CONCISE REPORT

The Effects of Alkyl-Lysophospholipids on Leukemic Cell Lines. I. Differential Action on Two Human Leukemic Cell Lines, HL60 and K562

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The action of an alkyl-lysophospholipid (ALP), ET180CH₃, on clonogenicity, ³H-TdR uptake, and cell numbers was tested in two human leukemic cell lines, HL60 and K562, and short-term human leukemic bone marrow cultures. ALP eliminated clonogenicity in HL60 but not in K562 cultures; ³H-TdR uptake and cell numbers were depressed at low concentrations of ET180CH₃ in HL60, but not K562 cultures. The action of the lysophospholipid analog on human leukemic bone marrow short-term cultures at low concentrations was similar to its action on HL60 cultures; clonogenicity and ³H-TdR uptake were depressed, but cell numbers were not significantly affected. The demonstration of differential action of ALP on two cell lines should significantly simplify the investigation of the mechanism of the reported differential action of ET180CH₃ on normal and leukemic cell membranes.

MATERIALS AND METHODS

The HL60 cell line was kindly supplied from passage 18 by Dr. Robert Gallagher of the Laboratory of Tumor Cell Biology, NCI, and has been passaged weekly in this laboratory since June 1978. The cell line has a doubling time of 24 hr and a cloning efficiency of 50%–60%. The K562 cell line was kindly supplied from passage 217 by Dr. Bismarck Lozzio, University of Tennessee, and has been passaged weekly since September 1977; the doubling time is about 24 hr, and cloning efficiency is between 40% and 60%. Clonogenic assays (CFU-C) were made in triplicate in semisolid double-layer agar cultures using 35 x 10 mm culture plates. Bottom layers of 0.5% agar in RPMI 1640 medium containing antibiotics and 20% fetal calf serum and supplemented with colony-stimulating activity from human placental conditioned medium (CSAM) were overlaid with cells in 0.3% agar and RPMI 1640 containing antibiotics and 20% fetal calf serum. Cell concentrations, stimulator concentration, and culture times were optimized for maximum cloning efficiency. HL60 cells were cloned at 2 x 10⁵/ml, K562 at 5 x 10⁵/ml, and human leukemic bone marrow cells at 10⁵/ml. Plates were examined for clonogenic activity (clusters of ~15 cells) on day 7. Agar plates were maintained in a standard CO₂ incubator.

Alkyl-lysophospholipid (ET180CH₃) was kindly supplied in solid form by Dr. Reinhard Andreesen of the Max Planck Institut für Immunobiologie. ALP was freely soluble in RPMI 1640 containing 20% fetal calf serum and stock solutions stored at -20°C were stable for at least 9 mo without loss of activity. ALP was added to 5-ml cultures in 25 sq cm flasks and incubated for 24, 48, or 72 hr prior to CFU-C assay. Before assaying, the ALP in the cultures was diluted to noncytotoxic levels, at least 50-fold.

The human placental conditioned medium used to stimulate colonies was prepared essentially as described by Burgess et al. Fresh human placental tissue was cut into 0.5-cm cubes; 100 ml RPMI 1640 culture medium per 30 cubes was added, and the tissue was incubated 4 days at 37°C in the presence of 7.5% CO₂. After decanting and centrifugation at 14,000 g for 20 min, the medium was dialyzed against 0.01 M sodium phosphate buffer, pH 6.8. Ammonium sulfate precipitation at 40% saturation and then 60% saturation was carried out. The 40%–60% ammonium sulfate fraction was dissolved in and dialyzed against 0.03 M Tris buffer, pH 7.4.

DNA synthesis was estimated by pulsing cells with ³H-TdR, followed by DNA extraction and liquid scintillation counting. Prior to pulsing, cultured cells were harvested, washed 3 times with serum-free culture medium (RPMI 1640) containing 50 µg/ml penicillin, 100 µg/ml neomycin, and 50 µg/ml streptomycin, and...
supplemented with $5 \times 10^{-3} \text{M} \beta$-mercaptoethanol. Two-tenths microCurie of tritiated thymidine (specific activity 20,000 Ci/mole), was added to 0.5-ml cultures in medium supplemented with 20% FCS, 18 hr prior to preparation of samples for counting. Cultures were started with the following cell numbers: HL60, $5 \times 10^5$ cells/ml; K562, $2 \times 10^5$ cells/ml; human bone marrow, $5 \times 10^5$ cells/ml. At appropriate times, usually after 24, 48, or 72 hr of incubation, 200 $\mu$ mole of "cold" TdR was added; cultures were precipitated and washed three times with methanol and then dried. RNA and protein were extracted enzymatically: 5 $\mu$g of RNAse and 150 $\mu$g pronase in a 0.1 M Tris buffer, pH 7.5, containing 0.1 M NaCl, was added to each tube; tubes were frozen and thawed rapidly 3 times and incubated 30 min at 37$^\circ$C. After spotting on filter discs, hydrolysates were washed 3 times with cold 5% TCA and once with 70% ethanol. Discs were transferred to scintillation vials and incubated 30 min at 50$^\circ$C in a 0.5-ml aliquot of tissue solubilizer (NCS reagent); 10 ml of a scintillation cocktail containing liquiflor (New England Nuclear, Boston, Mass.) was added and the vials counted in a Beckman Liquid Scintillation Counter. Efficiency for tritium under these conditions was 40%, and background was less than 2% of maximum incorporation.

RESULTS

The action of the alkyl-lysophospholipid, ET180CH3, in a time course experiment on the clonogenicity and $^3$H-TdR uptake in two cell lines and human bone marrow was tested. HL60, K562, and human leukemic (AML) bone marrow cells were cultured either 24, 48, or 72 hr in the presence of ET180CH3, and then tested for clonogenicity in semisolid agar cultures; the agar plates were counted on the seventh day for maximum efficiency. The depression of CFU-Cs in bone marrow and HL60 (Fig. 1B) is pronounced at a concentration of ET180CH3 of 5 $\mu$g/ml after a 24-hr pulse. Sharply contrasting to this depression is the relative resistance of clonogenicity and $^3$H-TdR uptake in K562 cultures to the cytotoxic action of ET180CH3 (Fig. 1A), even at relatively higher doses of ALP, 10 and 20 $\mu$g/ml. While a small reduction in CFU-Cs in K562 is seen, $^3$H-TdR uptake is unaffected; the small CFU-C reduction in K562 contrasts to the elimination of HL60 clonogenicity with 5 $\mu$g/ml of ALP after 72 hr of incubation.

The action of ALP in a dose-response experiment on clonogenicity in HL60, K562, and human leukemic (AML) bone marrow cells was tested after culturing either 24, 48, or 72 hr. Figure 2B graphs the CFU-C yield from cultures pulsed either 24, 48, or 72 hr with 0, 5, 10, or 20 $\mu$g/ml ET180CH3. Both HL60 and bone
marrow clonogenicity is depressed with only 5 μg/ml ET18OCH₃ within 24 hr of exposure to the drug, while K562 clonogenicity is resistant to ALP action, even at a concentration of 20 μg/ml for a 72-hr exposure. Clearly, clonogenicity in the cell lines HL60 and K562 in the presence of low concentrations of ET18OCH₃ is markedly different. The reduction of ³H-TdR uptake in subpopulations of HL60 and human leukemic bone marrow cells by the action of ET18OCH₃ is also pronounced and is sharply contrasted to the action of the drug on K562 cultures (Fig. 2A). While 5 μg/ml ET18OCH₃ is effective in depressing clonogenicity in HL60 by 24 hr of incubation, that concentration is equally repressive to ³H-TdR uptake only after pulsing 72 hr. Apparently, with low doses of ET18OCH₃, cells are still able to synthesize DNA for at least 18 hr, but are not capable of dividing enough to produce a recognizable clone in agar.

The depression of cell numbers by ET18OCH₃ in HL60, K562, and human leukemic bone marrow is shown in Fig. 3. The resistance of K562 to the cytotoxic action of ET18OCH₃ is again evident, using the depression of cell numbers as an index of action. The change in cell numbers seen in untreated bone marrow (AML) cells (Fig. 3) is unaffected by ALP; this information coupled with the demonstration of increased clonogenicity of bone marrow cells (Fig. 1B) indicates an increase in efficiency of cloning due to short-term leukemic bone marrow culturing. In contrast, the efficiency of cloning in HL60 remains constant (Figs. 3 and 1B), with both cell numbers and clonogenicity increasing in untreated cells.

The polarity of response of the two cell lines HL60 and K562 in ³H-TdR uptake and clonogenicity provides an experimental vehicle for the investigation of the mechanism of cytotoxic action of ET18OCH₃. Experiments to determine the mechanism of action are in progress.

**DISCUSSION**

Bone marrow is a mixture of developing and mature cell types, diverse in function. Studies of the mechanism of drug action on marrow are complicated by this diversity. The two homogeneous cell populations, HL60 and K562, representing two different stages in the development of mature white cells, constitute a model system for the investigation of the action of ALP on cell proliferation and differentiation.

We have confirmed the experiments of Andreesen et al., showing that ET18OCH₃ inhibits the uptake of ³H-TdR in bone marrow from leukemic patients. Importantly, we further demonstrated that ALP inhibits clonogenicity in cells from a leukemic patient and from a cell line derived from a leukemic patient,
reflecting the activity of ALP against the promyelocyte developmental stage in leukemias. And, also importantly, we demonstrated the resistance of a cell line derived from a leukemic patient to ALP judging by tritiated thymidine uptake and clonogenicity. The sensitive cell line, HL60, is a human promyelocyte line and the resistant line, K562, is a human undifferentiated blast cell line. The discovery of this differential response of two cell lines to ALP considerably simplifies further studies of the mechanism of action of alkyl-lysophospholipids on leukemic cells.

REFERENCES

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