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Cytotoxic Effects of 1,3-Bis(2 chloroethyl)-1-Nitrosourea (BCNU) on Cultured Human Glioblastomas[1] (39251)

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BCNU has produced temporary objective improvement in 7 of 18 patients with hopelessly recurrent glioblastoma (1). This compound is now commonly used in such cases, and is also used as an adjuvant to initial surgical removal. We report here the sequential morphologic changes in cultures of two human glioblastomas treated with graded doses of the agent.

Materials and methods. Tumor A-172 (Fig. 1A) is a glioblastoma cell line, courtesy of Dr. S. Aaronson, made available by the Naval Biological Research Center, Oakland, California. Except for the fact that it derived from a human glioblastoma, no information was available. It was in its 50th passage when received. It grew rapidly, and appeared relatively well differentiated, though the characteristic bizarre multinucleate cells were present. In stained coverslip preparations, it showed a textbook picture of glioblastoma multiforme. Tumor T98 (Fig. 2A), which was derived from a glioblastoma multiforme by Dr. Leonard Hayflick of Stanford University and which was kindly furnished by him, had been in culture for over 3 years. It also presented the picture of glioblastoma, but appeared much more anaplastic than A-172.

Cultures were set out, in groups of 8–10, in 4-cm glass petri dishes containing 3.0 × 2.5 cm coverslips and 5 ml of Eagle basal medium plus 10% fetal calf serum, with streptomycin and penicillin. BCNU was added 3 days later, when monolayers had formed. Concentrations used were 6, 18, 36, 48, and 60 μg/ml, obtained by dissolving 1 mg of BCNU powder in 0.03 ml of absolute alcohol, followed by 0.27 ml of sterile distilled water, and then by adding appropriate amounts of this solution to the 5 ml of culture medium. Untreated (control) cultures were included in each experiment, as well as alcohol-treated controls (which were identical with untreated controls). The progress of the cultures was followed with the inverted microscope, and coverslips were removed and stained 4–6 days after adding the agent. In two experiments, cultures were treated with a single dose, in the amounts noted; in two experiments the agent was given on 3 successive days (3, 4, and 5). In two experiments, treated and control cultures were trypsinized, transferred without additional treatment, and fixed 3 days later.

Results. Tumor A-172 (Table IA). When a single dose of 6 μg/ml was given, mitotic figures were as numerous as in the control cultures. At 18 μg/ml, mitotic activity was absent and there was some rounding and cell death. At 36 μg/ml, cell death appeared to be about 75% complete. When treated on 3 successive days, more severe damage occurred at 18 μg/ml (Fig. IB) and practically complete cell loss or death at 60 μg/ml (Fig. IC). Cultures transferred without further treatment showed rare mitotic figures when the original dose was 18 μg/ml. In the culture given 60 μg/ml, 2-minute foci with mitotic activity were seen in an otherwise practically empty coverslip.

Tumor 98 (Table IB). This highly anaplastic cell line was more resistant to BCNU, responding when treated on 3 successive days, and when transferred, only by reduction in population and in mitotic activity. A few rounded cells were found at the 18 μg/ml level (Fig. 2B). For an unknown reason, a more definite toxic reaction was found at the level of 60 μg/ml when given as a single dose (Fig. 2C).

Discussion. The usual human dose of BCNU is 100 mg/m² given iv on 3 successive days, this course being repeated at intervals of 6 weeks (1). The lowest dosage at which

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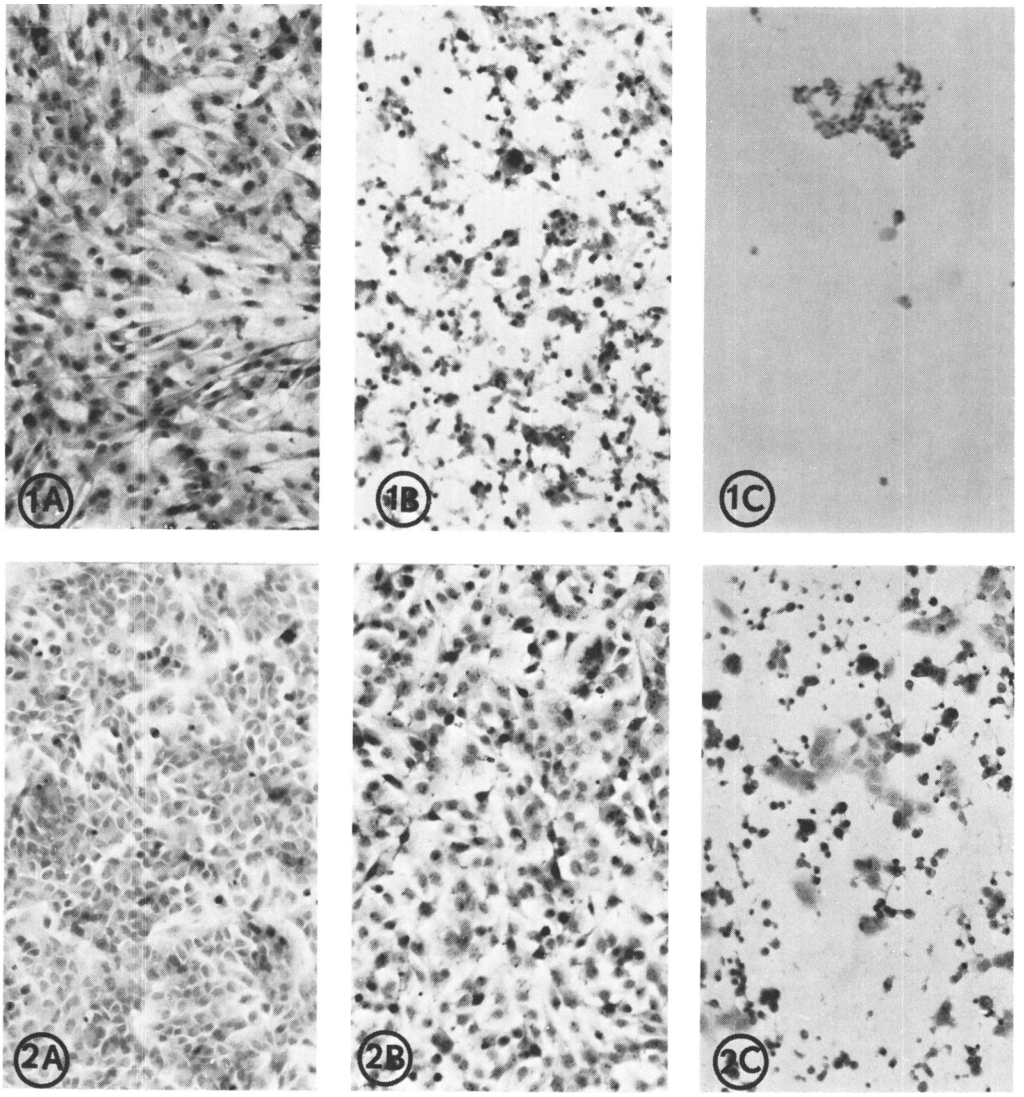

FIG. 1. Tumor line A-172: A, Control; B, 18 μg/ml of BCNU on 3 successive days; C, 60 μg/ml of BCNU on 3 successive days. × 100.

FIG. 2. Tumor line T-98: A, Control; B, 18 μg/ml of BCNU on 3 successive days; C, 60 μg/ml of BCNU given as a single dose. × 100.

we found an estimated 75% cell death was 18 μg/ml on 3 successive days, corresponding to about four times the usual human dose. The half-life of the drug in human serum is only 5 min, apparently because of rapid combination with the lipids in the brain and elsewhere.

In cultures, the lipids in the tumor cells and medium are in lower concentration than in the living animal. Other differences between cultured cells and cells growing as tumors in patients include the lack of immunologic response and the possibility of changes in cells after prolonged cultivation (though such changes are not seen histologically).

Because of such alterations in the milieu, Barker *et al.* (3) are not optimistic about *in vitro* sensitivity tests in brain tumors, but believe they may be useful for large-scale screening in a search for new agents. Our data show that cell lines from two tumors with the same original histologic diagnosis react differently to the same concentrations

TABLE I. EFFECTS OF GRADED CONCENTRATIONS OF BCNU ON TWO HUMAN GLIOBLASTOMA CELL LINES GROWN *IN VITRO*.

Tumor	Dosage schedule	Dose (μg/ml)	Population[a] (%)	Mitoses[b]	Rounding and clumping[c]	Cell death[d]
		0	100	++++	0	0
A-172-(IA)		6	100	++++	0	0
	Single dose	18	50	0	+	+[d]
		36	20	0	+++	+++
		48	10	0	+++	+++
		60	10	0	+++	+++
	Days 3, 4, and 5	0	100	+++	0	0
		18	20	0	++	+++
		60	2	0	++++	++++
	Duplicates transferred, no drug added	0	100	++++	0	0
		18	30	(+)	++	++
		60	1	(+)[e]	++++	++++
T 98 (IB)		0	100	++++	0	0
	Single dose	18	25	0	+	+
		60	4	0	++	+
	Days 3, 4, and 5	0	100	++++	0	0
		18	100	+	0	0
		60	40	(+)	(+)	0
	Duplicates transferred, no drug added	0	100	++++	0	0
		18	50	++	0	0
		60	20	+	(+)	0

[a] Population in control cultures, when coverslips were fixed, taken as 100%.
[b] ++++ means average of three to five mitoses per medium power field.
[c] ++++ means more than 99% of cells rounded and clumped.
[d] ++++ means 99% of cells washed away or appeared nonviable.
[e] Several mitoses in 2-min foci.

of a single drug (BCNU), their sensitivity being apparently related to the degree of anaplasia.

Barker *et al.* (2) using a glioma chemically induced in 150-g rats, have reported an increased life span of 84% when 10.4 mg/kg of BCNU was given ip on Days 9 and 16 after injection of the tumor cells. This dose was 80% of their 10% lethal dose, and translates to approximately 20 μg/ml of the fluid component of the rat.

Summary. Two cultured human glioblastoma cell lines were exposed to graded doses of BCNU, and the sequential cytoxic and cytocidal changes were studied histologically. Changes included cessation of mitotic activity, reduction in population, rounding and clumping of the cells, nuclear pyknosis, and cell death. The intensity of the changes was proportional to the dosage. With 3 daily doses of 18 μg/ml, the population in Tumor A-172 was only 20% of that in the controls, and 75% of the cells present appeared nonviable. This dosage is about four times that used in a single course in treating humans. After 3 doses at 60 μg/ml, cell death appeared complete, but transfer without additional treatment brought out 2-min foci of viable cells, with several mitoses. A more anaplastic glioblastoma, T98, was much less sensitive to BCNU, responding only by lowered population density, decreased mitoses and slight-to-moderate cytotoxicity in one experiment.

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