Response of cultured human glioblastomas to radiation and BCNU chemotherapy

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Individual and combined effects of ionizing radiation and chemotherapy with 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) on glial tumor cells were assessed in cultures derived from human glioblastomas. Drug and radiation exposures were performed on cell monolayers in 0.02 ml wells of microtest plates. Response to treatment was determined from serial observations on surviving populations in the original wells and comparisons with matched control cultures. Chemosensitivity was more variable than radiosensitivity in cell lines derived from five different glioblastomas: BCNU caused growth inhibition of 4% to 85% in doses of 8 μg/ml for 3 days compared to a 40% to 73% reduction after 400 rads of radiation. These findings were all significant statistically. Single doses above 600 to 800 rads appeared lethal, causing widespread loss of cells or transformation into giant forms that did not multiply. The dose-response curves after BCNU and radiation treatment of cultured glial tumor cells were exponential, demonstrating that both modalities affected a constant fraction of the exposed cell populations according to dose. The observations on radiosensitivity of human glial tumor cells conformed to the generally known responses of cultured normal and neoplastic mammalian cells to ionizing radiation. BCNU in the higher doses tested acted as a possible radiosensitizing agent to potentiate the lethal effects of radiation since an increased rate of cell loss was demonstrated in glioma cultures exposed to this drug and radiation compared to those treated only by irradiation. These results in a controlled experimental environment support the concept that a combination of BCNU and radiation therapy should increase the time of survival of patients with malignant gliomas.

KEY WORDS □ experimental brain tumor □ glioblastoma □ BCNU □ 1,3-Bis(2-chloroethyl)-1-nitrosourea □ radiation □ glial tumor cell culture □ radiosensitizing agent

The average life expectancy after a definitive operative diagnosis of glioblastoma multiforme is 3 to 6 months, with less than 20% of the cases remaining alive at 1 year and less than 10% at 2 years.18,20,28 It is generally believed that radiation therapy increases the average postoperative lifespan of patients with this disease18,28,48 but after 12 to 18 months there is little difference in the survival rate between the irradiated and nonirradiated groups.18,20 The most favorable results have been
reported after substantial tumor resections and high doses (5000 to 6000 rads) of radiation therapy, with 1-year survivals approaching 50%.

It has been suggested, however, that since the most serious cases, including the early postoperative deaths, do not reach the radiotherapist (about 20% of all cases), retrospective studies are biased in favor of radiation treatment. When survival times of patients with intracranial gliomas are calculated from the onset of symptoms, no differences are apparent between the surgical group and those who were also irradiated.

Recently, analysis of a prospective, controlled, randomized study of over 300 patients with glioblastomas or malignant cerebral gliomas has indicated that postoperative radiation (6000 rads, whole brain) does significantly increase the median survival after operation ($p < .001$). When patients who were entered in this collaborative study were randomized for additional treatment with 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) intravenously at 80 mg/M$^2$ per day for 3 days repeated bimonthly, the combination of postoperative radiation and BCNU chemotherapy resulted in an even greater increase in the survival time.

BCNU, one of a series of antitumor nitrosourea compounds, is extremely lipidsoluble and readily penetrates the brain and cerebrospinal fluid after parenteral administration. This drug has been highly effective against the L-1210 leukemia in mice and has completely inhibited the growth of a variety of transplantable animal tumors including a murine glioma. BCNU has greatly increased the lifespan of mice and rats bearing intracerebral gliomas. Alone and in combination with radiation treatment (2000 rads), BCNU significantly prolonged the survival of mice inoculated with ependymoblastoma cells intracranially, whereas radiation by itself did not appear effective.

In clinical trials, BCNU chemotherapy has produced improvement in roughly half of the patients with primary brain tumors having symptomatic recurrences and has significantly prolonged the postoperative survival time of nonirradiated patients with malignant cerebral gliomas.

These observations prompted the experimental studies reported in this paper concerning the individual and combined effects of ionizing radiation and BCNU on cell cultures derived from human glioblastomas. These investigations sought to establish some experimental basis to support the clinical notion that BCNU tends to augment the salutary response of malignant intracranial gliomas to radiation therapy.

Materials and Methods

Tumor Cell Lines

Tumor cells were derived from biopsied human brain (HB) tumors which had been diagnosed histologically as glioblastoma multiforme. Cells were maintained continuously in culture in Eagle's basal medium (BME) plus 10% fetal calf serum for periods up to 5 years; cultures were fed every 2 to 3 days and subcultured at 1- to 4-week intervals. The uncloned tumor cells were generally pleomorphic with round-to-oval deeply stained nuclei and occasional multinucleated forms. Cell morphology remained constant in succeeding subcultures. Compared to cell cultures of normal human brain tissue, glial tumor cells had faster growth rates (2 or 3 times), higher activities of acid phosphatase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase, greater susceptibility to the agglutinating effect of concanavalin A, and high sensitivity to infection with bovine enterovirus (BEV-1).

Experimental Procedures

Stock cultures of tumor cells were trypsinized, and approximately 10,000 cells were suspended in 1 ml of medium. Some 200 cells were inoculated into each of the 60 0.02 ml wells of microtest plates. The cells were attached to the plastic surface of the wells after overnight incubation at 37°C in 5% carbon dioxide and remained in situ throughout the experimental period. The plating efficiency varied less than 10% among the individual well cultures. BCNU in 100 mg amounts was dissolved in 2.5 ml of ethyl alcohol and further diluted with BME to the desired concentrations. These solutions of drug were freshly prepared for each experiment and used immediately. The final concentration of alcohol in the medium was 0.05%. The day after plating, drug-containing medium was substituted in the culture wells for a 1-day treatment or repeated on successive days for a 3-day treatment. Within several hours after
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the last exposure to BCNU, some of the treated and control cultures were fixed in methanol and stained with Giemsa for counting and determination of early cell losses. The remaining cell cultures were washed and allowed to recover in the same wells under standard conditions for an additional 7 or 10 days; well-counting was then repeated on similarly stained samples for assessments of the extent of cell growth.

Irradiations were carried out with a therapeutic unit* operated at 250 keV and 15 mA with added filtration of 1.0 mm copper. A treatment distance of 20 cm was selected to obtain a high dose rate and minimize the time the cell cultures were removed from the incubator. The variable collimator was removed from the x-ray tube head assembly for this reason, and the unit was calibrated for that geometry. A dose rate of 609.7 rads per minute was obtained for the 20 cm source-to-sample distance. The measured exposure rate in roentgens per minute was converted to rads per minute by employing the back scatter factor, National Bureau of Standards calibration factor, temperature and pressure correction factors, and the roentgen-to-rad correction factor for water. The glial tumor cell cultures were irradiated 1 or 4 days after plating and further incubated in the same wells for 10 or 7 more days, respectively, when they were fixed, stained, and counted.

The effects of treatment 1 week or more after exposure to BCNU or radiation, as observed from the relative decrease in cells compared to the number in control cultures, could be considered as a net reduction in cell proliferation and expressed in percent, from the following equation:

\[
\% \text{ growth inhibition} = \frac{\text{no. of cells/well, treatment} - \text{no. of cells/well, control}}{\text{no. of cells/well, control}} \times 100.
\]

All determinations reported herein represented an average of cell counts from three to five replicate well cultures for each treatment and control category. Microphotographs were made of the well cultures and were greatly enlarged to facilitate cell counting. Individual counts within the same groups of samples were in good overall agreement based on an analysis of their coefficients of variation (standard deviation/mean \(\times 100\)). Among the four main groups of experimental data presented, the median coefficient of variation ranged from 3.8% to 7.1%.

**Results**

**Sensitivity of glioblastoma cell cultures to BCNU and radiation**

The effects of BCNU, ionizing radiation, and a combination of both treatments were assessed in cell cultures of five human cerebral glioblastomas (Table 1). Four of the tumor cell lines had been continuously propagated up to 4 years (52 to 84 subcultures). The HB69 strain, in contrast, had been established for less than 3 weeks. Five replicate cultures of each cell line were exposed to BCNU 3 times in concentrations of 8 \(\mu\)g/ml on successive days and/or to a single 400 rad dose of radiation on the 4th day. These treatments were all sublethal, and some fraction of the exposed tumor cell population was invariably able to reproduce. Results were determined 11 days after plating from comparative cell counts in the treated and control cultures.

BCNU produced significant reductions in tumor cell growth ranging from 4% (\(p < .05\)) to 85% (\(p < .01\), all others). These findings demonstrated a widely varying drug sensitivity among the five glioblastoma strains tested. The effects of ionizing radiation on cell growth were more clustered, with inhibitions of 40% to 73% observed in the five cell lines (\(p < .01\)). Radiation was significantly more effective in the HB38 and HB8 cell lines, which were most resistant to the drug (\(p < .01\)). In the other three cell lines highly susceptible to BCNU, the drug effect was significantly greater than the response to radiation in each instance (\(p < .01\)). However, there was an overall correspondence in sensitivity to either BCNU or radiation; the three tumor cell lines most susceptible to the drug were also more radiosensitive, and the two relatively drug-resistant tumor cultures were also the least radiosensitive.

The combination of BCNU and radiation therapy proved superior to single treatments with drug or radiation in three tumor lines: HB38, HB8, and HB40 (\(p < .01\)). In the most

*Siemens Stabilipan therapeutic unit made by Siemens-Reininger AG, Erlangen, West Germany (Siemens Corporation, 186 Wood Avenue, South, Iselin, New Jersey 08830).
resistant HBna and HBn glioma cultures, the results of combination treatment were significantly better than the predicted additive effect of each treatment acting independently (p < .01, p < .001, respectively). This enhanced response to combination treatment was not found in the HBna cultures. Combination treatments were not significantly better than the results observed with BCNU alone in the HBna and HBn tumor cell lines, but were superior to radiation alone in each instance (p < .01).

BCNU: Effect of Dose and Number of Treatments

These and succeeding experiments utilized the long-term HBn tumor cell line which has been continuously maintained since 1968 and was only moderately sensitive to BCNU and radiation. All single doses of BCNU administered on the day after plating produced a significant decrease in cells by several hours later and a parallel suppression of cell growth after 10 days when compared to control cultures (p < .05, <.01) (Fig. 1). The effects of increasing dose were not at significant levels when only single treatments were done. Following three treatments with BCNU on successive days, a greater reduction in cells was evident shortly after the last dose, and there was a comparable decrease in cell numbers after a 7-day period of recovery. The slopes of the treatment and recovery curves after 3-day treatments describe an exponential decline, with increasing dose of drug. The various dose-response determinations were all at levels of significance compared to control observations (p < .05, 2 μg recovery; p < .01, all others). The slope of the recovery curve after three daily treatments was steeper than the corresponding curve measuring the reduction in cell numbers shortly after the last treatment. At 16 μg/ml/day × 3, BCNU produced an average cell loss of nearly 50%, and 1 week later there was a net reduction of cell numbers, or growth inhibition of 66%. The differences favoring a greater drug effect when recovery is measured proved significant (p < .01). Since viable cells cannot be readily distinguished from intact but dying cells by morphologic criteria, the determinations of growth inhibition were a more accurate reflection of drug effect. At the highest dose, drug-induced cytolysis was widespread, but a proportion of the cells remained drug resistant and were able to proliferate with a preponderance of giant forms (Fig. 2).

Radiation Dose and Sensitivity

Glial tumor cell cultures were exposed to single doses of radiation up to 1600 rads on the day after plating. Sensitivity was plotted as a function of dose and determined by the number of intact cells remaining in the culture wells on the 11th day after plating (Fig. 3). The curve describes an initial shoulder of relative insensitivity to the lowest doses of radiation, but the reduction in cell numbers compared to the controls becomes significant after a dose of 100 rads (p < .05). Between 200 and 800 rads there was an exponential decrease in the number of cells, indicating that a constant fraction of cells survived (or were killed) after each additional increment of radiation. The single dose increment which significantly decreased the number of cells remaining by a factor of 0.37 in the exponential portion of the curve, or Do, may be calculated at 300 rads (p < .05). When the radiation dose was administered in
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Fig. 2. Photomicrographs showing cytolytic and growth inhibitory effects of BCNU, 16 µg/ml, on glial tumor cells. Giemsa, × 170. A. Extensive cell loss, swelling, and fragmentation after 3 successive daily drug treatments. B. Control (for A). C. Reduced extent of growth and atypical cell morphology 1 week after third BCNU treatment under standard culture conditions. D. Control (for C).

equal fractions over 4 days totalling 200, 400, and 800 rads, the proportion of surviving cells approximately doubled and demonstrated the lesser lethal action of fractionated radiation on glioma cells (p < .05).

Cell cultures of the HB8 glioblastoma were more radiosensitive 1 day after plating than on the 4th day when the same dose of radiation was administered. A single dose of 400 rads on the 1st day produced a 62% decrease in the number of cells (from Fig. 3) compared to a 40% reduction when radiation was performed 3 days later in earlier experiments (Table 1). These differences in radiosensitivity can be correlated with the rate of tumor cell growth. The HB8 cell line has an initial doubling time of 2 days from plating. Subsequently, the rate of growth tapers off and from the 3rd to 6th day the doubling time increases to 4 days.

After single doses of 600 to 800 rads, more than 80% to 90% of the cells were destroyed. At these levels and above, the cells seemed

Fig. 3. Graph showing the effect of single doses of radiation administered 1 day after plating on the growth of glial tumor cells in well cultures. The fraction (f) of remaining cells is reduced to 37% by a dose of D₀ or 300 rads. Solid line indicates where the differences between successive experimental determinations are significant (p < .05), and the dashed line where they are not. Points indicate the average of three replicate samples.
irreversibly damaged and did not multiply (Fig. 4). Scattered among the residual giant spidered irradiated cells were globoid and irregular cell aggregates as well as cytolytic debris. These findings increasingly interfered with accurate cell-counting at the 800- and especially the 1600-rad dose levels, and persisting cell forms could not be distinguished from viable cells with retained reproductive capacities. Differences in results observed between 800 and 1600 rads were not significant.

**Kinetics of Combined Chemotherapy and Radiation in Glioblastoma Cell Cultures**

The combination of three different doses of BCNU and single shots of radiation to 1200 rads were compared to the effects of radiation alone in cell cultures of the moderately resistant HB8 glioma line (Fig. 5). Tumor cells were repeatedly exposed to BCNU on the 1st through 3rd days after plating and were irradiated on the 4th. Cell-counting was done after an additional week of culture under standard conditions. The configuration of the radiation dose curve was the same as the one determined previously, but the exponential portion of the curve had a more gradual slope. Cells were significantly reduced in number after 200 rads and after successive higher doses to 800 rads (p < .05). Along the linear declivity, 450 rads produced a decrease in the number of cells to 37%. These findings again confirmed the lower sensitivity of the HB8 glial tumor cells to radiation administered 4 days after plating and were correlated with a tapering off of the rapid log-phase of cell growth.

BCNU alone produced significant cell losses, increasing with dose to levels of nearly 50% at 16 μg/ml × 3 days (p < .05). Higher concentrations of drug at any given level of radiation gave a progressive decrease in the number of cells remaining (p < .05, except p < .1 between 4 and 8 μg at 800 rads). Increasing the amount of radiation with the same dose of drug produced further significant reductions in surviving cells along the initial exponential decline of the various curves, as indicated in Fig. 5 (p < .05). After combination treatments greatly reduced the remaining cell populations at progressively lower doses of radiation as the level of BCNU exposure was increased, each curve assumed a more gradual parallel slope where the effect of increased radiation was not significant.

The increasingly steep slopes of the initial phase of the survival curves indicated a higher rate of cell loss or kill when chemotherapy was combined with radiation, and this effect was related to the dose of the drug. The number of tumor cells was diminished to 37% after 280 rads when treated previously with BCNU at 8 μg/ml/day for 3 days. When this drug dose was doubled, the same decrease in tumor cell proliferation occurred after a radiation dose of 120 rads which by itself produced no significant reduction in cell numbers. This evidence of drug enhancement of radiation response proved highly significant when the tumor cells were exposed to the highest doses of BCNU. At these levels the effect of the combination was greater than the predicted additive effects of each treatment acting independently (p < .001). Results after BCNU at 8 μg/ml/day were also suggestive of an enhanced response to combination therapy between 50 and 400 rads, but not all points reached levels of confidence (p < .05). With the lowest dose of BCNU, the results of combined treatment were not significantly greater than would be expected from the sum of the two treatments acting independently.

### TABLE 1

**Effects of BCNU, radiation and combination on five human glioblastoma cell lines**

<table>
<thead>
<tr>
<th>Tumor (HB) &amp; No. of Subcultures (C)</th>
<th>HB6-C64</th>
<th>HB6-C64</th>
<th>HB6-C51</th>
<th>HB6-C51</th>
<th>HB6-C51</th>
<th>HB6-C51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1358</td>
<td>1033</td>
<td>370</td>
<td>306</td>
<td>538</td>
<td></td>
</tr>
<tr>
<td>BCNU, 8 μg/ml</td>
<td>1310 (4%)</td>
<td>684 (34%)</td>
<td>102 (72%)</td>
<td>69 (78%)</td>
<td>83 (85%)</td>
<td></td>
</tr>
<tr>
<td>x-ray 400 rads</td>
<td>793 (42%)</td>
<td>621 (40%)</td>
<td>131 (65%)</td>
<td>135 (56%)</td>
<td>145 (73%)</td>
<td></td>
</tr>
<tr>
<td>BCNU plus x-ray</td>
<td>703 (48%)</td>
<td>339 (67%)</td>
<td>69 (81%)</td>
<td>55 (82%)</td>
<td>62 (88%)</td>
<td></td>
</tr>
</tbody>
</table>

( ) = percent inhibition of growth compared to control.
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Fig. 4. A. Photomicrograph showing the effect of a single dose of 1600 rads on glial tumor cells administered 1 day after plating. The small number and morphological features of the spidery giant cells in the radiated wells appear the same at 3 and 10 days after treatment. Giemsa, × 170. B. Matched control culture after 11 days. Giemsa, × 170.

of the two independent actions. Allowing for the demonstrated differences in glial tumor cell radiosensitivity, depending on the day of treatment, essentially the same findings were observed when cultures were radiated first, on the day after plating, and subsequently exposed to BCNU.

Discussion

Present concepts of the relationship between radiation dose and mammalian cell response are largely derived from the pioneer in vitro studies of Puck and Marcus with a strain of HeLa cells originating from a human cervical carcinoma. Radiated cells may be lysed and disappear or they may persist as nonviable giant forms that can divide several times before disintegrating. Cells in radiated populations that by chance are missed by the ionizing rays or receive only sublethal damage subsequently exhibit an overall decrease in their rate of growth.

The multitarget survival curve after radiation of mammalian cells is characterized by an initial shoulder segment and then a straight exponential decline in cell numbers as the dose of x-ray is increased. The exponential feature of the dose-effect curve indicates that a specified amount of radiation kills a constant fraction of cells which is independent of the number of cells irradiated. The dose increment (D₀) needed to reduce the cell population to the fraction 1/e or 0.37, where e is the base of the natural logarithm system or 2.71828, has become a conventional parameter to describe the exponential slope of the radiation survival curve. Since cells can recover fully from sublethal effects of radiation in moderate doses within several hours, fractionated irradiation is less efficient than equivalent single doses with respect to cell killing.

In experimental radiobiology, cell survival or viability is equated to a demonstrated ability to continue multiplying after treatment and produce colonies of daughter cells. This conventional approach was not feasible.
in the present studies since glial tumor cells do not form colonies under our usual conditions of culture. Our results, nevertheless, follow closely the pattern established for mammalian cell survival curves with an initial shoulder segment succeeded by an exponential decrease in cell numbers up to 800 rads. When the remaining cells numbered less than 10% to 20%, the dose-effect relationship had a more gradual slope, or terminal "tail." Survival curves of homogeneous and mixed cell populations cannot be readily differentiated, but generally the slope will indicate the sensitivity of the most resistant subgroup after effective treatment. Biphasic survival curves with a decreased terminal rate of kill have been observed, for the most part, only after radiation of anoxic cell populations. Since our methods based on cell counting did not distinguish persisting non-viable cells from viable cells with intact reproductive capabilities, increasing difficulties in scoring were encountered after radiations above 600 rads or after radiation and chemotherapy produced widespread cytolysis. For these reasons we tended to attribute the slower rate of cell destruction observed after the original population was reduced to levels below 20% to experimental artifact. This interpretation was supported by recent growth promotion studies on the same HBs glioma cell line and the demonstration of colony formation from isolated cells after incubation with conditioned medium containing a colony stimulating factor. The effects of radiation appear lethal to these glioma cells at doses above 600 rads and colony formation is abolished.

Generally, inherent major biological variations in radiosensitivity have been considered slight or nonexistent among normal and neoplastic mammalian cells of diverse origin that have been studied. Increased radiosensitivity in our results was correlated mainly with a more rapid phase of glial tumor cell growth. Sensitivity to ionizing radiation is known to vary throughout the cell cycle, and resting cells appear more radioresistant. Some differences in radiosensitivity have been recorded, however, even among descendants of a single experimental cell line. Three sublines of a clone of HeLa cells had Do values between 112 and 149 rads in one laboratory, and related hamster cell sublines showed similar values ranging from 152 to 240 rads in another study. Thus, when differences in experimental design are also considered, the overall radiosensitivity of human glial tumor cells appears to be of the same order of magnitude as that found in other mammalian cell lines in vitro.

BCNU is considered to act like an alkylating agent which may affect all cells throughout the cell cycle to a greater or lesser degree. BCNU is known to be highly effective against cultured human glioma cells, acting in a dose-dependent fashion with complete cytolysis occurring at levels of 80 μg/ml. The dose effect curve determined for BCNU and glial tumor cells is exponential and conforms to the fractional kill hypothesis that a given dose of a given drug kills a constant fraction of cells, not a constant number. Thus, in vitro, the kinetics of glial tumor cell loss from radiation and after BCNU chemotherapy appear very similar in terms of a dose-related fractional rate of killing.

The selection of a 3-day treatment regimen for BCNU was made empirically, based on a similar dose schedule used clinically in the treatment of brain tumors. The range of doses used experimentally ranged from 1 to 8 times the doses used for human treatments in terms of body weight. The higher doses used in vitro, however, were of the same order of magnitude found effective in vivo against several experimental murine gliomas. Rapidly proliferating normal hematopoietic stem cells, in contrast, were 3 to 12 times more sensitive to BCNU in vivo than were the murine gliomas and a transplantable lymphoma, respectively. The overall response of tumor cells to chemotherapy, and to radiotherapy, presumably, is largely influenced by the kinetics of the surviving elements of the original population which are modified by treatment. Inhibition of regrowth may reflect a net reduction in the growth fraction among the surviving cells of an effectively treated population. From our data on cultivated human glial tumor cells, fractionation of the BCNU dose caused only equivocal differences in the extent of cell loss and inhibition of regrowth in comparison to single exposures to the same total amount of drug.

Cell cultures of different glioblastomas exhibited a wide range of sensitivity to BCNU. The differences observed most likely reflect...
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the inherent biological characteristics of the malignant cells. The relationship of chemosensitivity to the growth rate determined experimentally was not clearly defined. The HB₈ line, for example, had the fastest doubling time among five glioblastoma cell lines tested previously,⁶ but shows only moderate sensitivity to BCNU. Sensitivity of cultured glial tumor cells to steroids in toxic doses did not correlate with their rate of growth since the more resistant cells tended to regrow at faster rates after treatment.²⁴

Glioma cell lines that were comparatively more resistant to radiation and BCNU showed an increase in sensitivity when exposed to both treatments in combination. The responses observed, reflecting the outright killing and subsequent growth inhibition of surviving cells, proved significantly greater than the predicated additive effects of the drug and radiation considered as independent actions. The enhanced effect of combination treatment demonstrated with the higher doses of BCNU may indicate a potentiation of radiosensitivity by this drug in cultivated gliomas that are not highly susceptible to either therapeutic modality. Prior treatment with BCNU increased the slope of the initial exponential portion of the radiation survival curve (Fig. 5) which may be taken as the best indication of radiation sensitivity. Were the combined effects of BCNU and radiation additive in nature and non-interacting, the initial slopes would parallel one another and denote the two independent actions.¹³ The parallel effect evident in the terminal segments of the survival curves, as noted, reflected the same type of experimental artifact in all probability. Drug sensitization seemed proportional to the dose of BCNU. At maximum drug levels, small increments of radiation on the order of 100 rads produced a highly significant reduction in the number of surviving glioma cells.

Radiosensitization was first demonstrated in human and animal cells in vitro after incorporation of halogenated thymidine analogs into the DNA of the cells prior to irradiation.¹,¹² Enhanced radiosensitivity has also been described in human gliomas in vitro after prior exposure to 5-bromo-2'-deoxyuridine (B UdR).²⁹ These authors also reported promising clinical results in patients with malignant brain tumors treated by daily intraarterial infusions of B UdR during 4 to 7 weeks of radiation therapy, but this work was not confirmed subsequently.²¹ Negative clinical results were also reported in another study comparing treatment of patients with glioblastomas with 5-fluorouracil and/or radiation therapy.¹⁴ Thus, the clinical value of combining any form of chemotherapy and radiation for malignant neoplasms remains to be established.¹⁸

Many mammalian cells seem most radio-sensitive during mitosis and in the post-DNA-synthesis phase (G₂); whereas prior to and during the periods of DNA synthesis in the cell cycle (G₁, and early S phases) cells appeared more radioresistant.¹¹,⁸⁰ Although BCNU is believed to inhibit cells throughout the cell cycle, those in the S or G₀ resting phases may be more vulnerable to the effects of this drug.⁴,⁶² BCNU may reduce the tendency for cells surviving sublethal radiation exposure to repopulate at an accelerated rate.⁴² These potentially complementary effects of radiation and BCNU provide an explanation for the apparent action of this drug in sensitizing some of the more resistant glioblastoma lines to ionizing radiation under experimental conditions where cell growth is relatively constant and the therapeutic environment is uniform and controlled. These experimental results, in turn, seem to provide some basis for the clinical observation that patients with malignant gliomas who receive whole brain radiation (6000 rads) and continuous bimonthly BCNU chemotherapy have an extended postoperative survival.

Acknowledgment

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