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Toxicity of 5-Bromo 2'-Deoxyuridine to Malignant Lymphoid Cell Lines (39627)[1]

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The thymidine analog, 5-bromo 2'-deoxyuridine (BUDR) has diverse biological properties including radiosensitization (1-3), induction of latent viruses (4, 5), inhibition of expression of both tissue-specific differentiated traits (6, 7) and oncogenic potential of malignant cells (8, 9). BUDR is acutely cytotoxic to many cell cultures at concentrations above 10 μg/ml (10, 11). The data reported here show that various malignant lymphoid cell lines are sensitive to the toxic effects of BUDR at concentrations which are not cytotoxic to other cell types. A similar report describing the toxicity of BUDR to one line of mouse myeloma has recently appeared (12).

Material and Methods. *Cell lines.* MOPC 315 and MPC 11 are murine myelomas given to us by Dr. Herman Eisen and Dr. Matthew Scharf, respectively. S49.1 is a θ-positive murine lymphoma. S49.1TB.2 is a clone of S49.1 that can be grown in 30 μg of BUDR/ml. Both S49.1 and S49.1TB.2 were given to us by the Salk Instiute, La Jolla, Calif. RAG is a clone from a murine renal adenocarcinoma. CCRF/CEM is a lymphocytic cell line derived from the peripheral blood of a 4-year-old female with acute lymphoblastic leukemia. MMT is a murine mammary carcinoma. RAG, CCRF/CEM, and MMT were from the American Type Culture Collection, Bethesda, Md. L929 is a clone of mouse fibroblasts, and HeLa is derived from a human cervical adenocarcinoma. Both L929 and HeLa were purchased from Microbiological Associates, Bethesda, Md.

Cell cultures. All cultures were routinely carried in 250 ml (75 cm^2) Falcon tissue culture flasks containing 10 ml of media under a gas phase of 5% CO_2-95% air. They were fed 2 or 3 times weekly. Monolayer cultures were trypsinized with 0.25% trypsin and diluted into fresh flasks approximately once each week.

MOPC 315, MPC 11, L929, S49.1, and S49.1TB.2 were carried as suspensions in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 20% heat-inactivated horse serum kindly provided to us by the New York City Public Health Department. RAG cells were carried in DMEM with 10% fetal calf serum (Microbiological Associates, Bethesda, Md.). HeLa cells were grown in Minimal Eagle's Medium (MEM) containing 10% pooled human serum, or, when grown in suspension, in Ca^{++} and Mg^{++}-free MEM with 10% human serum. MEM and DMEM (both from Grand Island Biological Co., Grand Island, N.Y.) were fortified with nonessential amino acids. All media contained 100 units of penicillin, 100 μg of streptomycin, and 100 μg of gentamycin per ml.

Experimental procedure. Experiments were carried out in 2 ml cultures using 24 well Linbro plates. BUDR solutions of 1 mg/ml were made up weekly in Hanks' balanced salt solution and diluted into the appropriate media. All manipulations of BUDR or BUDR-containing solutions were done under Kodak 1A safelights; cultures of media containing BUDR were kept strictly in the dark.

Proliferation of cell cultures was assayed by determining the number of Trypan blue-excluding cells on the days indicated. All P values of ≤ 0.05 by the Student's t test are shown.

Nucleosides. BUDR and thymidine were purchased from Sigma Chemical Co., St. Louis, Mo. The nucleosides were shown to be pure when chromatographed on Silica thin-layer plates (Brinkmann Instruments, Long Island, N. Y.) using the following solvent systems: *n*-butanol (50%), acetic acid (25%), and water (25%); isopropanol

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(80%), water (20%), and 10^{-3} *M* EDTA; and distilled water alone.

Results. *Effect of BUDR on the proliferation of malignant lymphoid cell lines.* Growth of various malignant lymphoid cell lines in the presence of 0.10 or 1.0 μg of BUDR/ml results in marked toxicity, apparent by the third day of growth as shown in Figs. 1 and 2 for mouse myeloma MOPC 315. Cells seeded in prelog phase undergo approximately five doublings before reaching the saturation density of 1.5–2.0 × 10^6 cells/ml. In the presence of 1 μg of BUDR/ml, the growth rate is similar to that of the controls for approximately the first 24–48 hr in culture, after which there is a progressive drop in both total and viable cell counts. In BUDR-treated cultures, the percentage of live cells drops from over 90% on Day 1 to less than 20% on Day 5.

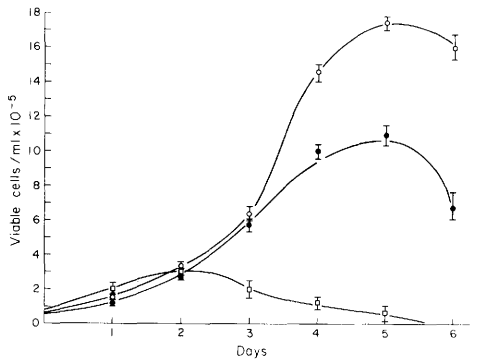

FIG. 1. Effect of 1.0 and 0.1 μg of BUDR/ml on the growth of MOPC 315. Triplicate 2 ml cultures were seeded at an initial cell density of 8 × 10^4 cells/ml. One plate was counted each day and discarded. Culture conditions were as described in Materials and Methods. 0 (○), 1.0 (□), and 0.1 (●) μg of BUDR/ml.

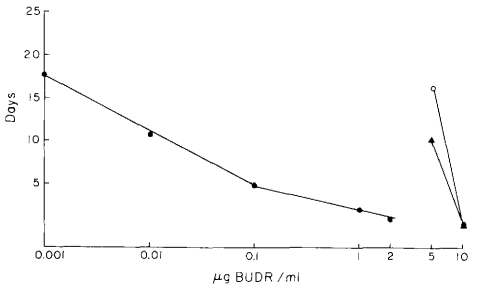

FIG. 2. Times necessary for the toxic effect of BUDR to MOPC 315 (●), HeLa (▲), and L929 (○). Times are mean values calculated from several growth experiments. Toxicity is defined as statistically significant inhibition of cell proliferation as compared to control cultures.

The growth curves of mouse myeloma MPC 11, mouse lymphoma S49.1, and human acute lymphoblastic leukemia CCRF/CEM follow qualitatively similar kinetics in the presence of BUDR.

Table I (Group I) shows the concentration of BUDR needed to cause a 50% inhibition of cell proliferation after 4 days of exposure to the analog. The doses range from 0.09 to 0.31 μg of BUDR/ml for the malignant lymphoid cell lines studied except for S49.1TB.2, which requires 87.0 μg of BUDR/ml.

Concentrations of 0.01 and 0.001 μg of BUDR/ml are toxic to MOPC 315 after repeated subcultures in its presence. As shown in Fig. 2, there is an inverse relationship between the time necessary for the expression of the toxic effect of BUDR and the amount of analog present.

Growth of other cell lines in the presence of BUDR. A variety of other cell types can be grown in BUDR concentrations that are extremely toxic to malignant lymphoid cell lines. Table I (Group II) shows that concentrations of BUDR ranging from 3.14 to 6.23 μg/ml are required for a 50% inhibition of proliferation. Concentrations of BUDR between 1 and 2 μg of BUDR/ml occasionally cause a transient inhibition of proliferation which is generally followed by a stimulation of growth compared to nontreated cultures.

TABLE I. CONCENTRATION OF BUDR REQUIRED TO CAUSE A 50% INHIBITION OF CELL PROLIFERATION IN 4 DAYS (ID_{50}).[a]

Group	Cell line	ID_{50} (μg/ml)
I	MOPC 315	0.09
	MPC 11	0.20
	CCRF/CEM	0.31
	S49.1	0.16
	S49.1TB.2	87.0
II	RAG	3.70
	L929	6.23
	HeLa (monolayer)	3.14
	HeLa (suspensions)	4.20
	MMT	4.25

[a] The 50% inhibitory doses (ID_{50}) of BUDR were determined from a graph of inhibition vs dose for 4 days of growth in the presence of the analog.

TABLE II. PROTECTION BY THYMIDINE AGAINST THE TOXICITY OF 5-BROMO 2'-DEOXYURIDINE TO MALIGNANT LYMPHOID CELL CULTURES.[a]

Condition	MOPC 315	MPC 11	S49.1	S49.1TB.2
Control	100	100	100	100
1 μg of BUDR/ml	37.2[b]	45.8[b]	64.1[b]	101
(1 μg of BUDR + 1 μg of thymidine)/ml	77.7[b]	83.6[c]	104	113[d]
1 μg of Thymidine/ml	95.0	109	107[b]	120[d]
(1 μg of BUDR + 10 μg of thymidine)/ml	85.7	104	103	113[c]
10 μg of Thymidine/ml	81.4	110[e]	103	119[b]

[a] Triplicate 2 ml cultures were seeded at an initial cell density of 7.5×10^4 cells/ml and grown as described in Material and Methods. Data are given as the percentage of viable control cells/milliliter.

[b] $P \leq 0.001$.

[c] $P \leq 0.005$.

[d] $P \leq 0.01$.

[e] $P \leq 0.025$.

Effect of thymidine on the toxic effect of BUDR to malignant lymphoid cells. As shown in Table II, the toxicity of 1 μg, of BUDR/ml to mouse myeloma cells is completely reversed by 10 μg of thymidine/ml. The effect on mouse lymphoma S49.1 is fully prevented by 1 μg of thymidine/ml. Thymidine alone either has no effect or slightly stimulates the proliferation of these cultures.

Discussion. Malignant lymphoid cell cultures are sensitive to the cytotoxic effects of BUDR at concentrations which are not harmful to other cell lines. This toxicity is dependent on both the concentration and duration of exposure to the analog.

Although the spectrum of acute toxicity at low levels of BUDR extends to two mouse myelomas, a mouse T-cell lymphoma, and a human acute lymphoblastic leukemia, reports of the continued high viability of human Burkitt lymphoma cells exposed to BUDR (13) suggest that the pronounced cytotoxicity observed here does not extend to all malignant lymphoid cell cultures.

The toxicity of BUDR at the concentrations used in this study is specific for malignant lymphoid cells of both T and B-cell origin. It is not dependent on the media and serum used or whether the cells grow as adherent or suspension cultures.

BUDR competitively replaces thymidine in DNA that is synthesized in its presence (11, 14). In most systems, the effects of BUDR are prevented by either blocking DNA synthesis (15) or by the addition of excess thymidine (12, 16–18). Our results indicate that the incorporation of BUDR into the DNA of malignant lymphoid cells may play a role in its toxic effect since the effect is prevented by the simultaneous addition of thymidine (Table II).

It has recently been shown that BUDR triphosphate is a powerful allosteric inhibitor of ribonucleotide reductase, causing a deficiency of deoxycytidine. Despite extensive incorporation of BUDR into the DNA of fibroblasts, the fibroblasts can grow in a BUDR concentration of 46 μg/ml if the media is supplemented with 45 μg of deoxycytidine/ml (19). We are presently studying the relationship between deoxycytidine deficiency and BUDR toxicity to lymphoid tumors at the low BUDR concentrations used in this study.

BUDR activates latent viral particles in many cell types (4, 5). Myelomas and lymphomas harbor latent viruses which may be susceptible to activation and associated cytotoxic effects. However, it is unlikely that viral activation is responsible for the effects reported here because exposure of MOPC 315 to BUDR does not cause the appearance of viral particles under examination with an electron microscope (16), and attemps to activate virus producton in myeloma cells with iododeoxyuridine concentrations as high as 20 μg/ml have not been successful (20).

Summary. Malignant lymphoid cell lines are unusually sensitive to the toxic effects of BUDR. Incubation of mouse myeloma MOPC 315 with 1 and 0.1 μg of BUDR/ml results in an acute toxic effect by Days 3 and 5, respectively. Subcultivation of MOPC 315 in 0.01 and 0.001 μg of BUDR/ml typically results in toxicity on Days 12 and 18, respectively. Similar patterns of acute

toxicity occur with mouse lymphoma S49.1, human leukemia CCRF/CEM, and mouse myeloma MPC 11. The dose of BUDR required to cause a 50% inhibition of proliferation (ID_{50}) ranges from 0.09 to 0.3 μg of BUDR/ml. In contrast, the ID_{50} for a variety of cell lines of alternate origins range from 3.14 μg of BUDR/ml for human cervical adenocarcinoma HeLa to 6.23 μg of BUDR/ml for mouse fibroblast L929. Fibroblasts L929 and HeLa cells were grown for 20 and 25 days, respectively, in 2 μg of BUDR/ml with no toxicity.

The toxicity of 1 μg of BUDR/ml to MOPC 315 and MPC 11 is partially prevented by 1 μg of thymidine/ml and completely prevented by 10 μg of thymidine/ml. Addition of 1 μg of thymidine/ml completely protects lymphoma S49.1 from the effects of 1 μg of BUDR/ml.

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