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Report

Eicosapentaenoic acid and sulphur substituted fatty acid analogues inhibit the proliferation of human breast cancer cells in culture

Farzaad Abdi-Dezfuli,[1]* Livar Frøyland,[2] Thor Thorsen,[1] Asbjøorn Aakvaag,[1] and Rolf K. Berge,[2]
*Department of Clinical Biology, Divisions of [1]Endocrinology and [2]Biochemistry, University of Bergen, Haukeland Hospital, N-5021 Bergen, Norway; *Present address: Eli Lilly Norge AS, Grenseveien 99, Oslo, Norway*

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Summary

Numerous studies have shown dietary fatty acids to influence the progression of several types of cancers. The purpose of the present investigation was to examine the influence of various types of fatty acids, including ω-3 fatty acids and a new class of hypolipidemic peroxisome proliferating fatty acid analogues, namely the 3-thia fatty acids, on MCF-7 human breast cancer cell growth. 3-thia fatty acids represent non-β-oxidizable fatty acid analogues in which a sulphur atom substitutes for the β-methylene group (3-position) in the saturated and unsaturated fatty acids.

The effects of increasing concentrations of palmitic acid, tetradecylthioacetic acid (a 3-thia fatty acid), eicosapentaenoic acid, docosahexaenoic acid, and two 3-thia polyunsaturated fatty acids on the proliferation of MCF-7 cells, maintained in serum-free culture, were studied. At the highest concentration of fatty acid used (64 μM) tetradecylthioacetic acid was found to be the most effective of all fatty acids tested in inhibiting cell growth, whilst palmitic acid and docosahexaenoic acid had no significant effect on cell growth. Thus, of the two dietary polyunsaturated ω-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, only eicosapentaenoic acid possesses an inhibitory effect on the proliferation of MCF-7 cells. In all cases the inhibitory effect of the fatty acid was found to be reversible.

Tetradecylthioacetic acid has been shown to be a potent peroxisome proliferator. It was, therefore, hypothesized that tetradecylthioacetic acid may inhibit the human MCF-7 cell growth by increasing the level of oxidative stress within the cell. However, use of agents which modify the cell's protective apparatus against oxidative stress had no influence on the inhibitory effect of tetradecylthioacetic acid.

These experiments indicate that tetradecylthioacetic acid inhibits cell growth by mechanisms which may be independent of oxidative status.

Introduction

Whilst the significance of dietary fat in the genesis and promotion of various pathological states, particularly cardiovascular disease, is well established, its influence in several types of human neoplasms, including breast cancer, remains unresolved. Nevertheless, numerous epidemiological studies have suggested an association between dietary fat and the incidence of breast cancer [1–3]. Furthermore, a great number of studies using animal cancer models (see ref. 4 for review), and investigations using hu-

Address for offprints: Rolf K. Berge, Department of Clinical Biology, Division of Biochemistry, University of Bergen, Haukeland Hospital, N-5021 Bergen, Norway; *Phone:* 47 5597 3098; *Fax:* 47 5597 3115

man breast carcinomas maintained in immunodeficient mice [5–10], have conclusively demonstrated an association between mammary neoplasia and both the level and type of fat consumed. The results of these studies suggest that, on the whole, diets containing a high proportion of ω-6 polyunsaturated fatty acids enhance tumor growth, whilst those rich in ω-3 polyunsaturated fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), suppress these processes.

Dietary ω-3 polyunsaturated fatty acids, predominantly of marine origin, have been shown to reduce the risk of coronary heart disease by decreasing the levels of both cholesterol and triacylglycerol in the blood [11–13]. Both EPA and DHA have been shown to increase peroxisomal β-oxidation of fatty acids in the liver [14, 15]. Increased peroxisomal β-oxidation, which generates hydrogen peroxide (H_2O_2), may therefore generate a state of oxidative stress if the level of H_2O_2 exceeds the peroxide catabolic capacity of the cell (Figure 1). Such circumstances may lead to cytotoxicity and, eventually, cell death due to free-radical (·OH) damage. Indeed, both EPA and DHA have been shown to inhibit cell growth in culture [16, 17]. However, precisely how this takes place is not clear [18]. As mentioned, one of the mechanisms by which these polyunsaturated fatty acids have been proposed to block cell growth is via the generation of oxygen radicals and the stimulation of intracellular lipid peroxidation (see ref. 19 for a review) (Figure 1).

In this regard, tetradecylthioacetic acid, CH_3-$(CH_2)_{13}$-S-CH_2-COOH, which is a member of a new class of hypolipidemic, sulphur-substituted fatty acid analogues [20] referred to generally as 3-thia fatty acids, has recently been shown by our group to inhibit MCF-7 breast cancer cell growth in culture [21]. Whilst this analogue has many of the chemical and physical characteristics of a normal saturated fatty acid, it can not undergo β-oxidation in the mitochondria or peroxisomes. As a consequence, the cell strives to compensate for this blockage by increasing its content of mitochondria and peroxisomes which, as a result leads to an increase in fatty acid oxidation of available normal fatty acids. Hence, it is viable to hypothesize that, as a potent peroxisome proliferator, tetradecylthioacetic acid

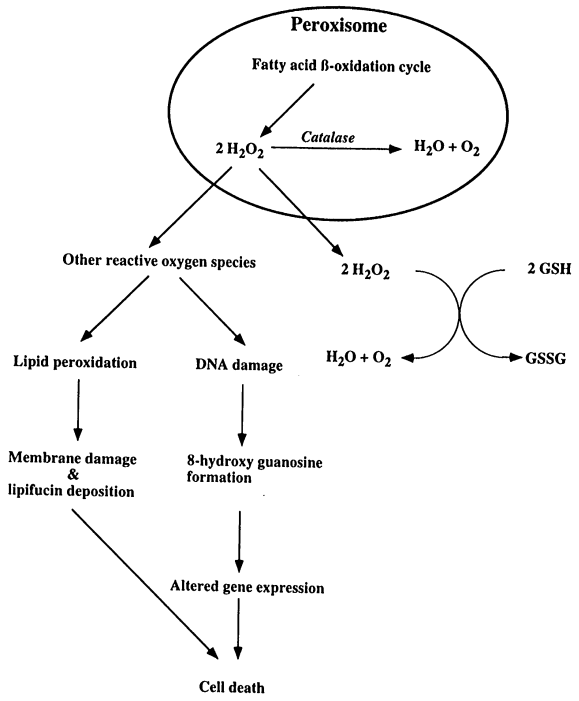

Figure 1. Postulated mechanism for the cytotoxicity of peroxisome proliferators. H_2O_2, hydrogen peroxide; GSH, glutathione (reduced form); and GSSG, oxidized glutathione.

may inhibit cell growth by generating an intracellular state of oxidative stress. The main aim of this study was, therefore, to investigate the possible mechanism(s) by which fatty acids modulate mammary neoplasia. With the above mentioned considerations in mind we (1) compared the effects of saturated and polyunsaturated ω-3 fatty acids to those of tetradecylthioacetic acid, (2) investigated the effect of sulphur substituted polyunsaturated ω-3 fatty acids, and (3) determined whether the effects of these compounds were reversed or intensified by antioxidants and oxidants, respectively.

Moreover, we measured the level of glutathione (GSH), an important antioxidant, and the levels of cysteine and cysteinylglycine, a substrate for GSH synthesis and a catabolic product of GSH, respectively. The oxidation of GSH occurs concomitantly to the reduction of hydroperoxides. The oxide-reduction may be spontaneous in the extent to which the thiol function of GSH chemically reacts with hydroperoxides at significant rates (see Figure 1). It is most likely that a lower level of GSH will result if

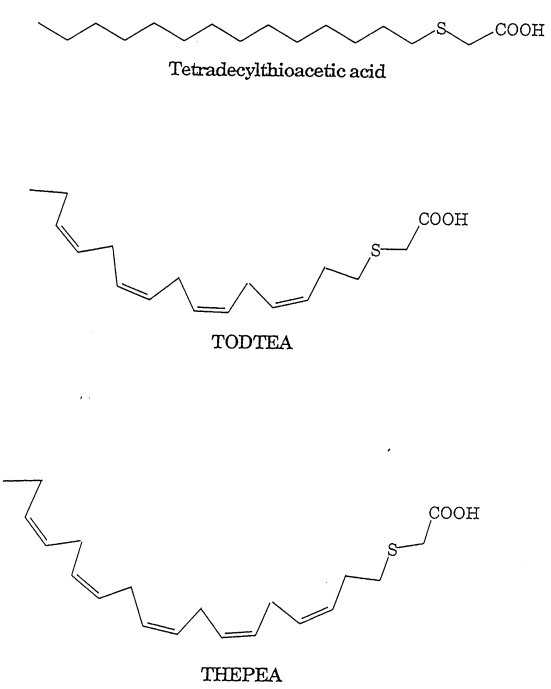

Figure 2. Representative structures of the various 3-thia substituted fatty acids.

the peroxisome proliferating fatty acids generate an increase in the oxidative stress.

Materials and methods

Chemicals

Palmitic (saturated), arachidonic (ω-6, polyunsaturated), eicosapentaenoic (ω-3, polyunsaturated), and docosahexaenoic (ω-3, polyunsaturated) acids were purchased from the Sigma Chemical Co. (St. Louis, MO); tetradecylthioacetic acid was synthesized as previously described [22]; and 3-thia octadeca-6,9,12,15-tetraenoic and 3-thia heneicosa-6,9,12,15,18-pentaenoic acids were a gift from Professor Skattebøl, Department of Organic Chemistry, University of Oslo (see Figure 2, for structures). Insulin and oestradiol-17β were obtained from Sigma, as were cysteamine-HCl (increases GSH) and 1-buthionine-(S, R)-sulphoximine (decreases GSH), α-Tocopherol acetate (antioxidant) was obtained from Norsk Medisinal Depot (Oslo, Norway).

Cells and culture conditions

The oestrogen-responsive human breast cancer epithelial cell line MCF-7 was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in a humidified (95%) atmosphere of 5% CO_2 and 95% air, maintained at 37 °C. The culture medium (standard medium) used throughout this investigation was comprised of Dulbecco's minimum essential medium (Gibco, Bio-Cult, Paisely, Scotland) to which had been added glutamine (2 mM), streptomycin (100 μg/ml), and penicillin (100 IU/l). MCF-7 cells were routinely sustained in standard medium supplemented with 10% fetal calf serum and the pH indicator phenol red (running medium).

Growth experiments

All experiments were carried out using 24-well cell culture plates (1.8 cm^2; Nunc). Initially, the cells were plated, i.e. allowed to attach to the well bottom, by incubating (for 24 h or 72 h) with plating medium which consisted of standard medium supplemented with charcoal-stripped (oestrogen depleted) fetal calf serum (5% final concentration), insulin (5 nM), and oestradiol (10 pM). Charcoal stripping of fetal calf serum was carried out as described previously [23]. Cells were seeded at a density of approximately 4×10^4 cells/0.5 ml plating medium/well. Following plating, the medium was replaced with serum-free medium [comprised of Dulbecco's modified Eagle's medium, without phenol red, containing non-essential amino acids (3%), bovine serum albumin (2.5 g/l), ethanolamine (2 μg/l), transferrin (36 mg/l), linoleic acid (1 mg/l), oleic acid (1 mg/l), palmitic acid (1 mg/l), hydrocortisone (40 nM), triiodothyronine (6 μM), d-biotin (14 μg/l), vitamin B-12 (14 μg/l), glutamine (2 mM), streptomycin (100 μg/ml), and penicillin (100 IU/l)] to which insulin (5 nM) and oestradiol (10 pM) had been added, and the cells were cultured for a further

24 h. On day 0 of the experiment, serum-free medium was replaced with experimental medium, which was comprised of serum-free medium supplemented with insulin (50 nM) and oestradiol (0.5 nM). The MCF-7 cell line used in this study has previously [21, 26], been shown to respond to oestradiol under similar serum-free culture conditions. Fatty acids, oestradiol, and α-tocopherol acetate were all dissolved in absolute ethanol, and insulin was dissolved in 1% (v/v) acetic acid before being added to the experimental medium; the volume of ethanol added was such that the final concentration of ethanol in the medium never exceeded 0.2%. Ethanol alone at the same final concentration was added to the control wells. Incubation was continued for six or nine days with the medium being changed every three days. Cell numbers were determined using a Coulter Counter® (Coulter Electronics, Luton, U.K.) following detachment by trypsination. The cell numbers presented represent the mean (± SEM) of six replicate wells, and statistical analysis was carried out using unpaired Student's t-test. The SEM of the cell doubling times presented in the Results ranged from 1 to 2%.

Determination of intracellular antioxidant status

The levels of glutathione, cysteine (a substrate for glutathione synthesis), and cysteinylglycine (a catabolic product of glutathione) in the MCF-7 cells receiving various treatments, were determined using a modification of the methods described by Djurhuus, Svardal, and Ueland [24], and Svardal, Mansoor, and Ueland [25]. Briefly, cells were removed from culture, washed twice with ice-cold phosphate-buffered saline (pH 7.4), and immediately set into ultra-freeze (– 85 °C). Parallel dishes receiving the same treatment were used for determining cell numbers. The frozen cells were eventually extracted with ice-cold sulphosalicylic acid (5%), and the precipitated proteins removed by centrifugation. The amounts of glutathione, cysteine, and cysteinylglycine in the acid extract were subsequently determined using the derivatives of these molecules with monobromobimane (Kosower's reagent) followed by analysis with reverse-phase HPLC.

Expression of results

Results are expressed as mean ± SEM. Treatment means were compared by one-way analysis of variance, single factorial model with multiple comparison, Fisher's PLSD using a statistical software StatView SE + Graphics™ on an Apple Macintosh.

Table 1. Effects of increasing concentrations of different saturated and polyunsaturated fatty acids and their 3-thia analogues on MCF-7 breast cancer cell growth. For each treatment, cells were initially seeded out and incubated for 72 h in medium containing oestradiol-deficient serum, and then cultured for 24 h in serum-free medium. Each value represents the $10^{-3} \times$ mean ($10^{-3} \times$ SEM) number of cells present in six separate wells.

Concentration (μM)	Cell numbers following six days of incubation with	
	Fatty acids	3-Thia analogues
	Palmitic acid	**Tetradecylthioacetic acid**
0	746 (10)[a]	746 (10)[a]
4	658 (12)	703 (6)[c]
8	657 (20)	669 (11)[c]
16	715 (18)	633 (22)[c]
32	642 (35)	581 (10)[c]
64	700 (15)	421 (14)[c]
	EPA	**TODTEA**
0	630 (12)	737 (8)
4	641 (6)	698 (4)
8	665 (14)	689 (8)
16	605 (15)	691 (20)
32	540 (6)[c]	577 (20)[c]
64	478 (14)[c]	540 (6)[c]
	DHA	**THEPEA**
0	583 (6)[b]	583 (6)[b]
4	603 (14)	615 (18)
8	615 (9)	589 (21)
16	626 (10)	501 (10)[c]
32	624 (13)	534 (10)[c]
64	599 (8)	504 (7)[c]

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; THEPEA, 3-thia heneicosa-6,9,12,15,18-pentaenoic acid; TODTEA, 3-thia octadeca-6,9,12,15-tetraenoic acid. [a,b] Both treatments were done in the same experiment and, therefore, only one control culture was set up. [c] Significantly lower ($p < 0.01$) than respective control (0 μM).

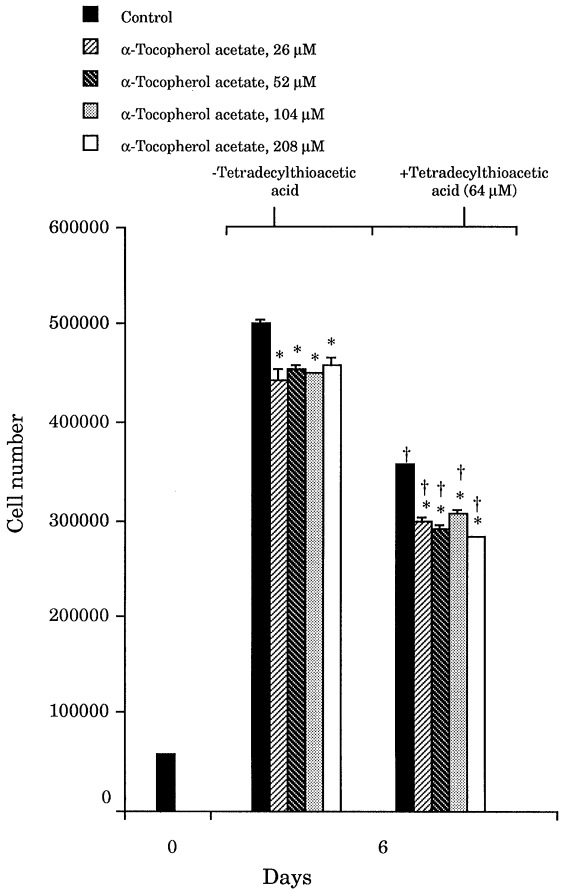

Figure 3. Effect of increasing concentrations of α-tocopherol acetate on MCF-7 cells cultured in the absence and presence of tetradecylthioacetic acid (64 μM). Cells were plated by incubating with plating medium for 24 h (seeding density ∼ 40000 cells/0.5 ml), and were subsequently cultured for 24 h with serum-free medium, before being cultured (day 0) in experimental medium for a further six days. Each bar represents the mean (± SEM) number of cells present in six separate wells. * Significantly ($P < 0.01$) lower than respective control. † Significantly ($P < 0.01$) lower than tetradecylthioacetic acid-free control.

Results

Effects of saturated and polyunsaturated fatty acids and their 3-thia fatty acid analogues on MCF-7 breast cancer cell growth

The effects of increasing concentrations of palmitic acid and tetradecylthioacetic acid on cell growth are shown in Table 1. Increasing concentrations of tetradecylthioacetic acid (4–64 μM) inhibited cell growth in a dose-dependent manner, such that at

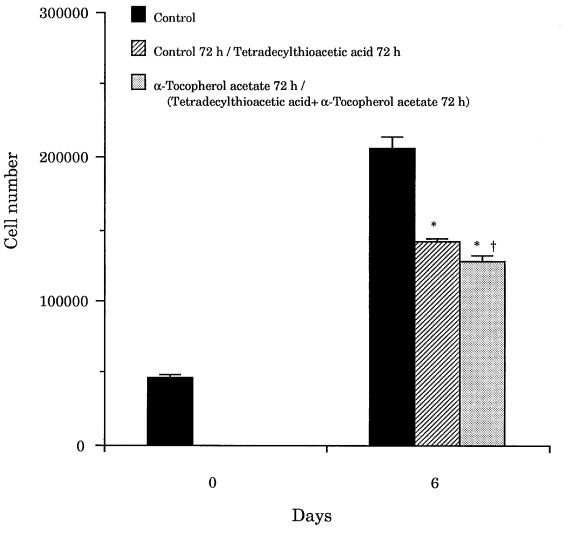

Figure 4. Effect of pre-incubation of MCF-7 cells with α-tocopherol acetate on the growth-inhibitory effect of tetradecylthioacetic acid. Cells were plated by incubating with plating medium for 24 h (seeding density ∼ 40000 cells/0.5 ml), and were subsequently cultured for 24 h with serum-free medium, before being cultured (day 0) in experimental medium; Control – cells were cultured in control medium for six days; Control 72 h/tetradecylthioacetic acid 72 h – cells were cultured in control medium for 72 h followed by 72 h incubation in the presence of tetradecylthioacetic acid (64 μM) for a further 72 h; α-Tocopherol acetate 72 h/(α-tocopherol acetate + tetradecylthioacetic acid) 72 h – cells were incubated with α-tocopherol acetate (105 μM) for 72 h and were subsequently cultured, for an additional 72 h, with medium containing both α-tocopherol acetate (105 μM) and tetradecylthioacetic acid (64 μM). Each bar represents the mean (± SEM) number of cells present in six separate wells. * Significantly ($P < 0.01$) lower than control. † Significantly ($P < 0.05$) lower than (control 72 h/tetradecylthioacetic acid 72 h).

the highest concentration of tetradecylthioacetic acid used, 64 μM, the cell doubling time was increased from 50 h to 73 h. In the case of palmitic acid, however, no such dose-dependent inhibition of growth was obtained (Table 1). The 3-thia polyunsaturated fatty acid analogues also produced a dose-dependent inhibition of cell growth.

The presence of 3-thia octadeca-6,9,12,15-tetraenoic acid (TODTEA) (64 μM) and 3-thia heneicosa-6,9,12,15,18-pentaenoic acid (THEPEA) (64 μM) in the culture medium resulted in an increase in the cell doubling time from 54 h to 74 h, and 52 h to 54 h, respectively. Of the two normal ω-3 polyunsaturated fatty acids included in the ex-

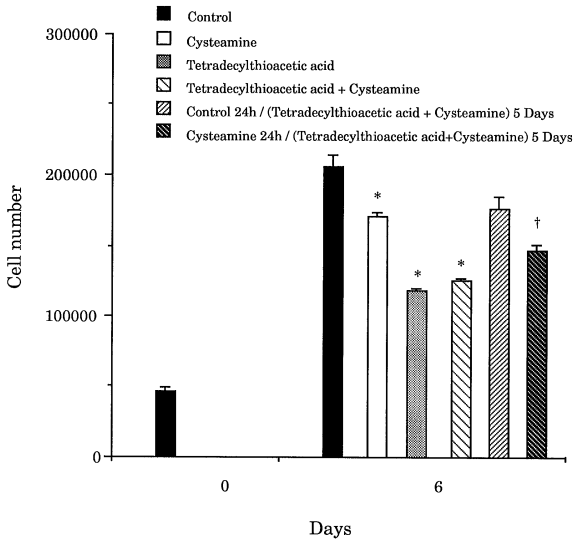

Figure 5. Influence of cysteamine on the growth-inhibitory effect of tetradecylthioacetic acid on MCF-7 cells. Cells were plated by incubating with plating medium for 24 h (seeding density ~ 40000 cells/0.5 ml), and were subsequently cultured for 24 h with serum-free medium, before being cultured (day 0) in experimental medium; Control – cells were cultured in control medium for six days; Cysteamine – cells were cultured in the presence of cysteamine (100 μM) for six days; Tetradecylthioacetic acid – cells were cultured in the presence of tetradecylthioacetic acid (64 μM) for six days. Tetradecylthioacetic acid × cysteamine – cells were cultured in the presence of tetradecylthioacetic acid (64 μM) and cysteamine (100 μM) for six days; Control 24 h / (tetradecylthioacetic acid + cysteamine) 5 days – cells were initially cultured in control medium for 24 h before being incubated with tetradecylthioacetic acid (64 μM) and cysteamine (100 μM) for a further five days; Cysteamine 24 h / (tetradecylthioacetic + cysteamine) 5 days – cells were initially cultured with cysteamine (100 μM) for 24 h before being incubated with tetradecylthioacetic acid (64 μM) and cysteamine (100 μM) for a further five days. Each bar represents the mean (± SEM) number of cells present in six separate wells. * Significantly ($P < 0.01$) lower than control. † Significantly ($P < 0.05$) lower than (control 24 h / (tetradecylthioacetic acid + cysteamine) 5 days).

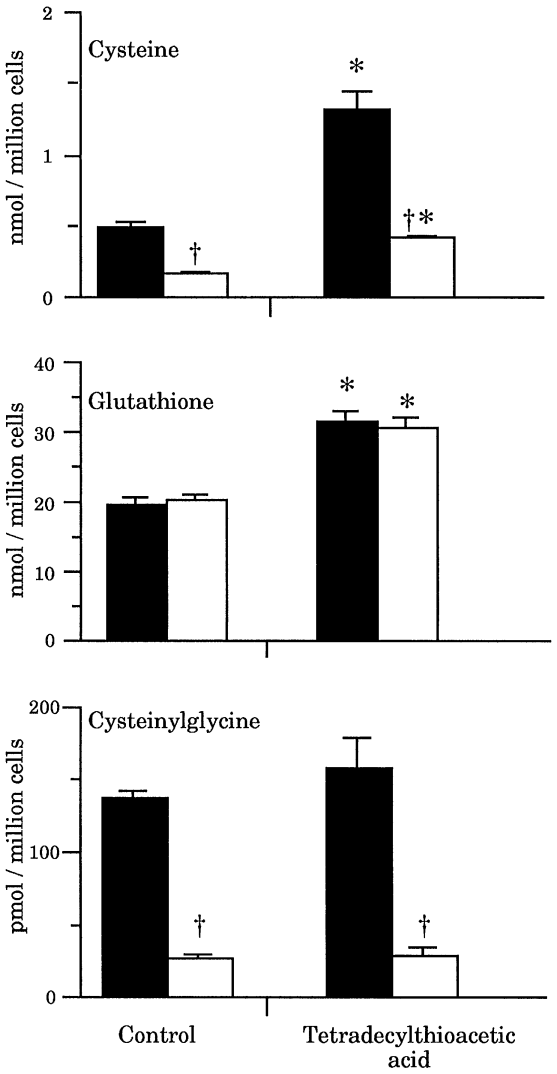

Figure 6. Effect of tetradecylthioacetic acid (64 μM) on total (i.e. reduced + oxidised + protein-bound; closed bars) and reduced (open bars) amounts of cysteine, glutathione, and cysteinylglycine in MCF-7 cells. The cells used in this experiment were run in parallel with tetradecylthioacetic acid treated cells shown in Figure 4. Each bar represents the mean (± SEM) values determined for cells present in six separate wells. * Significantly ($P < 0.01$) higher than value determined for control culture. † Significantly ($P < 0.01$) lower than total amount (reduced + oxidised + protein-bound) determined for respective treatment.

perimental medium, only eicosapentaenoic acid (EPA) was seen to have an inhibitory effect on cell growth, such that the inclusion of this fatty acid at a concentration of 64 μM in the culture medium increased the cell doubling time from 51 h to 55 h. Conversely, docosahexaenoic acid (DHA) had little or no effect on cell growth (Table 1).

MCF-7 cells were cultured, for six days, in the presence of the various fatty acids followed by replacement of the experimental medium by control medium and incubation for a further three days. The inhibitory effect of the fatty acids on cell growth was not permanent since changing the medium in experimental cultures to control medium resulted in continued growth (data not shown).

Influence of anti-oxidants on the inhibitory effect of tetradecylthioacetic acid on MCF-7 cell growth: modulation of glutathione metabolism

The effect of incubating MCF-7 cells with increasing concentrations of α-tocopherol acetate, in the absence and presence of tetradecylthioacetic acid is shown in Figure 3. α-Tocopherol acetate produced a slight, non-dose-dependent inhibition of cell growth, but in the absence and presence of tetradecylthioacetic acid. Similarly, the pre-incubation of cells with α-tocopherol acetate for three days, prior to culturing them with both tetradecylthioacetic acid and α-tocopherol acetate for a further three days, slightly enhanced the inhibitory effect of tetradecylthioacetic acid on cell growth (Figure 4). Growth was also slightly inhibited when cells were incubated with cysteamine for six days (Figure 5). Furthermore, there was no significant difference in the number of cells present in cultures containing tetradecylthioacetic acid compared to cultures of cells incubated with both tetradecylthioacetate acid and cysteamine. The incubation of cells with cysteamine for 24 h, prior to their exposure to tetradecylthioacetic acid in the presence of cysteamine for a further five days, did not remove the inhibitory effect of tetradecylthioacetic acid on cell growth.

Modifications of glutathione status concomitant with effects on cell growth were also observed. The effects of tetradecylthioacetic acid on glutathione metabolism in MCF-7 cells is shown in Figure 6. Glutathione was found to be completely in the reduced form in cells receiving control medium. Furthermore, tetradecylthioacetic acid was found to increase only this form of the molecule. This stimulatory influence of tetradecylthioacetic acid on the reduced form of glutathione appeared to occur via an effect on cysteine levels in the cell, since the amounts of both total and reduced cysteine increased significantly in comparison to control, whereas the levels of total and reduced cysteinylglycine in cells treated with tetradecylthioacetic acid were unchanged. In addition, the quantity of oxidized-protein-bound cysteine (amount of total cysteine minus reduced cysteine) was significantly higher in tetradecylthioacetic acid treated cells compared to control cultures (data not shown).

The increase in glutathione brought about by tetradecylthioacetic acid was blocked by α-tocopherol acetate (Table 2). Similarly, whereas cysteamine alone had no significant effect on the total cellular content of glutathione, in the presence of tetradecylthioacetic acid, cysteamine abrogated the stimulatory effect of tetradecylthioacetic acid (Table 2).

Whilst tetradecylthioacetic acid and the different antioxidants were found to influence intracellular glutathione metabolism, no correlation could be found between this effect of these compounds and

Table 2. Effects of tetradecylthioacetic acid and different antioxidants on total glutathione levels in MCF-7 cells. In this experiment, parallel cultures of cells receiving identical treatments were incubated for a total of six days in serum-free medium. Tetradecylthioacetic acid (64 μM), cysteamine (100 μM), and α-tocopherol acetate (105 μM) were included in the culture medium for the periods of time shown. Parallel determinations of total glutathione levels and cell numbers were subsequently determined using extraction followed by reverse-phase HPLC, and a Coulter Counter, respectively.

Treatment	Length of treatment	Total glutathione (nmol/10^6 cells) following 6 days of culture*
1. None (control	6 days	19.5 ± 1.1^a
2. Tetradecylthioacetic acid	6 days	31.6 ± 1.5^b
3. Cysteamine	6 days	22.9 ± 3.0^a
4. Tetradecylthioacetic acid + cysteamine	6 days	19.7 ± 1.4^a
5. None for 3 days followed by tetradecylthioacetic acid	3 + 3 days	30.9 ± 0.8^b
6. α-Tocopherol acetate for 3 days followed by tetradecylthioacetate acid + α-tocopherol acetate	3 + 3 days	19.2 ± 1.0^a

* Means ± SEM; Values with different superscripts are significantly ($P < 0.01$) different.

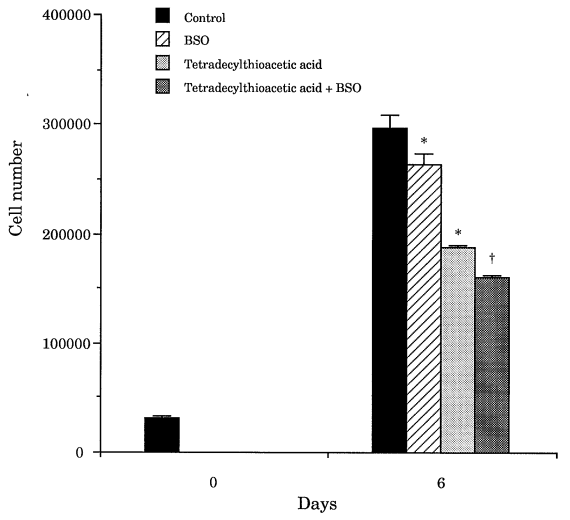

Figure 7. Influence of 1-buthionine-(S,R)-sulphoximine (BSO) on the growth-inhibitory effect of tetradecylthioacetic acid on MCF-7 cells. Control – cells were cultured in control medium for six days; Tetradecylthioacetic acid – cells were cultured in the presence of tetradecylthioacetic acid (64 μM) for six days; BSO – cells were cultured in the presence of BSO (20 μM) for six days. Experiment was carried out in an identical fashion to that described in the legend to Figure 2. Each bar represents the mean (± SEM) number of cells present in six separate wells. * Significantly ($P < 0.05$) lower than control. † Significantly ($P < 0.01$) lower than tetradecylthioacetic acid.

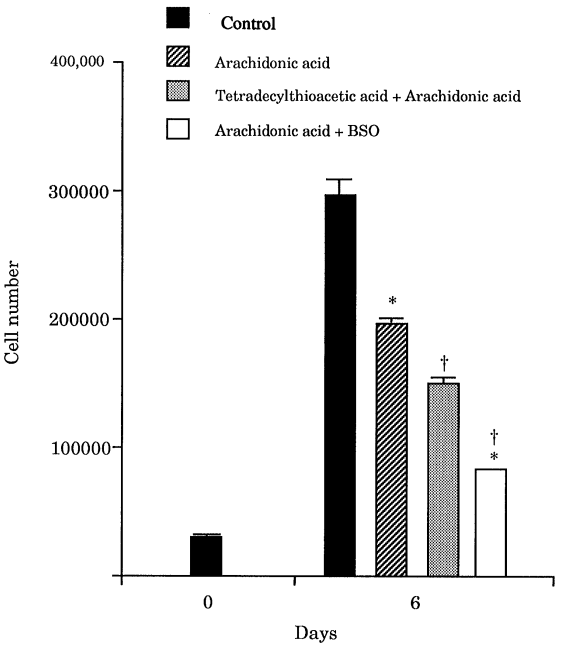

Figure 8. Influence of arachidonic acid on the growth of MCF-7 cells. Experiment was carried out in an identical fashion to that described in the legend to Figure 2. Each bar represents the mean (± SEM) number of cells present in six separate wells. * Significantly ($P < 0.01$) lower than cell number in control cultures. † Significantly ($P < 0.01$) lower than cell number in cultures treated with arachidonic acid alone.

their influence on cell growth. All inhibited cell growth by means other than via an effect on glutathione metabolism.

Influence of the pro-oxidant 1-buthionine-(S, R)-sulphoximine (BSO) and arachidonic acid on the inhibitory effect of tetradecylthioacetic acid on MCF-7 cell growth

Figure 7 shows the effect of BSO on cells grown in the absence and presence of tetradecylthioacetic acid. Whilst cell numbers in cultures containing tetradecylthioacetic acid plus BSO were slightly lower than those present in cultures containing only tetradecylthioacetic acid, the increased inhibition was most probably brought about by BSO itself and not through any action of tetradecylthioacetic acid. This is because BSO, on its own, inhibited the cells in control cultures to the same degree as that seen in cultures containing both tetradecylthioacetic acid and BSO.

The effect of culturing MCF-7 cells for six days with arachidonic acid (64 μM) in the presence and absence of BSO is shown in Figure 8. Arachidonic acid increased the cell doubling-time from 40 h for Control, to 52 h. This was increased to 56 h when tetradecylthioacetic acid was added to cultures containing arachidonic acid. However, there was a dramatic increase in the doubling-time for cells cultured with arachidonic acid when BSO was included in the culture medium, i.e. from 52 h in the presence of arachidonic acid to 90 h in the presence of both arachidonic acid and BSO.

Discussion

The influence of saturated and unsaturated fatty acids, including those used in this study, namely pal-

mitic acid and the two ω-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been the subject of intense research in the field of dietary modulation of mammary tumor biology. In the present study, at the highest concentration of fatty acid used, tetradecylthioacetic acid was found to be the most effective of all fatty acids tested in inhibiting cell growth, whilst palmitic acid and DHA had no significant effect on this process. In all cases, the inhibitory effect of individual fatty acids was found to be reversed upon their removal from the medium. Our group has previously demonstrated [21] that the inhibition in cell growth observed in cultures containing tetradecylthioacetic acid could not be overcome by the addition of increasing concentrations of insulin-like growth factor-I, insulin, or oestradiol, all of which are well known growth stimulatory agents for this cell line [26]. Thus, tetradecylthioacetic acid appears to abrogate MCF-7 cell growth via a pathway which is independent of the established means by which these growth promoting factors facilitate the proliferation of this cell line.

Recent studies by Gonzalez et al. [27] have revealed that the growth of human mammary carcinomas maintained in immunodeficient (nude) mice was significantly suppressed when the animals were fed a diet containing high levels of fish oil. This inhibitory effect was, in part, associated with an accumulation of lipid peroxidation products in the tumor tissue. It has been suggested that such an accumulation is due to increased incorporation of fish oil polyunsaturated fatty acids, in particular EPA and DHA, into the tumor cell membranes, which makes them susceptible to peroxidative damage, and leads ultimately to cell death [19]. However, the results obtained in this study do not support such an assertion. This is because whilst EPA inhibited cell growth to a small extent, DHA (which has a higher degree of unsaturation compared to EPA) had no effect on cell growth. Furthermore, tetradecylthioacetic acid, which is a saturated 3-thia fatty acid, was more potent than either of the two polyunsaturated 3-thia fatty acids. Likewise, other workers investigating the cytocidal potential of γ-linolenic acid (18:3, ω-6) and DHA have found that, whilst there was equal incorporation of these polyunsaturated fatty acids into the phospholipids of the human breast cancer cell line ZR-75-1, the levels of both lipid peroxidation and cell death were higher in cells treated with γ-linolenic acid than with DHA [28]. Arachidonic acid produced, in the present study, a marked inhibition of cell growth. This effect was dramatically amplified in the presence of 1-buthionine-(S,R)-sulphoximine (BSO). This suggests that, whilst it is unlikely that EPA and DHA inhibit cell growth via their incorporation in and subsequent peroxidative destabilisation of the cell membranes, the arachidonic acid induced suppression of cell growth may have been due, to a large extent, to lipid peroxidation. Such a proposal is supported by results obtained by Chow et al. [29] who showed that, whilst ω-6 polyunsaturated fatty acids, including arachidonic acid, exert their growth inhibitory effects mainly via lipid peroxidation, other mechanisms appear to be operating for the ω-3 polyunsaturated fatty acids. Interestingly, *in vivo* studies using highly purified EPA and DHA have revealed that, whilst EPA is hypotriacylglycerolemic, DHA is not [15, 30]. Consequently, it is possible that EPA blocks cell growth by interfering with pathways which involve triacylglycerol metabolism.

In a recent study which investigated the effect of feeding tetradecylthioacetic acid to rats over a two week period, it was discovered that the hepatic hydrogen peroxide (H_2O_2) and lipid peroxide contents in test animals were increased significantly over levels found in control animals [31]. However, that the inhibitory effect of tetradecylthioacetic acid on cell growth was due to an intracellular accumulation of harmful unstable peroxides, was not supported by results obtained in this study. This is because none of the agents used to boost the antioxidant defense systems in the cells produced a reversal of the inhibitory effect of tetradecylthioacetic acid on cell growth. Indeed, both α-tocopherol acetate and cysteamine, when added alone to cultures, were found to block cell growth. These observations are not without precedent, since other tocopherol esters [32] and cysteamine [33] have previously been shown to inhibit cell growth by dissimilar mechanisms. The inclusion of BSO, at a concentration known to deplete cells of glutathione [24], in cultures which had received tetradecylthioacetic

acid did not amplify the inhibition brought about by the 3-thia fatty acid. This challenges further the suggestion that tetradecylthioacetic acid induces a blockage in cell growth by stimulating the synthesis of harmful peroxides since, were this to be the case, then one would expect a dramatic amplification of the inhibitory effect of tetradecylthioacetic acid on cell growth in the presence of BSO. Nevertheless, the fact that tetradecylthioacetic acid increased intracellular glutathione synthesis demonstrates that tetradecylthioacetic acid can modulate one of the cells main antioxidant defense mechanisms. Whether this is in reaction to an increased oxidative status can only be conclusively determined by measuring the level of H_2O_2 and/or lipid peroxidation in the cells.

In vivo studies using tetradecylthioacetic acid and another sulphur-substituted fatty acid, hexadecadioic acid (3-thia dicarboxylic acid) have shown these fatty acid analogues to possess hypocholesterolemic properties [34]. This effect appears to be mediated primarily via the suppression of the activity of the rate-limiting enzyme in the synthesis of cholesterol, namely 3-hydroxy-3-methylglutaryl Coenzyme-A (HMG-CoA) reductase [35]. Data from different laboratories have revealed that non-sterol products down-stream to this key enzyme are critical for the initiation of DNA synthesis and cellular proliferation in mammalian cells [36–38], including human breast cancer cells [39–41]. Consequently, it is conceivable that tetradecylthioacetic acid suppressed cell growth by such a mechanism. This possibility is currently being investigated in our laboratory.

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