J. Food Sci. Nutr.
Vol. 1, No 1, p. 143~150(1996)

Therapeutic Potential of Membrane Fatty Acid Modification in Tumor Cells

-Review-

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Abstract

The membrane fatty acid composition of tumor cell can be modified either in cell culture by altering the lipid composition of the medium or during growth in animals by changing the dietary fat composition. These modifications are associated with changes in membrane physical properties and certain cellular functions, including carrier-mediated transport and enzyme contained within the membrane. Such effects influence the transport of nutrients and chemotherapeutic agents in cancer cells. Fatty acid modification also can enhance the sensitivity of the neoplastic cell to chemotherapy. The alteration in plasma membrane composition will be affected through dietary supplementations and the potential value to cancer patients could be a better understanding of the effects of diet on responsiveness of neoplasms to chemotherapy, i.e. cancer patients' chances for a "cure" can be improved by diet changes prior to treatment.

Key words: membrane fatty acid, tumor cell, dietary modification, chemotherapy

INTRODUCTION

The lipid molecules in biological membranes are arranged as a continuous double layer. This lipid bilayer provides the basic structure of the membrane and serves as a relatively impermeable barrier to the passage of ions and most water-soluble molecules. The most abundant lipids of biological membranes are the phospholipids. The typical phospholipid molecule has a polar head group and two hydrophobic hydrocarbon tails. The central core of fatty acid chains in lipid bilayer interacts with the proteins that penetrate into the membrane, including enzymes and transporters. Many different kinds of fatty acid chains normally make up the lipid bilayer. They usually contain an even number of carbon atoms, between 16 and 22. About 35 to 40% of fatty acids are saturated; the remainder are unsaturated and contain between 1 and 6 double bonds. Any change in the composition of the fatty acid chains can alter the structure of the lipid core and thereby has the potential to affect its normal barrier function, as well as the responsiveness of the integral proteins with which the core interacts.

A widely used method for investigating the role of lipids in biological membrane function is to alter the fatty acyl group composition of the membrane phospholipids. Most of the initial studies were done with microorganisms, erythrocytes, and hepatocytes(1-5). These works have been extended to tumor cells, and it was found that the tumor cell responded in a manner similar to a nonmalignant cell. Tumors obtain a substantial amount of fatty acid from the circulating free fatty acid of the host(6,7). Studies with Ehrlich ascites tumor cells indicated that extracellular fatty acids are rapidly taken up(6,8) and that there is a rapid turnover of intracellular phospholipid fatty acyl groups(9). The availability of free fatty acids regulates the rate of membrane fatty acid synthesis and chain elongation in the tumor cell. Tumor cells possess a very active chain elongation system that contributes significantly to the total fatty acid biosynthetic activity. The phospholipid structure formed with the changed composition of the circulating fatty acids may have different membrane properties and cell function in a rapidly growing tumor. The aim of this review is to assess the therapeutic potential of membrane fatty acid mod-

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ification in cancer cells. The present study is discussed about the membrane fatty acid modification of tumor cells, changes in membrane physical and functional properties by the alterations of fatty acid composition, and increased sensitivity of the neoplastic cells to certain forms of therapy.

FATTY ACID MODIFICATIONS

The fatty acid composition of biological membranes can be modified experimentally. This can be accomplished in tissue culture by altering the lipid composition of the growth medium or in the intact animal by changing the dietary lipid composition(10-14). Polyunsaturates have been focused to manipulate the fatty acid composition of cells. The polyunsaturated fatty acids normally present in animals can not be synthesized *de novo* and, therefore, are ultimately derived from the environment as opposed to saturated and monounsaturated fatty acids. There are two main types of polyunsaturates, the n-6(ω-6) type and n-3(ω-3) type. Linoleic acid(18 : 2) and arachidonic acid(20 : 4) are involved in n-6 polyunsaturated fatty acid. Linoleic acid is not synthesized by mammals and is, therefore, important dietary requirements. Plants can synthesize linoleic acid and are the original source of this fatty acid in our diet. Arachidonic acid is the substrate for prostaglandin and leukotrience synthesis in mammalian tissues. The member of n-3 polyunsaturated fatty acids are linolenic acid(18 : 3), eicosapentaenoic acid(20 : 5), and docosahexaenoic acid(22 : 6). Linolenic acid is synthesized by cold-water vegetation, and the fish that feed on these vegetations convert the 18 : 3 to the 20 : 5 and 22 : 6. Oils from cold-water, fatty fish such as mackerel are particularly rich in eicosapentaenoic acid and docosahexaenoic acid.

Substantial differences in the phospholipid fatty acid composition can be produced in Ehrlich ascites tumor cells by feeding fat supplemented diets to the tumor-bearing mice(15). The diets contained either sunflower seed oil, which has 70% linoleic acid, or coconut oil, which has 93% saturated fatty acid. There was no change in the membrane phospholipid or cholesterol content, while the plasma membrane fatty acid composition of the tumor cells was modified. Monoenoic acids made up for only 17% of the fatty acids with sunflower oil diet, whereas they made up for 36% with coconut oil diet. Conversely, polyenoic acids of the linoleate family accounted 38% of the fatty acids with sunflower oil diet but only 18% with coconut oil diet (10). Research(16) also clearly demonstrated that the plasma membrane fatty acid composition in L1210 murine leukemia cells was dependent upon the type of fat fed to the host animal. As in the case of the Ehrlich ascites tumor cells, what occurs is a substitution of phospholipid fatty acyl chains without any disruption in the usual membrane lipid bilayer structure. Fig. 1 shows the differences in the fatty acid composition of the L1210 plasma membranes when the cells were grown in mice fed the 2 different diets, polyunsaturated fat-rich(16% sunflower seed oil) diet or saturated fat-rich(16% coconut oil) diet. There was no appreciable change in the saturated fatty acid content of the plasma membrane, which accounts for about 40% of the membrane fatty acids. However, plasma membranes prepared from cells grown in animals fed the polyunsaturated sunflower oil diet contained almost 2-fold more polyenoic fatty acids than those prepared from cells grown in animals fed the saturated coconut oil diet. The membranes from cells grown in mice fed the coconut oil

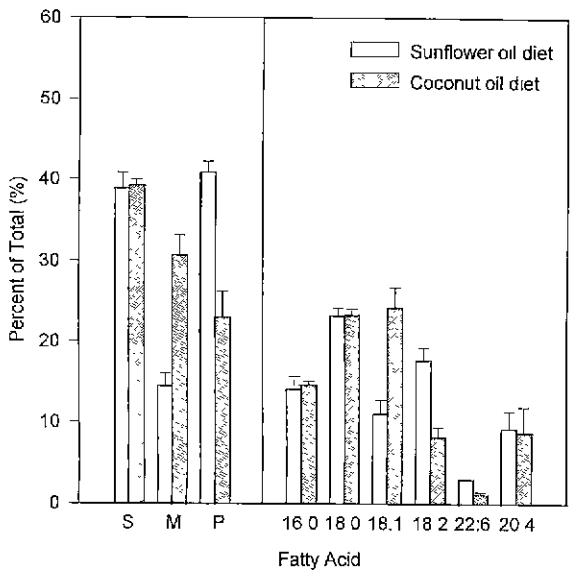

Fig. 1. Differences in plasma membrane fatty acid composition of the L1210 leukemia cells with dietary lipid modification.
The percentage composition is shown as the mean± S.E. of values from 3 separate membrane preparations. Left, distribution of fatty acids among the saturation classes. S: saturated, M: monoenoic, P: polyenoic. Right, values for individual acids The fatty acids are abbreviated as number of carbon atoms : number of double bonds.

diet contained 2-fold greater proportions of monoenoic fatty acids than control. Among the individual fatty acids, the main differences were an enrichment of oleic acid(18 : 1) in the coconut oil group and an enrichment of linoleate(18 : 2) in the sunflower oil group. There were 34% more unsaturated double bonds in the membranes from the cells grown on the polyunsaturated sunflower oil diet.

The dietary approach is also effective in modifying the membrane fatty acid composition of solid tumors. For example, there were large differences in the fatty acid composition of a transplanted mammary adenocarcinoma when the tumor-bearing mice were fed with diets containing corn oil as opposed to hydrogenated cottonseed oil(17). The phospholipid fatty acid composition in rat hepatoma 7288 CTC was modified by changes in dietary fat content(18). The plasma membranes lipid composition of murine $HSDM_1$ fibrosarcoma also have been modified in response to changes in the type of dietary fat(19).

For a precise evaluation of specific fatty acid substitutions, tumor cells grown in culture can be modified by the individual fatty acids. Many different types of fatty acid can be incorporated. Fatty acid substitutions occur in the plasma membrane(20) and microsomes of retinoblastoma cells(21,22). The extent of enrichment with a particular fatty acid is dependent upon the amount of the supplemental fatty acid added to the cell culture medium(23). In addition to allowing for more varied fatty acid substitutions, the cell culture approach also can produce more extensive enrichments than the dietary modification procedure. For example, when L1210 leukemia cells are enriched with 22 : 6, there are almost 3-fold more polyunsaturated fatty acid content of the phospholipids and a corresponding 58% reduction in monounsaturates(24).

The phospholipids in membrane of various tumor cells can be modified by supplementing with free fatty acids, and the cell growth inhibition was associated with the membrane fatty acid modification(25).

Zhu et al.(26) reported that the Ehrlich ascites carcinoma cells grown in ACR mice treated with linoleic acid exhibited appreciable changes in their fatty acid composition, and highly increased linoleic acid content. The linoleic acid also prolonged the life spans of the Ehrlich ascites carcinoma-bearing mice.

In our study, the fatty acid composition of AZ-521 human gastric cancer cells has been changed when linoleic acid was added to the culture media. The contents of linoleic acid(18 : 2), arachidonic acid(20 : 4) and docosahexaenoic acid(22 : 6) significantly increased, concomitantly the morpholagical change of the cells was observed(27). This fact implicated that the modified lipid composition of the cell membrane might affect regulations of other genes(28). The modification of the fatty acid composition of tumor cells also correlated with antitumor effect of linoleic acid(26).

MEMBRANE FLUIDITY

The fatty acid modifications in Ehrlich ascites carcinoma and L1210 leukemia cells produce physical changes of the plasma membrane as detected by electron spin resonance probes. The differences in the packing density of the membrane lipids, as indicated in the electron spin resonance order parameter, occur in the cells modified either by the dietary procedure (16,29) or in cell culture(20,30). Plasma membranes enriched in polyunsaturated fatty acids have the lowest order parameters, indicating more fluid lipid environment. The membrane fluidity is altered by the modifications in fatty acyl saturation since there is no appreciable difference in membrane cholesterol content, phospholipid head group composition, or fatty acid chain length. The polyenoic fatty acids decrease order and packing within the membrane lipid bilayer since their hydrocarbon chains are bent in a rigid configuration. This should reduce the magnitude of van der Waals interactions with surrounding acyl chains, lowering the melting temperature and increasing mobility. Such an effect was observed when the membrane phospholipids of Ehrlich ascites cells were enriched with either linoleic acid(18 : 2) or linolenic acid(18 : 3)(20). The membranes from L1210 leukemia cells grown in the mice fed sunflower oil had lower order parameter values than those grown in the mice fed the coconut oil diet(16).

Another electron spin resonance parameter is the temperature dependence of the rotational correlation time of the nitroxide spin-labeled molecule. Two discontinuities normally are noted in plasma membrane preparations, one at 31.5°C and the other at about 22°C. Discontinuities are used to identify the transition tem-

peratures at which conformational changes are believed to occur in the lipid bilayer of biological membrane. Changes of more than 5°C have been observed in the lower transition temperature(16,20,25). The temperature of this transition decreases with the increase of polyunsaturation of the plasma membrane. This also indicates that the membrane enriched with more unsaturated fatty acid has a greater fluidity. These results suggest that the fatty acid modifications produced in tumor cell membrane are sufficient to alter the fluidity of the plasma membrane, as measured with spin label probes.

EFFECTS ON MEMBRANE TRANSPORTERS AND ENZYMES

Many substances enter eukaryotic cells by combining with macromolecular carriers embedded in the membrane lipid bilayer(31). A number of different types of macromolecules(enzymes, transport systems) present in the membrane appear to be regulated by the composition of the surrounding lipids. Fatty acid modifications sufficient to alter the physical properties of certain regions of the plasma membrane may affect the function of membrane proteins located in these regions. Such effects may influence the uptake of nutrients and chemotherapeutic agents in neoplastic cells. The effects on carrier-mediated transport systems have been observed in several cases(16,21,22,32). Amino acid transport system is regulated by changes in the fatty acid composition of the cell membrane in Ehrlich ascites tumor cells. Changes in membrane fatty acid composition were associated with changes in membrane fluidity and in α-aminoisobutyric acid uptake process(32). Appreciable differences were noted when transport of the antineoplastic drug methotrexate by intact L1210 leukemia cells from animals fed the sunflower or coconut oil diets was compared(16). Table 1 illustrates the effect of lipid modifications on methotrexate transport. The K'_m(apparent K_m) for transport process by the cells from animals fed the polyunsaturated fat-rich sunflower oil diet was about 30% lower than the cells from mice fed the saturated fat-rich coconut oil diet. This value was statistically significantly different in the 2 diet groups($p<0.02$). However, the difference of V'_{max}(apparent V_{max}) for the transport process was not significant in the cells from

Table 1. Kinetic parameters for methotrexate transport

Diet	Kinetic parameters	
	K'_m[1] (μM)	V'_{max}[2] (pmol/5×10^7cells/min)
Sunflower	2.90±0.35	9.31±0.70
Coconut	4.10±0 10	11.75±1.08

[1]The values for two groups are significantly different($p<0.02$)
[2]The values for two groups are not significantly different ($p>0.05$)

animals fed diets containing the 2 different types of lipid. It was concluded that in the lower range of methotrexate concentrations, the cells having the more fluid membranes exhibited greater permeability to methotrexate and thus that changes in membrane lipid structure and physical properties brought about by fatty acid modification were sufficient to affect the entry of an antineoplastic drug into the L1210 cell. Results similar to these were obtained for carrier-mediated α-aminoisobutyric acid transport in fatty acid-modified Ehrlich ascites tumor cells(32). There were no significant differences in the K'_m values for the cells grown on the regular and sunflower oil diets, but the value was about twice as large for the cells grown on coconut oil diet($p<0.02$). There also was a slight increase in the V'_{max} value for the cells grown on coconut oil, but this difference was not significant ($p>0.05$). This indicated that increased membrane fluidity also was associated with a lower apparent K_m for the sodium-dependent component of α-aminoisobutyric acid transport but no change in apparent V_{max} for this process. The differences of the K'_m in the carrier-mediated transport systems explain that the structure of the carrier or of its binding site may be affected by changes in the composition of the surrounding fatty acyl tails. Alternatively, the accessibility of nutrients and chemotherapeutic agents to the binding sites of the carriers may be influenced by the surrounding lipid structure. No difference in V'_{max} suggests that the lipid alterations had no effect on the number of carrier molecules contained in the membranes of the cells that were enriched with polyunsaturated fatty acids or enriched with saturated fatty acids.

Lipid dependence has been demonstrated for enzymes that are contained in membranes(33,34). The activity of membrane-bound enzymes is controlled by the fatty acid composition of the membrane phospholipids(35-38).

Table 2. Activation energies for $(Na^+ + K^+)$-ATPase of plasma membranes with different fatty acyl compositions from Ehrlich ascites tumor cells

Activation energy (kcal/mol)	PM_C[1)]	PM_{SU}[1)]	PM_{TS}[1)]
Below T_{tr}[2)]	33.4	27.7	60.0
Above T_{tr}	18.0	19.7	21.8

[1)]PM_C, PM_{SU}, and PM_{TS} refer to plasma membranes from tumor cells grown in mice fed a regular chow diet, 16% sunflower oil diet, and 4% tristearin diet, respectively
[2)]Transition temperature

Alteration of the plasma membrane fatty acyl composition from Ehrlich ascites tumor cells produced changes in transition temperature and activation energy of membrane-bound $(Na^+ + K^+)$-ATPase(39). Activation energies of $(Na^+ + K^+)$-ATPase from Ehrlich ascites tumor cell membranes are given in Table 2. Energy of activation below the transition temperature was about twice as high for the plasma membrane of cells obtained from mice maintained on 4% tristearin diet (PM_{TS}) as for either 16% sunflower oil diet(PM_{SU}) or regular chow diet containing a mixture of fatty acids (PM_C). The values for PM_C and PM_{SU} were similar, in agreement with the fact that their fatty acyl compositions were quite similar. Energies of activation above the transition temperature were about the same in all three cases. It indicates that dietary lipid induced changes in the plasma membrane fatty acyl composition result in altered properties of the $(Na^+ + K^+)$-ATPase associated with these membranes. Other work (35) has also found that the fatty acyl groups and membrane fluidity are important for $(Na^+ + K^+)$-ATPase activity. Certain fatty acids supplementations of LM cell membranes increased the basal activity of membrane-bound adenylate cyclase(12). The differences between the basal activities of different supplemented membranes disappeared when the enzyme was solubilized. This suggested that the alteration in activity was a consequence of changes in the lipid/protein interactions rather than changes in the amount of the enzyme in the intact membrane.

EFFECTS ON CHEMOTHERAPY

Since the plasma membrane is the first barrier encountered by the drug, it is important to consider the interaction of anticancer drugs with cellular structure for understanding the cytotoxicity of these agents. Fatty acid modification might make the neoplastic cell more sensitive to chemotherapy because the membrane is a target of its action(40,41). P388 leukemia cells sensitive to adriamycin have a different phospholipid composition and membrane fluidity than resistant sublines(42, 43). The order parameter of the fatty acid spin probe for the measurement of membrane fluidity decreases in Sarcoma 180 ascites cells exposed to adriamycin and is dose-related(44).

The modulation of membrane fluidity with associated effects on membrane functions may be relevant to the cytotoxicity of adriamycin. Enrichment of L1210 murine leukemia cell membranes with polyunsaturated fatty acids considerably increases their sensitivity to the cytotoxicity of adriamycin(24,45). Fig. 2 shows the effect of six different fatty acids added to culture medium on adriamycin sensitivity. The survival of the cells determined using a soft agar clonogenic assay after exposure to 0.4μM adriamycin for 2 hours. Enrichment with polyunsaturated fatty acids resulted in enhanced sensitivity to adriamycin as compared to enrichment with monounsaturated fatty acids. The increased adriamycin cytotoxicity for the fatty acid-modified cells was directly proportional to the degree of unsaturation of the fatty acids selected for the modification. Cytotoxicity was least for oleate(18 : 1) and

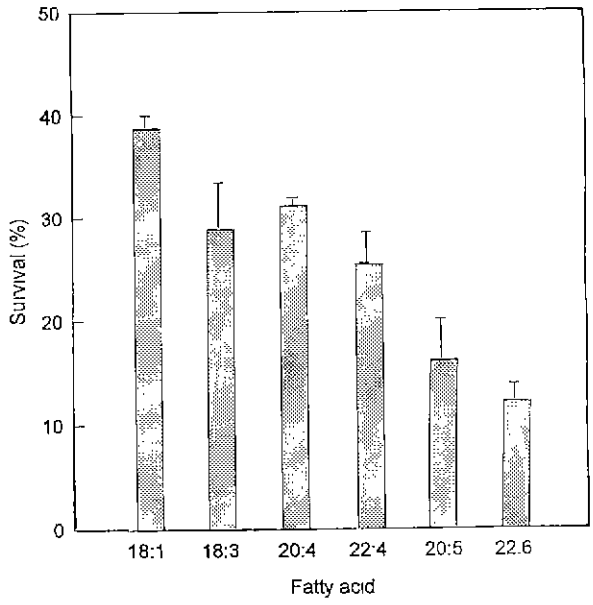

Fig. 2. Effect of fatty acids added to culture medium on adriamycin sensitivity.
The percentage of surviving cells is shown as the mean±S.E. of triplicate experiments.

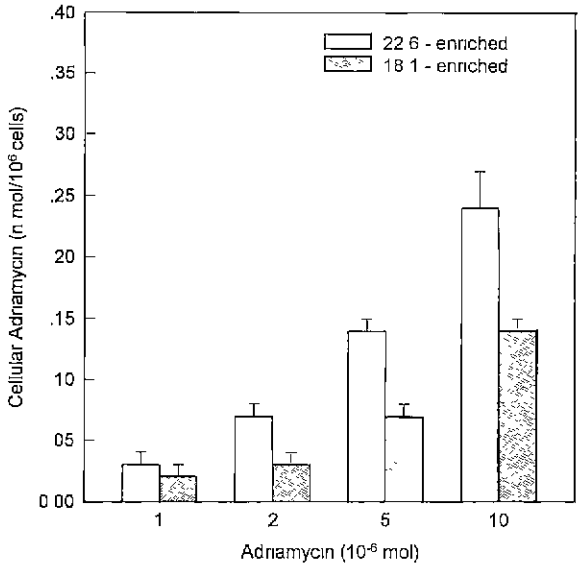

Fig. 3. Effect of fatty acid modification on cellular adriamycin accumulation.
Shown are the mean±S.E. of triplicates.

greatest for docosahexaenoate(22 : 6)-enriched cells. A study with different polyunsaturated fatty acids indicates that there is a high linear correlation of the number of double bonds per phospholipid fatty acyl chain and adriamycin cytotoxicity(24). The augmented adriamycin sensitivity of L1210 cells enriched with polyunsaturated fatty acids is due to increased cellular accumulation of the drug. Fig. 3 is the result of fluorescence assay to measure intracellular adriamycin in fatty acid-modified cells. It shows that the 22 : 6-enriched cells accumulated greater amounts of adriamycin as compared to 18 : 1-enriched cells at all concentrations above 1μM(24). The larger accumulation was probably is due to a greater rate of influx of adriamycin since there is no change in the rate of efflux. Adriamycin resistance is associated with enhanced active efflux of the drug in the P388 leukemia and human ovarian cell lines(46). The greater drug accumulation in the cells enriched with polyunsaturates could account for the enhanced cell-kill. It clearly indicates that an alteration of the chemical structure and physical properties of membranes affects sensitivity of a neoplastic cell to adriamycin. Thus, polyunsaturated fatty acid enrichment may be a good approach to sensitize the neoplastic cells to the cytotoxicity of anticancer drugs.

SUMMARY

Substantial modifications of the membrane fatty acid composition in a neoplastic cell can be produced. When the fatty acid composition is modified in a membrane, some of the saturated and monounsaturated fatty acyl groups are replaced by polyunsaturated groups without disrupting the basic structure of the membrane. The structural changes within the hydrophobic core of fatty acids in the lipid bilayer may influence the conformation and properties of some of the transport systems and enzymes contained within the membrane. This may affect their localization within specific lipid domains or modulate their function. Such effects may influence the response of the neoplastic cell to certain stimuli. The finding that the membrane lipid modifications that can be produced by diet during growth of the tumor are sufficient to influence antineoplastic drug transport or increase its sensitivity to certain forms of therapy is of considerable potential significance. Therefore, membrane fatty acid modification of neoplastic cells may be of potential value as a therapeutic approach designed to augment the cytotoxicity of other antineoplastic therapies. It is expected that dietary changes prior to chemotherapy may assist in improving the effectiveness of the chemotherapeutic treatment of cancer patients.

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(Received May 9, 1996)