The blood-brain and blood-tumor barriers: A review of strategies for increasing drug delivery[1]

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Drug delivery to brain tumors has been a controversial subject. Some believe the blood-brain barrier is not important, while others believe it is the major obstacle in treatment and have devised innovative approaches to circumvent it. These approaches can be divided into two categories: those that attempt to increase drug delivery of intravascularly administered drugs by manipulating either the drugs or capillary permeability, and those that attempt to increase drug delivery by local administration. Several strategies have been developed to increase the fraction of intravascular drug reaching the tumor, including intra-arterial administration, barrier disruption, new ways of packaging drugs, and, most recently, inhibiting drug efflux from tumor. When given intravascularly, all drugs have a common drawback: the body acts as a sink, and, even in the best situations, only a small fraction of administered drug actually reaches the tumor. A consequence is that systemic toxicity is usually the dose-limiting factor. When given locally, such as into the cerebrospinal fluid or directly into the tumor, 100% of an administered dose is delivered to the target site. However, local delivery is associated with variable and unpredictable spatial distribution and variation in drug concentration. The major dose-limiting factor of most local delivery methods will be neurotoxicity. The relative advantages and disadvantages of the different methods of circumventing the blood-brain barrier are presented in this review, and special attention is given to convection-enhanced delivery, which has particular promise for the local delivery of large therapeutic agents such as monoclonal antibodies, antisense oligonucleotides, or viral vectors. *Neuro-Oncology 2, 45–59, 2000 (Posted to Neuro-Oncology [serial online], Doc. 99-30, December 14, 1999. URL <neuro-oncology.mc.duke.edu>)*

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[3]Abbreviations used are as follows: AUC, area under the curve; BBB, blood-brain barrier; BTB, blood-tumor barrier; CED, convection-enhanced delivery; CSF, cerebrospinal fluid; HBBBD, hyperosmotic blood-brain barrier disruption; i.a., intra-arterial; K_1, blood-to-tissue transfer constant; k_2, tissue-to-blood efflux constant; k_3, tissue metabolism constant.

Introduction

The delivery of drugs to brain tumors has long been a controversial problem. In 1977, Vick et al. wrote in an editorial: "We believe that there is compelling evidence to suggest that the 'blood-brain barrier,' as it is generally conceived, is not one of the factors impeding the success of brain tumor chemotherapy." They went on to say that "dosage, route of administration, tumor cell uptake, metabolic fate within tumor cells, and the washout or sink effect of the extracellular space and CSF are the issues that will have to be studied" (Vick et al., 1977). Some clinicians have agreed with Vick et al. (Donelli et al., 1992; Stewart, 1994); however, on the whole, the belief that the BBB[3] and BTB prevent drugs from reaching brain tumors in sufficient concentrations to kill the tumor cells has motivated numerous attempts to increase the amount of drug that reaches the tumor. Many innovative methods have been used to try to increase drug delivery including, most recently, a method in which drugs are infused directly into brain tumors, a method referred to as convection-enhanced delivery (CED) (Bobo et al., 1994; Laske et al., 1997a; Lieberman et al., 1995). This review

attempts to place the various methods for increasing drug delivery into context. There is no ideal solution to this problem: each delivery method has advantages and disadvantages. Changes are also occurring in two other arenas that will have profound effects on brain tumor therapy. First, the study of drug effects and mechanisms of action, which is called pharmacodynamics, is evolving (Ali-Osman, 1999). Second, the development of new classes of therapeutic agents, such as monoclonal antibodies, antisense oligonucleotides, and receptor-linked toxins, introduces new conceptual problems in drug delivery because many of these compounds are large, which restricts even more severely entry into brain tumors from the vascular compartment (Jain, 1996, 1998).

There have been numerous recent reviews about increasing drug delivery across the normal BBB (Pardridge, 1998; Rapoport, 1996; Tamai and Tsuji, 1996). However, there are important differences between the normal BBB and the BTB. In this review, I will try to compare the extent to which the normal BBB and the variably abnormal BTB restrict the delivery of bloodborne therapeutic agents to brain tumors. I will then discuss the various methods that are being explored to increase the delivery of intravascular drugs to brain tumors. Finally, I will review alternative methods of drug delivery that completely circumvent the vascular compartment, including CED.

The BBB and BTB Barriers

The normal BBB is a formidable obstacle to the movement of most drugs from the blood into the brain. This is illustrated in Fig. 1, in which the permeability-surface area product for a series of compounds is shown in liver, lung, muscle, brain, and experimental brain tumors. In liver, sinusoidal capillaries exhibit almost no permselectivity (decreasing permeability with increasing molecular size), and as a consequence, drug delivery is little affected by molecular size. Muscle capillaries are continuous but have increased numbers of pinocytotic vesicles and variably competent interendothelial junctions, and as a consequence exhibit permselectivity. Normal brain capillaries are also continuous but have tight interendothelial junctions, few pinocytotic vesicles, and no fenestrations. The principle route by which drugs cross the BBB is by simple diffusion. The result is an astounding 8-log difference in the rate at which an immunoglobulin will cross a liver capillary and the rate at which one will cross a capillary in the brain.

In brain tumors, permeability is a complex topic. There are at least two major variables involved. The first variable concerns the tumor microvessel populations, that is, the BTB. We have recently presented evidence that there may be three distinct microvessel populations in brain tumors (Schlageter et al., 1999). The first consists of continuous, nonfenestrated capillaries like those of normal brain. Examples of brain tumors with this capillary population include experimental ethylnitrosourea-induced gliomas in animals (Blasberg et al., 1983), and in humans, grade 2 astrocytomas and many oligodendrogliomas. These tumors may not enhance with the contrast agents used with CT or MRI. The second microvessel population consists of continuous, fenestrated capillaries. Tumors with these microvessels exhibit increased permeability to small but not to large

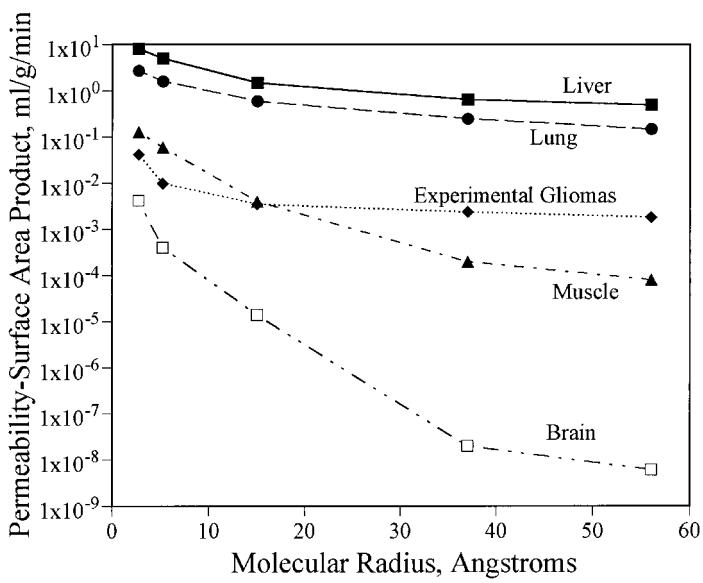

Fig. 1. The relationship between molecular size and capillary permeability. The relationship between the rate of entry (expressed as the permeability-surface area product, with units of ml g^{-1} min^{-1}) and molecular size is shown for five compounds (urea, sucrose, inulin, albumin, and IgG, arranged in increasing molecular size) in liver, lung, muscle, and brain capillaries (Parker et al., 1984; Taylor and Granger, 1984). The curve for experimental gliomas represents a compilation of data from RG-2 (Nakagawa et al., 1987) and D-54 MG gliomas (Blasberg et al., 1987). The capillaries of liver and lung do not exhibit permselectivity, whereas those of muscle and brain do exhibit permselectivity, that is, a decrease in the rate of transcapillary passage as a function of molecular size. The glioma capillaries do not exhibit significant permselectivity; however, the rate of transcapillary passage of albumin and IgG is lower than liver or lung capillaries because of a fewer number of gaps in the endothelial lining.

molecules. To identify this tumor population, permeability studies with two different-sized markers are required. With MRI, Ostrowitzki et al. beautifully illustrated differential permeability of a 9L rat glioma to gadopentetate (molecular mass = 0.5 kDa) and albumin-$(Gd\text{-}DTPA)_{30}$ (molecular mass = 92 kDa) (Ostrowitzki et al., 1998). Because studies in humans are generally not performed with two different-sized permeability markers, the prevalence of this tumor population in humans is not known. The third capillary population, represented in Fig. 1, contains interendothelial gaps that may measure as large as 1 μm; the RG-2 rat glioma (Nakagawa et al., 1987; Schlageter et al., 1999) and D-54 MG human glioma model (Blasberg et al., 1987) are examples. As shown in Fig. 1, these tumor models do not exhibit permselectivity for large molecules. However, Fig. 1 also points out that the permeability-surface area product of the gliomas is 2–3 log units less than that of the liver, which is a consequence of a smaller number of interendothelial gaps in the tumors than in the liver.

The second major variable with regard to capillary permeability involves the spatial distribution of the target capillaries. Although brain tumor capillaries may have increased permeability, like those of the RG-2 and D-54 MG tumors, permeability in brain surrounding tumor rapidly returns to normal brain values within a few millimeters of the tumor margin. If, as shown by Burger (1987), individual tumor cells may reside centimeters away from the edge of a tumor, spatial variability in capillary function will affect drug delivery to all brain tumors.

To understand the effects of changes in capillary permeability on drug delivery, it may be useful to introduce some general concepts. Once injected intravascularly, a drug mixes with the total body volume of blood, or in the case of water-soluble compounds that are not protein-bound, with total body plasma. Because a 70-kg human contains 3 liters of plasma, to achieve a starting plasma concentration of 1 unit of "ideal" drug per ml, 3000 units of drug must be given. As will be shown, this unpleasant fact will color all attempts to increase drug delivery associated with intravascular administration. The human body acts as an enormous sink in which the majority of intravascularly administered drug will be distributed, not to the brain tumor, but to other body tissues. Once mixed with total body plasma, the drug distributes throughout body tissues and is then eliminated. The time course of the mixing, distribution, and elimination of the drug in plasma is generally described by several different half-times, which represent the process of fitting the plasma concentration-time data to a multiexponential expression of the form:

$$C_p(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \qquad \text{(Eq. 1)}$$

where A, B, and C represent the y-intercepts and α, β, and γ represent the time constants, with units of min^{-1}. The time constants are related to the half-times by the expression

$$t_{1/2}^{\alpha} = \frac{\ln 2}{\alpha} \qquad \text{(Eq. 2)}$$

where the half-time has units of minutes. The integrated value of plasma concentration over time, usually referred to as the area under the curve (AUC_{PL}), represents the amount of drug passing through the brain or tumor vessels, that is, the amount of drug to which the brain or tumor vessels are exposed.

The time course of drug concentration over time in brain or brain tumor tissue is more complex. As we have discussed elsewhere (Blasberg and Groothuis, 1986), the concentration of ideal drug in tissue (Ci) (assuming passive transport across the capillary, no plasma or tissue binding, and first order metabolism and inactivation kinetics) is given by

$$Ci(T) = K_1 \int_0^T C_p(t) e^{-(k_2 + k_3)(T - t)} dt \qquad \text{(Eq. 3)}$$

where K_1 is the blood-to-tissue transfer constant (with units of ml g^{-1} min^{-1}), k_2 is the tissue efflux constant (with units of reciprocal time, min^{-1}), and k_3 is a metabolism or inactivation constant, also with units of reciprocal time. Equation 3 can be used to calculate the tissue drug concentration over time, and can be used to explore the impact of different values of K_1, k_2, and k_3 on tissue drug concentrations. Assuming that the drug is passively distributed across the BBB and not removed by other means, then K_1 and k_2 are related by the expression $\lambda = K_1/k_2$, where λ is the equilibrium distribution volume of the drug in the tissue.

It is very useful to have some expression of the efficiency of the drug delivery process, if for no other reason than to compare one experimental methodology for increasing drug delivery with another. Pardridge has used an expression called the pharmacokinetic rule to express the efficiency of drug entry (Pardridge, 1997):

$$\%ID/g = PS \times AUC \qquad \text{(Eq. 4)}$$

in which ID represents the percent of injected dose of drug delivered per gram of tissue, PS is the permeability surface area product (which for most water-soluble compounds is equal to K_1, with units of ml g^{-1} min^{-1}), and AUC is the plasma area under the curve (for which Pardridge uses the units %ID/min/μl). However, this expression does not consider drug efflux from tissue and/or metabolism. In this review, I will use two expressions to indicate the fractional efficiency of the drug delivery process. Both expressions contain a term in the numerator that refers to the concentration-time product of drug in brain or tumor tissue (AUC_B). The first expression, called the local exposure fraction, represents the fraction of drug removed from the blood to which the brain or tumor is exposed, that is, the blood circulating locally within the tissue:

$$\frac{AUC_B}{AUC_{PL}} = \frac{\begin{array}{c}\text{concentration} - \text{time product of drug} \\ \text{per g of brain or tumor tissue}\end{array}}{\begin{array}{c}\text{concentration} - \text{time product of drug} \\ \text{per ml of perfusing plasma}\end{array}} \qquad \text{(Eq. 5)}$$

Equation 5 expresses the fraction of drug removed from the plasma to which the tissue was exposed and is, therefore, an expression of local delivery efficiency. How-

ever, this is but a small fraction of the drug in the entire body. The second expression, called the total exposure fraction, incorporates the total body plasma volume:

$$\frac{AUC_B}{3000 \times AUC_{PL}} = \frac{\begin{array}{c}\text{concentration} - \\ \text{time product of drug per g} \\ \text{of brain or tumor tissue}\end{array}}{\begin{array}{c}\text{concentration} - \\ \text{time product of drug} \\ \text{per ml of perfusing plasma}\end{array}} \quad \text{(Eq. 6)}$$

The AUC_{PL} can be obtained from Equation 1 and AUC_B can be obtained from integrating Equation 3. Equation 6 reminds us that the fraction of drug entering a gram of brain or tumor is but one small fraction of drug circulating in the entire body, and that this route of delivery is inherently inefficient.

Equations 5 and 6 can be used to illustrate drug delivery to normal brain and brain tumor in what may be viewed as "best case" and "worst case" scenarios. The worst case scenario will almost always be represented by water-soluble drugs and the normal BBB, that is, the most restrictive situation. The best case scenario will be represented by lipid-soluble drugs and highly permeable tumors, such as those represented in Fig. 1 by the RG-2 and D-54 MG gliomas. In Table 1, the local and total exposure fractions are presented for four drugs with different permeabilities: methotrexate (K_1 = 0.0014), 5-fluorouracil (K_1 = 0.0096), aziridinylbenzoquinone (K_1 = 0.145), and 1,3-bis(2-chloroethyl)-1-nitrosourea (K_1 = 0.6 ml g^{-1} min^{-1}), and for two permeability scenarios, that of normal brain and that where permeability has increased 0.1 ml g^{-1} min^{-1} over normal brain. The data for the calculations were contained in a previous publication (Blasberg and Groothuis, 1986); the reader should be aware that this type of modeling is dependent on assumptions made about the different parameters. Nonetheless, Table 1 can be used to illustrate several different points. First, in the worst case scenario with tumor microvessel function like the normal BBB and a highly water-soluble drug (methotrexate), about 1% of the drug to which the tumor is exposed will enter the tumor. With a lipid-soluble drug like aziridinylbenzoquinone and normal BBB function, the local exposure fraction increases to 0.7; in other words, increasing lipid solubility does increase delivery to brain tissue. When tumor microvessel permeability increases by 0.1 ml g^{-1} min^{-1}, the fraction of methotrexate to which the tumor is exposed increases to 18%. A local increase in tumor permeability results in a potentially significant increase in drug delivery for a water-soluble drug. However, note that although the fractional removal across normal BBB is high for the lipid-soluble drugs aziridinylbenzoquinone and 1,3-bis(2-chloroethyl)-1-nitrosourea, the local exposure fraction does not increase significantly when permeability is increased (the increase is only 1–2 percentage points; Table 1). Increased drug delivery as a result of increases in capillary permeability will be more important for water-soluble drugs than for lipid-soluble drugs, for which permeability was not as great an issue in the first place.

It is important to remember that the rest of the body represents a large sink into which most of the drug is distributed. The fraction of drug entering tumor, compared with that circulating through the entire body, is miniscule in every case: <1% of circulating drug will reach the tumor, regardless of the drug's permeability and regardless of changes in capillary permeability (Table 1). Therefore, any method that is used to increase brain tumor permeability will still have to deal with the reality that most of the administered drug is distributed to the rest of the body, and so far as treatment of the brain tumor is concerned, wasted. In most instances, the limit on the total dose of drug that can be given is imposed by the tolerance of normal tissues, which is systemic toxicity in the case of drugs given systemically. Because the maximum dose that can be given to a patient is determined by factors external to treatment of the tumor (that is, systemic toxicity), we must accept as a starting principle that the administered drug dose cannot be increased, and we must search for alternative methods to increase the fraction of drug that reaches the tumor.

Table 1. Fractional delivery of four different chemotherapeutic agents across the BBB and BTB

	Permeability of normal brain		Permeability increase = 0.1 ml g^{-1} min^{-1}	
Drug	Local exposure fraction	Total exposure fraction	Local exposure fraction	Total exposure fraction
MTX	0.017[a]	0.00006[a]	0.18	0.00006
5-FU	0.11	0.00004	0.25	0.00008
AZQ	0.70	0.0002	0.72[b]	0.0002[b]
BCNU	0.54	0.0002	0.55[b]	0.0002[b]

Abbreviations: MTX, methotrexate; 5-FU, 5-fluorouracil; AZQ, azridinylbenzoquinone; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

Four drugs with different rates of brain entry, brain efflux, and plasma half-lives are shown. For each drug, the values represent the fraction of drug extracted by the tumor. The local exposure fraction represents the fraction of drug removed by 1 g of tumor from the plasma to which it was exposed (Equation 5). The total exposure fraction represents the fraction of drug removed by 1 g of tumor from the entire circulating volume of drug in plasma (Equation 6).

[a]The "worst case" scenario occurs when water-soluble drugs are crossing the normal BBB.

[b]The "best case" scenario occurs when lipid-soluble drugs cross capillaries in brain tumors with increased permeability. However, the two columns labeled total exposure fraction emphasize that the amount of drug removed by 1 g of tumor is very small compared with the total drug circulating in the body and indicates the magnitude of the sink effect of the rest of the body in brain tumor chemotherapy.

Table 2. Comparison of different methods of increasing delivery of intravascularly administered compounds to brain tumors

Method of increasing delivery	Principle advantages	Principle disadvantages	Limiting factor(s)
Chemical modification of existing drugs	Requirements are now understood for different delivery routes	Each new compound must be evaluated individually	BBB, BTB permeability, systemic toxicity
Prodrug	Utilizes inherent biochemical pathways in brain and tumor	Difference between BBB and BTB is not known	BBB, BTB permeability, systemic toxicity
Intra-arterial	First pass increase in AUC	Invasive, small increase in AUC_{BT}	BBB, BTB permeability, systemic toxicity
Hyperosmotic disruption	First pass increase in AUC and increased permeability	Invasive, differential effect on brain and tumor, short-lived	BBB, BTB permeability, systemic toxicity
Chemical modification	First pass increase in AUC and increased permeability	Invasive, no effect on brain, short-lived	BBB, BTB permeability
Receptor-mediated transport	Increased permeability	Low capacity system	BBB, BTB permeability
Inhibiting drug efflux	Decreased efflux, may be used with existing drugs	None known	None known
Avant-garde methods	Not yet known	Not yet known	Not yet known

Abbreviations: BBB, blood-brain barrier; BTB, blood-tumor barrier; AUC, area under the curve.

This table lists the different approaches that are available to increase the amount of drug in a brain tumor in cases where the drug is given either by i.v. or i.a. injection. The advantages and disadvantages are an expression of the author's opinion about the most prominent feature of each method. Details about each method may be found in the text.

Increasing Permeability of the BBB and BTB to Drugs Given Intravascularly

Table 2 summarizes many of the methods that are currently being used to manipulate the permeability of the BBB and BTB, as well as my own interpretation of their advantages and disadvantages. In light of the previous discussion, all of these methods share a major disadvantage: the bulk of administered drug will be lost to the rest of the body in a sink effect, and the maximum administered dose will almost always be determined by systemic toxicity.

Chemical Modification of Existing Drugs

Chemical modification of existing drugs may refer to several different approaches. First, an existing drug may be modified to make it fit a receptor in the BBB. This approach has been used with melphalan, in which nitrogen mustard (mechlorethamine) was linked to phenylalanine (Groothuis et al., 1992). Chemical modification may refer to linking an active protein (for example, growth factor) or a peptide to an antibody against a BBB receptor (Pardridge, 1995). More commonly, chemical modification refers to the process of making an existing drug more lipid soluble, with the intent of increasing BBB permeability. This approach has been used extensively with antiretroviral nucleoside analogs for treating AIDS, but has not been used much with brain tumor chemotherapeutic drugs. The use of increasingly accurate means to predict physicochemical properties offers encouragement that chemical modification will find wider use in modifying brain tumor drugs (van de Waterbeemd et al., 1998).

Prodrug Therapy

This refers to a special class of chemical modification in which a drug is chemically modified to increase its capillary permeability. Once in the brain, the prodrug undergoes an enzymatic reaction that returns the drug to its active state and with reduced BBB permeability (Sherman et al., 1991; Yoshikawa et al., 1999). These approaches, which have not yet been used for brain tumor chemotherapeutic drugs, offer an advantage by increasing K_1 in Equation 3 while k_2 and k_3 remain unchanged. This would increase the AUC_B, while AUC_{PL} remains unchanged. Although this increases the fraction of drug entering the tissue, it does not change total body exposure, and unfortunately, introduces the need to explore the pharmacokinetics and pharmacodynamics of each new compound.

Intra-Arterial Administration

This method of administration refers to the i.a. injection of a therapeutic agent, without the concomitant use of a barrier-modifying approach. The principle advantage of this method is that during the course of injecting a drug into an artery, the tissue perfused by that artery receives a higher plasma concentration during the first passage through the circulation (AUC_{PL} is increased). In a series of papers, Fenstermacher and coworkers theoretically evaluated the efficacy of intra-arterial delivery to the brain and brain tumors (Cowles and Fenstermacher, 1974; Eckman et al., 1974; Fenstermacher and Cowles, 1977; Fenstermacher and Gazendam, 1981). They evaluated the contributions of several variables and concluded that there were significant delivery advantages to i.a. administration in a setting where the rate of tumor blood flow is very low or where the rate of systemic transformation or excretion is very high, or in a unique situation where, having crossed the BBB or BTB, the drug binds to the tissue. Once the drug passes through the tumor microvasculature, it enters the systemic circulation and the pharmacokinetics are the same as for an i.v. administration. Thus, the ideal drug for i.a. administration is one

that rapidly crosses the BBB or BTB and is either bound to tissue elements or locally metabolized in the process of exerting its anticancer effect. There are practical problems associated with i.a. administration, however, including the need for the tumor to reside within the arterial distribution being infused and hemodynamic streaming of the administered drug. Perhaps the most obvious evidence that increased tissue drug concentrations can be achieved with i.a. administration is the increased incidence of local toxicity (Arafat et al., 1999). Nevertheless, because most drugs being used to treat brain tumors do not have the ideal qualities to take advantage of i.a. delivery, the results of clinical trials of i.a. chemotherapy in brain tumors show minimal, if any, improvement in survival (Dropcho et al., 1998; Gundersen et al., 1998; Hirano et al., 1998).

Hyperosmolar Blood-Brain Barrier Disruption (HBBBD)

This method involves the infusion of a hyperosmolar solution (usually 1.4 M mannitol) into a cerebral artery, generally followed by the intra-arterial administration of a drug. The principle features of this method are increased capillary permeability (caused by the hyperosmolar solution), followed by a first pass advantage from i.a. infusion: that is, K_1 is transiently increased, as is AUC_{PL}. This method was originally proposed by Rapoport (Rapoport and Thompson, 1973) and has been used extensively by Neuwelt and colleagues (Kroll and Neuwelt, 1998; Neuwelt and Dahlborg, 1989; Neuwelt et al., 1980; Neuwelt et al., 1983). Controversy about the use of this method arose because of differential susceptibility of the normal BBB as compared with the abnormal BTB. Whereas all studies demonstrated increased permeability of the normal BBB, some studies in animal tumors failed to demonstrate any increase in tumor permeability (Nakagawa et al., 1984); others showed transient permeability increases (Groothuis et al., 1990), and some reported significant permeability increases (Neuwelt et al., 1984). Most recently, Zunkeler et al. used PET to study the time course of BBB function in 13 patients with malignant astrocytomas after HBBBD (Zunkeler et al., 1996). They confirmed the differential effect of HBBBD: permeability was increased 1000% in brain and 60% in tumors. The half-time of the osmotic effect was 8.1 min in brain and 4.2 min in tumor. They modeled the effects of HBBBD on methotrexate, and concluded that concentrations above 1 μM, the minimal concentration required for an effect from methotrexate, would not be enhanced in tumor and would be enhanced only 10% in brain. Thus, HBBBD in combination with i.a. administration would be most useful for drugs that, once having crossed the BBB, bind to brain or tumor tissue and do not efflux back across the BBB. In addition, the differential effect of HBBBD on normal brain compared with that of tumor must be considered when evaluating neurotoxicity.

Chemical Modification of the BBB and BTB

The latest entry into the therapeutic arena is the use of chemical agents to disrupt the BBB (Black, 1995). The agents are mostly derivatives of normal vasoactive compounds and include bradykinin (Inamura and Black, 1994), interleukin-2 (Gutman et al., 1996), leukotriene C_4 (Black and Chio, 1992), and others (de Vries et al., 1996). The principle feature of these drugs is that they are given i.a. and followed by an i.a. injection of a therapeutic agent. RMP-7 is a commercially developed bradykinin analog that has reached clinical trials (Ford et al., 1998) and acts by increasing permeability at the intercellular junction (Sanovick et al., 1995). Studies with PET in 9 malignant gliomas have shown that permeability to ^{68}Ga-EDTA was increased 46 ± 42% in tumors, without a significant increase in tumor-free brain (Black et al., 1997). Another report suggests that RMP-7 can increase delivery across the normal BBB (Emerich et al., 1998). RMP-7 is generally administered over a 15-min period; the timing of administration of the chemotherapeutic drug relative to the RMP-7 has varied, e.g., as a bolus after RMP-7 or alternating chemotherapeutic drug administration with RMP-7. BBB function is restored rapidly after the RMP-7 infusion ends; continuing the infusion for over 20 min results in tachyphylaxis, with loss of responsiveness (Bartus, 1999). As with i.a. and HBBBD administration, the advantage with chemical modification of the BBB is limited to a fractional increase in the circulating drug to which the tumor is exposed, and still represents a vanishingly small fraction of the total body drug exposure. Like those previous techniques, chemical modification of the BBB will be ideal for a drug with defined properties, as discussed before. However, this ideal drug has yet to be developed.

Receptor-Mediated Transport

This is a very broad area because any receptor-mediated transport system in the BBB can be selected as a target. The considerations about drug delivery in a facilitated transport system are different from those involving simple diffusion. First, a facilitated transport system can be characterized by the Michaelis-Menten constants, K_m (the concentration at which the reaction velocity is half maximal), and V_{max} (the limiting velocity as the concentration approaches infinity). An important factor in the facilitated transport of drugs is the plasma concentration of the native substrate for the receptor. For example, phenylalanine crosses the BBB via the large neutral amino acid transport (LNAA) system; melphalan is a chemotherapeutic drug with a phenylalanine backbone and could therefore be transported by the LNAA system. However, the normal plasma levels of phenylalanine compete with melphalan to such a degree that the rate of melphalan transport into experimental gliomas is not increased (Groothuis et al., 1992). Lowering plasma phenylalanine levels increased the rate of melphalan entry into brain tumors, but only by a modest 8%. Most of the available transport receptors are low affinity, low capacity systems, such as those for the nucleosides, various peptides, transferrin, and insulin (Ermisch et al., 1993; Pardridge et al., 1995; Friden et al., 1996; Tamai et al., 1997). Although these transport systems have been used to increase brain delivery of oligonucleotides (Boado et al., 1998; Normand-Sdiqui and Akhtar, 1998),

nerve growth factor (Friden et al., 1993), and vasoactive intestinal peptide (Bickel et al., 1993), none of these transport systems has been explored to any extent in brain tumors.

Inhibiting Drug Efflux

Throughout most of the history of the BBB, movement across the capillary for most compounds without a transport system has been assumed to occur by simple diffusion: the process is passive and the rate that a compound crosses the barrier is the same in both directions, so that the process is symmetrical. Recent studies have begun to challenge this idea. In particular, P-glycoprotein represents an ATP-dependent efflux pathway that confers resistance to cancer cells by allowing them to move a variety of chemotherapeutic drugs out of the cell against a concentration gradient (Schinkel, 1999; Tsuji and Tamai, 1997). P-glycoprotein appears to be localized primarily to the luminal capillary membrane, and the number of drugs that are transported by this system may be quite large (Schinkel, 1999). Immunohistochemically, P-glycoprotein has been demonstrated in malignant glioma tumor cells (Leweke et al., 1998; von Bossanyi et al., 1997). Leweke et al. commented that tumor endothelial cells stained in 20 of 21 cases, and von Bossanyi et al. stated that tumor blood vessels were positive in 60% of tumors. PET has been used to demonstrate the reversal of P-glycoprotein pump function in mdr1a knock-out mice with cyclosporin A (Hendrikse et al., 1998). Since many chemotherapeutic drugs are transported by P-glycoprotein, this represents a logical and exciting target in brain tumor chemotherapy, especially because inhibition of P-glycoprotein function could occur at both the BTB and in tumor cells. Inhibiting P-glycoprotein would increase both extracellular and intracellular drug concentration (by decreasing k_2 in Equation 3), without increasing the amount of drug administered. This is an avenue of modifying brain tumor therapy that must be explored.

Avant-Garde Methods of Delivery

Included in this category are the newest methods for intravascular delivery, including the use of biodegradable nanoparticles (Schroeder et al., 1998; Song et al., 1997), liposomes (Allen and Moase, 1996; Sakamoto and Ido, 1993), monoglyceride-based systems (Chang et al., 1994), and magnetic microspheres (Chang and Bodmeier, 1997). Many of these novel approaches are evaluating different methods of giving drug-laden molecules that will preferentially accumulate in a tissue, usually combined with selective methods of perfusion, such as i.a. delivery. For example, Pulfer and Gallo delivered 1–2 μm i.a. magnetic aminodextran microspheres to RG-2 rat brain tumors and used a magnetic field to retain the particles within the tumor. They showed that this method resulted in significant accumulation of the particles as a result of surface charge and magnetic field (Chang and Bodmeier, 1997). These methods are too new to be applied to human brain tumors, but are representative of the diverse and novel directions that investigators are using to increase brain tumor delivery.

Summary of Intravascular Drug Delivery

A tremendous amount of progress has been made in the past few years in our understanding of drug delivery to brain tumors. This progress has not yet translated into impressive increases in survival of patients with brain tumors. There are major areas that remain to be explored and developed in the arena of intravascularly administered drugs. For example, because i.a. administration (with or without HBBBD or chemical modification) offers a unique first pass advantage, a search for drugs that have high lipid solubility and bind to brain tumor targets is likely to be profitable. Second, increasing tissue drug concentration by the use of efflux inhibitors is obvious: there will be no increase in total body exposure while potentially achieving significant increases in tumor drug concentration. However, the ultimate limiting factor for all intravascularly administered drugs is that of the potential toxicity from total body exposure. It seems unlikely that this important, and unfortunately, negative variable can be overcome.

Injection or Infusion of Drugs Directly into the Brain or Its Cavities

Table 3 summarizes the different approaches that are being used to administer drugs directly into the brain, thus bypassing the BBB. All of these routes and methods of administration drastically change the pharmacokinetic landscape. Instead of concern about crossing the formidable BBB or BTB, the drugs are administered directly into the CSF or brain extracellular space. Instead of needing to measure the influx rate, it is necessary to understand drug movement within the brain and to understand efflux mechanisms. In marked contrast to intravascular administration, 100% of the administered dose can be delivered using these methods of administration. The problem then becomes one of understanding the forces that control the movement of the drug within brain and tumor tissue. One or more of four forces will affect drug movement depending upon the particular mode of drug administration: bulk flow of CSF, bulk flow of brain interstitial fluid, bulk flow due to the infusion of a solution into the brain or CSF, and diffusion. Drug movement will also be variably affected by the amount of white matter in the target area as a result of the change from the highly tortuous environment of a gray matter structure (isotropic) to the highly oriented fiber pathways of white matter (anisotropic).

Intrathecal and Intraventricular Drug Administration

In this review, intrathecal drug administration refers to the administration of drug into the subarachnoid space, usually into the lumbar subarachnoid space, whereas intraventricular administration refers to injection or infusion into the lateral ventricle. They are discussed together because of the large number of features they have in common. The principle feature of drug administration by these routes is dominated by the bulk fluid flow of the

Table 3. Comparison of different methods for local drug delivery to brain tumors

Method of circumventing the BBB	Principle advantages	Principle disadvantages	Limiting factor(s)
Intrathecal	Easy access; ideal for therapy of meningeal disease	Not useful for parenchymal disease	Bulk flow rate of CSF
Intraventricular administration	Easy access; ideal for therapy of meningeal disease	Not useful for parenchymal disease	Bulk flow rate of CSF
Intratumoral injection	User control over administered dose; 100% reaches target	Invasive; distribution is diffusional	Unpredictable distribution; neurotoxicity
Intracavitary injection	User control over administered dose; 100% reaches target	Invasive; distribution is diffusional	Unpredictable distribution; neurotoxicity
Microdialysis	User control over administered dose; 100% reaches target	Invasive; distribution is diffusional	Unpredictable distribution; neurotoxicity
Biodegradable polymers	User control over administered dose; 100% reaches target	Invasive; distribution is diffusional	Unpredictable distribution; neurotoxicity
Convection-enhanced delivery	User control over administered dose; 100% reaches target	Invasive	Unpredictable distribution; neurotoxicity

Abbreviations: BBB, blood-brain barrier; CSF, cerebrospinal fluid.

This table lists the different approaches that are available to deliver therapeutic drugs and agents by local administration. The advantages and disadvantages are an expression of the author's opinion about the most prominent feature of each method. Details about each method may be found in the text.

CSF. The major source of CSF is from the choroid plexuses of the lateral and 4th ventricles; as much as another 30% may originate from the brain extracellular fluid. The direction of the flow is from the lateral ventricles into the 3rd and then 4th ventricles, out through the foramina of Luschke and Magendie, into the basal cisterns, around the convexities, and out of the subarachnoid space through the arachnoid granulations. In rats, when ^{14}C-sucrose was injected into the lateral ventricle, the sucrose distributed rapidly: within 5 min it was found in the basal cisterns, and by 1 h the sucrose had largely left the subarachnoid space (Ghersi-Egea et al., 1996). When the injected agent is metabolically active, it may enter selected cells (Rubertone et al., 1993). In humans, the normal volume of CSF produced is between 400 and 500 ml per day. Since the CSF spaces within the brain are about 150 ml in volume, there is a considerable bulk flow within this system, although the velocity varies tremendously from site to site. When isotopes are injected into the lumbar subarachnoid space, they travel up the spinal subarachnoid space and are found in the basal cisterns by 3 h. By 24 h they are found over the cerebral convexities. CSF flow from the lateral ventricles is directional; isotopes injected into the lumbar CSF do not normally enter the lateral ventricles. As a result of these bulk flow pathways, materials injected into the CSF will attain initial concentrations that are directly proportional to the concentration in the infusate. This route of administration is ideal for situations in which the target is within the subarachnoid space (for example, carcinomatous meningitis), or in which the target is close to the CSF brain interface. Carcinomatous meningitis has long been treated with intrathecal infusions, including the more recent use of monoclonal antibodies (Brown et al., 1996). However, the capacity of drugs to enter the brain extracellular space from the CSF is limited. Numerous studies have shown that entry into the brain is diffusional and that concentrations decline exponentially from the brain surface (Blasberg, 1977; Blasberg et al., 1977; Groothuis and Levy, 1997; Patlak and Fenstermacher, 1975). This route of administration is both impractical and inefficient for intraparenchymal brain tumors.

Intratumoral and Intracavitary Injection

These are discussed together and briefly because they are both limiting cases of convection-enhanced delivery, which is discussed below. These both represent local forms of drug administration in which the distribution will be determined initially by the rate and duration of the injection, and secondly by tissue bulk flow pathways and diffusion. The history of intratumoral injection was discussed by Tomita (1991), who recognized the potential usefulness of this approach as well as the importance of neurotoxicity as the limiting factor. Intratumoral and intracavitary injections are being widely explored as means of delivering large agents, such as viral vectors, growth factors, and cells (Bigner et al., 1998; Farkkila et al., 1994; Hsiao et al., 1997; Puri et al., 1996; Ram et al., 1997; Yang et al., 1997). All of the attributes discussed in the section on CED will also apply to these forms of drug delivery.

Microdialysis

Microdialysis is a method that employs the passive diffusion of a drug across a semipermeable membrane (de Lange et al., 1997; de Lange et al., 1999; Parsons and Justice, 1994). It can be used to both sample and deliver drugs to the surrounding tissue and has been used to sample extracellular drug concentrations in experimental brain tumors (Devineni et al., 1996; Nakashima et al., 1997). The principle features of microdialysis, when used as a drug delivery methodology, are that the tissue concentrations are a function of the drug concentration in the dialysate (which places control of the concentration in hands of the user) and that distribution of drug away from the dialysis catheter occurs by diffusion (Dykstra et

al., 1992). However, the normal BBB is disrupted immediately around the catheter, and stays disrupted for some time after catheter insertion, making drug efflux in this vicinity an additional problem when trying to understand local delivery kinetics (Groothuis et al., 1998; Morgan et al., 1996; Westergren et al., 1995). Because the principle factors in local tissue concentrations are diffusion and/or local brain bulk flow patterns, the volume of brain reached by microdialysis will be relatively small, and drug concentrations will, in general, decline exponentially away from the dialysis cannula. This method will be applicable when the volume of tissue that needs to be reached is small. The effect of normal brain bulk flow pathways on this delivery method remains to be studied.

Biodegradable Polymers

This represents a local delivery system in which biodegradable polymers are loaded with drug and implanted into brain or tumor tissue, resulting in timed-release of drug (Cao and Shoichet, 1999; Menei et al., 1997). The principle features of this system are that multiple polymer pellets or wafers can be implanted, which gives control over the spatial distribution, and that drug distribution away from the source occurs by diffusion, with a variable contribution from the inherent bulk flow pathways of the brain (Fung et al., 1998). This approach has been used in patients with malignant gliomas, where its safety and efficacy have been demonstrated (Brem et al., 1995; Olivi and Brem, 1994). As with microdialysis, the principle limiting feature of this method of drug delivery is that drug distribution occurs mainly by diffusion, in which drug concentrations fall exponentially away from the source and are generally limited to a few millimeters of tissue penetration (Fung et al., 1998).

Convection-Enhanced Delivery

This is a relatively new method of drug delivery to brain tumors. The principle feature of this delivery method is that a drug solution is infused directly into the brain. The concentration of the drug is independent of the infusion rate and duration, and the distribution of the drug is a function of the hydrostatic pressure of the infusion, as well as diffusion into surrounding tissue. From a delivery perspective, the only limiting factor to the drug concentration that can be delivered is the solubility of the drug. CED has been discussed from a theoretical perspective (Morrison et al., 1994), it has been explored in several animal models (Bobo et al., 1994; Groothuis et al., 1999; Laske et al., 1997a; Lieberman et al., 1995; Viola et al., 1995), and it has been used for delivery of a targeted toxin in human gliomas (Laske et al., 1997b). The distribution of the infused drug is highly dependent upon whether the drug is infused into a homogeneous gray matter structure or a structure containing fiber pathways. In the case of the infusion of a drug-containing solution into a homogeneous brain structure, the infusion initially produces a spherical volume in which the concentration of the drug is directly proportional to that of the infusate (Fig. 2b). After the infusion reaches equilibrium, at which point the amount of drug leaving across brain capillaries is equal to the amount being infused, the drug begins to diffuse away from the edge of the sphere, at concentrations that decline exponentially in accordance with the mathematics of diffusion (Fig. 2b). If the infusion encounters an organized white matter pathway, the rate of movement is either increased or decreased (depending upon the direction of the inherent bulk flow within that pathway) (Fig. 2c). Morrison stated that when the infusion is being made into a homogeneous isotropic medium, the radius of the central spherical component—the convective component—can be related to the infusion rate and the efflux constant of the drug being infused:

$$r_p = \sqrt[3]{2q_v/(4\pi K)} \qquad \text{(Eq. 7)}$$

where q_v is the infusion rate and K represents the steady-state efflux rate constant. Fig. 3 shows a plot of the radius of the convective component as a function of infusion rate and efflux constant. From reviewing Fig. 3,

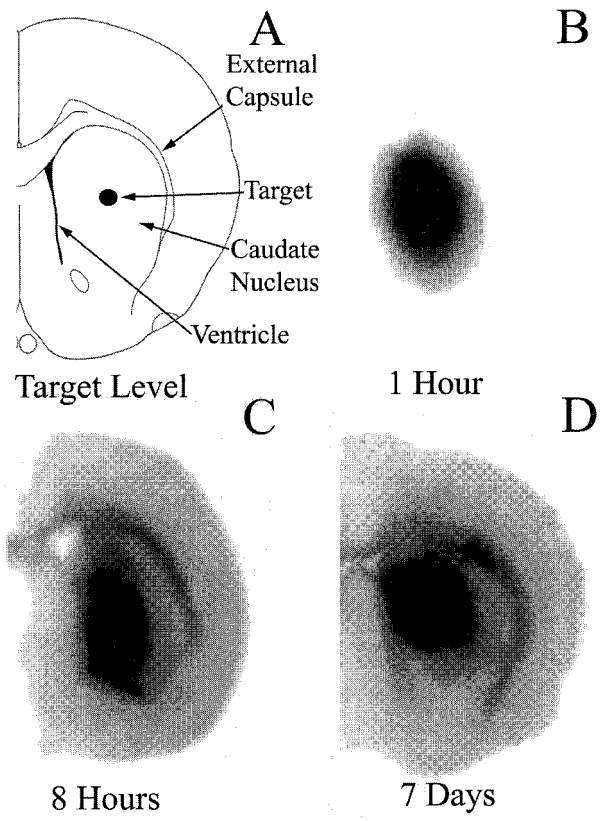

Fig. 2. The distribution of ^{14}C-sucrose in rat brain by convection-enhanced delivery. The diagram in the upper left (A) shows the target location (center of the caudate nucleus) as well as the organization of the brain at the target level. After a 1-h 0.5-μl/min infusion, the isotope is found in a spherical shape with even distribution across (B). By 8 h the isotope is moving into the external capsule (C). A similar appearance is found after a constant infusion over 7 days; the central convective component is clearly visible, surrounded by decreasing concentration from the edge (D). Note the isotope's rapid lateral movement down the external capsule (probably representing a normal brain bulk fluid pathway) but not into the corpus callosum. Infusion into rat brain for 7 days produced no neuropathologic changes other than those associated with catheter insertion.

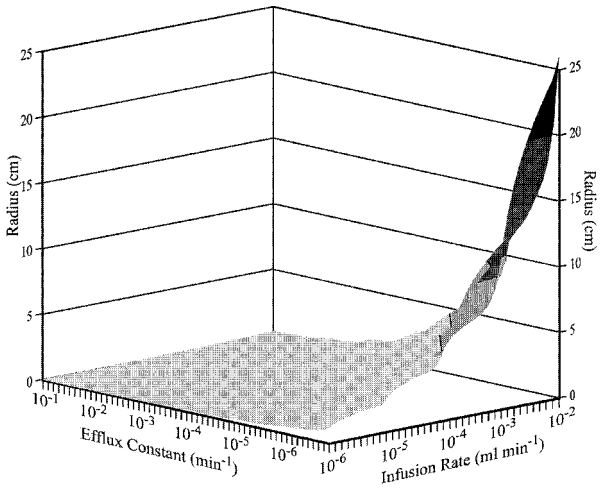

Fig. 3. Relationship between radius of convective CED component, infusion rate, and efflux rate. The radius of the inflection point between the convective and diffusional components of CED infusions is shown for infusion into a homogeneous brain structure (Equation 7). The values on the vertical axis represent the radius inside which the local concentrations remain uniform and outside of which drug concentrations decline exponentially. Changes for each 5-cm increment are represented by different shades of gray. The infusion rate was chosen from 1×10^{-6} to 1×10^{-2} ml per min^{-1}. The lower values are those seen with infusions from osmotic mini-pumps, while the higher values represent the upper limit of CED infusion rates in dogs. The efflux rate was chosen to vary from 1×10^{-1} to 1×10^{-7} min^{-1}. The volume of the convective component increases as a function of both increasing infusion rate and decreasing efflux rate.

which extends from infusion rates provided by osmotic mini-pumps (1 µl/h) to high infusion rates (10 µl/min), it is apparent that the volume of affected brain remains quite small for most infusion rates. Similarly, as the efflux constant decreases, indicating slower rates of crossing the BBB, the convection volume increases. Fig. 3 illustrates that CED is best suited for delivery of large compounds (with low efflux rates) and delivery at moderately high infusion rates, if the intent is to reach large brain volumes.

The shape of the volume of distribution at steady state will be highly dependent on the location in the brain that is being infused. Fig. 4 shows a CT scan of a dog brain receiving an infusion of iodinated contrast into white matter. The iodinated contrast rapidly distributes in the white matter of the ipsilateral hemisphere, with rapidly decreasing concentrations in the cortex on the affected side. Note that in both Figs. 2 and 4, the infused material does not cross the midline through the corpus callosum. This suggests that the direction of the normal bulk flow pathway within the corpus callosum is away from the midline, thus opposing the CED infusion component. In contrast to intravascularly administered drugs, where increased capillary permeability results in increased tissue concentrations (Table 1), the opposite effect will occur in CED infusions. Increased efflux across permeable tumor capillaries will reduce both the extracellular drug concentration and the volume of distribution. In Fig. 5, high concentrations of ^{14}C-sucrose were achieved by a CED infusion into an RG-2 glioma; but, at a distance of 2.4 mm from the infusion site, tissue concentrations decreased by several logarithmic units. Although studies have not been done in humans, it is easy to anticipate that the volume of distribution from a CED infusion will follow similar principles. Fig. 6 shows several levels of an MRI scan through a malignant glioma. Each set of scans shows a gadolinium-enhanced tumor margin (which can be considered nature's equivalent to a CED infusion) and a T_2-weighted image (which corresponds to the extracellular water originating from the tumor source). The

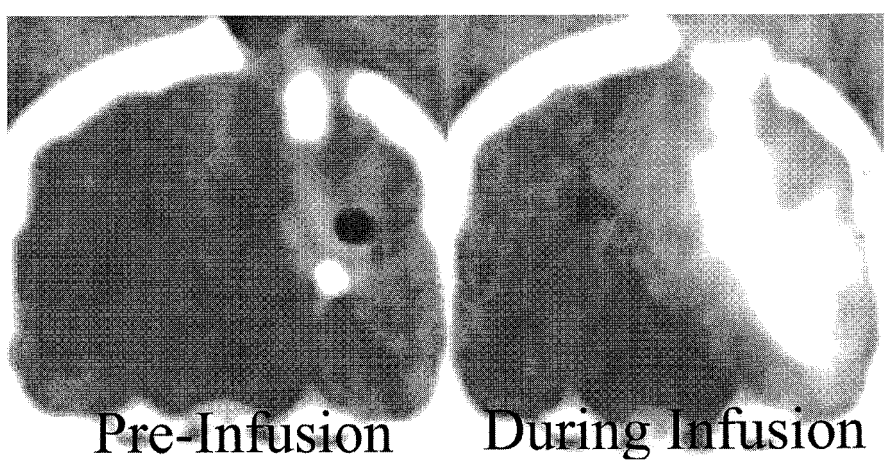

Fig. 4. Illustration of CED infusion in a dog. The figure on the left shows the CT scan at the start of a 3 µl min^{-1} infusion of Isovue (iopamidol) into the hemispheric white matter of a dog, while that on the right shows the same coronal level on the same dog after 10 days of infusion. The scan on the left contains an air bubble in the lateral ventricle; the cannula tip contains a metal marker (the high density circle) and a small amount of Isovue surrounding the catheter tip. In the scan on the right, the Isovue has distributed at a high concentration throughout the white matter of the ipsilateral hemisphere, with a rapid decline in concentration at gray-white interfaces. Note that the distribution of Isovue is limited to the ipsilateral hemisphere. No neuropathologic changes were observed as a result of the infusion.

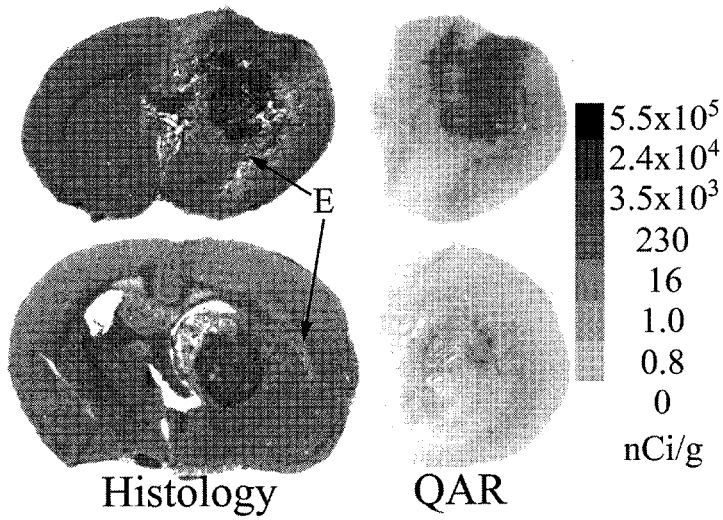

Fig. 5. Infusion of ^{14}C-sucrose into an RG-2 rat glioma. An infusion cannula was placed into the caudate nucleus of a Fisher-344 rat (target shown in Fig. 2A), and RG-2 cells were injected. Ten days later, ^{14}C-sucrose was infused at a rate of 10 μl/h for 7 days. Autoradiographic images were made (right images), and the sections were then stained with hematoxylin-eosin (left images). The top two images show that the ^{14}C-sucrose distributes in high concentration throughout the RG-2 tumor, extending into the edematous external capsule (E). However, the lower two sections, taken from 2.4 mm behind the infusion site, still contain tumor and show that the concentration of ^{14}C-sucrose is 100,000 times lower. These sections illustrate the problems associated with controlling drug delivery by convection-enhanced delivery.

shape and distribution of the edema are highly irregular and very dependent upon the brain structures encountered. Note that the edema fluid moves into the internal capsule but not into the thalamus or cortex. Notice also that the edema fluid does not cross the posterior limb of the corpus callosum, although it comes into contact with it at several levels.

A dominant feature of CED is that distribution of the drug is determined by the infusion parameters and the type of tissue being infused, whereas the concentration of drug in the infusate is unrelated to these parameters. The maximum concentration of the drug in the infusate will be determined by neurotoxicity within the central, convective component of the infusion, with variable concentrations in the anisotropic brain surrounding the central component, and concentrations 50 to 100 times lower in the diffusional component, furthermost from the infusion catheter. The ability to manipulate drug concentration in brain and tumor tissue with such ease, and to maintain these manipulations for long periods of time (Groothuis et al., 1999), brings a need for the development of entirely new concepts. For example, the limits with regard to the composition of the infusate need to be explored, such as pH, osmolarity, and ionic composition. Furthermore, how does one reliably study neurotoxicity? Are infusions into animal models going to be reliable predictors of toxicity in humans? Will differential neurotoxicity become an issue? It is possible that neurons, astrocytes, and oligodendrogliocytes will display different levels of susceptibility to many drugs. Will we see delayed neurotoxicity such as that seen with radiation? It will probably be best for many of the variables associated with neurotoxicity to be explored in animals before subjecting humans to CED infusions.

Summary of Drug Delivery that Circumvents the BBB

There has been an explosion of methodology involving local tissue drug delivery during the past 10 years. Some of these techniques, such as CED, fulfill the promise to circumvent the BBB entirely. In fulfilling this promise, these methods introduce new problems, such as the relationship between the spatial distribution of drugs and neurotoxicity, that need to be understood before these techniques are widely used. However, for viral vectors, monoclonal antibodies, antisense oligonucleotides, and other therapeutic agents that will be unable to cross the BBB in therapeutically effective amounts, CED may provide the necessary delivery tool.

The State of Drug Delivery to Brain Tumors–1999

If we return to the statement of Vick et al. at the beginning of this review, we can now state with certainty that the BBB and BTB are major factors limiting the access of many therapeutic agents to brain tumors; and, these barriers will become even more significant with the development of new molecular biological therapies that will involve large molecules, viruses, and even cells. It is also remarkable how prescient those authors were in identifying the other parts of the drug delivery process that must now be understood, and how little progress we have made in elucidating "tumor cell uptake, metabolic fate within tumor cells, and the washout or sink effect of the extracellular space and CSF" (Vick et al., 1977). For most of the past 20 years we have focused so much on

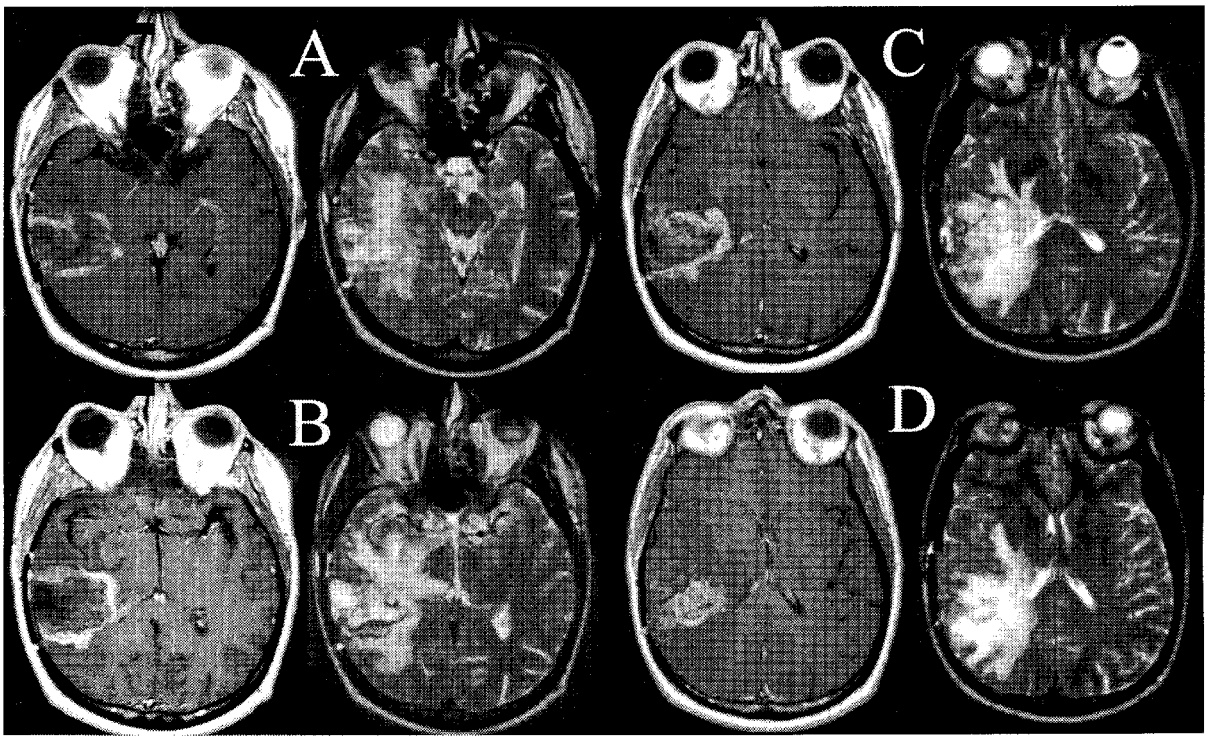

Fig. 6. Distribution of vasogenic edema in a malignant glioma. There are four pairs of scans extending through a tumor in the left temporal lobe, extending from the lowest (A) to the highest level (D). Each pair of scans contains a gadolinium-enhanced scan (left) and a T_2-weighted image (right). The gadolinium-enhanced scans show the area of abnormally permeable BBB. The T_2-weighted images show the distribution of edema (water) emanating from the tumor. The tumor may be considered a natural equivalent of a convection-enhanced delivery infusion. The resulting edema spreads extensively through white matter in the ipsilateral hemisphere, reaching forward in the internal capsule (levels C and D), but not penetrating gray matter structures such as the thalamus (levels C and D), or cortex (levels A–D). Note that the edema contacts the posterior limb of the corpus callosum in levels C and D, but does not extend into the corpus callosum, suggesting that there is a physiological barrier to the bulk movement of fluid in that direction.

ways to increase delivery across the BBB that we have failed to address these other issues, which now become the forefront in drug delivery methods such as CED. The arsenal from which to choose a drug delivery method is truly impressive. We must now study the other factors that limit effective brain tumor therapy. Perhaps the most pressing of these includes the need to develop effective laboratory tools for identifying the relationship between the concentration-time product of therapeutic agents and tumor cell kill, rather than relying on empirical testing in human subjects. It is most likely that the new century will see the development and application of tools for individualizing chemotherapy for brain tumors and other solid human tumors.

References

Ali-Osman, F. (1999) Human glioma cultures and *in vitro* analysis of therapeutic response. In Berger, M.S., and Wilson, C.B. (Eds.),*The Gliomas*. Philadelphia: W.B. Saunders Co. pp. 134–142.

Allen, T.M., and Moase, E.H. (1996) Therapeutic opportunities for targeted liposomal drug delivery. *Adv. Drug Deliv. Rev.* **21**, 117–133.

Arafat, T., Hentschel, P., Madajewicz, S., Manzione, J., Chowhan, N., Davis, R., Roche, P., Afif, I., Roque, C., Meek, A., and Magdy, S. (1999) Toxicities related to intraarterial infusion of cisplatin and etoposide in patients with brain tumors. *J. Neurooncol.* **42**, 73–77.

Bartus, R.T. (1999) The blood-brain barrier as a target for phamacological modulation. *Curr. Opin. Drug Discov. Dev.* **2**, 152–167.

Bickel, U., Yoshikawa, T., Landaw, E.M., Faull, K.F., and Pardridge, W.M. (1993) Pharmacologic effects *in vivo* in brain by vector-mediated peptide delivery. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2618–2622.

Bigner, D.D., Brown, M.T., Friedman, A.H., Coleman, R.E., Akabani, G., Friedman, H.S., Thorstad, W.L., McLendon, R.E., Bigner, S.H., Zhao, X.G., Pegram, C.N., Wikstrand, C.J., Herndon, J.E., II, Vick, N.A., Paleologos, N., Cokgor, I., Provenzale, J.M., and Zalutsky, M.R. (1998) Iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with recurrent malignant gliomas: Phase I trial results. *J. Clin. Oncol.* **16**, 2202–2212.

Black, K.L. (1995) Biochemical opening of the blood-brain barrier. *Adv. Drug Deliv. Rev.* **15**, 37–52.

Black, K.L., and Chio, C.C. (1992) Increased opening of blood tumour barrier by leukotriene C4 is dependent on size of molecules. *Neurol. Res.* **14,** 402–404.

Black, K.L., Cloughesy, T., Huang, S.-C., Gobin, Y.P., Zhou, Y., Grous, J., Nelson, G., Farahani, K., Hoh, C.K., and Phelps, M. (1997) Intracarotid infusion of RMP-7, a bradykinin analog, and transport of gallium-68 ethylenediamine tetraacetic acid into human gliomas. *J. Neurosurg.* **86,** 603–609.

Blasberg, R.G. (1977) Methotrexate, cytosine arabinoside, and BCNU concentration in brain after ventriculocisternal perfusion. *Cancer Treat. Rep.* **61**, 625–631.

Blasberg, R.G., and Groothuis, D.R. (1986) Chemotherapy of brain tumors: Physiological and pharmacokinetic considerations. *Semin. Oncol.* **13,** 70–82.

Blasberg, R.G., Patlak, C.S., and Shapiro, W.R. (1977) Distribution of methotrexate in the cerebrospinal fluid and brain after intraventricular administration. *Cancer Treat. Rep.* **61,** 633–641.

Blasberg, R.G., Kobayashi, T., Horowitz, M., Rice, J., Groothuis, D.R., Molnar, P., and Fenstermacher, J.D. (1983) Regional blood-to-tissue transport in ethylnitrosourea-induced brain tumors. *Ann. Neurol.* **14,** 202–215.

Blasberg, R.G., Nakagawa, H., Bourdon, M.A., Groothuis, D.R., Patlak, C.S., and Bigner, D.D. (1987) Regional localization of a glioma-associated antigen defined by monoclonal antibody 81C6 in vivo: Kinetics and implications for diagnosis and therapy. *Cancer Res.* **47,** 4432–4443.

Boado, R.J., Tsukamoto, H., and Pardridge, W.M. (1998) Drug delivery of antisense molecules to the brain for the treatment of Alzheimer's disease and cerebral AIDS. *J. Pharm. Sci.* **87,** 1308–1315.

Bobo, R.H., Laske, D.W., Akbasak, A., Morrison, P.F., Dedrick, R.L., and Oldfield, E.H. (1994) Convection-enhanced delivery of macromolecules in the brain. *Proc. Natl. Acad. Sci. U.S.A.* **91,** 2076–2080.

Brem, H., Piantadosi, S., Burger, P.C., Walker, M., Selker, R., Vick, N.A., Black, K., Sisti, M., Brem, S., Mohr, G., Muller, P., Morawetz, R., and Schold, S.C. (1995) Placebo controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. *Lancet* **345**, 1008–1012.

Brown, M.T., Coleman, R.E., Friedman, A.H., Friedman, H.S., McLendon, R.E., Reiman, R., Felsberg, G.J., Tien, R.D., Bigner, S.H., Zalutsky, M.R., Zhao, X.G., Wikstrand, C.J., Pegram, C.N., Herndon, J.E., II, Vick, N.A., Paleologos, N., Fredericks, R.K., Schold, S.C., Jr., and Bigner, D.D. (1996) Intrathecal ^{131}I-labeled antitenascin monoclonal antibody 81C6 treatment of patients with leptomeningeal neoplasms or primary brain tumor resection cavities with subarachnoid communication: Phase I trial results. *Clin. Cancer Res.* **2,** 963–972.

Burger, P.C. (1987) The anatomy of astrocytomas. *Mayo Clin. Proc.* **62**, 527–529.

Cao, X., and Shoichet, M.S. (1999) Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders. *Biomaterials* **20,** 329–339.

Chang, C.-M., and Bodmeier, R. (1997) Binding of drugs to monoglyceride-based drug delivery systems. *Int. J. Pharm.* **147**, 135–142.

Chang, H.-K., Gendelman, R., Lisziewicz, J., Gallo, R.C., and Ensoli, B. (1994) Block of HIV-1 infection by a combination of antisense tat RNA and TAR decoys: A strategy for control of HIV-1. *Gene Ther.* **1,** 208–216.

Cowles, A.L., and Fenstermacher, J.D. (1974) Theoretical considerations in the chemotherapy of brain tumors. In Sartorelli, A.C., and Johns, D.G. (Eds.), *Handbook of Experimental Pharmacology*. Vol. 38. Berlin: Springer Verlag. pp. 319–329.

Cree, I.A., and Kurbacher, C.M. (1997) Individualizing chemotherapy for solid tumors: Is there any alternative? *Anticancer Drugs* **8,** 541–548.

de Lange, E.C.M., Danhof, M., de Boer, A.G., and Breimer, D.D. (1997) Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood-brain barrier. *Brain Res. Rev.* **25,** 27–49.

de Lange, E.C.M., de Boer, A.G, and Breimer, D.D. (1999) Microdialysis for pharmacokinetic analysis of drug transport to the brain. *Adv. Drug Deliv. Rev.* **36**, 211–227.

de Vries, H.E., Blom-Roosemalen, M.C.M., van Osten, M., de Boer, A.G., van Berkel, T.J.C., Breimer, D.D., and Kuiper, J. (1996) The influence of cytokines on the integrity of the blood-brain barrier *in vitro*. *J. Neuroimmunol.* **64,** 37–43.

Devineni, D., Klein-Szanto, A., and Gallo, J.M. (1996) *In vivo* microdialysis to characterize drug transport in brain tumors: Analysis of methotrexate uptake in rat glioma-2 (RG-2)-bearing rats. *Cancer Chemother. Pharmacol.* **38**, 499–507.

Donelli, M.G., Zucchetti, M., and D'Incalci, M. (1992) Do anticancer agents reach the tumor target in the human brain? *Cancer Chemother. Pharmacol.* **30**, 251–260.

Dropcho, E.J., Rosenfeld, S.S., Vitek, J., Guthrie, B.L., and Morawetz, R.B. (1998) Phase II study of intracarotid or selective intracerebral infusion of cisplatin for treatment of recurrent anaplastic gliomas. *J. Neurooncol.* **36,** 191–198.

Dykstra, K.H., Hsiao, J.K., Morrison, P.F., Bungay, P.M., Mefford, I.N., Scully, M.M., and Dedrick, R.L. (1992) Quantitative examination of tissue concentration profiles associated with microdialysis. *J. Neurochem.* **58**, 931–940.

Eckman, W.W., Patlak, C.S., and Fenstermacher, J.D. (1974) A critical evaluation of the principles governing the advantages of intra-arterial infusions. *J. Pharmacokinet. Biopharm.* **2**, 257–285.

Emerich, D.F., Snodgrass, P., Pink, M., Bloom, F., and Bartus, R.T. (1998) Central analgesic actions of loperamide following the transient permeation of the blood brain barrier with Cereport (RMP-7). *Brain Res.* **801**, 259–266.

Ermisch, A., Brust, P., Kretzschmar, R., and Ruhle, H.-J. (1993) Peptides and blood-brain barrier transport. *Phys. Rev.* **73**, 489–527.

Farkkila, M., Jaaskelainen, J., Kallio, M., Blomstedt, G., Raininko, R., Virkkunen, P., Paetau, A., Sarelin, H., and Mantyla, M. (1994) Randomised, controlled study of intratumoral recombinant γ-interferon treatment in newly diagnosed glioblastoma. *Br. J. Cancer* **70**, 138–141.

Fenstermacher, J., and Gazendam, J. (1981) Intra-arterial infusions of drugs and hyperosmotic solutions as ways of enhancing CNS chemotherapy. *Cancer Treat. Rep.* **65** (Suppl. 2), 27–37.

Fenstermacher, J.D., and Cowles, A.L. (1977) Theoretic limitations of intracarotid infusions in brain tumor chemotherapy. *Cancer Treat. Rep.* **61**, 519–526.

Ford, J., Osborn, C., Barton, T., and Bleehen, N.M. (1998) A phase I study of intravenous RMP-7 with carboplatin in patients with progression of malignant glioma. *Eur. J. Cancer* **34**, 1807–1811.

Friden, P.M., Walus, L.R., Watson, P., Doctrow, S.R., Kozarich, J.W., Backman, C., Bergman, H., Hoffer, B., Bloom F., and Granholm, A.-C. (1993) Blood-brain barrier penetration and *in vivo* activity of an NGF conjugate. *Science* **259**, 373–377.

Friden, P.M., Olson, T.S., Obar, R., Walus, L.R., and Putney, S.D. (1996) Characterization, receptor mapping and blood-brain barrier transcytosis of antibodies to the human transferrin receptor. *J. Pharmacol. Exp. Ther.* **278**, 1491–1498.

Fung, L.K., Ewend, M.G., Sills, A., Sipos, E.P., Thompson, R., Watts, M., Colvin, O.M., Brem, H., and Saltzman, W.M. (1998) Pharmacokinetics of interstitial delivery of carmustine, 4-hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer implant in the monkey brain. *Cancer Res.* **58**, 672–684.

Ghersi-Egea, J.F., Finnegan, W., Chen, J.L., and Fenstermacher, J.D. (1996) Rapid distribution of intraventricularly administered sucrose into cerebrospinal fluid cisterns via subarachnoid velae in rat. *Neuroscience* **75**, 1271–1288.

Groothuis, D.R., and Levy, R.M. (1997) The entry of antiviral and antiretroviral drugs into the central nervous system. *J. Neurovirol.* **3**, 387–400.

Groothuis, D.R., Warkne, P.C., Molnar, P., Lapin, G.D., and Mikhael, M.A. (1990) Effect of hyperosmotic blood-brain barrier disruption on transcapillary transport in canine brain tumors. *J. Neurosurg.* **72**, 441–449.

Groothuis, D.R., Lippitz, B.E., Fekete, I., Schlageter, K.E., Molnar, P., Colvin, O.M., Roe, C.R., Bigner, D.D., and Friedman, H.S. (1992) The effect of an amino acid-lowering diet on the rate of melphalan entry into brain and xenotransplanted glioma. *Cancer Res.* **52**, 5590–5596.

Groothuis, D.R., Ward, S., Schlageter, K.E., Itskovich, A.C., Schwerin, S.C., Allen, C.V., Dills, C., and Levy, R.M. (1998) Changes in blood-brain barrier permeability associated with insertion of brain cannulas and microdialysis probes. *Brain Res.* **803**, 218–230.

Groothuis, D.R., Ward, S., Itskovich, A.C., Debrescu, C., Allen, C.V., Dills, C., and Levy, R.M. (1999) Comparison of ^{14}C-sucrose delivery to the brain by intravenous, intraventricular, and convection-enhanced intracerebral infusion. *J. Neurosurg.* **90**, 321–331.

Gundersen, S., Lote, K., and Watne, K. (1998) A retrospective study of the value of chemotherapy as adjuvant therapy to surgery and radiotherapy in grade 3 and 4 gliomas. *Eur. J. Cancer* **34**, 1565–1569.

Gutman, M., Laufer, R., Eisenthal, A., Goldman, G., Ravid, A., Inbar, M., and Klausner, J.M. (1996) Increased microvascular permeability induced by prolonged interleukin-2 administration is attenuated by the oxygen-free-radical scavenger dimethylthiourea. *Cancer Immunol. Immunother.* **43**, 240–244.

Hendrikse, N.H., Schinkel, A.H., de Vries, E.G.E., Fluks, E., Van der Graaf, W.T.A., Willemsen, A.T.M., Vaalburg, W., and Franssen, E.J.F. (1998) Complete *in vivo* reversal of P-glycoprotein pump function in the blood-brain barrier visualized with positron emission tomography. *Br. J. Pharmacol.* **124**, 1413–1418.

Hirano, Y., Mineura, K., Mizoi, K., and Tomura, N. (1998) Therapeutic results of intra-arterial chemotherapy in patients with malignant glioma. *Int. J. Oncol.* **13**, 537–542.

Hsiao, M., Tse, V., Carmel, J., Tsai, Y., Felgner, P.L., Haas, M., and Silverberg, G.D. (1997) Intracavitary liposome-mediated p53 gene transfer into glioblastoma with endogeneous wild-type p53 *in vivo* results in tumor suppression and long-term survival. *Biochem. Biophys. Res. Comm.* **233**, 359–364.

Inamura, T., and Black, K.L. (1994) Bradykinin selectively opens blood tumor barrier in experimental brain tumors. *J. Cereb. Blood Flow Metab.* **14**, 862–870.

Jain, R.K. (1996) Delivery of molecular medicine to solid tumors. *Science* **271**, 1079–1080.

Jain, R.K. (1998) The next frontier of molecular medicine: Delivery of therapeutics. *Nat. Med.* **4**, 655–657.

Kroll, R.A., and Neuwelt, E.A. (1998) Outwitting the blood-brain barrier for therapeutic purposes: Osmotic opening and other means. *Neurosurgery* **42**, 1083–1100.

Laske, D.W., Morrison, P.F., Lieberman, D.M., Corthesy, M.E., Reynolds, J.C., Stewart-Henney, P.A., Koong, S.-S., Cummins, A., Paik, C.H., and Oldfield, E.H. (1997a) Chronic interstitial infusion of protein to primate brain: Determination of drug distribution and clearance with single-photon emission computerized tomography imaging. *J. Neurosurg.* **87**, 586–594.

Laske, D.W., Youle, R.J., and Oldfield, E.H. (1997b) Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat. Med.* **3**, 1362–1368.

Leweke, F., Damian, M.S., Schindler, C., and Schachenmayr, W. (1998) Multidrug resistance in glioblastoma: Chemosensitivity testing and immunohistochemical demonstration of P-glycoprotein. *Pathol. Res. Pract.* **194**, 149–155.

Lieberman, D.M., Laske, D.W., Morrison, P.F., Bankiewicz K.S., and Oldfield, E.H. (1995) Convection enhanced distribution of large molecules in gray matter during interstitial drug infusion. *J. Neurosurg.* **82**, 1021–1029.

Menei, P., Venier-Julienne, M.C., and Benoit, J.P. (1997) Drug delivery into the brain using implantable polymeric systems. *STP Pharm. Sci.* **7**, 53–61.

Morgan, M.E., Singhal, D., and Anderson, B.D. (1996) Quantitative assessment of blood-brain barrier damage during microdialysis. *J. Pharmacol. Exp. Ther.* **277**, 1167–1176.

Morrison, P.F., Laske, D.W., Bobo, H., Oldfield, E.H., and Dedrick, R.L. (1994) High-flow microinfusion: Tissue penetration and pharmacodynamics. *Am. J. Physiol.* **266** (1 Pt 2), R292–R305.

Nakagawa, H., Groothuis, D.R., and Blasberg, R.G. (1984) The effect of graded hypertonic intracarotid infusion on drug delivery to experimental RG-2 rat gliomas. *Neurology* **34**, 1571–1581.

Nakagawa, H., Groothuis, D.R., Owens, E.S., Fenstermacher, J.D., Patlak, C.S., and Blasberg, R.G. (1987) Dexamethasone effects on ^{125}I-albumin distribution in experimental RG-2 gliomas and adjacent brain. *J. Cereb. Blood Flow Metab.* **7**, 687–701.

Nakashima, M., Shibata, S., Tokunaga, Y., Fujita, H., Anda, T., Arizono, K., Tomiyama, N., Sasaki, H., and Ichikawa, M. (1997) *In vivo* microdialysis study of the distribution of cisplatin into brain tumour tissue after intracarotid infusion in rats with 9L malignant glioma. *J. Pharm. Pharmacol.* **49**, 777–780.

Neuwelt, E.A., and Dahlborg, S.A. (1989) Blood-brain barrier disruption in the treatment of brain tumors: Clinical implications. In Neuwelt, E.A. (Ed.), *Implications of the Blood-Brain Barrier and Its Manipulation.* Vol. 2. New York: Plenum Medical Book Co. pp. 195–262.

Neuwelt, E.A., Frenkel, E.P., Diehl, J., Vu, L.H., and Hill, S. (1980) Reversible osmotic blood-brain barrier disruption in humans: Implications for the chemotherapy of malignant brain tumors. *Neurosurgery* **7**, 44–52.

Neuwelt, E.A., Balaban, E., Diehl, J., Hill, S., and Frenkel, E. (1983) Successful treatment of primary central nervous system lymphomas with chemotherapy after osmotic blood brain barrier opening. *Neurosurgery* **12**, 662–671.

Neuwelt, E.A., Barnett, P.A., and Frenkel, E.P. (1984) Chemotherapeutic agent permeability to normal brain and delivery to avian sarcoma virus induced brain tumors in the rodent: Observations on problems of drug delivery. *Neurosurgery* **14**, 154–160.

Normand-Sdiqui, N., and Akhtar, S. (1998) Oligonucleotide delivery: Uptake of rat transferrin receptor antibody (OX-26) conjugates into an *in vitro* immortalised cell line model of the blood-brain barrier. *Int. J. Pharm.* **163**, 63–71.

Olivi, A., and Brem, H. (1994) Interstitial chemotherapy with sustained release polymer systems for the treatment of malignant gliomas. *Recent Results Cancer Res.* **135**, 149–154.

Ostrowitzki, S., Fick, J., Roberts, T.P.L., Wendlend, M.F., Aldape, K.D., Mann, J.S., Israel, M.A., and Brasch, R.C. (1998) Comparison of gadopentetate dimeglumine and albumin-(Gd-DTPA)$_{30}$ for microvessel characterization in an intracranial glioma model. *J. Magn. Reson. Imaging* **8**, 799–806.

Pardridge, W.M. (1995) Vector-mediated peptide delivery to the brain. *Adv. Drug Deliv. Rev.* **15**, 109–146.

Pardridge, W.M. (1997) Drug delivery to the brain. *J. Cereb. Blood Flow Metab.* **17**, 713–731.

Pardridge, W.M. (1998) CNS drug design based on principles of blood-brain barrier transport. *J. Neurochem.* **70**, 1781–1792.

Pardridge, W.M., Kang, Y.-S., Buciak, J.L., and Yang, J. (1995) Human insulin receptor monoclonal antibody undergoes high affinity binding to human brain capillaries *in vitro* and rapid transcytosis through the blood-brain barrier *in vivo* in the primate. *Pharm. Res.* **12**, 807–816.

Parker, J.C., Perry, M.A., and Taylor, A.E. (1984) Permeability of the microvascular barrier. In Staub, N.C., and Taylor, A.E. (Eds.), *Edema.* New York: Raven Press. pp. 143–188.

Parsons, L.H., and Justice, J.B., Jr. (1994) Quantitative approaches to *in vivo* brain microdialysis. *Crit. Rev. Neurobiol.* **8**, 189–220.

Patlak, C.S., and Fenstermacher, J.D. (1975) Measurements of dog blood-brain transfer constants by ventriculocisternal perfusion. *Am. J. Physiol.* **229**, 877–884.

Puri, R.K., Hoon, D.S., Leland, P., Snow, P., Rand, R.W., Pastan, I., and Kreitman, R.J. (1996) Preclinical development of a recombinant toxin containing circularly permuted interleukin 4 and truncated Pseudomonas exotoxin for therapy of malignant glioma. *Cancer Res.* **56**, 5631–5637.

Ram, Z., Culver, K.W., Oshiro, E.M., Viola, J.J., DeVroom, H.L., Otto, E., Long, Z., Chiang, Y., McGarrity, G.J., Muul, L.M., Katz, D., Blaese, R.M., and Oldfield, E.H. (1997) Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat. Med.* **3**, 1354–1361.

Rapoport, S.I. (1996) Modulation of blood-brain barrier permeability. *J. Drug Target.* **3**, 417–425.

Rapoport, S.J., and Thompson, H.K. (1973) Osmotic opening of the blood-brain barrier in the monkey without associated neurological deficits. *Science* **180**, 971.

Rubertone, J.A., Woo, D.V., Emrich, J.G., and Brady, L.W. (1993) Brain uptake of thallium-201 from the cerebrospinal fluid compartment. *J. Nucl. Med.* **34**, 99–103.

Sakamoto, A., and Ido, T. (1993) Liposome targeting to rat brain: Effect of osmotic opening of the blood-brain barrier. *Brain Res.* **629**, 171–175.

Sanovick, E., Bartus, R.T., Friden, P.M., Dean, R.L., Le, H.Q., and Brightman, M.W. (1995) Pathway across blood-brain barrier opened by the bradykinin agonist, RMP-7. *Brain Res.* **705**, 125–135.

Schinkel, A.H. (1999) P-glycoprotein, a gatekeeper in the blood-brain barrier. *Adv. Drug Deliv. Rev.* **36**, 179–194.

Schlageter, K.E., Molnar, P., Lapin, G.D., and Groothuis, D.R. (1999) Microvessel organization and structure in experimental brain tumors: Microvessel populations with distinctive structural and functional properties. *Microvasc. Res.* **58**, 312–328.

Schroeder, U., Sommerfeld, P., Ulrich, S., and Sabel, B.A. (1998) Nanoparticle technology for delivery of drugs across the blood-brain barrier. *J. Pharm. Sci.* **87**, 1305–1307.

Sherman, M.E., Erozan, Y.S., Mann, R.B., Kumar, A.A.J., McArthur, J.C., Royal, W., Uematsu, S., and Nauta, H.J.W. (1991) Stereotactic brain biopsy in the diagnosis of malignant lymphoma. *Am. J. Clin. Pathol.* **95**, 878–883.

Song, C.X., Labhasetwar, V., Murphy, H., Qu, X., Humphrey, W.R., Shebuski, R.J., and Levy, R.J. (1997) Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery. *J. Controlled Release* **43**, 197–212.

Stewart, D.J. (1994) A critique of the role of the blood-brain barrier in the chemotherapy of human brain tumors. *J. Neurooncol.* **20**, 121–139.

Tamai, I., and Tsuji, A. (1996) Drug delivery through the blood-brain barrier. *Adv. Drug Deliv. Rev.* **19**, 401–424.

Tamai, I., Sai, Y., Kobayashi, H., Kamata, M., Wakamiya, T., and Tsuji, A. (1997) Structure-internalization relationship for adsorptive-mediated endocytosis of basic peptides at the blood-brain barrier. *J. Pharmacol. Exp. Ther.* **280**, 410–415.

Taylor, A.E., and Granger, D.N. (1984) Exchange of macromolecules across the microcirculation. In Renkin, E.M., and Michel, C.C. (Eds.), *Handbook of Physiology*. Sect. 2, Vol. IV, Part 1. Bethesda, Md.: American Physiological Society. pp. 467–520.

Tomita, T. (1991) Interstitial chemotherapy for brain tumors: Review. *J. Neurooncol.* **10**, 57–74.

Tsuji, A., and Tamai, I. (1997) Blood-brain barrier function of P-glycoprotein. *Adv. Drug Deliv. Rev.* **25**, 287–298.

van de Waterbeemd, H., Camenish, G., Folkers, G., Chretien, J.R., and Raevsky, O.A. (1998) Estimation of blood-brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. *J. Drug Target.* **6**, 151–165.

Vick, N.A., Khandekar, J.D., and Bigner, D.D. (1977) Chemotherapy of brain tumors: The "blood-brain barrier" is not a factor. *Arch. Neurol.* **34**, 523–526.

Viola, J.J., Agbaria, R., Walbridge, S., Oshiro, E.M., Johns, D.G., Kelley, J.A., Oldfield, E.H., and Ram, Z. (1995) *In situ* cyclopentenyl cytosine infusion for the treatment of experimental brain tumors. *Cancer Res.* **55**, 1306–1309.

von Bossanyi, P., Diete, S., Dietzmann, K., Warich-Kirches, M., and Kirches, E. (1997) Immunohistochemical expression of P-glyoprotein and glutathione S-transferases in cerebral gliomas and response to chemotherapy. *Acta Neuropathol.* **94**, 605–611.

Westergren, I., Nystrom, B., Hamberger, A., and Johansson, B.B. (1995) Intracerebral dialysis and the blood brain barrier. *J. Neurochem.* **64**, 229–234.

Yang, W., Barth, R.F., Adams, D.M., and Soloway, A.H. (1997) Intratumoral delivery of boronated epidermal growth factor for neutron capture therapy of brain tumors. *Cancer Res.* **57**, 4333–4339.

Yoshikawa, T., Sakaeda, T., Sugawara, T., Hirano, K., and Stella, V.J. (1999) A novel chemical delivery system for brain targeting. *Adv. Drug Deliv. Rev.* **36**, 255–275.

Zunkeler, B., Carson, R.E., Olson, J., Blasberg, R.G., DeVroom, H., Lutz, R.J., Saris, S.C., Wright, D.C., Kammerer, W., Patronas, N.J., Dedrick, R.L., Herscovitch, P., and Oldfield, E.H. (1996) Quantification and pharmacokinetics of blood-brain barrier disruption in humans. *J. Neurosurg.* **85**, 1056–1065.