Membrane Lipid Modification and Sensitivity of Leukemic Cells to the Thioether Lipid Analogue BM 41.440

Eric S. Petersen, Eric E. Kelley, Edward J. Modest, and C. Patrick Burns

ABSTRACT

Since the ether lipid anticancer drugs are membrane targeted, we examined the effect of membrane lipid structural alteration on their cytotoxicity. Enrichment with docosahexaenoic acid increased the sensitivity to the thioether lipid BM 41.440, compared to control cells enriched with oleic acid. The effect was dependent upon drug concentration, time, and the extent of cellular fatty acid enrichment. Other polyunsaturated fatty acids had a similar effect, which was proportional to the degree of unsaturation of the molecule inserted. Depletion of cellular glutathione with buthionine sulfoximine increased the sensitivity to ether lipid, but prooxidants such as Fe²⁺ and antioxidants such as vitamin E had little effect. The addition of serum to the incubation medium markedly diminished the cytotoxicity of ether lipids for cells modified with both docosahexaenoic acid and oleic acid, probably due to binding of the drug to serum components. The toxicity of another ether lipid, 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, was not affected appreciably by membrane alteration. Drug uptake studies with a radiolabeled BM 41.440 analogue, 1-[³H]hexadecylthio-2-ethyl-rac-glycero-3-phosphocholine, demonstrated no difference in transport at early time points and no difference in accumulation up to 60 min. We conclude that increases in cellular and/or membrane fatty acid polyunsaturation heighten the cytotoxic effect of a membrane-active ether lipid. The effect is not due to a change in drug transport or accumulation. It may be related to a change in oxidative events. These observations provide further confirmation of the membrane being the target of ether lipid action, using biochemical rather than morphological techniques. Most importantly, this observation offers a potential innovative approach to therapy.

INTRODUCTION

The ether lipid anticancer drugs have been a focus of investigation because of their novel membrane target. They are in clinical trials for therapy (1-4) and for tumor imaging (5). The interest in these compounds has grown as the number of different described pharmacological and biochemical effects on tumor cells has expanded. These effects have included in vitro and in vivo antitumor cytotoxicity (4, 6-8), induction of differentiation in animal and human leukemia cell lines (9-12), activation of host macrophages (13, 14), and enhancement of the effect of hyperthermia (15-17). These drugs affect several membrane properties, producing changes in membrane fluidity (18-20), inhibition of protein kinase C activity (21-26), modulation of phospholipid metabolism (27-31), alteration of membrane nuclear magnetic resonance spectra (32), morphological changes showing membrane damage (6, 33, 34), inhibition of growth factor-dependent inositol phosphate calcium signaling (35), inhibition of phosphati-dylinositol-specific phospholipase C (36), elevation of intracellular calcium (37), inhibition of Na⁺,K⁺-ATPase and sodium pump activity (38, 39), inhibition of epidermal growth factor binding (40), and inhibition of arachidonate release (21). These agents do not affect DNA directly (41, 42) and are independently additive (43) or synergistic (44) with DNA-active agents. These membrane-active properties provide some insight into the yet unknown mechanism of action of these drugs.

The composition of membrane lipids can be influenced considerably by the fatty acids added to the growth medium, since essential polyunsaturated fatty acids are obtained from the environment (45, 46). Fatty acid modification can increase the sensitivity of L1210 cells to doxorubicin (47, 48), influence the flux of antineoplastic drugs including doxorubicin (48), methotrexate (49), mitoxantrone (50), and melphalan (51) in neoplastic cells, and increase the rate of differentiation of HL-60 cells induced by all-trans-retinoic acid (52). We reasoned that alteration of the membrane target for the ether lipid group of antineoplastic drugs offered an opportunity to gain insights into the mechanism of action. An augmentation of sensitivity would also offer therapeutic possibilities. Furthermore, there is evidence that membrane constituents influence the cytotoxicity of membrane-active drugs. Chabot et al. (53) found that the sensitivity to ET-18-OCH₃ may depend on the cellular content of endogenous ether lipids. In addition, the cholesterol content of cellular membranes modulates the uptake of ether lipids and their cytotoxicity (54-56); it has been suggested that the high content of cholesterol may explain the inherent resistance of K562 cells (55). Diomedes et al. (55, 56) demonstrated that experimental reduction of membrane cholesterol content results in increased ether lipid cytotoxicity. Because of the membrane-active properties of this class of drug and the observation that modulation of the membrane target of their action influenced cytotoxicity, we undertook a study of the effect of cellular fatty acid modification on the sensitivity of a leukemia cell line to ether and thioether lipids.

MATERIALS AND METHODS

Cells, Lipids, Chemicals, and Media. L1210 murine lymphocytic leukemic cells were obtained from the American Type Culture Collection and maintained in continuous cell suspension at 37°C in RPMI 1640 medium. The L1210 cultures were supplemented with 5% heat-inactivated FBS. All cultures were maintained in a humidified atmosphere of 5% CO₂:95% air. BM 41.440 (ilmofosine) was a generous gift from Dr. Dieter Herrmann (Boehringer Mannheim GmbH, Mannheim, Germany) and from Dr. Wolfgang Berdel (Free University of...
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Berlin, Berlin, Germany); ET-18-OCH₃ (edelfosine) was kindly provided by Dr. R. Nordström (Medmark Pharma GmbH, Munich, Germany). Fatty acids were obtained from Nu Chek Prep (Elysian, MN). Fatty acid standards and Chromosorb were from Supelco Inc. (Bellefonte, PA). Boron trifluoride, D,L-α-tocopherol aceate (vitamin E), BSO, and butylated hydroxytoluene were obtained from Sigma Chemical Co. (St. Louis, MO). RPMI 1640 was purchased from Gibco Laboratories (Grand Island, NY), FBS and equine serum from HyClone Laboratories (Logan, UT), and agar from Difco Laboratories (Detroit, MI).

Fatty Acid Modification and Lipid Analysis. Modification of cellular fatty acid composition was accomplished by growing cells for 2 days in medium supplemented with either 22:6, 18:1, or other fatty acids in selected experiments. Specific quantities of the sodium salts of each fatty acid were added dropwise to the FBS. Free fatty acid content was determined (57). The stock of modified serum was diluted to 640 μM and further diluted to prepare the growth medium so that the concentration in the final medium was 32 μM. These concentrations were chosen as the optimal ones to bring about modification without cell toxicity from the fatty acids per se. After 2 days (42–46 h) of exponential growth in the modified media, the cells were washed with phosphate-buffered saline and extracted with CHCl₃/CH₃OH (2:1, v/v). The fatty acids were then methylated for 1 h at 95°C with acetonitrile:14% BF₃ in methanol (58) and the methyl esters of the fatty acids were separated by gas chromatography using a 2-mm x 1.9-m glass column packed with 10% SP-2330 on 100/120 mesh Chromosorb W-AW (Supelco). Peak areas were quantified and identified by comparison to retention times of known standards. We have previously documented that supplementation of growth medium of L1210 cells with the fatty acids used in these experiments results in appreciable enrichment in the membrane by the added fatty acid (45, 48, 52).

Drug Exposure and Clonogenic Survival Assay. Incubations with drugs and cofactors were carried out in RPMI 1640 medium with or without serum supplement. Survival was assayed with a clonogenic assay using 0.3% soft agar and RPMI 1640 supplemented with 20% equine serum, as previously described (59). After an incubation period of 7–10 days, colonies of >50 cells were counted with an inverted microscope. Survival of cells exposed to BM 41.440 or ET-18-OCH₃ was expressed as a percentage of an appropriate control.

Transport and Accumulation Studies with Radiolabeled Ether Lipids. The thioether phospholipid analogue ET-16S-OC₂H₅ was selected for the uptake study because this compound is a closely related structural isomer of BM 41.440 with precisely the same molecular weight and atomic composition, with the only difference being the transposition of two atoms at position 2, i.e., the oxygen ether and the distal carbon atom. The atomic configuration at positions 1 and 3 is identical. Fig. 1 shows this minor difference in structure. [9,10-³H₂]ET-16S-OC₂H₅ (specific activity, 56 Ci/mmol; purity, >96% by thin layer chromatography) provided by Dr. Steven Wyrick (University of North Carolina, Chapel Hill, NC) (60) was stored in absolute ethanol until immediately before use and was then dried with Na₂ and taken up in BSS (132 mm NaCl, 5 mm KCl, 1 mm MgSO₄, 16 mm Na₂HPO₄, 5.6 mm glucose, pH 7.2). Uptake of thioether was ascertained using our previously published methods (48–50). Briefly, cells (2 x 10⁶/ml in BSS) were incubated with labeled drug at 37°C. At the experimental times, rapid separation was accomplished by centrifugation at 15,600 x g for 2 min in an Eppendorf 5412 centrifuge through 0.3 ml of ice-cold n-butylphthalate:corn oil (3:5:1, v/v) layered beneath 0.5 ml of ice-cold BSS. The supernatant was aspirated and the tip of the 1.5-ml conical microtube containing the pellet was severed and placed in a scintillation vial, which contained 0.5 ml of tissue solubilizer (NCS; Amersham Corporation, Oakville, Ontario, Canada). After a period of 30 min in the tissue solubilizer, 5 ml of BudgetSolve scintillation cocktail (Research Products Inc.) were added and the radioactivity was determined using a liquid scintillation counter.

Experiments to attempt to differentially remove drug adsorbed on the surface of the plasma membrane and to distinguish it from internalized drug were based on studies described by Bazill and Dexter (61). Briefly, L1210 cells (5 x 10⁶/ml) were incubated with 0.0156 μM [⁹⁹ᵐ⁻¹⁰¹] ET-16S-OC₂H₅ in BSS at 37°C for 2 min. An aliquot taken for determination of total radioactivity was filtered through a Whatman GF/A glass fiber filter and washed five times with 1 ml of BSS. The remainder of the cell suspension was centrifuged for 1 min at 3600 x g and the supernatant, containing the radiolabeled drug that was not associated with cells, was removed. The cells were resuspended in ice-cold BSS containing 20% FBS and were incubated on ice for 45 min. The cells were then collected on glass filters as described above and washed, and the radioactivity was determined. The amount of drug detected after exposure of the cells to the albumin contained in the FBS was compared with total cellular radioactivity. The difference between the two is a measure of an exchangeable pool of drug which could represent that drug bound loosely to the plasma membrane; however, we could not eliminate the possibility that internalized drug was being removed by efflux during FBS exposure.

RESULTS

Lipid Modification. There was considerable modification of the fatty acid composition of L1210 phospholipids grown in medium supplemented with 18:1 or 22:6 (Table 1). As compared to unmodified cells grown in unsupplemented medium, the cells grown in 22:6 contained an 8-fold higher percentage of 22:6, a 2-fold increase in total polyunsaturates, and an increase in the mean number of double bonds per fatty acid. There were also significant increases in 16:0, 18:0, 18:1, total monounsaturates, and total saturates. The cells grown in 18:1-enriched medium were similar to unmodified cells. Overall, these changes are similar to those reported previously in this cell line (48, 59, 62).

Sensitivity of Lipid-modified L1210 Leukemia to BM 41.440. To assess sensitivity of fatty acid-modified leukemia cells to either lipids, L1210 cells were incubated with BM 41.440 for 8 h at concentrations of drug up to 30 μM. Cells enriched in 22:6 were compared with controls enriched in 18:1. Fig. 2 shows that the cells enriched with the highly polyunsaturated fatty acid 22:6 were more sensitive to the ether lipid than were those enriched with 18:1, which results in a lipid composition similar to that of unmodified cells (59). The difference in survival was evident at all concentrations studied except for 20 μM. The concentration-dependence differences were maximal at 5 and 7.5 μM; at concentrations above 10 μM differences were difficult to detect since cell survival was <10%.

A kinetic study using 7.5 μM BM 41.440 is shown in Fig. 3. There were highly significant differences at 4–12 h of drug exposure; the statistical significance of the difference was particularly high at 8 h.

Effect of Extent of Fatty Acid Modification. Since modification of L1210 cells in 22:6-supplemented medium increased their sensitivity to BM 41.440, we next examined whether the amount of 22:6 in the growth medium influenced sensitivity to the drug. Concentrations of fatty acid were chosen that were nontoxic. We previously showed in another leukemic cell line that higher concentrations of fatty acid in the growth medium

Fig. 1. Structural formulas for the radiolabeled thioether phospholipid [⁹⁹ᵐ⁻¹⁰¹] ET-16S-OC₂H₅ and BM 41.440.
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Table 1. Fatty acid composition of L1210 cell phospholipids

L1210 cells were grown for 2 days in medium supplemented with 18:1 or 22:6 at 32 μM. Cells were washed and extracted with CHCl3:CH3OH (2:1, v/v). Phospholipids in the lipid extracts were isolated using silicic acid chromatography. After alkaline hydrolysis, fatty acids in the saponifiable fraction were methylated and the methyl esters were separated by gas-liquid chromatography. Values are the mean and SE of at least three determinations.

<table>
<thead>
<tr>
<th>Fatty acid composition (%)</th>
<th>Unmodified</th>
<th>18:1-modified</th>
<th>22:6-modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.8 ± 0.4</td>
<td>2.8 ± 0.4</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>16:0</td>
<td>12.2 ± 1.3</td>
<td>13.7 ± 0.7</td>
<td>23.1 ± 0.8a</td>
</tr>
<tr>
<td>18:0</td>
<td>10.6 ± 0.4</td>
<td>10.0 ± 0.4</td>
<td>21.4 ± 1.0b</td>
</tr>
<tr>
<td>18:1</td>
<td>56.0 ± 0.8</td>
<td>51.5 ± 1.5</td>
<td>20.3 ± 0.8a</td>
</tr>
<tr>
<td>20:4</td>
<td>3.6 ± 1.3</td>
<td>1.4 ± 0.1</td>
<td>2.0 ± 0.6b</td>
</tr>
<tr>
<td>22:6</td>
<td>3.4 ± 0.3</td>
<td>4.1 ± 0.4</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Others</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>2.0 ± 0.2b</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>13.3 ± 2.6</td>
<td>15.8 ± 2.8</td>
<td>27.6 ± 2.4a</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>61.3 ± 1.0</td>
<td>57.5 ± 1.8</td>
<td>25.6 ± 0.8b</td>
</tr>
<tr>
<td>Saturated</td>
<td>24.6 ± 1.8</td>
<td>26.6 ± 1.2</td>
<td>46.8 ± 1.6b</td>
</tr>
<tr>
<td>Double bonds</td>
<td>13.3 ± 2.6</td>
<td>15.8 ± 2.8</td>
<td>27.6 ± 2.4a</td>
</tr>
<tr>
<td>a Significantly different from unmodified at P &lt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Significantly different from 18:1-modified at P &lt; 0.05.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Mean number of double bonds per fatty acid.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Effect of Glutathione Depletion Using BSO. The prooxidant BSO was tested at a concentration previously demonstrated in our laboratory to deplete cellular glutathione in L1210 cells (64). Cells were modified with 22:6 for 48 h and during the last 24 h 100 μM BSO was present. The survival after 4 h of exposure to BM 41.440 or another ether lipid, ET-18-OCH3, is shown in Table 2. There was heightened sensitivity to BM 41.440 at 20 μM and to ET-18-OCH3 at 10 μM. Lower concentrations of BM 41.440 were insufficient to demonstrate a difference; higher concentrations of ET-18-OCH3 led to a majority of the cells being killed in this experiment whether or not BSO was present. Lower concentrations of BSO (25 μM) and of the two ether lipids were insufficient to affect survival.

Effect of Serum on Ether Lipid Cytotoxicity. L1210 cells enriched with 22:6 were exposed to 10 μM BM 41.440 for 4 h in the presence of serum at concentrations of 0, 1, 5, and 10%. Fig. 6 shows the effect of the FBS on clonogenic survival. In the presence of BM 41.440, L1210 cell survival was zero in medium that was serum free or contained only low concentrations of

obtained by longer incubation times are associated with greater extents of modification of cell phospholipids (63). Cells were modified in medium containing 22:6 at 5, 10, 16, or 32 μM prior to exposure to 10 μM BM 41.440; the clonogenic survival was then compared (Fig. 4). The cell survival decreased as the amount of 22:6 in the medium increased. The survival of cells modified with 32 μM 22:6 was significantly lower than that of cells modified with any of the other concentrations.

Effect of Other Fatty Acids. Since cells enriched with the highly polyunsaturated fatty acid 22:6 had heightened sensitivity to BM 41.440, we examined the sensitivity of cells enriched with fatty acids of lesser degrees of polyunsaturation (Fig. 5). The sensitivity increased incrementally as the number of double bonds in the enriching fatty acid increased. The effects of all fatty acids, except 18:3ω3 and 18:3ω6, were significantly different from those of 18:1. In addition, there was a difference in the two 18:3 fatty acids, since 18:3ω6 rendered the cells more sensitive than did 18:3ω3 (P = 0.02).
Fig. 5. Sensitivity of L1210 cells enriched with fatty acids of various degrees of polyunsaturation. Cells were grown for 2 days in medium containing 32 μM fatty acids prior to exposure to 20 μM BM 41.440 for 8 h. Survival was measured in a clonogenic assay. Cells modified with each fatty acid except 18:3 were significantly more sensitive (18:1 versus 18:3, not significant; versus 18:3<sup>6</sup>, P = 0.08; versus 20:4, P = 0.01; versus 20:5, P = 0.000005; versus 22:6, P = 0.0000001).

Table 2. Effect of glutathione depletion using BSO on ether lipid cytotoxicity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control</th>
<th>BM 41.440</th>
<th>ET-18-OCH&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>+BSO</td>
<td>100 ± 2*</td>
<td>100 ± 4</td>
<td>63 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>-BSO</td>
<td>71 ± 11</td>
<td>89 ± 6</td>
<td>36 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Mean and SE of at least three determinations. All values were normalized to percentage of specific control value, which was set to 100%.

serum. However, at 5–10% serum the toxicity of this moderately low concentration of ether lipid was abrogated, and the survival approached that of controls containing neither ether lipid nor serum. In the absence of BM 41.440, serum facilitated the growth of L1210 cells 3–4-fold. There was a similar effect on ET-18-OCH<sub>3</sub> cytotoxicity (using 10 μM drug for 4 h, there was 0% survival in 0% FBS versus 69% survival in 5% FBS). The protective effect of serum probably results from competitive binding of these lysophospholipid derivatives to albumin in the serum: it is known that there is high affinity binding to serum albumin and less cell uptake of ET-18-OCH<sub>3</sub> in the presence of serum (61, 65). For this reason, most experiments were performed in serum-free medium.

Transport and Accumulation Studies. The uptake of [3H]ET-16S-OC<sub>2</sub>H<sub>5</sub> by both 22:6- and 18:1-enriched L1210 cells was studied at nine time points between 15 s and 60 min (Fig. 7). Initial uptake (Fig. 7, inset) was linear for longer than 60 s and then reached a plateau after 2 min. Most importantly, there was no difference in association of radiolabel with the cells enriched in polyunsaturated 22:6 and the monounsaturated 18:1. When the drug uptake was studied at 2 min using 5 μM [3H]ET-16S-OC<sub>2</sub>H<sub>5</sub>, there was likewise no difference in the cell-associated radioactivity in 22:6-enriched cells (8.3 ± 0.6 dpm/1000 cells) and 18:1-enriched cells (7.7 ± 0.1 dpm/1000 cells; P = 0.41).
The rapid association of drug with the plasma membrane measured experimentally at 0°C was also not significantly greater in the 22:6-enriched cells (7.3 ± 2.1 pmol/10⁸ cells for the 22:6-modified cells versus 7.41 ± 2.1 pmol/10⁸ cells for the 18:1-modified cells). It has already been demonstrated that the radiolabel remains attached to unmetabolized drug and that there is little metabolism of the drug in cell culture (66–68).

Experiments were performed to compare cellular [³H]ET-18-OCH₃ before and after incubation in a serum-containing medium. Since the ether lipids bind avidly to albumin (61, 65), it was hoped that this might allow differentiation of external (albumin-extractable) from internal (albumin-unextractable) drug. Incubation in medium with 20% serum for 45 min at 0°C extracted 94% of the radiolabeled drug from the 22:6-enriched cells (5.08 ± 0.21 pmol/5 × 10⁸ cells for untreated cells versus 0.31 ± 0.0007 pmol/5 × 10⁸ cells after albumin incubation) and 90% from the 18:1-enriched cells (5.1 ± 0.17 pmol/5 × 10⁸ cells for untreated cells versus 0.51 ± 0.03 pmol/5 × 10⁸ cells after albumin incubation). Most importantly, the more sensitive 22:6-enriched cells did not contain more drug after incubation in serum.

Effect of Fatty Acid Modification on Cytotoxicity of ET-18-OCH₃. L1210 cells were modified with 22:6 or 18:1 and exposed for 4 or 8 h to 2.5, 5, 7.5, 10, 15, 20, or 30 μM ET-18-OCH₃. Survival in a clonogenic assay was not significantly affected by 22:6 enrichment. The sensitizing effect of BM 41,440 may be the result of the presence of the more lipophilic thio group in the sn-1 position or the additional CH₂ in the sn-2 position, compared to ET-18-OCH₃.

DISCUSSION

Our observation that a change in the lipids of tumor membranes influences ether lipid sensitivity lends further support to the central importance of a membrane target for these drugs. However, it does not clarify the relative importance of distinct membranes since our modification model results in changes in the lipids of diverse intracellular membranous structures as well as the surface membrane (69). It also does not allow identification of the mechanism by which the drug and membrane interaction leads to cell death. There are several ways that changes in membrane lipids could influence the proposed mechanisms. First, fluidization of membranes by insertion of polyunsaturated fatty acids (49, 59) would be additive with the known ability of the ether lipids to decrease membrane order (18–20). A similar additive effect could occur if physical changes such as alterations of nuclear magnetic resonance spectra (32) or membrane surface ultrastructure (33, 34), proposed as mechanisms for ether lipid action, are crucial to cytotoxicity. Second, possible mechanisms such as alteration of phospholipid metabolism (27–31), interference with phosphatidylinositol signaling (35, 36) or affinity of phospholipases, or other enzymes, for substrate (36), or arachidonate release (21) would each be influenced since lipid modification-induced changes in the polyunsaturated fatty acids would alter the phospholipid substrate. Third, an alteration of the lipid phase could affect proteins and receptors known to be affected by ether lipids, such as protein kinase C (11, 22–25), Na⁺,K⁺-ATPase and sodium pump activity (38, 39), or binding to receptors such as those for epidermal growth factor (40).

One possible mechanism to explain the increased sensitivity of the 22:6-enriched L1210 cells to ether lipids is heightened susceptibility to lipid peroxidation. The chemical structure of this class of antineoplastic agents does not suggest that a free radical would be generated directly as a result of metabolism of the drug. However, it is possible that free radicals are generated indirectly as a secondary event of membrane damage and that membrane fatty acids with increased numbers of double bonds are more susceptible to this secondary damage. In support of this possibility is the fact that cytotoxicity was increased by the prooxidants Fe²⁺ plus ascorbic acid and by glutathione depletion. In addition, we have shown that ether lipids increase membrane lipid peroxidation (70).

One possible explanation for the increase in sensitivity of the L1210 cells enriched in polyunsaturated fatty acids would be an augmentation of cellular uptake and accumulation of the ether lipid drug. There is precedence for this. For example, Storch and Munder (65) observed greater accumulation of radiolabeled ET-18-OCH₃ by a sensitive fibrosarcoma cell line, compared to a resistant counterpart. Hoffman et al. (66) showed that more radiolabeled ET-18-OCH₃ was incorporated into sensitive HL-60 cells, compared with resistant K562 or mature neutrophils. Bazill and Dexter (61) found greater uptake by sensitive WEHI-3B and HL60 cells. However, one study (67) reported that MDCK cells, which are relatively resistant to ether lipid cytotoxicity, incorporate more ether lipid than HL-60 cells, and another study (56) found no correlation of ether lipid uptake and sensitivity of human leukemia cell lines. Furthermore, Diomede et al. (55) showed that the uptake of radiolabeled ET-18-OCH₃ could be modulated down by prior incorporation of cholesterol into the cell. Daniel et al. (68) reported that resistant leukemia cell lines took up less ether lipid than did sensitive counterparts. Our results demonstrate that there is no change in initial transport or accumulation of drug resulting from enrichment with the polyunsaturated fatty acid that would explain the increased cytotoxic sensitivity. Furthermore, experiments with cells preloaded with radiolabeled ether lipid showed no less removal of drug from the sensitive 22:6-enriched cells, compared to the less sensitive 18:1-enriched cells, by 20% FBS added to the medium. In those experiments, the removal of the majority of drug after incubation in a medium that contained FBS could be due to (a) removal of a large amount of externally bound drug, (b) removal of ether lipid contained in the outer bilayer, or (c) efflux of internalized drug during the 45-min incubation. We cannot differentiate between those possibilities, but the high proportion of drug extracted from the cells by serum suggests that efflux is a mechanism at least partly responsible. The identification of experimentally derived cells that have a difference in sensitivity to ether lipids which is not explained by a disparity in drug uptake is important. Other mechanisms must be implicated, and one of these possibilities is a dissimilarity in susceptibility to oxidative stress.

Our observations suggest that it might be possible to utilize membrane lipid modification as an adjunct to ether lipids in the therapy of human neoplasms. Polyunsaturated fatty acids in the form of fish oil capsules containing purified steam-stripped menhaden fish oils (71), liquid-formula diets (72), or oils given as dietary supplements (73) can be given to humans safely, and these can modify the lipids of normal human tissues. For selected needs, suspensions of acylglycerol emulsions can be administered i.v. Therefore, it is likely that human cancers could also be modified in vivo by special therapeutic diets. Since many tissues of the host are affected when fatty acid modification is attempted (74), such replacement may also increase toxicity. However, tissues are modified to different extents (74), so it
REFERENCES


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