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ORIGINAL ARTICLE

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Quaternary ammonium analogs of ether lipids inhibit the activation of protein kinase C and the growth of human leukemia cell lines

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Abstract *Purpose*: ET-18-OCH_3 (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) is a representative of the first generation of antitumor ether lipids and is a model in the development of new compounds including a series of quaternary ammonium analogs (QAA). In the present study, we evaluated the QAA as inhibitors of cell growth and studied their mechanism of action. *Methods*: We compared the effects of the QAA on the proliferation of human leukemia cell lines which are sensitive (HL-60) or resistant to ET-18-OCH_3 (HL-60R and K562). In addition we used cell fractionation and enzymatic assays to determine the effects of QAA on protein kinase C (PKC) translocation in response to 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). *Results*: The QAA and ET-18-OCH_3 were approximately equally effective inhibitors of HL-60 cell growth. However, the QAA were more effective inhibitors of K562 and HL-60R cell proliferation. The HL-60R cells, which were selected for resistance to ET-18-OCH_3, were also resistant to BM 41.440 which is structurally similar. In serum-free medium, the phosphorus-containing compounds (ET-18-OCH_3, BM 41.440 and He-PC) were much more effective inhibitors (8–20-fold) of the K562 cell line while the activities of the QAA were only moderately increased (1.2–2.3-fold). When serum albumin was added to the serum-free medium, the K562 cells became resistant to ET-18-OCH_3, suggesting that albumin is responsible for the differential sensitivity. The QAA compounds, which inhibit PKC activity in vitro, inhibited cell proliferation. However, a QAA which did not inhibit PKC did not inhibit cell proliferation. The phorbol ester TPA stimulates PKC translocation and causes HL-60 cell differentiation to adherent macrophage-type cells. The QAA inhibited TPA-induced cell differentiation and PKC translocation indicating that they also inhibit PKC in intact cells. *Conclusions*: The cellular effects of the QAA appear to be due to inhibition of PKC. In addition, these data indicate that albumin, which is important as a mediator of the uptake of ET-18-OCH_3, has only a small effect on the uptake of QAA. Together these data indicate that the QAA are potential anticancer agents, showing a significant ability to inhibit growth of leukemia cell lines that are resistant to ET-18-OCH_3.

Key words Ether lipids · Protein kinase C · Leukemia cells

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Introduction

ET-18-OCH_3 (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) is a representative of the first generation of antitumor ether lipids and selectively inhibits the growth of neoplastic cells both in vitro and in vivo [11, 21, 30]. At low concentrations, ET-18-OCH_3 inhibits cell proliferation [10]. However, at higher concentrations, ET-18-OCH_3 can cause direct cytotoxicity to nonproliferative cells [10]. Since ET-18-OCH_3 is selective toward leukemia cells, sparing normal bone marrow cells, the ether-lipids are particularly promising for use in bone marrow purging [3, 16, 22, 32, 40, 41]. The observations that ET-18-OCH_3 is selectively cytotoxic to neoplastic cells has led to the development of several new analogs. BM 41.440, SRI 62-834 and He-PC represent a second generation of ether-linked lipids which

have shown selectivity toward tumor cells and have been evaluated in clinical trials [19, 25, 39].

In contrast to many other anticancer drugs, the ether lipids do not induce DNA damage and are not mutagenic [2]. ET-18-OCH_3 has been shown to inhibit several enzymes including protein kinase C, phospholipase C, CTP:phosphocholine cytidylyltransferase, phosphatidylinositol-3-kinase and coenzyme A-independent transacylase [4, 7, 8, 12–14, 17, 18, 28, 29, 35, 43]. However, the relative importance of these activities has been difficult to determine owing to the number of enzymes inhibited and the relative lack of structural specificity of the ether lipids. Thus, the precise target for the ether lipids remains undefined and it is probable that multiple targets are involved in the total spectrum of biological activities.

Some tumor cell lines show a high sensitivity to the growth inhibitory effects of ether lipids, while others are relatively resistant [36]. Recently, a difference in cellular drug uptake has been proposed by several authors as the explanation for the different sensitivity to ether lipids [1, 20, 26, 33]. It has been shown that if the treatment occurs in serum-free medium the cytotoxic activity of ET-18-OCH_3 is increased and the difference between sensitive and resistant cells is dramatically decreased [33]. These findings suggest that a serum component is responsible for the differential sensitivity.

We have synthesized a series of quaternary ammonium analogs (QAA) of ET-18-OCH_3, in the effort to develop analogs with an enhanced PKC inhibitory effect and antineoplastic activity [24]. These compounds are analogs of alkylglycerols which incorporate an ''inverse choline'' moiety into their structure. All of these compounds, except the analog CP-37, have a PKC inhibitory activity in a cell-free assay comparable to that of ET-18-OCH_3 [24]. Like the other antitumor ether phospholipids, they inhibit the activation of PKC by acting as competitive inhibitors of activation by phosphatidylserine [24].

In the present study, we found that the new QAA analogs were more active than the previously described ether lipids in inhibiting the growth of resistant cells. In addition, they inhibited TPA-induced cellular effects including HL-60 cell differentiation and PKC translocation, suggesting that they act on the PKC pathway. Our results also indicate that serum albumin is responsible for the differential sensitivity of the HL-60 and K562 cell lines, suggesting that the resistance of K562 cells is regulated primarily by cellular incorporation.

Materials and methods

Chemicals

ET-18-OCH_3 was from Medmark Pharma (Munich, Germany). BM 41.440, SRI 62-834 and He-PC were gifts to Dr. Ed Modest from Boehringer Mannheim (Mannheim, Germany), Sandoz Research Institute (East Hanover, N. J.) and Dr. H. Eibl (Göttingen, Germany). The MTT kit, Cell Titer 96 Cytotoxicity Assay was from Promega (Madison,Wis.). Bovine serum albumin and histone type IIIS were from Sigma (St. Louis, Mo.). Phosphatidylserine and 1,2-diacylglycerol were from Serdary Research Laboratories (London, Ontario, Canada). DEAE-Sephacel was from Pharmacia (Uppsala, Sweden). Coomassie blue for protein measurement was from Pierce (Rockford,Ill.).

Cell culture

HL-60 promyelocytic leukemia cells and K562 chronic myelogenous leukemia cells were obtained from the American Type Culture Collection (Rockville, Md.). The HL-60R subline was previously selected in our laboratory by incubation with increasing concentrations of ET-18-OCH_3 [33]. All cell lines were cultured in RPMI-1640 medium supplemented with 2 m*M* L-glutamine, 50 units/ml penicillin G, 50 μg/ml streptomycin sulfate, and 10% heat-inactivated fetal bovine serum. Chemicals for cell culture were obtained from Gibco (Grand Island, N.Y.).

Inhibition of cell growth

Cells in exponential growth phase were seeded into 96-well plates (5×10^4 cells in 100 μl/well) and incubated with different concentrations of the test compound for 24 h at 37 °C. Then the MTT dye solution was added and the cells were incubated again at 37 °C. After 4 h, the solubilization solution was added and incubated overnight at room temperature. Absorbance at 590 nm was read, using a reference wavelength of 610 nm, with a scanning microtiter plate spectrophotometer.

In selected experiments, the cells were incubated with the compounds for 1 h in centrifuge tubes at 37 °C, then centrifuged and washed with sterile phosphate-buffered saline, resuspended in fresh medium and seeded into a 96-well plate (5×10^5 cells in 100 μl/well). The dye solution was added after 20 h of incubation at 37 °C. For the experiments in serum-free medium, cells in exponential growth were washed once with phosphate-buffered saline and resuspended in serum-free medium or medium containing different concentrations of serum albumin immediately before treatment.

Preparation of cytosolic and membrane extracts

K562 cells (5×10^5/ml) were seeded in T75 flasks and treated with the ether lipids overnight. The cells were then counted with trypan blue, centrifuged and resuspended in fresh medium (2–3×10^6/ml). Half of the samples were incubated with 100 n*M* TPA for 5 min in a 37 °C waterbath. The cells were then centrifuged, washed once with ice-cold phosphate-buffered saline and resuspended in 2 ml ice-cold 20 m*M* Tris-HCl, pH 7.5, 2 m*M* EDTA, 50 m*M* β-mercaptoethanol, 1 m*M* phenylmethylsulfonyl fluoride and 50 μg/ml leupeptin. The protease inhibitors phenylmethylsulfonyl fluoride and leupeptin were added immediately before use from 100 × stocks, respectively, in ethanol and distilled water. In all the following steps the samples were on ice. The cells were sonicated using a probe sonicator 3 × 10 s and centrifuged (8000 rpm for 2 min) to remove nuclei and unbroken cells. The supernatant was subsequently centrifuged at 100 000 *g* for 1 h at 4 °C. The supernatant (cytosolic fraction) was stored at 4 °C. The pellet was resuspended in 2 ml of the same buffer plus 0.5% Triton × and sonicated. After 30 min in ice, the samples were centrifuged again at 100 000 *g* for 1 h. The supernatant represented the solubilized membrane fraction.

PKC from the cytosolic or membrane fraction was partially purified by anion exchange chromatography. The sample was applied to a 0.8 × 4 cm DEAE-Sephacel column that had been previously equilibrated with 20 m*M* Tris HCl, pH 7.5, 2 m*M* EDTA, 50 m*M* β-mercaptoethanol, 1% sucrose, 2 m*M* phenylmethylsulfonyl fluoride. The samples were eluted with the same buffer containing 120 m*M* NaCl. The protein concentration was determined by the method of Bradford [9], with bovine serum albumin as a standard.

Protein kinase C assay

Kinase activity was determined by the transfer of phosphorus-32 from ^{32}P-ATP (1 μCi/tube) to histone. The reaction was started by adding 50 μl sample to 195 μl of the reaction mixture (25 m*M* Tris HCl, pH 7.5, 10 m*M* $MgCl_2$, 40 μg histone, 10 μ*M* ATP, 0.6 m*M* $CaCl_2$, 20 μg/ml phosphatidylserine, 2 μg/ml 1,2-diacylglycerol and 1 μCi ^{32}P-ATP). The histone was used from a 1 mg/ml stock in water and made fresh every 7–10 days. The ATP was prepared as a stock of 2.5 mg/ml and made fresh every time. The phosphatidylserine and 1,2-diacylglycerol were stored in chloroform and each was dried under nitrogen and resuspended in water by sonication before they were added separately to the reaction mixture. The reaction was carried out for 20 min at 30 °C and halted by the addition of 50 μl bovine serum albumin from a stock of 10 mg/ml and 1 ml of 25% ice-cold trichloroacetic acid. The tubes were kept on ice and then filtered in a Millipore vacuum box using 0.45-μm membrane filters (Gelman Sciences, Ann Arbor, Mich.). The filters were washed with 6 ml trichloroacetic acid and air dried. The radioactivity bound to the filters was determined in 3.5-ml Ecolume by scintillation counting. PKC activity is reported as picomoles of ^{32}P-ATP transferred to the histone per minute per milligram of protein. The blank value (no sample in the reaction mixture) was subtracted from all the sample values. Every sample was tested in the presence or absence of the PKC activators phosphatidylserine, 1,2-diacylglycerol and Ca^{2+}. The activity measured in the presence of the activators was two to ten times higher than the activity measured in the absence of the activators.

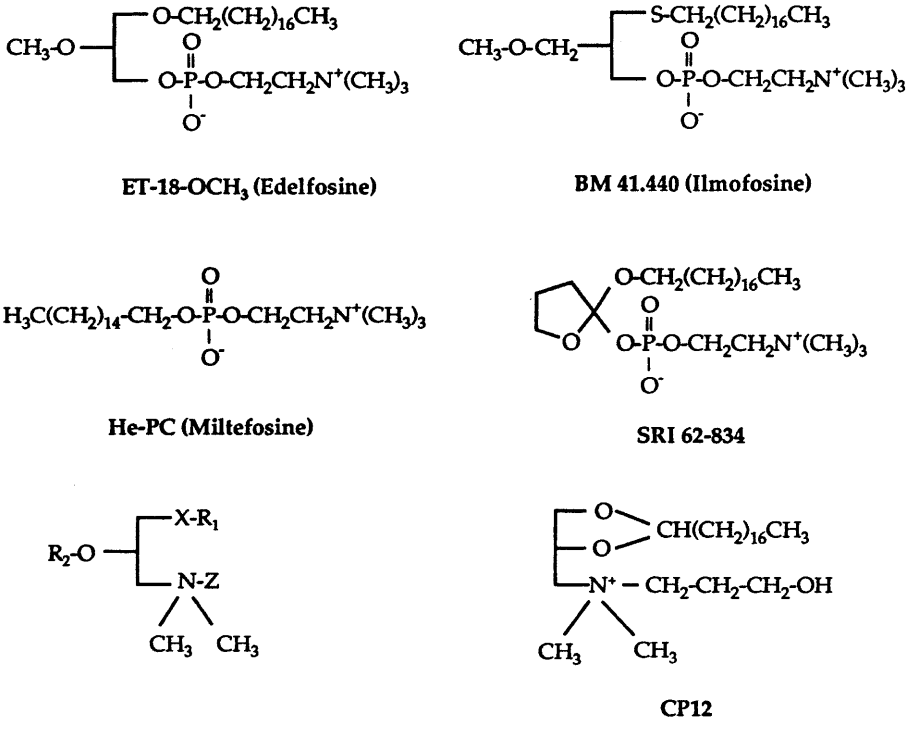
Differentiation

TPA-induced macrophage differentiation of HL-60 cells was determined by cell adherence. Cells in exponential growth phase were seeded in six-well plates (200 000 cells/well), treated with 20 n*M* TPA for 50 min and incubated in fresh medium at 37 °C. The ether lipids, when present, were added 10 min before TPA treatment and incubated for a total of 1 h. After 2 days, the cells in suspension were harvested, and the adherent cells were washed once with phosphate-buffered saline and then scraped with a rubber policeman. Cells were counted with trypan blue in a hemocytometer. The proportion of differentiated cells is expressed as a percentage of the total cells that were adherent.

Results

Growth inhibition

We tested the QAA for their capacity to inhibit the growth of two leukemia cell lines, HL-60 and K562. We also used an HL-60 subline resistant to ET-18-OCH_3 (HL-60R). These assays were conducted in comparison with ET-18-OCH_3, BM 41.440, SRI 62-834 and He-PC, which have been reported previously and served as a reference. The structures of the different compounds are shown in Fig. 1. The viability of the cells based on



CP 7 X=S, R1=$C_{16}H_{33}$, R2=CH_3, Z= CH_2CH_2OH
CP 66 X=S, R1=$C_{16}H_{33}$, R2=CH_3CH_2, Z= CH_2CH_2OH
CP 13 X=S, R1=$C_{16}H_{33}$, R2=CH_3CH_2, Z= $CH_2CH_2CH_2OH$
CP 19 X=S, R1=$C_{16}H_{33}$, R2=CH_3, Z= CH_3
CP 37 X=O, R1=Naphthyl, R2=CH_3, Z= CH_2CH_2OH
CP 10 X=S, R1=$C_{18}H_{37}$, R2=CH_3, Z= $CH_2CH_2CH_2OH$

Fig. 1 Structures of the phosphorus-containing ether lipids and the quaternary ammonium analogs

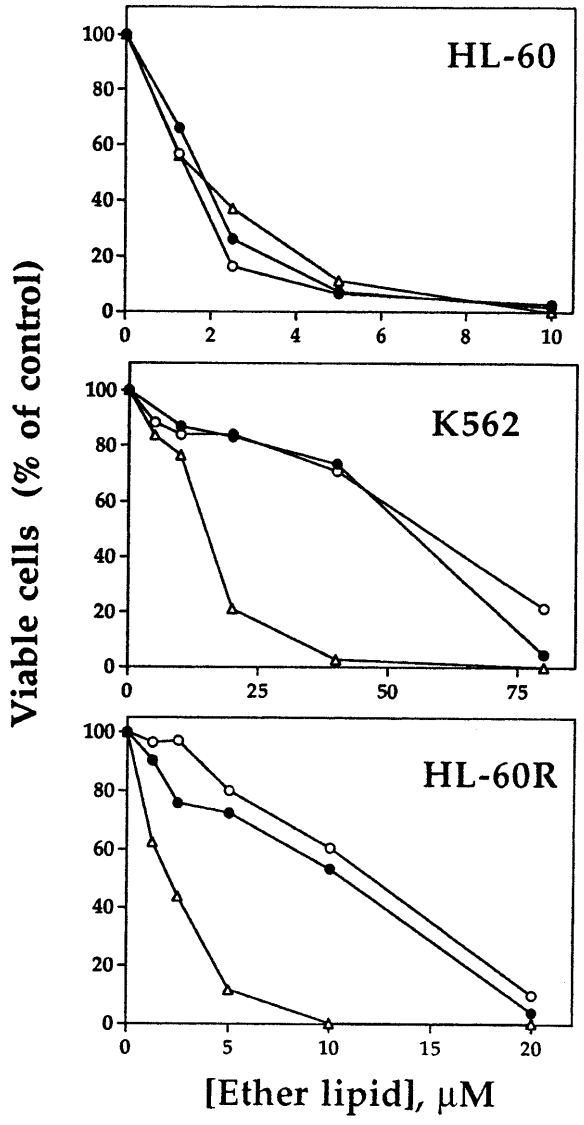


Fig. 2 Effects of ether lipids on the growth of HL-60, K562 and HL-60R cell lines in RPMI-1640 with 10% fetal bovine serum. Cells were incubated for 24 h with the ether lipid prior to determination of viability using the MTT assay. The results are expressed as percentages of an untreated control. The data presented are the mean ± SE of four replicates from a representative of 2–21 experiments (● ET-18-OCH_3, ○ BM 41.440, △ CP-19)

trypan blue exclusion before testing was >90%. MTT viability experiments were done with each of the three cell lines to ensure that under the conditions used (an initial concentration of 50 000 cells/well and incubation for 24 h) the absorbance was proportional to the number of cells. We found a linear relationship in the range of 250–200 000 cells/well (data not shown).

In the HL-60 cell line, which is sensitive to ether lipids, no difference in sensitivity to ET-18-OCH_3, BM 41-440 and CP-19 was observed (Fig. 2). Each of the compounds caused a significant reduction in the number of viable cells. However, the resistant cells showed a strikingly different profile of inhibition. Both the K562 and the HL-60R cells were resistant to the structurally similar phosphorus-containing ether lipids ET-18-OCH_3 and BM 41-440, but were sensitive to CP-19 (Fig. 2). Thus, resistance to the phosphorus-containing ether lipids did not confer cross-resistance to the QAA.

Table 1 Effects of ether lipids on the growth of HL-60, K562 and HL-60R cell lines. Cells were incubated with the test compounds for 24 h in RPMI-1640 with 10% fetal bovine serum. Cell viability was determined using the MTT assay. Values are the concentration of drug (μM) that reduced the number of cells to 50% of the untreated control (IC_{50}), and are the means ± SE of at least eight determinations from two separate experiments (*NT* not tested)

Analog	HL-60	K562	HL-60R
ET-18-OCH_3	2.0 ± 0.18	57.9 ± 3.2	10.5 ± 0.9
BM 41.440	1.5 ± 1.26	58 ± 2	13.5 ± 1.5
SRI 62-834	2.0 ± 0.22	56.6 ± 1.5	14.5 ± 1.5
He-PC	9.7 ± 1.45	>100	NT
CP-13	1.5 ± 0.27	13.7 ± 0.88	NT
CP-19	1.9 ± 0.2	14 ± 1	3.5 ± 1.5
CP-10	2.9 ± 0.52	16 ± 0.88	5.9 ± 0.35
CP-7	2.1 ± 0.32	14 ± 1	4 ± 1.25
CP-66	2.8 ± 0.62	16 ± 1.53	NT
CP-12	2.8 ± 0.05	25 ± 0.01	9 ± 2
CP-37	>20	>80	NT

Most of the ether lipids tested were growth inhibitory with IC_{50} values for HL-60 cells varying between 1.5 μM and 2.9 μM (Table 1). He-PC was less effective with an IC_{50} of 9.7 μM. The exception was CP-37, which does not inhibit PKC [24]. CP-37 was not growth inhibitory at the highest concentrations tested (IC_{50} >20 μM). When K562 cells were used, higher doses were required for growth inhibition (Table 1). All the QAA were effective against K562 cells, with IC_{50} values significantly lower than for ET-18-OCH_3 (Table 1). CP-37 was again an exception, with an IC_{50} >80 μM. We also used a resistant cell line (HL-60R) which has been selected in our laboratory by incubating the cells with increasing concentrations of ET-18-OCH_3 [33]. The HL-60R cell line was fivefold more resistant to ET-18-OCH_3 than the original HL-60 cell line (IC_{50} 10.5 μM compared with 2 μM), but only about twofold more resistant to the QAA (IC_{50} varying between 3.5 μM and 9 μM). A summary of the growth inhibition by all the compounds is given in Table 1.

Both HL-60 and K562 cell lines showed greater sensitivity to ET-18-OCH_3 in serum-free medium (Fig. 3). However, the sensitivity of the K562 cell line was much more influenced by the removal of serum than HL-60 cells. In K562 cells, the IC_{50} of ET-18-OCH_3 decreased from 58 μM to 7 μM in the absence of serum. In HL-60 cells, the IC_{50} decreased by less than half. Since K562 was the cell line most influenced by treatment in the absence of serum, it was used to determine which component of the serum was responsible for the difference in sensitivity. When bovine serum albumin was added to serum-free medium there was a decrease in the sensitivity to ET-18-OCH_3 (Fig. 4). The degree of resistance increased with increasing albumin concentration up to

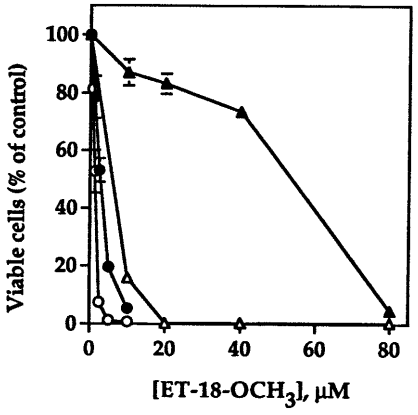


Fig. 3 Effect of fetal bovine serum on growth inhibition of K562 and HL-60 cells by ET-18-OCH_3. Cells were treated with ET-18-OCH_3 for 24 h in the presence or absence of 10% fetal bovine serum. The values shown are the mean of four replicates from a representative one of two experiments (*triangles* K562 cells, *circles* HL-60 cells; *open symbols* serum-free medium, *filled symbols* serum-containing medium)

2.5 mg/ml, which is the average albumin concentration in 10% fetal bovine serum (Fig. 4). This suggests that ET-18-OCH_3 was bound to albumin and that this binding was important in determining the differential sensitivity to the ether lipids. The effect of serum on the sensitivity of K562 cells to ether lipids is shown in Table 2. The QAA were somewhat more effective (1.2- to 2.3-fold) in the absence of serum. However, the activities of He-PC, SRI 62-634, ET-18-OCH_3 and BM 41.440 were dramatically increased (8- to > 19-fold) in the absence of serum. These results indicate that the QAA were not bound to serum albumin. It is interesting to note that He-PC, which had a very low activity

Table 2 Inhibition of K562 cell proliferation by ether lipids in the presence or absence of fetal bovine serum. The values are the concentration of drug (μM) that reduced the number of cells to 50% of the untreated control (IC_{50}). The ratio between the IC_{50} of the serum-containing and the serum-free medium is given in the right column. The cells were cultured as described in the legend to Fig. 3. Values are means ± SE of four replicates of a representative of two to four experiments

Analog	Serum	Serum-free	Ratio
ET-18-OCH_3	58 ± 3.2	7 ± 1.5	8
BM 41.440	58 ± 2	6.3 ± 0.3	9
SRI 62-834	56.5 ± 1.5	4.3 ± 0.3	13
He-PC	> 100	5.5 ± 2.5	> 19
CP-19	14 ± 1	8.5 ± 1.5	1.6
CP-10	16 ± 1.5	11.5 ± 4.5	1.4
CP-7	18 ± 2.5	10 ± 2	1.8
CP-66	14.5 ± 1.5	12 ± 4	1.2
CP-12	25 ± 0.1	10.5 ± 1.5	2.3
CP-37	> 80	> 80	

against K562 in complete medium, became much more effective in serum-free medium (IC_{50} of 5.5 μM versus > 100 μM).

When HL-60 cells were treated with high concentrations of ether lipids for 1 h, the QAA were much more active than ET-18-OCH_3 (Fig. 5) or BM 41.440 (data not shown). Therefore, not only were the QAA more effective in resistant leukemia cell lines, but using the appropriate protocol they could be more active in sensitive leukemia cell lines (HL-60). Also in this protocol (1 h treatment plus 20 h in fresh medium) all the compounds became more toxic if the treatment occurred in serum-free medium (Table 3). ET-18-OCH_3 was the compound most affected (38-fold difference versus 7–8-fold difference for the QAA) (Table 3). These results indicate that the QAA may be useful in bone marrow purging because of their effectiveness in short-term exposures.

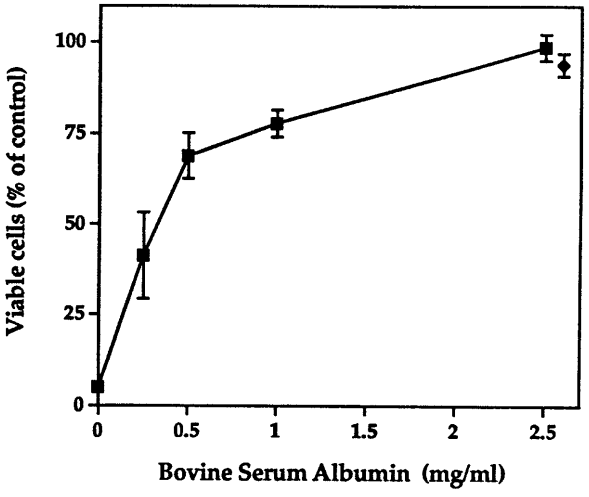


Fig. 4 Effect of bovine serum albumin on the growth inhibition of K562 cells by ET-18-OCH_3. Cells were incubated for 24 h with 10 μM ET-18-OCH_3 in serum-free medium containing different concentrations of bovine serum albumin (the *diamond* represents treatment in complete medium). The values are the means ± SE of four replicates from one of two separate experiments

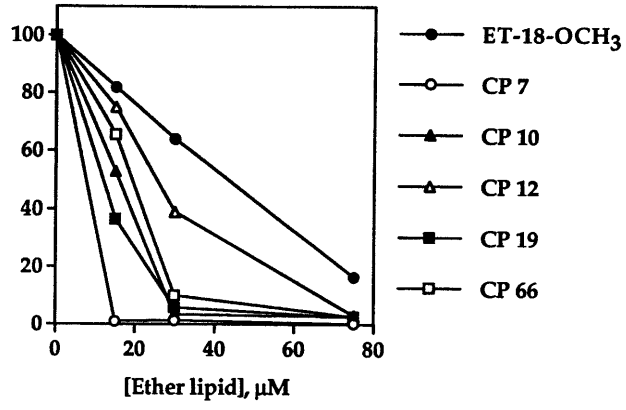


Fig. 5 Effect of short-term treatment with ether lipids on the survival of HL-60 cells. Cells were treated for 1 h in serum-containing medium, then washed in phosphate-buffered saline, resuspended in fresh medium and grown for 20 h in a 96-well plate. The values are means and SE of four replicates from a representative one of three experiments

Table 3 Comparison of the growth inhibition of HL-60 cells by ether lipids in the presence or absence of serum. HL-60 cells were treated for 1 h with the test compound, as described in the legend to Fig. 5, in the presence or absence of fetal bovine serum. The cells treated in serum-free medium were resuspended in complete medium for the last 20 h. The IC_{50} values presented are the means ± SE of two to four separate experiments with four replicates each. The ratio between the IC_{50} with and without serum is shown the right column

Analog	Serum	Serum-free	Ratio
ET-18-OCH_3	5 ± 7.6	1.2 ± 0.5	38
CP-7	9.7 ± 0.3	1.4 ± 0.4	7
CP-10	13.5 ± 1.5	1.6 ± 0.1	8

PKC activity

Since the QAA analogs inhibit PKC in vitro, we determined the effects of CP-19 on the translocation of PKC in intact cells. In K562 cells, PKC was predominantly cytosolic (89%) and was translocated to the membrane by stimulation with TPA (Fig. 6). Pretreatment with CP-19 inhibited TPA-induced translocation (Fig. 6). In these experiments, CP-19 inhibited the growth of K562 cells by 20% (4 μ*M*) or 40% (8 μ*M*). The viability of the cells was 93% and 77%, respectively.

Differentiation

To determine whether the QAA could inhibit a PKC-dependent cellular response, we studied the effect of CP-10 and CP-19 on TPA-stimulated HL-60 cell differentiation. HL-60 cell differentiation to macrophage-like cells is characterized by adherence to the culture dish. Pretreatment of HL-60 cells with CP-10, CP-19 and ET-18-OCH_3 inhibited TPA-stimulated HL-60 cell differentiation (Fig. 7). Together these results indicate that the QAA can inhibit PKC translocation and inhibit the response of cells to TPA which requires PKC.

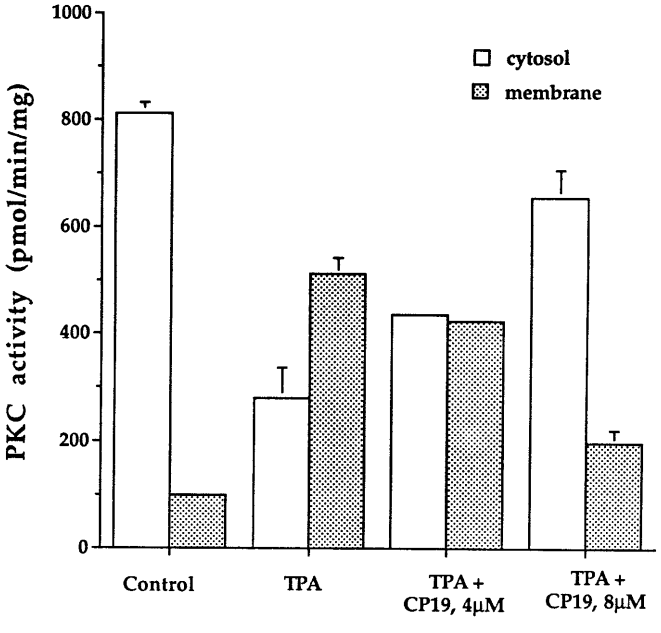


Fig. 6 Inhibition of TPA-induced PKC translocation. K562 cells were incubated overnight with CP-19 (4 or 8 μ*M*) and then stimulated for 5 min with 100 n*M* TPA. PKC activity was measured in the cytosolic and solubilized membrane fractions as described in Materials and methods. The values presented are the mean and range of two replicates from one of two similar experiments

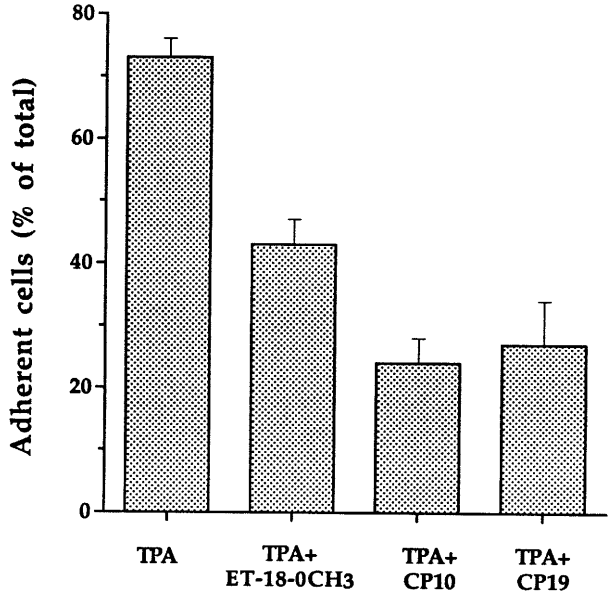


Fig. 7 Inhibition of TPA-induced HL-60 cell differentiation. HL-60 cells were incubated with the ether lipid (75 μ*M* ET-18-OCH_3 and 30 μ*M* CP-10 or CP-19) for 10 min before the 50-min incubation with 20 n*M* TPA. Adherence was measured after 48 h as described in Materials and methods. In untreated cells, < 1% cells were adherent. The values presented are the means ± SE of 2–12 experiments

Discussion

In the present work we demonstrated that a novel group of QAA of ET-18-OCH_3 inhibited PKC activation in intact cells and inhibited the growth of human leukemia cell lines. These compounds were especially effective against HL-60R and K562 cell lines, which were resistant to the previously described ether lipids. Therefore, these compounds may be useful in the treatment of leukemias that are resistant to ET-18-OCH_3.

In addition, we found that the growth inhibitory activity of the QAA was less influenced by serum than was the activity of ET-18-OCH_3 and BM 41.440. Several authors have suggested that differences in uptake may be the reason for different sensitivity to ether lipids [1, 20, 33, 34]. Our results suggest that the ether lipids bound to albumin cannot be easily incorporated into resistant cells. We found that in serum-free conditions all the ether lipids were much more effective as growth inhibitory compounds and their activity decreased with

increasing concentration of albumin in the medium. HePC, which is almost exclusively bound to albumin [23], showed a very low toxicity in serum-containing medium, and it showed the most dramatic decrease in IC_{50} when the treatment occurred in serum-free medium (IC_{50} 5.5 μM versus >100 μM). The fact that the activity of the QAA was not influenced as much by the presence or absence of albumin in the medium suggests that these compounds are not bound to serum albumin to such a high degree as the other ether lipids. We found that they were much more effective than ET-18-OCH_3 after 1-h treatment in HL-60 cells, while 24-h treatment with the ether lipids produced comparable growth inhibitory effects.

PKC is the intracellular receptor for the tumor-promoting phorbol esters [5, 6]. It is known that TPA binds to specific sites on PKC and causes translocation of PKC from the cytosol to the plasma membrane, where it can be activated after binding phosphatidylserine and Ca^{2+}. This is one of the first events in TPA-induced differentiation of HL-60 cells to macrophage-type cells [15, 37, 38]. ET-18-OCH_3 inhibits PKC activity in vitro and antagonizes the effects of TPA in intact cells [12, 14, 27] In MCF-7 cells, ET-18-OCH_3 inhibits PKC at concentrations that inhibit cell proliferation [44]. The inhibition of both PKC translocation and HL-60 cellular differentiation, by QAA indicates that these compounds, like ET-18-OCH_3, inhibit PKC in intact cells. The finding that CP-37, which does not inhibit PKC, did not inhibit the growth of any cell line, supports the hypothesis that PKC inhibition is involved in the growth-inhibitory effect of the ether lipids. However, others have shown that PKC is only one of the enzymes inhibited by ET-18-OCH_3 [7, 8, 35, 43] and Salari et al. [31] have shown that the effects of ether lipids on PKC do not correlate with cytotoxicity in some leukemia cell lines. We have recently shown ET-18-OCH_3 inhibits PKC translocation in response to cytosine arabinoside and enhances cytosine arabinoside-induced apoptosis [42]. The observation that these effects can be reproduced by inhibiting PKC with antisense techniques argues for a role of PKC in this effect of ET-18-OCH_3. Thus, the ether lipids may be effective in growth inhibition by a combination of their effects on multiple pathways.

Differences between sensitive and resistant cells seem to be mostly a result of a different rate of drug uptake [33]. In fact, when most of the drug was free in the medium (serum-free condition) the difference in sensitivity between HL-60 and K562 cell lines was reduced. Furthermore, the resistant cells were sensitive to the QAA, which seemed to be less bound to albumin than ET-18-OCH_3.

The quaternary ammonium analogs of ether lipids represent a new class of antineoplastic agents, and could be used in the treatment of leukemias refractory to the currently used ether lipids. Because they are very active against leukemic cells after short-term treatment, they may be particularly useful in bone marrow purging.

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