Perturbations of Cellular Acylation Processes by the Synthetic Alkyllysophospholipid 1-O-Octadecyl-2-O-methylglycero-3-phosphocholine Do Not Correlate with Inhibition of Proliferation of MCF7 and T84 Cell Lines

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ABSTRACT

We have investigated the hypothesis that the antiproliferative effect of 1-O-octadecyl-2-O-methylglycerol-3-phosphocholine (ET-18-OCH₃) is mediated through the inhibition of cellular acylation processes that control the unsaturated fatty acid complement of phospholipids. The effect of ET-18-OCH₃ on the incorporation of radiolabeled oleic, linoleic, and arachidonic acids into MCF7 and T84 phospholipids was investigated. Incubation of MCF7 cells with fatty acids and 2.75 µg/ml ET-18-OCH₃, which inhibited the proliferation of the cells after 8 h, resulted in decreased incorporation of fatty acids into a number of phospholipids, notably phosphatidylcholine; however, increased incorporation of fatty acids into other phospholipids was also observed. After 12 h incubation with the alkyl-lysophospholipid, differences in the distribution of newly incorporated fatty acids into the phospholipid classes were observed without any effect on the total amount of fatty acid incorporated. Incubation of MCF7 cells with 5 µg/ml ET-18-OCH₃, which caused a cessation in proliferation, had a similar effect on the incorporation of the fatty acids into the phospholipids, but the redistribution of newly incorporated fatty acids in the phospholipids was accompanied by a decrease in the amount of associated radiolabeled fatty acid. Incubation of T84 cells with the labeled fatty acids and 3.5 µg/ml ET-18-OCH₃, which significantly decreased proliferation after 8 h, resulted in decreased incorporation of oleic acid into phosphatidylcholine and increased incorporation of oleic, linoleic, and arachidonic acids into phosphatidylethanolamine, prior to the decrease in proliferation. After 12 h incubation with alkyl-lysophospholipid, significant increases in the total amount of labeled oleic and arachidonic acids incorporated in the phospholipid fraction were observed. These results clearly indicate that the antiproliferative effect of ET-18-OCH₃ in MCF7 and T84 cells is not dependent on inhibition of acylation processes and the above hypothesis may not be applicable to all alkyl-lysophospholipid-sensitive cells.

INTRODUCTION

It is now well established that a number of synthetic ether phospholipids collectively referred to as ALPs² possess antineoplastic activities in vivo and in vitro (1, 2). Although their mechanism(s) of action have yet to be established, a number of hypotheses have been postulated (1, 3). One proposed mechanism is an interference of ALP in the cellular processes by which cells maintain a required complement of unsaturated fatty acids in their membrane phospholipids (4). The inhibition of fatty-acy-CoA acyltransferase is envisaged to lead to a decreased unsaturated/saturated fatty acid ratio in cellular phospholipids that inhibits cell growth (4–6). Lysophospholipid acyl-CoA acyltransferases, the enzymes that catalyze the esterification of unsaturated fatty acids, have been suggested to be the target for ALP in cells, with the enzymes in more sensitive cells having a greater affinity for the ALP than the enzymes in less sensitive cells (6).

There is evidence suggesting that the inhibition of acylation by ALP is restricted to acyl-CoA:1-acylglycerophosphocholine acyltransferase, the enzyme responsible for the acylation of LPC to PC. Thus incubation of ET-18-OCH₃ resulted in a decrease in the incorporation of oleic or linoleic acid into PC in ALP-sensitive cells whereas no decrease was observed in ALP-resistant cells (4, 5). ET-18-OCH₃ decreased the incorporation of LPC into PC in HL60, an ALP-sensitive human leukemic cell line, but had no effect on K562, an ALP-resistant cell line (7). Support has also been obtained from a comparison of kinetic data of the LPC acyltransferase activities in an ALP-sensitive fibrosarcoma cell line, Meth A cells, and bone marrow-derived macrophages which are resistant to ALP (6).

The formulation of the hypothesis implicating the inhibition of acylation by ALP as the mechanism of its antiproliferative effect in cancer cells is based on a very limited number of cells and, as far as we are aware, has not been investigated in any epithelial cancer cell line. The studies reported in this and the accompanying paper (8) were designed to investigate the link between the antiproliferative effect of ALP and inhibition of cellular acylation processes in a number of human epithelial cancer cell lines.

MATERIALS AND METHODS

Cell Culture. MCF7 and T84 cell lines were obtained from the American Type Culture Collection (Rockville, MD) and cultured in media ( Gibco) supplemented with 10% fetal bovine serum (Collekt Gold; Flow Laboratories). MCF7 was grown in Dulbecco’s modification of Eagle’s medium and T84 in a 1:1 mixture of Ham’s F-12 nutrient mixture and Dulbecco’s modification of Eagle’s medium. The cells were cultured in T75 flasks (Falcon) at 37°C in 5% CO₂/95% humidified air atmosphere.

Effect of ALP on Cell Proliferation. Cells were seeded into 6- or 24-well plates and grown to log phase. Subsequently the medium was removed and replaced with fresh medium containing the required concentration of ET-18-OCH₃ (Medmark Chemicals, Gruenewald, Germany) in experimental wells and no ET-18-OCH₃ in control wells. The increase in cell number was determined at selected times by trypsinizing the cells and estimating the cell number with a Model ZM Coulter Counter (Coulter Electronics).

Effect of ET-18-OCH₃ on Fatty Acid Incorporation into Cellular Phospholipids. Cells were grown to log phase in 6-well dishes (Falcon). The medium was removed and replaced with one containing the required concentration of ET-18-OCH₃ in experimental wells and medium without ET-18-OCH₃ in control wells. Tracer amounts of radiolabeled fatty acids in ethanol (7.5 µCi) were added as follows: [3H]oleic acid, 1 µCi/well; [3H]arachidonic acid, 0.75 µCi/well; and [14C]linoleic acid, 0.15 µCi/well. The cells were then placed in a CO₂ incubator at 37°C. At different times subsequent to the addition of the label, each well was washed twice with 1.5 ml of 0.1% fatty acid free bovine serum

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3 The abbreviations used are: ALP, alkyl-lysophospholipid; LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid; ET-18-OCH₃, 1-O-octadecyl-2-O-methylglycero-3-phosphocholine.
albumin and trypsinized. The cells were transferred into 15-ml polypropylene tubes and dispersed with a 21-gauge needle. Aliquots (100 µl) were taken for determination of cell number with a Coulter Counter. The rest of the cells were pelleted by centrifugation. The cells were extracted once with 5 ml chloroform/methanol (1/1) and twice with chloroform/methanol (2/1). The extracts were combined in silated tubes and solvents were removed with nitrogen. Chloroform, methanol, and 0.9% KCl were added to give a biphasic system of chloroform/methanol/0.9% KCl (4/2/3). After thorough mixing and centrifugation, the upper phase was removed and the lower phase was stored at -20°C.

Phospholipids were separated by thin-layer chromatography on Whatman K6 plates activated at 110°C for 1 h with a solvent system of chloroform/methanol/water/acetic acid (50/37.5/2.5/3.5 by volume) (9). This system allowed the complete separation of LPC, SM, PC, LPE, PS, PI, PE, and PA. Authentic phospholipid markers (Serdary Laboratories, London, Ontario, Canada) were added to aliquots of the lipid extracts to aid in visualization. After development, the phospholipids were visualized with iodine and scraped into scintillation vials. Water (1 ml) and 10 ml scintillant (Ecolite; ICN) were added to each vial and the radioactivity was determined by scintillation counting.

RESULTS

Effect of ET-18-OCH₃ on Proliferation of MCF7 and T84 Cells. The main aim of this study was to investigate whether ET-18-OCH₃ affects the proliferation of MCF7 and T84 cells by inhibiting cellular acylation processes. It was therefore necessary to identify the earliest times subsequent to incubation with the ALP at which a decrease in cell proliferation was apparent. Preliminary experiments indicated that MCF7 and T84 cells were quite sensitive to the drug; therefore, the dose-response growth curves for MCF7 and T84 cells were conducted with ET-18-OCH₃ concentrations of 5 µg/ml or less. These experiments led to the selection of drug concentrations of 3.5 µg/ml for T84 cells and 2.75 µg/ml for MCF7 cells. The time-dependent growth inhibition of MCF7 and T84 at these concentrations are shown in Fig 1. A significant decrease (P < 0.01) in growth of both cells was apparent 8 h after incubation with the drug; however, the cells were still proliferating at the end of the 12-h incubation period, albeit at much slower rates than control cells. Also shown in Fig. 1 is the effect of 5 µg/ml ET-18-OCH₃ on the growth of MCF7 cells. At this concentration, proliferation was completely inhibited; however, the viability of the cells as measured by the exclusion of trypan blue was unaffected at the end of the incubation. The proliferation rate of such cells 48 h after incubation in ALP-free medium was 2.6-fold less than that of control cells. Thus incubation with ALP had severely damaged the ability of the cells to divide. The sensitivity of MCF7 cells to the growth-inhibitory effects of ET-18-OCH₃ observed in this study are very different from that reported by Kosano and Takatani (3) who observed no effect on the growth of MCF7 cells incubated with up to 25 µg/ml ET-18-OCH₃ for 12 h. We have no explanation for these conflicting results. The possibility that the MCF7 cells in each study represent different sublines cannot be discounted. We did not observe any morphological differences between cells incubated in ALP and controls.

Effect of ET-18-OCH₃ on Incorporation of Fatty Acids into MCF7 Phospholipids. In view of differences in the distribution of fatty acids in phospholipid classes, three fatty acids with different degrees of unsaturation were used to monitor acylation processes in all the major phospholipids. MCF7 cells growing in log phase were incubated with the fatty acids in the presence or absence of ET-18-OCH₃ for selected periods (0.5–12 h). The amount of radiolabeled fatty acid associated with each phospholipid class was expressed as dpm/10⁶ cells and used as an index of the extent of incorporation. Although the incorporation of fatty acids into the 8 phospholipid classes was monitored at each time point, we have not displayed the results obtained for LPC, SM, and LPE in the figures due to the low incorporation of radiolabeled fatty acids into these phospholipids and for the sake of clarity. Any significant differences in the label incorporated into LPC, SM, and LPE between control and experimental cells are described in the text.

The possibility that the MCF7 cells in each study represent different sublines cannot be discounted. We did not observe any morphological differences between cells incubated in ALP and controls.

Two different concentrations of ET-18-OCH₃, one of which decreased proliferation (2.75 µg/ml) while the other caused a cessation in proliferation (5 µg/ml), were examined for their effects on the acylation processes in MCF7 cells. Oleic acid was rapidly taken up by MCF7 cells and incorporated mainly into PC; the PE and PA fractions were also significantly labeled (Fig. 2). Five h after incubation with 2.75 µg/ml ET-18-OCH₃, a 20% decrease in the amount of oleic acid esterified into PC was observed, which was maintained for the duration of the incubation. ET-18-OCH₃ inhibited incorporation of oleate into PS by 34% 8 h after initiation of incubation. Other changes caused by the ALP were increased incorporations of oleate into PE and PA of 47% and 40%, respectively, relative to controls, beginning 3 h after incubation with the drug. At the higher concentration of ET-18-OCH₃ (5 µg/ml), the decreased incorporation of oleate into PC was between 40 and 50% and about 30% for PS (Fig. 2B). These reductions were observed 3 h after incubation with ET-18-OCH₃ compared to 5 h for the lower drug concentration. The increased esterification of oleate into PE and PA was also observed at the higher concentration of ET-18-OCH₃. The magnitude of increase over the controls ranged from 23 to 45% and was observed after 5 h. The increased incorporation of [3H]oleic acid into PA occurred 1.5 h after addition of ET-18-OCH₃ and was 50–90% higher than the quantities incorporated into control cells. Although incorporation of oleic acid into LPE was low [less than incorporation into PI (Fig. 2B)], a significant decrease [P < 0.005] of 40–63% in incorporation of oleic acid into LPE was observed between 3 and 12 h after incubation with ET-18-OCH₃.

MCF7 cells rapidly incorporated [14C]linoleate into their phospholipids with the majority going into PC followed by PE and PA (Fig. 3). Three h after incubation with 2.75 µg/ml ET-18-OCH₃, the esterification of linoleate into PC was inhibited about 20% and into PS by 25% (Fig. 3A). Incorporation of linoleic acid into PE was increased 20–26% by ET-18-OCH₃ after 5 h. Experiments with 5 µg/ml ET-18-OCH₃ revealed changes of a similar magnitude to those described above for PC, PS, and PE (Fig. 3F); however, these occurred at earlier times, namely 1.5 h for PC and PS and 3 h for PE, than were
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Fig. 2. Effect of ET-18-OCH³ on incorporation of oleic acid in MCF7 cellular phospholipids. MCF7 cells were incubated with oleic acid in the presence (○, □, △, ○, △) or absence (○, □, ○, △, ○) of 2.75 μg/ml ET-18-OCH³ (A) or 5 μg/ml ET-18-OCH³ (B). Lipids were extracted at different times and separated into PE (○), PI (△), PS (○), and PA (△). Values are the mean of three separate determinations. Asterisks, statistically significant differences of at least P < 0.05 between cells incubated with ET-18-OCH³ and controls.

Fig. 3. Effect of ET-18-OCH³ on incorporation of linoleic acid in MCF7 cellular phospholipids. MCF7 cells were incubated with linoleic acid in the presence (○, □, △, ○, △) or absence (○, □, ○, △, ○) of 2.75 μg/ml ET-18-OCH³ (A) or 5 μg/ml ET-18-OCH³ (B). The phospholipid classes depicted are PC (○), PE (△), PI (△), PS (○), and PA (△). Values are the mean of three separate determinations. Asterisks, statistically significant differences between cells incubated with ET-18-OCH³ and controls of at least P < 0.05.

observed with 2.75 μg/ml ET-18-OCH³. A decrease in incorporation into PA after 12 h was also observed.

The results of experiments conducted with arachidonic acid are displayed in Fig. 4. The distribution of arachidonate into MCF7 phospholipids was quite distinct from those of oleate and linoleate. The incorporation of arachidonate into PE and PI was similar at earlier periods but after 5 h, there was greater esterification of arachidonate into PE than into PC. Relative to experiments with oleate and linoleate, the PA fraction was not as highly labeled, but esterification into the PI fraction was much greater. Incubation of MCF7 cells with 2.75 μg/ml ET-18-OCH³ resulted in a 25-30% reduction in arachidonate incorporation into PE and PI after 5 h (Fig. 4A). The only other significant change caused by ET-18-OCH³ was a 30% increase in label in PI after 12 h incubation. Incubation of MCF7 cells with 5 μg/ml ET-18-OCH³ resulted in a decrease in the esterification of arachidonate into PC (30-35%), PS (25%), and PA (30%) after incubation with the ALP (Fig. 4B). A 20% reduction in [3H]arachidonate associated with PE was observed 3 and 5 h after incubation with the drug, but by 8 h no differences were discernible between control and experimental cells. The effect of 5 μg/ml ET-18-OCH³ on arachidonate incorporation into PI was similar to that of PE. The ALP decreased incorporation of [3H]arachidonate into PI between 1.5 and 5 h; however, incorporation was similar between the experimental and control groups after 8 h.

Fig. 4. Effect of ET-18-OCH³ on incorporation of arachidonic acid in MCF7 cellular phospholipids. MCF7 cells were incubated with arachidonic acid in the presence (○, □, △, ○, △) or absence (○, □, ○, △, ○) of 2.75 μg/ml ET-18-OCH³ (A) or 5 μg/ml ET-18-OCH³ (B). The phospholipid classes depicted are PC (○), PE (△), PI (△), PS (○), and PA (△). A 20% reduction in [3H]arachidonate associated with PE was observed 3 and 5 h after incubation with the drug, but by 8 h no differences were discernible between control and experimental cells. The effect of 5 μg/ml ET-18-OCH³ on arachidonate incorporation into PI was similar to that of PE. The ALP decreased incorporation of [3H]arachidonate into PI between 1.5 and 5 h; however, incorporation was similar between the experimental and control groups after 8 h.

Effect of ET-18-OCH³ on Incorporation of Fatty Acids into T84 Phospholipids. The effect of ET-18-OCH³ on acylation processes in T84 cells was conducted with a drug concentration of 3.5 μg/ml, which decreased proliferation after 8 h but did...
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Fig. 4. Effect of ET-18-OCH₃ on incorporation of arachidonic acid in MCF7 cellular phospholipids. MCF7 cells were incubated with arachidonic acid in the presence (●, ○, ▲, ■) or absence (□, △, ◆, ●) of 2.75 μg/ml ET-18-OCH₃ (A) or 5 μg/ml ET-18-OCH₃ (B). The phospholipid classes depicted are PC (●), PE (○), PI (▲), PS (◆), and PA (■). The values are the mean of three separate determinations. Asterisks, statistically significant differences between cells incubated with ET-18-OCH₃ and controls of at least P < 0.05.

Fig. 5. Effect of ET-18-OCH₃ on incorporation of oleic, linoleic and arachidonic acids in T84 cellular phospholipids. T84 cells were incubated with [3H]oleic acid (△), [3H]linoleic acid (○) or [3H]arachidonic acid (■) in the presence (●, ○, ▲, ■) or absence (□, △, ◆, ●) of 3.5 μg/ml ET-18-OCH₃. Incorporation of radioactivity into PC (●), PE (○), PS (◆), PI (▲), and PA (■) were determined at different times. The values are the mean of three separate determinations. Asterisks, statistically significant differences between cells incubated with ET-18-OCH₃ and controls of at least P < 0.05.

not cause its cessation or affect the viability of the cells. The acylation processes in T84 were investigated with oleic (Fig. 5A), linoleic (Fig. 5B), and arachidonic acids (Fig. 5C). As can be seen in Fig. 5A, most of the oleic acid was esterified to PC at all the times examined. ET-18-OCH₃ reduced esterification of oleate into PC by 15% between 1.5 and 8 h. In contrast, incorporation into PE increased by 30% after 3 h and by 18% into PA after 8 h.

In cells incubated with linoleic acid, although the majority of label was also found in the PC fraction at all times (Fig. 5B), ET-18-OCH₃ did not affect the esterification of the fatty acid into the PC fraction. Significant changes caused by the ALP were a 47% increase in [14C]linoleic acid associated with PE after 3 h, which decreased to 20% after 12 h, and a 22% increase in linoleate incorporated into PA after 12 h incubation with ET-18-OCH₃.

Experiments with arachidonic acid (Fig. 5C) revealed a greater incorporation of the fatty acid into phospholipids other than PC in contrast to that observed for oleic and linoleic acids (Fig. 5, A and B). Incubation with ET-18-OCH₃ did not significantly affect the incorporation of arachidonic acid into PC until after 12 h, when an increase of 20% was observed over controls. In fact, the drug did not decrease the incorporation of [3H] arachidonate into any major lipid but rather increased incorporation into PE by 20% after 5 h, into PS by 25%, into PA by 56%, and into PI by 31% after 12 h. Although labeling into LPE was slight (similar to that of PA), a 30% decrease in incorporation of arachidonate into LPE was observed 1.5 h after incubation with ET-18-OCH₃.

Effect of ET-18-OCH₃ on the Distribution of Newly Incorporated Fatty Acids. In view of the perturbations in acylation processes caused by ET-18-OCH₃, the distribution of newly incorporated fatty acids in the phospholipids in cells incubated with and without ET-18-OCH₃ were analyzed at the end of the
incubation period. In MCF7 cells incubated with 2.75 µg/ml ET-18-OCH₃ and oleic acid (Table 1A), a 21% decrease in the proportion of label in PC and a 22% decrease in LA were observed, in addition to a 48% increase in PE and a 34% increase in PA relative to controls. Incubation of MCF7 cells with 5 µg/ml of ALP, the proportion of newly esterified oleate in PC and PS and a 19% decrease (P < 0.05) was observed in experiments with arachidonic acid incubated with 5 µg/ml ET-18-OCH₃. Significant decreases were found in the proportion of label associated with LPC (52%), SM (33%), PC (26%), and LPE (36%) while significant increases of 22 and 14% were observed with PI and PE, respectively. Analysis of the total radioactivity incorporated into the phospholipids showed no significant difference between the controls and cells incubated with 2.75 µg/ml ET-18-OCH₃. In cells incubated with 5 µg/ml of the ALP, the proportion of newly esterified arachidonate associated with the phospholipids was 15% less (P < 0.05) relative to controls. Similar analysis with T84 cells (Table 2) showed a 13% increase in the proportion of newly esterified oleic acid associated with the PE fraction in cells incubated with ET-18-OCH₃ relative to controls. In experiments with arachidonic acid decreases of 37 and 42% were observed in LPE and PC with increases of 7 and 28% in PI and PA, respectively, as a result of incubation with ET-18-OCH₃. Significant increases of 16% for oleate (P < 0.05) and 22% for arachidonate (P < 0.005) were observed in the total quantity of the labeled fatty acids incorporated into the total phospholipid fraction of T84 cells incubated with ET-18-OCH₃. ET-18-OCH₃ did not affect the distribution of newly esterified linoleic acid or the quantity of linoleic acid incorporated into the total phospholipid fraction in T84 cells. 

DISCUSSION

The inhibition of LPC acyl-CoA acyltransferase by ALP has been postulated to be crucial in the inhibition of cancer cell growth.
growth. It has been suggested that this causes a decrease in the unsaturated fatty acid content in the cells which results in loss of viability (4-6). If this is indeed the mechanism by which ET-18-OCH₃ inhibits growth of MCF7 and T84 cells, one would expect decreased incorporation of fatty acids into the PC fraction isolated from cells incubated with inhibitory concentrations of ET-18-OCH₃. Furthermore, such inhibition should occur prior to the decrease in cell proliferation. Our studies with MCF7 cells utilized two concentrations of ET-18-OCH₃, one of which (2.75 μg/ml) inhibited proliferation after 8 h, while the other, 5 ng/ml, caused a complete cessation of proliferation. At both drug concentrations, one of the major effects was a reduced incorporation of oleic, linoleic, and arachidonate into PC. At the lower drug concentration of 2.75 μg/ml where a decrease in proliferation could be monitored, the reduced incorporation of the fatty acids into PC occurred prior to the decrease in proliferation. Considered in isolation the above observations would implicate the inhibition of LPC acyl-CoA acyltransferase in the antiproliferative effect of ET-18-OCH₃ in MCF7 cells; however, ET-18-OCH₃ interfered in the acylation processes of phospholipids other than PC. An increased incorporation of oleate into PE and PA was observed earlier than the decrease into PC. An increased incorporation of linoleate into PE was also observed. This increased incorporation could conceivably offset any reduction in the fatty acid into PC and thereby prevent drastic changes in the unsaturated/saturated ratio in the membranes. This supposition is supported by the observation that after 12 h incubation with ET-18-OCH₃ (2.75 μg/ml), in spite of changes in the distribution of the newly esterified fatty acids, there were no differences in the quantity of labeled fatty acids associated with the phospholipids of cells incubated with or without ALP. Thus incubation of MCF7 cells with 2.75 μg/ml ET-18-OCH₃ did not result in a net loss of unsaturated fatty acid incorporated but rather caused a redistribution of the fatty acids among the phospholipid classes. However, incubation of MCF7 cells with 5 μg/ml ET-18-OCH₃ resulted in reductions of 15 and 20% in the quantities of oleate and arachidonate incorporated into the phospholipid fraction, signifying a net decrease in incorporation of these fatty acids into the phospholipids relative to controls. Since 5 μg/ml ET-18-OCH₃ completely inhibited proliferation, it is likely that the reduced incorporation of fatty acid in cellular phospholipids was a consequence of the lack of growth rather than its cause. The most convincing evidence that inhibition of acylation of LPC acyl-CoA acyltransferase may not be a fundamental process in the antiproliferative action of ET-18-OCH₃ comes from our studies with T84 cells. Despite the rapid incorporation of linoleic and arachidonic acids into the PC fraction of these cells, incubation with concentrations of ET-18-OCH₃ which inhibited proliferation after 8 h did not affect the incorporation of either fatty acid into PC. In fact, the only significant changes that occurred with each fatty acid prior to cessation of growth was an increase in the incorporation into PE. A decreased incorporation of oleic acid into PC caused by ET-18-OCH₃ was observed between 1.5 and 8 h, but at the end of the incubation no differences were apparent between the control and experimental groups. This suggests the existence of compensatory mechanisms that overcome the initial decrease. In any case, if ET-18-OCH₃ inhibits the incorporation of oleate into PC but increases the incorporation of even more unsaturated fatty acids like linoleate and arachidonate into other phospholipids, the net effect is unlikely to be a reduction in the proportion of unsaturated fatty acids in the cellular membranes. Indeed the ET-18-OCH₃-induced decrease in incorporation of oleic and linoleic acids into PC reported in Meth A cells was accompanied by increased incorporation of the fatty acids into PE (4). Our results with T84 cells indicate that the net effect of incubation with ET-18-OCH₃ for 12 h was an increase in the quantities of arachidonate and oleic acids incorporated into the phospholipid fraction. Thus even though ET-18-OCH₃ inhibits proliferation in T84 cells, its net effect appears to be an activation of cellular acylation processes rather than their inhibition. The results of our studies with MCF7 and T84 cells lead us to conclude that perturbations in the cellular acylation processes are unlikely to be key events in the mechanism of inhibition of growth of these cells by ET-18-OCH₃; therefore, the hypothesis linking the antiproliferative effect of ALP with its interference in cellular acylation may not be applicable to all cells.

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