Lipids as anti-cancer agents

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In the early 1970s, three independent laboratories observed antitumor effects of certain ether lipids for the first time. P.G. Munder and colleagues (1) examined synthetic analogs of 2-lysophosphatidylcholine (2-LPC) and reported prophylactic and therapeutic efficacy of certain alkyl-lysophospholipid (ALP) analogs in some allogeneic and syngeneic mouse transplantation tumors. These first-generation ALP originally were synthesized as a new class of biological response modifiers with a higher metabolic stability than 2-LPC. K. Ando and colleagues (2) reported on therapeutic effects of certain alpha-glyceryl ethers in Ehrlich ascites tumor. B. Boeryd and colleagues (3), meanwhile, described therapeutic activity of methoxy-substituted alkyl glycerols in some metastasizing sarcomas induced by methyl-cholantrene in mice.

Whereas the naturally occurring alpha-glyceryl ethers and alkyl glycerols so far have not led to the systematic development of anticancer drugs with applications in clinical oncology, this attempt is under way with different synthetic ALP analogs and their derivatives. Extensive reviews on recent developments in this research have been published (4,5). The following is a brief summary of the more significant aspects of this work.

During an investigation of the influence of ALP analogs on cellular immunity, strong antitumor effects of some of these compounds were first observed in the allogeneic Ehrlich ascites tumor in mice. In these experiments, ALP analogs with long aliphatic side chains in the *sn*-1 position of the glycerol molecule and metabolically stable substitution in the *sn*-2 position, such as 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-

OCH_3), have shown the highest prophylactic and therapeutic efficacy. Systemic therapy with ET-18-OCH_3, starting 1–9 days after tumor inoculum, led to significant survival of the tumor-bearing animals, as compared to controls.

We have simultaneously done extensive studies on anaplastic Lewis lung carcinoma in mice. This tumor spontaneously metastasizes to the lungs even if the primary tumor lesion is surgically removed. Thus, this model offers the unique opportunity to study the effect of experimental therapeutics on the growth of the primary tumor lesion, in an adjuvant chemotherapeutic situation in the presence of occult micrometastasis after the surgical removal of the primary tumor and on already established metastasis. Systemic treatment with ET-18-OCH_3 and similar ALP analogs protected animals from lung metastasis even if treatment was started more than a week after surgery. In the classical situation of adjuvant chemotherapy, when the compounds were given shortly after surgical removal of the primary tumor, the treated groups showed 40–60% survivors, whereas about 90% of the animals died from lung metastases in the control groups.

Further therapeutic screening of the first-generation ALP analogs in different laboratories revealed that methyl-cholantrene-induced fibrosarcomas, the sarcoma S 180 J, the myelomas MX 5563 and MPC 11, the M 1 leukemia, the B 16 melanoma and other mouse tumor models are sensitive to these lipids. However, we and others could not show striking therapeutic efficacy in other mouse tumor and leukemia systems such as in the AKR leukemia, in radiation-induced lymphomas or in L 1210 leukemia.

Rat tumor models that are sensitive to the therapeutic action of ALP analogs include autochthonous methylnitrosourea-induced mammary carcinomas and dimethylbenzanthracene-induced leukemias. On the other hand, some chemically induced rat colonic adenocarcinomas seem to be rather resistant.

Some of the compounds—such as the ALP analog ET-18-OCH_3 or the thioether-phospholipid BM 41.440, which contains a 1-*S*-alkyl ether linkage in the *sn*-1 position of the molecule—have been also tested for therapeutic activity in xenotransplanted human tumors growing in athymic (nu/nu) mice. Considerable growth retardation of some gynecologic tumors such as ovarian carcinomas, ovarian sarcomas and endometrial adenocarcinomas, as well as a lung cancer, was found under systemic therapy with some of these compounds. However, other xenotransplanted human tumors such as testicular cancers or melanomas have been found to be resistant.

In summary, there is considerable prophylactic and therapeutic activity of some ALP analogs in various mouse and rat tumor models.

Mode of action

During the early treatment studies, it became evident that the antineoplastic activity of some ALP analogs in vivo might be partially mediated by cytotoxic macrophages. In a number of studies aimed at assessing the importance of cytotoxic macrophages as mediators of ALP effects, we and others could show that macrophages after incubation with these lipids not only are cytotoxic in vitro to neoplastic cells from a variety of histologies, but they also can be used in an adoptive cell transfer for successful treatment of syngeneic tumor and metastasis developments in vivo (for details, see references 4,5). The putative involvement of other cell types of cellular host resistance such as lymphocytes could not yet be demonstrated beyond doubt.

After it had become evident that macrophages are involved in mediating the antineoplastic action of ALP analogs, some direct effects of these drugs on tumor cells were also observed. Perhaps the most striking observation was that ALP analogs with an ether linkage in the *sn*-1 position of the glycerol moiety and a metabolically stable substitution at the *sn*-2 position were directly antiproliferative and cytotoxic in vitro at micromolar concentrations when coincubated with neoplastic cells for more than 1 day. However, *sn*-1 ester analogs were ineffective within this dose range, regardless of changes made in the *sn*-2 position of the molecule.

These antiproliferative and cytotoxic effects were first studied in detail by Andreesen et al. using cells of various forms of human leukemia and also could be shown in single-cell suspensions derived from a wide variety of human lymphomas and solid tumors (for literature, see references 4, 5). This activity is independent of the type of assay used. Scanning electron microscopy revealed that destruction of the outer cell membrane occurs. The facts that the cytotoxicity observed is a slow and progressive process, that *sn*-1 linked ester-lysophospholipids have not shown the same activity in a similar dose range, and that the effect could also be observed in cell culture experiments using serum-containing medium rule out a direct detergent effect as the basis of cell membrane destruction.

Although a multitude of experimental studies have been performed, the molecular mechanisms of the cytotoxic action of these ether lipids at present are poorly understood and still controversial (4,5). However, there is agreement that cellular uptake and accumulation of these compounds are crucial early steps in a cascade of events leading to cell death. With the development and testing of ALP-type ether lipids, the outer cell membrane has evolved as an interesting new target for experimental cancer chemotherapy. This could stimulate new research approaches beyond those concerned with ether lipids.

There also is good experimental evidence that the ALP analogs show a variety of other direct effects on neoplastic cells even when tested at subtoxic dose levels. Honma and coauthors in Japan have extensively studied the morphological and functional induction of differentiation of leukemic blasts by various of these structures (for references, see 4,5). Furthermore, in an attempt to understand the striking antimetastatic effect of various ether lipids, studies done by Storme et al. (for references, see 4,5) showed impressive anti-inva-

sive activity of ET-18-OCH_3 and BM 41.440 in an in vitro model in which malignant MO_4 cells were confronted with precultured fragments of embryonic chick cardiac muscle or lung fragments.

State of the art

Recently, four international scientific meetings focused on ether lipid analogs and other membrane-active chemotherapeutic agents. The first two were held in Göttingen, West Germany (6,7); one of these was the First International Symposium on Ether Lipids in Oncology (7). The American Association for Cancer Research held a minisymposium on Ether Lipid Analogs and Other Membrane-active Chemotherapeutic Agents during its 29th annual meeting in New Orleans (8). The American Oil Chemists' Society held a sequence of sessions dealing with the topic during its 1988 annual meeting held in Phoenix (9). All four meetings presented an enormous body of data on recent developments in this area of research. The following includes a brief summary of major advances reported at these meetings.

Several studies, dealing with the accumulation, intracellular fate and metabolism of ether lipids such as ET-18-OCH_3 in neoplastic cells, show that these compounds are metabolically stable and are only slowly degraded (if at all), for example, by phospholipase C. Further studies have clearly shown inhibition of protein kinase C and related transmembrane signaling, as well as opening of voltage-dependent calcium channels whose activity is modulated by protein kinase C. Whether these effects share responsibility for the cytotoxicity of the compounds remains to be established.

With the first-generation ALP analogs, and particularly ET-18-OCH_3 as a reference structure, many laboratories have embarked on the chemical synthesis and the screening of a variety of structurally related compounds which promise antineoplastic activity. Among structures that show promising in vitro and in vivo antitumor action are 1-thioether phospholipid analogs such as BM 41.440 (Boehringer Mannheim), cyclic analogs of ET-18-OCH_3 such as SRI 62-834 (Sandoz), 2-alkoxyalkyl or 2-alkoxyalkenyl analogs (Eli Lilly), alkyl-ethylene-glycophospholipids (Takeda Chemicals) and 1-*N*-alkylamide analogs of ALP. Other structures, such as various *sn*-2 analogs of platelet activating factor (PAF) and alkyl-linked lipoidal amines (CP 46,665; Pfizer), show in vitro antitumor properties, but seem to be less promising in vivo for various reasons.

Based on the hypothesis that degradation of certain ALP analogs by a phospholipase C is required for the generation of toxic metabolites, Eibl and coworkers have synthesized a series of alkylphosphocholines, such as hexadecylphosphocholine (D 18506, ASTA-Werke). The investigators showed impressive therapeutic in vivo activity for D 18506 in a breast cancer model in rats. Furthermore, within a series of analogs, there was a direct correlation between apparent therapeutic activity and in vitro cytotoxicity on one side and susceptibility of the agents to degradation by phospholipase C.

Our recent work has concentrated on chemical conjugates of ether lipids and other cytotoxic drugs, such as nucleoside analogs. In cooperation with C.I. Hong, we have shown that *sn*-3 lipid conjugates of arabinoside-cytosine (ara-C), when tested in vivo on various leukemias and solid tumor models in mice, have a comparatively higher therapeutic activity than the parent compounds ara-C and the ether lipids alone or equimolar mixtures of both. A 1-*S*-alkyl ether moiety in the lipid represents a significant advantage in comparison to an ester-linked lipid as part of the conjugate. This was clearly documented in the case of a syngeneic mouse leukemia.

Of particular interest is the observation by Andreesen et al. that some ALP analogs affect the secretion and activity of interleukin-1 and interleukin-2. This not only provides some explanation for other biological activities of these lipids such as radioprotection (10), but also draws parallels to other biologically active ether phospholipids, such as the endogenous platelet activating factor (PAF).

PAF has been investigated in great detail in recent years. It acts as a hormone-like mediator in various physiological and pathophysiological situations (11). Besides the fact that ether lipids are normal constituents of cellular membranes and are present in human plasma (4), the PAF molecule is one of three ether lipid structures with known or putative endogenous biological mediator activity. The other two are an ether-linked glycerophosphoserine called "modulator" which plays a role in the activation/transformation of glucocorticoid receptor (12), and certain alkyl-linked diglyceride analogs which have been described as negative regulators of protein kinase C activity after stimulation by diacylglyerols (13).

Although scientists have speculated for some time that ALP offers a unique approach to marrow purging because of its selective antineoplastic cytotoxicity, no systematic studies were done until Vogler and coauthors discovered in experiments with mice that ET-18-OCH_3 clearly exerts selective cytotoxic effects in eliminating leukemic WEHI-3B cells from normal bone marrow progenitor cells, within a simulated remission bone marrow, which results in cures of mice after radiation and syngeneic transplantation of these purged bone marrow samples. Subsequently, several laboratories have clearly demonstrated selective cytotoxicity of ALP analogs against leukemic blasts, in comparison with normal human bone marrow progenitor cells in various assay systems in vitro. This has prompted us to start a clinical phase I/II study to assess the safety and efficacy of autologous bone marrow transplantation after supralethal chemoradiotherapy in acute leukemia, using remission marrows treated in vitro with ether lipids as purging agents.

The ALP ET-18-OCH_3 was introduced into first clinical phase I pilot trials by our group (for further references, see 4,5). Currently, there are three lipids in clinical trials in West Germany for treatment of cancer and leukemia. ET-18-

OCH_3 is given to patients with nonsmall cell lung cancer per os in a multi-institutional phase II drug efficacy study. A multi-institutional phase I drug safety trial with BM 41.440 given orally has been recently completed, and this drug is ready to enter phase II drug efficacy trials in a wide spectrum of neoplastic diseases. Hexadecylphosphocholine is currently being studied in a phase II trial for the topical treatment of skin metastasis in patients with breast cancer and in a phase I trial in an oral formulation. These early clinical studies have shown encouraging tumor responses in small numbers of patients treated. Thus, further clinical testing of these agents and of other lipid analogs on a larger scale is clearly indicated.

Future perspectives

There is increasing evidence that certain ether lipids and their derivatives represent a new group of drugs that have proven active in the experimental therapy of cancer and leukemia. This also is supported by the results of the first clinical trials.

The activity of some of these structures is partially mediated through nonspecific host resistance cells. In addition, they exert direct effects on neoplastic cells: they are cytotoxic, they are anti-invasive, and they induce differentiation. Although the molecular mechanisms leading to these direct effects are yet poorly understood, accumulation of the agents in neoplastic cells, disturbances in lipid metabolism and subsequent destruction of cell membranes seem to be important. Thus, cellular membranes have evolved as a new target for experimental cancer therapy.

Modern lipid synthesis and lipid chemistry have developed molecules which can be studied as cytotoxic drugs either alone or in combination with other cytotoxic agents or biological response modifiers. Close cooperation between organic chemists, biochemists, tumor biologists, pharmacologists and clinical oncologists will be required to further develop this field so that biologically active lipids can be added to our armory of drugs against malignant disease.

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