Radiosensitization of Brain Tumor Cells with a Thymidine Analogue (Bromouridine)*

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TREATMENT of malignant brain tumors has been one of the biggest problems in neurosurgery. If surgical removal is not feasible, two other ways of treatment are available at present, namely, radiation therapy and chemotherapy. Chemotherapy has not yet produced satisfactory results. Radiation therapy is variably effective in some kinds of brain tumor, such as medulloblastoma, ependymoma, pinealoma, and pituitary adenoma. If a way could be found to enhance the radiosensitivity of these and other brain tumors, it would make radiotherapy much more valuable. Many such agents have been examined. Bagshaw classified these agents into four groups according to the mode of action: 1) sensitization, 2) augmentation, 3) potentiation, and 4) additivity.

We have been greatly interested in one of thymidine analogues that strongly enhances the radiosensitivity of the cells, 5-bromo-2'-deoxyuridine (bromouridine or BUdR), which belongs to Bagshaw's group 1 (sensitization). In this agent, only the methyl radical of the 5th position of the pyrimidine ring of thymidine is replaced by bromine, as shown below.

\[
\text{Pyrimidine} \quad \begin{array}{c}
N_1=CH \\
H-C \quad N_2=CH \\
\end{array}
\]

\[
\begin{array}{ccc}
\text{O} & \text{C} & \text{O} \\
\text{N} & \text{C} & \text{N} \\
\text{N} & \text{C} & \text{Br (I, Cl)} \\
\text{Ribose} & \text{Deoxyribose} & \text{Deoxyribose} \\
\text{Uridine} & \text{Thymidine} & \text{BUdR} \\
\end{array}
\]

The BUdR is picked up by the deoxyribo-

nucleic acid (DNA) of the dividing cells instead of the thymidine; the sensitivity to irradiation of the cells that have incorporated the BUdR into DNA increases about two to three times, as measured by a single exposure to irradiation in vitro in such experimental tumors as D93S, D98Az, H. Ep. 1 (human epidermoid carcinoma of the cervix), ascitic P-388 (lymphocytic leukemia), L cell, Hepatoma 129, and E. coli. The BUdR, however, has almost no cytotoxic or anti-metabolic effects on unirradiated cells. This fact aroused a keen interest from a viewpoint of radiotherapy. Malignant tumors have higher mitotic rates and, accordingly, are thought to be much more vigorous in synthesizing DNA than normal tissues. Therefore, the rate of uptake of the BUdR into DNA should be much higher in tumors than in surrounding normal tissues, and the destructive effect of radiation could be enhanced selectively in the tumor cells.

Experimental Studies

Uptake of BUdR into the Human Brain Tumor Cell in Vitro. There have been no reports concerning incorporation of BUdR into brain tumor cells. We therefore wanted to confirm that this was possible with human brain tumor cells cultured by trypsinization monolayer method, using BUdR-3H (0.156 \(\mu\)g/ml or 1.56 \(\mu\)g/ml in the medium). It was confirmed that BUdR-3H was in fact taken up into the nuclei of cultured brain tumor cells such as those from glioblastoma, ependymoma, astrocytoma, oligodendroglioma, meningioma, and meningoarcoma. This was observed in five or six experiments with each tumor by radioautography. Figure 1 shows cultured cells of a case of astrocytoma; here radioautography was done after 2 days of incubation. As is seen, the black grains were concentrated in the cell nuclei.

The labelling index of BUdR-3H was found to be about the same as that of thymidine-3H; this fact seemed to indicate that BUdR was taken up by dividing cells at about the same rate as that for thymidine.

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Brain Tumor Radiosensitization by Bromouridine

Radiosensitizing Ability of BUdR in Vitro.

We had no established strains of brain tumor cell lines in the laboratory and were doubtful that we could do a quantitative study using primary cultures of brain tumors. However, since good constant growth had been obtained by the trypsinization monolayer technique in primary culture of brain tumors, we applied this simple and reliable method to investigating the effect of BUdR in vitro. The method used was as follows:

After obtaining a good proliferation of brain tumor cells in culture, we added 10 to 40 μg/ml of BUdR to the medium and incubated the bottles at 37°C for a period corresponding to the specific generation time of the tumor cells already obtained from the studies with labelling index of thymidine-3H. Then we irradiated the bottles with various amounts of x-ray (500 to 8000 r) in serial divided bands. Control bottles without BUdR were also irradiated at the same time. After the irradiation, the bottles were incubated at 37°C for 7 to 20 days. The materials were then fixed and stained. We determined the effect of the irradiation on the cells by observing the population of the cells and their morphological changes.

Figure 2 shows the effect on cells grown from an oligodendroglioma. As seen in Fig. 2 (center), the effect of simple irradiation was usually slight. In the BUdR plus irradiation group (Fig. 2 right), however, pyknosis of the nuclei, decrease of stainability, and fibrous change of the cells were noted. The cell population was markedly reduced in this group.

These experiments show that cells that have incorporated BUdR in vitro are much more sensitive to irradiation. We would like to emphasize that BUdR alone had no in-

Fig. 2. Effect of BUdR on radiosensitization of tumor cells. Left: Oligodendroglioma incubated for 48 days in the medium (80% Eagle L+20% NCTC 109+20% calf serum) with 20 μg/ml of BUdR. The characteristics of the cells were almost the same as the cells cultured without BUdR. Giemsa, ×100. Center: The same oligodendroglioma cultured for 48 days without BUdR, then given 8000 r of x-ray. Giemsa, ×100. Right: The same oligodendroglioma cultured for 48 days with 20 μg/ml of BUdR, then given 8000 r of x-ray. Giemsa, ×100.