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BRAIN TUMORS IN ANIMALS: PAST, PRESENT AND FUTURE

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The Past: Surgery, Irradiation and Chemotherapy

The major goals of therapy for a brain tumor have been to control secondary effects, such as increased intracranial pressure or cerebral edema, and to eradicate the tumor or reduce its size. Beyond general efforts to maintain homeostasis, palliative therapy for dogs or cats with a brain tumor has consisted of glucocorticoids for edema reduction, and in some cases (e.g., lymphoma), for retardation of tumor growth. Some animals with a brain tumor will demonstrate dramatic improvement in clinical signs for weeks or months with sustained glucocorticoid therapy. Should seizure therapy be needed, phenobarbital or bromide are the drugs best suited for the control of generalized seizures.

Three major methods of therapy for a brain tumor have been available for use in dogs and cats: surgery, irradiation, and chemotherapy.

Surgery. In association with the availability of CT and MRI, and the development of advanced neurosurgical, anesthetic, and critical care techniques, complete or partial surgical removal of intracranial neoplasms has been practiced with increasing frequency. Neurosurgical intervention is an essential consideration in the management of intracranial neoplasms of cats or dogs, whether for complete excision, partial removal, or biopsy.

Radiation Therapy. The use of radiation therapy for the treatment of primary brain tumors of dogs and cats is well established. Irradiation may be used either alone or in combination with other treatments. Radiation therapy also is recommended for the treatment of secondary brain tumors. Metastases, pituitary macroadenomas or macrocarcinomas, and skull tumors have been successfully managed by means of either radiation therapy alone or as an adjunct to surgery. Lymphoma may also be sensitive to radiation therapy.

Chemotherapy. Traditionally, cytotoxic drugs have had a limited role in the treatment of dogs or cats with brain tumors, and progress in the development of truly effective chemotherapeutic protocols for humans or companion animals has been slow. Several factors affect the use of chemotherapeutic agents for the treatment of brain tumors in dogs or cats. The first, unique to the brain, is that the blood-brain barrier may prevent exposure of all or some of the tumor to a chemotherapeutic agent injected parenterally. Second, tumor cell heterogeneity may be such that only certain cells within a tumor are sensitive to a given agent. Third, a tumor may be sensitive only at dosages that are toxic to the normal brain or other organs

The Present: Therapeutic Delivery Strategies for Canine Brain Tumors

Development of novel therapeutic strategies to combat primary brain tumors has followed closely behind elucidation of the basic molecular and genetic mechanisms underlying both tumorigenesis and subsequent progression. Despite the wealth of data documenting successful treatment of experimental tumors, translation into the clinical setting has been slow. Many existing therapeutics are rendered ineffective in the treatment of brain tumors due to the inability to effectively deliver and sustain them within the brain. The major obstacle to therapeutic delivery via the vascular route (following either orally administration or direct vascular administration) is the blood brain barrier (BBB).

Transport across the brain vascular endothelium is essentially trans-cellular, therefore the ideal substance to be transported should be:

1. Small (< 400Da)
2. Lipophilic (lipid soluble)
3. Non-polar at physiological pH
4. Non-protein bound

Unfortunately, a majority of chemotherapeutic agents are large positively charged, hydrophilic molecules. Many therapeutic molecules such as cyclosporine, doxorubicin and vincristine have poor BBB penetration despite being lipophilic (cyclosporine A is more lipophilic than diazepam). This is the result of additional “barriers” such as high levels of degrading enzymes within the endothelial cells, and high concentrations of efflux transporter proteins such as P-glycoprotein, multiple organic anion transporter proteins (MOAT) and multi-drug-resistance proteins (MRP).

In addition to barriers preventing movement of therapeutic agents from the blood into the brain parenchyma, mechanisms are also present to limit movement into the cerebrospinal fluid (CSF). Passage of substances through the arachnoid membrane is prevented by tight junctions and is generally impermeable to hydrophilic molecules. While the capillaries of the choroids plexus are fenestrated, non continuous and allow free movement of small molecules, the adjacent choroidal epithelial cells form tight junctions preventing the passage of most macromolecules. An active organic acid transporter system in the choroid plexus also is capable of driving therapeutic organic acids such as penicillin or methotrexate, back into the blood from the CSF. Entry of drugs into the CSF does not necessarily guarantee that they will reach the interstitial fluid in the brain, suggesting the presence of the so-called CSF-brain barrier, mainly attributed to insurmountable diffusion distances required to equilibrate CSF with brain interstitial fluid.

Although the BBB may be inconsistently compromised in tumor vasculature a variety of obstacles still restrict delivery of therapeutic agents. Tumor microvascular supply often is heterogeneous and chaotic, with significant areas

of inefficient or poor blood flow, vascular shunting, blind ending vessels etc resulting in erratic distribution of drugs if they are able to penetrate the BBB.

Improving delivery of therapeutic agents to brain tumors in the face of these obstacles has focused on the following areas of research.

1. Improve entry through the BBB by modification of therapeutic drugs.
    a. Increase influx.
    b. Decrease efflux.
    c. Utilization of carriers/receptors.
2. Disruption of the BBB.

A variety of approaches have been used to disrupt BBB integrity including:

    a. Chemical (often toxic), DMSO, ethanol, aluminium, irradiation, hypertension, hypercapnia, hypoxia
    b. Osmotic agents such as mannitol and arabinose.
    c. Biochemical agents such as leukotriene C4, bradykinin, histamine etc.

3. Circumventing the BBB.

Using non-vascular delivery of therapeutic agents directly into the CNS is appealing in many ways. Apart from removing the BBB as a restriction to delivery of many potent anticancer therapies, targeting the drugs directly potentially reduces systemic toxicity, degradation and immunological stimulation (particularly with protein and virally based therapies). However strategies are generally more invasive requiring craniotomy or insertion of catheters.

    a. Intraventricular/intrathecal infusion.
    b. Wafers/microspheres/microchips
    c. Delivery from biological tissues (Gene Therapy):

    Delivery of therapies directly from living cells within the brain or tumor itself can provide sustained levels of drugs in specific targeted regions. The two main strategies examined to date are:

        i. Implantation of transfected cell lines.
        ii. Transduction of resident CNS cells or brain tumor cells with gene therapy constructs

4. Interstitial delivery.

Both gene therapies and direct acting drugs, such as chemotherapeutics, can be delivered directly into tumor or brain parenchyma. AAV vectors carrying thymidine kinase suicide constructs and antiangiogenic agents have been shown to be efficacious in both in vitro and in vivo models, and direct injection into canine primary brain tumors has been done. Results in clinical tumors however have been disappointing mainly due to limited distribution of the therapy beyond the local region of the injection site.

The Future: Biopsy and Convection Enhanced Delivery

Biopsy remains the sole method available for the definitive diagnosis of brain tumor type in cats or dogs, and is an essential step prior to consideration of any type of therapy. However, biopsy is not always attempted because of practical considerations, such as cost and morbidity. The most recent advance in the biopsy of brain tumors of dogs and cats has been the modification of a CT-guided stereotactic brain biopsy system for use in cats and dogs. This CT-guided stereotactic biopsy system provides a relatively rapid and extremely accurate means of tumor biopsy, with a low rate of complications. Cytological evaluation of brain tumor smear preparations, rapidly fixed in 95% alcohol and stained with hematoxylin and eosin, may be done within minutes of biopsy collection. Diagnostically accurate information from this rapid technique is generally available from both primary and metastatic nervous system tumors.

Convection enhanced delivery (CED) is a local delivery technique that utilizes a bulk-flow mechanism to deliver and distribute macromolecules over clinically relevant volumes of targeted tissue. Unlike local injection techniques, CED uses a pressure gradient established at the tip of an infusion catheter that pushes the infusate through the interstitial space. Volumes of distribution of infused molecules are significantly increased compared to local injection or surgical implantation methods that rely primarily on diffusion and are limited by concentration gradients and molecular weight of the delivered substance. Distribution of infusates over centimeters, rather than millimeters, have been reported in a variety of experimental model systems using CED. Real time *in vivo* imaging of CED is an essential consideration if adequate drug distribution is to be confirmed ante mortem. Additionally, the ability to detect and minimize distribution or leakage of drugs to normal tissues during delivery has the potential to significantly decrease toxicity and increase therapeutic index. Several surrogate marker systems have been described, facilitating image-guided CED, including magnetic resonance imaging (MRI) systems utilizing T2 imaging correlated with ^{123}I-labelled serum albumin single photon emission computed tomography (SPECT), and liposomes co-labeled with gadolinium. Liposomes are phospholipid nanoparticles composed of a bi-layered membrane capable of encapsulating a variety of therapeutic molecules. Liposomal encapsulation of a variety of drugs, including chemotherapeutics, has been shown to result in prolonged half-life, sustained release, and decreased toxicity. CED of liposomes, containing therapeutic drugs, directly into targeted brain tissue offers several advantages over systemic delivery of unencapsulated drug, including bypassing of the blood-brain barrier, increased volume of distribution within the target tissue, and increased therapeutic index as a result of both liposomal encapsulation and minimal systemic exposure. Irinotecan/CPT-11 is a camptothecin derivative and topoisomerase I inhibitor with activity against a variety of cancer types, including brain tumors. We previously reported the efficacy and safety of and efficacy and safety of direct delivery of liposomally encapsulated camptothecin analogs in rodent models of glioma. Translation of this promising therapeutic approach into clinical trials will require demonstration

of the safety and efficacy of combined real time gadolinium based imaging and liposomally encapsulated CPT-11 treatment in a large animal model system. The advantages of a canine model system over established rodent and primate models are several and include the ability to investigate aspects of feasibility and toxicity on a scale relevant to human clinical patients, and the unique potential to investigate CED efficacy, and adverse effects in large, spontaneously occurring tumors.

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