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Review

# Anticancer mechanisms and clinical application of alkylphospholipids ☆

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ABSTRACT

Synthetic alkylphospholipids (ALPs), such as edelfosine, miltefosine, perifosine, erucylphosphocholine and erufosine, represent a relatively new class of structurally related antitumor agents that act on cell membranes rather than on DNA. They selectively target proliferating (tumor) cells, inducing growth arrest and apoptosis, and are potent sensitizers of conventional chemo- and radiotherapy. ALPs easily insert in the outer leaflet of the plasma membrane and cross the membrane via an ATP-dependent CDC50a-containing 'flippase' complex (in carcinoma cells), or are internalized by lipid raft-dependent endocytosis (in lymphoma/leukemic cells). ALPs resist catabolic degradation, therefore accumulate in the cell and interfere with lipid-dependent survival signaling pathways, notably PI3K-Akt and Raf-Erk1/2, and de novo phospholipid biosynthesis. At the same time, stress pathways (e.g. stress-activated protein kinase/JNK) are activated to promote apoptosis. In many preclinical and clinical studies, perifosine was the most effective ALP, mainly because it inhibits Akt activity potently and consistently, also in vivo. This property is successfully exploited clinically in highly malignant tumors, such as multiple myeloma and neuroblastoma, in which a tyrosine kinase receptor/Akt pathway is amplified. In such cases, perifosine therapy is most effective in combination with conventional anticancer regimens or with rapamycin-type mTOR inhibitors, and may overcome resistance to these agents. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.



## 1. Introduction: development of clinically useful ALPs

Synthetic metabolically stable analogs of lysophosphatidylcholine (LysoPC) are widely used as biological response modifiers. Already in the late 1960s, Hansjörg Eibl chemically replaced the glycerol-linked C1 ester bond in lysoPC by an ether linkage, and added another ether-linked methyl group at the C2 position [1]. The resulting ether lipid, 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phophocholine (named edelfosine, ET-18-$OCH_3$) (Fig. 1) appeared a potent immune modulator and an effective inhibitor of (tumor) cell proliferation [2–4]. The cytotoxic effect of edelfosine has been evaluated in a large variety of both tumor (leukemic and solid) and normal cell types, showing a high degree of selectivity towards tumor cells [5–8]. In addition to this potent anti-tumor activity in vitro, edelfosine has demonstrated in vivo efficacy in tumor models in both mouse [6,9,10] and rat [11,12] However, clinical use of edelfosine has remained limited [13,14]. In fact, the only clinically relevant application of edelfosine at this moment is for purging purposes of bone marrow in acute leukemia patients [15,16].

A number of other ether analogs of lysoPC subsequently followed prototypic edelfosine as promising anticancer agents (Fig. 1). We conveniently abbreviate these compounds collectively as ALPs, although they are formally classified in two groups, i.e. alkyl-lysophospholipids and alkylphospholipids. A thio-ether variant of edelfosine, 1-hexadecyl-thio-2-methoxymethyl-*rac*-glycero-3-phosphocholine (ilmofosine) has demonstrated both in vitro and in vivo anti-tumor activity in a variety of tumors, but lacked clinical activity in patients [17–20]. Similar to edelfosine, gastrointestinal toxicity was the most frequently observed and dose-limiting side effect of ilmofosine.

Eibl and Unger, in the late 1980s, identified hexadecylphosphocholine (miltefosine), lacking a glycerol moiety (Fig. 1), as the minimal structural requirement for the antitumor activity of ALPs [21]. Unlike most ALPs, miltefosine is metabolized by phospholipases to a substantial degree, yielding (non-toxic) choline, phosphocholine and

*Abbreviations:* ALP, alkylphospholipid; ASK1, apoptosis signal-regulating kinase 1; CT, CTP:phosphocholine cytidylyltransferase; DISC, death-inducing signaling complex; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinase; JNK, c-Jun N-terminal kinase; ErPC, erucylphosphocholine; ErPC3, erucylphosphohomocholine (erufosine); FADD, Fas-associated protein with death domain; c-$FLIP_L$, cellular FLICE (FADD-like interleukin 1β-converting enzyme)-inhibitory protein long form; MAPK, mitogen-activated protein kinase; MM, multiple myeloma; PC, phosphatidylcholine; PI, phosphatidylinositol; $PIP_2$, phosphatidylinositol-4,5 bisphosphate; $PIP_3$, phosphatidylinositol-3,4,5 trisphosphate; PI3K, phosphatidylinositol 3-kinase; PH, pleckstrin homology domain; PLC, phospholipase C; PKD, protein kinase D; ROS, reactive oxygen species; SAPK, Stress-activated protein kinase; SHIP-1, SH2 (Src homology 2)-domain-containing inositol phosphatase-1; SMS, sphingomyelin synthase; mTOR, mammalian target of rapamycin; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand

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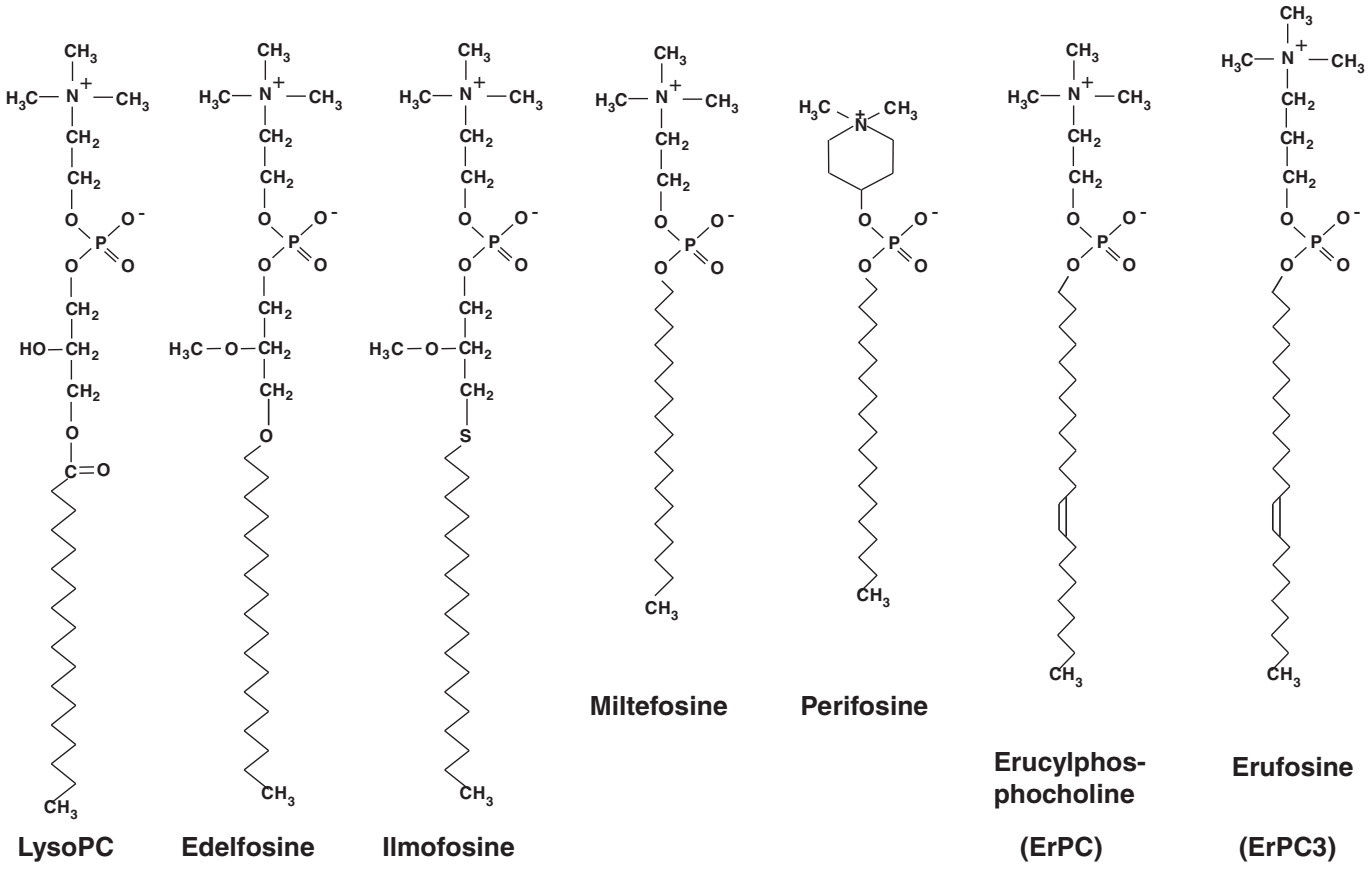


**Fig. 1.** Chemical structures and commercial names of synthetic clinically relevant alkyl-phospholipids (ALPs), metabolically stable analogs of natural lysophosphatidylcholine (LysoPC). In the alkyl-lysophospholipid-prototype edelfosine and its thio-ether derivative ilmofosine, the glycerol backbone is maintained. The other ALPs lack the glycerol backbone, the alkyl chain being esterified directly to the phosphate group. Miltefosine (hexadecylphosphocholine) is the prototype of this group of compounds. In perifosine, the choline headgroup is substituted by a heterocyclic methylated piperidyl residue. Erucylphosphocholine (ErPC) differs from miltefosine only by a longer chain length ($C_{16} \rightarrow C_{22}$) and the introduction of a ω-9 cis-double bond. Erufosine (ErPC3; erucylphosphohomocholine) differs from ErPC by one additional methyl group in the choline headgroup, yielding higher solubility in aqueous solutions.

1,2-diacylphosphatidylcholine after systemic treatment [22,23]. Nevertheless, miltefosine displays potent antitumor activity in vitro [23–25] and in some tumor models [26]. Importantly, due to its hemolytic effect, miltefosine could not be administered intravenously [27] but only as an oral or topical formulation. Unfortunately, gastrointestinal toxicity and lack of activity of miltefosine in patients with advanced soft tissue sarcoma [28], metastatic colorectal cancer [29] and squamous cell carcinoma of the head and neck [30] prevented its further development as an oral anti-cancer agent. When applied topically, however, miltefosine was effective in the treatment of skin metastases of breast cancer [31]. Subsequent phase II trials confirmed these observations and reported objective responses to topical miltefosine treatment in patients with cutaneous lymphoma [32] and cutaneous breast cancer metastases [33]. In addition to their application as anticancer agents, ALPs have shown activity against protozoal disease [34], and miltefosine is now widely used as an effective oral treatment against visceral leishmaniasis [35,36].

Yet another structural ALP modification, aimed at improving therapeutic potency and metabolic stability, was obtained by replacing the choline moiety in miltefosine by a heterocyclic piperidine group, yielding octadecyl-(N,N-dimethyl-piperidino-4-yl)-phosphate (perifosine; D-21266; Fig. 1) [37]. Perifosine showed cytotoxic effects against a wide range of tumor cell types in vitro and showed favorable pharmacokinetics [38]. Clinically relevant plasma concentrations could be reached in mice after single oral dosing. Perifosine was not metabolized and displayed only slow elimination, with a terminal half-life of approximately 140 h. In three different types of xenografted squamous cell carcinomas, high levels of tumor accumulation were measured [38]. Upon oral administration perifosine showed a dose-dependent anti-tumor effect in a variety of preclinical models [37–39]. Perifosine was tested in a large number of clinical studies (reviewed in [40,41]) and was well tolerated. However, phase II clinical trials of perifosine as a single agent on recurrent prostate cancer, adenocarcinomas, and melanomas have been disappointing. Its anticancer activity, due to inhibition of the Ser/Thr kinase Akt, is now most successfully exploited in combination with other anti-cancer regimens [41–43] (see Section 4).

Erucylphosphocholine (ErPC) and its homolog erufosine (ErPC3; erucylphospho-N,N,N-trimethylpropylammonium) (Fig. 1) represent the most recent members of the ALP family [44] with promising clinical potential. With a longer 22 carbon chain and a ω-9 cis-double bond, they lack hemolytic- and myelotoxicity due to the formation of lamellar instead of micellar structures in aqueous solutions and are therefore suitable for intravenous administration. This resulted in significant tumor remissions in experimental rat gliomas and mammary carcinomas at relatively low doses and with reduced gastrointestinal toxicity [45,46]. Importantly, ErPC3 and especially ErPC are superior to other ALPs in their ability to cross the blood–brain barrier and accumulate in brain tissue [47,48]. In view of the positive response of brain tumors both in vitro [44] and in vivo [45], ErPC and ErPC3 should be considered as promising ALPs, with a potential application in the treatment of patients with otherwise poorly responsive primary or metastatic brain tumors. No clinical data on these compounds are available yet.