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Short communication

Effect of ether lipids on mouse granulocyte-macrophage progenitor cells

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Summary. In this study we determined the potential bone marrow toxicity of the ether lipid derivatives 1-0-octadecyl-2-0-methyl-rac-glycero-3-phosphocholine (OcMe-G-3-PC), 1-0-hexadecyl-propanediol-2-phosphocholine (He-Pr-2-PC), and hexadecylphosphocholine (He-PC). OcMe-G-3-PC inhibited the proliferation of mouse granulocyte-macrophage progenitor cells (GM-CFCs) at a dose of 1 μg/ml, whereas He-Pr-2-PC and He-PC started to inhibit the growth of hemopoietic precursors at 5 μg/ml. In contrast to this finding, NMRI mice given 10 mg/kg i.v. daily for 4 weeks and 20 or 30 mg/kg for 5 days showed no bone marrow toxicity. We conclude that the dose-dependent toxic effects observed in vitro are within the physiological tolerance in vivo.

Introduction

Both naturally occurring and synthetically prepared ether lipids show a variety of biological properties such as activation of macrophages [16, 17], platelet aggregation and serotonin release [1, 2, 20], inhibition of neoplastic cell invasion in normal tissue [21], influence on diffusion characteristics of biological membranes [22, 23], induction of differentiation of malignant cells [13, 14], and cytotoxicity against tumor cells in vitro and in vivo [3, 5, 9, 17, 18]. One of the intriguing and promising aspects of tumor chemotherapy with ether lipids is that, unlike essentially all other antitumor agents, these compounds are incorporated into and interact with the cell membrane, not with the nucleus. In addition, some selected ether lipids have been shown to display selective antineoplastic activity against leukemic cells compared with normal bone marrow progenitor cells in vitro [19, 25] and against the methylnitrosourea (MNU)-induced rat mammary carcinoma in vivo [5, 10, 11, 18]. In view of increasing evidence of the antineoplastic effect of some ether lipids, we deemed it important to investigate possible hematotoxic side effects. Three selected ether lipids were investigated for possible toxic effects on bone marrow cells in vitro and in vivo.

Materials and methods

1-0-Octadecyl-2-0-methyl-rac-glycero-3-phosphocholine (OcMe-G-3-PC), 1-0-hexadecyl-propanediol-2-phosphocholine (He-Pr-2-PC), and hexadecylphosphocholine (He-PC) were synthesized in our laboratory as previously described by Eibl [7, 8].

Isolation of murine bone marrow cells. Femurs of mice were removed and denuded under aseptic conditions. Bone marrow cells were flushed out with a 24-gauge needle. The cells were suspended in RPMI-1640 medium (Biochrom, Berlin, FRG) and counted in a Neubauer chamber prior to culturing.

Preparation of WEHI-3B conditioned medium. The murine myelomonocytic cell line WEHI-3B was grown in RPMI-1640 medium supplemented with 20 m*M* HEPES, 4 m*M* L-glutamine, 200 μg/ml streptomycin/penicillin (Biochrom, Berlin, FRG), and 10% fetal calf serum (Gibco, Karlsruhe, FRG) until the cells were in a logarithmic growth phase. The culture medium was then changed and the cells were incubated for 2 to 3 additional days. Thereafter, the cell medium was removed, centrifuged, and dialyzed against distilled water for 3 days, with daily changes of the dialyzing fluid.

Agar cultures. The agar cultures were prepared according to the method of Metcalf [15]. In short, 1 ml agar culture medium consisting of equal volumes of double-strength Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Karlsruhe, FRG) and 0.6% Bacto-Agar (Difco; Detroit, Michigan, USA) in distilled water, 50,000 bone marrow cells, 10% WEHI-3B conditioned medium as a growth factor source, and either ether lipid solution or phosphate-buffered solution (PBS) as a control was plated in 35-mm petri dishes. The plates were incubated at 37° C under a fully humidified atmosphere and 6.5% CO_2. Colonies were scored after 7 days; only those containing >40 cells were taken into account.

Animal experiments. Female NMRI mice (8–12 weeks old, 25–30 g) were used in the study. Ether lipids were dissolved at varying concentrations in 0.9% NaCl containing 0.5% (w/v) bovine serum albumin. Aliquots of 200 μl ether lipid solution or 0.9% NaCl were injected via the tail vein. The mice were divided in three groups: one group received a dose of 10 mg/kg daily for 4 weeks (5 days/week), and the second and third groups were treated with daily injections of 20 and 30 mg/kg, respectively, for 5 days. The mice were killed 5 days after the termination of treatment

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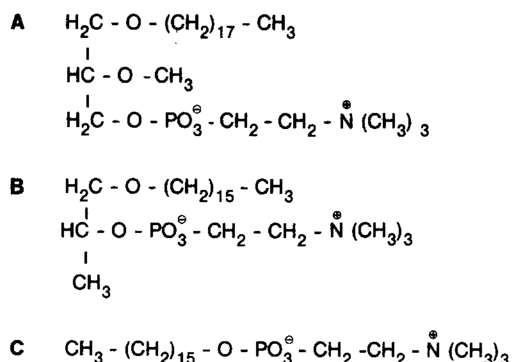

Fig. 1. Chemical structures of the various ether lipids. *A*, 1-0-Octadecyl-2-0-methyl-rac-glycero-3-phosphocholine (OcMe-G-3-PC); *B*, 1-0-hexadecyl-propanediol-2-phosphocholine (He-Pr-2-PC); *C*, hexadecylphosphocholine (He-PC)

and the bone marrow was withdrawn for stem-cell culture experiments.

Results and discussion

The organ most frequently affected by cancer chemotherapeutic agents is the bone marrow. Because of differences in the peripheral blood half-life of erythrocytes, leukocytes, and platelets, the latter two are obviously most frequently involved in early suppression by chemotherapy [12]. Therefore, the inhibition of in vitro colony formation of granulocyte-macrophage progenitor cells (GM-CFCs) may represent a relevant, sensitive reference to toxic effects of antineoplastic agents.

The chemical structures of the ether lipid derivatives used in this study are given in Fig. 1. There are three reasons why these compounds were selected for the investigation of possible hematotoxic side effects. First, they represent main classes in a broad spectrum of structural variations of cytotoxic ether lipids; second, they can inhibit tumor growth in vitro and in vivo [3, 5, 9, 17, 24]; and third, two of the selected ether lipids have recently been introduced into clinical phase I trials [4, 24].

Table 1 summarizes the in vitro effects of these derivatives on granulocyte-macrophage colony formation in normal murine bone marrow progenitor cells. The compounds display a concentration-dependent inhibition of colony formation of GM-CFCs. The inhibitory effect of OcMe-G-3-PC was observed even at a concentration of about 1 μg/ml, whereas He-PC and He-Pr-2-PC revealed inhibition at a concentration of about 5 μg/ml. Thus, in comparison with OcMe-G-3-PC, the latter two compounds are about 5 times less toxic. This may partially be explained by a difference in the length of the alkyl chain; it is known that alkyllysophospholipids with a C18 chain are more toxic to leukemic cells in vitro than those with a C16 chain [17].

In the in vivo experiments, the mice were given daily i.v. doses of 10 mg/kg ether lipids for 4 weeks or 20 or 30 mg/kg for 5 days, respectively. In contrast to the results obtained in vitro, no inhibition of colony formation could be observed (Table 2). In fact, even a moderate increase in the blood leukocyte count was noticed after the end of therapy in the mice treated with 20 or 30 mg/kg (Table 3). As the leukocytes appeared to be mature in blood-smear specimens, an activation of the marginal leukocyte pool might have been responsible for this observation.

It is noteworthy that a daily oral dose of 4 mg/kg He-PC given for 4 weeks is sufficient to reduce significantly the growth rate of dimethylbenzanthracene (DMBA)-induced mammary carcinomas in the rat [11]. Doses of 10 mg/kg and more can effect a regression of the tumor throughout the period of treatment [11], independent of its size at the start of treatment.

The difference between toxic levels in vitro and in vivo are not fully understood. One reason could be that ether lipids are metabolized in vivo and cleared rapidly. However, it has been shown that He-PC catabolism in mice occurs very slowly [6]. The most plausible explanation may be the lack of the natural environment of bone marrow progenitors under in vitro conditions, which makes these immature cells more susceptible to any challenge. In con-

Table 1. In vitro effect of ether lipids on clonogenicity of normal bone marrow granulocyte-macrophage progenitors

Compound	Number of colonies[a] (% of control)				
	Concentrations of ether lipids (μg/ml)				
	0.1	1.0	5.0	10.0	20.0
OcMe-G-3-PC	92±8.8	66±5.5	15±7.7	5± 3.6	2± 0.7
He-PC	90±0.7	91±4.4	63±5.3	25±11.4	17±11.5
He-Pr-2-PC	98±4.0	98±2.1	61±6.1	30± 8.1	19± 3.5

[a] Means ± SD of 3–5 independent experiments

Table 2. In vivo effect of ether lipids on clonogenicity of normal bone marrow granulocyte-macrophage progenitors

Compound	Number of colonies[a] (% of control)		
	Ether lipid dose (mg/kg)		
	10	20	30
OcMe-G-3-PC	87± 6.4	93± 1.5	79± 6.6
	93± 3.6	73± 9.1	158±11.5
	88±13.7	106± 6.6	135± 2.1
He-PC	101± 3.0	84±14.5	101± 7.3
	118±10.5	89± 3.4	148± 7.3
	73± 1.5	84± 6.5	70±15.1
He-Pr-2-PC	125±10.9	107±10.8	142± 8.7
	98± 8.4	152±18.1	157± 8.2
	76± 7.8	117± 4.9	141±10.4

[a] Data represent the means ± SD of triplicate cultures. Mice were treated i.v. with 10 mg/kg ether lipids for 4 weeks and with 20 or 30 mg/kg for 5 days

Table 3. White cell count after i.v. injection of different ether lipids

Injected compound (mg/kg)	Total white cell count ($\times 10^9$/l)		
	OcMe-G-3-PC	He-PC	He-Pr-2-PC
10	2.4±0.6	2.7±0.6	3.0±0.3
20	6.4±0.3	6.2±0.9	5.9±1.7
30	4.6±1.0	4.4±1.5	6.1±0.9

[a] Data represent the means ± SD of triplicate determinations. Controls: 3.2±1.6

clusion, the absence of in vivo hematotoxicity and simultaneous activity against tumor cells should offer a great advantage for the use of cytotoxic ether lipids in cancer therapy.

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