Current Trends in the Chemotherapy of Brain Tumors with Special Reference to Glioblastomas*

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Glioblastomas account for one out of every four brain tumors, yet despite efforts to treat them by total operative removal, radiation therapy, and administration of a variety of chemotherapeutic agents, we know of no instance in which such treatment has resulted in cure. However, surgical experience does indicate that the most radical operative procedure that is consistent with maintenance of neurological function accomplishes more for patients, in terms of survival, and achieves a lower operative mortality and better postoperative condition than does a less extensive operation. In most large series, patients harboring glioblastomas have a median postoperative survival of 6 months, and few survive beyond 18 months. Postoperative radiation therapy usually lengthens survival by several months, and is believed by some authors to allow long-term survival. However, others maintain that radiation improves neither the quality nor the duration of survival in patients with glioblastomas. Because occasional longer-term survival follows treatment by operation alone, long-term survival of isolated cases treated by any adjuvant therapeutic modality lacks significance.

In 1964, one of us (CBW) reviewed reports of brain tumor chemotherapy appearing during the preceding 12 years. As judged by clinical improvement during the course of therapy, several antitumor agents were beneficial in approximately one-half of the treated patients. In a more recent review, Batzdorf found no significant difference in the survival of 145 operative patients treated with chemotherapeutic agents and 43 patients undergoing operation alone with or without postoperative radiation. While chemotherapy may improve the quality of survival, apparently to date it has failed to lengthen the lives of patients with glioblastomas to any statistically significant degree.

The neurosurgeon's recently acquired interest in the chemotherapy of brain tumors stems from a recognition of two facts. First, with the exception of a few low-grade and favorably situated gliomas, tumors arising within the brain have defied surgical cure. Second, for the first time cancer chemotherapists can speak in realistic terms about the cure of solid tumors outside the brain.

In the following review we will discuss certain factors unique to brain tumor chemotherapy and principles of chemotherapy applicable to the central nervous system. A historical review of specific agents and routes of their administration will serve as a background for a discussion of brain tumor chemotherapy in the light of presently available agents and past experience with them.

Anatomical Characteristics of Glioblastomas

Glioblastomas infiltrate the surrounding brain and almost invariably are larger and more extensive than suspected by clinical and radiological findings. At autopsy, at least one-half of the glioblastomas are found to involve both cerebral hemispheres by extension across cerebral commissures, primarily the corpus callosum. The high frequency of bilateral involvement has important implications for surgical removal as well as regional and local chemotherapy.

Cerebral edema of some degree invariably surrounds the periphery of glioblastomas. Ultrastructural characteristics of this edema indicate its localization predominantly in the extracellular space and white matter. Such studies further indicate that at least some of the extracellular fluid originates from within astrocytes, a rupture of cell membranes releasing the fluid into the extracellular compartment. Edema not only contributes to the

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space-occupying effects of the tumor, but represents disruption of the normal barrier mechanisms imposed between the vascular compartment and extracellular space within the brain. Present diagnostic methods do not permit clear distinction between edema and neoplastic tissue, and reduction in the volume of either element reduces the total mass effect of the tumor-edema complex. Any therapeutic maneuver resulting in temporary clinical improvement, particularly when survival is not lengthened also, must be suspected of favorably influencing edema rather than the neoplasm. Disruption of barrier mechanisms in the vicinity of the tumor has further implications regarding the delivery of a chemotherapeutic agent to the tumor cells and exposure of the edematous brain to agents excluded from undamaged brain by intact barriers.

Glioblastomas possess a complex vascular supply. Hardman studied the angio-architecture of gliomas in a classic paper and his observations have been supplemented by subsequent postmortem studies. Conventional arteriography fails to give an entirely accurate picture of tumor blood supply because injection alters normal flow and contrast media are viscous and heavy. Blood vessels within a glioblastoma are characterized by tortuosity, aneurysmal dilatation, glomeruloid sprouts, endothelial proliferation, and medial hypertrophy. Although Hardman found no true arteriogenous anastomoses, capillary dilatation and sinusoidal enlargement produced a similar effect. Characteristics of blood vessels within a tumor become significant whenever chemotherapy by intravascular administration is considered. Arteriography clearly indicates an abundance of blood vessels within many glioblastomas, and rapid transit of blood through such tumors is a second common characteristic. However, by analogy with cerebroarteriovenous malformations, the presence of large blood vessels and a rapid rate of blood flow offer no assurance of adequate perfusion through surrounding tissues, whether normal or neoplastic.

Exploitation of Differences Between Brain and Brain Tumors

In treating tumors within the brain, as elsewhere, the narrow margin of safety between the minimal effective therapeutic dose and the maximal tolerated tissue dose limits the effectiveness of both radiation and chemotherapy. Even when cytotoxic action can be intensified by various potentiating factors, this small therapeutic index usually persists due to a relative lack of specificity of these agents for the tumor cell. The rationale of combining different therapeutic modalities is based on the assumption that simultaneous administration of two or more agents acting at different sites and stages of cellular activity should result in inhibition of a greater total number of tumor cells than would result from administration of a single agent.

With the exception that some tumor cells depend upon an exogenous source of asparaginé, the nutritional requirements of tumor cells differ in no significant way from those of normal cells, and apparently both types of cells handle these nutrients similarly along the same biosynthetic pathways. Surprisingly, in vitro studies suggest that the respiratory rates of benign brain tumors are high whereas those of glioblastomas are significantly lower. With no apparent distinction, anticancer drugs inhibit the respiration of normal brain and brain tumor cells, and this lack of specificity in vitro corresponds to clinical and pathological evidence of the toxicity of many anticancer drugs on normal nervous tissue.

Hexose provides the endogenous energy reserve in normal white matter, whereas in vitro studies show that glioblastomas continue to produce lactate at a steady rate after prolonged anaerobiosis. This lactate production far exceeds that expected from hexose breakdown by way of the Embden-Meyerhof pathway. Preliminary studies in our laboratory suggest that glial cells may be asparagine-dependent, but with this possible exception, metabolic differences between normal brain and brain tumors cannot be exploited in our present stage of knowledge. Even the finding of an exploitable metabolic difference might be disappointing, since cytologic and cytochemical evidence indicates that within the heterogeneous group of tumors classified as glioblastomas marked differences exist not only between tumors but also within an individual tumor.

Intracellular synthesis of deoxyribonu-
Cleic acid (DNA) is a preliminary step in cell division. Consequently, DNA synthesis is a characteristic of dividing cells and is altogether absent within cells incapable of cell division. Animal studies provide no evidence of DNA synthesis in neurons under any circumstances. In contrast, neuroglial nuclei do incorporate tritiated thymidine indicating that a low degree of mitotic activity continually takes place in the glial population of adult rats. Except for the small amount of mitotic activity seen within microglia, most activity occurred in oligodendroglia. Experimental work to date suggests that glial cell loss balances the production of new cells by a low degree of mitotic activity, in other words, the neuroglial cell population turns over slowly in normal adult neural tissue. Because more or less rapid cell division is an inherent feature of brain tumors, as of tumors elsewhere, antimetabolic agents and other drugs that interrupt or arrest cell division would seem ideally suited to brain tumor chemotherapy. However, to date, this theoretically attractive approach has met with limited success, either because direct neurotoxic effects occur or because effective local drug levels are associated with intolerable systemic toxicity.

The differences between normal and neoplastic glia might be accentuated by potentiating factors such as hyperoxygenation and hyperthermia. The response of many normal and neoplastic tissues to ionizing radiation is enhanced under conditions of increased oxygen tension, and therefore, because biological effects of alkylating agents are similar to those produced by radiation, administration of oxygen under pressure has been used to enhance the antitumor effectiveness of alkylating agents. Hyperoxygenation achieved by infusion by hydrogen peroxide greatly enhances the uptake of radioactive compounds by brain tumors, but this principle has not been applied to brain tumor chemotherapy with alkylating agents. Elevation of temperature increases most metabolic enzymatic processes and, further, hyperthermia alone is known to be harmful to neoplastic cells. Elevated tissue temperature increases the binding of alkylating agents in isolated perfusion systems and this technique has been employed in brain tumor chemotherapy. While hyperoxygenation may be applicable to brain tumor chemotherapy, the therapeutic use of hyperthermia, whether local or general, appears ineffective.

Cell-Generation and Tumor-Doubling Times

Cell-Generation Time. The period between two successive cell divisions, known as the “cell-generation time,” varies widely among different normal and neoplastic tissues; neoplastic cells do not necessarily have more rapid generation times than normal cells of the same type. The period during which a cell is actively synthesizing DNA, the “S” phase, occupies a variable fraction of the total cell cycle, being, in many rapidly dividing tissues, in the order of one-third of the cell cycle.

The duration of the “S” phase becomes critical in the dosage schedules of antimetabolites because these agents affect only cells synthesizing nucleic acids and have no effect on cells in other stages of the cell cycle. Because cell division within a tumor occurs asynchronously, an antimetabolite must be available to the tumor cell population during a period corresponding to at least one complete cell cycle. On the other hand, cell generation times become relatively unimportant in the application of alkylating agents because these drugs are toxic to cells throughout their entire cycle.

Although knowledge of cell-generation times of glial tumors would enhance an approach to their treatment by chemotherapeutic agents, available information in this regard is fragmentary and probably misleading. The one in vivo study of a human glioblastoma was based on a labeling index of 0.6%, and calculations made on this basis gave an unreasonably long generation time of 1½ to 2 months. Cells derived from many normal and neoplastic tissues complete their cell cycle in vitro under favorable conditions on an average of 24 hours, exact values varying somewhat on the type of cell and the culture conditions. Using tissue slices, Kury and Carter obtained a cell-generation time of 2 to 5 days for a malignant astrocytoma, a value difficult to accept. Studies in our laboratory using two techniques (tritiated thymidine uptake and cinemomicroscopy) gave cell-generation times in the range of 24 hours for glioblastomas in cell culture. At present we are at-
tempting to determine the duration of the "S" phase in glioblastomas by using a double label in vivo and in vitro.

**Tumor-Doubling Time.** The period in which a tumor doubles its volume is referred to as the "tumor-doubling time." The number of cells in a tumor is the most fundamental measure of tumor size, and tumor volume is proportional to the number of tumor cells only if the mean effective volume per cell remains constant during growth. For the majority of solid tumors, tumor-doubling time greatly exceeds cell-generation time. Reasons for this difference are: 1) a variable portion of cells within a solid tumor are in a non-proliferating phase; and 2) tumor-doubling time lengthens as the tumor becomes larger, probably as a result of nutritional factors and effects of crowding. To speak of doubling time as an expression of growth has great importance from the viewpoint of evaluating drug effectiveness. At present, tumor-doubling time can be applied with reliance to palpable tumors suitable for measurement and to tumors that can be visualized, as those in the lung. Until we have some means of measuring the volume of glial tumors and of separating tumor volume from the volume of invaded and edematous brain, we cannot begin to think in terms of modifying brain tumor-doubling time by chemotherapy.

**Blood-Brain and Blood-Tumor Barriers**

**Blood-Brain Barrier.** The movement of substances across capillary walls into the brain depends upon particle size, lipoid solubility, chemical dissociation, protein-binding, and stereospecific transport mechanisms. In general, drugs that are lipoid-soluble and unassociated at body pH rapidly enter brain and CSF. For example, methotrexate, which is lipoid-insoluble, is excluded from the brain by an intact blood-brain barrier, and the barrier therefore protects the normal brain. The normal barrier can be circumvented by introducing agents directly into CSF because, for most substances, no barrier exists between CSF and brain extracellular fluids.

**Blood-Tumor Barrier.** One might reasonably ask: Is there a blood-tumor barrier within glial neoplasms? The fact that glial tumors are composed of neoplastic cells does not mean that component astrocytes have lost their original metabolic properties and vascular relationships. Present evidence of barrier phenomena within brain tumors comes largely from studies with radioactive isotopes. The localization of isotopes within a tumor depends upon two factors: 1) passage of the substance through the capillary wall (the blood-tumor barrier); and 2) accumulation and retention of the substance in tumor cells or in interstitial spaces within the tumor. The retention of blood-borne substances within a brain tumor is related to factors such as tumor vascularity, metabolic activity of tumor cells, and the extent of the interstitial space. Necrotic areas within a tumor accumulate substances in a manner similar to that of damaged brain where the barrier has undergone interruption. Information at hand suggests that glial tumors do possess barriers to the extravascular passages of some substances, but in glioblastomas the barrier bears little similarity to an intact blood-brain barrier. Even within a tumor, extravascular accumulation of blood-borne substances may reflect regional biochemical characteristics of the neoplastic cells rather than local differences in membrane permeability.

The blood-brain barrier is subject to deliberate and reversible alterations. For example, hypercapnia, either primary or secondary, impairs the barrier whereas hyperoxia alone does not. Prolonged hyperventilation alters the normal barrier, probably through the mechanism of ischemic hypoxia. Contrast agents commonly used in cerebral angiography affect the barrier and further damage areas with a pre-existing injury. Brain cooling alters the blood-brain barrier, an alteration that is predictable and completely reversible (Baldwin, M., personal communication). Exactly how the preceding factors affect the blood-tumor barrier remains unknown. The preferential effect of 1-methyl-1-nitrosourea on animal tumors implanted in brain, in contrast to tumors implanted subcutaneously, at least suggests that blood-tumor barriers may be influenced by their environment, a consideration that adds further complexity to the problem of delivering drugs to their desired site of action.
Present Obstacles to Successful Brain Tumor Chemotherapy

Apart from deficient knowledge of biological and biochemical features of human glial tumors, the brain-tumor chemotherapist recognizes three other barriers to effective experimentation and treatment: 1) lack of a suitable animal model; 2) lack of a reliable means of drug selection for individual tumors; and 3) lack of a rapid system for precise measurement of tumor response.

Studies using animal models have contributed significantly to advances in cancer chemotherapy. Animal models have served their greatest usefulness in the screening of promising drugs, in the development of routes of administration, and in the prediction of effective dose schedules. An outstanding example of information provided by animal models is experience with L 1210 murine leukemia. This model not only served for the screening of antileukemic agents but also allowed prediction of the effective dose schedule for maintenance therapy in acute leukemia of childhood. A limited number of studies have been conducted using induced mouse gliomas but, because of the heterogeneous tumor cell types and the limitations imposed by a tumor that is more nearly like a metastatic than a primary tumor, this model has contributed little to clinical chemotherapy. Work in our laboratory using a transplanted rat sarcoma has shown the superiority of intra-arterial over intravenous infusion of vinblastine sulfate, and of intrathecal over systemic administration of methotrexate. The value of this model is sharply limited, and at present we are developing an improved model using a nitrosourea-induced rat glioma.

The problem of selecting an effective drug for an individual tumor is not unique to brain-tumor chemotherapy. Cancer chemotherapists select an initial drug on the basis of reported and personal experience with tumors of the same histological type. With many solid tumors this prediction of effectiveness can be made with considerable accuracy, but should the initial drug prove ineffective others can be tried.

Such a trial-and-error method of drug selection fails for glioblastomas because rapid progression of the tumor precludes adequate trial of more than one agent. Past experience indicating a small number of favorable responses to a variety of agents can be interpreted in two ways: 1) either a small number of tumors will respond to any one of a spectrum of agents; or 2) more likely, any given agent is effective against a relatively small number of tumors with limited cross-sensitivity to other agents. In the latter instance, drug selection in the individual case assumes paramount importance. Routine histologic studies have not provided a guide to therapy. To date, in vitro studies, however attractive in principle, cannot allow prediction of tumor responsiveness. Until we have an agent effective against more than a small proportion of glioblastomas, our inability to select an initially effective drug will remain a major stumbling block to effective brain-tumor chemotherapy.

Finally, our inability to measure tumor mass, and therefore response or non-response to therapy, is possibly the greatest obstacle to successful brain tumor chemotherapy. The vagaries of angiography, brain scan, and measurement of intracranial pressure render each of these examinations unreliable means of defining tumor mass. At present, effectiveness of chemotherapy is judged by two criteria: 1) the clinician's judgment, which is too unscientific to serve as more than a rough estimate of tumor behavior; and 2) length of survival which, while satisfying the statistician, provides no guidance to the clinician charged with treating the individual patient. A biochemical test reflecting some metabolic activity of the tumor would be an ideal solution to the problem, and logically this metabolic product might exist in the cerebrospinal fluid. Workers in Milan have measured desmosterol, a cholesterol precursor, in the CSF of patients bearing a variety of brain tumors, and their results suggest that CSF levels of desmosterol or some related sterol may furnish a biochemical index to the neoplastic glial cell population. Measurement of glycoprotein levels in CSF, although they are abnormal in patients bearing primary brain tumors, appears less promising.

Agents Used in Brain Tumor Chemotherapy

Many chemotherapeutic agents have re-
ceived more or less extensive trial in the treatment of brain tumors. A number have been used against metastatic tumors, but these results cannot be used to predict the effectiveness or ineffectiveness of these agents in the treatment of glial tumors.

The agents mentioned below have been used in the management of glial tumors, predominantly glioblastomas. Agents will be grouped according to their route of administration. The agents that have been administered by more than one route (methotrexate) will be discussed separately in appropriate sections.

**Drugs Delivered by Systemic Administration**

*Mithramycin*. In 1965, Kennedy, *et al.*, 41 reported nine glioblastomas treated with Mithramycin. They observed objective improvement in five of the nine patients with improvement lasting up to 5 months. Although highly toxic in tissue culture by present-day standards, Mithramycin is not effective against most experimental tumors and would not pass screening tests now used for experimental animal tumors. 42 In this respect, Mithramycin is a unique antitumor agent because the regressions observed when it is used against a variety of clinical tumors do not correlate with the poor results obtained against standard screening experimental tumors. Its mechanism of action is believed to be an inhibition of the synthesis of ribonucleic acid.

A more recent report of this agent contains results obtained in 64 patients; 33% of those receiving 20 mg or more of Mithramycin were alive at the end of 18 months. 81 Today, this drug is being used in a Cooperative National Study. Judged by preliminary statistical analysis, this agent, although benefiting certain patients, adds little to results achieved by operation and conventional radiotherapy.

**Vincristine Sulfate**. Vincristine has been used to treat a number of childhood tumors. It has toxic effects similar to those of vinblastine sulfate, another vinca alkaloid, but the two drugs show little or no cross-resistance. 59 Vincristine has prominent toxic effects, of which neurological damage is the most serious. 10 Lassman, *et al.*, 50 in a study of clinical and in vitro responses to vincristine, reported striking results in six children, two with medulloblastomas and four with astrocytomas. Five of nine adults with glioblastomas derived benefit from the drug, which was administered by weekly intravenous injections, 0.05 mg/kg. 49 A more recent report confirms the effectiveness of vincristine against medulloblastomas. 47 Although this experience is small, the drug's effectiveness against such tumors justifies its use in recurrent tumors.

**BCNU** *(1,3-bis(2-chloroethyl)-1-nitrosourea)*. Being highly soluble in lipids, BCNU appears simultaneously in the CSF and bloodstream following intravenous or oral administration. Its half-life in plasma is less than 15 minutes *in vivo*, 55 its fate in CSF is less clearly defined. BCNU's ability to penetrate the blood-brain barrier and its effectiveness against intracerebral-implanted L 1210 leukemia in mice stimulated early interest in this synthetic compound. 11, 93 Laboratory studies have shown no correlation between cytotoxicity in mammalian cell culture and the antileukemic activity of nitrosoureas in animal systems. BCNU exhibits cross-resistance to several alkylating agents, and its biochemical mechanism of action appears to be related to that of alkylating agents. 93 When compared with other alkylating agents, BCNU produces the greatest increase in survival time and in the number of survivors among mice-bearing intracerebral leukemic implants. 10

Clinical trials have shown the effectiveness of BCNU in a variety of tumors outside the central nervous system, in particular Ewing's sarcoma, Hodgkin's disease, and malignant melanoma. 17, 65, 82 Although it possesses ideal pharmacological characteristics of a chemotherapeutic agent for tumors within the central nervous system, a single clinical trial by Walker (Walker, M., personal communication) gave disappointing results. However, Walker used a weekly dose of 60 mg per square meter of body surface, a dosage schedule that may not be optimal. Marrow toxicity limits drug dosage, and because the nadir occurs 4 weeks after administration, use of the drug demands cautious administration. On the basis of experience with Hodgkin's disease, 17 an effective and safe schedule is 125 mg per square meter of body surface on 3 consecutive days in courses spaced at intervals of 4 to 6 weeks.
This drug has many appealing features, and a single discouraging clinical trial should not limit further exploration of its effectiveness against primary brain tumors.

**Intra-Arterial Administration**

Several drugs have been administered through the carotid artery by single injection, by intermittent or continuous infusion, and by perfusion using one or both carotid arteries and one or both internal jugular veins. In theory, intra-arterial administration should achieve a high drug concentration within a tumor supplied by this artery. The advantage of a high local drug concentration might be further enhanced with those agents for which there is a systemic antidote such as sodium thiosulfate for nitrogen mustard and citrovorum factor for methotrexate.

Although attractive in theory, several problems are encountered with intracarotid drug administration: variability of blood supply to different parts of a tumor as shown by inequality of dye distribution; varying patterns of drug distribution related to position of the catheter within the carotid artery; and a variety of complications such as catheter occlusion, arterial thrombosis, local infection, and intolerable local tissue toxicity.13,21,22,25,58,63,90,101 Because the nature of the cerebral circulation precludes its strict isolation, perfusion chemotherapy of brain tumors offers little if any advantage over direct intra-arterial administration. In general, therapeutic results of intra-arterial chemotherapy have not differed remarkably from those achieved by systemic chemotherapy.

**Nitrogen Mustard.** Present-day brain-tumor chemotherapy began with intra-arterial administration of nitrogen mustard,26 and, of the alkylating agents, nitrogen mustard appears to be the most effective. The largest experience with intra-arterial nitrogen mustard was reported by Owens.72-74,76 His and other published results suggest significant antitumor effects in occasional patients, but the small number of favorable responses has not encouraged other investigators to continue its use.77

**Methotrexate.** Methotrexate is an antimetabolite largely excluded from the normal central nervous system by barrier mechanisms. It blocks the reduction of folic acid to tetrahydrofolic acid, and its effects can be neutralized by the administration of citrovorum factor.4,29,103 Citrovorum factor, like methotrexate, fails to enter the CSF in appreciable amounts, and several investigators have effectively combined intra-arterial administration of methotrexate with concomitant systemic administration of citrovorum factor. Laboratory studies have shown the relationship of cellular levels of folic acid reductase to acquired resistance to methotrexate, although, in vitro, the development of resistance to methotrexate has no apparent effect on the sensitivity of the cells to other agents. Cellular resistance afforded by excess folic acid reductase is explained by immobilization of methotrexate in an irreversible equation.28,40

Continuous intra-arterial infusion of methotrexate with concomitant intermittent systemic administration of citrovorum factor thus has a rational basis, and one report of this mode of therapy suggests its effectiveness against some brain tumors, as judged by objective improvement.103 However, this report is less than convincing, and further use of intra-arterial methotrexate holds little promise. Our limited experience with protracted intra-arterial infusion of methotrexate in primary and metastatic tumors led us to discontinue its use 2 years ago (Wilson, C. B. and Norrell, H. A., Jr., unpublished results).

**Vinca Alkaloids.** Two derivatives of the periwinkle plant, *Vinca rosea* (Linn), have similar chemical structures and chemotherapeutic spectra.96 These two agents, vincristine sulfate and vinblastine sulfate, have had extensive clinical trial by intra-arterial administration.16,62,75,106 Objective improvement has been observed in approximately one-half of the treated patients, and drug toxicity has been acceptable. Of the two agents, vincristine appears slightly superior. However, the difficulties attending long-term intra-arterial administration and the modest success observed to date give little reason to recommend wider clinical trial of the vinca alkaloids.

**Bromouridine (BUdR).** Bromouridine is a halogenated pyrimidine analog incorporated into cellular DNA as a substitute for thymidine, the methyl radical in the pyrimidine being replaced by bromine. After incorporating BUdR, cultured cells become two to
three times more radiosensitive to single exposures. Sano, et al.,\(^{91}\) recognized four advantages of \(\text{BUdR}\) as a radiosensitizing agent for brain tumor radiotherapy: 1) normal cells within the central nervous system do not engage in DNA synthesis and therefore do not incorporate \(\text{BUdR}\); 2) an intact blood-brain barrier protects normal brain from \(\text{BUdR}\); 3) even in high concentrations \(\text{BUdR}\) has few toxic manifestations; and 4) more than 80% of the drug is dehalogenated by the liver within 1 hour following intravenous administration.

Earlier in vitro studies had shown that incorporation of \(\text{BUdR}\) by cultured cells was greatly enhanced by small amounts of methotrexate added to the culture medium. Methotrexate’s enhancement of \(\text{BUdR}\) incorporation was believed to represent inhibition of cellular biosynthetic mechanisms rendering the cell more dependent upon exogenous thymidine for DNA synthesis. To achieve maximal incorporation of \(\text{BUdR}\) by tumor cells, then, Sano, et al., administered \(\text{BUdR}\) by intracarotid infusion in combination with methotrexate.

Their method of therapy began with a decompressive craniotomy followed by open insertion of a polyethylene catheter into the internal carotid artery 2 to 4 weeks later. \(\text{BUdR}\) was administered by continuous carotid infusion in amounts of 600 to 1000 mg per day combined with 1 to 5 mg of methotrexate in normal saline. After 1 to 2 weeks of infusion, a 6-week course of radiation therapy was begun for a total of 6000R tumor dose. \(\text{BUdR}\) infusion was discontinued during the final 2 or 3 weeks of radiation. The principal toxic manifestation was a leukopenia. That this was reversed by omitting methotrexate from the infusion suggests that methotrexate was given in quantities sufficient to depress bone marrow. While methotrexate was used primarily to achieve greater \(\text{BUdR}\) uptake by tumor cells, it probably had two additional effects: 1) a direct antitumor effect, since the dose was sufficient to depress bone marrow, and 2) its own radiosensitizing effect (methotrexate is known to sensitize \(\text{HeLa}\) cells to radiation).\(^8\)

The rationale of combining methotrexate with \(\text{BUdR}\) should be reconsidered, and the sequence of \(\text{BUdR}\) and radiation should be examined carefully in view of the known division delay caused by ionizing radiation.\(^{24}\)

The results obtained by Sano, et al., with combined \(\text{BUdR}\)-methotrexate-radiation therapy are contained in Table 1. Of prime interest are the five patients with highly malignant gliomas who survived beyond 24 months. Objective improvement observed in many patients surviving for shorter periods (many of whom are presently alive) supports their claim for the effectiveness of this form of therapy. Regardless of the role assigned to any one of the three therapeutic modalities used, the results achieved by these investigators are most impressive and will undoubtedly stimulate others to pursue similar courses of combined radiation and chemotherapy.

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<td><strong>Follow-up of patients with malignant hemispheric gliomas treated with bromouridine, methotrexate, and radiation</strong></td>
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* Designated according to the time since operation (for previously untreated tumors) or since the time of recurrence (for tumors previously treated by operation and radiation).

† Five died before treatment was completed.

The triethylene thiophosphoramide (thio-TEPA) and 5-Fluorouracil (5-FU). Davis and Shumway\(^{24}\) used thio-TEPA to treat 97 patients harboring metastatic brain tumors, 31 from the lungs and 66 from the breast. They reported near-miraculous responses, but this report has received no subsequent confirmation. Although useful in the treatment of tumors outside of the central nervous system, 5-FU has not been given adequate clinical trial in the treatment of brain tumors. Undoubtedly, it has been tried in several pilot studies, and a presumed failure to observe antitumor action has discouraged wider trial.\(^{30}\)

**Drugs Delivered by Intrathecal Administration**

Current evidence indicates the virtual absence of a barrier mechanism between cere-
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brosplinal fluid and extracellular fluid within the central nervous system. The intrathecal administration of drugs normally excluded by the blood-brain barrier follows as a logical pharmacological maneuver. Direct intrathecal drug administration also permits the use of substances that, if given intravenously, would be too rapidly detoxified in extracerebral tissues. Intrathecal drug administration has the added theoretical advantage of providing high local concentration with less risk of systemic toxicity, a consideration of particular importance if the drug is detoxified by or bound to blood proteins. However, the location of a tumor deep within the brain would present an obstacle to the use of a drug whose effectiveness depends upon its diffusion through normal or near-normal brain. In order to reach the depths of cerebral tissue a drug must possess a diffusion coefficient favoring rapid transit through the complex extracellular compartment of intact brain, a consideration less important for tumors adjacent to pial or ependymal surfaces. Rational application of intrathecal chemotherapy, therefore, depends upon two assumptions: 1) free communication between CSF and brain extracellular fluid; and 2) access of the drug to the tumor either by surface contact or by diffusion through intact brain. We have little information concerning the movement of drugs through the interstitial spaces of a tumor, and this matter demands investigation. The final interphase might be termed the extracellular fluid-tumor barrier with the critical membrane residing at the surface of the tumor cell. Our experience with tumor cells in vitro would indicate that this membrane barrier permits entry of some, if not all, of the chemotherapeutic agents in current use.

Distribution of a drug within the CSF following intrathecal administration assumes major importance. Studies with labeled compounds have defined patterns and rates of CSF flow. Bulk flow of CSF appears to be little affected by body position, substances introduced by lumbar injection moving cephalad at rates related directly to the total volume of fluid injected. Under normal circumstances, a substance injected into the lumbar subarachnoid space does not flow into the ventricular system. Although flow patterns are affected by pathological changes associated with brain tumors and related operative procedures, the importance of proper drug distribution demands that special techniques be applied to assure movement of a drug to its site of action.

Three drugs possess characteristics suiting them for intrathecal administration: methotrexate, 8-azaguanine, and thio-TEPA. Other agents, such as vinblastine sulfate, have severe toxic reactions when injected into the CSF in minute amounts. Methotrexate has received the most extensive trial, and our present knowledge of intrathecal chemotherapy is based largely on this experience.

Methotrexate. When introduced directly into the CSF, methotrexate enters the brain by diffusion across ependymal and pial surfaces. Within the central nervous system it is handled like inulin both being lipid-insoluble substances that are neither metabolized nor actively transported. Methotrexate is removed from the central nervous system by bulk flow of CSF at a rate largely determined by hydrostatic forces. In addition, recent in vitro studies suggest that methotrexate is actively transported from CSF to blood by an energy-dependent mechanism through the epithelial surface of the choroid plexus. Because it is lipid-insoluble and highly ionized, systemically administered methotrexate is largely excluded from the normal central nervous system. Consequently, its intrathecal administration achieves CSF levels greatly in excess of those obtained by its systemic administration. Recent studies indicate the persistence of low levels of methotrexate in CSF for as long as 3 weeks, but the significance of low drug concentrations is uncertain for two reasons: 1) the amount of methotrexate remaining in tissues is not obviously and directly related to the duration of its effects; and 2) the correlation between CSF drug levels and inhibition of tumor cells within the brain parenchyma is unknown. At the present time, we know of no evidence concerning the relative merits of a continuous low concentration versus intermittent high concentrations of methotrexate in the treatment of brain tumors.

Because methotrexate acts by inhibiting the conversion of folic acid to tetrahydrofolate acid, its systemic effects can be neutralized by the administration of citrovorum factor. Citrovorum factor, like methotrexate, fails to enter CSF in appreciable amounts,
and on this basis Rall, et al., suggested the combined use of intrathecal methotrexate and systemic citrovorum factor. Although we have used this combination in one patient without success, concomitant administration has not received adequate clinical trial. When injected into CSF, methotrexate has little neurotoxicity, even in amounts (up to 4 mg/kg) that produce serious systemic toxicity. In 1967, we reported six patients treated by intrathecal administration of methotrexate. To assure adequate drug distribution, the methotrexate was injected into a one-way shunt incorporating a reservoir. In the initial series, five of the six patients were improved. Later, six patients were added to that series. One patient with a brain-stem glioma improved but subsequently died. Two patients, both harboring medulloblastomas, showed unquestionable improvement. Two patients in the original report received methotrexate by intermittent intrathecal injection for periods of 22 and 24 months, and these patients are being reported in a separate paper.

In 1968, Newton, et al., reported their series of 44 childhood tumors treated by intrathecal methotrexate. They introduced the drug directly into the lumbar or cisternal subarachnoid space, into the cerebral ventricles, or into the tumor bed. Single injections of 0.25 mg/kg were repeated for five to seven doses on successive days, and patients showing response to treatment received additional courses. Objective improvement occurred often, but tended to be brief. Five patients survived and were living 5 years after potentially lethal tumor recurrence. On that dosage schedule, systemic drug absorption produced gastrointestinal, mucocutaneous, and hematologic toxicity.

Rubin, et al., at the National Institutes of Health, treated a series of nine patients by spinal fluid perfusion using methotrexate. Perfusion was done one to two times per week with a perfusate containing 500 gamma per ml. In five of seven patients with glioblastomas, treatment produced symptomatic improvement without objective change in tumor size.

Long-term intrathecal administration of methotrexate is both practicable and safe, and a certain number of brain tumors, medulloblastomas in particular, will respond to this mode of therapy. On the basis of effective dosage regimens in leukemia and in view of new information on tumor kinetics and the fate of methotrexate in CSF, maintenance therapy should be continued for an indefinite period of time. However, without concomitant administration of citrovorum factor, treatment with intrathecal methotrexate is limited by systemic toxicity. The unquestioned effectiveness of intrathecal methotrexate against certain tumors should prompt investigation of different dose schedules, and efforts should be made to obtain information on the movement of citrovorum factor across the blood-tumor barrier. We predict that methotrexate will assume an important role in brain tumor chemotherapy.

8-Azaguanine. This agent is a guanine analog. Certain tumor cells require guanine for nucleic acid synthesis, in contrast to normal cells which form their nucleic acids from simple precursors. The drug 8-azaguanine has its greatest inhibitory effect on rapidly growing tissues, and the extent of cell inhibition generally parallels the rate of cell division. Normal human brain cells are rich in 8-azaguanine deaminase, an enzyme responsible for converting 8-azaguanine to 8-azaxanthine. Unlike 8-azaguanine, 8-azaxanthine has no growth-inhibiting effects. Glioblastoma cells contain a negligible amount of 8-azaguanine deaminase and as a consequence represent a vulnerable cell population within an organ whose normal cells are protected by normally present levels of the enzyme.

The drug must be applied locally because blood levels are too low and too evanescent to be effective. In cell culture, glioblastoma cells are inhibited by prolonged exposure to a concentration of 700 gamma/ml, and blood levels attained by intravenous administration have reached a maximal level of only 50 gamma/ml at the end of 2 minutes.

Selverstone has applied this agent locally. Preliminary studies in his laboratory confirmed the high levels of azaguanine deaminase in normal brain and its virtual absence in human glioblastomas. In addition, he found that tumor cells possessed a specific phosphorylase needed for the activation of 8-azaguanine.

Rubin, et al., conducted preclinical toxic-
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ity studies and reported preliminary results with intrathecal perfusion of 8-azaguanine. Dogs and cats survived ventricular perfusions at drug levels of 700 gamma/ml, and it was suspected that the optimal therapeutic concentration in man might be in the order of 1000 gamma/ml. At the time of their report Rubin, et al., had treated only one patient, without apparent benefit.

The desirable pharmacological characteristics of 8-azaguanine and its acceptable toxicity when introduced into the CSF justify additional studies on its application as a chemotherapeutic agent. Because the technical complexities of ventricular perfusion present practical difficulties, the drug might be given by a simpler method such as the Heyer-Pudenz shunt that we have used.65

Thio-TEPA. Smith reported treating eight patients with this agent administered intrathecally or directly into a neoplastic cyst.5 Results were uncertain although no ill effects were observed. This report gives little encouragement for further study of this agent.

Summary and Conclusions

We have reviewed the current status of chemotherapy for malignant gliomas. Whatever chemotherapeutic advances are made in the immediate future, the neurosurgeon's role in the treatment of brain tumors will remain central because the opportunity for complete tumor eradication is best when the volume of tumor is smallest. Consequently, chemotherapy will always be most effective when used as an adjunct to radical surgical removal. Medulloblastomas, brain-stem gliomas, and low-grade ependymomas generally respond favorably to radiation. For this group of radiosensitive tumors, chemotherapy may not be justified as an adjuvant to operation but should be reserved for post-radiation tumor recurrences. Glioblastomas are quite another matter. Radiation therapy achieves few long-term survivals and no cures, and adjuvant chemotherapy, with or without concomitant radiation therapy, deserves our continued consideration.

At this point of time, three forms of brain tumor chemotherapy hold reasonable promise:

1. Vincristine sulfate. Intravenous administration of this agent is effective in at least some recurrent medulloblastomas.

2. Radiation therapy combined with BUdR and methotrexate. Promising results have been achieved with this therapy against malignant gliomas, although the high cost of BUdR and present limitations on its availability impose practical restrictions.

3. Intrathecal methotrexate. This drug is both safe and effective in certain tumors, most notably recurrent medulloblastomas.

Some of the agents that have been discarded as ineffective should perhaps be given further trial, since improved dose schedules and different routes of administration might produce entirely different results. If neural tumors require exogenous asparagine, administration of asparaginase may be of value. Of the several potentially useful agents presently under investigation, BCNU seems the most promising.

References


40. JUKES, T. H., and BROQUIST, H. P. Sulfonylureas and folic acid antagonists. In: Meta-
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