Influence of Coenzyme A-independent Transacylase and Cyclooxygenase Inhibitors on the Proliferation of Breast Cancer Cells

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ABSTRACT

Recent studies have demonstrated that arachidonic acid (AA) may serve as an important signal that blocks cell proliferation of certain neoplastic cells. The current study was conducted to determine whether disruption of AA homeostasis influences breast cancer cell proliferation and death. Initial experiments revealed that inhibition of AA remodeling through membrane phospholipids by inhibitors of the enzyme, coenzyme A-independent transacylase (CoA-IT), attenuates the proliferation of the estrogen receptor-negative, MDA-MB-231, and estrogen receptor-positive, MCF-7 breast cancer cell lines. This growth inhibition was accompanied by a marked accumulation of AA in both free fatty acid and triglyceride forms, a marker of intracellular AA stress within mammalian cells. Cell cycle synchronization experiments revealed that the CoA-IT inhibitor, SB-98625, blocked MDA-MB-231 cell replication in early to mid G1 phase. Time-lapse video microscopy, used to observe the changes in cell morphology associated with apoptosis, indicated that SB-98625 treatment induced early rounding and occasional blebbing but not late apoptotic events, blebstering, and lysis. The cyclooxygenase inhibitors, NS-398 and indomethacin, were found to be less potent blockers of cell proliferation and poor inducers of cellular AA accumulation than CoA-IT inhibitors in these breast cancer cell lines. Finally, AA provided exogenously blocked the proliferation of MCF-7 cells, and this effect could be attenuated in MCF-7 cells overexpressing the glutathione peroxidase gene, GSHPx-1. Taken together, these experiments suggest that disruption of AA remodeling in a manner that increases intracellular AA may represent a novel therapeutic strategy to reduce cancer cell proliferation and that an oxidized AA metabolite is likely to mediate this effect.

INTRODUCTION

Over the past four decades, it has been firmly established that the modulation of AA levels is intimately linked to cellular function and human disease (1–6). Recent studies suggest that AA is an important mediator of cellular events that control mitogenesis and apoptosis (7–15). These studies raise the hope that modulation of AA metabolism represents a novel strategy to attenuate cancer cell proliferation and thereby reduce tumor development. A consistent finding fueling this concept is that NSAIDs that block COX activity are effective in reducing the incidence of colon tumors in both humans and rodents (16–21). Two forms of COX, COX-1 and COX-2, are encoded by separate genes producing protein products that have ~60% amino acid sequence homology (22) and are considered the constitutive and the inducible forms, respectively. Several studies have revealed that COX-2 is overexpressed in transformed cells and tumors (23–29). An association of COX-2 with cancer is strongly supported by experiments that show that COX-2 knockout mice have a markedly reduced number of intestinal polyps in a murine model of adenomatous polyposis (APC1716 knockout; Ref. 30).

A key question generated but left unanswered by these data are: how does diminished COX expression alter tumor growth? It is known that overexpression of COX-2 in intestinal epithelial cells prevents them against apoptosis (31–33). COX-2 expression has also been linked to the promotion of angiogenesis, enhanced expression of bcl-2, and decreased expression of transforming growth factor β2 and E-cadherin (33–37). Human colon cancer cells stably transfected with COX-2, when compared with control vector or parental cells, are more invasive, and the increased invasiveness can be reversed by treatment with NSAIDs (26). It is also known that NSAIDs can induce apoptosis in several cell lines and tumors (38–41). All of these studies suggest that the overexpression of COX-2 favors the survival of certain cells, thus enhancing tumorigenesis.

Most investigators have assumed that the antineoplastic effects of NSAIDs are attributable to decreased eicosanoid production. However, Chan et al. (42) have recently suggested an alternative mechanism to account for NSAID effects in cancer. They found that NSAID treatment of colon tumor cells results in a marked increase in intracellular AA levels which, in turn, stimulates the conversion of sphingomyelin to ceramide. These data suggest that an increase in free intracellular levels of AA, and not a decrease in prostaglandin production, may mediate apoptosis after NSAID treatment of colon cancer cells. Consistent with this observation, we have demonstrated recently that increasing intracellular AA levels above a critical threshold in HL-60 cells increases ceramide formation and apoptosis (43). Furthermore, Finstad et al. (44) demonstrated that either AA or eicosapentaenoic acid could block HL-60 cell proliferation in a prostanoïd-independent manner primarily by promoting apoptosis or cell differentiation. Together, these studies suggest that maintaining intracellular AA levels below a critical threshold may be necessary to prevent the apoptosis of some cells.

When AA enters a cell or is mobilized from intracellular phospholipids by phospholipases, there are selective pathways that transport AA back into phospholipids, thereby maintaining very low intracellular concentrations of AA. In fact, measurable quantities of AA cannot be detected in most resting mammalian cells (45). There are as many as 20 different arachidonate-containing phospholipid molecular species in any given neoplastic cell. AA moves through these different molecular species in a cyclic fashion, requiring the activity of several enzymes (45). One of these enzymes is the CoA-independent transacylase, which transfers an acyl moiety between the two different classes of phospholipids. Specifically, the acyl group from a phospholipid donor is transferred to a lyso-phospholipid in the absence of CoA or the production of a free fatty acid intermediate. The current study tests the hypothesis that maintaining intracellular AA concentrations below a critical threshold is important for preventing apoptosis of breast cancer cells using COX inhibitors and a novel set of inhibitors of the CoA-IT enzyme. This study suggests that enzymes
that control intracellular AA levels may represent novel therapeutic targets to block tumorigenesis.

MATERIALS AND METHODS

Materials. DMEM, penicillin-streptomycin, glutamine, and FBS were purchased from Life Technologies, Inc. (Gaithersburg, MD). HBSS was purchased from Life Technologies, Inc. (Grand Island, NY). The enzyme inhibitors N-(2-cyclohexyloxy-4-nitrophenyl)-methanesulfonamide (NS-389) and indomethacin were purchased from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA). Fatty acids were purchased from Cayman Chemical Co. (Ann Arbor, MI). [5,6,8,9,11,12,14,15-14C]AA ([14C]AA) was purchased from American Radiolabeled Chemical, Inc. (St. Louis, MO). Phosphatidylcholine, phosphatidyl ethanolamine, phosphatidylserine, and phosphatidylinositol standards were purchased from Serdary Research Labs (Englewood Cliffs, NJ). Uniplate Silica Gel G TLC plates were purchased from Analtech, Inc. (Newark, NJ). Ecolume scintillation cocktail was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). NP40 was purchased from Accurate Chemical and Scientific Corp. (Westbury, NY). Mono-, di- and tri-linolein glyceride standards, essentially fatty acid-free HSA, DMSO, propidium iodide, RNase, sodium chloride, sodium citrate, sodium azide, Trizma-base, hydroxyurea, and trypan blue were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents (high-performance liquid chromatography grade) and other general reagents were purchased from Fischer Scientific (Norcross, GA). The CoA-IT inhibitor, SB-98625 [diethyl-7-(3,4,5-triphenyl-2-oxo-2,3-dihydroimidazol-1-yl)heptapeptide phosphonate] was synthesized in our laboratory, and SB-45905 [2-[2-[3,4,5,6-tetrahydrophencyl]phenyl]-4-[3-(trifluoromethylenyl)phenyl]-4,5-dichlorobenzene sulfonic acid] was a generous gift from Dr. James Winkler (Smithkline Beecham, King of Prussia, PA).

Cells and Cell Culture. MDA-MB-231 cells were maintained in DMEM containing heat-inactivated 10% FBS, 100 units/ml penicillin-streptomycin, and 2 mM L-glutamine; MCF-7 cells were maintained in DMEM/F12 medium containing heat-inactivated 10% FBS, 100 units/ml penicillin-streptomycin, and 2 mM L-glutamine, and MCF-7/H6 cells were maintained in DMEM/F12 medium containing heat-inactivated 10% FBS, 100 units/ml penicillin-streptomycin, and 2 mM L-glutamine, and MCF-7/H6 cells were maintained in DMEM/F12 medium containing heat-inactivated 10% FBS, 0.5 mg/ml G-418, 2 mM L-glutamine; MCF-7 cells were passaged weekly from confluent populations by trypsinization at concentrations of 1.0 × 10^5 cells/flask (75 cm^2), 4.0 × 10^5 cells/flask (25 cm^2), and 2.0 × 10^6 cells/flask (75 cm^2). The flask was placed on an Axiovert 135 inverted phase contrast microscope from Zeiss (Thornwood, NY) equipped with an enclosed stage heater and air recirculator device that maintained a constant temperature (37°C) and CO_2 atmosphere (5:95). Cells were illuminated with red light, and images were collected using a 100 CCD camera from DAGE-MTI (Michigan City, IN) and a model 6740 time lapse video recorder from Panasonic (Suwanee, GA) at a fixed rate of 1 frame every 10 s (final time lapse, 600:1).

Cell Cycle Analysis. Cells were plated into several subconfluent populations and treated with different concentrations of an inhibitor or fatty acid or an equivalent volume of the delivery vehicle (ethanol or DMSO). After 18 h of treatment both adherent and nonadherent cells were pooled and prepared for analysis by flow cytometry. After centrifugation and removal of the old medium, cells were washed with PBS (pH 7.4). The pellet was resuspended in 100 μl of PBS and fixed by adding 2 ml of cold 70% ethanol solution (70:30 ethanol:water, v/v; -20°C). The cells were incubated at -20°C for 10 min and then centrifuged and resuspended in 100 μl of PBS. The cells were stained with a propidium iodide solution prepared by adding NP40 (0.6% v/v) and 36 μg/ml RNase to a 1× dilution of a 20× stock (1.1688 g of sodium chloride, 2.130 g of sodium citrate, and 0.100 g of propidium iodide in 100 ml of water, pH 7.6 using acetic acid). Data were collected using a Coulter Epics XL-MCL flow cytometer (Hialeah, FL) and analyzed with the Verity:ModFit program (version 5.2) from Verity Software House, Inc. (Topsham, ME). Each sample was analyzed using at least 20,000 events corrected for debris and aggregate populations. The percentage of inhibition was determined as a decrease in the percentage of cells in S phase as compared with controls.

Cell Cycle Synchronization. MDA-MB-231 cell populations were maintained at confluence for 24 h in medium depleted of serum to produce a G_0-G_1 cell cycle arrest. These cells were replated into several subpopulations (5 × 10^3 cells/cm^2) using fresh medium to induce their release into an active G_1 phase for 12 h, after which fresh medium containing hydroxyurea (2 mM) was added. This synchronized the cells near the G_1-S phase border. After 12 h, the cells were washed twice with medium to remove the hydroxyurea and allowed to grow in medium containing either delivery vehicle or inhibitor. Cells were harvested and prepared for flow cytometry as described above at various time points after being released from the block and treated.

Equilibrium Labeling of Glycerolipids with [3H]AA and Analysis of Labeled Products. Cellular glycerolipids were labeled to isotopic equilibrium with [3H]AA (2.0 μM at 200 μCi/mmol) with a confluent population of MDA-MB-231 cells. Specifically, [3H]AA in 200 μl of HBSS containing HSA (0.25 μg/ml) were added to the cells (1μCi/1 million cells) for 30 min at 37°C. The cells were then washed 3× with HBSS containing HSA and incubated for an additional 24 h in untreated medium under normal growth conditions. The cells were harvested and replated at subconfluent populations (5 × 10^5 cells/flask) in medium containing a CoA-IT or cyclooxygenase inhibitor or delivery vehicle. At the indicated times, the cells were harvested after washing 2× with HBSS containing HSA (0.25 μg/ml) and cellular lipids extracted by the method of Bligh and Dyer (49). Total radioactivity incorporated into lipids was determined by liquid scintillation spectroscopy using a fraction of the extract. Labeled lipids were combined with cold glycerolipid standards (10 μg each of phosphatidylcholine or phosphatidyl ethanolamine, monoglyceride, diglyceride, triglyceride, and free AA), spotted on silica gel G TLC plates, and developed using hexane:ethyl ether:formic acid (90:60:6, v/v). Radiolabeled products were visualized using a System 2000 Imaging system from BioScan (Washington, DC) and brief iodine staining. These products were then collected, and the radioactivity was determined by liquid scintillation spectrosopy.

RESULTS

Effect of Remodeling Inhibitors on the Growth of Breast Cancer Cells. Initial experiments in this study were designed to determine whether blocking AA remodeling would have effects on cells derived from solid tumors (estrogen receptor negative, MDA-MB-231, and positive, MCF-7, breast cancer epithelial cells) similar to those described previously in HL-60 cells. Addition of the CoA-IT inhibitor (25 μM SB-98625 for 72 h) to MDA-MB-231 or MCF-7 cells resulted in a 60 and 80% reduction in the exponential growth rates, respectively (Fig. 1). Although growth rates were reduced, the viability of both cell types remained >90% as determined by trypan blue exclusion. Similar results were observed with a structurally distinct CoA-IT inhibitor (SB 45905).^4^ Our previous study using HL-60 cells showed that the blockage of proliferation by CoA-IT inhibitors was accompanied by marked apoptosis. However, the aforementioned experiment suggested that apoptosis was not occurring in either of the breast cancer cell lines. Apoptosis was further examined using time-lapse video microscopy. Treatment of MDA-MB-231 cells with 25 μM SB-98625 resulted in the prevention of cell division after 24 h and induced only the

^4 Unpublished data.
28, 41, and 31% of cells were found in G1, S, and G2-M phases of the cell cycle, respectively (Fig. 2). At 25 μM SB-98625, the majority of control cells had reached G2-M of a second cell cycle. Reduction in the percentage of cells in S phase with a concomitant increase of cells in G1 was observed with SB-98625 (25 μM; Fig. 4). These data provide evidence that inhibition of AA remodeling blocks cell cycle progression in a manner nearly identical to that observed with the SB-98625 (25 μM; Fig. 4). Blockage of AA remodeling by the CoA-IT inhibitor led to a marked redistribution of AA from phospholipids to neutral lipid pools. Further analysis of cellular neutral lipids revealed that almost all of the radioactivity was associated with triglycerides. Within 72 h after adding the inhibitor, ~7% of the total cellular AA had shifted from phospholipids to triglycerides. In a separate set of experiments, radioactivity was analyzed in the lipid extract from both the cells and the growth medium combined. Here there was a significant increase in the radioactivity associated with free AA when control cells (6.8 ± 2.2 of total counts) were compared with CoA-IT inhibitor-treated cells (10.8 ± 2.0 of total counts).

Effect of CoA-IT Inhibition on Cell Cycle Progression. To begin to determine where CoA-IT inhibition influences cell growth, the cell cycle phase distribution was assessed by flow cytometry after treatment of MDA-MB-231 cells with the CoA-IT inhibitor, SB-98625 (25 μM), for 24 h. In asynchronous, rapidly proliferating cell populations, 28, 41, and 31% of cells were found in G1, S, and G2-M phases of the cell cycle, respectively (Fig. 2A). SB-98625 induced a dose-dependent reduction in the percentage of cells in S phase with a concomitant increase of cells in G1. At 25 μM, >75% of cells arrested in G1 of the cell cycle with only 14% of cells in S phase (Fig. 2C). Increasing the concentration of the inhibitor to 50 μM did not further influence cell cycle progression.

Subsequent experiments were designed to determine more precisely the point in the cell cycle that was influenced by CoA-IT inhibition. MDA-MB-231 cells were synchronized in late G1 of the cell cycle using hydroxyurea (as described in “Materials and Methods”) and then released in the presence or absence of the CoA-IT inhibitor (Fig. 3). By 3 h, the majority of both control and SB 98625-treated cells had moved into S phase of the cell cycle. Additionally, both treated and control cells moved from S phase to G2-M at similar rates. It was not until near the end of the first cell cycle/beginning of the second cycle that there was a marked difference between treated and control cells. At this point (12 h), control cells readily moved from G2-M to G1 and into a second cell cycle, whereas treated cells slowed their movement into early G1. By 24 h, treated cells had accumulated in G1 while the majority of control cells had reached G2-M of a second cell cycle. These data provide evidence that inhibition of AA remodeling blocks the cell cycle somewhere in early G1 before the hydroxyurea checkpoint. Similar experiments using exogenously provided AA revealed that high concentrations of AA (≥200 μM) blocked cell cycle progression in a manner nearly identical to that observed with the SB-98625 (data not shown).

Effect of Blocking AA Remodeling on the Distribution of Cellular AA. To determine the influence of CoA-IT inhibition on the distribution of AA in cellular lipid pools, MDA-MB-231 cells were labeled to isotopic equilibrium with [3H]AA and then treated with SB-98625 (25 μM; Fig. 4). Blockage of AA remodeling by the CoA-IT inhibitor led to a marked redistribution of AA from phospholipids to neutral lipid pools. Further analysis of cellular neutral lipids revealed that almost all of the radioactivity was associated with triglycerides. Within 72 h after adding the inhibitor, ~7% of the total cellular AA had shifted from phospholipids to triglycerides. In a separate set of experiments, radioactivity was analyzed in the lipid extract from both the cells and the growth medium combined. Here there was a significant increase in the radioactivity associated with free AA when control cells (6.8 ± 2.2 of total counts) were compared with CoA-IT inhibitor-treated cells (10.8 ± 2.0 of total counts).
Comparison of the Antiproliferative Effects of NSAIDs and CoA-IT Inhibitors on Breast Cancer Cell Lines. The next set of studies compared the effects of COX inhibitors (indomethacin, preference for COX-1, and NS-398, selective for COX-2) to a CoA-IT inhibitor (SB-98625) in MDA-MB-231 and MCF-7 cells (Fig. 5). The results demonstrate that SB-98625 is a more potent inhibitor of breast cancer cell proliferation with an IC50 of 16 μM for MDA-MB-231 cells and an IC50 of 10 μM for MCF-7 cells. Both indomethacin and NS-398 were less potent, with IC50s of 100 μM in both cell breast cancer cell lines. These COX inhibitor concentrations are well above

Fig. 3. Effect of the CoA-IT inhibitor, SB-98625, on the cell cycle progression of synchronized MDA-MB-231 cells. Cells were synchronized using hydroxyurea as described in “Materials and Methods.” Cells were released from the hydroxyurea block in the presence of either control vehicle (ethanol, 0.05%) or inhibitor (25 μM) and incubated for up to 24 h. At various time points, the cells were harvested and prepared for flow cytometry analysis as described in “Materials and Methods.”

Fig. 4. Incorporation of [3H]AA into triglycerides of MDA-MB-231 cells treated with the CoA-IT inhibitor, SB-98625. MDA-MB-231 cells labeled to isotopic equilibrium with [3H]AA were incubated with 0.05% ethanol (□) or 25 μM SB-98625 (■) for up to 72 h. Cellular lipids were extracted and then separated by TLC. Radioactivity was determined by scintillation counting of isolated zones. Values are reported as the percentage of total radioactivity per lane at each time point and represents the means of three separate experiments, and comparisons were made using the paired two-tailed Student’s t test; bars, SE.* P < 0.05; ** P < 0.01.
their IC\textsubscript{50} for prostaglandin inhibition in other cell types. For indomethacin, the value is closer to that reported for lipooxygenase inhibition (50).

**Influence of COX Inhibitors on the Distribution of Cellular AA.**
A potential explanation for the reduced potency of COX inhibitors (when compared with CoA-IT inhibitors) is that intracellular AA levels do not rise as drastically by blocking COX. Table 1 shows the accumulation of AA within triglycerides after treatment of cells with indomethacin, NS-398, SB-98625, or exogenous AA. Although there was a dose-dependent increase of AA within triglycerides after indomethacin treatment, the concentrations of either COX inhibitor required to increase levels of labeled triglycerides were much higher than their IC\textsubscript{50} for inhibiting COX. The exogenous addition of 100 \mu M AA resulted in the movement of \~50\% of the [\textsuperscript{3}H]AA from phospholipids into triglycerides. This result demonstrates that the \textit{de novo} synthesis pathway (45) of AA incorporation is up-regulated when cells are exposed to high concentrations of AA. Together, these data support a correlation between the capacity of an AA remodeling inhibitor to raise free AA levels with its capacity to block cell proliferation.

**Influence of Free Fatty Acids on Cell Proliferation.** The results described above suggest that raising intracellular AA levels above a critical threshold will prevent the proliferation of MDA-MB-231 cells and MCF-7 cells. The next set of experiments determined whether exogenously provided fatty acids would influence breast cancer cell proliferation. Fig. 6A illustrates that MCF-7 cell proliferation is more sensitive to exogenous AA when compared with the MDA-MB-231 cells with IC\textsubscript{50} values of 125 and 225 \mu M, respectively. This is consistent with the slightly lower IC\textsubscript{50} for the CoA-IT inhibition in MCF-7 cells.

A previous study by Chen et al. (51) showed that loosely coupled enzymes such as cytochrome P-450 can generate reactive oxygen species such as superoxide and hydrogen peroxide, and these products can react with certain 20 carbon polyunsaturated fatty acids to produce antineoplastic products. To examine whether this process might be important for the antiproliferative effects of AA in breast cancer cells, we exposed MCF-7 cells that stably overexpress the major cytoplasmic glutathione peroxidase enzyme, GPX-1 (EC 1.11.1.9), to exogenous AA. The transfected cell line produces 25-fold more GPX-1 activity than control MCF-7 cells and shows a significant decrease in susceptibility to oxidants, such as hydrogen peroxide and quinone doxorubicin (52). Fig. 6B shows that the cells overexpressing AA resulted in the movement of \~50\% of the [\textsuperscript{3}H]AA from phospholipids into triglycerides. This result demonstrates that the \textit{de novo} synthesis pathway (45) of AA incorporation is up-regulated when cells are exposed to high concentrations of AA. Together, these data support a correlation between the capacity of an AA remodeling inhibitor to raise free AA levels with its capacity to block cell proliferation.

**Table 1.** Effect of SB-98625, COX inhibitors, and AA on the distribution of [\textsuperscript{3}H]AA in MDA-MB-231 cells.

<table>
<thead>
<tr>
<th>Addition to growth medium</th>
<th>Inhibitor</th>
<th>[\textsuperscript{3}H]AA in cellular triglycerides, % of total dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% ethanol</td>
<td>control</td>
<td>4.0 ± 0.9</td>
</tr>
<tr>
<td>10 \mu M indomethacin</td>
<td>COX-1→COX-2</td>
<td>5.3 ± 1.4</td>
</tr>
<tr>
<td>50 \mu M indomethacin</td>
<td>COX-1→COX-2</td>
<td>6.9 ± 2.0</td>
</tr>
<tr>
<td>25 \mu M SB-98625</td>
<td>CoA-IT</td>
<td>9.3 ± 1.2</td>
</tr>
<tr>
<td>100 \mu M AA</td>
<td>control</td>
<td>49.2 ± 4.1</td>
</tr>
<tr>
<td>0.1% DMSO</td>
<td>control</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>10 \mu M NS-398</td>
<td>COX-2</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>50 \mu M NS-398</td>
<td>COX-2</td>
<td>4.6 ± 1.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} P < 0.005.  
\textsuperscript{b} P < 0.05.
aa were less sensitive to aa than control MCF-7 cells with
~2-fold shift to the right in the dose-response curve with IC_{50} of 210
and 125 μM, respectively. These data suggest that the production of
lipid oxidants may play a role in the capacity of AA to influence cell
proliferation.

**Discussion**

Epidemiology studies demonstrate that inhibitors of COX can re-
duce the risk of colon cancer, and to a lesser extent, the risk of breast
cancer (29). Additionally, Cox-1 and Cox-2 are overexpressed in
human breast cancer tissue. In the studies outlined here, we test the
hypothesis that inhibitors of Cox and CoA-independent transacylase
share the capacity to raise intracellular AA levels above a critical
threshold. The results suggest that: (a) both Coa-IT inhibitors, and
to a lesser extent COX inhibitors, block cell proliferation; (b) the Coa-
IT-induced block occurs in the early to mid-G_1 phase of the cell cycle;
(c) the capacity of Coa-IT or COX inhibitors to block cell proliferation
correlates well with their ability to induce the accumulation of
AA within triglycerides (a marker of high AA levels); (d) addition of
high concentrations of exogenous AA blocks the proliferation of
breast cancer cells; and (e) AA accumulation-induced cell cycle arrest
is reversed, at least in part, by overexpression of GPX-1.

Because of the increased potency of Coa-IT relative to COX
inhibitors, the current study focused on the cellular events associated
with Coa-IT inhibition in breast cancer cells. In previous studies with
HL-60 cells, it has been difficult to separate effects of Coa-IT
inhibitors on blocking cell cycle progression versus the events asso-
ciated with apoptosis (45). However, in breast cancer cells, Coa-IT
inhibitors block cell cycle progression within 20 h and induce only the
earliest stages of apoptosis at later time points, according to criteria set
forth by Collins et al. (47) using time-lapse video microscopy. Thus,
it was possible to determine precisely the influence of Coa-IT inhi-
bition on different parameters of cell proliferation in this cell type.
Coa-IT inhibitors caused a dose-dependent accumulation of cells in
G_1, concomitant with a marked reduction of cells in S and G_2-M.
Synchronization experiments revealed that Coa-IT inhibition traps
breast cancer cells in early to mid-G_1 before the hydroxyurea check-
point. These experiments raise the interesting question of whether this
is the point where AA, which accumulates as a result of blocking
remodeling, might induce signal transduction events that inhibit cell
cycle progression. Future studies will be necessary to define the
molecular events that link the accumulation of intracellular AA to
distal signals mediating cell cycle progression and apoptosis.

The fate of AA that occurs as a result of blocking arachidonate-
phospholipid remodeling was also examined in this study. The most
striking change that occurred as a result of Coa-IT inhibition was the
accumulation of AA within cellular triglycerides. A shift of AA into
glycerolipid pool under conditions of high intracellular AA concen-
trations has been observed in several cell types (44). Mammalian
cells, when presented with high concentrations of AA, have the
capacity to incorporate the bulk of the AA during de novo glycerolipid
biosynthesis, and much of this AA ultimately resides within cellular
triglycerides (53, 54). The fact that there is a marked increase in free
AA in the supporting culture medium after Coa-IT treatment suggests
that high concentrations of free AA are produced and released by cells
under these conditions. Taken together, the current study suggests that
as AA remodeling between phospholipids is blocked, the capacity of
the cell to control free AA levels is reduced or lost, and the resulting
free AA is incorporated into triglycerides via the de novo synthesis
pathway.

Exogenously provided AA was less potent than Coa-IT inhibitors
at blocking cell proliferation. It is unlikely that large quantities of
exogenous AA reach subcellular locations, where it can act as a
mediator of the cell cycle progression or apoptosis. Rather, there are
very efficient acylation pathways (deacylation/reacylation or the de
novo pathway) that incorporate exogenous AA into cellular glycer-
olipids, thereby maintaining low levels of intracellular AA. Inhibition
of the cell cycle in synchronization experiments using exogenously
provided AA revealed that high concentrations of AA were needed to
produce a similar G_1 arrest in MDA-MB-231 cells using SB98625. It
may be that high concentrations of exogenously provided AA are
required to raise the levels of intracellular AA to those observed with
Coa-IT inhibitors. This is especially the case when exogenous AA is
added to media containing 10% FBS. FBS can act effectively to
partition AA away from the cells. Interestingly, MCF-7 cells were
more sensitive to exogenous AA than MDA-MB-231 cells. Our pre-
liminary data studies suggest that this may be attributable to the
inability of MCF-7 cells to incorporate the high levels of AA into
triglycerides and, thus, rid themselves of intracellular AA. Data
obtained with MCF-7 cells overexpressing glutathione peroxidase are
consistent with those of Chen et al. (51) and suggest that AA reacts with
reactive oxygen species to produce antineoplastic products. Stud-
ies are currently under way in our laboratory to identify these impor-
tant product(s).

Together, this study further emphasizes the potential for enzyme
inhibitors (COX and Coa-IT) that raise intracellular AA levels to
influence neoplastic cell proliferation and survival. In particular, this
study points out that in some neoplastic cells (breast cancer epithelial
cells), COX inhibitors have only a modest capacity to raise AA levels
and influence neoplastic cell growth, whereas another class of inhib-
itor (AA-phospholipid remodeling) is potent in both regards. This
suggests that Coa-IT inhibitors could be important therapeutic tools
to prevent neoplastic cell proliferation. A recent study in colon cancer
cells suggests that a key molecular event that links COX inhibition to
the attenuation of cell proliferation and apoptosis is the accumulation
of AA within the cell (42). Also in HL-60 cells, we have shown that
inhibitors of AA-phospholipid remodeling induce a marked accumu-
lation of AA and have further demonstrated that this AA initiates
ceramide synthesis with subsequent apoptosis (43). Our data reveal
that a better understanding of the crucial connections between AA
metabolism and cell proliferation may provide new opportunities to
use old drugs (which have been developed over the past three decades
for inflammation) and point the way for the discovery and develop-
ment of new drugs to block tumorigenesis.

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**References**

eicosanoid mediators in normal physiology and human diseases. FASEB J., 9:


3. Petroni, A., Blasevich, M., Papini, N., La Spada, P., and Galli, C. Changes in
arachidonic acid levels and formation in lipid synthesis in the human neuroblastoma
SK-N-BE during retinoic acid induced differentiation. J. Neurochem., 67:
549–546, 1996.

Alterations of potassium channel activity in retinal Muller glial cells induced by

5. Reese, E. A., Wu, Y. K., Witzneritz, A., Homko, C., Yao, J., Borenstein, M., and
Sloskey, G. Dietary polyunsaturated fatty acid prevents malformations in offspring


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