Differential Permeability of a Human Brain Tumor Xenograft in the Nude Rat: Impact of Tumor Size and Method of Administration on Optimizing Delivery of Biologically Diverse Agents

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ABSTRACT
To assess how to maximize drug delivery to intracerebral tumors and surrounding brain, this study examined the effects of route and method of administration and tumor size on the distribution of three agents in a nude rat intracerebral tumor xenograft model. Aminoisobutyric acid (M, 103), methotrexate (M, 454), and dextran 70 (Mr, 70,000) were administered i.v. or intra-arterially (i.a.) with or without osmotic blood-brain barrier disruption (BBBD) at 8, 12, or 16 days after tumor cell inoculation (n = 72). A 2.2- to 2.5-fold increase in delivery to tumor and surrounding brain was observed when i.a. was compared with i.v., and a 2.5- to 7.6-fold increase was observed when BBBD was compared with the saline control. The combined effect of i.a. administration and BBBD was to increase delivery 6.3- to 16.7-fold. The greatest benefit of BBBD was seen in animals with 8-day tumors, whereas BBBD had less benefit in improving delivery to intracerebral tumor and brain around tumor as the tumors grew larger. Regional delivery decreased as the molecular weight of the agent increased. Based on these results, we suggest that i.a. administration of antitumor agents may be adequate to obtain initial responses in large, very permeable, intracerebral tumors. However, in smaller, less permeable tumors or after an initial response to treatment, there may be a significant therapeutic advantage to i.a. agent administration and BBBD.

INTRODUCTION
Increased BBB permeability, as demonstrated by image enhancement, is suggestive of a malignancy (1) in the CNS. However, BTB permeability is variable and complex (2, 3). Clinical studies reported by Chamberlain et al. (4) have demonstrated that up to 30% of patients with highly anaplastic astrocytomas had nonenhancing lesions (i.e., no breakdown of the BBB) on computed tomography. Similarly, nonenhancing tumors on computed tomography or magnetic resonance scan have been described in 10% of patients with cerebral lymphoma, either at the time of diagnosis or recurrence (5). In such situations, CNS penetration of systemically administered therapeutic agents is limited. Other factors that may impede drug delivery to tumors include the variable and uneven distribution of tumor vasculature and high interstitial pressure that opposes movement of drugs, especially larger molecules, from vessels (6-9).

Based upon serial positron emission tomography scan evaluation of patients with primary CNS lymphoma, Ott et al. (10) reported that, although the main bulk of tumor often has increased permeability prior to initiation of therapy, within 5 weeks of chemotherapy treatment, the permeability can return to the levels present in normal brain. As a consequence of the diminished drug delivery that results from this decreasing tumor permeability, response to chemotherapy is often transient. Additional clinical evidence in support of the need for increased drug delivery to brain tumors was reported by Stewart (11). If the treatment of tumors that bridged two arterial circulations, one infused with IA chemotherapy and one not, there was tumor regression in the i.a.-treated region and tumor progression in the portion not receiving i.a. infusion (11). Zünkeler et al. (12), in a recent positron emission tomography scan study of osmotic BBB opening in glioma patients, observed a substantial increase in permeability in brain but only a modest increase in permeability within the tumor. Based on this, they suggest that delivery with i.a. administration without osmotic BBBD may be adequate to maximize drug delivery to tumor. On the other hand, in primary CNS lymphoma where the enhancing tumor rapidly disappears, increasing chemotherapy delivery with osmotic BBBD has been shown to result in a durable response without radiotherapy and without cognitive loss (13, 14). Preclinical support for a therapeutic advantage of osmotic BBB-enhanced drug administration has been reported recently as well (15). Rats with F98 intracerebral gliomas were treated with...
boron neutron capture therapy after i.v., i.a., or i.a. with BBBD administration of the boronated compound. Each method of boron delivery was associated with an increase in median survival time over untreated controls, with the greatest prolongation in survival time seen in animals that had BBBD. Consequently, it may be theorized that with a markedly abnormal BBB, such as is present in many large intracranial malignant tumors, there may be adequate drug delivery in tumor with i.a. administration to obtain an initial response. However, in smaller tumors and in those that are responding to chemotherapy, as well as in tumor-infiltrated surrounding brain, the BBB may be a limiting factor for adequate chemotherapeutic delivery (16, 17). This issue may be further magnified when large molecular weight therapeutic agents, such as biologically specific proteins (i.e., monoclonal antibodies), are administered (18–20).

The present report evaluates the differential permeability to agents of varying molecular weight in an intracerebral tumor xenograft model in the nude rat. We have described previously increasing permeability with increasing tumor size and tumor permeability differences to several agents ($R^2$, 0.964) with a large range of molecular weights (21). The present report describes the effects of two methods to increase tumor delivery and/or tumor permeability: i.a. (right internal carotid artery) agent administration and BBBD (right hemisphere; Refs. 22–27). In an LX-1 human small cell lung carcinoma intracerebral and SQ1 model, three water-soluble agents (molecular weights ranging from 103 to 70,000) were evaluated after i.v. or i.a. administration, with and without BBBD. Three different time points after tumor cell inoculation were chosen, which reflect very different in vivo tumor sizes. The objective was to more precisely determine when i.a. administration and BBBD could be applied beneficially, as related to biologically different agents and varying tumor size.

MATERIALS AND METHODS

Animal Tumor Model. Female athymic nude rats from a colony maintained at the Oregon Health Sciences University were used for all studies. LX-1 human small cell lung carcinoma cells were grown in culture and harvested as described previously (28). Cell viability was >85%, as determined by trypan blue exclusion. For intracerebral implantation, the rats were anesthetized with i.p. ketamine (50 mg/kg) and xylazine (2.0 mg/kg). The head was shaved and immobilized in a stereotaxic frame. A 2-cm midline incision was made to expose the frontal bone on the right. A 2-mm burr hole was made over the right hemisphere, with location defined by stereotactic coordinates: 0, Bregma; lateral, −0.31 cm (right); and vertical, −0.65 cm (down from skull surface). Anatomical location was deep in the caudate-putamen. A 27-gauge needle and a 100-μl syringe were used to inject 10 μl of the cell suspension (8 × 10⁶ cells). This LX-1 intracerebral tumor model has permeability characteristics similar to a variety of rat brain tumors reviewed by Blasberg et al. (29). Each animal also received 500 μl of the cell suspension (4 × 10⁷ cells) inoculated s.c. into the right flank.

BBBD. BBBD in the rat was performed using the technique reported previously (28). Briefly, animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and a catheter was tied into the right external carotid artery for retrograde infusion. Ten min before BBBD, Evans blue (2%, 2 ml/kg) and fluorescein (10%, 0.12 ml) were administered i.v. to provide a visual basis for tumor excision and to verify the success of BBBD. Mannitol (25%), warmed to 37°C, was infused retrograde for 30 s, with cephalad flow into the right internal carotid artery at a rate of 0.09 ml/s. In control studies, saline (0.9% NaCl) instead of mannitol was infused at an identical rate and volume. Animals that either did not have a tumor or did not have at least a 2+ (good) disruption in normal brain on a scale of 0–3+ (30) were repeated (about one-third of the animals).

Differential Permeability. This study was designed to evaluate the effect of tumor growth, i.a. agent administration, and BBBD on intracerebral tumor permeability to different agents. In tumor-bearing animals, three agents of varying molecular weights were given as a bolus injection i.a. (right internal carotid artery) or i.v. (right femoral vein) immediately following mannitol or saline. The agents used were [14C]AIB ($M$, 103), [3H]MTX ($M$, 454), and [3H]DEX70 ($M$, 70,000). Three different time points of tumor progression were evaluated for each agent (8, 12, and 16 days), which corresponds to tumors that are ~10 mm³ at 8 days to greater than 100 mm³ at 16 days (21). Experimental groups were randomly established by agent, time of progression, and treatment. Animals were anesthetized with sodium pentobarbital and prepared for BBBD. A catheter was also placed in the right femoral artery for plasma collection. Immediately after mannitol or saline infusion, 10 μCi of the appropriate agent were administered, and plasma samples were collected immediately and at 2, 4, 6, 8, and 10 min after mannitol or saline. All animals were sacrificed immediately after the last plasma sample was obtained.

At sacrifice, the brain and SQ1 were removed, and the following samples were obtained for scintillation counting: ICT, BAT (2–3-mm edge adjacent to tumor), ipsilateral BDT, contralateral normal brain (LH), and SQ1. All samples were corrected for quench and background activity obtained from tissue controls. Agent delivery into tumor and surrounding brain was determined by calculating the percentage of accessible tissue space (ml/g). As described previously (21, 29, 31), the percentage of accessible tissue space is a ratio of the radioactivity per gram of tissue divided by the radioactivity per milliliter of plasma at sacrifice, multiplied by 100. This calculation takes into consideration both intravascular and extravascular components. Thus, if the tissue measurement is greater than the plasma measurement, the value will exceed 100%.

Data Analysis. The experimental design followed a factorial arrangement with agents (AIB, MTX, and DEX70), route of administration (i.v. and i.a.), and BBBD (with and without) being the main factors. Six animals were allocated to each of the 12 primary treatment combinations. All allocations were made randomly. Of primary importance in the present study were the effects of routes of administration, BBBD, and agents. Of secondary interest was the effect of tumor progression (time). Therefore, of the six animals per treatment group, two animals were studied on each of three different days (8, 12, or 16 days after inoculation). For estimating the rate of change (slope) of tissue permeability, an unbiased estimate, based on six animals, was obtained for each route of administration, BBBD, and agent combination by fitting a least squares linear regression model using indicator variables for each agent, route, and disruption.
Table 1  Summary of regression coefficients from the least squares linear regression analysis of log percent accessible tissue space

The estimated value of the percentage of accessible tissue space for any agent in any tissue under any treatment condition at any time between 8 and 16 days after inoculation can be calculated by adding the regression coefficient of the factors (main and interactions) to the baseline regression coefficient for a given agent and determining the antilog (see “Example calculation”). AIB (M, 103), [3H]MTX (M, 454), or [14C]DEX70 (M, 70,000) was administered i.v. or i.a. after saline or BBBD at 8, 12, and 16 days after inoculation of LX-1 cells. Animals were sacrificed 10 min after agent administration. Measurements of the percentage of accessible tissue space (dpm/g tissue)/dpm/ml plasma at sacrifice) were expressed on a log scale. Listed are the regression coefficients for the main factors (time, i.a. administration, and BBBD treatment) with the isolated modification of the main factors in specific tissues (interactions). Only additive factors and interactions that were significant in at least one tissue region were evaluated in the final models and are shown above.

| Agent delivery (mUg) was calculated in ICT, BAT, BDT, LH (as outlined in Table 1), and SQT after i.v. or i.a. administration of AIB, MTX, or DEX70, with or without BBBD, at 8, 12, or 16 days after tumor cell inoculation. Raw data were log transformed and model fitted using a least squares linear regression model for each tissue (R^2, 0.88–0.97).

Baseline drug delivery data (i.v. without BBBD) correlated well with a previous study, which evaluated differential permeability of these same agents when given only i.v. (21). The estimated values for delivery in ICT of these agents were very similar between the two studies. As in the previous report, differential permeability was demonstrated with significant differences in delivery between the agents in all tissue samples (P ≤ 0.0002). When averaged across postinoculation time and treatment groups, tissue values for AIB were 2–3-fold greater than MTX and 10–40-fold greater than DEX70. Values for MTX were 4–20-fold greater than DEX70 (Fig. 1).

Based on the analysis of calculations, there are three major data findings: (a) the effects of i.a. agent administration and BBBD (method of delivery); (b) the effect of the number of days after inoculation (tumor size); and (c) the effect of combinations of study factors (additional interactions). The estimated value of delivery for any agent in any tissue under any treatment condition at any time between 8 and 16 days after inoculation can be calculated from Table 1. Listed are the effects of tumor progression (time), i.a. administration, and BBBD treatment with the isolated modification of these factors in specific tissues (interactions). Only significant additive factors and interactions are listed in Table 1.

Method of Delivery Effects. The largest and most significant result was demonstrated with i.a. administration and

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Example calculation:

% accessible tissue space in ICT at 16 days after MTX given i.a. with BBBD:

Baseline: 1.307
i.a.: 0.398
BBBD: 0.320
Time × BBBD (total 8 days): -0.208

Antilog 1.817 = 65.61% accessible tissue space

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a Numbers in parentheses,  P values for each coefficient.
b NE, not evaluated in the model due to lack of significance.
Based on Table 1, the estimated percentage of accessible tissue space (ml/g) values for AIB (A), MTX (B), and DEX70 (C) are shown at 8 days after inoculation of LX-1 human small cell lung carcinoma for four treatment groups: i.v. (IV) or i.a. (IA) agent administration after normal saline or BBBD. Least squares regression analyses of log-transformed data from multiple time point measurements were used to determine estimates ($R^2$, 0.90–0.97).

**BBBD treatment.** For all agents, there was a significant increase in delivery with i.a. agent administration compared with i.v. administration ($P < 0.0001$) in ICT (2.5-fold), BAT (2.4-fold), and BDT (2.2-fold; Fig. 1). There was also a significant increase with BBBD treatment ($P < 0.0001$) in ICT (2.5-fold), BAT (5.8-fold), and BDT (7.6-fold; Figs. 1 and 2). Thus, the combination effect (product) of i.a. administration and BBBD was an increase in delivery of ~6.3-fold in ICT, 13.9-fold in BAT, and 16.7-fold in BDT.

**Tumor Size Effects.** The effect of time (tumor progression) on delivery was not a significant factor by itself in any tissue except SQT, where there was a 36% decrease in delivery between 8 and 16 days after inoculation. During progression, the SQT undergoes marked cystic degeneration. In SQT, i.a. agent administration was associated with an additional decrease in delivery of 23% ($P < 0.05$) over the 8-day experimental period of tumor progression (time × i.a. interaction), although the SQT was not exposed to direct i.a. agent administration. There was also a time effect for all agents with BBBD treatment (time × BBBD interaction) in ICT ($P = 0.0518$; Fig. 2) and BAT ($P = 0.0483$), resulting in a decline in the beneficial effect of BBBD of 38% in ICT and 46% in BAT from 8 to 16 days after inoculation.
Additional Interaction Effects. Three other isolated instances of interactions in conjunction with BBBD, regardless of route, occurred with specific agents in specific tissues, the biological relevance of which is unclear. After MTX administration and BBBD (MTX × BBBD interaction), there was a 50% decrease in the increased effect of BBBD in ICT (P < 0.05). Consequently, there was only a 1.25-fold increase in MTX delivery in ICT compared with the overall 2.5-fold effect of BBBD. After AIB administration and BBBD (AIB × BBBD interaction), there was an additional increase of 73% in BAT (P < 0.05), resulting in an overall increased effect of BBBD of 9.9-fold. In SQ1, the effect of AIB × BBBD showed a decrease of 22% in SQ1 (P < 0.05; Fig. 1 and Table 1).

The plasma curves (dpm/ml) over the 10-min experimental period were evaluated by agent and treatment. At the 0- and 2-min sample points, average plasma levels were significantly (P < 0.05) higher (25–45%) for saline-infused animals compared with BBBD animals. Thereafter, levels were nearly equal. Average plasma levels were also higher (~23%) for i.v. compared with i.a. When comparing agents, DEX70 had the highest levels and the slowest clearance. AIB and MTX had similar clearance rates. Average plasma levels were significantly different between all three agents; dpm/ml over the experimental period was 1–2 × 10^7 for DEX70, 1–6.5 × 10^6 for AIB, and 2–8.5 × 10^5 for MTX.

DISCUSSION

It has been shown that there is BBB heterogeneity within a given intracerebral tumor (34–36) and also differential permeability of a given tumor to agents with different molecular weights (19, 21, 37, 38). Generally, there is increasing tumor permeability with increasing tumor size (21, 37, 39), and there may be decreasing permeability with decreasing tumor size (10). Decreasing permeability with time after therapy may partially explain the transient nature of some chemotherapeutic responses (10).

In the present report, we evaluated i.v. and i.a. administration and BBBD treatment in the context of increasing tumor size and different molecular weight agents. To quantify delivery, the percentage of accessible tissue space was calculated. As described previously, the percentage of accessible tissue space correlates with a time-dependent functional measure of capillary permeability (permeability × capillary surface area), as measured in a previous study with the same biologically diverse agents (21). Changes in intravascular content did not account for measured differences in this model. There were minimal differences in intravascular plasma volume, determined as described previously (21). After i.v. administration, plasma volume was 1–2% in brain samples and 3–4% in SQ; after i.a. administration, plasma volume increased 1–3% in ipsilateral brain and ICT but had no effect in contralateral brain or SQ. Preliminary clinical data\(^4\) looking at cerebrospinal fluid drug levels indicate similar results with i.v., i.a., and i.a. + BBBD as reported here.

i.a. administration increased delivery regardless of baseline permeability. Comparing delivery to ICT, BAT, and BDT for each agent, large differences were found in the baseline values between these tissues, but very similar values were found following BBBD with the smaller agents AIB and MTX. The tumor: normal ipsilateral brain ratio (ICT: BDT) for i.v. AIB and MTX (at 8 days) changed from a baseline of 5.1 and 6.3 to 1.04 and 0.97 after BBBD, respectively. Thus, the tumor and normal ipsilateral brain levels were nearly equal after BBBD. This may reflect a technical balance and limit of osmotic barrier disruption in this model and demonstrates that a partial natural disruption of the BTB is initially nearer this limit than the totally

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intact BBB (29). The ICT:BDT for i.v. DEX70, in contrast to AIB and MTX, changed from a baseline of 1.58 to 0.53 after BBBD. The smaller DEX70 ratios may reflect the decreased convection associated with macromolecules, as reported by Jain (40).

When i.a. agent administration and BBBD were combined, there was 6–17-fold increase in delivery in ICT, BAT, and BDT. These effects were not seen in LH or SQT. For unclear reasons, there was a negative interaction of MTX and BBBD in ICT as well as a positive interaction of AIB and BBBD in BAT. These isolated effects are incongruous with the rest of the study and may be due to animal variability and/or related to tumor size. However, for all agents, the effect of BBBD was reduced by 38% in ICT (P = 0.05) and by 46% in BAT (P = 0.05) with tumor progression (time) between 8 and 16 days after inoculation. This can be appreciated in Fig. 2 and corresponds to the negative regression coefficient seen for time × BBBD in Table 1. Tumor progression between 8 and 16 days corresponds to a tumor volume increase of ∼10 mm³ to >100 mm³, as reported previously (21). Even at 8 days after inoculation, these tumors were of sufficient size to be visually apparent, with a large portion showing a necrotic center (21). The differences in the effects of BBBD may be much more dramatic with smaller, even microscopic, tumors where barrier integrity is even less compromised, compared with the large 16-day tumors. Therefore, when intracerebral tumors are large, it may be possible to obtain an initial tumor response with i.a. administration alone. This scenario is consistent with our current clinical experience and is compatible with a model in which brain tumor chemotherapy is “barrier independent.” As tumors respond to chemotherapy or in smaller tumors, however, the data observed in the present report strongly support the suggestion that chemotherapy delivery is “barrier dependent,” and it becomes advantageous to optimize drug delivery to both tumor and tumor-infiltrated surrounding brain. This may be especially important when considering the source-sink relationship between tumor and brain (23, 41). BBBD may be particularly advantageous clinically in tumors such as diffusely infiltrating gliomas or lymphomas. Supporting the recent report by Zünkeler et al. (12), there was a marked advantage in delivery to BAT as well as to BDT with i.a. administration with BBBD (12).

As reported previously by Barnett et al. (21), the permeability of LX-1 intracerebral tumor is an order of magnitude less for DEX70 than it is for either AIB or MTX (21). This present report shows that the relative increase in delivery to ICT with i.a. administration and BBBD for DEX70 is of the same order of magnitude as with AIB and MTX (6.3-fold), although the baseline permeability for DEX70 is more than one log less. This also held true for BAT, BDT, and SQT compared with the smaller molecular weight agents, despite the fact that DEX70 has a much slower plasma clearance than the other two agents. Thus, although the BBB and BTB exhibit size-dependent differential permeability to different molecular weight agents, the relative effects of i.a. and BBBD appear to be the same. Therefore, as was shown previously in the intracerebral human carcinoma 417D model, delivery to tumor and surrounding brain is significantly more difficult for large molecular weight agents than for smaller water-soluble agents (30).

In summary, this study demonstrates: (a) i.a. agent administration with BBBD dramatically increases delivery to ICT and surrounding brain; (b) BBBD becomes less effective in ICT and BAT with increasing tumor size; and (c) there are marked differences between different molecular weight agents (differential permeability), regardless of treatment. The role of osmotic BBBD may be most important in smaller and/or less permeable tumors, in tumors that exhibit an initial response to chemotherapy, and in tumor-infiltrated surrounding brain, particularly when administering larger molecular weight agents.

REFERENCES


