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Chapter 6

Alkyl-lysophospholipids as anticancer agents and enhancers of radiation-induced apoptosis

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ALKYL-LYSOPHOSPHOLIPIDS AS ANTICANCER AGENTS AND ENHANCERS OF RADIATION-INDUCED APOPTOSIS

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Synthetic alkyl-lysophospholipids (ALPs, also referred to as ether-phospholipids) have been studied as antitumor agents for more than a decade. Classical examples of these ALPs include 1-O-octadecyl-2-O-methyl-rac-glycerol-3-phosphocholine (ET-18-OCH₃; Edelfosine) and hexadecylphosphocholine (HePC; Miltefosine). Unlike most currently available chemotherapeutic drugs that target the nuclear DNA, ALPs exert their action at the plasma membrane level, where they interfere with mitogenic signal transduction pathways. Whereas malignant cells are highly sensitive to the lethal action of ALPs, normal cells remain relatively unaffected, illustrating the potential selective antitumor properties of this class of drugs. Recently, ALPs have regained interest because of their capacity to induce apoptosis in various tumor cell lines. Moreover, in combination with other (conventional) anticancer regimens, ALPs seem to cause an additive and sometimes synergistic cytotoxic effect. These biologic properties make ALPs attractive drugs for further clinical evaluation. The present review discusses recent insights into the models of action of ALPs, their interaction with ionizing radiation, and clinical application.

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Alkyl-lysophospholipids, Lipid metabolism, Signal transduction, Radiotherapy.

INTRODUCTION

Synthetic alkyl-lysophospholipids (ALPs) constitute a heterogeneous group of unnatural lysophospholipids with different biologic effects, including inhibition of tumor cell invasion (1), induction of cell differentiation (2), and apoptosis (3–7). Whereas the majority of the conventional anticancer drugs may cause severe side effects due to bone marrow suppression, ALPs are known to exert minimal hematologic toxicity (3, 4, 6, 7). Also normal resting vascular endothelial cells are not affected by ALPs (7). This selective antitumor effect was not limited to human cell lines but was also observed in primary tumor cell cultures from cancer patients (4).

The prototype of these ALPs is the platelet-activating factor analog ET-18-OCH₃. It is characterized by the presence of an ether-linked methyl group at position α-2 of the glycerol backbone (Fig. 1A). Later it was found that the glycerol moiety is not an essential structural element for the observed antitumor activity and that alkylphosphocholines have similar, if not better, potencies. Hexadecylphosphocholine (HePC), which contains an ester bond, belongs to this new generation of ALPs (Fig. 1B).

Originally, it was thought that the growth inhibitory effect of ALPs was indirect and mediated by macrophages activated by these compounds (8). Subsequent studies clearly showed that ALPs cause direct destruction of tumor cells and that this effect was associated with disturbance of the membrane phospholipid metabolism (9, 10). Many studies have been undertaken to identify the primary target(s) for the antineoplastic effects of ALPs (See below).

Several ALPs have entered clinical studies for a variety of indications. For instance, ET-18-OCH₃ has been found useful as a purging agent in autologous bone marrow transplantation (11, 12). Encouraging clinical results have also been obtained with HePC as a topical drug for the treatment of skin metastases of breast cancer (13–15) and cutaneous malignant lymphoma (16). Prolonged oral administration of HePC, which shows only minimal myelotoxicity, proved to be effective in the treatment of visceral leishmaniasis, a systemic protozoal infection (17). A recently developed structural analog of HePC, octadecyl-(1,1-dimethyl-piperidino-4-yl)-phosphate (D-21266; Perifosine) appeared to be more active and better tolerated upon systemic administration in preclinical studies than HePC (18). This latter ALP compound is currently undergoing Phase I evaluation as an

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oral drug at The Netherlands Cancer Institute and elsewhere (19).

**Effect of ALPs on signal transduction**

Although the precise mechanism responsible for ALP-induced cytotoxicity is unknown, the plasma membrane has been identified as the primary site of action of these compounds (20). In living cells, the natural membrane phospholipids undergo a continuous rapid turnover, which is essential for the signaling pathways that allow cells to survive. The enzymes involved in this lipid turnover basically include all known intracellular phospholipases (types C, D, and A), transacylases, and biosynthetic enzymes. Some of these enzymes (the catalytic ones) are essential for generating lipid second messengers (e.g., diacylglycerol, phosphatidic acid, arachidonic acid, and derivatives), especially when cells are activated by growth factors. Other anabolic enzymes then serve to replenish the pool of degraded phospholipids to allow renewed receptor-induced phospholipase-mediated degradation. Thus, the signaling-associated phospholipid turnover of phosphatidylinositol (PI), phosphatidylcholine (PC), and sphingomyelin occurs in cycles, i.e., hydrolysis and resynthesis. Owing to their single long alky chain, ALPs readily insert into the outer leaflet of the plasma membrane, where their other bonds (as in ET-18-OCH₃) or alternative ester bonds (as in HePC) make them relatively resistant to phospholipase activities (21). ALPs interfere with membrane phospholipid turnover in a broad sense: ET-18-OCH₃ and HePC inhibit phosphoinositide-specific phospholipase C and its associated diacylglycerol and inositol 1,4,5-triphosphate (IP₃) formation (22–25). Perhaps even more importantly, both ET-18-OCH₃ and HePC also inhibit PC turnover at the level of PC degradation as well as PC resynthesis (26–28). The latter inhibition occurs at the level of cytidylyltransferase (CT) (29), the rate-determining step in PC biosynthesis. Baburina and Jackowski (30) studied the role of CT in ALP-induced apoptosis directly. By increasing CT expression via a tetracycline-responsive promoter, the cytotoxic effect of ALP was reversed. Acylation of exogenously supplied lysoPC circumvented the requirement for CT activity by providing an alternate route to PC (30).

These data support the view that interruption of PC synthesis at the CT step by ALPs results in an imbalance that accounts, at least in part, for the apoptotic action of ALPs. Other effects of ALPs on membrane-associated activities include the inhibition of arachidonate release by phospholipase A₂ (31), inhibition of lyso-phospholipid acylation in general (32), and inhibition of Na⁺, K⁺-ATPase and sodium pump activity (33).

ALP-induced interference with phospholipid metabolism affects the generation of lipid second messengers as discussed above. This may subsequently lead to inhibition or activation of specific downstream signal transduction pathways. Indeed, one of the well-documented effects of ALPs involves inhibition of the mitogen-activated protein kinase (MAPK or extracellular signal-regulated kinase [ERK] pathway (34). In many cell systems, mitogenic signaling is believed to depend on prolonged activation of MAPK/ERK (35) and is generally associated with increased activities of phospholipases C and D, as well as protein kinase C (PKC) (36). ALP-induced inhibition of MAPK/ERK has been reported to occur at different levels and has included a reduction of IP₃ formation, resulting in attenuation of intracellular Ca²⁺ mobilization (18, 24) (Fig. 2) and inhibition of phospholipase C (PLC), phospholipase D (PLD), and PKC activity (37–39). Another downstream target of ALP action is the stress-activated protein kinase (SAPK or c-Jun N-terminal kinase [JNK]-c-Jun pathway, which has been shown to be important in apoptosis induction (40–43). Mollinedo et al. (44) showed in human leukemic cells that ET-18-OCH₃ increased c-Jun and c-fos gene expression, leading to activation of the AP-1 transcription factor. The involvement of the SAPK/JNK pathway in ALP-induced apoptosis was further demonstrated by using antisense oligonucleotides directed against c-jun, which blocked ET-18-OCH₃-induced apoptosis (45). These findings are in line with results from our laboratory. We showed that the three ALPs: ET-18-OCH₃, HePC, and D-21266, not only prevented activation of the MAPK/ERK pathway but also activated the SAPK/JNK cascade with rapid kinetics (7) (Fig. 3A, B). Furthermore, overexpression of a dominant-negative mutant allele of c-jun abrogated ALP-induced apoptosis in human leukemic cells (7). More recently, much attention has been given to the phosphatidylinositol 3-kinase-Akt/ PKB survival pathway (46). This pathway is activated in response to growth factors and mitogens and has been shown to inhibit apoptosis induced by a variety of stress factors, including growth factor withdrawal, UV irradiation, and C2-ceramide (47–49). Preliminary data from our laboratory indicate that ALPs efficiently block insulin-induced Akt/PKB phosphorylation (Fig. 3C). Whether the inhibitory effect on the PI3K-Akt/PKB pathway is important for ALP-induced apoptosis remains to be established.

Thus, ALPs affect multiple signal transduction pathways originating from the plasma membrane. Which biologic response will prevail following ALP treatment depends on the relative contribution of each of these signaling systems (Fig. 4).
ALPs as anticancer agents

**Interaction of ALPs with ionizing radiation**

There is growing clinical evidence that the combined use of different treatment modalities, such as radiotherapy and chemotherapy, reduces local recurrence rates, improves survival, and limits normal tissue toxicity (50–52). The introduction of a new generation of biologic response modifiers in clinical studies may lead to a further improvement of the therapeutic results. Based on their favorable biologic and pharmacologic profiles, ALPs would be suitable candidates to fulfill this expectation. Several groups of investigators have studied the interaction between ALPs and chemotherapeutic drugs or ionizing radiation in vitro (7, 38, 53, 54). In HL-60 human leukemic cells treated with cytosine arabinoside (ara-C), ET-18-OCH₃ decreased cell viability in a dose-dependent fashion (38). Furthermore, ET-18-OCH₃ strongly increased apoptosis in these cells pretreated with differentiation-inducing concentrations of ara-C. In a rat sarcoma model, HePC alone had no antineoplastic effect on the tumor but enhanced the chemotherapeutic effect of cyclophosphamide (53). Additive cytotoxic effects were

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**Fig. 2.** Inhibition of lysophosphatidic acid (LPA)- and bradykinin (Bk)-induced Ca²⁺-mobilization in A431 cells by Et-18-OCH₃. (A), Calcium mobilization was determined by measuring the increase in fluorescence of the fluorochrome Indo-1-AM upon Ca²⁺ binding. (B), LPA- and Bk-induced calcium mobilization was inhibited by Et-18-OCH₃ in a dose-dependent fashion. Data represent mean ± SEM of 5 independent experiments.

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**Fig. 3.** Effect of ET-18-OCH₃ on the MAPK/ERK (A), SAPK/JNK (B), and Akt/PKB (C) pathways in A431 cells. (A), ET-18-OCH₃ inhibits LPA- and EGF-induced MAPK/ERK activity. MAPK/ERK activity was determined by using phospho-specific antibodies against p42 and p44 MAPK. (B), ET-18-OCH₃ activates the SAPK/JNK pathway. SAPK/JNK activity was determined by an in vitro kinase assay, using Gst c-Jun (1-135) as substrate. (C), ET-18-OCH₃ inhibits insulin-induced Akt/PKB activity. Akt/PKB activity was determined by using phospho-specific (Ser473) antibodies against Akt/PKB. Wortmannin also prevents insulin-induced Akt/PKB phosphorylation, indicating PI3K-dependency in this system.

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**Fig. 4.** Proposed model of the modulation of pro- and anti-apoptotic protein kinase pathways by ALPs.
also found in human carcinoma cells when ET-18-OCH₃ or HePC was combined with radiation (54). Both compounds reduced radiation-induced clonogenic cell survival in a supra-additive manner. We have tested the effect of three different ALPs (ET-18-OCH₃, HePC, and D-21266) on radiation-induced apoptosis in two types of human leukemia cells (7). All three compounds strongly enhanced radiation-induced apoptosis. This effect was additive for HePC and D-21266 and synergistic for ET-18-OCH₃. Two types of normal vascular endothelial cells were resistant to ALP-induced apoptosis, even after relatively high concentrations (7).

It remains to be established how ALPs enhance radiation-induced cytotoxicity. Ionizing radiation may not only target the nuclear DNA but like ALPs also interact with the plasma membrane and initiate similar pro-apoptotic signal transduction pathways (7, 55). Moreover, ALP-induced inhibition of mitogenic signaling at the level of PKC may also enhance radiation-induced apoptosis. Several studies support this latter possibility. Activation of PKC by growth factors or phorbol ester has been shown to protect endothelial cells from undergoing radiation-induced apoptosis (56). Conversely, pharmacological inhibition of PKC enhances cellular radiosensitivity (57).

**CONCLUDING REMARKS**

In summary, many studies, both experimental and clinical, have been performed to better describe the pharmacologic, biologic, and toxicologic characteristics of ALPs. The efficient and selective antitumor effect of these drugs, as well as their capacity to enhance radiation-induced cell kill, make ALPs attractive candidates for introduction in clinical radiotherapy.

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