Review

Biochemical effects of lipids on cancer therapy

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The fatty acid composition of cancer cells can be modified during growth in culture or in animals. Substantial changes can be produced in the degree of unsaturation of the membrane phospholipids. Enrichment with polyunsaturated fatty acids makes leukemia cells more susceptible to lipid peroxidation and increases lipid radical formation in response to oxidant stress, ether lipids, and photodynamic therapy. Increases in polyunsaturation also make leukemic lymphoblasts more sensitive to the anthracycline drug doxorubicin, the thioether lipid ilmofosine, and photodynamic therapy with Photofrin. Enrichment with docosahexaenoic acid also slows the growth of the L1210 cells by facilitating differentiation. These findings suggest that polyunsaturated fatty acid supplementation may make certain forms of cancer treatment more effective. Parinaric acid, which contains conjugated double bonds, is cytotoxic to several kinds of human tumor cells through a mechanism that appears to involve oxidant stress. It is not known yet whether any of these lipid-based approaches to cancer therapy will show selectivity for malignant cells. Until this is resolved, the potential clinical utility of these approaches remains uncertain. (J. Nutr. Biochem. 5:114–123, 1994.)

Keywords: fatty acids; membranes; lipid peroxidation; free radicals; anticancer agents

Introduction

The fatty acids present in mammalian tissues are obtained either from dietary fat or through biosynthesis. All the biologically important saturated and monounsaturated fatty acids can be synthesized completely from acetyl CoA. Therefore, if the diet does not provide enough of these fatty acids, synthesis increases to maintain an adequate supply for tissue function. By contrast, the two classes of polyunsaturated fatty acid that normally occur in mammalian tissues, the omega-6 and omega-3 fatty acids, cannot be completely synthesized. All of these polyunsaturates are ultimately derived from the diet. If an adequate dietary supply is not available, the tissues eventually become deficient in these polyunsaturated fatty acids. This may interfere with membrane function or the production of vital lipid mediators.

The same principles apply to malignant cells, which derive all of their omega-6 and omega-3 polyunsaturated fatty acids from the circulation of the tumor-bearing host. Studies with murine ascites tumors indicate that the circulating lipid is provided by free fatty acid and triglyceride-rich lipoproteins.[1,2] The polyunsaturated fatty acids are supplied either preformed or as precursors that are elongated and further desaturated by the cancer cells.[3] Therefore, the type and amount of polyunsaturated fatty acid available to a tumor depends on what is present in the circulation of the host, which, in turn, ultimately depends on the dietary fat intake. Because some of the phospholipid in tumor cells undergoes rapid turnover,[4] certain membrane domains should respond quickly to changes in the fatty acid composition of the extracellular fluid.

These findings suggest that it should be possible to change the fatty acid composition of a tumor, especially the polyunsaturated fatty acids, by altering the dietary fat intake of the tumor-bearing host. Such changes might alter the membrane or growth properties of the cancer cell or increase its sensitivity to cytotoxic agents and thereby offer some therapeutic advantages. This hypothesis has been summarized in several reviews.[5–7] *Figure 1* is a diagrammatic representation of the biologic effects of fatty acid modification. The lipid modification using specialized diets or regional perfusion, as well as the ensuing chemotherapy or hyperthermia, are represented with heavy arrows. The consequent biologic events that are potentially altered by the modification and therefore susceptible to this therapeutic approach are shown using thin arrows. This figure summarizes the central issues of our hypothesis regarding the use of fatty acid enrichment in cancer treatment.

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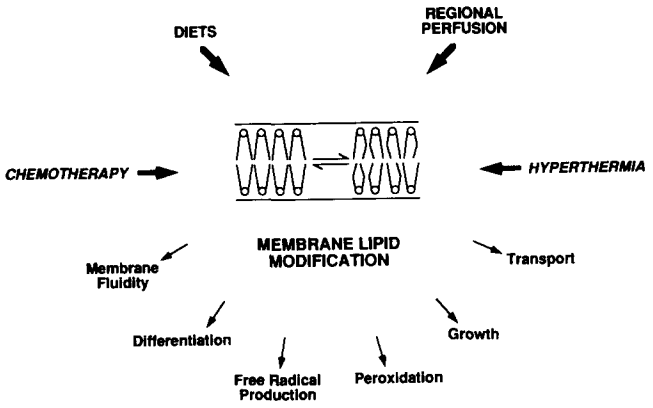

Figure 1 Diagram showing overall hypothesis of therapeutic approach. Interventions are shown at top and middle (heavy arrows), and biologic events affected are below (thin arrows).

In our model, membrane fluidity is increased by enriching the membranes with polyunsaturated fatty acids. This change in membrane order, which can be demonstrated by electron spin resonance studies on cells modified in tissue culture or in vivo[5,6] has the potential of influencing many biologic properties of neoplastic cell membranes. These properties influence the manner in which the cell responds to its environment and to anticancer therapies. For example, the activity of membrane-bound enzymes, binding of ligands, and transport of compounds across the plasma membrane can be modulated by the biophysical changes resulting from experimental lipid modification.[5–7] It is likely that there are changes in cell and membrane properties that are as yet undefined, and these may influence the complex responses of cells to certain cytotoxic drugs, higher temperatures, and differentiation agents. Fatty acid modification also affects eicosanoid production, and this could affect growth rate, production of and response to cytokines, and cell-cell interaction. Recently, we demonstrated that enrichment of cell membranes with polyunsaturated fatty acids results in heightened oxidative state, and this may explain the response to therapies known to be mediated by free radicals. Fatty acid modification could improve responses to conventional cancer therapy through all of these mechanisms.

Our initial studies demonstrated that tumor cells could be highly enriched in linoleic acid, an omega-6 polyunsaturate, by feeding tumor-bearing mice diets enriched in sunflower oil.[8] The fatty acid compositional changes that occurred were of sufficient magnitude to alter some of the physical and transport properties of the plasma membrane.[9–11] This work was extended subsequently to cancer cells grown in culture to produce more precise fatty acid modifications.[12]

The purpose of this review is to describe the recent developments in this lipid-based approach to cancer treatment. A trend that has become apparent is a shift in emphasis from membrane structure-function relationships to mechanisms involving oxidant stress and lipid peroxidation.[13]

Fatty acids as direct cytotoxic agents

Dietary studies

The growth of tumors in animals is inhibited by diets rich in omega-3 fish oils,[14–18] and this is in contrast to the growth stimulation of tumors in animals eating diets rich in vegetable oils, which contain omega-6 polyunsaturates, especially linoleate (18:2ω6).[19–22] The effect is not specific to any one omega-3 fatty acid because various dietary fatty acids containing 3-6 double bonds inhibit tumor growth.[14,18,23] Saturated fatty acids have also been studied. Tricaprylin fed to rodents with implants of experimental lymphoma resulted in toxicity to tumor cells.[24] In the same study, intrahepatic implants of carcinoma treated with intraperitoneal sodium caprylate and subcutaneous implants of hepatoma treated with transdermal injections of caprylate demonstrated tumor lysis but only limited damage to normal tissue.

Human tumors transplanted into nude mice ingesting diets rich in omega-3 fatty acids are also inhibited.[25–29] The effect is not limited to one type of tumor because the growth of breast,[14,15,28,29] prostate,[25,26] and colon[17] cancers have been slowed. However, diets rich in fish oils had no inhibitory effect on an experimental lymphoma;[30] this could be related to the fact that the omega-3 fatty acid decreased tumor expression of the immunophenotypic marker CD4, which may influence the immune response in vivo. Although most omega-6 fatty acids seem to facilitate tumor growth, dietary γ-linolenic acid (18:3ω6) has an inhibitory effect on some neoplasms.[28]

Several studies have also reported an effect of omega-3 fatty acid-enriched diets on establishment and growth of tumor metastasis in animals. Mice fed diets containing a high level of fish oil had several-fold fewer lung metastases from a transplantable colon cancer when compared with animals fed the omega-6–rich safflower oil.[17] Likewise, rats fed fish oil-enriched diets had fewer lung metastasis from a mammary tumor as compared with animals fed corn oil, but the effect was present only when the diet contained 16% fish oil and not 21% fish oil.[31] Conversely, omega-6 fatty acids enhanced metastasis of human breast cancer in a mouse model.[22]

There is also considerable evidence that fish oils exert an inhibitory effect on experimental tumorigenesis. This effect likely occurs at the post-initiation phase.[32] This literature has been reviewed recently.[33–36]

Cell culture studies showing cytotoxicity

Saturated fatty acids may destroy neoplastic cells in culture.[37] Omega-3 and omega-6 polyunsaturated fatty acids may also kill tumor cells when added in sufficient concentration to the growth medium of cells in tissue culture.[38–41] This cytotoxic effect has been reported for many types of tumors,[36] and the results are consistent with the dietary studies reported above. The cell killing is a direct function of concentration, however, the critical level required varies from tumor to tumor.[40,42] Concentrations of fatty acids can be identified that are cytotoxic for tumor cells but do not kill non-neoplastic cells or revertant cell lines.[39,40,42–44] This observation is important because selectivity is an essential feature of clinically useful anticancer agents. Pertinence to cancer therapy is indicated by the fact that increased tumorigenic phenotype is directly correlated with increased sensitivity to polyunsaturated fatty acids.[43,45] The mechanism for this selectivity is unknown, but neoplastic cells are known to have differences in membrane lipid composition. This baseline difference or their rapid proliferative state may result in more effective lipid modification in neoplastic cells. Alternatively, the oxidation-antioxidant status

brought about by the fatty acid modification may differ in neoplastic as compared with normal cells.

Mechanism

There are several biologic mechanisms that could account for the cytotoxicity of fatty acids. The growth-enhancing effect of omega-6 fatty acids could be due to increased prostaglandin levels because prostaglandin inhibitors, such as indomethacin, can prevent the tumor stimulation.[21,46] Similarly, the inhibitory effect of the omega-3 fatty acids could result from a decrease in dienoic prostaglandins or an altered eicosanoid profile.[47] Cyclooxygenase inhibitors failed to reverse the inhibitory effect of γ-linolenic acid on neuroblastoma cells, suggesting that prostaglandins are not the major mediator of its cytotoxicity.[41]

An alternative explanation is suggested by the reports of changes in oncogene expression brought about by fatty acids in some studies of mammary tissue,[48,49] but not in others.[50] Another possible mechanism is related to oxidative events because the fatty acids vary in extent of polyunsaturation and, therefore, in susceptibility to oxidation; this is developed in detail in other sections of this review.

Feasibility of using fatty acids alone as anticancer agents

We carried out studies designed to modify the fatty acid composition of cultured murine and human neoplastic cells.[51–54] Modification requires incubation of cells with media containing 10 to 40 μM fatty acid for 2 to 5 days. We have not generally observed appreciable changes in cell viability, clonogenic efficiency, or experimental tumor growth rate. This may be due to the fact that we have chosen concentrations of fatty acid that are sufficient to modify the fatty acid composition but are not high enough to damage the cells.

Although there are exceptions, especially with omega-6 fatty acids,[36,46] the concentrations of fatty acid required in many studies to destroy tumor cells and demonstrate selectivity are higher than one can expect to obtain from dietary supplementation. In addition, binding proteins in the serum or tumor and the presence of antioxidants in the body may abrogate the cytotoxicity. In this regard, the presence of serum ameliorates the effect of fatty acids,[55] probably because albumin and other serum proteins bind fatty acids. These considerations make it less likely that fatty acids can be highly effective alone as anticancer agents in the treatment of human cancer.

Some clinical trials have been undertaken. The limited data from cancer patients given capsules containing γ-linolenic acid (plus antioxidants and other fatty acids in some cases) is not promising[56] or is inconclusive.[57,58] However, randomized trials of diets in which the experimental fatty acids represent a large percent of ingested fat are needed to adequately test the hypothesis that dietary fatty acids alone will be effective in treating human cancer.

We conclude that dietary fatty acid therapy alone will not likely be sufficient to bring about the log-kill necessary for effective anticancer therapy in humans.

Effect of lipids on cellular differentiation

Abnormalities of cellular differentiation are thought to be a fundamental defect of acute leukemia and other malignancies. There is evidence that the differentiation signal originates in the plasma membrane.[54,59,60] Because a change in membrane lipid composition or synthesis occurs as the process of maturation proceeds,[61–64] it seems likely that membrane lipids are involved. Furthermore, arachidonic acid and ceramide have been implicated as mediators of chemically induced differentiation of the HL-60 leukemia cell.[65,66]

Because of this evidence that membrane lipids may have an important role in the differentiation process and its abnormalities in malignancy, we examined the effect of membrane fatty acid composition on the rate of cellular differentiation. HL-60 human myeloblastic leukemia cells have the capacity to terminally differentiate when exposed to retinoic acid. We grew HL-60 cells in media supplemented with low doses of docosahexaenoic acid (22:6ω3) or, for comparison, oleic acid (18:1ω9). The phospholipids of the cell membranes became enriched in the supplemental fatty acid after 1 to 2 days of growth. Cells enriched with 22:6 differentiated at an accelerated rate when exposed to retinoic acid as compared with those enriched with 18:1 or unmodified cells.[54] Specifically, 24 hours after the addition of retinoic acid, the HL-60 cells enriched with the highly polyunsaturated fatty acid had greater superoxide anion radical production and nitroblue tetrazolium reduction. These are measures of differentiation. Most dramatically, the percentage of cells in the G_1 or G_0 phase of cell cycle at 24 hours, as a measure of differentiation-related growth arrest, was >70% for cells enriched in polyunsaturates compared with <40% for those enriched in monoenoics and <50% for unmodified cells. Because the cells grown in 22:6 contained 50% more polyunsaturated fatty acids and almost one-third more double bonds, it seems likely that the polyunsaturated fatty acid enrichment and accelerated differentiation are related. This accelerated differentiation also had an appreciable effect on growth rates, and the cells enriched in polyunsaturates showed a differentiation-associated slowing of growth.

Other laboratories have studied the effects of lipids on differentiation. Prior supplementation of the growth media of cultured colon cancer cells with 22:6 facilitated differentiation induced by butyrate.[67] Fatty acids in the growth medium also modulated expression of the adipose differentiation-related aP2 gene.[68] Lipids are involved in differentiation in other ways. Butyric acid, a short chain saturated fatty acid, induces differentiation.[69] Furthermore, 1,2-diacylglycerol mediates hexamethylene bisacetamide-induced erythropoiesis in erythroleukemia cells.[70]

These observations on differentiation from our laboratory and others could explain why diets rich in the highly unsaturated fish oil fatty acids slow tumor growth and retard carcinogenesis. They could also be of use in the design of differentiation therapy, an approach that is becoming important clinically.[71,72] At a more basic level, these studies provide further evidence that the differentiation signal may be generated by the lipids of the plasma membrane.

Facilitation of chemotherapy

Introduction and concept

Because it seems unlikely that dietary fatty acids alone will be sufficient for effective anticancer therapy in humans, we

explored the possibility of combining them with traditional chemotherapy. There was some reason to believe that prior exposure of neoplastic cells to fatty acids would result in an enhanced effect of drugs. For example, enrichment of the membranes of L1210 leukemia cells with polyunsaturated fatty acids resulted in a decrease in K_m for the methotrexate transporter[11] and of the transition temperature for melphalan uptake carrier.[73] In addition, the passive uptake of mitoxantrone was increased.[74] These effects could be the consequence of a change in membrane fluidity that results from fatty acid enrichment[11] or, in the case of methotrexate and melphalan, a change in positioning of the drug carrier within the lipid bilayer. In addition, polyunsaturated fatty acid supplementation resulted in increased accumulation of another important drug, doxorubicin.[53] These observations suggested a rationale for additive or synergistic effects of membrane lipid modulation when combined with chemotherapy.

Effect of polyunsaturation on doxorubicin sensitivity

To test the effect of membrane enrichment with polyunsaturated lipids on drug sensitivity, we chose doxorubicin (Adriamycin) because it is frequently used in the treatment of human cancer. The sensitivity of L1210 leukemia cells, as measured in a clonogenic assay, was enhanced considerably by prior enrichment of the cells with the highly polyunsaturated fatty acid 22:6, as compared with cells grown in unsupplemented media or cells enriched with the fatty acid 18:1, which has only one double bond.[52] The increased sensitivity was a function of time and drug concentration and occurred at doxorubicin levels that can be obtained in humans. The accentuated drug sensitivity increased as the concentration of fatty acid used to supplement the medium was increased. Because the content of 22:6 in the cells increased three to four fold after growth in the supplemented media, it seems likely that the changes in cellular fatty acid composition drastically altered the sensitivity of the tumor cell to doxorubicin. Furthermore, when cells were enriched with one of six fatty acids having different degrees of polyunsaturation, there was a direct relationship between degree of polyunsaturation and doxorubicin sensitivity.[53] A future objective is to test the effect of dietary fatty acids on sensitivity of the tumor to doxorubicin in vivo.

This modulation of sensitivity could be a result of a change in physical properties of the membranes brought about by replacement of saturated with unsaturated fatty acids, thereby affecting uptake as discussed above. Alternatively, the increased number of double bonds could increase susceptibility to oxidative events. In this regard, doxorubicin metabolism results in the generation of a free radical.[75] We have not yet explored this possibility in detail for doxorubicin, but there is considerable evidence that the ether lipid class of drugs initiates important oxidative events as discussed below.

Data from other laboratories have also suggested the feasibility of an antitumor strategy based on lipid modification. Borgeson et al. reported that human mammary tumors grown in nude mice were more susceptible to the anticancer drugs doxorubicin and mitomycin when the animals were fed fish oil.[27] Krokan et al. studied the sensitivity of human cancer cell lines.[76] Pretreatment with 22:6 increased the toxicity of doxorubicin in glioblastoma, but not lung cancer nor HeLa cell lines. Schick et al. found that the presence of monocarboxylic acids was necessary for certain xanthate derivatives to be cytotoxic to some but not all cell lines.[77] Prior incubation of resistant but not sensitive small cell lung carcinoma cell lines with 22:6 increased their sensitivity to doxorubicin.[78] Timmer-Bosscha et al. studied small cell lung carcinoma cells lines and found that resistant but not sensitive lines became more sensitive to cisplatin after culturing with 22:6.[79]

Possible clinical applications

These observations have important clinical implications. 22:6 was selected for many of these studies because of its very high degree of unsaturation. Normally, however, 22:6 accumulates in many parts of the central nervous system, especially in excitable membranes. This raises the possibility that brain tumors may be especially good targets for therapy involving 22:6 supplementation. The increased doxorubicin sensitivity of glioblastoma cells enriched with 22:6 is consistent with this possibility.[76]

Studies in culture indicated that human Y79 retinoblastoma cells cannot be enriched to any greater extent than human skin fibroblasts if exposed directly to 22:6.[3] However, when supplemented with linolenic acid (18:3ω3), a precursor of 22:6, the retinoblastoma cells accumulated much more 22:6 than the skin fibroblasts. This suggests that in attempting to target 22:6 enrichment to the central nervous system, it may be more effective to use an omega-3 fatty acid precursor as a supplement rather than 22:6 itself. Recent studies indicate that primary cultures of astrocytes also convert omega-3 fatty acid precursors to 22:6.[80] Therefore, while omega-3 precursors may be more effective in targeting 22:6 enrichment to certain parts of the nervous system, this approach is unlikely to be selective for only the malignant neural cells.

Patients with neoplasms of many types could ingest defined diets containing variable degrees of polyunsaturated fats. This could be done with formula diets in a clinical research unit or by supplementing their usual diets with capsules or slurries of fatty acid ethyl esters or triglycerides rich in the required fatty acid prior to administration of systemic or oral anticancer agents. Alternatively, isolated portions of the body could be perfused with triglyceride emulsions or liposomes containing the proper fatty acid, followed by systemic or perfused chemotherapy. These observations also suggest the possibility that patients on certain chemotherapy regimens should avoid extremes of dietary fat intake to prevent abrogation or unsuspected acceleration of the effect of administered chemotherapy.

Response to oxidant stress

Polyunsaturated fatty acids are the main intracellular substrate for lipid peroxidation. Therefore, it was reasonable to assume that tumor cells enriched in polyunsaturated fatty acid might have an increased response to oxidant stress. This was tested initially in the U937 monocytic leukemia cell line.[81] U937 cell suspensions were grown in a medium supplemented with 22:6 or, as a control, with the same concentration of a monounsaturated fatty acid, 18:1. After 2 days, this medium was removed and the cells washed to

remove any adherent fatty acid. Aliquots of each type of cells were taken for fatty acid analysis by gas-liquid chromatography. Other aliquots were exposed to oxidant stress and assayed by electron paramagnetic resonance (EPR).

The chromatography data indicated that the lipids of the cultures supplemented with 22:6 contained 28% 22:6, and 41% of the total fatty acid was polyunsaturated. By contrast, those supplemented with 18:1 contained only 2% 22:6, and only 21% of their fatty acids were polyunsaturated.

Oxidant stress was induced by the addition of $FeSO_4$ in a medium that contained chelating-resin–treated phosphate buffer and a spin trap, α-(4-pyridyl 1-oxide)-N-*tert*-butylnitrone (POBN). As shown in *Figure 2,* an intense POBN free radical spin adduct spectrum was detected when the cells were enriched with 22:6 *(Figure 2D),* but not in the control cells supplemented with 18:1 *(Figure 2C)* or grown without any fatty acid supplement *(Figure 2B).* Radical adducts also were not detected if Fe^{2+} was not added to the EPR medium *(Figure 2A).*

Subsequent studies indicated that L1210 murine leukemia cells enriched with 22:6 also produced an intense radical adduct spectrum when subjected to oxidant stress.[82] This is consistent with the observation that 22:6-supplemented L1210 cells produce increased amounts of thiobarbituric acid reactive substances and ethane.[83]

These results suggest that one of the effects of polyunsaturated fatty acid enrichment of cancer cells is to increase the tendency to form lipid radicals when they are subjected to a given level of oxidant stress. This may be at least partly responsible for the increased cytotoxicity produced by doxorubicin or hyperthermia in L1210 cells when they contain increased levels of polyunsaturated fatty acids.[51,52]

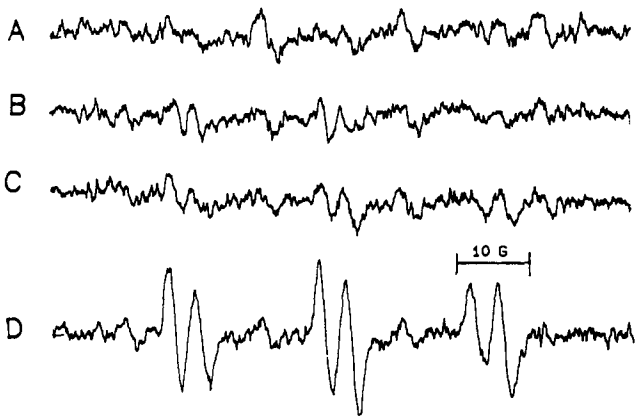

Figure 2 Polyunsaturated fatty acid enrichment increases lipid radical formation in cultured U937 human monocytic leukemia cells exposed to oxidative stress. The cells were grown in media containing 5% fetal bovine serum and either no supplemental fatty acid, 10 μM 18:1, or 10 μM 22:6. After 2 days, the cells were isolated from these media, washed, and resuspended in chelating resin-treated phosphate buffer. The final mixture contained 10 mM POBN and 4×10^6 cells in a total volume of 0.5 mL. Following transfer to a quartz flat cell, 80 μM $FeSO_4$ was added, and the EPR spectra was recorded at room temperature with a Bruker ESP 300 spectrometer operating at 9.79 GHz and 100 kHz modulation frequency. (A), cells enriched with 22:6 but no $FeSO_4$ added; (B), cells grown in medium without supplemental fatty acid, exposed to $FeSO_4$; (C), cells enriched with 18:1, exposed to $FeSO_4$; (D), cells enriched with 22:6, exposed to $FeSO_4$.[81]

Ether lipids

The ether lipids are a new class of anticancer agents that are of particular interest because they represent a different strategy for killing neoplastic cells. They have no direct effect on DNA and are additive[84] or synergistic[85] with drugs that interfere with DNA synthesis. These agents are membrane targeted, as might be expected, because their chemical structure is that of a lysophospholipid derivative, similar to platelet activating factor. They contain an ether group in the *sn*-1 position, a short chain hydrocarbon at the *sn*-2 carbon, and a phosphocholine group similar to that present in phosphatidylcholine at the *sn*-3 carbon. The prototype compound is edelfosine *(Figure 3);* derivatives have substitutions in diverse parts of the molecule, especially in the *sn*-1 position. They are currently undergoing clinical trials in Europe for patients with solid tumors. Ether lipids are particularly promising as drugs for use in vitro to remove residual neoplastic cells from bone marrow collections prior to reinfusion during autologous bone marrow transplantation for treatment of lymphoma and leukemia.[86] There is evidence that these lipid derivatives may damage neoplastic cells to a greater extent than normal cells, thereby providing the selectivity necessary for effective anticancer therapy.[87,88]

The cytotoxicity of ether lipids has been linked to many mechanisms of action, but the central one remains unidentified. They have been reported to interfere with inositol phosphate calcium signaling,[89] disturb phospholipid metabolism,[88,90] modulate protein kinase C activity,[91,92] induce cellular differentiation,[92-95] activate macrophages,[88] or inhibit phosphatidylinositol-specific phospholipase C.[96]

We developed methods that allow modification of the fatty acids contained in plasma and intracellular membranes of cancer cells.[5,51] We hypothesized that modification of the membrane target of ether lipid drugs would influence cytotoxicity and provide further insights into the mechanism of action of this interesting class of anticancer drugs. Prior enrichment of L1210 murine leukemia cells with 22:6 resulted in a 39% increase in cytotoxicity of the thioether lipid, ilmofosine (BM 41.440).[97] The effect was dependent on extent of fatty acid enrichment, drug concentration, and time. This provides a potential new approach to therapy; however, the mechanism is not well understood. Studies with radiolabeled drug demonstrate that the effect on cytotoxicity is not due to a change in drug transport or accumulation,[97] but oxidative events remain a possibility.

Because of a related observation that glutathione depletion with buthionine sulfoximine heightened sensitivity, we exam-

EDELFOSINE

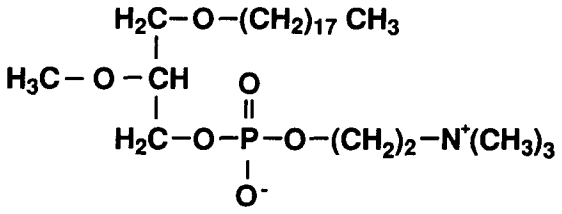

Figure 3 Chemical structure of the parent compound ether lipid edelfosine (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine).

ined the effect of members of the ether lipid class on cellular lipid peroxidation. Edelfosine, the prototype ether lipid, and ilmofosine induced peroxidation of the lipids of L1210 leukemia cells in a time-dependent and drug-concentration–dependent manner, as measured by increased thiobarbituric acid reactive substance and ethane generation.[83] The peroxidation was best detected when the cells had been previously enriched with polyunsaturated fatty acids, and it was especially pronounced in the presence of oxidative cofactors such as Fe^{2+}. There was an inverse correlation of cell survival with peroxidation, suggesting that the cytotoxicity and peroxidation might have a linked biological relationship.

This suggestion of drug-induced oxidative stress was investigated further by measuring changes in cellular oxidative events. The generation of ascorbate free radical from ascorbic acid added to the incubation medium can be used as a general measure of cellular oxidative state.[98] After the addition of edelfosine to medium containing L1210 cells, 100 μM ascorbic acid and 20 μM Fe^{2+}, there was a two- to three-fold increase in ascorbate radical detected by EPR and spin trapping, indicating heightened cellular oxidative stress *(Figure 4)*.[82,83] This increase lasted about 200 seconds before returning to baseline, which indicates a burst of free radical production and short radical half-life.[82]

Except for these observations, the role of the membrane lipids in the ether lipid-induced heightened oxidative stress remained undefined. We recently detected a lipid-derived free radical from L1210 cells enriched in 22:6 and have demonstrated that the ether lipid edelfosine enhanced the generation of this radical.[82] The radical is detected in real time from live cells after the addition of the drug to the incubation medium. It has the spectral characteristics of an alkyl radical. The chemical structure of ether lipids suggests that metabolism of the drug is not responsible for the free radical generation, so we hypothesize that it is a result of an interaction of membrane polyunsaturated fatty acids with the drug during an event that both generates a radical and leads to cell damage. This process occurs within minutes, prior to detectable cytotoxicity. The ability to monitor free radical events, either through measurement of the oxidative state or of the specific lipid-derived species, can be applied to other anticancer drugs that may have oxidative components to their mechanisms of action. These include doxorubicin, mitomycin, vincristine, or bleomycin.

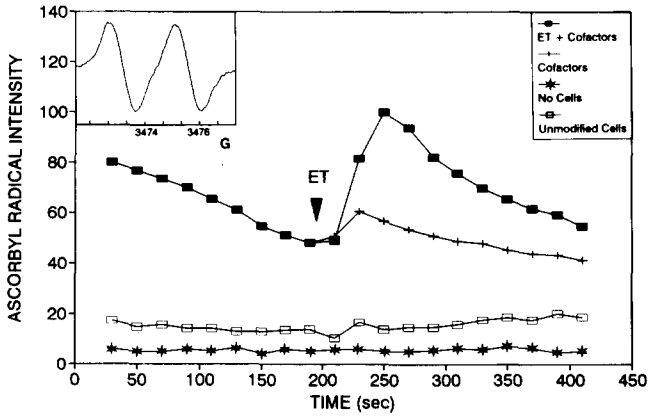

Figure 4 Effect of edelfosine on ascorbate free radical intensity generated by 22:6-enriched L1210 cells. Shown are the ascorbate radical, monitored by EPR, during incubation with Fe^{2+} (20 μM) and ascorbic acid (100 μM). At the arrow, 40 μM edelfosine (ET + Cofactors) or diluent (Cofactors) was added. Also shown are the radical intensity in cells not previously enriched with supplemental fatty acid (unmodified cells) and in the absence of cells (No cells) when medium and cofactors were present and edelfosine was added at the arrow. *Inset*, representative ascorbate radical doublet on EPR spectrum (a^H = 1.8 G).[83]

Conjugated fatty acids

There are naturally occurring polyunsaturated fatty acids that have conjugated systems of double bonds. These conjugated fatty acids are much more unstable than ordinary polyunsaturated fatty acids, which have a single methylene carbon between each pair of double bonds. Parinaric acid, a conjugated fatty acid that contains four double bonds (9,11,13,15-18:4), has been used as a probe in biological studies because it enters cells, localizes in membranes, and is fluorescent.[99,100]

We originally tested parinaric acid to determine whether it might be more effective than ordinary polyunsaturated fatty acids in enhancing therapeutic modalities that generate free radicals. However, studies with parinaric acid in the U937 human leukemia cell line demonstrated that this conjugated fatty acid was itself cytotoxic in the concentration range of 1–4 μM.[101] These concentrations of parinaric acid also inhibited the growth of HL-60 cells, another undifferentiated human leukemia cell line, and the Y-79 human retinoblastoma cell line. An oxidative mechanism appears to be involved because the cytotoxic effect of 1.5 μM parinaric acid in U937 cells is almost completely inhibited by the addition of 1.5 to 3.0 μM butylated hydroxytoluene.[101] Small amounts of parinaric acid are taken up by the U937 cells. This may make the intracellular lipids more susceptible to ordinary levels of oxidative stress to the point of producing a cytotoxic effect.

A similar type of antitumor effect has been obtained with conjugated derivatives of linoleic acid (CLA). These fatty acids contain 18-carbons and a conjugated pair of double bonds. When rats are fed CLA, the development of mammary tumors induced by dimethylbenz(a)anthracene is reduced, and the final tumor incidence and tumor weight is decreased.[102] The maximum antitumor effect was obtained when 1% CLA was present in the diet. CLA also has an antioxidant effect, but concentration-dependence data indicate that this cannot fully explain the antitumor action.

Although still at a very preliminary stage, the initial results with CLA and parinaric acid suggest that conjugated polyunsaturated fatty acids may have promise as antitumor agents. Additional studies are needed to define the mechanism of action of this class of compounds and determine their therapeutic potential.

Photodynamic therapy

Malignant tumors can be destroyed by increasing their sensitivity to light. This process requires a photosensitizing drug that localizes in the tumor, a visible light source, and oxygen to elicit cellular damage. Because light fails to penetrate to great depths, photosensitization has been especially studied for the treatment of superficial cancers of the skin or tumors accessible by fiber optic devices via endoscopy, such as

those of the bronchus, urinary bladder, or esophagus. Deeper tumors can be treated by exposure of the tumor bed to light during surgery. There is also promise for this approach in bone marrow purging prior to transplantation.[103] The clinical use of photodynamic therapy has been reviewed recently.[104]

There is in vitro evidence that this type of antitumor therapy is more toxic to malignant cells as compared with nonmalignant cells.[103] In vivo there is selectivity in part due to the fact that the drugs are inactive in the dark and therefore do not affect internal organs. In addition, there may be increased drug uptake by some,[105] but not all[106] malignant cells. However, photodynamic therapy may kill tumors in a large part by interfering with vascular structures[107] because endothelial cells are highly sensitive to oxidative stress.[108,109] In addition, there is enhanced thromboxane release into the serum when photodynamic therapy is directed at rat tumors compared with tissues in the animal not having tumors; furthermore, indomethacin inhibited vascular stasis and cytotoxicity of photodynamic therapy, suggesting that thromboxane production is associated with these events.[110]

Photodynamic therapy is cytotoxic because of the absorption of visible light by the systematically administered photosensitizing drug localized in the tumor. With light exposure, the drug converts to an excited triplet state, and subsequently this excitation energy is transferred to oxygen to form singlet oxygen.[111,112] Thereafter, lipid peroxidation occurs and manifests chemically as production of thiobarbituric acid reactive substances[113] via the generation of lipid hydroperoxides.[113-115] The fact that glutathione peroxidase protects cells from photosensitization is further evidence that lipid peroxidation is central to the cytotoxicity.[114]

The sensitizing drugs most commonly used for experimental studies contain a porphyrin ring structure, e.g., benzoporphyrin, hematoporphyrin derivative, or for clinical studies, the more purified Photofrin (QLT Phototherapeutics, Inc, Vancouver, British Columbia, Canada). The dye merocyanine 540 has also been studied in vitro for its photosensitizing properties.[116,117] New photosensitizing drugs are being tested.[105,118-120]

Our recent work in this area has been based on the use of membrane lipid polyunsaturation to enhance photodynamic therapy. Most photosensitizing drugs are hydrophobic and localize in membranes,[116] including those of mitochondria or lysosomes.[105] The lipid peroxidation induced by photodynamic therapy makes it likely that membrane polyunsaturated lipids are prominent targets and that free radical mechanisms play a major part. Therefore, we used the EPR spin trapping technique that allows us to detect lipid-derived free radicals using POBN as the spin trap.[81,82] L1210 cells were exposed to visible light (tungsten bulb) in the presence of Photofrin and oxidative cofactors Fe^{2+} plus ascorbic acid.[121,122] A POBN/lipid radical spin adduct was detected *(Figure 5)*. The extent of radical production was considerably greater than in the dark or without Photofrin. In the absence of Fe^{2+}, Photofrin photosensitization resulted in low levels of radical formation. However, during light exposure a build up of lipid hydroperoxides apparently occurred because the subsequent addition of Fe^{2+} initiated a three-fold higher burst of radical formation as compared to that when Photofrin, light and Fe^{2+} are present simultaneously. In addition, increasing the oxidizability of membranes by growing the

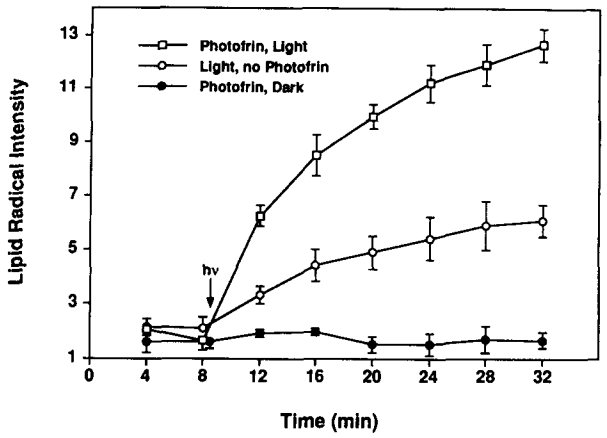

Figure 5 The generation of lipid-derived free radical from photodynamic therapy. Shown is the EPR signal intensity of the radical produced from L1210 cells (2×10^6/mL) with Fe^{2+} (5 μM), ascorbate (100 μM) and POBN spin trap (25 mM). Light was turned on at hν. Shown are the mean and SEM of three experiments.[122]

L1210 cells in fatty acid-supplemented media to enrich their lipids with polyunsaturated fatty acids resulted in greater lipid radical formation and lower cell survival. Free radical production was inversely correlated with cell survival, suggesting that free radical-mediated peroxidation events are central to Photofrin photosensitivity. Our findings demonstrate the importance of membrane lipid-derived free radical-mediated peroxidation in cellular photodynamic cytotoxicity. Prooxidant conditions and increased polyunsaturation of the fatty acids enhance these peroxidative processes and may have clinical utility.

Conclusions and perspective

As illustrated in *Figure 1*, the fatty acid composition of cancer cells is subject to variation, depending on the type of fatty acid available in the circulation of the host. Therefore, the fatty acid composition of a tumor, including the membrane lipid bilayer, depends to some extent on the dietary fat. It should be possible to alter the composition, especially the type and amount of polyunsaturated fatty acid by changing the dietary fat intake, administration of fat supplements, or in the case of solid tumors, regional perfusion with fat emulsions.

Experimental studies indicate that enrichment of leukemic lymphocytes with polyunsaturated fatty acids increases the cytotoxicity of certain therapeutic modalities, including doxorubicin, an anthracycline drug, and hyperthermia. These therapeutic modalities generate free radicals. Leukemia cells enriched in 22:6 generate more lipid radicals when they are exposed to iron-induced oxidant stress, antitumor ether lipids, or photodynamic therapy in the presence of hematoporphyrins. Taken together, these results suggest that increases in polyunsaturation may make cancer cells more susceptible to antitumor treatment modalities that produce free radicals.

Polyunsaturated fatty acids containing conjugated systems of double bonds also appear to be effective in cancer treatment. These fatty acids have a direct cytotoxic effect and also may act as tumor suppressors.

While these results are promising, the critical issue of whether lipid-based treatment strategies have any selectivity for neoplastic cells, as opposed to the host tissues, has not been thoroughly evaluated. Such information is needed to assess the therapeutic potential of the lipid modification approach. Another uncertainty relates to the fact that all of the currently available in vivo tumor lipid modification data are from rodents. While similar modifications have been produced in the human U937 monocytic leukemia and Y79 retinoblastoma cells, this has only been done in tissue culture, and it is uncertain whether clinically significant tumor lipid modifications can be produced in humans. Such studies can easily be done, however, because the necessary diets, fat supplements, and parenteral lipid emulsions already are in use and do not appear to be harmful to normal individuals. Studies demonstrating antitumor effectiveness and safety are needed in experimental animals, however, before it will be justified to extend lipid-targeted therapy to human cancer.

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