Pharmacological Inhibitors of Mammalian Fatty Acid Synthase Suppress DNA Replication and Induce Apoptosis in Tumor Cell Lines

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Abstract

Pharmacological inhibitors of the anabolic enzyme, fatty acid synthase (FAS), including the natural product cerulenin and the novel compound c75, are selectively cytotoxic to cancer cells via induction of apoptosis, apparently related to the tumor cell phenotype of abnormally elevated fatty acid synthetic metabolism. As part of a larger effort to understand the immediate downstream effect of FAS inhibition that leads to apoptosis, the effects of these inhibitors on cell cycle progression were examined. Both FAS inhibitors produce rapid, profound inhibition of DNA replication and S phase progression in human cancer cells. The dose responses for fatty acid synthesis inhibition and DNA synthesis inhibition are similar. The kinetics of both effects are rapid, with fatty acid synthesis inhibition occurring within 30 min and DNA synthesis inhibition occurring within 90 min of drug exposure. Meanwhile, apoptotic changes are not detected until 6 h or later after inhibitor exposure. Fatty acid synthetic pathway activity and the magnitude of DNA synthesis inhibition by FAS inhibitors are increased in parallel by withdrawal of lipid-containing serum from the cultures. The mechanism of DNA synthesis inhibition by cerulenin is indirect, because expression of certain viral oncoproteins rescues DNA synthesis/S phase progression in cerulenin-exposed cells. The data suggest a direct linkage at a regulatory level, between fatty acid synthesis and DNA synthesis in proliferating tumor cells.

Introduction

The antimitabolite, cerulenin, is a natural product of cephalosporium caerulens. Cerulenin, (2R,3S)-2,3-epoxy-4-oxo-7,10-trans,trans-dodecadienamide, has been known since the 1960s as a specific inhibitor of fatty acid synthesis across a broad phylogenetic spectrum (1, 2). Cerulenin irreversibly inhibits the enzyme, FAS, by binding covalently to the active site cysteine of the β keto acyl synthase moiety, which performs the condensation reaction between the elongating fatty acid chain and each successive acetyl or malonyl residue. We have recently developed a novel small molecule inhibitor of FAS, called c75, with comparable inhibitory effects on fatty acid synthesis.

Cerulenin and c75 are selectively cytotoxic to human cancer cells, apparently related to the tumor cell phenotype of abnormally elevated fatty acid synthetic metabolism (3, 4). Exposure of human cancer cells to cerulenin or c75 triggers apoptosis (5). However, little is known of the immediate downstream effects of FAS inhibition that precede activation of the cell death program in tumor cells. Cerulenin has been reported previously to produce inhibition of DNA synthesis in bacteria, in addition to its well-characterized effects on fatty acid synthesis, but the reported work did not distinguish a specific inhibitory effect on DNA synthesis from a more general cytotoxic or cytostatic effect (6). We report here that two chemically distinct FAS inhibitors produce rapid, profound inhibition of DNA replication and S phase progression in human cancer cells that precedes their cytotoxic effect. This result is consistent with an indirect mechanism secondary to fatty acid synthesis inhibition.

Materials and Methods

Cell Lines and Culture Conditions. With the following exceptions, cell lines were obtained from the American Type Culture Collection. RKO colon carcinoma cells and stably transfected RKO clones were provided by Dr. M. Kastan (7). The stably transfected MCF7 clone was provided by Dr A. Fornace (8). Cells were cultured in DMEM with 10% fetal bovine serum (HyClone). HL60 cells were cultured in RPMI with 10% fetal bovine serum (HyClone). Both FAS inhibitors produce rapid, profound inhibition of DNA replication and S phase progression in human cancer cells.

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3 The abbreviations used are: FAS, fatty acid synthase; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; HPV, human papillomavirus.

4 F. P. Kabajda, E. S. Pizer, N. S. Mani, and C. A. Townsend. Synthesis and Anti-Tumor Activity of a Novel Inhibitor of Fatty Acid Synthase, submitted for publication.

Results and Discussion

Several lines of evidence suggest linkage between tumor cell proliferation and elevated fatty acid synthesis (9, 13). To determine whether cell cycle progression is altered after inhibition of fatty acid

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synthesis, we measured incorporation of thymidine into DNA after exposure of tumor cells to cerulenin or c75 (Fig 1, A and B). These experiments demonstrated that fatty acid synthesis inhibitors produce a profound inhibition of DNA replication within 2 h. Because S phase typically requires at least 6 h, this effect is consistent with inhibition of S phase progression rather than inhibition of the G1-S transition. Fig. 1, A and B, demonstrates that the dose responses of fatty acid synthesis and DNA synthesis to cerulenin and c75, 2 h after drug exposure, are similar for HCT116 colon carcinoma cells. Cerulenin (10 μg/ml) and c75 (10 μg/ml) both produce inhibition of DNA synthesis to <10% of control levels. Pulse labeling at later time points confirmed that DNA synthesis remained inhibited to <1% of control levels at 8 and 18 h after drug exposure (Fig. 1C).

To determine how rapidly fatty acid synthesis and DNA synthesis are inhibited by these drugs, suspension cultures of HL60 promyelocytic leukemia cells adapted to serum-free medium were metabolically labeled with [U-14C]acetic acid or [3H]thymidine after exposure to drug and serially sampled to determine labeling rates. Inhibition of fatty acid synthesis was very rapid, with divergence of labeling between control and drug treated cells occurring between 15 and 30 min (Fig. 2A). Thymidine labeling demonstrated partial inhibition of DNA synthesis during the first 60–90 min after drug exposure, with maximal inhibition occurring after 60 min of exposure to c75 or after 90 min of exposure to cerulenin (Fig. 2B). The fatty acid synthesis and DNA synthesis rates during each time interval after addition of drug, determined as a percentage of control, are shown below the graphs. Meanwhile, fragmentation of chromosomal DNA, a definitive sign of apoptosis, was not detectable until at least 6 h after drug exposure (11). Similar kinetics of apoptosis induction were observed in HL60 cells using detection of altered plasma membrane phospholipid packing by decoration with merocyanine 540 as the indicator of early entry into apoptosis (data not shown; Ref. 12). The rate at which apoptosis occurs is variable among cell lines, with most carcinoma lines that we have studied requiring more than 6 h after drug exposure before bright staining with merocyanine 540 occurs (data not shown).

We have observed previously that adaptation of HL60 cells from medium containing 10% fetal bovine serum to serum-free, fatty acid-free medium produces elevation of FAS enzyme expression (9). Metabolic labeling of endogenously synthesized lipids performed in medium with 10% fetal bovine serum or in serum-free, fatty acid-free

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**Fig. 1.** Inhibition of fatty acid synthesis and of DNA replication by FAS inhibitors have similar dose responses. In A and B, triplicate cultures of HCT116 colon carcinoma cells were exposed to cerulenin or c75 for 4 h. During the last 2 h, cells were pulse labeled with either [3H]thymidine or [U-14C]acetic acid as described in “Materials and Methods.” Label incorporation is shown as a percentage of control. In C, similar pulse labeling with [3H]thymidine was performed from 6 to 8 h or from 16 to 18 h after drug exposure. Label incorporation is shown as dpm per 5 × 10⁶ cells. Bars, SE.
medium reveals comparable modulation of fatty acid synthesis pathway activity, probably because cells deprived of the exogenous lipid available in serum increase endogenous fatty acid synthesis to produce membrane lipids required for growth (Table 1). Despite an ~20-fold difference in overall pathway activity in the two media, both cerulenin and c75 inhibit fatty acid synthesis to approximately one-half of control levels in both media. However, the fractional inhibition of DNA synthesis by cerulenin or c75 is much greater in serum-free, fatty acid-free medium than in medium with 10% fetal bovine serum (~20% of control levels versus ~80% of control). When the same experiment was performed in 5% serum, an intermediate level of DNA synthesis inhibition occurred (data not shown). The parallel modulation of fatty acid synthesis pathway activity and sensitivity to DNA synthesis inhibition suggest a functional linkage between inhibition of fatty acid synthesis and S-phase arrest. Of note, carcinoma cells do not demonstrate comparable plasticity in their response to serum and exogenous lipid concentrations.

Replication of DNA in mammalian cells is a highly complex process that requires coordinate activity of numerous metabolic pathways for nucleotide synthesis, active basal replication machinery, and S-phase cyclins and cyclin-dependent kinases (14). The early genes of
Fig. 3. Expression of certain viral oncogenes abrogates the inhibitory effect of cerulenin on DNA replication. Triplicate cultures of the indicated cells were exposed to cerulenin for 4 h. During the last 2 h, cells were pulse labeled with either [U-14C]acetic acid (A, C, and E) or [3H]thymidine (B, D, and F) as described in "Materials and Methods." Label incorporation is shown as a percentage of control. Bars, SE.
certain DNA tumor viruses are able to abrogate higher order S-phase regulation and facilitate passage of infected cells through S phase to accomplish viral replication (15). To broadly distinguish between cellular targets that are directly required for DNA replication and those that indirectly affect replication, we compared the effect of FAS inhibitors on cells before and after introduction of three viral oncogenes.

Expression of viral oncogenes from SV40 or HPV relieved the inhibitory effect of cerulenin on DNA replication, although fatty acid synthesis inhibition was similar. CV-1, a monkey kidney cell line, is the parent of COS-1, a stably transfected, transformed line that expresses SV40 T antigen (16). The effects of cerulenin on this pair of cell lines were as follows. The dose-dependent inhibition of fatty acid synthesis was similar for CV-1 cells and COS-1 cells, indicating that the drug is able to enter the cells and inactivate FAS comparably (Fig. 3A). The dose-dependent inhibition of DNA synthesis in CV-1 cells was similar to that of HCT116 colon carcinoma cells. However, the inhibitory effect of cerulenin on DNA synthesis in COS-1 cells was markedly reduced, with thymidine incorporation remaining at 66% of control levels after exposure to cerulenin (10 μg/ml; Fig. 3B). Similar results were obtained when RKO colon carcinoma cells were compared with stably transfected RKO clones that expressed HPV 16 E6 or E7 genes (Fig. 3, C and D) and when MCF7 breast carcinoma cells were compared with a stably transfected MCF7 clone that expressed the HPV 16 E6 gene (Fig. 3, E and F). The RKO-HPV16 E6 clone shown in Fig. 3 is representative of three clones tested. An RKO clone transfected only with neomycin phosphotransferase behaved similarly to the parent cell line but with slightly delayed drug response kinetics (not shown). The differential effects of c75 on DNA replication in this panel of cell lines was variable (not shown).

The mechanism(s) by which cerulenin and c75 inhibit DNA replication remain to be elucidated. However, the above data support the model that DNA replication is inhibited as a consequence of fatty acid synthesis inhibition. Two chemically distinct FAS inhibitors both inhibit DNA replication with dose responses similar to their effects on FAS. The inhibition of DNA replication follows rapidly after fatty acid synthesis inhibition and comodulates with fatty acid synthesis pathway activity in response to serum. The mechanism likely does not involve a direct effect of drug on the DNA molecule or on the basal replication machinery. Rather, inhibition probably occurs at the level of higher order cell cycle regulation and may involve p53 function, because expression of SV40 T antigen, HPV 16 E6, or HPV16 E7 abrogated the inhibition of DNA replication by cerulenin. This differential effect on DNA synthesis occurred despite similar fatty acid synthesis inhibition in parent and viral oncogene-expressing cells. Our model postulates a direct mechanistic linkage between fatty acid synthesis and DNA synthesis. This model proposes a novel regulatory pathway in proliferating mammalian cells, which could have a major role in maintaining normal cellular growth. An analogous growth arrest response in bacteria, the stringent response, cooperatively inhibits its macromolecular synthesis after a variety of nutrient deprivation stresses, including cerulenin exposure (17), and similar physiological effects occurred after restriction of phospholipid synthesis (18). The importance of this effect in the ultimate cytotoxic outcome of fatty acid synthesis inhibition in human cancer remains to be determined.

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References


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