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TUMORS IN THE CENTRAL NERVOUS SYSTEM

CURRENT ISSUES AND LESSONS FOR THE INTERNIST

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Considerable skepticism exists concerning the potential for cure for true parenchymal lesions when such lesions are beyond the highly demarcated scope of surgery or radiation. The significant successes of chemotherapy (with or without adjunctive aid of surgery or radiation) have been ascribed to unusual biologic features of the tumors. Thus, in Hodgkin's disease the good results have been ascribed to the relatively low cell burden of true malignant cells (i.e. a modest number of Reed-Sternberg cells in a largely reactive matrix), or in choriocarcinoma in women to a biologically "foreign" tissue, or in small cell undifferentiated of the lung to low bulk cell mass with relatively high vascular volume and atypical growth pattern. The recent remarkable curative successes in some parenchymal tumors, such as testis, have provided a new challenge and focus on factors that affect adequate control of neoplastic lesions.

Malignant tumors in the central nervous system (CNS) provide a particularly exciting arena for our attention. Although primary neoplasms in the CNS are of low incidence (approximately 1.5% of all new cancer cases in the U.S.), the mortality rates have progressively increased to approximately twice that of 1940.
In addition, metastatic carcinoma to the central nervous system has a significant incidence and is an important factor in therapeutic failure for a variety of systemic tumors. In addition to the magnitude of the problem, the two potentially curative moieties (surgery and radiation) have made little impact on the outcome of these tumors over the past 30 years. Since these two approaches are destined to limited success because of the problems of injury to the normal brain, the current review will focus on reasons for our therapeutic limitations, and we will consider future treatment strategies.

CURRENT STATUS OF THERAPY FOR TUMORS IN CNS

Review of the extensive therapeutic trials for tumors in the central nervous system quickly identifies the dismal results and lack of important progress over the past 3 decades. In 1960, Taveras (1) reviewed the previous radiation therapy experience with 420 histologically confirmed cases of malignant CNS tumors and noted that 11.9% were alive at 12 months and 3.7% survived 24 months. Two exhaustive compendia of therapy reports, that of Shapiro and Ausman (2) which evaluated studies prior to 1969, and that from Levin and Wilson's program (3) extending to 1980, noted that the median survival time following surgery and radiation therapy was still in the range of 7.5-9.5 months. Even more grim are the results of therapy for metastatic neoplasms into the CNS, with a median survival of approximately 4 months after therapy (4).

Two very recent reports that encompassed extensive multi-institutional participation help focus the current status of treatment. As shown in Table 1, an extensive study by the Brain Tumor Study Group (5) has again confirmed the value of post-operative radiation as a means of modest prolongation of expected life span. In addition, this study examined what has been considered to be the best drug for brain tumors, the nitrosourea BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea [carmustine]) because it is lipophilic and can penetrate the blood-brain barrier (6), as well as a recent derivative, methyl CCNU (1-[2-chloroethyl)-3-[4-methylcyclohexyl)-1-nitrosourea[semustene]). The combination of radiation therapy plus BCNU resulted in a median survival of 51 weeks and only 27% of the patients alive at 18 months, a very modest gain over that achieved with radiation therapy alone. This study further highlights the problems in the interpretation of outcome of therapy. Although the Brain Tumor Study Group consists of highly sophisticated investigators, many of the cases treated had to be eliminated; hence, the category "valid" cases (Table 1). For instance, nearly 10% of the cases on review did not have a malignant glioma. Some cases had to be eliminated because they did not get the treatment to which they were randomized, and some were given treatment in addition to that in the protocol (5). Even with that careful analysis, tumor heterogeneity was present since 84% of the patients had glioblastoma multiforme, 11% had anaplastic astrocytoma, and 5% had "other" tumors. Furthermore, these percentages were not consistent in each treatment group.

TABLE 1

RANDOMIZED MULTI-INSTITUTIONAL MULTI-MODALITY THERAPY TRIALS

Group (Ref.)	Number of Patients Entered	Number Evaluable	Treatment Options	Number of Cases	Number of Valid Cases	Median Survival - Weeks -	Percentage Alive at 18 Months %
Brain Tumor Study Group (5)	467	358	Methyl CCNU (semustine)	111	81	24	10
			Radiation therapy (6000 rads/7 wk)	118	94	36	15
			BCNU plus radiation Rx (carmustine)	120	92	51	27
			Methyl CCNU plus radiation Rx	118	91	42	23
Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group	626	535	Radiation Rx (6000 rads/7 wk) (whole brain)	148		40	19
			Radiation Rx plus radiation boost (1000 rad)	105		34	22
			Radiation Rx plus BCNU (for 2 yrs)	165		40	29
			Radiation Rx plus methyl CCNU (for 2 yrs) plus DTIC (imadazole carboxamide)	136		40	26

Also shown in Table 1 is the recent report of an extensive study of 626 patients by the Radiation Therapy Oncology Group and the Eastern Cooperative Oncology Group (6). Again, it is difficult to identify significant therapeutic gain beyond that achieved by radiation therapy. Nevertheless, this study added some very important observations that are critical in the evaluation of therapy studies. Thus, they demonstrated that through examination of the initial histologic characterization of the grade of the tumor by the commonly applied grading criteria of Kernohan, correlative patterns of prognosis could not be identified. Utilizing the criteria of Nelson and Tsukada (7), the diagnostic classification became more consistent. This grading system is:

Nelson and Tsukada Histologic Classification of Astrocytoma (7)

(1) Astrocytoma: Uniform cells with moderate density and rare mitoses.
(2) Astrocytoma with Anaplastic Foci: Multifocal or diffusely cellular with nuclear pleomorphism, increased cell density and increased mitoses. Vascular prominence and no necrosis.
(3) Glioblastoma Multiforme: The features of astrocytoma with anaplastic foci plus one or more foci of coagulation necrosis involving tumor cells.

The relevance of histologic grading in the interpretation of the clinical results is emphasized by their observation that patients with anaplastic astrocytoma had a median survival of 27 months, whereas those with glioblastoma multiforme had a median survival of only 8 months (6). Age also was a very critical parameter in evaluating treatment results. In patients under the age of 40 years, 64% survived 18 months, whereas only 20% of the patients in the 40-60 year age range lived that long. In the over 60-year age group, only 8% survived 18 months. Thus, the need for careful criteria in each aspect of the study is clear. Therefore, the evaluation and interpretation of slightly different, but by no means significantly better, results with high dose radiation (8) or combination programs (9,10,11) are exceedingly difficult.

It should be emphasized that in select subsets of patients with malignant tumors in the CNS, improved survival has been a by-product of our more intensive treatment programs. This has been particularly true for pineal region tumors where the microsurgical approach followed by cytoreductive chemotherapy has placed this heretofore almost incurable group into a frequently curable status (12). Medulloblastoma, a rare adult lesion, is now more effectively managed by our improved radiotherapy and multimodality treatment approaches (13,14,15). Even one rare form of metastatic cancer, choriocarcinoma in women, has had improved survival results with multimodality therapy (16).

CRITERIA FOR THERAPEUTIC RESPONSES IN CNS TUMORS

The major expression of therapeutic efficacy in virtually all of the studies is survival. Since significant functional impairment results from our therapy (Table 2)(17,18,19), alternative criteria for the evaluation of treatment must be included in the analysis.

TABLE 2

CHRONIC SEQUELAE OF CURRENT CNS TREATMENT PROGRAMS

1. Progressive leukoencephalopathy

2. Structural brain changes (computed tomography)
   - Dilatation of ventricles
   - Widening of subarachnoid spaces
   - Altered brain density
   - Intracerebral calcification

3. Neuropsychologic (behavioral) impairment

4. Second malignancies

Two additions to our present use of survival data are of value. First, the performance status of the patient (see Table 3) at the institution of the therapy program is clearly a factor in all studies where it has been analyzed (5,6).

TABLE 3

EVALUATION OF FUNCTIONAL PERFORMANCE STATUS*

Grade	Criteria
0	Fully active, able to carry on all predisease performance without restriction (Karnofsky: 90-100%).
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature; e.g. light house work or office work (Karnofsky: 70-80%).
2	Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours (Karnofsky: 50-60%).
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours (Karnofsky: 30-40%).

* Cancer 52:767-772, 1983.

Since a goal of therapy is a functional survival, the effects of therapy and the function of the patient become important parameters in the measurement of therapeutic efficacy. A broader set of criteria to quantify the goals of therapy have been enunciated by the European Brain Tumor Study Group (EORTC), and these parameters of evaluation (9) are an excellent addition:

1. Prolongation of survival time
2. Rate and length of objective remissions
3. Prolongation of the symptomatic "tumor-free" interval
4. Number of long-term survivors

FACTORS AFFECTING SUCCESS WITH TREATMENT MODALITIES

The primary limitation to surgical success is the remarkable loss of brain mass required to remove most tumors, with formidable resultant morbidity. Nevertheless, the trials of more extensive surgical removal have demonstrated that "debulking" results in a [Gompertzian] shift in the growth curve, allowing for a higher growth fraction with presumed increased sensitivity to multi-modality (radiation, chemotherapy) therapy (20,21). To date, however, the application of more intensive radiation therapy, including a variety of high linear energy methods (e.g. fast neutrons, heavy ions, pi mesons) have not significantly improved therapeutic results. The pattern of reproliferation of malignant gliomas following therapy has clearly demonstrated the biologic importance of the area of brain adjacent to tumor (the so-called BAT), since it appears to be the critical re-proliferating site (22).

Cancer cell heterogeneity has been considered a particularly difficult factor in malignant tumors in the CNS (23). However, cellular heterogeneity in malignant neoplasms has been known since the turn of the century, when morphologic differences among cells within a given tumor were described. Poste (24) has brilliantly reviewed the evidence, repeatedly demonstrated, for remarkable heterogeneity in the expression of a "myriad" of phenotypic properties by tumor cells in both primary and metastatic lesions from the same host. These include such features as karyotype, antigenicity, immunogenicity, biochemical properties, growth behavior, and cellular susceptibility to chemotherapeutic agents. Indeed, such extensive diversity has led Nowell to provide evidence for a proposal that the generation of cell variants is an inevitable and fundamental feature of progressive tumor growth (25). Thus, there appears no absolute basis to ascribe special attributes or specific limitations that affect the therapy of CNS neoplasms.

One special aspect of tumors in the central nervous system is the issue of adequacy of drug delivery to the tumor. Since successful chemotherapy critically requires satisfaction of the steep dose response curve common to malignant neoplasms (26,27), the ability to deliver cytotoxic drugs to tumors in the CNS becomes an important issue. Although a commonly expressed view in the past, well

enunciated by Vick and Bigner (28), has been that the known blood-brain barrier is not a factor in CNS tumors, that thesis no longer has universal acceptance (21,29), and the role of the blood-brain barrier has taken on new and important significance in the examination of therapeutic trials in CNS tumors.

THE BLOOD-BRAIN BARRIER

The blood-brain barrier separates the blood from the brain and cerebrospinal fluid (CSF). The "barrier" is effected by vascular endothelial cells that are connected by so-called "tight junctions", thereby constraining intercellular diffusion. Not all areas in the brain have this type of endothelial lining, and there are regions without such a barrier (Table 4).

TABLE 4

NON-BLOOD-BRAIN BARRIER REGIONS

1.) Ependymal epithelia (lining of cerebral ventricles)
2.) Pineal body
3.) Posterior lobe of pituitary
4.) Area postrema
5.) Median eminence
6.) Wall of optic recess
7.) Eminentia saccularis

These sites, in general, do not have continuous endothelia with tight junctions, but rather have fenestrated endothelia allowing transcapillary exchange of protein and other solutes. These sites are rather special regions that do not share the ordinary functions of the brain. The primary feature of these sites is that they are involved in secretion of hormonal or chemoreceptor moieties. The tight junctions limit solute exchange to the transcellular route. Thus, the permeability and transport characteristics of cells govern ingress of material. As expected, lipid-soluble solutes easily penetrate these [cellular-plasma membrane] barriers, whereas lipid insoluble enter less well. The "barrier" is actually a relative phenomenon, rather than an absolute one, and appears to function as a series of interfaces between brain and blood. Variable patterns of entry apply to the brain and the CSF, and the entry rates to these two compartments are different for a variety of substances.

The ability to manipulate the blood-brain barrier for physiologic studies or clinical intervention developed from early Swedish investigations of permeability of cerebral vessels and attempts to enhance radiologic examination of the central nervous system (30,31,32). Those early observations were developed into a critical analytic physiologic model by Rapoport (33), who recognized that the hypertonic solutions used to "open" the vascular bed in the brain actually functioned to shrink endothelial cells so that the retracted cell membranes induce tension on the tight junctions and widen

("open") them. Rapoport's extensive and brilliant studies examined the potential reversibility of barrier "opening" and the time course of increased permeability and provided evidence that the tight junction widening conformed to a graded (rather than all-or-none) response, depending upon the degree of osmotic shrinkage (33). For example, the threshold for osmotic barrier opening to Evans blue-albumin (mol. wt. 68,500) by carotid perfusion of NaCl is 1.2 osmolal, whereas 0.7 osmolal solutions can permit entry of sucrose or inulin.

THE BLOOD-BRAIN BARRIER (BBB) AS A FACTOR IN CHEMOTHERAPY DELIVERY TO CNS TUMORS

As expressed above, a popular thesis in neuro-oncology has been that the "barrier hypothesis" is unimportant, since the BBB "is not intact in malignant tumors metastatic to the CNS" (28). This view is supported by observations that the vessels inside metastatic cerebral tumors often have a fenestrated and discontinuous endothelium that is characteristic of the vessels of the tissue of the primary tumor (34). Similar fenestrated and gap junctions have also been seen in gliomas (34).

In spite of these studies, the evidence suggests that the actual status of the barrier is far more complex. A working hypothesis is that there may be loss of the BBB in the center of the tumor, but that the tumor periphery has different characteristics. To describe this difference, this area has sometimes been called the "brain around tumor" (BAT). An example was shown by Levin et al (35), in studies of the uptake of methotrexate by intracerebral ependymoblastomas. In the area of normal brain the methotrexate level was 3.4% of the plasma level, whereas in the center of the tumor it was 32% of the plasma level. The methotrexate concentration at the brain-tumor interface was only 13% of the plasma level. In a similar manner, Tator (36), in studies on the uptake and distribution of intravenously administered radiolabeled methotrexate in mice bearing intracerebral implants of ependymoblastoma, observed that almost all the cells in the central mass of the tumor were heavily labeled, whereas cells at the periphery of the mass and those infiltrating into adjacent brain showed scanty labeling.

Shapiro et al, using C^{14}-labeled alpha aminoisobutyric acid (AIB), have reported a great deal of variability in the degree of BBB disruption in experimental brain tumors. They reported a nearly normal BBB in small tumors and in the BAT (37). Similarly, Groothuis et al (38,39) have studied chemically and virally induced CNS tumors in animal models and have found that intravascular peroxidase penetrates these tumors in the CNS in a variable manner. Relative to the question of drug entry, it is of note that when these same tumors are placed in subcutaneous tissue, peroxidase freely penetrates the tumor parenchyma.

Walker (40) provides the view that not only is the BBB intact, but that a "sink effect" may further contribute to the low concentration of drugs at the periphery of the tumor. This expresses the concept that "extra" drug that enters the periphery of the tumor will rapidly diffuse away into drug-free surrounding brain because of a "mild disruption" of the BBB.

The clinical thesis that a drug delivery problem exists in CNS tumors was emphasized by Benjamin et al (41), who described the progressive increase in the size of the brain metastases concomitant with systemic tumor regression following the administration of adriamycin. Posner (42) recently stated that, "....there are several reports in the literature indicating that drugs that appear to be successful in treating systemic metastases are ineffective against the same tumor when it is in the brain."

Whether these observations are the result of only a partially impaired barrier at the periphery of the tumor or are due to the "sink effect," or are a result of both factors, remains to be determined. Nonetheless, it is difficult to explain the responsiveness of metastatic tumors (including metastatic breast carcinoma, oat cell carcinoma, and testicular carcinoma) to systemically administerd chemotherapy and the failure of the neural metastasis to respond to that therapy without invoking drug delivery as part of the explanation.

ISSUES IN THE REGULATION OF DRUG ENTRY INTO THE CENTRAL NERVOUS SYSTEM

It is reasonably evident that the BBB is really a regulatory interface between the blood and the nervous system. The regulatory function of the barrier is superimposed on base line permeability restrictions, and all of these parameters govern the composition of the microenvironment of the neurons, axons, and glia. Ions, proteins and large lipid-insoluble non-electrolytes cross mainly through lateral intercellular spaces and the tight junctions between endothelial cells. Water and most non-electrolytes cross the plasma membranes, although the exact pathways are not certain.

The two main factors determining drug entry from blood into the CNS are molecular weight and lipid solubility. The BBB normally prevents the passage of ionized water-soluble drugs with a molecular weight greater than 180 daltons (43). Most currently available and effective chemotherapeutic agents have molecular weights between 200 and 1200 daltons (i.e. methotrexate, 455; daunorubicin, 544; and cytoxan, 261). Thus, on a molecular weight basis, the passage of many chemotherapeutic agents is impeded by the blood-brain barrier.

Of at least equal importance in drug entry into the CNS is the lipid solubility. There is a linear correlation between the octanol/water coefficient of chemotherapeutic agents and their cerebrovascular permeability. Methotrexate, which has a pH of 4.7

and is 99.8% ionized at a blood pH of 7.4, is very lipid insoluble. As a result, the normal CSF/plasma ratio of this drug is only 0.02 (33).

The entry of macromolecular water soluble nutrients and drugs when the BBB is intact is further regulated by a number of transport mechanisms. The cerebrovascular endothelium can transport (by facilitated, stereospecific, saturable mechanisms) substances that are involved in and capable of serving a regulatory role in brain metabolism.

Methods to Disrupt the Blood-Brain Barrier

Irreversible disruption of the BBB is found in a number of pathological states, such as trauma, tumors, heavy metal poisoning, oxygen deprivation and inflammation (33). In addition, barrier opening and brain edema may occur following a number of insults which alter regulation of cerebral blood flow. For instance, Westergaard (44) demonstrated enhanced barrier permeability to horseradish peroxidase following the acute induction of hypertension with intravenous aramine. Although sometimes reversible, barrier disruption induced by hypertension is often accompanied by pathological changes (i.e., hypertensive hemorrhage).

From a therapeutic point of view, it is quite clear that BBB disruption must be reversible to be useful. The techniques available for the reversible induction of BBB permeability are quite limited. Hypercarbia was shown by Cameron (45) to disrupt the BBB, and this disruption appears to be reversible. MacDonnell et al (46) have demonstrated reversible BBB disruption following intravenous 5-FU administration, but no barrier opening following parenteral administration of other cytotoxic agents such as methotrexate, cyclophosphamide, and vincristine. Unfortunately, we have been unable to reproduce these results. To date, BBB disruption by intracarotid infusion of hypertonic solutions is the best documented and most thoroughly evaluated method available for reversible disruption.

Reversible Osmotic Modification ("Opening") of the Blood-Brain Barrier

In 1970, Rapaport demonstrated that the blood-brain barrier could be "opened" by perfusion through the carotid artery of 2M urea without permanent injury of the animal (47). Subsequent studies demonstrated that reversible osmotic BBB "opening" is a threshold event relative to osmolality and to the duration of the infusion (48). In addition, the barrier remains open for less than an hour (48,49). The rate of osmotic induction has been shown to be exceedingly critical to achieve barrier modification (48,49,50). The reversibility of blood-brain barrier modification has been demonstrated by evaluating the entry of Evans blue-albumin (M.W. 68,000 daltons) sequentially following the induction of barrier

modification with an intracarotid hypertonic mannitol infusion (48,49,50). The primary physiologic sequelae to barrier modification is a transient 1.5% increase in brain water (48,51).

Extensive evaluation of this hypertonic mannitol induced modification of the blood-brain barrier has now been done. The brilliant physiologic observations of Rapoport et al (33,47,48,51) have been serially extended by Neuwelt and coworkers (49,50) to develop a model to examine the role of blood-brain barrier modification as a therapeutic device for the management of neoplasms in the central nervous system (52,53,54,55,56). These studies developed methods and evaluated findings first in a murine model (49,50,59,61,61,63,64, 65,66), and the observations were then extended to a canine model (49,50,52,53,55,57,60) prior to beginning each of the studies in man (54,56,57,58,67,68,69).

OBSERVATIONS RELATED TO BLOOD-BRAIN BARRIER MODIFICATION

Physiologic, pharmacologic, and neuropathologic correlative studies have been possible with the murine and canine models of blood-brain barrier modification. The following describe some of the important findings that have resulted from studies in this system:

Ability to Selectively Deliver Agents to Specific CNS Sites Following Blood-Brain Barrier Modification:

An important aspect of the studies of drug delivery following blood-brain barrier modification is that it allows one to restrict the delivery of drug to those parts of the brain where tumor is present (49,52,55,62,63,69). Our recent development of a procedure to carry out blood-brain barrier modification in the posterior fossa provided very firm evidence that drug delivery could be restricted to highly specific areas of the brain (60). These studies demonstrated that this procedure did not result in altered brain stem function (60), and that the same parameters of effective delivery described above were applicable to the posterior fossa (Figure 1). Finally, it must be emphasized that the ability to enhance safe delivery to the posterior fossa was critical to any future clinical application of the barrier modification method in light of the common involvement of this area with tumors in the CNS.

Development of Evaluable Successful Clinical (Non-Invasive) Parameters of Blood-Brain Barrier "Opening"

The animal studies provided evidence that the application of an osmotic (hypertonic [25%] mannitol) bolus "opened" the blood-brain barrier, since Evans blue (administered I.V. prior to the procedures) left the vascular compartment and stained the brain in the areas of brain exposed to the osmotic change. Evidence that

other moieties also gained access to tissues was shown by brain methotrexate levels 100-fold increased over that in the not modified portion of brain (49,52).

These studies further led to the application of a sensitive, non-invasive correlative parameter of the localization, extent, and degree of barrier opening by means of computed tomography (49,52,55). CT scans permitted a careful mapping of the areas affected (Figure 2), and the use of CT numbers permitted a semi-quantitated analysis of the degree of barrier opening which correlated well with the tissue measurements (e.g. methotrexate levels) in brain (52,53). The application of these methods provided a convenient non-invasive characterization and quantification of the blood-brain barrier modification studies in man (54,56,57).

Characteristics of Cytotoxic Agent Pharmacokinetics After Blood-Brain Barrier Modification:

Methotrexate was chosen as the initial drug for study because excellent tissue methods for its measurement existed, the drug was known to be reasonably tolerated by brain tissue, it did not cause an arachnoiditis, and modest tumor responses had been previously recorded. Barrier modification was shown to be associated with an approximate 100-fold increase in tissue concentration of methotrexate in the area of brain subjected to hyperosmolar mannitol. This represented approximately a 20-fold increase in amount of drug delivered over direct intracarotid infusion (49,55).

Serial measurements of methotrexate (MTX) concentration in the CSF after barrier modification was shown to be an inconsistent and unreliable measure of the actual tissue levels of MTX in brain (55). Tissue measurements of MTX confirmed that the "penetration" or extent of MTX in the brain tissue was to the deepest structures supplied by the vascular bed involved (55).

Problems and Complications of Blood-Brain Barrier Modification in Man

The utilization of blood-brain barrier modification in man has been well tolerated (54,56,57,59). The only notable complication has been seizures following barrier modification. In the first 53 cases studied, seizures occurred 11 times. As described above, the success, pattern, and degree of barrier modification have been monitored by CT scan following the procedure. Since the iodinated contrast agent (meglumine iothalamate) is, as are all such materials, epileptogenic, we evaluated the role of the CT studies as a factor in this complication. Analysis demonstrated that 8 of 20 patients who had seizures following osmotic blood-bain barrier modification had been monitored by CT scan studies. In 7 of these patients, we carried out subsequent barrier modification, but we monitored the effectiveness of the barrier opening by radionuclide scans, and in

only one was a seizure seen and it was focal (56,57,59). Although the radionuclide scan does not provide the sensitivity or spatial resolution of the CT scan, we now use it in any patient with suspected seizure activity.

Effects of Adrenal Cortical Steroids and Osmotic Blood-Brain Barrier Modification on Cytotoxic Agent Delivery to Tumor:

Adrenal cortical steroids are commonly used in patients with brain tumors, since their use can result in a rapid and dramatic effect on the symptom complex present. The steroid response appears to be the result of decreased vasogenic cerebral edema in and around tumors in the brain. Steroids are known to reduce the amount of contrast enhancement on CT scans and decrease the uptake of radionuclide as defined by imaging. Since these effects define "decreased vascular permeability", a consideration related to steroid administration is that it could reduce the delivery of chemotherapeutic agents to malignant lesions in the brain.

Using an avian sarcoma virus (ASV) induced malignant glioma model, studies of the effect of adrenal cortical steroids and osmotic blood-brain barrier modification on the delivery of methotrexate to normal brain and tumor was examined (62). In animals bearing the ASV-induced glioma, barrier modification resulted in significantly increased delivery of methotrexate to the tumor and the brain around tumor (BAT) compared to the non-modified hemisphere or control animals. The administration of adrenal cortical steroids in doses commonly used to manage patients with tumors in the CNS, resulted in a significant decrease in methotrexate delivery to the tumor even when attempted enhancement with barrier modification was performed.

These studies provided two important observations (62). First, the identification of an existent blood-brain barrier in a CNS tumor model was established, and the proof that enhanced cytotoxic drug delivery was achieved by blood-brain barrier modification was documented (62). Second, these studies demonstrate that steroid administration, a common therapeutic approach in tumors in the CNS in man, results in significant interference in cytotoxic drug delivery to tumor even when enhanced delivery methods are utilized (62).

APPLICABILITY OF THE OSMOTIC BLOOD-BRAIN BARRIER MODIFICATION APPROACH IN MAN:

The development of a safe, reproducible, reversible technique for blood-brain barrier modification in the animal models was translated to studies in man. This sequence made it quite simple to define the clinical characteristics and parameters of blood-brain barrier modification in man (54), and demonstrates the procedure to be reversible and safe (56). An early finding in these studies in man was the identification of multiple tumor nodules after barrier

modification in a patient with metastatic carcinoma to the CNS when at least some of the nodules were not seen on CT scan without barrier modification (56). This provided the first evidence in man that at least a partial blood-brain barrier exists in malignant tumors in the CNS.

The studies in man demonstrated that, like the canine pattern, the cerebrospinal fluid concentration of drug was an inconstant and invalid measurement of the effective drug delivery to brain tissue (58). The clinical lesson from that observation is that chemotherapy delivery for carcinomatous or leukemic meningitis is best done by non barrier modified methods (i.e. intrathecal or intraventricular routes). In addition, drug which gained access to brain tissue with barrier modification remained for a longer duration of time than drug which gained access in the absence of barrier modification (58,68).

Clinical Results: Treatment of CNS Lymphoma and Malignant Glioma with Blood-Brain Barrier Modification and Multiagent Chemotherapy:

Clinical studies employing blood-brain barrier modification initially began as a Phase I trial of safety and feasibility. These have now expanded to a Phase II trial of efficacy and randomized clinical trials (i.e. multiagent chemotherapy in absence or with blood-brain barrier modification) are presently beginning.

Table 5 describes a compilation of studies in the Phase II trial. It should be emphasized that, except for three of the patients with CNS lymphoma, all of the patients treated have had a history of initial surgical biopsy (and frequently decompression), post surgical radiation to maximum tolerated doses and, in some, chemotherapy was given, with subsequent recurrent tumor. As shown in Table 5, a clinical response required objective decrease in the tumor size and improved neurologic function. In the patients with glioblastoma who had such a response, clear prolongation of life has been recorded.

TABLE 5

OBJECTIVE RESPONSE IN PATIENTS TREATED WITH COMBINATION CHEMOTHERAPY IN ASSOCIATION WITH OSMOTIC BLOOD-BRAIN BARRIER DISRUPTION

Tumor Type	Number Treated[a]	Responders[b]	Stabilization[c]	No Response[d]
Glioblastoma	10	4	1	5
Primary CNS lymphoma	6	5	1	
Oligodendroglioma	1			1
Medulloblastoma	1	1		
Ependymoblastoma	1	1		
Metastatic lung carcinoid	1		1	
Metastatic breast (adenocarcinoma)	1	1		
TOTALS	21	12	3	6

[a] Patients receiving at least three courses of combination chemotherapy.
[b] Regression in tumor size (50%) as evidenced by CT scan and/or clinical improvement (includes patients who initially responded with subsequent progression of disease).
[c] CT scan unchanged with no clinical deterioration.
[d] Progression in tumor size/clinical deterioration.

The longest such patient is now at 48 months; one at 24 months; one at 20 months; one at 16 months, and the rest over 9 months but not yet at one year.

Since radiation therapy is known to reduce our ability to gain drug entry into the CNS, and since recent clinical review of the cumulative national experience in primary CNS lymphoma managed by radiation therapy documented a mean survival of only 14 months (in the very best series), we have approached primary CNS lymphoma differently. Since the results with radiation therapy are not only dismal but are also associated with decreased mentation, we have treated patients with primary CNS lymphoma with multiagent chemotherapy delivered with barrier modification following diagnosis (and the patients' informed consent). The rationale to this approach in these patients was based on our early evidence that this type of therapy resulted in very dramatic response within three weeks (69) and our knowledge that we could treat response failures with radiation at any later time. Only one patient with primary CNS lymphoma has required a boost to a residual lesion deep in the hindbrain region.

An interesting ancillary finding in the patients with CNS lymphoma who were studied with barrier modification was the evidence of more tumor than had been recognized on the CT scans done without barrier modification (69). Extensive finger-like projections of these tumors characterizes the very much greater involvement of the brain than is clinically anticipated.

The multiagent chemotherapy approach is now being approached on a randomized basis to evaluate the role of blood-brain barrier modification as a factor in tumor response. In addition, new drugs are also being examined in Phase II trials.

EVIDENCE THAT DRUG DELIVERY IS AFFECTED BY BLOOD-BRAIN BARRIER MODIFICATION IN MAN:

Strong clinical support for the thesis that some degree of intact barrier exists in patients with tumors in the CNS is provided by our current observations in three patients, one with carcinoma of the breast metastatic to the CNS, one with glioblastoma, and one with a primary CNS lymphoma (microglioma)(70). All achieved significant tumor regression when chemotherapy was given with osmotic blood-brain barrier "opening" to enhance drug access to the lesion. However, these same patients demonstrated concurrent development of new tumor nodules in portions of the central nervous system distant from regions where osmotic barrier modification was induced.

Case #1

R.L., a 24-year old woman, was referred with a history of right modified radical mastectomy for infiltrative ductal adenocarcinoma (estrogen receptor positive) with no lymph node involvement. She received local radiation. Metastases to the lung were identified nine months post initial diagnosis, and she was treated with chest wall radiation and anti-estrogen (tamoxifen) therapy, followed by oophorectomy. Seventeen months after the mastectomy, a brain metastasis in the cerebellum was documented, and whole head radiation (5000 rads) was administered. This lesion resolved, but five months later a new cranial metastasis was identified in the area of the left quadrageminal plate (Fig. 3A), and she was referred to the Oregon Health Sciences University for chemotherapy in association with barrier modification.

She underwent her first course of chemotherapy in conjunction with barrier modification via the left vertebral artery six months after her cranial radiation. She had marked clinical improvement following her first treatment and steroids were discontinued. She underwent two additional courses of therapy over the next three months, with progressive reductions in her chemotherapy due to myelosuppression (i.e. a 75% reduction of cyclophosphamide and elimination of procarbazine). A CT scan prior to her fourth treatment revealed greater than a 90% reduction in the tumor mass in the tectal region (Fig. 3B), with resultant decompression of the cerebral aqueduct and

a decrease in her ventriculomegaly. Unfortunately, her serial CT scans also showed two new lesions (Fig. 3C): one just lateral to the left lateral ventricle and a second in the right parietal cortex. Despite continued treatment with markedly reduced doses of chemotherapy, her new CNS lesions as well as her systemic disease progressed. Her tectal lesion, however, did not recur. She subsequently expired one year from documentation of her cranial disease. No post mortem examination was permitted.

Case #2

C.F., a 42-year old man, underwent surgical decompression for a right posterior temporal glioblastoma. He was treated with radiation (5040 rads to the entire brain: 6660 rads to the tumor). Following radiation, the patient's CT scan showed a persistent large enhancing lesion that was unchanged from the preoperative studies, although there was less shift of normal structures. Three months after the completion of radiation, the patient had increasing visual symptoms and he required large doses of steroids.

The patient underwent nine barrier modification procedures in association with methotrexate, cytoxan and procarbazine administration over 12 months. Drug induced myelosuppression required some delay between procedures, and the advent of herpes zoster of the left eye also resulted in a temporary cessation of therapy. The patient did have improvement in his symptoms. It was possible to discontinue the dexamethasone, and his papilledema resolved. The CT scan documented a decrease in the mass effect and tumor enhancement (Figs. 4A and B).

Twelve months after initiation of chemotherapy (18 months following surgery), the patient developed left-sided weakness and severe neck pain, a recurrence of the symptoms that led to his initial presentation. A myelogram (Fig. 4C) revealed a block in the lower cervical spine and evidence of other, more caudal, subarachnoid tumor seeding. The cerebrospinal fluid (CSF) myelin basic protein was not elevated. Palliative radiation therapy to his cervical spine was instituted. A CT scan obtained at the time of progression of cervical spine lesion revealed little evidence of mass effect or enhancement, but some increase of low density in the area of his original tumor. He subsequently expired one month following documentation of seeding of his tumor. A request for post-mortem examination was refused.

Case #3

H.F., a 67-year old woman, was admitted to another institution with a three-week history of headaches and progressive hemiparesis (8). A CT scan revealed an enhancing lesion in the right basal ganglia area with marked edema and ventricular shift; biopsy led to the diagnosis of primary CNS lymphoma. She was treated with cranial radiation

(5,000 rads to the entire brain; 6,000 rads to tumor), and subsequent CT scan demonstrated neither mass effect nor tumor enhancement. Her left hemiparesis slowly resolved.

Three months later, her hemiparesis recurred and the CT scan revealed a mass in the opposite (left) cerebral hemisphere with ventricular compression (Fig. 5A). The patient was referred for evaluation. No systemic disease was found. A myelogram was normal; however, the CSF contained large non-cleaved cells consistent with large cell lymphoma.

Blood-brain barrier modification was carried out in the left cerebral region via the internal carotid artery, and chemotherapy was given. The cerebrospinal fluid methotrexate concentration four hours after the infusion (750 mg) was 1.7×10^{-5}M. Three days after treatment the CSF had no malignant cells. A CT scan at four weeks showed an 80-90% decrease in the size of the left cerebral lesion. Unfortunately, this CT also identified a lesion in the right cerebral hemisphere adjacent to the lateral ventricle at the site of original tumor (Fig. 5B). She was then treated with four more courses of chemotherapy, given with barrier modification via the internal carotid arteries performed on alternate sides 24 hours apart. She is presently alert, oriented, and ambulatory with a walker. Her current CT scans reveal no evidence of residual tumor in either cerebral hemisphere.

These observations provide further evidence of an extant blood-brain barrier in malignant tumors in the CNS in man, and suggest that this barrier is a factor in our therapy of such tumors. Since the chemotherapy in these three patients with very different types of tumors resulted in an objective decrease in tumor size, "drug resistance" as the explanation for treatment failures for tumors in the CNS does not appear valid. It seems likely that drug delivery to tumor in these present cases was seriously affected by a partially or completely intact blood-brain barrier.

FUTURE THERAPEUTIC APPROACHES IN CNS NEOPLASMS

The described availability of a significant drug delivery method by blood-brain barrier modification emphasizes the value of some other additions to the clinical approach to neoplasms in the CNS.

CLINICAL APPLICATION OF BIOLOGIC MARKERS FOR CANCER IN THE CEREBROSPINAL FLUID

A variety of moieties (generally polypeptides, glycoproteins or enzymes) are made or released from neoplasms. These, when measurable, in a body fluid are frequently termed "tumor markers" and provide important parameters of tumor activity in a variety of systemic neoplasms. In general, these moieties are either tumor associated products or embryonal (so-called oncofetal) gene

products. Although they are not tumor specific, they provide a valuable clinical tool in diagnosis and the response to therapeutic intervention in a variety of tumors. These markers gain greater specificity and importance when found in cerebrospinal fluid since, unlike serum, they are found in increased levels largely in the presence of tumors. Table 6 denotes the most common and clinically relevant tumor associated moieties that may be found in the cerebrospinal fluid (71,72,73,74).

The utilization of such markers as a serial parameter of therapeutic responsiveness has increased value now that effective therapy exists for some of the tumors in the CNS (75).

Utilization of Hypoxic Cell Radiosensitizers to Enhance Radiation or Chemotherapeutic Effectiveness:

It is well established that the concentration of molecular oxygen is important in the response of cells (in vitro and in vivo) to radiation and to certain chemotherapeutic agents (76). For instance, cells radiated under conditions of hypoxia (less than 10^{-6} mole O_2) require a three-fold increment in dose to achieve some level of cell kill in normally oxygenated cells (less than 30 micromolar O_2)(Aston et al). Hypoxia in focal areas of solid tumors has long been known. The biology of this hypoxic protection of neoplastic cells against the cytotoxic effects of the radiation or chemotherapy has been recognized as a serious limiting factor in therapeutic efficacy (77). The mechanisms of hypoxia appear to relate primarily to cellular density and microvascular bed. The common scenario is that although the therapy kills a substantial proportion of the aerated cells, the resistant hypoxic cells are capable of proliferation and "treatment failure" is recognized. As reviewed above, this failure of local control of tumor is classical for neoplastic lesions in the central nervous system.

TABLE 6
CEREBROSPINAL FLUID MARKERS OF CNS TUMORS

Marker	Biologic Source		CSF Normal Level	Tumor Cell Type	Clinical Observations
	Type	Normal			
Alpha-feto-protein [AFP]	Onco-fetal antigen	Fetal: Yolk sac Liver g.i. tract	0	Primary: Yolk sac tumors Endodermal sinus tumors Pineal germinoma Metastatic: Testicular CA	Excellent clinical parameter
Beta-sub-unit of human chorionic gonado-trophin [beta-HCG]	Onco-fetal antigen	Placenta	< 1.5 I.U.	Pineal germinoma Metastatic: testis choriocarcinoma	Excellent clinical parameter
Carcino-embry-onic antigen [CEA]	Onco-fetal antigen		Not detectable	Leptomeningeal carcinomatosis esp. lung	Of partic-ular value in meta-static CA; esp.to lepto-meninges. Of no val-ue when serum conc.
Poly-amines		Nucleic acid Metabolic products	Putrescine: 298pmol/ml Spermidine 240pmol/ml	Medulloblastoma Glioblastoma	Excellent as marker of recur-rent medullo-blastoma.
Lactic dehydro-genase [LDH]	Enzyme		Isoenzyme 5: < 15%	Leptomeningeal carcinomatosis -Esp. breast, lung, and melanoma	Sensitive marker of lepto-meningeal carcinoma-tosis.
Beta-glucur-onidase	Enzyme		<45 mU/L	Leptomeningeal carcinomatosis -Breast, lung, melanoma	Good as marker of lepto-meningeal disease.
Desmos-terol		Inter-mediate precursor of cholesterol	Not detectable.	Glioblastomas	

The rationale for the development of "hypoxic cell sensitizers" is based upon the evidence that molecular oxygen has the ability to sensitize living cells to radiation (78,79). These radiosensitizers are electron affinic compounds that function as oxygen "mimics". The popular conceptual mechanism for this effect is that ionizing radiation (or certain cytoreductive chemotherapeutic agents) produces free radicals which destroy chemical bonds and produce auto-oxidative chain reactions. These molecular lesions would ordinarily be subjected to cellular repair processes, but such repair is prevented by the available oxygen due to formation of organic peroxides.

A variety of nitrofurans, clinically used as antibacterial and antiprotozoal drugs, have been shown to provide in vitro radiosensitization against hypoxic cells (80). For the agents that were initially examined, the active moiety appeared in the nitro group of the nitroheterocylic structure. Both nitronidazole and misonidazole have been entered into clinical trials (81,82,83). As shown in Table 7, the initial clinical trials have not documented a significant increase in median survival. Nevertheless, this entire family of agents poses a new potential in the exploration of tumor cell kill in CNS neoplasms in man.

The toxicity of these sensitizers is shown in Table 8. The observation that the neurotoxicity to these agents was less (approximately 15%) in patients with tumors in the CNS than other sites led to the identification of dexamethasone as a "rescue" measure to decrease this side effect of therapy (84).

NEW AGENTS AND NEW APPROACHES:

1.) Re-examination of "Old" Drugs:

The present observations pose the requirement that we re-examine the "neuro"-pharmacology of the spectrum of available chemotherapeutic agents. Classically, neurotoxicity and neurologic sequelae have not been a significant problem during the chemotherapy of systemic neoplasms, because most drugs did not gain access to the CNS. The present studies demonstrate the delivery of high concentrations of drug to selective parts of the brain, thereby increasing the likelihood of toxicity. Studies are currently in progress to define the CNS pharmacokinetics and potential neurotoxicity of drugs delivered following blood-brain barrier modification. Parenthetically, it should be emphasized that one concern that compels caution in such studies is the concern that findings identified in the animal model do not accurately translate into events in man.

TABLE 7

CLINICAL TRIALS OF HYPOXIC CELL RADIOSENSITIZER

Tumor Type	Study	No. Patients	Evaluable Patients	Radiation	Agent	Comment
Malignant gliomas (10)	RTOG 78-01	54	35	Whole brain 400R:Mon 150R:T,Th,F x 6 wks +180R boost x 5. Total dose 6000 rads.	Misonidazole: 2.5g/m^2 qwk x 6 = 15g/m^2	Survival equivalent to radiation alone.
Metastases to brain (10)	RTOG 78-12	40	34	600R BIW x 3 wks. = 3600 rads	Misonidazole: 2.0g/m^2 BIW x 3 wks= 12g/m^2	Equal to best previous RTOG results.
Malignant gliomas (7)	Edmonton	61		3 arms: 1.) 6000 rads		median survival 29 wks
				2.) 3890 rads +	Metronidazole 6g/m^2	20 wks
				3.) 3890 rads +	Misonidazole 1.25g/m^2	28 wks
Malignant gliomas (7)	BTSG 77-02	+400		4 arms: Radiotherapy +	BCNU	Median survivals 40-48 wks No difference between arms
				Radiotherapy +	Misonidazole	
				Radiotherapy +	BCNU + Misonidazole	
				Radiotherapy + streptozotocin		
Malignant gliomas (7)	MRC	380		Multiple Rx arms:		No survival differences
Glioblastoma	Yale	19		Rad:4200 rad +	metronidazole	Median survival: 9.4 mos

TABLE 8

HYPOXIC CELL RADIOSENSITIZER (MISONIDAZOLE) TOXICITY

Acute: Gastrointestinal

- nausea & vomiting (approx. 50%)

Neurotoxicity

Grade	Peripheral	Central
1	Objective sensory changes or mild paresthesias or decreased reflexes	Mild lethargy or confusion
2	Moderate paresthesias or pain or detectable weakness or absent reflexes	Moderate lethargy or confusion or seizure
3	Severe paresthesias or pain or severe weakness	Severe lethargy or confusion uncontrolled seizures
4	Paralysis	Coma

2.) Examination and Evaluation of New Agents:

In addition to studies of the entire spectrum of drugs, new agents always represent a hope in oncology. The critical requirement for lipid solubility to penetrate the blood-brain barrier has led to specific attempts to develop such agents. Aziridinylbenzoquinone, commonly called AZQ (2,5-diazeradenyl-3,6-(carboethoxyamino)-1,4-benzoquinone) is the most promising recent agent (85). This agent has sufficient lipid solubility to permit some CNS penetration with the required aqueous solubility to allow formulation for administration. The major toxicity of AZQ in the preliminary studies is myelosuppression. The clinical efficacy of AZQ is still unproven.

3.) Use of Xenograft Model to Evaluate Chemotherapeutic Effectiveness:

The athymic ("nude") mouse has provided an opportunity to study the growth and behavior of human tumor cells in vivo under controlled conditions (86). The preliminary data from such studies indicates that the differential sensitivity to chemotherapeutic agents has a pattern that reflects the known clinical effectiveness of the individual drug; for instance, imadazole carboxamide (DTIC) in malignant melanoma and cyclophosphamide in breast cancer. Since one serious consideration in all of the therapy of brain tumors has been "drug resistance of the tumor", the exploitation of such a model provides an opportunity to evaluate the response of human tumors to drug exposure.

In order to characterize differences in drug delivery from drug sensitivity, we have chosen such a model for the examination of human tumors. Since the size of the nude mouse precludes blood-brain barrier modification studies, we have utilized the athymic rat in our initial studies. The rat model shares with the nude mouse many properties associated with immunologic deficiency, including the acceptance of allografts and xenografts, lack of response of splenic lymphocytes to T-cell mitogens, and enhanced susceptibility to a number of infectious agents. In our early studies, we have examined human tumor explants to the brain and to subcutaneous sites to evaluate differences in drug delivery and tumor cell response. In spite of the transplant nature (and therefore presumed lack of barrier) of the tumor into the CNS, differences in drug response at these two sites appear evident (87).

4.) Evaluation of Monoclonal Antibodies as a Vehicle for Enhanced Drug Delivery with Blood-Brain Barrier Modification:

Utilizing hybridoma technology, John Minna and coworkers at the NIH have developed a panel of monoclonal antibodies, and his interest has particularly focused on those directed against small cell carcinoma of the lung. In conjoint collaboration with Minna, we have begun to evaluate the delivery of tumor specific monoclonal antibodies to human tumors grown in the nude rat as both subcutaneous and intracerebral sites. Since these antibodies are particularly

suitable for labeling with high energy, short range isotopes, the consideration for enhanced and specific targeted drug delivery is posed by this opportunity to "piggyback" the isotope to the antibody. In our preliminary studies (87) of the human small cell carcinoma of the lung grown intracerebrally and subcutaneously, we have examined some of the characteristics of this panel of antibodies and their effects. We have shown that the tumor specific monoclonal antibodies fail to cross the blood-brain barrier in significant quantity in both the normal and the small cell carcinoma of lung-brain tumor bearing animals, even when the antibodies are given by the intra-arterial route. By contrast, excellent delivery was achieved following blood-brain barrier modification, and good tissue binding noted. In addition, unlike the circumstance with methotrexate and a variety of other chemotherapeutic agents, the delivery of these monoclonal antibodies is ten-fold greater when these are administered via the vertebral artery than via the internal carotid artery after blood-brain barrier modification (87). Current studies are in progress to examine the delivery, permeability, localization and binding, and dose response characteristics of these monoclonal antibodies in animals with an intact BBB and in the presence of barrier opening (and simultaneous subcutaneous) model using radiolabeled tumor-specific monoclonal antibody.

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