is highly expressed in tumor tissues [for a review see ref. (21)]. Celecoxib, a Cox-2 specific inhibitor, both prevents tumor formation and caused regression of pre-existing tumors in APCmin mouse models (22). SC-58125, also a Cox-2 specific inhibitor, induces apoptosis in vitro (23), and blocks tumor formation in vivo (24). However, other data with Cox-1 knockout APCmin mice indicate that deletion of Cox-1, in addition to Cox-2, reduces the number of intestinal polyps in these mice (25). Mofezolac, a Cox-1 specific inhibitor, was equally as effective as the Cox-2 specific inhibitor nimesulide, at inhibiting the number of aberrant crypt foci in rats fed azoxymethane, a model for chemoprevention, indicating both Cox-1 and Cox-2 contribute to tumor formation in vivo (26). In addition, APC gene knockout mice (APC1309) fed these drugs also showed a similar reduction in the number of colorectal polyps indicating Cox-1 is also important in tumorigenesis (26). Furthermore, Cox-1, but not Cox-2 mRNA and protein is over-expressed in human ovarian cancers when compared with normal ovarian tissue (27), SC-560, but not the Cox-2 specific inhibitor Celecoxib, inhibited arachidonic acid-induced vascular endothelial growth factor (VEGF) expression in the ovary (27). Therefore, specific
Cox-1 inhibitors such as SC-560 and mofezolac may have chemo-preventative effects in the colon (26,28), breast (29) and potentially in the ovary (27).

The chemo-preventative activity of NSAIDs directed against colorectal cancer may, in part, be independent of Cox (28,30-32). Inhibition of cell proliferation and the induction of apoptosis are believed to be responsible, in part, for the chemo-preventative effects of NSAIDs illustrated in many recent reviews (14-18). SC-560 and Celecoxib were both effective at inhibiting the growth of Cox-deficient HCT-15 colon cancer xenografts in nude mice and induced apoptosis in vitro (28). Furthermore, Zhu et al. examined the molecular and structural requirements for the induction of apoptosis by Cox-2 specific inhibitors in prostate cells (33). They concluded that the induction of apoptosis was independent from the structural requirements for Cox inhibition. Therefore, both Cox-dependent and independent mechanisms are probably involved in the chemo-preventative activity of these compounds.

This laboratory has proposed the hypothesis that the chemo-preventative activity of Cox inhibitors is mediated, in part, by altering gene expression (12). Sulindac sulfide is a potent chemo-preventative drug against colorectal cancer (2-6). It is also a significant modulator of gene expression in colorectal cancer cells and these effects are probably linked to the chemo-preventative activity of sulindac sulfide at least in vitro (12). In this report, we have investigated the effect of selective Cox-1 and Cox-2 specific inhibitors on apoptosis, growth of colorectal cancer cells on soft agar, and the expression of the same genes modulated by sulindac sulfide in colorectal cancer cells (12). We used the Cox-1 specific inhibitor, SC-560 and the Cox-2 specific inhibitor, SC-58125 (Table I), which have reported chemo-preventative activity against colorectal (24,28,34) and breast cancer cells (29) in xenograft models.

Materials and methods

Chemicals

Chemicals were purchased from Sigma Chemical Company (St Louis, MO) unless otherwise noted. SC-560 and SC-58125 were from Cayman Chemical Company (Ann Arbor, MI) and were dissolved in DMSO and prepared fresh weekly.

Cell line and reagents

Cell lines were purchased from ATCC (Rockville, MD) and were maintained at 37°C/5% CO2. Cell culture reagents were from Life Technologies (Rockville, MD). Human colorectal carcinoma HCT-116 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 10 mg/l gentamicin (complete media). Human colorectal carcinoma SW-480 cells were maintained in EMEM medium, which contained 15% fetal bovine serum (FBS), 10 mg/l gentamicin and sodium pyruvate (complete media).

Cell culture treatments

Cells were plated at 50% confluency in complete media overnight for recovery and treated in serum-free media containing vehicle, SC-560 or SC-58125 followed by RNA or protein isolation. Vehicle treatments consisted of 0.1% DMSO in serum-free media.

Cell proliferation assay

Cell proliferation was measured using the MTS colorimetric assay by Promega (Madison, WI), which estimates the number of viable cells in proliferation. Briefly, 500 cells/well were plated in complete media in 96-well tissue culture dishes overnight. Cells were treated with various concentrations of vehicle or NSAID in complete media in a final volume of 0.1 ml complete media. Cell viability was measured daily for 5 days at 490 nm in an ELISA plate reader following the addition of 0.02 ml MTS ‘Aqueous One’ solution per well and a 1 h incubation at 37°C/5% CO2. Each experiment was carried out in quadruplicate and repeated two times. Percent viability is calculated relative to vehicle treated controls using the mean OD 490 ± SEM. A representative experiment from day 5 is shown.

Cell death detection

Cell death was measured using the Nuclear Matrix Protein ELISA kit from Oncogene Research Products (San Diego, CA). Briefly, 0.1 ml of media from cells treated for 30 h in media containing 2% FBS and vehicle, SC-560 or SC-58125 were spun down at 2000 r.p.m. for 10 min. The supernatant was collected and used undiluted according to the protocol provided.

Measurement of DNA content and apoptosis

The DNA content for vehicle or NSAID-treated SW-480 and HCT-116 cells were determined by fluorescence-activated cell sorting (FACS). Cells were plated at 50% confluency in 6-well plates overnight then treated in media containing 2% FBS and vehicle, SC-560 or SC-58125 were spun down at 2000 r.p.m. for 10 min. The supernatant was collected and used undiluted according to the protocol provided. The DNA content from day 5 is shown.

Measurement of DNA content and apoptosis

The DNA content for vehicle or NSAID-treated SW-480 and HCT-116 cells were determined by fluorescence-activated cell sorting (FACS). Cells were plated at 50% confluency in 6-well plates overnight then treated in media containing 2% FBS and vehicle, SC-560 or SC-58125 were spun down at 2000 r.p.m. for 10 min. The supernatant was collected and used undiluted according to the protocol provided. The DNA content from day 5 is shown.

Soft agar cloning assay in the presence of NSAIDs

Soft agar assays were performed to compare the clonogenic potential of colorectal cancer cells in semisolid medium. HCT-116 and SW-480 cells were resuspended at 6000 cells in 2 ml of warm, complete media containing 0.35% agarose and the final concentration of NSAID or vehicle tested in the appropriate media and plated on top of 1 ml of 0.5% agarose in 6-well plates in triplicate repeated twice. Plates were incubated for 3 weeks at 37°C/5% CO2. Cell colonies were visualized following an overnight stain with 0.5 ml of p-iodonitrotetrazolium violet at room temperature then image captured using

Table I. Comparison of cyclooxygenase inhibitors

<table>
<thead>
<tr>
<th>IC-50:</th>
<th>Sulindac sulfide (47)</th>
<th>SC-560 (48)</th>
<th>SC-58125 (49)</th>
</tr>
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<tbody>
<tr>
<td>Cox-1</td>
<td>0.2 μM</td>
<td>0.009 μM</td>
<td>&gt;10.0 μM</td>
</tr>
<tr>
<td>Cox-2</td>
<td>14.0 μM</td>
<td>6.3 μM</td>
<td>0.07 μM</td>
</tr>
<tr>
<td>Cox-2/Cox1</td>
<td>70</td>
<td>700</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Gene expression by selective Cox inhibitors

Cell proliferation and apoptosis
To determine if selective Cox inhibitors inhibit cell proliferation, HCT-116 cells were treated with vehicle or various concentrations of SC-560 or SC-58125 in complete media for 5 days. At 100–200 μM concentrations, SC-560 and SC-58125 significantly inhibited cell growth in a concentration-dependent manner (Figure 1A). To verify that the concentrations and conditions used to treat cells with SC-560 and SC-58125 in subsequent experiments were non-toxic to the cells, cell death using an NMP ELISA assay, which measures the concentration of a nuclear protein released into the media, was performed. Both compounds were not toxic according to NMP ELISA at or below 50 (SC-560) or 100 μM (SC-58125) (data not shown).

HCT-116 cells were treated with various concentrations of SC-560 or SC-58125 for 24 and 30 h in media containing 2% serum and apoptosis was greater at 30 h (data not shown). Relative fold-increase in apoptosis was measured by Annexin-V followed by propidium iodine staining using FACS analysis. SC-560 and SC-58125 induced apoptosis at the low, non-toxic concentrations chosen. A significant induction of apoptosis was seen in HCT-116 cells treated for 30 h with 25 and 50 μM SC-560, which occurred in a concentration-dependent manner, as compared with vehicle-treated controls (Figure 1B). SC-58125 also induced apoptosis in HCT-116 cells after a 30 h treatment albeit to a lesser extent than SC-560 even at higher (100 μM) concentrations.

Inhibition of colorectal cancer cell growth on soft agar
Subsequently, we determined if SC-560 and SC-58125 would inhibit the growth of cells on soft agar. Growth of HCT-116 cells on soft agar was inhibited following treatment with various concentrations of SC-560. SC-58125 also inhibited the growth of these cells on soft agar, however, to a lesser degree even at higher concentrations (Figure 2). Thus, SC-560 was more effective than SC-58125, which required higher concentrations.

NSAIDs regulate the expression of several genes related to cell growth and apoptosis
One explanation for NSAIDs chemo-preventative activity is alterations in gene expression. Previously, we reported that the potent chemo-preventative drug sulindac sulfide, a NSAID that inhibits the growth of HCT-116 cells on soft-agar, altered the expression of eight genes in a concentration-dependent manner that may be linked to this drug’s chemo-preventative activity (12). They include the induced genes: NAG-1, ATF3, C/EBP, NAG-3 and the repressed genes INSIG1, MSX1, MAD2 and NRG-1. We determined if SC-560 and SC-58125 also regulated the expression of these genes at the mRNA level as measured by real-time RT-PCR. The real-time RT-PCR data presented for each gene is from the time point with the greatest fold induction or repression measured following treatment with SC-560 (8 h genes include C/EBP, NAG-1, ATF-3 and INSIG1, which were similarly induced at 24 h, while the 24 h genes include NAG-3, MSX1, MAD2 and NRG-1). This is consistent with the results observed by microarray analysis following treatment of HCT-116 cells with sulindac sulfide as reported previously (12). To ascertain if changes in gene expression were dependent on the concentration used, we treated HCT-116 cells with various concentrations of SC-560.
and SC-58125. As estimated by real-time RT-PCR, all of these genes were regulated by SC-560 in a concentration-dependent manner and was nearly optimal at 10 μM except for ATF3, which was significantly more induced at 25 μM (Figure 3A and B). In particular, the transcription factors C/EBPβ, ATF3 and MSX1 plus NAG-1, a gene belonging to the TGF-β superfamily linked to the pro-apoptotic and antitumorigenic activity of NSAIDs in vitro (36) and chemo-preventative activity in animal models (37), were all regulated by SC-560 in this study. ATF3 and NAG-1 were the two most avidly expressed genes, while INSIG1 and NRG-1 were the most repressed. Only NAG-3, an EST, was poorly expressed following treatment with SC-560.

In contrast, except for ATF3, SC-58125 poorly altered the expression of these genes indicating that SC-58125 is less effective than SC-560 at modulating the expression of these genes (Table II), which is in general agreement with the in vitro pro-apoptotic and antitumorigenic effects of these drugs in this study. At higher concentrations of SC-58125, which had minimal effect on apoptosis and growth on soft agar relative to SC-560, modulation of these genes did occur (Figure 3C and D).

Effects of SC-560 on protein expression

Western blots were performed to determine if SC-560 significantly altered protein expression of the five genes that had antibodies available. NAG-1, ATF3 and C/EBPβ protein expression were induced in a concentration-dependent manner and protein expression appeared to be in general agreement with mRNA levels measured by real-time RT-PCR. Similarly, MAD2 protein expression was repressed according to western blotting and real-time RT-PCR in HCT-116 cells (Figure 4).

Fig. 1. Cox specific inhibitors inhibit cell proliferation and induce apoptosis. (A) HCT-116 cells were treated for 5 days in media containing 10% FBS plus vehicle, 10, 100 or 200 μM SC-560 (left panel) or vehicle, 10, 100 and 200 μM SC-58125 (right panel) as indicated. Following treatment, cells were measured for cell proliferation at OD 490 as illustrated in the Materials and methods. (B) HCT-116 cells were treated for 30 h in media containing 2% FBS plus vehicle, 10, 25 or 50 μM SC-560 (left panel) or vehicle, 10, 50 or 100 μM SC-58125 (right panel) as indicated. Apoptosis was then measured using FACS analysis as illustrated in the Materials and methods. *Statistical significance is according to ANOVA with Fisher’s LSD method for pairwise comparisons (P < 0.05) level of significance from a representative experiment.
Gene expression by selective Cox inhibitors

Sulindac sulfide is currently used to suppress the development of adenomatous polyps in patients with FAP (19) and because Cox-2 is often elevated in tumors, most of the attention has been focused recently on the use of Cox-2 specific inhibitors as chemo-preventative agents. However, Cox-1 specific inhibitors have recently gained increased attention as they also inhibit tumor formation in animal models. Most traditional Cox inhibitors are more potent inhibitors of Cox-1 than Cox-2. Thus, it appears both Cox-1 and Cox-2 play a role in the development of tumors. This conclusion is supported by Narko et al., who demonstrated that cells transfected with Cox-1 became tumorigenic when injected into nude mice (38). Cox-1 knockout mice show reduced intestinal tumorigenesis in APC\textsuperscript{min} mice models (25), and are also resistant to chemically induced tumors, an indicator of chemo-preventative activity (39). Recently, over-expression of Cox-1, but not Cox-2 protein and mRNA expression were seen in ovarian cancers while the expression of Cox-1 correlated with the expression of various angiogenic factors, which was inhibited by Cox-1 specific inhibitors (27). Thus, Cox-1 specific inhibitors, in addition to traditional NSAIDs, and Cox-2 specific inhibitors may be beneficial in the prevention of various cancers.

Previously, we reported that sulindac sulfide altered the expression of a number of genes (12) including NAG-1, a member of the TGF-\(\beta\) superfamily with pro-apoptotic and antitumorigenic activity \textit{in vitro} (36) and chemo-preventative activity in xenograft mouse models \textit{in vivo} (37). Here we were interested in determining if specific NSAIDs with reported chemo-preventative properties also altered the expression of the same genes we found modulated by sulindac sulfide. Therefore, biological indicators such as the induction of apoptosis and the ability to inhibit growth on soft agar were first confirmed in our model, then changes in gene expression by selective Cox inhibitors was examined in two colorectal cancer cell lines. The Cox-1 selective inhibitor, SC-560 and the Cox-2 specific inhibitor, SC-58125 were chosen as reports in the literature indicate both of these drugs have chemo-preventative properties in cell culture models \textit{in vitro} and in animal models \textit{in vivo}.

SC-560 was more potent than SC-58125 at enhancing apoptosis, inhibiting the growth of cells on soft agar and modulating gene expression \textit{in vitro}. The activity of SC-560 was comparable in potency to sulindac sulfide. SC-560 appears to avidly alter apoptosis, growth on soft agar and the expression of sulindac sulfide modulated genes that were linked previously to its chemo-preventative activity. SC-58125 had only modest activity in the biological assays reported here and required higher concentrations to alter gene expression. Thus, in HCT-116 cells, these genes may play an important role in eliciting chemo-preventative effects of NSAIDs such as SC-560 illustrated here and sulindac sulfide reported previously (12). The chemo-preventative activity of SC-58125 seen in animal models is not linked conclusively to the regulation of these genes and is probably linked to its inhibition of Cox-2 activity (23,24). However, SC-58125 enhanced the expression of NAG-1, as well as ATF3, which was the most avidly induced gene in this study indicating multiple mechanisms are probably involved. SC-58125 increased the mRNA expression of MSX1 and INSIG1, two genes whose expression was either not changed or repressed by sulindac sulfide and SC-560.

MSX1 protein expression was induced as estimated by western blotting in HCT-116 cells, however, the mRNA was repressed by SC-560 according to real-time RT-PCR in this cell line. The reason for this discrepancy is not known. NAG-1 was avidly induced in a dose-dependent manner by SC-560 in HCT-116 cells.

**Gene expression in SW-480 cells**

Since HCT-116 cells are devoid of Cox activity, we chose to determine if these genes were also modulated by SC-560 at the mRNA level and protein level in another cell line, SW-480 cells, which express Cox-1 but only low levels of Cox-2. Following treatment of SW-480 cells with SC-560, NAG-1, C/EBP\(\beta\) and ATF3 protein expression were up-regulated in SW-480 cells (Figure 5A). MAD2 was repressed at the protein level according to western blotting, however, MSX1 protein levels were somewhat increased following treatment with SC-560 (Figure 5B) similar to that seen in the HCT-116 cells. SC-58125 has little effect on the protein expression of these genes even at high concentrations (data not shown).

Changes in mRNA levels by real-time RT-PCR confirmed the fact that the expression of seven of these eight genes was modulated by SC-560 in this cell line (Table III). Only MSX1 was not altered at the mRNA level by real-time RT-PCR in SW-480 cells, following treatment with 25 \(\mu\)M SC-560. Even at high (100 \(\mu\)M) concentrations, SC-58125 was a relatively weak modulator of these genes in SW-480 cells (Table III). Changes in the mRNA expression of these genes by SC-560 were also verified by northern blot analysis (Figure 6). With the exception of MSX1, the northern blot analysis was in agreement with the real-time RT-PCR data indicating that SC-560 modulates these genes at the mRNA level. The reason for this discrepancy is not known.

**Fig. 2.** Cox specific inhibitors repress clonogenic growth on soft agar. HCT-116 cells were treated with various concentrations of SC-560 or SC-58125 as indicated and incubated for 3 weeks. Colony forming units were counted electronically on a personal computer equipped with IPLab version 3.0 (Scanalytics, Inc., Fairfax, VA). * Statistical significance is according to ANOVA with Fisher’s LSD method for pairwise comparisons (\(P < 0.05\)) level of significance from a representative experiment.
Fig. 3. Cox specific inhibitors alter mRNA gene expression in HCT-116 cells. Fold induction (A and C) or repression (B and D) following treatment with SC-560 (A and B) or SC-58125 (C and D). Results are fold change over time-matched vehicle-treated controls.

Table II. Comparison of gene modulation by SC-560 and SC-58125 in HCT-116 cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Induced: 10 μM SC-560</th>
<th>10 μM SC-58125</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAG-3</td>
<td>1.5 ± 0.32</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>C/EBPβ</td>
<td>2.5 ± 0.23</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>NAG-1</td>
<td>3.3 ± 0.13</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>ATF-3</td>
<td>2.9 ± 0.12</td>
<td>0.26 ± 0.02</td>
</tr>
</tbody>
</table>

Values are fold change ± SEM over time-matched vehicle-treated controls. Cells were treated for 8 or 24 h at the concentration indicated as illustrated in the Materials and methods. Bold values are statistically significant according to a two-sided t-test. A separate control was used for each NSAID.

Fig. 4. SC-560 modulates protein expression in HCT-116 cells. Western blots of NAG-1, ATF3, C/EBPβ and MAD2 were performed as indicated in the experimental procedures. HCT-116 cells were treated in SFM for 24 h containing vehicle (lane 1), or 10, 25, 50 and 100 μM SC-560 (lanes 2-5). Blot was stripped and re-probed with actin.
in HCT-116 cells, respectively. Thus, changes in gene expres-
sion by Cox inhibitors appear to be complex and dependent on
the structural character of the specific Cox inhibitor rather than
its ability to selectively inhibit Cox-1 or Cox-2. This is in
agreement with Zhu et al. who reported that NSAIDs induce
apoptosis independent of their ability to inhibit Cox (33).

As illustrated here and elsewhere, evidence is mounting that
gene regulation may play a part in the chemo-preventative
effect of NSAIDs (28,30,31,36,40,41). This is the first report
showing changes in gene expression of a variety of genes
by selective Cox inhibitors. Furthermore, changes in the
expression of a variety of genes, in particular, immediate-
early genes such as the transcription factors C/EBPβ and
ATF3 as seen with SC-560 may be an important event in the
apoptotic cascade and they occur prior to the induction of
apoptosis, supporting the conclusion that these genes are part
of the apoptotic events rather than the result of apoptosis.

Several of these genes are believed to be important in the
response to NSAIDs including NAG-1, which is induced by
NSAIDs (36) and repressed in human colorectal tumors (37).
Treatment of mice with the pro-drug sulindac, which is con-
verted to sulindac sulfide, increases the expression of NAG-1
in mice tissues (37). Similarly, NRG-1 is repressed by NSAIDs
and induced in tumors (42). Furthermore, the transcription
factors ATF3 and MSX1 as well as MAD2, are linked to cell
cycle progression. In addition, C/EBPβ binds to the promoter
of p21WAF1/CIP1, which is a powerful cell cycle inhibitor,
thereby inducing its expression (43). Both C/EBPβ and
ATF3 are members of the basic leucine zipper family of
transcription factors and are considered immediate early
genes most often equated with involvement in the stress res-
ponse. ATF3 forms heterodimers with C/EBPβ regulating the
expression of Gadd153 and several other growth-regulating
cellular promoters and even heterodimerizes with Gadd153,
resulting in down regulation of ATF3 and C/EBPβ-mediated
gene regulation (44). Tetracycline inducible over-expression
of ATF3 suppresses cell growth and slows down cell cycle
progression from G1 to S phase (45).

INSIG1 and MSX1 are two genes identified as repressed by
sulindac sulfide in SW-480 cells (12) and at the mRNA
level by SC-560 in HCT-116 cells in this study. While these
genes are not modulated by sulindac sulfide in HCT-116 cells
(12), they were induced following treatment with SC-58125
in HCT-116 cells in this study and over-expression of MSX1
induces G1 phase arrest in ovarian cancer cells indicating
another possible mechanism by which NSAIDs may
function (46).

In conclusion, the Cox-1 specific inhibitor SC-560 induces
apoptosis and inhibits the growth of human colorectal cancer
cells on soft agar, an in vitro indicator of tumorigenicity, and is
a powerful modulator of gene expression, mimicking the

| Table III. Comparison of gene modulation by SC-560 and SC-58125 in SW-480 cells |
|-----------------------------------|--------------|--------------|
| Induced:                          | SW-480 cells | SW-480 cells |
| NAG-3                             | 25 µM SC-560 | 100 µM SC-58125 |
| C/EBPβ                            | 1.9 ± 0.21   | 0.7 ± 0.01   |
| ATF3                              | 4.2 ± 0.03   | 1.7 ± 0.06   |
| NAG-1                             | 3.1 ± 0.07   | 1.4 ± 0.03   |
| Actin                             | 3.1 ± 0.09   | 0.97 ± 0.09  |
| Repressed:                        | SW-480 cells | SW-480 cells |
| MSX1                              | 1.2 ± 0.03   | 1 ± 0.03     |
| INSIG1                            | 0.2 ± 0.01   | 1.2 ± 0.08   |
| MAD2                              | 0.8 ± 0.01   | 0.66 ± 0.01  |
| NRG-1                             | 0.56 ± 0.02  | 0.7 ± 0.05   |

Values are fold change ± SEM over time-matched vehicle-treated controls. Cells were treated for 8 or 24 h at the concentration indicated as illustrated in the Materials and methods. Bold values are statistically significant according to a two-sided *t*-test.
effects seen by the potent chemo-preventative drug sulindac sulfide. In contrast, the selective Cox-2 inhibitor, SC-58125 was a weak modulator of gene expression although it modestly induced apoptosis and inhibited growth on soft agar in this study. Together, the data lead us to believe that in addition to Cox-2, Cox-1 is important to the inhibition of colorectal cancer by NSAIDs, and one mechanism that probably explains, at least in part, the chemo-preventative activity of NSAIDs is modulation of gene expression. Thus, the mechanisms by which Cox inhibitors exert their chemo-preventative activity in colon are complex and may involve changes in the expression of multiple genes in addition to inhibition of progtaglandin production. Further studies using animal models are required to better understand the chemo-preventative activity of these compounds.

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References


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