REVIEW

Ether Lipids in Cancer Chemotherapy

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Abstract. There is considerable evidence that certain ether lipids such as 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine represent a new class of antineoplastic agents. These ether lipids have been shown to be cytotoxic for a wide variety of tumors. The site of action of ether lipids appears to be the cell membrane, where they inhibit the biosynthesis of phosphatidylcholine as well as the activity of protein kinase C. However, it remains uncertain that these effects represent the only mechanism for the cytotoxic action of these ether lipids. Further experiments to elucidate the molecular mechanisms of the cytotoxic action of these compounds are necessary. Clinical phase I pilot trials have been completed showing encouraging tumor response in a small number of patients treated. Some of these ether lipids are selectively cytotoxic to leukemic cells sparing of normal cells within a certain dose range. Thus, they are uniquely suitable for purging residual leukemic cells from remission marrow used for autologous bone marrow transplantation. A phase I/II study of autologous bone marrow transplantation in acute leukemia using bone marrow cells treated with ether lipids is in progress. (*Keio J Med* 39 (2): 75–78, June 1990)

Key words: cell membrane, alkyl-lysophospholipids

Introduction

Certain ether lipids and their derivatives such as alkyl-lysophospholipids and 1-thioether phospholipids represent a new class of anti-neoplastic agents in experimental therapy of cancer.^{1,2} This is also supported by the results of the first clinical trials.³ The activity of these agents is partially mediated through nonspecific host resistance cells.⁴ In addition, they possess direct effects on neoplastic cells; they are cytotoxic, antiinvasive and effective in inducing cell differentiation.^{5–7} Although the molecular mechanisms leading to these direct effects are yet poorly understood, accumulation of the agents in neoplastic cells, disturbing lipid metabolism and subsequently destroying cell membranes seems to be crucial for their cytotoxicity.^{8,9} Thus, cell membranes have evolved as a new target for experimental cancer therapy. Extensive reviews on recent developments in this research area have been published.^{1,2} The following is a review of the significant aspects of this research area.

Preclinical Studies

Alkyl-lysophospholipids (ALP), analogs of naturally occurring lysophosphatidylcholine (2-LPC), were originally synthesized as a new class of biological response modifiers with a higher metabolic stability than 2-LPC.¹⁰ 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.¹⁰ 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-OCH₃) (Fig. 1) have shown the highest prophylactic and therapeutic efficacy. In addition, systemic therapy with ET-18-OCH₃ starting 1–9 days after tumor inoculum led to significant survival of the tumor-bearing animals, as compared to controls.

Further extensive studies were performed with the anaplastic Lewis lung carcinoma in mice.¹¹ This tumor spontaneously metastasizes to the lungs even if the pri-

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Fig. 1 Structural formula of ET-18-OCH₃

mary tumor lesion is surgically removed. Thus, this model offers the unique opportunity to study the effects 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-OCH₃ and similar ALP analogs protected animals from lung metastasis even if treatment was started more than a week after surgery.¹¹ 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.¹¹

Further therapeutic screening of the first generation ALP analogs in different laboratories revealed that methyl-cholantrene induced fibrosarcomas, the sarcoma S180J, the myeloma MX 5563 and MPC11, the M1 leukemia, the B16 melanoma and other mouse tumor models are sensitive to these lipids.^{1,2,12} However, we and others could not show striking therapeutic efficacy in some other mouse tumor and leukemia systems such as the AKR leukemia and L 1210 leukemia.^{1,2}

Rat tumor models that are sensitive to the therapeutic action of ALP analogs include autochthonous methyl-nitrosourea-induced mammary carcinomas and dimethylbenzanthracene-induced leukemias.¹³ On the other hand, some chemically induced rat colonic adenocarcinomas seem to be rather resistant.¹⁴

Some of the compounds — such as the ALP analog ET-18-OCH₃ 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 gynecological tumors such as ovarian carcinomas, ovarian sarcomas and endometrial adenocarcinomas was found under systemic therapy with some of these compounds.¹⁵ However, other xenotransplanted human tumors have been found to be resistant.¹⁶

Vogler and co-authors demonstrated selective cytotoxic activity of ET-18-OCH₃ in their experiments with mice.¹⁷ Lethally irradiated mice were injected with normal bone marrow cells containing 1–2% leukemic cells (WEHI-3B) to simulate a remission marrow after the cells were incubated with various concentrations of ET-18-OCH₃ *in vitro*. All the mice given cells not treated with ET-18-OCH₃ succumbed to leukemia, whereas there was a dose-related increase in survival in those transplanted with ET-18-OCH₃ treated cells. 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*.^{5,18}

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.^{4,11} 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 a variety of neoplastic cells, but they also can be used in an adoptive cell transfer for successful treatment of syngeneic tumor and metastasis development *in vivo*.¹¹ 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 in the sn-2 position were directly antiproliferative and cytotoxic *in vitro* at micromolar concentrations when coincubated with neoplastic cells for more than 1 day.^{1,2,19} However, sn-1 ester analogs were ineffective within this dose range, regardless of changes made in the sn-2 position of the molecule.^{1,2,19}

These antiproliferative and cytotoxic effects were first studied in detail by Andreesen et al.¹⁹ 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.^{20–22} This activity is independent of the type of assay used. Scanning electron microscopy revealed that destruction of the outer cell membrane occurs.^{20,22} 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 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 are still controversial. However, there is an agreement that cellular uptake and accumulation of these compounds are crucial early steps in a cascade of events leading to cell death.⁹

There is also 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 coworkers have extensively studied the morphological and functional induction of differentiation of leukemic cells by various ALP analogs.⁷ Furthermore, in an attempt to understand the striking anti-metastatic effect of various ether lipids, studies done by Storme et al. showed impressive anti-invasive activity of ET-18-OCH₃ and BM 41.440 in an *in vitro* model, in which malignant MO₄ cells were confronted with pre-cultured fragments of embryonic chick cardiac muscle or lung fragments.⁶

Several studies, dealing with the accumulation, intracellular fate and metabolism of ether lipids such as ET-18-OCH₃ in neoplastic cells, show that these compounds are metabolically stable and are only slowly degraded if at all, for example, by phospholipase C.²³ Further studies have clearly shown inhibition of protein kinase C activity and related transmembrane signaling, as well as opening of voltage dependent calcium channels, which is modulated by protein kinase C.²⁴ Whether these effects share responsibility for the cytotoxic action of these ether lipids remains to be established.

Development of New Ether Lipids

With the first generation ALP analogs, and particularly ET-18-OCH₃ 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. Structures that show promising *in vitro* and *in vivo* antitumor action are 1-thioether phospholipid analogs such as BM 41.440 (Boehringer Mannheim),^{20,25,26} alkyl-ethylene-glycophospholipids (Takeda Chemicals)²⁷ and 2-acetamide analogs of ALP.²⁸ Other structures, such as various sn-2 analogs of platelet activating factor (PAF) and alkyl-linked lipoidal amines (CP46, 665; Pfizer), show *in vitro* antitumor properties, however, some of them such as the CP-46, 665 seem to be less promising *in vivo* for various reasons.^{29,30}

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.³¹ 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 vitro* in various leukemia 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.^{32,33} An 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 a syngeneic mouse leukemia.³³

Clinical Studies

ET-18-OCH₃ was introduced into first clinical phase I pilot trials by our group.³ Currently, there are three lipids in early clinical trials in West Germany for treatment of cancer and leukemia. ET-18-OCH₃ has been given to patients with non-small cell lung cancer per os in a multi-institutional phase II drug efficacy study.³⁴ 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.³⁵ Hexadecylphosphocholine is currently being studied in a phase II trial for the topical treatment of skin metastases in patients with breast cancer and in a phase I trial in an oral formulation.³⁶ 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.

A clinical phase I/II study to assess the safety and efficacy of autotransplantation after supralesional chemotherapy in acute leukemia using remission marrows treated with ether lipids *in vitro* is currently underway, however, the data are too early to draw any final conclusions.

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