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Antileukemic effect of alkyl phospholipids

I. Inhibition of proliferation and induction of differentiation of cultured myeloid leukemia cells by alkyl ethyleneglycophospholipids

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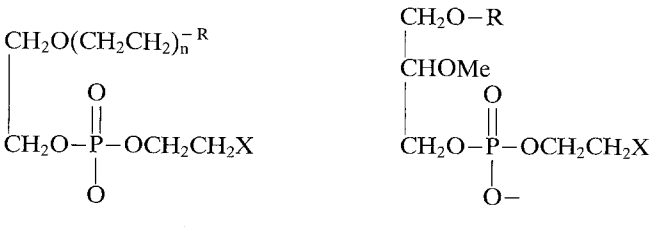
Summary. *Various alkyl ethyleneglycophospholipids, i.e., alkyl phospholipids, with ethyleneglycol or its congener in place of glycerol as a molecular backcone, were synthesized and their effects on cell proliferation and differentiation of cultured human (HL-60) and mosue (Ml) myeloid leukemia cells were studied. On incubation with alkyl ethyleneglycophospholipids, proliferation of both cell lines was inhibited and the cells were induced to differentiate into morphologically and fuctionally mature granulocytes and macrophages. Among the compounds tested, dodecyl ethyleneglycophospholipid with a pyridinioethyl group was the most effective in induction of differentiation of both cell lines.*

Introduction

Several human and mouse myeloid leukemia cells can be induced to differentiate in vitro into mature macrophages and granulocytes by various inducers [2, 11, 12, 15, 16]. The M1 myeloid leukemia cell line, established from an SL mouse, and the HL-60 cell line, from a patient with acute promyelocytic leukemia, have been extensively investigated, and results have shown that differentiation of the cells is closely associated with a change in phospholipid metabolism [3, 5, 7, 8, 10]. Moreover, alkyl lysophospholipids (ALPLs, Fig. 1), which might cause a serious disturbance of phospholipid metabolism, induced differentiation of M1 cells and HL-60 cells into macrophages and granulocytes [9].

Syngeneic mice inoculated with myeloid leukemia M1 cells all died of leukemia, but their survival was significantly enhanced by injection of an inducer of differentiation [6, 11, 14]. These findings suggest that inducers of differentiation might be effective in the therapy of leukemia. Preliminary experiments, however, showed that ALPLs had considerable side-effects in SL mice syngeneic to M1 myeloid leukemic cells. Since inducers causing few side-effects must be found for clinical use, we examined the effects of various alkyl phospholipids on differentiation of myeloid leukemia cells and found an effective class of alkyl phospholipids.



AEPL, I ALPL, II

Fig. 1. Structures of alkyl ethylene glycophospholipids (AEPLs) and alkyl lysophospholipids (ALPLs)

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Materials and methods

The alkyl phospholipid analogs used were synthesized in our laboratories by a modification of the method described elsewhere [18].

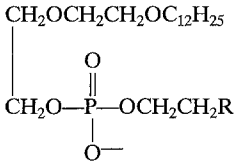
Cells and cell culture. Human promyelocytic leukemia HL-60 cells were maintained in PRMI-1640 medium supplemented with 15% fetal calf serum as previously described [1]. M1 cells were kept in Eagle's minimum essential medium with twice the usual concentrations of amino acids and vitamins and supplemented with 10% heat-inactivated calf serum [6, 9].

Assay of the properties of differentiated cells. Nitroblue tetrazolium reduction was assayed as reported previously [4]. The percentage of cells containing intracellular blue-black formazan deposits was then determined by examination of at least 300 cells. For assay of phagocytic activity, cells were incubated for 5 h with a suspension of polystyrene latex particles (2 μl/ml of serum-free culture medium; average diameter, 1 μm; Dow Chemical Co.). Then the cells were washed three times with phosphate-buffered saline (138 m*M* NaCl-2.7 m*M* KCl-8 m*M* Na_2HPO_4-1.5 m*M* KH_2PO_4, pH 7.4), and the number of phagocytic cells among at least 300 viable cells was counted [9]. Lysozyme activity was determined by a lysoplate method with lysoplates containing 1% agar, $^1/_{15}$ *M* sodium phosphate buffer (pH 6.6), 50 m*M* NaCl, and heat-killed *Micrococcus lysodeikticus* (0.5 mg/ml) [13]. The percentages of cells that were morphologically similar to mature granulocytes and macrophages were determined in smears treated with May-Grünwald-Giemsa stain.

Results

To find new and more effective inducers, we synthesized various types of alkyl phospholipids and examined their effects in inducing differentiation of myeloid leukemia cells into mature granulocytes and macrophages.

Table 1. Effects of dodecyl ethyleneglycophospholipids on growth and differentiation of HL-60 cells

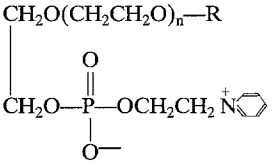


Compound no.	R	GD_{50}[a] (μg/ml)	Morphological[b] differentiation (% of mature myeloid cells)
III	$\overset{+}{N}H_3$	5.8 ± 0.4	13 ± 2
IV	$\overset{+}{N}(CH_3)_3$	3.5 ± 0.3	14 ± 2
V		3.6 ± 0.3	28 ± 3
VI		5.1 ± 0.4	9 ± 2
VII		6.8 ± 0.4	11 ± 2
VIII		7.5 ± 0.5	3 ± 1
IX		5.2 ± 0.3	8 ± 2
X		5.6 ± 0.4	7 ± 1

[a] GD_{50}, concentration resulting in half the number of control generations ± SD

[b] Mature myeloid cells, myelocytes, metamyelocytes, and banded and segmented neutrophils. HL-60 cells were cultured with the GD_{50} concentration of each compound for 6 days

Table 2. Effects of alkyl ethyleneglycophospholipids with a pyridinioethyl group on growth and differentiation of HL-60 cells



Compound no.	*n*	R	GD_{50}[a] (μg/ml)	Morphological[b] differentiation (% of mature myeloid cells)
V	1	$C_{12}H_{25}$	3.5 ± 0.3	28 ± 3
XI	1	$C_{13}H_{27}$	2.6 ± 0.2	27 ± 3
XII	1	$C_{14}H_{29}$	1.2 ± 0.2	24 ± 2
XIII	1	$C_{15}H_{35}$	1.5 ± 0.2	18 ± 3
XIV	1	$C_{18}H_{35}$	1.4 ± 0.2	7 ± 2
XV	1	$C_{18}H_{37}$	1.2 ± 0.2	12 ± 2
XVI	0	$C_{15}H_{31}$	1.6 ± 0.2	15 ± 2
XVII	0	$C_{14}H_{29}$	2.8 ± 0.3	8 ± 1
XVIII	0	$C_{18}H_{37}$	1.2 ± 0.2	6 ± 1
XIX	2	$C_{12}H_{25}$	2.5 ± 0.3	8 ± 2
XX	4	$C_{12}H_{25}$	2.6 ± 0.3	7 ± 1

[a] GD_{50}, concentration resulting in half the number of control generations ± SD

[b] Mature myeloid cells, myelocytes, metamyelocytes, and banded, and segmented neutrophils. HL-60 cells were cultured with the GD_{50} concentration of each compound for 6 days

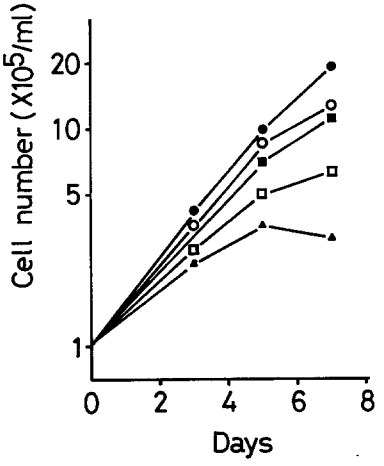


Fig. 2. Effect of dodecyl ether, V, on growth of HL-60 cells. HL-60 cells were treated with various concentrations (μg/ml) of V. (●) 0; (○) 1; (■) 2; (□) 4; (▲) 6

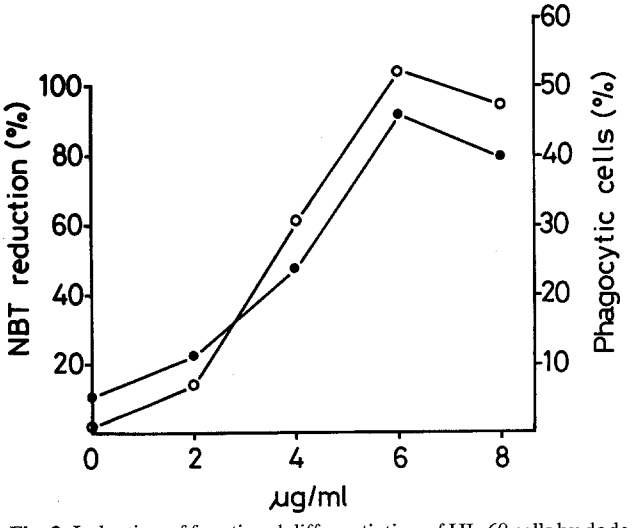


Fig. 3. Induction of functional differentiation of HL-60 cells by dodecyl ether, V. HL-60 cells were cultured for 5 days in medium containing V. (●) nitroblue tetrazolium reduction; (○) phagocytosis

Alkyl ethyleneglycophospholipids (EAPLs, Fig. 1) are novel synthetic phospholipids which differ from natural phosphoglycerides in that they contain neither fatty acid ester or glycerol but possess one long-chain alkyl ether group (R-) as a non-polar tail and ethyleneglycol, diethyleneglycol, or its congener as a molecular backbone. They also contain phosphoric acid esterified to a substituted or unsubstituted aminoalcohol as a polar head group.

In the course of screening, we found that AEPLs, like ALPLs, induced differentiation of human promyelocytic leukemia HL-60 cells and that in general they had less acute toxicity to mice than ALPLs. Then we examined the effects of various AEPLs (compounds III–XX) on differentiation of HL-60 cells (Tables 1 and 2). The growth-inhibiting effects of the compounds were examined by determining the concentrations of the drugs required to reduce the cell number to half that of untreated cultures (GD_{50}).

Table 1 shows that dodecyl ethyleneglycophospholipid with a pyridinioethyl group (V) inhibited the growth of HL-60 cells as effectively as the corresponding choline analog (IV),

Table 3. Induction of morphological differentiation of HL-60 cells by compound V

Compound V (μg/ml)	Cell number[a] (× 10^5/ml)	Cell type (%)		
		Myeloblasts and promyelocytes	Myelocytes and metamyelocytes	Banded and segmented neutrophils
0	19.2	98	2	0
1	15.6	91	9	0
3	12.6	76	21	3
5	6.4	36	57	7
7	3.2	11	75	14

[a] HL-60 cells were cultured with various concentrations of compound V for 6 days

Table 4. Induction of morphological and functional differentiation of M1 cells by compound V

Compound III (μg/ml)	Cell number[a] (× 10^5/ml)	Lysozyme activity (U/10^7 cells)	Phagocytic cells (%)	Morphological change (%)[b]
0	11.8	2.2	0	1.0
2	8.7	7.5	4	9.2
4	6.3	16.2	17	38.3
6	4.0	24.5	26	47.5
8	2.2	19.6	24	63.1

[a] M1 cells were cultured for 4 days
[b] Cells in an intermediate stage of differentiation, mature granulocytes, and macrophages

but was more effective than the latter in inducing differentiation of HL-60 cells. Among the pyridinioethyl compounds tested, the dodecyl V, tridecyl XI, and tetradecyl XII ethers were the most effective in inducing differentiation. However, compound XI had more acute toxicity to mice than compounds V and XII. When these compounds were given as a single dose of 2 mg IP, all mice that received tridecyl ether, XI, died within 2 days, but four of five mice that received tetradecyl ether, XII, survived. The GD_{50} value of XI was also higher than that of XII (Table 2). Dodecyl ether, V, was less toxic than tetradecyl ether, XII, and mice tolerated it when it was given as repeated injections of 2 mg.

Figure 2 shows the effect of V on growth of HL-60 cells. This compound inhibited cell growth appreciably at a concentration of more than 1 μg/ml. When HL-60 cells were cultured without inducers most of them were promyelocytic, whereas when the cells were cultured with increasing concentrations of V mature granulocytes appeared and increased in number (Table 3). We next examined the ability of V to induce phagocytosis and reduction of nitroblue tetrazolium dye by HL-60 cells, which are typical functional markers of differentiation of these cells (Fig. 3). Results showed that induction by V was dose-dependent, with a maximum at a concentration of 6 μg/ml.

Mouse myeloid leukemia M1 cells were also induced to differentiate into macrophages and granulocytes by treatment with V (Table 4). V had a similar effect on M1 cells to that on HL-60 cells.

Discussion

The relation of the structure of alkyl phospholipids to their ability to induce differentiation of myeloid leukemia cells was studied to allow some insight into the nature of the cellular target sites. Phosphatidic acid, phosphatidylinositol or phosphatidylserine analogs had no influence on cell growth or differentiation, even when used at the effective concentrations of the pyridinioethyl phosphate analogs (data not shown). All the compounds found to induce cell differentiation were phospholipids with O-alkyl groups of various chain lengths and a quaternary ammonium group (Table 1). Although analogs with relatively short alkyl chains ($C_{12}H_{25}$-, $C_{13}H_{27}$-) and with a pyridinio group slightly inhibited cell proliferation, analogs with a dodecyl group strongly induced cell differentiation.

Previous experiments showed that 1-O-tetradecyl lysophosphatidylcholine (ALPL, II, R = $C_{14}H_{29}$, X = $\overset{+}{N}Me_3$) is effective at a concentration as low as 1 μg/ml in inducing differentiation of M1 cells and HL-60 cells, and that this compound has no effect on proliferation and differentiation of normal mouse bone marrow cells at a concentration as high as 20 μg/ml [9]. The selective effect of the compound on leukemia cells may be attributed to the lack of activity of the 1-O-alkyl cleavage enzyme in most tumor cells, unlike in normal cells [17]. However, this ALPL has significant side-effects, and in particular, impairs excretory processes when injected IP into mice. AEPLs had similar types of side-effects, but their toxicities were much lower than those of ALPLs. Thus they appear to be more effective for leukemia therapy.

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