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Antileukemic effect of alkyl phospholipids

II. Prolongation of survival times of leukemic mice by alkyl ethyleneglycophospholipids

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Summary. *Alkyl ethyleneglycophospholipids induced differentiation in vitro of mouse myeloid leukemia M1 cells into mature granulocytes and macrophages. The compounds also prolonged the survival of syngeneic SL mice inoculated with M1 cells. Although in mice with florid leukemia these compounds alone scarcely affected survival, administration of dodecyl ethyleneglycophospholipid with pyridinioethyl as a polar group plus actinomycin D significantly prolonged survival.*

Introduction

Mouse myeloid leukemia M1 cells are leukemogenic to syngeneic SL mice, but they can be induced by various inducers to differentiate in vitro into mature granulocytes and macrophages, and in syngeneic mice to lose their leukemogenicity [4, 7, 8]. In a diffusion chamber in syngeneic SL mice, myeloblastic leukemia M1 cells were induced to differentiate into mature granulocytes and macrophages and their proliferation rate decreased [4]. We recently showed that M1 cells were induced to undergo differentiation in the host even under conditions in which leukemic cells could interact directly with the host cells [5, 7]. Moreover, M1 cells in the peritoneal cavity of syngeneic SL mice were stimulated to differentiate into mature granulocytes and macrophages when the mice were treated with some inducers of differentiation [5].

Resistant M1 cells that could not differentiate in vivo were much more leukemogenic than sensitive cells that could be induced to differentiate [4, 7]. Some inducers of cell differentiation significantly enhanced the survival times of mice inoculated with sensitive M1 cells, but scarcely affected the survival times of mice inoculated with resistant M1 cells [7]. On the basis of these results, we suggested that treatment of leukemia in vivo with inducers of differentiation could be a new approach to the control of leukemia [7].

In the present experiments we examined the possible therapeutic effects on leukemia in mice inoculated with M1 cells of alkyl ethyleneglycophospholipids (AELPs) that induced M1 cells to undergo terminal differentiation in vitro.

Materials and methods

Animals. Inbred SL strain mice were maintained as previously reported, and mice 6–8 weeks old were used for experiments [4].

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Cells. M1 cells were isolated from a spontaneous myeloid leukemia in an SL mouse. The cells were cultured in suspension in Eagle's minimum essential medium with twice the normal concentrations of amino acids and vitamins, and supplemented with 10% heat-inactivated calf serum [4, 8]. The cells were cultured at 37° C in a humid atmosphere of 5% CO_2 in air.

Administration of AEPLs and actinomycin D. The alkylphospholipids used were synthesized in our laboratories by a modification of the method described elsewhere [11]. The compounds used in the present experiment were 2-[2-(dodecyloxy)ethoxy]ethyl 2-pyridinioethyl phosphate, compound V, and 2-[2-(tetradecyloxy)ethoxy]ethyl 2-pyridinioethyl phosphate, compound XII [6]. They were dissolved in 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 0.2 ml volumes of the solutions were injected into mice IP three times a week. Actinomycin D was purchased from Sigma Chemical Co, St. Louis, USA. Stock solution (0.5 mg/ml) was prepared in absolute ethanol. Mice were injected IP with 0.2 ml of alkylphospholipid solution containing 2 µl of the stock solution of actinomycin D.

Growth of M1 cells in bone marrow of syngeneic mice. M1 cells were injected IV into the tail vein of SL mice at 10^6 cells per mouse. At various times after the inoculation bone marrow cells were removed from the femur, and their cellular composition was determined on smears stained with May–Grünwald–Giemsa, by examination of at least 400 cells.

Statistical analysis. Differences between values were evaluated by an unpaired two-tailed *t*-test and were considered significant if the *P* value was less than 5%.

Results

Prolongation by AEPLs of survival times of mice inoculated with M1 cells

Syngeneic SL mice inoculated IP with 3×10^5 M1 cells received AEPLs IP three times a week, the first injection being given 1 day after tumor challenge. AEPLs significantly prolonged the survival of mice inoculated with M1 cells (Fig. 1): all the untreated mice inoculated with M1 cells died by day 30 after the inoculation, the mean survival time being

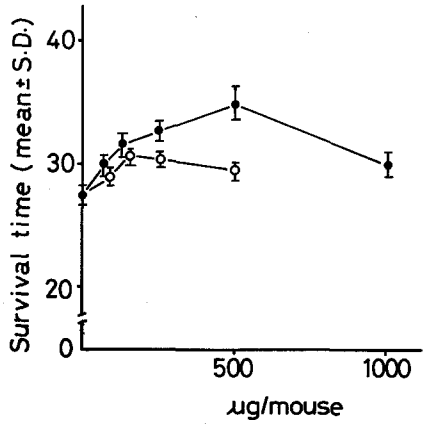

Fig. 1. Effects of various doses of AEPLs on the survival of mice inoculated with M1 cells. Mice received compound V (●) or compound XII (○) IP three times a week. *Points* are means for 10–14 mice; *bars* indicate SD

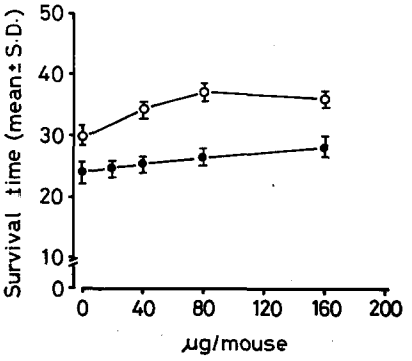

Fig. 2. Effects of various doses of compound V with or without actinomycin D on the survivals of mice with florid leukemia. Mice were inoculated IV with 10^6 M1 cells. Treatment with compound V with (○) or without (●) 1 μg actinomycin D was started 21 days after inoculation of M1 cells. *Points* are means for 30–37 mice; *bars* indicate SD

Table 1. Development of myeloid leukemia in SL mice inoculated with M1 cells

Day after inoculation	Cell type of bone marrow (% of nucleated cells)							
	Blasts	Promyelocytes	Myelocytes and metamyelocytes	Stab and segmented neutrophils	Eosinophils	Lymphocytes	Erythroid cells	Others
0	7 ± 3	11 ± 4	18 ± 4	27 ± 5	12 ± 4	9 ± 2	11 ± 3	5 ± 1
11	6 ± 2	13 ± 3	23 ± 5	22 ± 4	8 ± 3	11 ± 2	8 ± 2	9 ± 3
16	17 ± 3	8 ± 2	24 ± 6	20 ± 3	5 ± 2	9 ± 2	7 ± 2	10 ± 3
21	61 ± 7	6 ± 2	8 ± 2	13 ± 3	6 ± 2	3 ± 1	2 ± 1	1 ± 1

M1 cells were inoculated into the tail vein of SL mice at 10^6 cells per mouse. Bone marrow smears were made on the days indicated. More than 400 cells were examined. Averages (± SD) from four separate experiments are shown

about 27 days, whereas the mean survival time of mice treated with 125 μg compound XII was about 31 days and that of mice treated with 500 μg compound V was about 35 days.

Effect of administration of AEPLs plus actinomycin D on the survival of leukemic mice

For studies on the effect of AEPLs on the survival of mice with florid leukemia, 10^6 M1 cells were injected into the tail vein. Since bone marrow smears made 21 days later showed that more than 60% of the bone marrow nucleated cells were myeloblastic (Table 1), we used these animals as mice with florid leukemia.

When treatment with AEPLs was started 21 days after IV inoculation of 10^6 M1 cells no prolongation of survival was observed (Fig. 2), even with high doses of AEPLs (data not shown).

We previously reported that M1 cells could be sensitized to some inducers of differentiation by treatment with actinomycin D at a low concentration that did not affect the viability of the cells [9]. Figure 3 shows the effect of compound V on induction of phagocytic activity, a typical marker of differentiated cells,

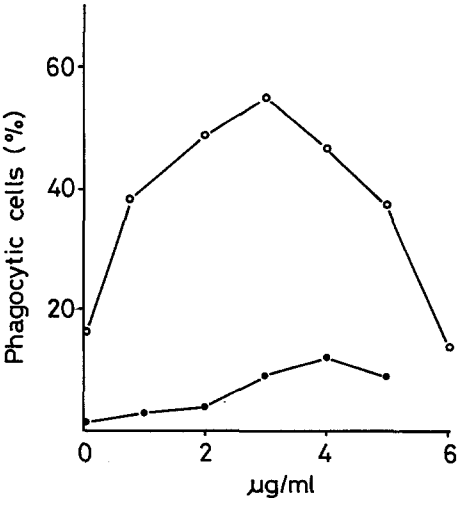

Fig. 3. Synergistic effects of compound V and actinomycin D on induction of phagocytosis in less sensitive M1 cells. Phagocytic activities of cells treated with compound V alone (●) or compound V and 1 ng/ml actinomycin D (○) for 2 days

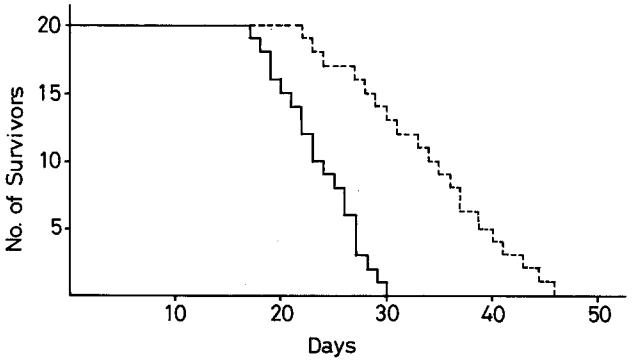

Fig. 4. Survivals of leukemic mice injected with compound V plus actinomycin D. *Solid line* untreated leukemic mice; *broken line*, leukemic mice treated with 80 μg of compound V plus 1 μg actinomycin D

in less sensitive M1 cells (clone DS-1-1). After treatment with compound V, about 10% of the cells were phagocytic, whereas after treatment with compound V plus 1 ng/ml actinomycin D 60% of the cells were phagocytic. These results show that actinomycin D and AEPLs have synergistic effects.

On the basis of this in vitro observation, we examined the effect of actinomycin D plus compound V on survival of leukemic mice. The survival of leukemic mice was significantly enhanced by treatment with compound V plus 1 μg actinomycin D (Figs. 2 and 4).

Discussion

Alkyl lysophospholipids (ALPLS) inhibit or retard tumor growth in vivo [2, 10]. Intravenous, intracutaneous or oral administration of ALPLs had a protective effect against the development of metastases, although when given SC or IP they have so far been ineffective [1]. We previously examined the effect of ALPLs on survival of mice inoculated with M1 cells. In this syngeneic leukemia model, IP or PO treatment with various ALPLs caused only slight prolongation of survival (data not shown). Berdel et al. reported tha the LD_{50} values of ALPLs were much higher when these compounds were bound to serum proteins and that in this form their hemolytic effects was inhibited [2]; when ALPLs were given as solutions in 5% albumin they showed significant therapeutic activity against the growth of various tumors.

In the present work, we tried to find antileukemic drugs with few side-effects. We selected the effective compounds from a variety of synthetic alkyl phospholipids by measuring their activities for inducing differentiation of human and mouse myeloid leukemia cells, but not their cytotoxic activities [6]. From compounds that induced differentiation, we then selected those with less acute toxicity in mice. Among about 100 compounds, we found that dodecyl ethyleneglycophospholipid with a pyridinioethyl group, compound V, was the most suitable. The compounds in this group showed extremely low hemolytic activity: the hemolytic activity of V was less than 1/100th that of 1-O-octadecyl lysophosphatidylcholine (data not shown). Therefore, IP administration of compound V without binding with serum protein had a protective effect against myeloid leukemia.

The previous and present results show that low concentrations of drugs used in cancer chemotherapy, such as actinomycin D, adriamycin, and daunomycin, can act synergistically with inducers of differentiation of M1 cells [3, 9]. These findings are of therapeutic significance, because leukemia cells tend to become spontaneously resistant to an inducer of cell differentiation. Among the AEPLs tested the most effective inducer of differentiation (compound V) was the most effective in prolonging survival of leukemic mice, but not the most cytotoxic compound. Therefore, the effect of alkylphospholipids on survival may be partly due to stimulation of in vivo differentiation of M1 cells.

Combination therapy with alkylphospholipid and actinomycin D was definitely more effective than therapy with alkylphospholipid or actinomycin D alone. These results suggest that AEPLs could well be useful in treatment of human patients with myeloid leukemia.

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