Cancer Chemother Pharmacol (2002) 49: 187–193
DOI 10.1007/s00280-001-0399-x

ORIGINAL ARTICLE

Binqiu Liu · Yingqiang Wang · Kerry L. Fillgrove
Vernon E. Anderson

Triclosan inhibits enoyl-reductase of type I fatty acid synthase in vitro and is cytotoxic to MCF-7 and SKBr-3 breast cancer cells

Received: 6 June 2001 / Accepted: 11 October 2001 / Published online: 9 January 2002

Abstract *Background and purpose:* Human type I fatty acid synthase has been proposed as a chemotherapeutic target for the treatment of breast cancer based on the inactivation of human β-ketoacyl synthase activity by cerulenin. Triclosan, a common antibiotic, functions by inhibiting the enoyl-reductase enzymes of type II fatty acid synthases in susceptible bacteria. If triclosan is an inhibitor of human fatty acid synthase and if inhibition of fatty acid synthase is toxic to breast cancer cell lines, triclosan could prove to be a lead compound for the treatment of breast cancer. Consequently, the inhibitory activity of triclosan against vertebrate type I fatty acid synthases and its effects on breast cancer lines in cell culture were investigated. *Methods:* The inhibitory activities of triclosan against human and goose fatty acid synthases and each of the partial reactions were investigated using spectrophotometric assays. The ability of triclosan at various concentrations to inhibit growth and reduce the viability of MCF-7 and SKBr-3 cells in culture was evaluated. *Results:* Kinetic studies showed triclosan to be a slow binding inhibitor of human and goose type I fatty acid synthase and to inhibit the partial activity of enoyl-reductase with IC_{50} values between 10 and 50 μM. Triclosan at similar concentrations was also shown to inhibit both viability and growth of MCF-7 and SKBr-3 cells in culture. *Conclusions:* The results corroborate the hypothesis that fatty acid synthase may be a target of breast cancer chemotherapy and suggest that inhibitors of the enoyl-reductase partial activity of fatty acid synthase may have chemotherapeutic potential.

Keywords Triclosan · Enoyl-reductase · Fatty acid synthase · Enzyme inhibition · Breast cancer · Cell culture

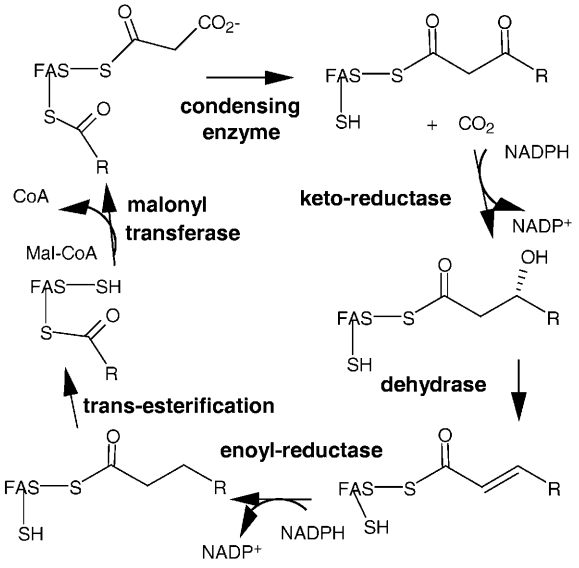
B. Liu · Y. Wang · K.L. Fillgrove · V.E. Anderson (✉)
Department of Biochemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4935, USA
E-mail: anderson@biochemistry.cwru.edu
Tel.: +1-216-3682599
Fax: +1-216-3683419

Introduction

The de novo synthesis of saturated fatty acids in mammalian cells is accomplished by type I fatty acid synthase (FAS), a homodimer of 250 kDa monomers. In de novo synthesis of fatty acids, two carbons derived from malonyl-CoA are added to the nascent fatty acid that is covalently linked as a thioester to FAS in a reaction catalyzed by the condensing enzyme. The keto intermediate is reduced by two equivalents of NADPH as part of the cycle of six reactions shown in Scheme 1. Separate catalytic domains of FAS corresponding to the condensing enzyme, keto-reductase, dehydrase and enoyl-reductase have been identified [2, 25, 29, 33] and have some homology with the comparable enzymes of type II FAS.

FAS is overexpressed in some breast cancer cell lines, constituting as much as 28% of the soluble protein in SKBr-3 cells [9, 30], while being minimally expressed in humans consuming a Western diet [32]. The inactivation of FAS has been shown to induce apoptosis in breast cancer cells, and consequently to be a potential chemotherapeutic target [12, 13, 14, 22]. Inactivation of type I FAS has been shown to result in an anticancer effect in the treatment of tumor xenografts [23].

The most common "specific" inactivator of FAS is cerulenin, a fatty acid amide with an α,β-epoxide, that has been demonstrated to alkylate an active site cysteine in the β-ketosynthase active site of both type I and type II FAS [7, 19]. Recently, the ability of a series of cerulenin analogues to inhibit fatty acid synthesis and to prevent palmitoylation of H-*ras* and N-*ras* has been investigated, with the finding that the anticancer activity correlates more closely with the ability to inhibit palmitoylation [15]. We have identified triclosan as an inhibitor of the enoyl-reductase activity of type I FAS and have demonstrated that it is effective in cell culture against SKBr-3 and MCF-7 breast cancer cell lines. The inhibition of human FAS by a different mechanism and at a different active site of FAS supports the proposition that type I



Scheme 1

FAS may be a chemotherapeutic target and additionally suggests that inhibition of any of the enzymatic activities of this multifunctional enzyme may be effective.

Materials and methods

Materials

Triclosan, 2,4,4′-trichloro-2′-hydroxydiphenyl ether, was obtained from Protameen Chemicals (Totowa, N.J.). Acetyl-CoA, malonyl-CoA, acetoacetyl-CoA, crotonoyl-CoA and NADPH were from Sigma. Frozen goose uropygial glands were obtained from Grimaud Farms (Aurora, Calif.) and FAS isolated as described previously [4]. Human FAS from SKBr-3 cells was partially purified by detaching SKBr-3 cells grown to subconfluence in 100-mm dishes by trypsinization. The cells were pelleted, washed four times with cold phosphate-buffered saline (PBS, pH 7.2), resuspended in homogenization buffer (0.25 *M* sucrose, 10 m*M* DTT, 1 m*M* EDTA, 0.1 mg/ml trypsin inhibitor, pH 7) at a cell to buffer ratio of 1:4 and homogenized using a Potter-Elvehjem homogenizer at +4°C. The homogenate was centrifuged at 100,000 *g* at +4°C for 1 h and the supernatant concentrated using a Centricon 10 ultrafiltration device (Amicon).

Cell lines and culture conditions

Both MCF-7 cells and SKBr-3 cell lines were obtained from the American Type Culture Collection. The MCF-7 cells were maintained in MEM with 10% fetal bovine serum, 2 m*M* L-glutamine and 10 U penicillin/10 μg streptomycin at 37°C in an atmosphere containing 5% CO_2. The SKBr-3 cells were maintained in McCoy's 5a medium with 10% fetal bovine serum, 15 m*M* L-glutamine and 10 U penicillin/10 μg streptomycin at 37°C in an atmosphere containing 5% CO_2.

FAS inhibition

The overall activity of FAS was assayed based on the oxidation of NADPH (100 μ*M*) dependent on acetyl-CoA (25 μ*M*) and malonyl-CoA (100 μ*M*) in phosphate buffer, pH 7.4, monitored at 340 nm. Triclosan, added from an ethanol stock solution, inhibited the overall activity of FAS in a time- and concentration-dependent manner. Because of the curvature in the uninhibited assay during the time course, the triclosan inhibition was characterized by varying the time of preincubation of triclosan with FAS and NADPH and monitoring the initial rate of NADPH reduction on the addition of the CoA substrates. The onset of inhibition could be characterized by a pseudo-first-order rate constant by plotting $\ln(A/A_0)$ versus time, where A is the inhibited initial steady-state rate and A_0 is the uninhibited initial steady-state rate.

Inhibition of partial reactions

The FAS reaction comprises four enzymatic activities that change the chemical form of the thioester substrate: condensing enzyme, 3-keto reductase, 3-hydroxyacyl dehydrase and enoyl-reductase. Each of these reactions, and the ability of triclosan to inhibit each of them, can be assayed separately. The condensing enzyme activity is monitored by the production of triacetic lactone from malonyl-CoA at 280 nm [10, 27]. The β-ketoacyl-reductase is assayed as the acetoacetyl-CoA-dependent oxidation of NADPH monitored at 340 nm [33]. The α,β-enoyl reductase activity is monitored as the crotonyl-CoA-dependent oxidation of NADPH at 340 nm [29]. All spectrophotometric assays were performed with a Hewlett-Packard 8452 diode array under thermostatic control at 25°C. Because of the time-dependent inhibition of the overall reaction, the triclosan was incubated for 10 min with the enzyme and NADPH prior to the addition of the thiolester substrates.

Cell growth inhibition assays

A 20 mg/ml stock solution of triclosan was prepared in DMSO. Both the MCF-7 and SKBr-3 cells, which were harvested during exponential growth, were plated at 2×10^5 cells/well in six-well plates. After attachment, the medium was replaced by fresh medium containing a series of dilutions of triclosan. The cells were incubated for a further 1–4 days. After removal of the supernatant, the cells were incubated with 0.05% trypsin/0.53 m*M* EDTA at 37°C for 5 min, washed once in normal medium, spun at 700 *g* for 5 min, and resuspended in PBS. Cell counting was performed using a hemocytometer and light microscope. Alternatively, after removal of the supernatant from the cell culture, the cells were washed once in PBS, fixed in 100% methanol for 10 min, stained with 0.5% crystal violet in 20% methanol for 10 min, and washed with tap water. After air-drying, the stained cells were solubilized in 1% SDS. Absorbance of the retained crystal violet was determined at OD_{595}. The cell density is reported as the ratio of triclosan-treated cells to control cells expressed as a percentage. The inhibitory concentration at the 50th percentile of cell growth (IC_{50}) was determined.

Cell viability assay

Cell viability was determined using a trypan blue exclusion assay. Detached cells were collected from the supernatant, and attached cells were collected by incubation with 0.05% trypsin in 0.53 m*M* EDTA at 37°C for 5 min. Both detached and attached cells were mixed, washed and resuspended in normal medium. Cells were stained with 0.2% (w/v) trypan blue and incubated for 2 min at room temperature. Viable cells were not stained by trypan blue while dead cells were. Viability was expressed as the percentage of viable cells in relation to the sum of viable and nonviable cells.

Results

Triclosan inhibition of FAS

Triclosan was an effective inhibitor of FAS as shown in Fig. 1. The inhibition was time-dependent, suggesting

slow binding inhibition [20]. Under the assay conditions the IC_{50} was between 10 and 20 μM. An exact IC_{50} was difficult to obtain because it was a function of the time that the inhibitor was preequilibrated with the enzyme. The inhibition was reversible. If inhibitor and enzyme were incubated for 30 min at 100 μM triclosan and then diluted 100-fold into assay buffer, active enzyme was recovered (data not shown). Both goose uropygial gland FAS and human FAS from SKBr-3 cells were inhibited by triclosan in a similar manner. The determination of the pseudo-first-order rate constants for the time-dependent inhibition of both goose and human enzymes are shown in Fig. 2. The rate constants were 15 and 4.7 $M^{-1}s^{-1}$, respectively.

Triclosan inhibition of the partial reactions

The inhibition by triclosan of the partial reactions catalyzed by FAS were examined by standard assays. The only partial reaction inhibited by triclosan was the α,β-enoyl-reductase activity. The IC_{50} for triclosan in this reaction was 90 μM with 1.0 mM Cr-CoA as the substrate. Since we could not approach saturation with crotonyl-CoA, it was not possible to determine if triclosan was a competitive inhibitor of crotonyl-CoA reduction. The key result is that triclosan was capable of specifically inhibiting the enoyl-reductase partial reaction of type I FAS.

Inhibition of MCF-7 and SKBr-3 cell growth by triclosan

Triclosan inhibited the proliferation of MCF-7 cells in both a time- and a dose-dependent manner, as shown in Fig. 3. The growth inhibition became obvious at a triclosan concentration of 5 μg/ml on the 2nd day of culture. The growth inhibition was also quantified by determining the extent of staining with crystal violet by measuring the OD_{595} of solubilized cells. The results shown in Fig. 4A reinforce the time- and concentration-dependence of the inhibition. The IC_{50} decreased from 8 μg/ml for a 1-day incubation to 4 μg/ml for 3- and 4-day incubations with MCF-7 cells. The growth inhibition of SKBr-3 cells on exposure to triclosan required slightly higher concentrations in this assay, as shown in Fig. 4B.

To test the viability of all of the cells, a trypan blue exclusion assay was utilized. As shown in Fig. 5, treatment with triclosan reduced the viability of MCF-7 cells in the same time- and concentration-dependent manner as observed in the other growth inhibition studies.

Morphological changes in MCF-7 cells

The growth inhibition was accompanied by morphological changes in the MCF-7 cells as shown in Fig. 6. Representative colonies and/or cells in the presence of

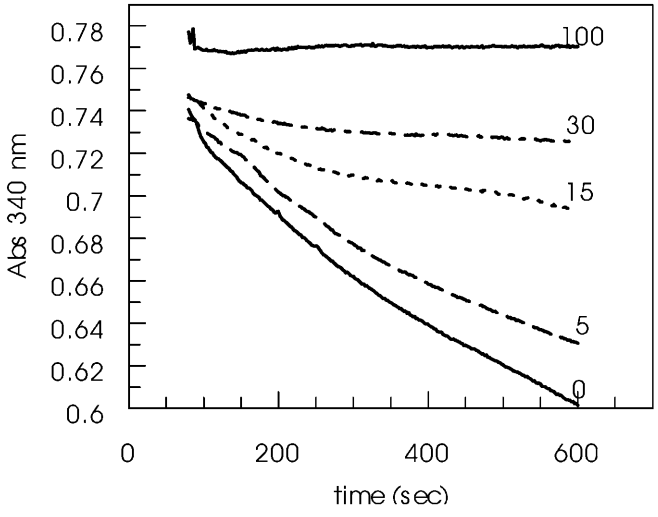


Fig. 1 Time-dependent inhibition of FAS by triclosan. The inhibition of oxidation of NADPH dependent on acetyl-CoA and malonyl-CoA by goose uropygial gland FAS was monitored as a function of triclosan concentration. Increasing concentrations (in micromolar, shown above each line) resulted in greater curvature of the time courses and, at higher concentrations, complete inhibition of FAS activity. The slow onset of inhibition made determination of a precise K_i impractical

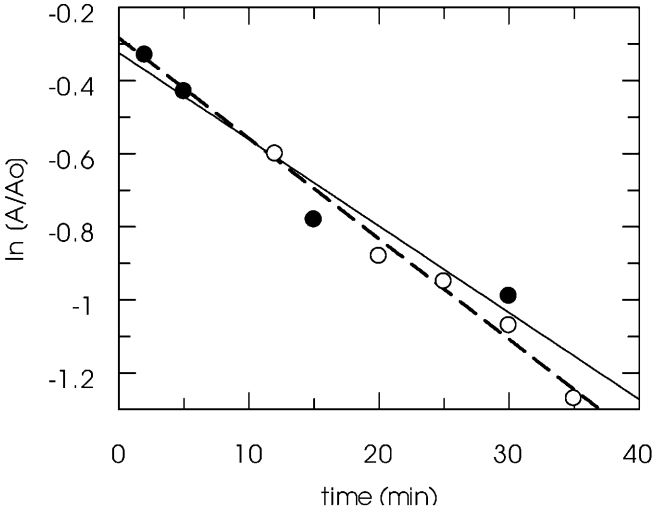


Fig. 2 Pseudo-first-order rate constants for the apparent inactivation of FAS by triclosan. Preincubation of triclosan with FAS resulted in apparent first-order inactivation. Data for goose uropygial gland and human FAS are shown with preincubation for various times with either 30 μM triclosan for the goose enzyme (*filled circles*, *solid line*), or 100 μM for the enzyme isolated from SKBr-3 cells (*open circles*, *dashed line*). Linear regression provided pseudo first-order rate constants for inactivation of 0.024 and 0.028 min^{-1}, which correspond to second-order rate constants for the association of triclosan with the goose and human enzymes of 15 and 4.7 $M^{-1}s^{-1}$, respectively

various concentrations of triclosan on the 3rd day of exposure are shown. The morphological changes following exposure to triclosan were dependent on the triclosan concentration.

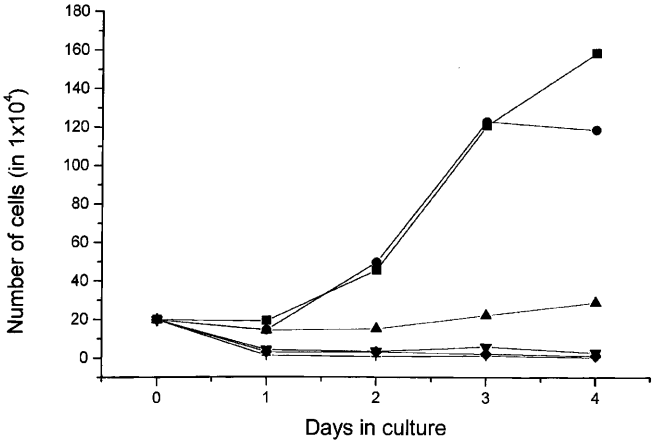
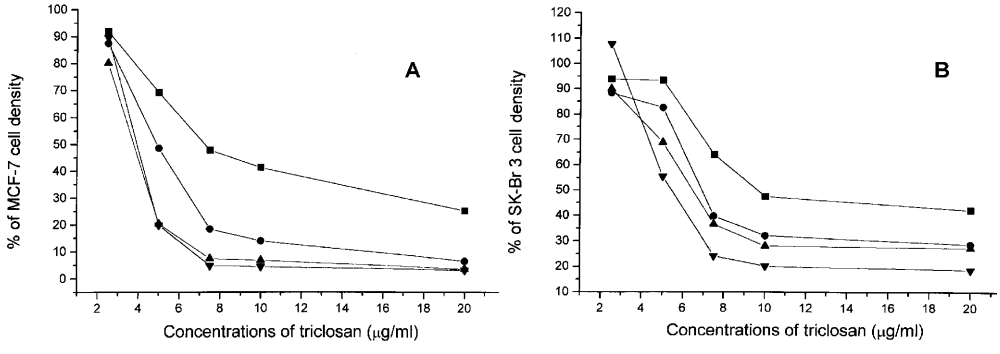


Fig. 3 Growth inhibition curves of MCF-7 cells treated or not treated with triclosan evaluated using cell counting. Each line represents one concentration of triclosan. MCF-7 cells were seeded at 2×10^5 per well into six-well plates on day 1. After attachment (about 8 h) the medium was replaced by fresh medium containing a series of triclosan dilutions followed by incubation for 1, 2, 3 and 4 days. Growth inhibition was dependent on both triclosan concentration and incubation time. All the cells were collected as described on days 1, 2, 3 and 4. The normal growth curve of MCF-7 cells is shown (*squares*), along with the effects of triclosan at 2.5 (*circles*), 5 (*triangles*), 7.5 (*inverted triangles*), 10 (*diamonds*), and 20 μg/ml (*crosses*)

Fig. 4A, B Effects of triclosan on MCF-7 (**A**) and SKBr-3 (**B**) cells. The growth of the breast cancer cell lines was evaluated by crystal violet staining monitored at 595 nm, as described in Materials and methods. Exponentially growing cells were replated into a six-well plate at 2×10^5 cells/well. After attachment, the medium was replaced by fresh medium containing different concentrations of triclosan in DMSO followed by incubation for 1, 2, 3 and 4 days. Cells were washed, fixed and stained with crystal violet. Absorbance of solubilized stain was measured at 595 nm. The percentage cell density was calculated from the ratio of triclosan-treated cells to control cells. The data from different days are indicated as (*squares* day 1, *circles* day 2, *triangles* day 3, *inverted triangles* day 4)



Discussion

Triclosan has been in use for over 30 years as an antibiotic in soaps mouthwashes and other oral dentifrices. Recently, the mechanism of antibiotic action has been identified as the specific inhibition of type II enoyl-reductases [8, 17]. Crystallographic studies have suggested that the 2-phenoxyphenol moiety resembles an enol intermediate in the reaction, interacting favorably with the nicotinamide of the dinucleotide cosubstrate as well as the enzyme active site [16, 24, 26, 28]. Triclosan has a very high affinity for these bacterial type II FAS enoyl-reductases and this is enhanced by the presence of the oxidized dinucleotide substrate, the K_D values being in the low picomolar range [31].

Comparisons of the human FAS protein sequence or its enoyl-reductase domain [5, 6, 11] with *E. coli* or *Brassica napus* FabI sequences using BLAST [1] have revealed no identifiable regions of homology between

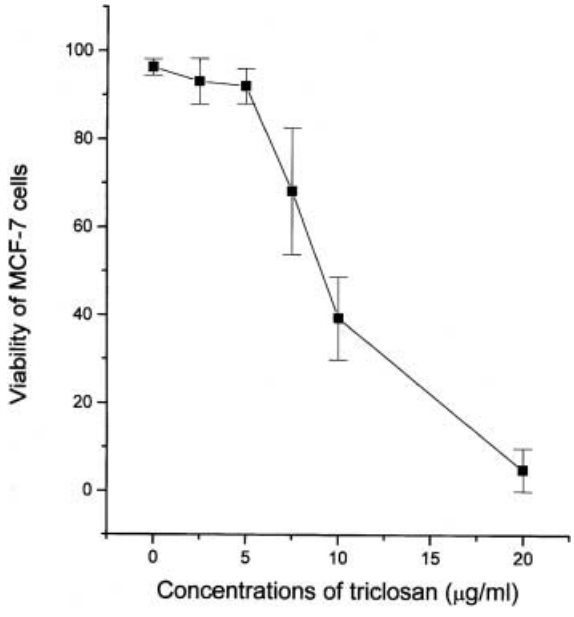


Fig. 5 Triclosan reduced the viability of MCF-7 cells. The viability of MCF-7 cells incubated with increasing concentrations of triclosan for 3 days was determined by trypan blue exclusion. The averages and standard deviations from three independent experiments are shown

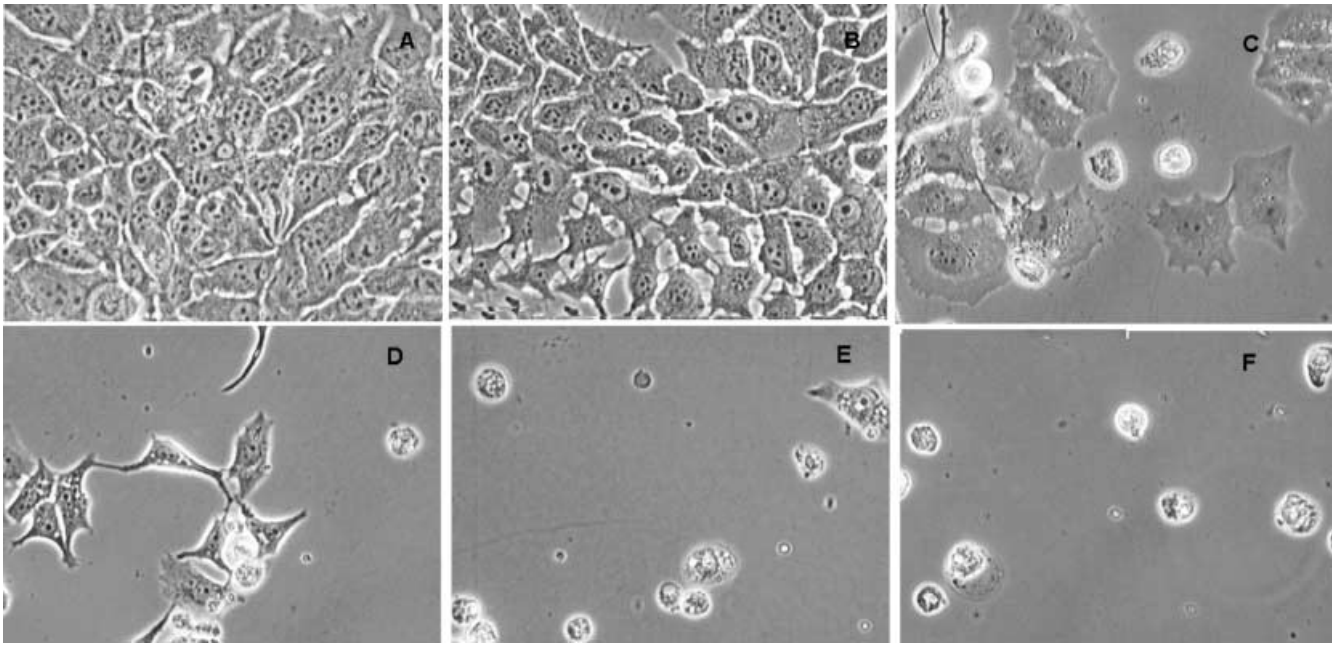


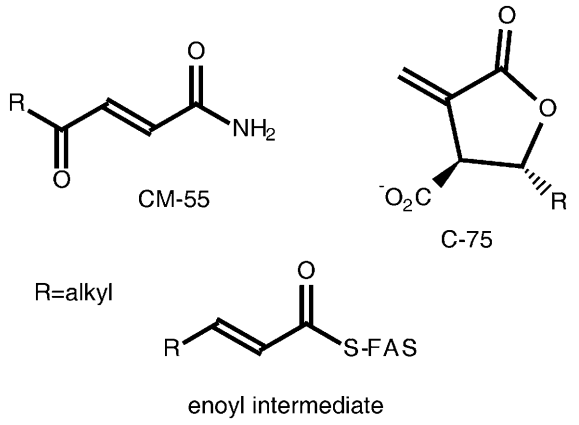
Fig. 6A–F Morphological changes in MCF-7 cells treated and not treated with triclosan on day 3. MCF-7 cells (2×10^5 cells/well) were grown on a six-well plate and treated with different concentrations of triclosan. After 3 days of incubation the cells were examined by inverted phase contrast microscopy. MCF-7 cells growing (**A**) in normal medium or (**B–F**) in normal medium augmented with triclosan at 2.5 (**B**), 5 (**C**), 7.5 (**D**), 10 (**E**) and 20 μg/ml (**F**)

these different enzymes. However, there is significant homology with numerous enoyl-reductase domains of multifunctional polyketide synthases and type I FAS from *Mycobacterium tuberculosis*. The reaction mechanism of the two classes of FAS enoyl-reductases is anticipated to be similar based on the identical chemical reactions catalyzed. If triclosan is mimicking a common intermediate in the reduction reaction, it has the potential to be a potent inhibitor of the enoyl-reductase of type I FAS. Our studies showed triclosan to be an inhibitor of both the overall reaction of goose and human type I FAS and the enoyl-reductase partial reactions. The affinity was six orders of magnitude lower than that measured for triclosan binding to the $NADP^+$ complex of the *E. coli* enzyme, which represents an interaction at least 8 kcal/mol less favorable. This large difference in binding energy may reflect steric differences in the active sites of the different enoyl-reductases, which would suggest that other 2-hydroxydiphenylethers could be more effective inhibitors of the type I enzyme.

The inhibition of human FAS by triclosan would be of only modest mechanistic interest if it were not for the observation that inhibition or inactivation of FAS in breast cancer cells is cytotoxic and induces apoptosis. These observations suggest FAS is a potential chemotherapeutic target [12, 13, 14]. Two lines of evidence indicate that the inhibitors may not be functioning by inhibiting FAS. First, the requirement for de novo palmitate synthesis in the presence of serum palmitate is undemonstrated, and second exogenous palmitate is unable to rescue cells treated with FAS inactivators [9]. All of the current inhibitors and inactivators of human FAS target a cysteine at the β-ketoacyl synthase active site. These alkylating reagents have the potential to be nonspecific, and a stronger correlation with inhibition of *ras* palmitoylation than with fatty acid synthesis has been reported [15].

The fact that triclosan inhibited human FAS by a completely different mechanism from the previously described inactivators but was still cytotoxic in cell culture with MCF-7 and SKBR-3 cells supports the contention that FAS is a potential chemotherapeutic target for the treatment of breast cancer. The inhibition of FAS is attributable to the inhibition of the enoyl-reductase activity. While these two effects do not have to be identical, the inhibition of human FAS with an IC_{50} of about 50 μ*M* and the concentration yielding 50% growth inhibition (5 μg/ml or 17 μ*M*) are comparable.

Our results suggest that enoyl-reductase may be an alternative target site for pharmaceutical intervention. The inhibition by triclosan is specific, as the cytotoxicity of 3-chlorophenol with both cell lines is over 100-fold less (data not shown). In spite of inhibiting human and goose FAS, triclosan has been shown to have minimal toxicity in rats, dogs and baboons when included in the diet at less than 100 mg/kg per day, and only minor pharmacological effects at up to 1000 mg/kg per day over months of treatment [3]. Additionally, enoyl-reductase is a particularly attractive target because inhibition at this site will increase the concentration of the enoyl thiolester intermediate. This α,β-unsaturated intermediate resembles both CM55 [21] and C75 [14] as shown in Scheme 2.



Scheme 2

Similar α,β-unsaturated thiolesters are inactivators of numerous enzymes, functioning as Michael acceptors [18]. Because of the minimal homology of the FAS and *E. coli* enoyl-reductases, we anticipate that varying the substituents of the 2-phenoxyphenol may yield a more effective inhibitor of the human FAS enoyl-reductase.

Acknowledgements The assistance of Grimaud Farms (California) in obtaining fresh goose uropygial glands and rapidly freezing them is gratefully acknowledged. This work was supported in part by NIH grant GM-36562. K.L.F. was supported by an NIH Metabolism Training Grant, DK 07319.

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403
2. Amy CM, Witkowski A, Naggert J, Williams B, Randhawa Z, Smith S (1989) Molecular cloning and sequencing of cDNAs encoding the entire rat fatty acid synthase. Proc Natl Acad Sci U S A 86:3114
3. Bhargava HN, Leonard PA (1996) Triclosan: applications and safety. Am J Infect Control 24:209
4. Buckner JS, Kolattukudy PE (1976) One-step purification and properties of a two-peptide fatty acid synthetase from the uropygial gland of the goose. Biochemistry 15:1948
5. Chang SI, Hammes GG (1989) Homology analysis of the protein sequences of fatty acid synthases from chicken liver, rat mammary gland, and yeast. Proc Natl Acad Sci U S A 86:8373
6. Chirala SS, Huang WY, Jayakumar A, Sakai K, Wakil SJ (1997) Animal fatty acid synthase: functional mapping and cloning and expression of the domain I constituent activities. Proc Natl Acad Sci U S A 94:5588
7. Funabashi H, Kawaguchi A, Tomoda H, Omura S, Okuda S, Iwasaki S (1989) Binding site of cerulenin in fatty acid synthetase. J Biochem 105:751
8. Heath RJ, Yu YT, Shapiro MA, Olson E, Rock CO (1998) Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. J Biol Chem 273:30316
9. Hennigar RA, Pochet M, Hunt DA, Lukacher AE, Venema VJ, Seal E, Marrero MB (1998) Characterization of fatty acid synthase in cell lines derived from experimental mammary tumors. Biochim Biophys Acta 1392:85
10. Jayakumar A, Tai MH, Huang WY, al-Feel W, Hsu M, Abu-Elheiga L, Chirala SS, Wakil SJ (1995) Human fatty acid synthase: properties and molecular cloning. Proc Natl Acad Sci U S A 92:8695
11. Joshi AK, Witkowski A, Smith S (1997) Mapping of functional interactions between domains of the animal fatty acid synthase by mutant complementation in vitro. Biochemistry 36:2316
12. Kuhajda FP, Pasternack GR (1998) Chemotherapy for cancer by inhibiting the fatty acid biosynthetic pathway. United States Patent no. 5,759,837
13. Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, Pasternack GR (1994) Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proc Natl Acad Sci U S A 91:6379
14. Kuhajda FP, Pizer ES, Li JN, Mani NS, Frehywot GL, Townsend CA (2000) Synthesis and antitumor activity of an inhibitor of fatty acid synthase. Proc Natl Acad Sci U S A 97:3450
15. Lawrence DS, Zilfou JT, Smith CD (1999) Structure-activity studies of cerulenin analogues as protein palmitoylation inhibitors. J Med Chem 42:4932
16. Levy SB, McMurry LM (1999) Enoyl-ACP (acyl carrier protein) reductase-interacting substances in antimicrobial screening. Patent no. WO 99/37800
17. McMurry LM, Oethinger M, Levy SB (1998) Triclosan targets lipid synthesis (letter). Nature 394:531
18. Mishra PK, Drueckhammer DG (2000) Coenzyme A analogues and derivatives: synthesis and applications as mechanistic probes of coenzyme-A ester-utilizing enzymes. Chem Rev 100:3283
19. Moche M, Schneider G, Edwards P, Dehesh K, Lindqvist Y (1999) Structure of the complex between the antibiotic cerulenin and its target, beta-ketoacyl-acyl carrier protein synthase. J Biol Chem 274:6031
20. Morrison JF, Walsh CT (1988) The behavior and significance of slow-binding enzyme-inhibitors. Adv Enzymol Relat Areas Mol Biol 61:201
21. Ohno T, Awaya J, Kesado T, Nomura S, Omura S (1974) Mechanism of action of CM-55, a synthetic analogue of the antilipogenic antibiotic cerulenin. Antimicrob Agents Chemother 6:387
22. Pizer ES, Jackisch C, Wood FD, Pasternack GR, Davidson NE, Kuhajda FP (1996) Inhibition of fatty acid synthesis induces programmed cell death in human breast cancer cells. Cancer Res 56:2745
23. Pizer ES, Wood FD, Heine HS, Romantsev FE, Pasternack GR, Kuhajda FP (1996) Inhibition of fatty acid synthesis delays disease progression in a xenograft model of ovarian cancer. Cancer Res 56:1189
24. Qiu X, Janson CA, Court RI, Smyth MG, Payne DJ, Abdel-Meguid SS (1999) Molecular basis for triclosan activity involves a flipping loop in the active site. Protein Sci 8:2529
25. Rangan VS, Joshi AK, Smith S (2001) Mapping the functional topology of the animal fatty acid synthase by mutant complementation in vitro. Biochemistry 40:10792
26. Roujeinikova A, Levy CW, Rowsell S, Sedelnikova S, Baker PJ, Minshull CA, Mistry A, Colls JG, Camble R, Stuitje AR, Slabas AR, Rafferty JB, Pauptit RA, Viner R, Rice DW (1999) Crystallographic analysis of triclosan bound to enoyl reductase. J Mol Biol 294:527
27. Spencer JB, Jordan PM (1992) Purification and properties of 6-methylsalicylic acid synthase from *Penicillium patulum*. Biochem J 288:839
28. Stewart MJ, Parikh S, Xiao G, Tonge PJ, Kisker C (1999) Structural basis and mechanism of enoyl reductase inhibition by triclosan. J Mol Biol 290:859
29. Stoops JK, Wakil SJ (1981) Animal fatty acid synthetase. A novel arrangement of the beta-ketoacyl synthetase sites comprising domains of the two subunits. J Biol Chem 256:5128
30. Thompson BJ, Smith S (1982) Lack of coordinated regulation of lipogenic enzymes in a human breast cell line, SKBr3. Biochim Biophys Acta 712:217
31. Ward WH, Holdgate GA, Rowsell S, McLean EG, Pauptit RA, Clayton E, Nichols WW, Colls JG, Minshull CA, Jude DA, Mistry A, Timms D, Camble R, Hales NJ, Britton CJ, Taylor IW (1999) Kinetic and structural characteristics of the

inhibition of enoyl (acyl carrier protein) reductase by triclosan. Biochemistry 38:12514
32. Weiss L, Hoffmann GE, Schreiber R, Andres H, Fuchs E, Korber E, Kolb HJ (1986) Fatty-acid biosynthesis in man, a pathway of minor importance. Purification, optimal assay conditions, and organ distribution of fatty- acid synthase. Biol Chem Hoppe Seyler 367:905
33. Wong H, Mattick JS, Wakil SJ (1983) The architecture of the animal fatty acid synthetase. III. Isolation and characterization of beta-ketoacyl reductase. J Biol Chem 258:15305