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Fatty acid synthesis inhibitors were studied as a means to treat breast cancer *in vivo*. A nude mouse model of MDA-435 was validated and the fatty acid synthesis inhibitor, C-75, was shown to substantially inhibit the growth of MDA-435 in a local intraperitoneal treatment model. Studies were carried out addressing the mechanism of C-75 which, while not yet conclusive, have shown a degree of fatty acid synthase inhibition. Additional candidate compounds for *in vivo* analysis have been identified.

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FOREWORD

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Introduction

In the last several years, fatty acid synthesis in general, and FAS (EC2.3.1.85) in specific have assumed importance in the diagnosis and potentially in the treatment of cancer. Levels of fatty acid synthase protein and message are increased in certain subsets of breast, colorectal, prostate, ovarian, and other cancers. In some instances, such as in breast, prostate and ovarian cancer, FAS elevation may portend worsened prognosis (1-8); in others, such as colorectal cancer, FAS elevation appears constitutive (9). Whenever FAS is elevated, increases in the amount of the enzyme are accompanied by corresponding increases in the flux of labeled acetate through the fatty acid synthetic pathway (10), indicating that tumor-associated FAS is fully functional. Endogenously synthesized fatty acids appear to be essential to the viability of those tumor cells displaying increased FAS. Several studies in vitro demonstrated that inhibition of FAS resulted in cell death (10-12). When the enzyme is inhibited, susceptible tumor cells undergo apoptosis (12). More recently, one study demonstrated successful treatment in vivo of OVCAR-3 by a FAS inhibitor (13). Because increased amounts of FAS appear to mark increases in activity of the endogenous fatty acid synthetic pathway, diagnostic analysis of enzyme levels may identify subsets of tumors uniquely susceptible to antineoplastic therapy based upon inhibition of fatty acid synthesis.

The goals of the work performed pursuant to this grant revolve around a recently discovered putative fatty acid synthase inhibitor, C-75, that exhibits anti-tumor

activity in in vitro systems. These goals are: [1] to study C-75 in vivo in human breast cancer xenograft models; [2] to characterize the mechanism of action of C-75 in terms of its effects on fatty acid metabolism; and [3] to determine the interplay between dietary lipid intake and the anti-tumor effects on C-75.

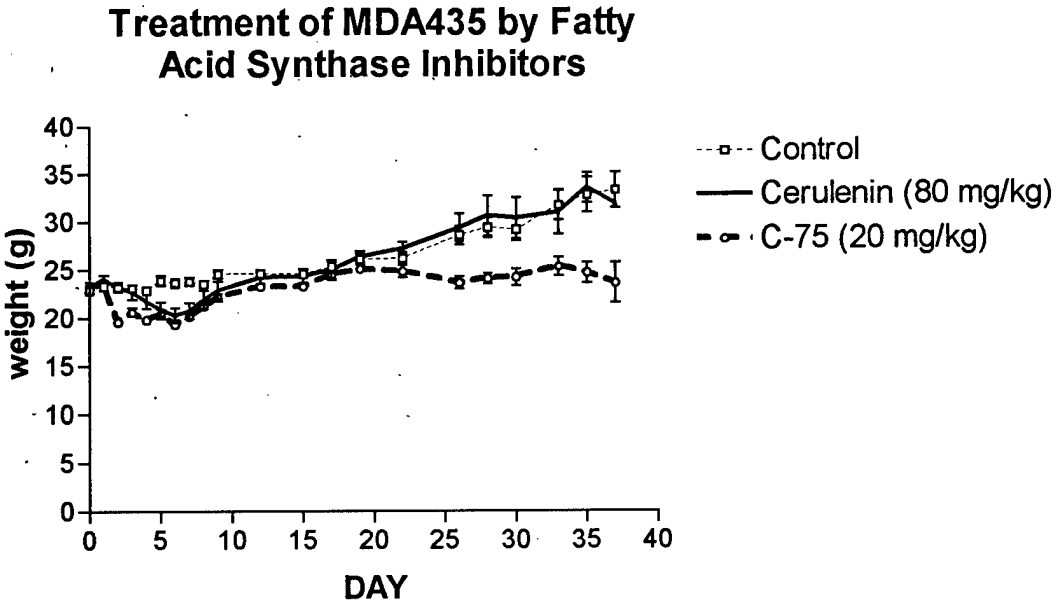
Body

Dose Escalation Toxicity Studies. Initial experiments were performed to determine the maximum tolerated dose of C-75 in nude mice. These studies determined that doses of 20 mg/kg given on alternate days was well tolerated. Larger doses resulted in animal death. The maximum length of therapy has not yet been determined.

Optimization of MDA-435 model. The MDA-435 model was validated over a broad range of tumor inoculum size. A dose of 2 x 106 cells/mouse i.p. was the minimum dose to result in 100% tumor takes. In accordance with this model, tumor became detectable by approximately 20 days post inoculation as determined by weight gain and clinically apparent ascites. Mice accumulated the maximum permissible tumor burden between 35 and 40 days.

Activity of C-75 in vivo. The following experiment demonstrates the activity of C-75 in vivo. Groups of 5 mice were inoculated on Day 0 with 2.0 x 106

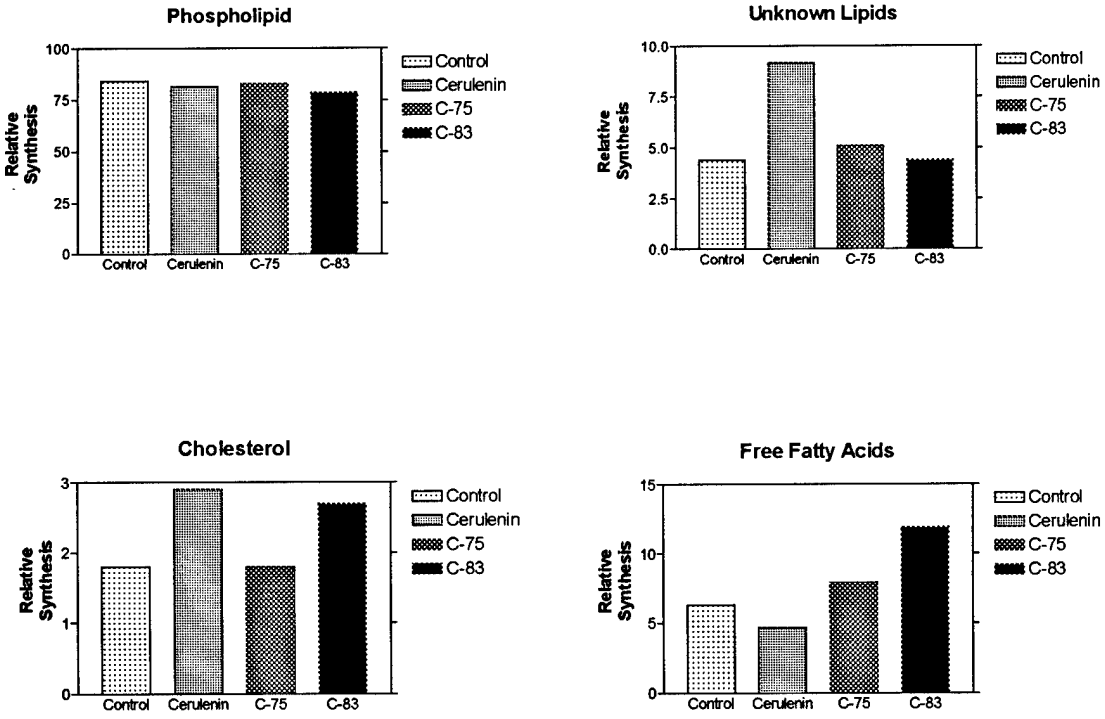
MDA-435 cells in 200 µl unsupplemented RPMI-1640. Control mice were treated 200 µl of vehicle control on Days 1, 2, 3, 4, and 5. A second group received cerulenin on days 1, 2, 3, 4, and 5. A third group of mice were treated with C-75 every other day on days 1, 3, 5, and 7. Mouse weights were monitored every day during treatment, and every other day thereafter. The experiment was terminated on Day 37 when a number of mice reached the maximally permissible weight.



The data clearly show that C-75 produces a profound anti-tumor effect in excess of that observed with cerulenin.

Mechanism of C-75 action. The following data summarize experiments to determine the distribution of ^{14}C-acetate label among cellular lipids following treatment of breast cancer cells. SkBr3 cells were plated in 24-well plates to a density of 2 x10^5 cells/well in 1 ml of medium. After overnight incubation,

cerulenin, C-75, a related compound, C-83, or vehicle (DMSO) were added to sets of three wells to a final concentration of 10 μg/ml. Cells were then incubated for 2 hours. ^{14}C-acetate (20 μCi) were added to each well and the incubation was continued for an additional 2 hours. The Medium was then removed and cells were washed and then extracted with 2:1 chloroform/methanol (1 ml/well). The extracts were washed and then 10 μl aliquots were counted to determine total incorporation into each fraction. Cerulenin and C-75 incorporated 88% and 82% of the controls respectively. Distribution of the ^{14}C-acetate among lipid classes was determined by thin layer chromatography. For each extract, 10 μl were spotted onto a 20 x 20cm silica gel H plate. After drying, the solvent the plate was developed in 90:10:1 hexane/diethyl-ether/acetic acid allowing solvent front to equilibrate at the top of the plate. Distribution of radioactivity was determined by phosphoimaging and quantitated using Image-Quant software by integration of the fractions in each lane.



The data show that cerulenin and C-75 do not affect the proportion of lipids in the phospholipid pool. Whereas cerulenin yields increases in an unknown lipid, C-75 does not. The relative proportions of lipids in cholesterol is slightly less for C-75 than for cerulenin. Overall, however, the distribution of label among various lipid classes does not differ markedly between cerulenin and C-75. In enzyme assays, C-75 produces a dose-dependent but incomplete inhibition of fatty acid synthase.

Other. As part of an ongoing effort to identify other compounds that inhibit fatty acid synthesis with potential anti-tumor activity *in vivo*, a screening program continues. To date 113 compounds have been tested in the a breast cancer cell culture assay for activity. Twenty-two compounds have shown some activity in the range of 20 to 2.5 μg/ml. Ten of these compounds showed direct inhibition of fatty acid synthase in an *in vitro* assay system in a range of 10 to 2 μg/ml.

Conclusions

1. Fatty acid synthase inhibitors such as C-75 are active against breast cancer cells in an *in vivo* model.

2. The mechanism of action is consistent with inhibition of fatty acid synthase.

3. Other inhibitory compounds may become available in the near future.

References

1. Kuhajda, F.P., Piantadosi, S., and Pasternack, G.R. Haptoglobin-related protein (Hpr) epitopes in breast cancer as a predictor of recurrence of the disease. *N. Engl. J. Med.*, 321:636-641, 1989.
2. Shurbaji, M.S., Pasternack, G.R., and Kuhajda, F.P. Expression of haptoglobin-related protein in primary and metastatic breast cancer. *Am. J. Clin. Pathol.*, 96:238-242, 1991.
3. Shurbaji, M.S., Kuhajda, F.P., Pasternack, G.R., and Thurmond, T.S. Expression of oncoantigen 519 (OA-519) in prostate cancer is a potential prognostic indicator. *Am. J. Clin. Pathol.*, 97:686-691, 1992.
4. Epstein, J., Carmichael, M., and Partin, A. OA-519 (Fatty Acid Synthase) as an independent predictor of pathologic stage in adenocarcinoma of the prostate. *Urology*, 45:81-86, 1995.
5. Jensen V., Ladekarl M., Holm-Nielsen P., et al. The prognostic value of oncogenic antigen 519 (OA-519) expression and proliferative activity detected by antibody MIB-1 in node-negative breast cancer. *J Pathol.* 176:343-352, 1995.
6. Alo P.L., Visca P., Marci A., et al. Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. *Cancer* 77:474-482, 1996.
7. Gansler, T.S., Hardman, W., Hunt, D., Schaffel, S., and Hennigar, R.A., Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. *Human Pathol.* In press.

8. Martin, A.W., Corrigan, G.A., Lear, S.C., Kuhajda, F.P., Pasternack, G.R. The prognostic significance of expression of OA-519 in infiltrating ductal carcinoma of the breast. *Breast Cancer Research and Treatment* 19:213, 1991.

9. Rashid, A., Pizer, E.S., Moga, M., Milgraum, L.Z., Zahurak, M., Pasternack, G.R., Kuhajda, F.P., and Hamilton, S.R. Expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. *Am. J. Pathol.* 150:201-208, 1997.

10. Kuhajda, F.P., Jenner, K., Wood, F.D., Hennigar, R.A., Jacobs, L.B., Dick, J.D., and Pasternack, G.R. Fatty acid synthesis: A potential selective target for antineoplastic therapy. *Proc. Natl. Acad. Sci. (USA)* 91:6379-6383, 1994.

11. Pizer, E.S., Wood, F.D., Pasternack, G.R., and Kuhajda, F.P. Fatty acid synthase (FAS): A target for cytotoxic antimetabolites in HL60 promyelocytic leukemia cells. *Cancer Res.* 56:745-751, 1996.

12. Pizer, E.S., Jackish, C., Wood, F.D., Pasternack, G.R., Davidson, N.E., and Kuhajda, F.P. Inhibition of fatty acid synthesis induces programmed cell death in human breast cancer cells. *Cancer Res.* 56:2745-2747, 1996.

13. Pizer, E.S., Wood, F.D., Heine, H.S., Romantsev, F.E., Pasternack, G.R., and Kuhajda, F.P. Inhibition of fatty acid synthesis delays disease progression in a xenograft model of ovarian cancer. *Cancer Res.* 56:1189-1193, 1996.