Antioxidants Reduce Cyclooxygenase-2 Expression, Prostaglandin Production, and Proliferation in Colorectal Cancer Cells

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Abstract

Increased expression of cyclooxygenase (COX) and overproduction of prostaglandins (PGs) have been implicated in the development and progression of colorectal cancer (CRC). Recent observations suggest that reactive oxygen intermediates play a role in tumor cell growth regulation and expression of the inducible COX, COX-2. We therefore evaluated the effects of various antioxidants on COX expression and cellular growth in the human CRC cell line HCA-7. The antioxidants pyrrolidinedithiocarbamate (PDTC), N-acetylcysteine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and U74006 decreased PG production, intracellular redox status, and cellular growth in a concentration-dependent manner. The decrease in cellular growth was associated with the induction of apoptosis. Unlike the selective COX inhibitors 1-[(4-methylsulfonyl)phenyl]-3-trifluoromethyl-5-[(4-fluorophenyl)pyrazole (SC 58125) and (2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS 398) that inhibit COX-2 catalytic activity, these antioxidants decreased COX-2 expression at the transcriptional level. Combined treatment of HCA-7 cells with PDTC and SC 58125 resulted in an additive decrease in PG levels and anchorage-dependent and -independent growth. Furthermore, whereas antioxidants or SC 58125 reduced tumor growth in vivo, coadministration of PDTC and SC 58125 resulted in actual tumor regression. These results suggest that combined therapy with NSAIDs and antioxidants might be useful in the prevention and/or treatment of CRC.

Introduction

CRC is the second leading cause of cancer mortality in Western societies. Epidemiological studies have reported up to a 50% decrease in the relative risk of CRC in persons who regularly ingest aspirin or other NSAIDs, suggesting that these drugs are effective cancer chemopreventive agents (1). NSAIDs exert their pharmacological effect by inhibiting COX, the enzyme responsible for PG synthesis. However, prolonged use of drugs such as aspirin frequently results in untoward gastrointestinal side effects, which has led to the development of more selective COX inhibitors (2).

Two isoforms of COX, COX-1 and COX-2, have been identified. Although they both catalyze the formation of the unstable PG enol, COX-2 expression is markedly elevated in most colorectal adenomas (5). Furthermore, COX-2 but not COX-1 expression is markedly elevated in most colonic tumors in azoxymethane-treated rats and in intestinal adenomas from multiple intestinal neoplasia (Min) mice (6, 7). Although the proposed role of COX-2 in tumor development and progression has not been fully elucidated, numerous studies have demonstrated that selective COX-2 inhibitors can significantly reduce polypl formation and tumor growth in vivo (8, 9).

Alterations in oxidant tone have been associated with perturbations of cell growth and transformation. Irani et al. (10) reported that activation of H-Ras in fibroblasts led to an elevation of superoxide production, which was associated with increased cellular proliferation. More recently, we have demonstrated a relationship between intracellular redox status and CRC cell growth (11). Numerous genes and their enzyme products, including COX-2, are regulated by cellular redox status. For example, nitric oxide and peroxynitrite, the coupling product of nitric oxide and superoxide, have been shown to increase COX-2 activity in the murine macrophage cell line RAW 264.7 (12). Additionally, interleukin 1 and LPS-induced COX-2 expression has been reported to be decreased after antioxidant treatment of rat mesangial cells and alveolar macrophages, respectively (13, 14). We have therefore evaluated the effect of various antioxidants on intracellular redox status and COX-2 expression and function in human CRC cell line HCA-7 and correlated these changes with effects on cellular proliferation. HCA-7 cells express COX-1 and COX-2 and produce large amounts of PGs (15). We report herein that antioxidants significantly decrease PG production and proliferation in this cell line. The decrease in proliferation is due to an induction of G1 cell cycle arrest and/or apoptosis. In addition, we examined the potential additive interaction between antioxidants and SC 58125 in vitro and in vivo models of CRC. Whereas the administration of SC 58125 alone reduced tumor growth in vivo, the combination of an antioxidant and SC 58125 caused significant tumor regression. Therefore, these data suggest that coadministration of antioxidant(s) and selective COX-2 inhibitor(s) may be useful in the prevention and/or treatment of CRC.

Materials and Methods

Materials. Cell culture reagents were purchased from Life Technologies, Inc. (Grand Island, NY). Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise stated. [3H]Thymidine was purchased from Amersham (Arlington Heights, IL). Polyclonal antibodies to human COX-1 and COX-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antioxidant lazaroid U74006 (tirilazad mesylate) was a gift from John McCall (Upjohn-Pharmacia Pharmaceuticals, Kalamazoo, MI). SC 58125 was generously provided by Peter Isakson (G. D. Searle and Co., St. Louis, MO), and NS 398 was purchased from Cayman Chemicals.
Antioxidants and COX Expression

Cell Culture and Cell Growth Assays. HCA-7 cells were grown as described previously (15). Effects of selected agents on DNA synthesis were evaluated by \([\text{\textsuperscript{3}H}]\)thymidine incorporation. HCA-7 cells \((1 \times 10^5 \text{cells/filter})\) were plated on 24-mm Transwell filters \(0.4 \mu \text{m} \text{pore size; Costar, Cambridge, MA}\) in DMEM, and experiments were performed when cells reached 100% confluence. Cells were serum-starved for 48 h before the addition of test agents. After a 21-h incubation, cells were pulsed with \([\text{\textsuperscript{3}H}]\)thymidine \((1 \mu \text{Ci/well})\) for an additional 3 h. The relative amount of radioactivity incorporated into trichloroacetic acid-insoluble material was determined by scintillation counting in aqueous fluor (Amersham). Results were normalized to cell number that was determined in replicate wells for each condition, and all results presented are representative of at least three separate experiments.

To assess anchorage-independent cell growth, HCA-7 cells \((1 \times 10^5 \text{cells/35-mm dish})\) were plated in DMEM supplemented with 1% \((v/v)\) fetal bovine serum and 0.4% \((w/v)\) agar in the presence or absence of the selected agent. The colony number was quantified after 10 days using an Omnicon image analyzer. Colonies greater than 50 \(\mu \text{m}\) in diameter \((approximately 50 \text{cells})\) were scored as positive. Values are representative of three experiments carried out in triplicate.

Flow Cytometric Analysis. Unsynchronized HCA-7 cells were exposed to different concentrations of selected agent for 24 h, and the cell cycle profile was determined as described previously (11). Detection of apoptotic cells by flow cytometry was performed using the ApopTag plus \textit{in situ} apoptosis detection kit \((Oncor, Gaithersburg, MD)\).

Alterations in Intracellular Redox Status. Intracellular redox status (as determined by endogenous H\(_2\)O\(_2\) levels) was performed as described previously using 5 \(\mu\)M DHR as a specific fluorescent dye probe \((Molecular\,Probes,\,Inc.,\,Eugene,\,OR;\,Ref.\,11)\).

Determination of PGs. Serum-starved HCA-7 cells \((1 \times 10^5 \text{cells/filter})\) were treated with selected agent(s) \((24\,h)\). Arachidonic acid \((>99\%\,pure;\,Nu\,Chek\,Prep.,\,Inc.,\,Elysian,\,MN)\) was added for the last 30 min of the incubation to a final concentration of 20 \(\mu\)M. Medium was removed from the basolateral surface of the cells. PGE\(_2\) was measured by a stable isotope dilution assay using gas chromatography/negative ion chemical ionization mass spectrometry (15).

Western Blot Analysis of COX Proteins. Total cell extracts were prepared and denatured by boiling for 5 min in SDS-polyacrylamide sample buffer. Total cell extract \((100 \mu\)g) was resolved by 12% SDS-PAGE and electrotransferred to Immobilon-P membrane \((Millipore,\,Bedford,\,MA)\). The membrane was then incubated with 5% nonfat dry milk in PBS for 1 h at room temperature and subsequently incubated with antibodies against COX-1 or COX-2 \((0.1 \mu\text{g/ml})\) overnight at 4°C. Protein proteins were visualized with enhanced chemiluminescence \((Amersham)\).

Northern Blot Analysis. Polyadenylated mRNA was isolated from treated HCA-7 cells as described previously (11). mRNA \((3 \mu\text{g})\) was separated by electrophoresis through 1% \((w/v)\) agarose-formaldehyde gels and blotted onto 0.2-\(\mu\text{m}\) nitrocellulose membranes. Hybridizations were performed with human-specific probes labeled by RNA polymerase-directed reverse transcription \((18,19)\) or random primer extension \((COX-1)\) or \((COX-2)\); kindly provided by Dr. Raymond N. DuBois, Nashville, TN). 1B15 was used as a constitutive cDNA probe to assure equivalent loading and transfer of RNA.

Nuclear Run-On Assay. HCA-7 nuclei were prepared in NP40 lysis buffer \((10 \text{mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM MgCl}_2, \text{and 0.5\% (v/v) NP40})\). Nuclei were isolated from cells in serum-free medium alone \((control)\) or with selected agents for the indicated intervals. Nuclear run-on transcripts were labeled as described previously (16). Equivalent amounts of radioactivity were added to each nitrocellulose filter and hybridized for 4 days at 42°C in the presence of 50% \((v/v)\) formamide. Filters were prepared by blotting 5.0 \(\mu\text{g}\) of COX-1, COX-2, or 1B15 cDNA inserts \((as\,well\,as\,BKSII\,plasmid\,DNA)\). Posthybridization washes were performed at 42°C using 0.1\% \((w/v)\) SSC, 0.1% \((w/v)\) SDS, and 1 \(\text{mM}\) EDTA.

Tumorigenicity in Vivo. HCA-7 cells \((1 \times 10^5)\) suspended in 0.2 ml of PBS were injected s.c. between the scalp of male athymic mice \((BALB/c\,nu/nu;\,Harlan\,Sprague\,Dawley\,Inc.,\,Indianapolis,\,IN)\). When tumors reached a mean size of 150 \(\text{mm}^3\), animals received three-weekly i.p. injections of either SC 58125 \((5 \text{mg/kg})\), PDTC \((100 \text{mg/kg})\), a combination of both agents, or saline for 5 weeks. Tumor volumes were estimated weekly as described previously (11). In preliminary experiments, a series of single doses of SC 58125 and PDTC were administered over a 30-day period to establish the LD\(_{90}\) and an effective route of administration.

Statistical Analyses. Paired \(t\) tests were used to test for significant differences between treated and untreated cells in growth assays. Tests of hypotheses concerning the relationships between DHR fluorescent changes, \([\text{\textsuperscript{3}H}]\)thymidine incorporation, and PG release or the overall therapeutic efficacy of agents used in vivo were carried out using the restricted maximum likelihood mixed effect model. All tests of significance were two-sided, and differences were considered statistically significant when \(P\) values were < 0.05.

Results

Effect of Antioxidants on Intracellular Redox Status, Cell Proliferation, and PG Levels in HCA-7 Cells. Initially, we addressed the ability of antioxidants \((U74406,\,NAC,\,PDTC,\,and\,Trolox)\), selective COX-2 inhibitors \((NS\,398\,and\,SC\,58125)\), a nonselective COX inhibitor \((indomethacin)\) or a non-COX modulator \((acetaminophen)\) to affect the growth, PG production, and redox status of HCA-7 cells \textit{in vitro}. As shown in Table 1, antioxidants inhibited cellular proliferation, as measured by a concentration-dependent reduction in \([\text{\textsuperscript{3}H}]\)thymidine incorporation and cell number \((data\,not\,shown)\). As previously reported, indomethacin, SC 58125, and NS 398 exerted similar growth-inhibitory effects in these cells \((15)\), whereas cellular proliferation was unaffected by acetaminophen.

To determine whether these effects on proliferation correlated with the antioxidant properties of these compounds on the modulation of PG production, we quantified the intracellular redox status \((by\,measuring\,DHR\,fluorescence\,as\,an\,indicator\,of\,endogenous\,H_2O_2\,levels)\) and PGE\(_2\) levels in parallel cultures over a 24-h period. As shown in Table 1, antioxidants dose-dependently decreased endogenous H\(_2\)O\(_2\), an event that correlated with a decrease in PGE\(_2\) levels. These cellular changes \((H_2O_2\,production\,and\,PGE_2\,levels)\) were significantly correlated with the observed decrease in cellular proliferation \((P < 0.001)\). This statistically significant correlation demonstrates a link between the ability of antioxidants to alter intracellular redox status, cellular proliferation, and PGE\(_2\) production. In distinct contrast, H\(_2\)O\(_2\) levels failed to correlate with PGE\(_2\) production and proliferation in HCA-7 cells treated with either nonselective or selective COX-2 inhibitors, except at very high concentrations.

Antioxidants Inhibit COX-2Expression at the Transcriptional Level. The effect of antioxidants and NSAIDs on COX-1 and COX-2 expression in HCA-7 cells was then determined. Initial studies showed that PG production was maximally inhibited at 8 h after antioxidant or NSAID treatment \((data\,not\,shown)\); thus, COX-1 and COX-2 mRNA were measured at this time point. PDTC dose-dependently reduced COX-2 but not COX-1 mRNA and protein levels \((Fig.\,1,\,A\,and\,B)\). Similar decreases in COX-2 mRNA and protein were observed after treatment of HCA-7 cells with U74406, NAC, or Trolox. Because previous reports have demonstrated that PDTC can modulate nuclear factor \(\kappa\)B and CAAT/Enhancer Binding Protein \((C,EBP)\) \(\beta\) DNA-binding activities \((11,17)\), and recognition sites for these factors are located within the COX-2 gene promoter \((18)\), the mechanism by which COX-2 mRNA was reduced by PDTC was further investigated by nuclear run-on transcription assays. Fig. 1C shows that the treatment of HCA-7 cells with 50 \(\mu\)M PDTC significantly decreased COX-2 transcription by 1 h of treatment. This suppression of COX-2 transcription persisted even after 24 h of treatment with PDTC \((data\,not\,shown)\). Treatment of HCA-7 cells with U74406 \((10^{-6}\,\text{M})\) or NAC \((50 \mu\text{M})\) exhibited similar reductions in COX-2 mRNA transcripts \((data\,not\,shown)\), indicating that antioxidants decrease COX-2 activity at the transcriptional level. In contrast, the selective COX-2 inhibitor SC 58125, which inhibits COX-2 activity by binding directly to the catalytic site, failed to alter COX-2 transcriptional activity, although a slight decrease in COX-2 protein was noted at 50 \(\mu\)M \((Fig.\,1,\,A-C)\). Treatment of cells with acetaminophen failed to alter COX-1 or COX-2 expression.

Antioxidants Decrease HCA-7 Cell Growth \textit{in Vitro} and \textit{in Vivo}. To examine the effect of antioxidants on tumorigenicity, HCA-7 cells were initially treated with various antioxidants or SC 58125 in a soft

STUDIES WERE THEN PERFORMED TO EXAMINE COMBINED TREATMENT OF PDTC AND SC 58125 ON ANCHORAGE-INDEPENDENT GROWTH OF HCA-7 CELLS. AS SHOWN IN FIG. 2, COMBINED TREATMENT RESULTED IN A FURTHER DECREASE (APPROXIMATELY 60%) IN HCA-7 CELL GROWTH. THIS EFFECT WAS ACCOMPANYED BY AN ADDITIONAL REDUCTION IN [3H]THYMIDINE INCORPORATION AND PGE2 LEVELS, SUGGESTING THAT INHIBITION OF PG SYNTHESIS IS ASSOCIATED WITH DECREASED TUMORIGENESIS.

WE NEXT EXAMINED THE THERAPEUTIC EFFICACY OF PDTC ALONE (100 mg/kg) OR IN COMBINATION WITH SC 58125 (5 mg/kg) IN VIVO BY TREATING ATHYMIC MICE BEARING HCA-7 TUMOR XENOGRAFTS. AFTER THE ESTABLISHMENT OF PALPABLE TUMORS (MEAN TUMOR VOLUME, 150 mm3), ANIMALS RECEIVED EITHER PDTC, SC 58125, BOTH AGENTS, OR NORMAL SALINE ONCE PER WEEK. AS SHOWN IN FIG. 3, PDTC OR SC 58125 GIVEN INDIVIDUALLY SIGNIFICANTLY SLOWED TUMOR GROWTH OVER THE 5 WEEKS OF TREATMENT IN COMPARISON WITH SALT-TREATED CONTROLS, THOUGH TUMOR GROWTH WAS NOT PREVENTED BY EITHER SINGLE AGENT. HOWEVER, TREATMENT OF ANIMALS WITH THE COMBINATION OF PDTC AND SC 58125 LED TO A CESSION OF TUMOR GROWTH, AND BY WEEK 5 OF TREATMENT, THERE WAS ACTUAL TUMOR REGRESSION. CESSATION OF TREATMENT RESULTED IN A SMALL BUT SIGNIFICANT INCREASE IN TUMOR GROWTH OVER THE FOLLOWING 16 WEEKS (965 ± 125 mm3).

DISCUSSION

THESE STUDIES DEMONSTRATE THAT ANTIOXIDANTS, IN ADDITION TO NSAIDS, MODULATE PG PRODUCTION IN THE CRC CELL LINE HCA-7. IN CONTRAST TO THE SPECIFIC COX-2 INHIBITOR SC 58125, HOWEVER, NUCLEAR RUN-ON EXPERIMENTS DEMONSTRATE THAT THE ABILITY OF ANTIOXIDANTS TO DECREASE PGs IS REGULATED AT THE LEVEL OF COX-2 GENE EXPRESSION. ASSOCIATED WITH THE DECREASE IN PG LEVELS WERE SIGNIFICANT REDUCTIONS IN H2O2 PRODUCTION AND PROLIFERATION, SUGGESTING A DIRECT RELATIONSHIP BETWEEN THE ABILITY OF ANTIOXIDANTS TO DECREASE EICOSANOID SYNTHESIS, ALTER INTRACELLULAR REDOX STATUS, AND AFFECT GROWTH IN HCA-7 CELLS. THE ABILITY OF THESE COMPOUNDS TO DECREASE PROGAGON PRODUCTION AND PROLIFERATION IS LIKELY DUE TO THEIR ANTIOXIDANT PROPERTIES AS OPPOSED TO OTHER PHARMACOLOGICAL EFFECTS, BECAUSE WE STUDIED AGENTS THAT VARIED WIDELY WITH REGARD TO CHEMICAL AND PHYSICAL PROPERTIES. WHEREAS THE ABILITY OF ANTIOXIDANTS TO DECREASE PG PRODUCTION IN HCA-7 CELLS SEEMS TO BE DUE TO A REDUCTION IN COX-2 GENE TRANSCRIPTION, ANTIOXIDANTS REGULATE EICOSANOID SYNTHESIS IN SEVERAL WAYS. IT HAS BEEN REPORTED PREVIOUSLY IN THE MACROPHAGE CELL LINE RAW 264.7 THAT ENHANCED EICOSANOID SYNTHESIS IS DEPENDENT ON THE INTRACELLULAR FORMATION OF PEROXYNITRITE, A POTENT OXIDANT GENERATED FROM NITRIC OXIDE AND SUPEROXIDE (12). SUPEROXIDE DISMUTASE MIMETICS OR NITRIC OXIDE SYNTHASE INHIBITORS DECREASE PG PRODUCTION IN THIS CELL LINE. PEROXYNITRITE LIKELY AFFECTS PG SYNTHESIS BY DIRECTLY ALTERING THE CATALYTIC ACTIVITY OF COX. FURTHERMORE, IT HAS BEEN REPORTED THAT VITAMIN E AND OTHER ANTIOXIDANTS DECREASE THROMBOXANE FORMATION IN HUMAN PLATELETS BOTH IN VITRO AND IN VIVO, PROBABLY BY DIRECTLY INHIBITING THE FORMATION OF THE COX-DERIVED BICYCLOENDOPEROXIDE INTERMEDIATE PGH2 (19, 20). ON THE OTHER HAND, SAKAMOTO ET AL. (21) COULD FIND NO EFFECT OF VITAMIN E ON PG SYNTHESIS IN LPS-STIMULATED RAT MACROPHAGES. IN ADDITION TO INHIBITING THE CATALYTIC ACTIVITY OF COX, TETSUKA ET AL. (13) HAVE REPORTED THAT ANTIOXIDANTS DECREASE COX LEVELS VIA A POSTTRANSCRIPTIONAL MECHANISM IN RAT MESANGIAL CELLS. FURTHERMORE, IN ALVEOLAR MACROPHAGES, HEMPEL ET AL. (14) HAVE REPORTED THAT ANTIOXIDANTS DECREASE COX-2 GENE TRANSCRIPTION, LEADING TO A DECREASE IN EICOSANOID SYNTHESIS. IN THE PRESENT STUDIES, WE HAVE FOUND THAT COX-2 LEVELS ARE REGULATED BY ANTIOXIDANTS AT THE TRANSCRIPTIONAL LEVEL. NONETHLESS, IT IS ALSO CONCEIVABLE THAT ANTIOXIDANTS REGULATE EICOSANOID SYNTHESIS IN HCA-7 CELLS BY INHIBITING THE CATALYTIC ACTIVITY OF COX OR POSTTRANSCRIPTIONAL REGULATION OF mRNA STABILITY.

UNLIKE PREVIOUS REPORTS IN WHICH ANTIOXIDANTS HAVE BEEN SHOWN TO REGULATE EICOSANOID PRODUCTION IN VARIOUS CELL LINES, WE HAVE FOUND A LINK BETWEEN THIS BIOCHEMICAL PERTURBATION AND A FUNCTIONAL RESPONSE IN HCA-7 CELLS, SPECIFICALLY, INHIBITION OF GROWTH. HOW ANTIOXIDANTS AFFECT
tumor cell growth is an area of intense interest. In this regard, we have previously reported that antioxidants inhibit growth in CRC cell lines, including HCT 116 and HCT 15, by induction of p21(WAF1/CIP1) through a mechanism involving CAAT/Enhancer Binding Protein (C/EBP) β independent of p53 (11). However, this pathway is independent of PG synthesis, because neither HCT 15 cells nor HCT 116 cells express detectable COX protein, and neither generates eicosanoids. A decrease in cell proliferation can be attributed to either growth arrest or cell loss due to apoptosis. Growth of HCA-7 cells in the presence or absence of selected agents for 24 h, followed by propidium iodide staining of cells and subsequent flow cytometric analysis, revealed that selected antioxidants and COX-2 inhibitors induced a significant accumulation of cells in the G1 phase of the cell cycle and/or the appearance of a sub-G1 peak in a dose-dependent manner (data not shown). As shown in Table 1, treatment of HCA-7 cells with antioxidants at high concentrations led to an increase in the

Fig. 1. Effect of PDTC or SC 58125 on COX expression in HCA-7 cells. A, Western blot of HCA-7 cells after PDTC or SC 58125 treatment for 24 h. COX-2 but not COX-1 protein levels are decreased by PDTC in a concentration-dependent manner. B, Northern blot of HCA-7 cells treated for 8 h as described above. 1B15 is shown as a control for equivalent loading and transfer. C, nuclear run-on analysis of COX transcription in HCA-7 cells after treatment with SC 58125 (50 μM) or PDTC (50 μM) for 0, 1, and 2 h. Nascent mRNA transcripts were elongated with RNA polymerase and 250 μCi of [32P]UTP. Equal counts were added to nitrocellulose membranes containing 5 μg of plasmid DNA, COX-1, COX-2, or 1B15 cDNA inserts.

Fig. 2. Effects of PDTC and SC 58125 on HCA-7 cell growth and PGE2 levels in vitro. A, soft agar colony formation was measured by seeding HCA-7 cells in agar supplemented with either medium alone (control), PDTC (25 μM), SC 58125 (25 μM), or both agents. Colonies were scored after 10 days of growth at 37°C. Values are representative of three experiments carried out in triplicate. * PDTC or SC 58125 significantly decreased anchorage-independent cell growth compared to that of untreated cells (P < 0.01). △, treatment with PDTC and SC 58125 significantly decreased anchorage-independent cell growth compared to that of cells treated with the individual agent (P < 0.05). B, HCA-7 cells were grown in Transwell filters and were serum-starved for 48 h. Cells were then treated with PBS (control), PDTC (25 μM), SC 58125 (25 μM), or PDTC and SC 58125. A 3 h pulse of [3H]thymidine (1 μCi/ml) was administered 21 h after agent treatment, and trichloroacetic acid-precipitable counts were determined. All experiments were performed in triplicate and repeated three times. Values are expressed as mean ± SE. * PDTC or SC 58125 significantly decreased DNA synthesis compared to that of untreated cells (P < 0.05). △, treatment with PDTC and SC 58125 significantly decreased DNA synthesis compared to that of cells treated by the addition of either single agent alone. C, under identical experimental conditions as those described in B, a 30-min pulse of arachidonic acid (20 μM) was administered before media were collected. PGE2 release into the medium was quantified as described in Table 1. Results are expressed as nanograms of PGE2/105 cells. * PDTC or SC 58125 significantly decreased PGE2 release compared to that of untreated cells (P < 0.05). △, PDTC and SC 58125 significantly decreased PGE2 synthesis compared to that of cells treated by the addition of either single agent alone (P < 0.01).

4 R. Chinery, R. J. Coffey, and J. D. Morrow, unpublished observations.
number of TUNEL-positive cells, indicating that the sub-G1 peak was most likely due to the induction of apoptosis. In addition, HCA-7 cells treated with NSAIDs displayed apoptotic effects, as has been reported for other intestinal epithelial cell lines (22, 23).

Thus, our studies have now provided evidence that a second pathway, presumably involving PG production, seems to be operative in the inhibition of CRC tumor growth by antioxidants. The relationship between PG production and the induction of p21WAF1/CIP1, however, remains to be determined.

An important aspect of the present study is that the combined administration of antioxidants and SC 58125 to HCA-7 cells in vitro results in an additive decrease in PG production and cell growth compared to cells treated with either agent alone. These observations are supported by in vivo experiments in athymic mice that show that PDTC combined with SC 58125 inhibits HCA-7 tumor xenograft growth to a greater extent than either agent alone. Furthermore, in these studies, tumor size actually decreases in mice receiving both agents. These results would suggest an additive or synergistic effect between antioxidants and NSAIDs to inhibit eicosanoid synthesis leading, presumably, to cell death. We have previously reported that antioxidants enhance the cytotoxicity of chemotherapeutic agents toward CRC tumors in athymic mice (11). It would thus be of interest to determine whether an additional cytotoxic effect is observed when athymic mice bearing CRC tumors are given NSAIDs in addition to antioxidants and chemotherapy agents.

In summary, the present studies report that antioxidants modulate PG production, COX expression, and cellular growth in the human CRC cell line HCA-7. In addition, antioxidants combined with NSAIDs inhibit tumor cell growth in vitro and in vivo to a greater extent than either agent alone and, in fact, cause a decrease in tumor burden in athymic mice. These observations may provide an impetus to determine whether combination therapy with NSAIDs and antioxidants is beneficial in the prevention and/or treatment of human CRC.

References


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