THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 4, Number 1, 1998, pp. 87–99
Mary Ann Liebert, Inc.

PERSPECTIVE

Some Biological Actions of Alkylglycerols from Shark Liver Oil

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ABSTRACT

Shark liver oil has been used for over 40 years as both a therapeutic and preventive agent. The active ingredients in shark liver oil have been found to be a group of ether-linked glycerols known as alkylglycerols. Initial clinical use was for treating leukemias, and later to prevent radiation sickness from cancer x-ray therapy. Studies over the last 30 years have shown that alkylglycerols are multifunctional. The level of natural alkylglycerols rises within tumor cells, apparently in an effort to control cell growth. Recent studies indicate that the activation of protein kinase C, an essential step in cell proliferation, can be inhibited by alkylglycerols. This action suggests a competitive inhibition of 1.2-diacylglycerol by alkylglycerols. Further studies on the immunostimulatory action of alkylglycerols suggest a primary action on the macrophage. The process of macrophage activation has been demonstrated with both synthetic and natural alkylglycerols. While the exact mechanism has not been found, both an autocrine and paracrine system have been suggested. Shark liver is a major natural source of alkylglycerols, which have no known side effects in dosages of 100 mg three times a day. The information presented in this article suggests that alkylglycerols may be used both as an adjunct therapy in the treatment of neoplastic disorders and as an immune booster in infectious diseases.

INTRODUCTION

The recent upsurge of interest in alternative medicine suggests that more information about the natural products be made available to physicians who show a proclivity for this branch of medicine. Shark liver oil has been used for many years in Europe as an adjunct treatment for several types of cancer (Brohult, 1963). Some of the active ingredients in shark liver oil known as alkylglycerols have been extensively studied both *in vivo* and *in vitro*. Many synthetic analogs of these ether compounds have been made and investigated. The purpose of this article is to bring to the attention of the physician some of the scientific support for the

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use of these compounds both as adjunct therapy and as a prophylactic, or preventive measure against disease states characterized by neoplasia, immune dysfunction, or inflammation.

The alkylglycerols are a naturally occurring group of lipids characterized by an ether linkage in their molecular structure (Weltzien and Munder, 1983). These compounds have been known and studied for over 50 years, mainly for their ability to reduce radiation damage, suppress tumor growth, increase hemopoesis, and to accelerate wound healing (Bodman and Maisin, 1958; Watkins and Giffin, 1933; Caldwell et al., 1945; Ghys, 1960; Brachwitz et al., 1987; Werbach, 1994). In the last 10 years the role of the alkylglycerols in stimulating the human immune system has become apparent and is receiving increased attention from investigators. One major effect of the alkylglycerols on the immune system appears to center in the activation of macrophages. A second cellular effect appears to be the inhibition of protein kinase C in proliferative reactions. In this article our purpose is to introduce the subject to the reader by reviewing briefly: (1) the chemistry of the alkylglycerols; (2) the data supporting macrophage activation; and (3) the possible cellular actions of alkylglycerols with protein kinase C.

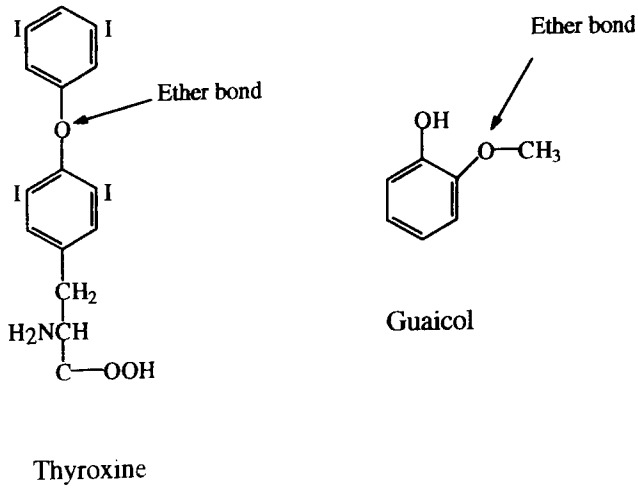


FIG. 1. Two examples of natural compounds that have ether bonds.

THE OCCURRENCE, CHEMISTRY, AND ABSORPTION OF THE NATURAL ALKYLGLYCEROLS

The ether bond is the key to understanding the unique functioning of the alkylglycerol. This bond, C—O—C, is found throughout nature in many important biological compounds, the most familiar of which is thyroxin from the thyroid gland. In the plant world, guaicol is another familiar ether linked substance. These two structures are diagrammed below in Figure 1. Glycerol ethers are quite widespread having been isolated from many life forms and in many different molecular arrangements. The basic structure of these compounds consists of a glycerol molecule with one or more of the hydroxyl groups being replaced by long chain fatty acids. The bonding of three fatty acid molecules to the glycerol molecule with an ester linkage is known as a triglyceride, or common fat. The ester linkage is characterized by —COO group where one oxygen is linked in a double bond to a carbon atom. A typical triglyceride and alkylglycerol are shown in Figure 2.

While the chemistry of the alkylglycerols in many animals and plants has been studied quite extensively, we shall limit our discussion to the main ether lipids found in shark liver oil. A note, however, on the composition of shark liver oil is in order. Natural or unprocessed shark liver oil contains high levels of vitamins A and D along with other constituents besides the ether lipids (see Table 1).

In commercial preparations of the alkylglycerols extracted from shark liver oil, many of these components are removed, or reduced in amount in the extraction process. In this article, therefore, when we speak of shark liver oil derived alkylglycerols, we are referring to a highly refined end product containing only the alkylglycerols. Typical analysis of commercial capsules reveals the presence of alkylglycerols (major component triglycerides, free fatty acids, vitamin A, Ω-3 fatty acids, squalene, vitamin E, squalamine, and trace amounts of minerals such as iron, copper, and zinc.

One of the major sources of natural alkylglycerols is the liver of the Greenland shark, *Somnious microcephalus*, which contains up to 50% of alkylglycerols. Other sources include the elasmobranch fish such as the small shark,

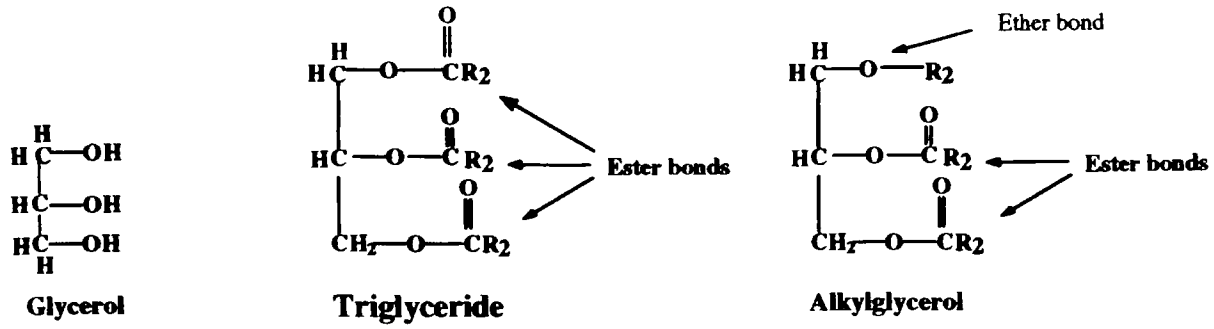


FIG. 2. Examples of glycerol-type compounds. Note that the three carbon glycerol molecule is the basic structure for the other two molecules. Note also that the triglyceride contains only ester-linked fatty acids while the alkylglycerol contains one ether linked fatty acid.

Chimaera monstrosa, and the dog fish, *Squalus acanthias*. Cod liver oil and certain mollusks are additional sources of alkylglycerols (Hallgren and Larsson, 1962a). These compounds are found in various organs of the animals studied, including bone marrow fat, spleen liver, plasma and erythrocytes, and in milk (Holmberg et al., 1962; Hallgren and Larson, 1962b). They are isolated from the unsaponifiable fraction of the oils obtained from the animals. Tsujimoto and Toyama were the first investigators to report the presence of ether lipids in shark liver oil (Tsujimoto, 1932; Tsujimoto and Toyama, 1922). Three alkylglycerols obtained from the Greenland shark are: batyl alcohol, chimyl alcohol, and selachyl alcohol. The chemical structure of these compounds is given below in Figure 3. Note that while chimyl and batyl alcohol are saturated chains, selachyl alcohol contains one unsaturated bond. Note also that all the ether bonds are on the number 3 carbon of glycerol. In the natural state the alkylglycerols are usually present as ester compounds, with carbons 1 and 2 esterified with C_{16} or C_{18} saturated fatty acids.

A fourth compound, a methoxy substituted alkylglycerol, makes up about 3.0% of the total alkylglycerols found in shark liver oil (Fig. 4). This compound appears to have potent biological activity both as an immune stimulant and as an antineoplastic agent (Hallgren et al., 1974).

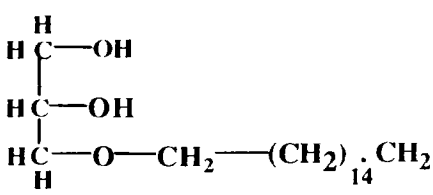
ABSORPTION AND METABOLISM

After oral ingestion of ether lipids absorption occurs from the intestine. About 95% of radioactive (C_{14} labeled) chimyl alcohol is absorbed in the intestine with 5% being excreted in the feces in one study (Bergstrom and Blomstrand, 1956). In the gastrointestinal tract, a large proportion of the ingested ether lipids in the form of 1-O-alkylglycerols are cleaved at the ether bond with the alkyl moieties giving rise to fatty acids. The remainder is incorporated into 1-O-alkyl-2,3-diacyl-*sn*-glycerols and 1-O-alkyl-2-acyl-*sn*-glycerol and 1-O-alkyl-2-acyl-*sn*-glycerol 3-phosphoethanolamines (Blomstrand and Ahrens, 1959; Paltauf, 1971). Absorption and fate of ether lipids is a separate topic, and those readers with a particular inter-

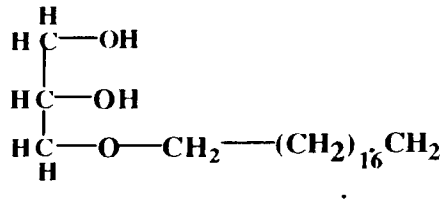
TABLE 1. PERCENTAGE COMPOSITION OF ALKOXYGLYCEROLS FROM VARIOUS SOURCES (BY WEIGHT)

Alkylglycerol	*Human bone marrow*	*Human milk*	*Liver oil Greenland shark*
14:0	2.0		
15a			07
16:0	29.4	3.9	9.1
16:1	trace	10.8b	
17.0	7.6	3.6	3.6
18:0	24.6	22.8	2.8
18:1	16.7	33.89	59.4b
18:2	1.4	1.6	
18:3			
19.a	6.1	2.4	1.5
20:0	2.9	1.6	
20:1	3.2	2.3	6.2
22:0	0.7	0.7	
22:1	5.1	3.4	2.2
24	2.1		

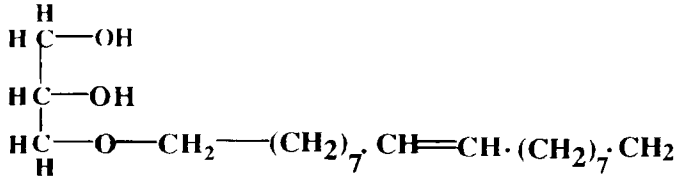
From Hallgren and Larsson (1962b). The number of carbon atoms in the first column refers to the long-chain component of the molecule. The number after the colon denotes the number of double bonds. Greenland shark liver oil contains 3–4% methoxy-substituted alkoxy-glycerols. Adapted from Brohult et al.



Chimyl Alcohol



Batyl Alcohol



Selachyl Alcohol

FIG. 3. Three naturally occurring alkylglycerols. Note that the ether linkage is on the number 3 carbon, designated *sn* 3. (*sn* means standard nomenclature and is used in glycerol chemistry.)

est are referred to the following articles: Bandi and Mangold, 1973; Weber, 1985; Paltauf, 1971.

THE ROLE OF THE MACROPHAGE IN THE IMMUNE SYSTEM

Early in the investigation and study of ether lipids, it was evident that there was an augmentation of the immune system seen in animals that ingested these compounds. The mechanism of action, however, was unknown. Recently it was discovered that the macrophage is a key cell in the initiation and operation of many immune functions. The macrophage is part of an extremely versatile and highly regulated cellular system. Possessing microbicidal activity, macrophages are equipped to recognize and destroy both intracellular and extracellular replicating invaders, whether or not these invaders are procaryotic or eucaryotic types. They are important scavengers for effector cells and molecules of host origin, as well as for exogenous compounds that they can take up, degrade and detoxify, or contain. They are known to regulate a large number of body functions including iron and lipid metabolism. The large number of secretory products generated by the macrophage helps to regulate other cells, including the fibroblasts and cells involved in the formation of myeloid component in the bone marrow (Adams and Hamilton, 1984; Cohn, 1982).

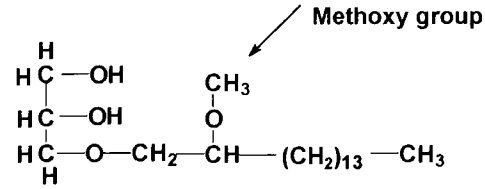


FIG. 4. The structure of the methoxy substituted alkylglycerol. This molecule has been found to have potent anticancer activity. It makes up only a small percentage of the alkylglycerols in shark liver oil, approximately 3%.

Obviously such powerful cells need to be tightly controlled, and so they are. The resident macrophages in the tissues are usually downregulated to a high degree. The process of activation, which we shall discuss in detail, comes from a variety of extracellular stimuli. It has been found, over the years, that the macrophage is a multipotential cell with the capacity to develop in many ways depending on

the specific signal it receives. This system is far from being fully understood, but the control of this delicate balance of activities is essential to life, so it has become a prime area of intensive investigation.

The macrophages originate from precursor cells in the bone marrow and pass into the circulation as monocytes. They remain in the blood stream for several hours and then migrate to various tissues where they are transformed into macrophages. The macrophage is an activated monocyte, and is easily distinguished from the smaller neutrophil by its size and characteristic nucleus. While both cells are phagocytic, the macrophage has a far greater potential for killing bacteria. A greater cytoplasmic volume and more cytoplasmic organelles are present in the macrophage, including more lysosomes, microtubles, microfilaments and Golgi membranes. After leaving the bloodstream the macrophage will enter the peritoneal cavity, other serous cavities, and the red pulp of the spleen and the lymph nodes.

The concept of macrophage activation has many meanings for immunologists, but historically the meaning refers to changes in the macrophage that result in induction of enhanced antimicrobial activity that is essential for host survival (Nathan 1983, 1986b; Nathan et al., 1979). Two distinct mechanisms of activation are currently recognized: paracrine and autocrine. Paracrine activation requires that lymphocytes recognize antigens and produce soluble glycoproteins that activate the macrophage (Mackaness, 1970), essentially an adaptive mechanism transferrable by lymphocyte in a highly specific induction. The expression of activity by the macrophage is nonspecific. Defects in this paracrine pathway are associated with serious disease states of which acquired immune deficiency is an example (Murray, 1986).

In the autocrine system, microbial products can induce the production of compounds that can activate macrophages. There is a larger number of these cellular products produced by the inflammatory reaction, the most well characterized of which is the interferon (IFN-γ) or tissue necrosis factor-γ (Shparber and Nathan, 1986). No known diseases are identified with a defect in this pathway. A specific surface receptor for IFN-γ exists, and although immediate second messengers have not been identified, alterations in calcium metabolism appears to be one critical factor (Adams and Hamilton, 1987). The ability of macrophages to recognize neoplastic cells is of special interest to many investigators and is a key concept to this article. There are many other functions of macrophages that have been reported (Adams and Hamilton, 1984) but one of the best documented is the "oxidative burst," or the ability of the macrophage to secrete hydrogen peroxide, a reactive oxygen species (Nathan and Tsunawaki, 1986). This system is very effective for killing microbes.

The concept of immune activation embodies many intercellular and molecular events (Elsbach, 1977). The lymphocytes, particularly the T-helper cells, play a key role in activating the macrophage. In-resident macrophages in most tissues are usually not activated with respect to their surface active receptors. It may take several stages for them to be fully activated. For example, will an inflammatory response in tissues evoke a reaction that changes the C3 complement on the surface of the macrophage to fully empower the cell to engulf and destroy bacteria, or red blood cells. They are then further activated to a high killer state by secretions from T lymphocytes, one of which includes gamma interleukin.

Surface changes on macrophages that have become fully activated include alteration of receptors and other surface components. The result is a formidable cell capable of secreting 60 different substances concerned in acute and chronic inflammatory reactions. It is the activation of this cell by natural alkylglycerols that is the topic of this article. The macrophage activated by soluble mediators produced by immune T lymphocyte against specific antigens is able to kill any infectious agent regardless of the organism that stimulated the immune T lymphocyte. Activated macrophages show increased enzyme synthesis activity and increased metabolism. In addition, they are known to produce both superoxide anion and hydrogen peroxide as killing agents, as mentioned earlier. The ability to turn on this cell to a state of high activation is a potent means of augmenting the immune defense (Elsbach,

1977). Inflammation is a major trigger for the activation of the macrophage and the byproducts of cellular destruction produced in the inflammatory process are key components in the activation process.

MACROPHAGE ACTIVATION BY ALKYLGLYCEROLS

A standard method to detect macrophage activation is ingestion of foreign matter by the macrophages, either red cells or zymosan particles (Bianco et al., 1975). Mouse peritoneal macrophages can be activated *in vivo* by dodecylglycerol, a synthetic ether lipid, and by batyl alcohol, a naturally occurring ether lipid. For this activation to occur *in vitro*, a study by Homma and Yamamoto (1990) showed that the process required the presence of both B cells and T cells and the existence of an additional serum factor. These same investigators found that natural batyl alcohol was more effective at dosages below 100 ng per mouse then was dodecylglycerol in producing macrophage activation. In their *in vitro* studies it was found that B cells treated with alkylglycerols released and transmitted a factor to T cells, which in turn, modified the factor into a form that ultimately activated the macrophage. This study was later repeated and it was found that vitamin D_3 binding protein was required for activation (Yamamoto et al., 1991).

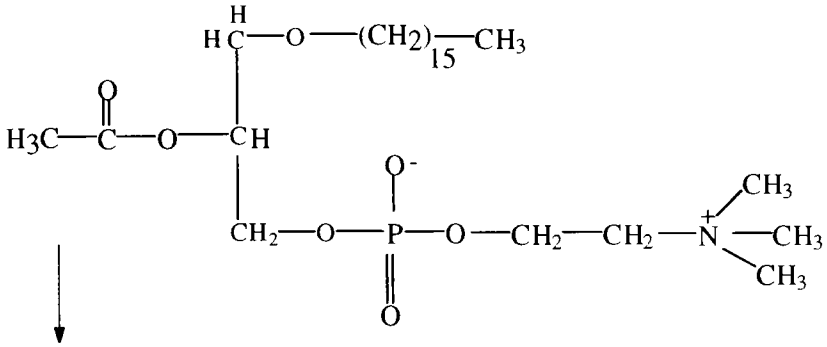
Inflamed cancerous tissue releases alkyllysophospholipids and other alkylglycerols that are degradation products of alkyl phospholipids and alkyl neutral lipids. These compounds are found in cancerous tissue in high concentration, but are very low in normal tissues. One of these products, dodecylglycerol (DDG), is one of the most potent macrophage activators known (Ngwenya and Yamamoto, 1985, 1986; Yamamoto et al., 1988; Adams and Hamilton, 1984). The use of the natural *sn*-3-octylglycerol, or batyl alcohol, found in shark liver oil produced the same effect as DDG (Hallgren et al., 1974). Yamamoto and colleagues (1987) suggested that the end action of alkylglycerol may be the same mechanism that triggers activation of macrophages. One possible action could be the detergent-like activity that could increase the fluidity of the cell membrane. Enhanced fluidity allows greater freedom of movement of the membrane, thereby increasing the opportunity for reactions. An alternative possible mechanism would be the conversion of alkylglycerols by monoglycerol kinases to compounds that are similar to platelet-activating factor (PAF), that is alkylglycerolysophosphates (Yamamoto et al., 1987).

There is evidence that oral intake of natural alkylglycerols results in higher levels of plasmalogens in the erythrocytes in human subjects. (Plasmalogens are ether compounds that are formed in the metabolic pathway of phosphoglycerides. They differ from phosphoglycerides in that they contain an ether linkage on the C-1 of the glycerol molecule). It is known that plasmalogens protect animal cell membranes against oxidative stress. One of the plasmalogens is known is PAF (Pinckard, 1985; Schlondorff and Nuewirth, 1986). This compound has numerous functions, one of which appears to be macrophage activation (Demopoulos et al., 1979; Lee and Snyder, 1985, Lewis et al., 1983; McManus, 1986; O'Flaherty and Wykle, 1983). The conversion of PAF to the lyso-form releases acetate that is a known stimulant to macro-phage activation (Mencia-Heurta et al., 1981; Wykle et al., 1986; Blank et al., 1981). The chemistry of this reaction is diagrammed in Figure 5. In rat studies, batyl alcohol is incorporated into all tissues except brain tissue, and this action is a stereo-specific incorporation (Das et al., 1992). It has been shown that alkylglycerols are present in human and cow's milk (Hallgren et al., 1974; Hallgren and Larsson, 1962b). Because the neonate has not developed a mature immune system (Quie, 1990) the prospect of transmitting immune functional components in the milk is a practical way to provide some protection for the newborn (Migliore and Jolles, 1988; Orga et al., 1977). In one study, lactating rats were fed alkylglycerols dissolved in corn oil. The composition of the alkylglycerols was similar to that found in shark liver oil in that batyl alcohol, chimyl alcohol, and selachyl alcohol were the major constituents (Migliore and Jolles, 1988). The findings from this study showed that while peripheral blood granulocytes were elevated, there was no elevation of peripheral

lymphocytes. Plasma levels of immunoglobulins were elevated in those pups whose dams were fed alkylglycerols but not in the controls. Both immunoglobulin G (IgG) and immunoglobulin M (IgM) were elevated to a significant degree.

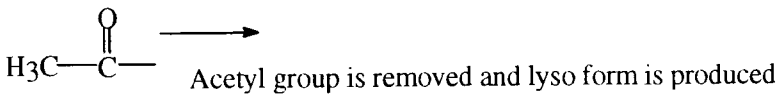
Alkylglycerols and alkyl-lysophospholipids stimulate macrophages not only to develop increased phagocytic ability, but also to convey extracellular cytolysis through IgG receptor binding of macrophages to target cells. The cytolytic property is important in the treatment of neoplastic diseases. This concept is supported by a number of studies with both natural alkylglycerols and alkyl derivatives of lysophopholipids shown to have direct cytotoxic effects on tumor cells *in vivo* (Andreesen et al., 1978; Berdel et al., 1980, 1982; Modelell et al., 1979). Tumoricidal activity requires higher dosages of these agents to be effective as compared with the level needed to macrophage-mediated cytocidal activity. The advantage of the dual beneficial effects of macrophage activation and cytotoxicity for malignant cells is a point worth considering (Yamamoto et al., 1987).

In a controlled study Oh and Jadhav (1994)

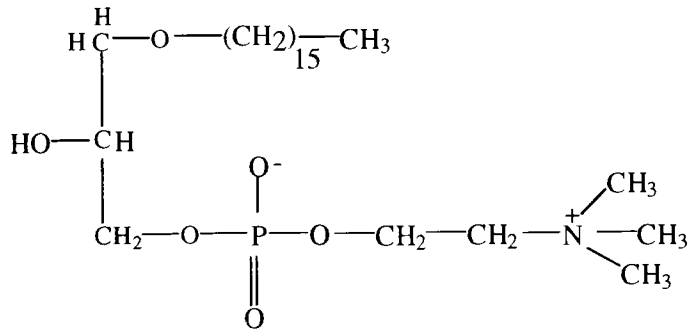


Platelet Activating Factor (PAF) (This is the active form)

(1-O-alkyl-2acetyl-sn-glycero-3-phosphocholine)



Acetyl group is removed and lyso form is produced



Lyso PAF (This is the inactive form)

(1-O-alkyl-2acyl-sn-glycero-3-phosphocholine)

FIG. 5. The structure of platelet activating factor (PAF). Note that the acetate group on the number 2 carbon is needed for the active form. The inactive form is called the lyso-form (Wardlow et al., 1986).

used alkylglycerol supplemented diets containing 30% chimyl, 28% batyl, and 42% selachyl glycerol. This proportion was chosen as it closely resembles the alkylglycerols found in human milk (Linman, 1960). Three groups of rats were fed various levels of total alkylglycerols from 10, 50, 250 ppm solubilized in corn oil. The results of this study confirmed the positive effects of alkylglycerols on the immune system. There was an increase in the alkylglycerol content in milk of the dams (mothers). This finding is of interest in that glyceryl ethers are found in fetal tissues and in rapidly reproducing tissues such as bone marrow (Hallgren et al., 1974). The level of alkylglycerols increased by 36% for the 10 ppm group, 94% for the 50 ppm group, and 417% for the 250 ppm group, respectively. These data suggest that the alkylglycerols are well absorbed by the intestinal mucosal cells.

Of great significance was the increase in plasma IgG levels in the dams, with the effect maximized at the 50 ppm level, and a further increase in IgG levels was noted as the pups suckled, suggesting that the increase in IgG levels may have been due to the immune stimulation by the alkylglycerols fed to the mothers.

Plasma IgM levels were also increased in the pups from the rats fed alkylglycerols while the control group had very low levels of IgM. In the dams fed various levels of alkylglycerols, there was a proportional increase in the plasma IgM levels as the amount of fed alkylglycerols increased from a multiple of 3 for the 10 ppm group, to a multiple of 9 for the 250 ppm group. While there was an increase in peripheral granulocytes in the pups, there was no increase observed in the peripheral lymphocytes (Oh and Jadhav, 1994).

THE SECOND MESSENGER CONCEPT AND THE EFFECT OF ALKYLGLYCEROL ON PROTEIN KINASE C

Cells receive signals from other cells primarily through receptors on the cell membrane. This topic is the subject of intense investigation, generating a voluminous literature, yet many details are still unknown. The fundamental sequence appears to follow a pattern in which a specific substance reacts with a specific membrane-bound receptor, which in turn sets up a series of reactions that require a second messenger. This messenger initiates a second series of kinase reactions with eventual phosphorylation and activation of a specific protein, such as an enzyme that has a definitive action. A well-known compound in one of these sequences is cyclic adenosine monophosphate (cAMP). All reactions of cAMP, regardless of end result, modify the activities of a specific group of enzymes through the action of protein kinases that are cAMP-dependent. Protein kinases transfer the terminal adenosine triphosphate (ATP) to the amino acids serine, threonine, or tyrosine residue on substrate molecules. These phosphorylated enzymes are much more active than the unphosphorylated enzymes. The cAMP-dependent enzyme reactions have many biological effects. Glucose conversion from glycogen, for example, is the most studied reaction, and is used as a textbook model (Darnell et al., 1990). There are two other second messengers that follow the same sequential pattern, but have different end results. One of these is Ca^{2+} and the other is 1,2 diacylglycerol, both of which are related to the metabolism of inositol phosphates, precursors of second messengers.

The level of Ca^{2+} ion in the cytosol is usually maintained below 0.2 μM. Calcium adenosine triphosphatases (ATPases) pump Ca^{2+} ions across the plasma membrane to the cells exterior, or to the lumens of the endoplasmic reticulum, or into Ca^{2+} storage vesicles within the cell. A rise as small as 1 μM in cytosolic Ca^{2+} will trigger responses such as are listed in Table 2. It is by another second messenger that many of the increased levels of Ca^{2+} arise, namely 1,4,5 inositol triphosphate (O'Rourke et al., 1985). The precursor of 1,4,5 inositol triphosphate also generates diacylglycerol, which works synergistically with Ca^{2+} to activate protein kinase C (Nishizuka, 1984). See Figure 6 for an outline of this system. It is the action of diacylglycerol that appears to be affected by the alkylglycerols.

Protein kinase C is widely distributed in the body organs and tissues, with the highest level in the brain (Nishizuka, 1984). While the activation of protein kinase C is biochemically de-

TABLE 2. CYTOTOXICITY OF TUMORS AND LEUKEMIAS OF HUMAN ORIGIN IN VITRO USING SYNTHETIC ANALOGS OF ALKYLGLYCEROLS

Type of disease	*Sensitive*	*Resistant*
Prostate carcinomas	2	
Testicular senunoma	1	
Teratocarcinomas	3	
Bladder carcinomas	2	
Hypernephroid carcinomas	17	2
Astrocytomas	2	
Glioblastomas	1	
Meningioma	1	
Medulloblastoma	1	
Carcinoma of the ovary	1	
SCLCA	1	
NSCLC	1	
NHL/B	1	
NHL/T	1	
AMML	4	
AML	3	
ALL	2	
HL-60	1	
K-562	1	
CML/BC	5	
TOTAL	51	2

ASCLC, small-cell lung cancer; NSCLC, non-small cell lung cancer NHIIB (T), non-Hodgkin lymphoma of B (T)-cell origin AMML, acute myelomonocytic leukemia AML, acute myeloid leukemia ALL, acute lymphocytic leukemia CML/BC, chronic myeloid leukemia/blast crisis. (Adapted from Berdel, 1987).

pendent on Ca^{2+} it is physiologically independent of Ca^{2+} Protein kinase C is normally an inactive soluble cytosolic protein. Through the action of Ca^{2+} ions, protein kinase C binds to a leaflet of the plasma membrane where it is activated by 1,2-diacylglycerol (Darnell et al., 1990). The many functions of protein kinase C include its activation by tumor promoters such as the phorbol esters (Castagna et al., 1982). Tumor promoters are mainly lipid soluble compounds obtained from plant sources that play a role in transforming a normal cell into a malignant cell with resultant uncontrolled growth. Protein kinase C is a receptor for phorbal esters and activation. The ability of alkylglycerols to inhibit the inactivation of protein kinase C is an important finding.

Studies using a malignant canine tumor (Madin-Darby kidney tumor) showed that alkylglycerols generated by the tumor cells were potent inhibitor of protein kinase C (Warne et al., 1995). These investigators found that cellular growth stopped when the level of alkylglycerol rose. Adding 1-O-dodecyl-*sn*-glycerol resulted in an inhibition of phorbol ester stimulated translocation of protein kinase C

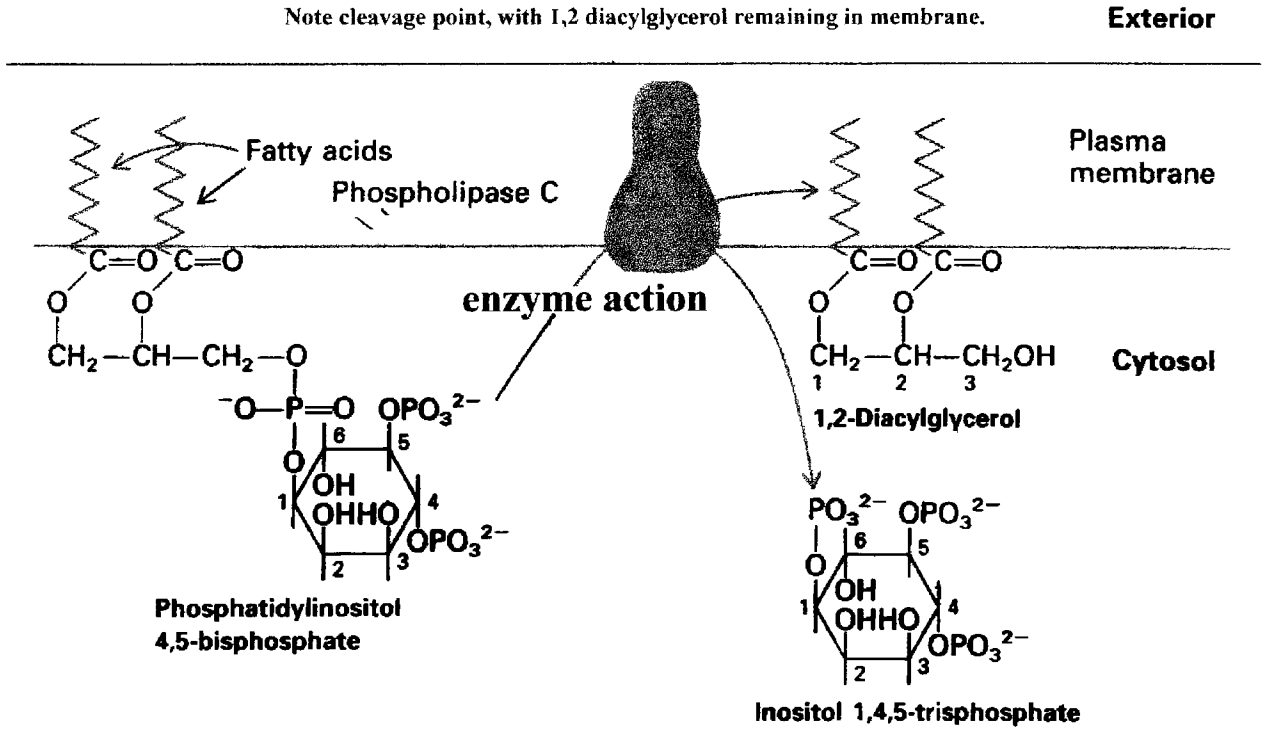


FIG. 6. Schematic of the action of 1,2-diacylglycerol production from phosphatidylinositol 4,5 bisphosphate. It is the production of 1,2-diacylglycerol that drives protein kinase C.

from the cytosol to the membrane fraction, and a decrease in the membrane associated activity of protein kinase C. A study using a lipophosphoglycan from Leishmania parasites showed this material to inhibit protein kinase C. Fractionation of the lipophosphoglycan revealed the active portion was in the lipid fraction and proved to be 1-O-alkylglycerol (McNeely et al., 1989). The alkylglycerols were found to be potent and specific inhibitors of the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (or TPA) on arachidonic acid metabolism. This inhibition was believed to be due to inhibition of protein kinase C (Robinson et al., 1996). A 12-carbon chain alkylglycerol was found to be the most effective molecule for inhibition of TPA-stimulated arachidonic acid release. However, an 18-carbon chain alkylglycerol is reported to be a more effective inhibitor of protein kinase C directly (McNeely et al., 1989).

CLINICAL STUDIES

Clinically, the alkylglycerols have been used for many years both to treat cancer patients and to alleviate radiation-associated tissue damage (Brohult, 1963). A review of the efficacy of alkylglycerols in various human tumors showed that a large number of these tumors were sensitive to a number of synthetic ether lipids (Berdel et al., 1982). In Europe, alkylglycerols have been used since 1934, beginning with extracts of bone marrow fat to treat granulocytopenia (Marberg and Wikes, 1938). These investigators showed later that it was the unsaponifiable fraction of bone marrow that was effective in increasing the leukocytes. One of their cases reported was leukopenia induced by radiation treatment (Marberg and Wikes, 1937, 1938).

Continuing studies over the next 30 years identified batyl alcohol as an active component that produced both erythrocytosis and granulocytosis (Linman, 1969). In 1977 oral alkylglycerols were given to cancer patients undergoing radiation treatment; the results of these studies confirmed earlier reported studies (Brohult, 1963).

In a series of studies on the effects of alkylglycerols on the frequency of injuries after radiation therapy for cervical cancer, the investigators found a 60% decrease in incidence of radiation associated injuries. The injuries were classified from mild (grade I) to severe (grade IV) and ranged from subjective discomfort and minimal soft tissue inflammation to rectal and intestinal fistulas. The authors concluded that the alkylglycerols had two effects: inhibition of tumor growth and protection against tissue damage after radiation (Brohult et al., 1977). In subsequent studies the same investigators found a reduced mortality in patients with uterine cancer treated with alkylglycerols prior to radiation therapy. The tumor regression was remarkably higher for patients younger than 60 years of age with advanced stages of cancer. Patients with less advanced stages did not do better than the untreated controls, who had lower levels of alkylglycerols (Brohult et al., 1986). This finding is consistent with the observation that tumor cells produce alkylglycerols in great amount over time (Warne et al., 1995).

It is of interest that alkylglycerols are effective in reducing radiation damage, as this would suggest an antioxidant role for these ether-linked compounds. It is well established that ionizing radiation destroys tissue by ionizing water to form hydroxyl radicals (Halliwell and Gutteridge, 1993). The hydroxyl radical is the most powerful oxidizing agent discovered so far and, as such, it has been associated with many disease states including DNA damage and aging (Halliwell and Gutteridge, 1993). A recently published study showed that chimyl alcohol was effective in reducing the reperfusion/ischemia injury associated with myocardial injury (Mulik et al., 1994). This observation would suggest further clinical studies with alkylglycerols as a prophylactic against myocardial damage. The mechanism may be due to the antioxidant effects of the alkylglycerols because there are no other ways to prevent the damaging effects of hydroxyl radical than to inhibit its formation or to quench it as soon as it is formed (Halliwell and Gutteridge, 1993).

DOSAGE

Currently, there are several manufacturers of shark liver oil capsules. It is important to check

on the purity as well as the standardization of the contents of alkylglycerols. The usual dosage forms are capsules with 250 to 500 mg of shark liver oil containing 20% of alkylglycerols, which means there are 50 to 100 mg of available alkylglycerols. The recommended daily intake will vary with the disorder. As an adjunct therapy to traditional cancer treatments and for prophylactic and therapeutic measures in immune deficiency, the appropriate dosage would be three to a maximum of six 500-mg capsules per day. As an immune booster and for the purposes of prevention in less severe cases, one standard 250-mg capsule taken two to three times a day has proven to be a useful measure. Depending on age and size, children may be given one or two standard 250-mg capsules for the same purpose. For young children or anyone who has difficulty in swallowing capsules the capsule may be punctured and the contents given on food.

SUMMARY

Many articles have been published over the last 30 years exploring and reporting the activity of the alkylglycerols. An excellent summary of the work by Brohult was published in 1963 on the use of refined shark liver oils in radiation treatment (Brohult, 1963), and in 1987 a comprehensive report on the use of alkylglycerols in oncology was published pointing out their tumoricidal activity (Baumann, 1987). Some natural and synthetic ether lipids exhibit strong antitumor activity and are being used in cancer therapy (Brachwitz et al., 1987; Brohult et al., 1986; Weltzein and Munder, 1983). Work on the use of shark liver oil as growth promoters was published as early as 1960 (Brohult, 1960). More recent studies are focusing on the use of alkylglycerols as immunomodulators and immunostimulators, as reported in this article. The alkylglycerols appear to work primarily on the cell membrane to activate macrophages, and some of these agents may be degradation products of alkylglycerols as we see in lysophospholipids. Increased particle ingestion by macrophages results in antibody-mediated tumoricidial activity and increased superoxide generation.

The inhibitory action of the alkylglycerols on protein kinase C suggests a strong point of attack against proliferative diseases. It is interesting that the alkylglycerols can be precursors of the PAF, which has numerous biological effects. The role of alkylglycerol in cancer prevention, infection control, and modification of aging rate and protection against heart disease are all potential uses of these agents both in the natural and synthetic forms.

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