Surface properties, more than size, limiting convective distribution of virus-sized particles and viruses in the central nervous system

MICHAEL Y. CHEN, M.D., ALAN HOFFER, M.D., PAUL F. MORRISON, PH.D., JOHN F. HAMILTON, M.D., PH.D., JEFFREY HUGHES, PH.D., KURT S. SCHLAGETER, PH.D., JEONGWU LEE, PH.D., BRANDON R. KELLY, B.S., AND EDWARD H. OLDFIELD, M.D.

Surgical Neurology Branch and the Division of Bioengineering and Physical Science, ORS National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland; and Department of Neurosurgery, Virginia Commonwealth University School of Medicine, Richmond, Virginia

Object. Achieving distribution of gene-carrying vectors is a major barrier to the clinical application of gene therapy. Because of the blood–brain barrier, the distribution of genetic vectors to the central nervous system (CNS) is even more challenging than delivery to other tissues. Direct intraparenchymal microinfusion, a minimally invasive technique, uses bulk flow (convection) to distribute suspensions of macromolecules widely through the extracellular space (convection-enhanced delivery [CED]). Although acute injection into solid tissue is often used for delivery of oligonucleotides, viruses, and liposomes, and there is preliminary evidence that certain of these large particles can spread through the interstitial space of the brain by the use of convection, the use of CED for distribution of viruses in the brain has not been systematically examined. That is the goal of this study.

Methods. Investigators used a rodent model to examine the influence of size, osmolarity of buffering solutions, and surface coating on the volumetric distribution of virus-sized nanoparticles and viruses (adenovirus-associated viruses and adenoviruses) in the gray matter of the brain. The results demonstrate that channels in the extracellular space of gray matter in the brain are large enough to accommodate virus-sized particles and that the surface characteristics are critical determinants for distribution of viruses in the brain by convection.

Conclusions. These results indicate that convective distribution can be used to distribute therapeutic viral vectors in the CNS.

Key Words • gene therapy • brain • convection • drug delivery • virus • rat

Gene therapy in solid tissues requires transfection of a therapeutic gene into a clinically useful volume of tissue. This is particularly challenging in the CNS because of the BBB. Findings in previous studies have demonstrated that intrathecal, intraventricular, intravascular, and acute parenchymal injections can distribute virus-sized vectors only to limited volumes of brain tissue in small animals.25,28,30 Intrathecal and intraventricular injections deposit therapeutic agents directly into CSF, which has several limitations. Both of these delivery approaches depend on diffusion, in which distribution is limited by the molecular weight and concentration of the infused agent and is associated with an exponential loss of concentration beyond the CSF–tissue interface. In addition, any diffusion into brain tissue that occurs must overcome any outflow of CSF from the brain, as well as pass through the subependymal or pia mater layers. Small molecules such as sucrose will distribute a few millimeters despite these obstacles,9,10,26 however, larger particles, such as unmetabolized proteins and viruses, have a much greater difficulty penetrating this deeply into tissue within a reasonable time.9,26,28 As a result, these techniques have not been successful in the clinical setting.

Intravascular delivery, the least invasive delivery approach, is the simplest procedure used to deliver drug to solid tissues, including the CNS. Nevertheless, this method fails in the delivery of large molecules or vectors, because channels through the BBB are small enough to inhibit the passage of even small macromolecules. Hence no report exists of robust delivery of virus-sized molecules into the brain after intravascular injection. Additionally, this method does not lend itself to targeting a specific region of the brain.

Another approach for distribution of macromolecules
that may enhance distribution of virus-sized particles in solid tissues is CED. Convection-enhanced delivery relies on bulk flow to carry macromolecules through the tissue extracellular space. Instead of being restricted by the BBB, CED uses this barrier to its advantage by restricting the access of infused agents to the systemic circulation. Unlike diffusion, which relies on a concentration gradient, CED depends on an externally generated pressure gradient to distribute a relatively homogeneous concentration of compounds to discrete anatomical targets of various sizes.

This method of delivery should also carry particles significantly farther than can be achieved using diffusion alone. Furthermore, by delivering the complete dose at the target site, smaller quantities of agent may be administered than required for systemic dosing, thus reducing systemic exposure and toxicity relative to other modes of delivery. Successful and safe use of convective delivery of macromolecules in the laboratory and clinic has been demonstrated (NIH protocol No. 13445-21). For these reasons, CED is a suitable candidate for the delivery of gene therapy vectors to clinically relevant regions of the CNS.

The objectives of this study were to determine the feasibility of using convective delivery of viral vectors into gray matter and to examine systematically the important variables associated with it. We measured the distributions of virus-sized nanoparticles (polystyrene nanospheres), adenovirus particles, and endovascular vectors in rat striata, and compared them with the dispersion of albumin, a 69-kD inert protein that is small relative to viruses. Albumin is a marker compound that can be delivered to large volumes of brain by convection and is transported without significant retardation or short-term loss through the extracellular milieu. In this report we demonstrate that convection also can be used to distribute particles the size of AAV and that CED can distribute AAV and adenovirus into the gray matter of the CNS. The results indicate that, within the size range of these particles, surface properties can be dominant factors for distribution of viruses by CED and, like albumin, perfusion of a relatively large volume of brain with viruses should be achievable with CED. Thus, use of this simple delivery approach can potentially achieve intracerebral distribution of gene transfer vectors for laboratory and clinical use.

Materials and Methods

Animal Preparation

One hundred forty-four female Sprague–Dawley rats, weighing 250 to 300 g each, were used in the experiments. All procedures were performed in accordance with the regulations of the Animal Care and Use Committee of the NINDS and the NIH Radiation Safety Committee.

Polystyrene Nanosphere Particles as Surrogates of Viral Vectors

Because extensions of cell bodies have the potential to amplify the distribution of transgene expression well beyond the boundaries of vector distribution, viral distribution may significantly differ from the region of transgene expression, especially in the CNS. To isolate the measurement of particle delivery and distribution rather than detection of the distribution of viral expression, polystyrene nanospheres were used to model viral particles. Through control of particle size, surface properties, and the osmolarity of buffering solutions, the use of nanospheres permitted isolation of variables that may influence the convective movement of particles through the interstitial space.

We used polystyrene nanospheres measuring 20, 40, 100, and 200 nm in diameter (Bang Laboratories, Fishers, IN). We chose this size range to cover the wide spectrum of viral sizes used in gene therapy. The initial surface charge densities of standard (20-, 40-, 100-, and 200-nm), super-low-charged (40-nm), and high-charged (100-nm) nanospheres were 0.01 ± 0.001, 0.002, and 0.043 carboxyl groups/Å², respectively. Nanospheres were radiolabeled by incorporation of 14C-phenanthrene (specific activity 55 mCi/mmol; American Radiolabeled Chemicals, St. Louis, MO). Stock nanospheres were diluted to 4% solids with ultrapure water and 24 mmol 3-3-cholamidopropyl dimethylammonio-1-propanesulfonate. The nanosphere solution was incubated for 24 hours in a mixture of 14C-phenanthrene (4 mg/ml) and dimethyl sulfoxide. Following incorporation of 14C-phenanthrene, the bead solutions were dialyzed (Slide-A-Lyzer cassettes, 10,000-MW pores; Pierce Chemical Co., Rockford, IL) four times with solutions of PBS that had ionic concentrations that sequentially increased from 75 to 290 mOsm. The final dialytes contained less than 3% of the total radioactivity and were stable for at least 1 month after production. Some nanospheres from each group were incubated overnight in a 4.5% solution of BSA. Because no polystyrene nanospheres smaller than 20 nm were available, the distributions of the viruses and nanospheres were compared with 14C-BSA (specific activity 0.024 mCi/mg; New England Nuclear, Boston, MA). The concentration of nanospheres in the infusate was approximately 5 × 10^14 particles/ml and, unless otherwise indicated, the nanospheres were suspended in 290 mOsm PBS.

Selection of Viral Vectors

Type 2 AAV-CMV-GFP (10^11 particles/ml) was obtained from the laboratory of Dr. Muszuka (University of Florida, Gainesville FL). Type 5 Ad-CMV-GFP (10^12 particles/ml) was purchased from Quantum Biotecnologies (Montreal, QB, Canada). The AAV was suspended in a solution of 4% lactated Ringer solution (290 mOsm), whereas the adenovirus was suspended in 200 units/ml 4% sucrose, and 2 mM MgCl₂. To determine if the albumin would have an effect on distribution, 40% albumin was mixed 1:7 with the adenoviral suspensions.

Adeno-associated virus and adenovirus were labeled with CY5 Alexa 546 (Molecular Probes, Eugene, OR) and CY3 PA33000 (Amersham Pharmaceuticals, Piscataway, NJ), respectively, by using kits and following the manufacturers’ instructions. Purification of fluorescently labeled virus was accomplished using gel permeation chromatography (AAV) or cesium banding (adenovirus). To ensure that there was no unbound fluorescent label, spectroscopy was performed on the CY5-AAV ultrafiltrate. To verify that the AAV remained labeled once infused, sections of the rat brain were homogenized and incubated for 24 hours. Specimens were then centrifuged through a 30,000-D microcon filter (Millipore, Billerica, MA) and analyzed for signal intensity (< 5%). The ultrafiltrate was infused into animals as a control.

For transfection efficiency experiments, U2OS human osteosarcoma cells (2 × 10⁶ cells/well suspended in either 1 × PBS or 1 × PBS with 4.5% BSA) were exposed to Ad-GFP-BGal (multiplicity of infection of 2, 5, or 10) for 10, 30, or 60 minutes. After centrifugation, the cells were separated from the medium and resuspended in fetal bovine serum with Dulbecco modified Eagle medium. Counts for cells expressing GFP were performed 2 days after infection.

Assessment of Aggregation

One disadvantage of working with nanospheres is that, at salt concentrations higher than those characteristic of interstitial fluid, particularly in combination with divalent cations (as in CSF), the particles tend to aggregate. We investigated this to occur during CED in distributional channels characterized by the diameter of a single nanosphere, aggregate formation would inhibit particle movement. Accordingly, a baseline study involving dynamic light scattering (DLS-Protein Solutions, Viscotek Europe Ltd., Crowthorne, Berkshire, United Kingdom) was initiated to measure the hydrodynamic radii of particles and to determine whether any aggregation was indi-
Convective distribution of viral vectors in the rat brain

cated in vitro by increases in the radii in the isotonic or subisotonic domains. Measurements were made of stock nanospheres and phenanthrene-loaded nanospheres (~5 × 10^11 particles/ml) in 29 mOsm PBS, 290 mOsm PBS, artificial CSF, and artificial CSF with 0.1% or 4.5% albumin.

**Animal Experiments**

A protocol listing all animal procedures was approved by the NINDS Animal Care and Use Committee and all animal procedures met the NIH criteria for animal experimentation. The method we used for stereotactic injection of suspensions into the rat striatum has been previously described. Briefly, the animals were anesthetized using an intraperitoneal injection of 1 ml/kg ketamine (100 mg/ml) containing 1% Rompun (Bayer AG, Leverkusen, Germany) and were placed in a stereotactic frame. A syringe with a 32-gauge needle was lowered into the striatum according to the following coordinates specified in a rat stereotactic atlas: anteroposterior +0.5 mm, lateral +2.8 mm, and depth −5.5 mm from the bregma. Unless otherwise specified, 4 μl of the various infusates was infused at 0.1 μl/minute for all experiments. (We chose this relatively slow rate because in previous studies undertaken in our laboratory we found that convective delivery of albumin at a rate of 0.1 to 0.5 μl/minute did not cause backward leakage of the infusate along the catheter track. Moreover, because the particles were significantly larger than albumin, it was thought that the more conservative rate would further decrease the possibility of backward leakage.) For experiments that did not involve the assessment of viral gene expression, the animals were immediately killed and their brains were removed and snap frozen in isopentane at −20°C. The brain specimens were stored at −70°C. For adenoviral experiments involving viral gene expression, animals were kept alive 3 days before being killed. For toxicity studies in which we examined the effect of infusate osmolarity the animals were also kept alive for 3 days.

**Tissue Analysis**

Coronal brain sections were serially sectioned at 20-μm thickness on a cryostat (−18°C). One of every five sections was mounted on saline-coated slides. For quantitative autoradiography, these slides were exposed (4 hr/30,000 cpm/μl bead solution) to emulsion film (Biomax MR; Eastman–Kodak, Rochester, NY). The autoradiograms were analyzed using NIH Image version 1.62 (created by W. Rasband, NIH, Bethesda, MD). Optical densities were correlated to radioactive standards with known tissue equivalents. Boundaries of infusion were defined by a threshold of approximately 10% of the maximal signal. The area from each slice was multiplied by the slice thickness of the five slices (0.1 mm) and this was summed to determine the V_d.

Green fluorescent protein was visualized using a FluoView microscope (Olympus Corp., Tokyo, Japan) with an argon laser and a 2 × objective. Confocal images were captured using FluoView software and analyzed using NIH Image software. Areas of viral gene expression were manually outlined and summed to determine the V_d. The distribution of the AAV and adenovirus capsids was examined using a charge-coupled device camera to capture images on an epitofluorescence microscope (Carl Zeiss, AG, Oberkochen, Switzerland). The area of distribution within each slice was evaluated using NIH Image software with a threshold of 10% of maximal signal intensity, and the volume was reconstructed as stated earlier.

**Statistical Analysis**

Analysis of variance and the Fisher protected least significant difference test were used to determine statistical significance. A probability level less than 0.05 was deemed significant. The tests were performed using a Power Macintosh 9600/300 (Apple Computer, Cupertino, CA) with Statview statistical software (SAS Institute, Cary, NC).

**Results**

**Coated and Uncoated Nanospheres in Vitro**

Dynamic light scattering measurements revealed that the particle radii did not change significantly in 20 mOsm PBS, 290 mOsm PBS (isotonic solution), artificial CSF, artificial CSF with 0.1% albumin (a concentration similar to that of CNS interstitial albumin), or artificial CSF with 4.5% albumin (the concentration of serum albumin). In nanospheres (20, 40, 100, and 200 nm in diameter), which were mixed in artificial CSF with 4.5% albumin there was a mean increase in the radius of 7.3 nm, indicating not aggregation but the formation of a monolayer coat of albumin (6.5–7 nm in diameter) around the nanosphere.

**Isotonic polystyrene nanospheres have restricted mobility in vivo.** Having established the absence of aggregation in response to changes in the osmolality of CSF in vitro, we examined the mobility of polystyrene nanospheres in rat striata. The distributions of carboxylated polystyrene nanospheres with identical charge densities and diameters of 20, 40, 100, or 200 nm were compared with the distribution of 14C-labeled albumin (Figs. 1 and 2 upper). The V_d of the nanospheres rapidly decreased as the particle size increased, with an apparent maximum diameter threshold of 40 nm for convective distribution of these particles into the extracellular space of the CNS. The V_d of the 20-nm diameter spheres (diameter similar to AAV) was approximately half the V_d of albumin. Nevertheless, particles with diameters of 40 nm or larger demonstrated minimal distribution (a 20-fold less V_d) into the gray matter compared with 14C-albumin (Figs. 1 and 2 upper).

**Effects of Altering Particle Characteristics**

After determining that CED did not lead to the wide distribution of polystyrene nanospheres, we examined the stratal distribution of GFP expression by adenovirus (80–90 nm diameter) containing the CMV promoter and the GFP gene (Ad-CMV-GFP) for a comparison with the results obtained using the nanospheres (Figs. 2 upper and 3). To our surprise the volume of GFP expression after infusion of 4 μl
Fig. 2. Upper: Bar graph showing the V_d of nanospheres of graded sizes, virus capsids, and adenoviral gene expression following CED of 4 μl infusate into the rat striatum. Bovine serum albumin is a 7-nm-diameter macromolecule that freely distributes in the extracellular space. The hatched columns indicate particles. Columns representing particles coated with albumin before convective infusion are indicated by “alb.” The V_d of AAV capsid, adenovirus (Adeno) capsid, and adenoviral gene expression are also shown. Surface properties, more than size, influence distribution. Note the greater distribution of adenovirus capsids compared with AAV capsids, despite the much smaller size of AAV, and that the V_d of the AAV capsids significantly exceeds the V_d of particles of a similar size. Increasing the V_d of the AAV capsids by twofold nearly doubles the V_d (p < 0.05). Pretreatment with albumin enhances the distribution of nanospheres.
Convective distribution of viral vectors in the rat brain

**AAV**

Capsid

**Adenovirus**

Capsid

GFP Expression

Fig. 3. Distribution of fluorescent virus capsids and gene expression. After stereotactic convective distribution of 4 μl of AAV (diameter 23 nm) and adenoviruses (diameter 80–90 nm) with fluorochromes attached to their capsids (CY5-aAV) into rat striata, animals were immediately killed and the distribution of the virus was compared with the distribution of adenoviral gene expression (Ad-CMV-GFP; animals killed after 3 days). A confocal microscope with a 2 × lens was used to visualize labeled capsids and GFP. Thin yellow bar = 1 mm.

of Ad-CMV-GFP was similar to the $V_d$ of an equal amount of $^{14}C$-albumin. Based on particle size alone, the $V_d$ of nanospheres with smaller or equivalent diameters (20, 40, and 100 nm) should be at least as large as the adenovirus (80–90 nm in diameter), yet the $V_d$ of the nanospheres were markedly smaller. This raised the possibilities that aggregation was occurring in vivo, the particles were adsorbing to elements of the interstitial matrix, and/or that factors other than those relating to size or adsorption were influencing particle mobility in the extracellular space. Consequently, a further investigation into the effects of particle coatings (to block aggregation or matrix adsorption) and osmolarity of buffering solutions was undertaken.

**Particles Coated With Albumin**

Nanospheres measuring 20, 40, 100, and 200 nm were coated with a monolayer of albumin (confirmed by dynamic light scattering) by exposing them overnight to a 4.5% albumin solution. Infusions (four for each size) produced $V_d$ for 40-, 100-, and 200-nm diameter nanospheres that were significantly larger than that which occurred with their uncoated counterparts, although the $V_d$ of $^{14}C$-albumin were still less than the $V_d$ of $^{14}C$-albumin (Fig. 2 upper and lower).

The ionic strength of buffer does not alter particle distribution. Despite alterations in surface coating, the distribution of 100 nm nanospheres was considerably less than the distribution of Ad-CMV-GFP (80–90 nm diameter). One possible reason for this discrepancy was the difference in the ionic strengths of the buffers that suspended the polystyrene particles and the virus (290 and 145 mOsm, respectively). To examine this possibility, the osmolarity of buffering solutions was varied to determine its effect on the distribution of convectively distributed macromolecules and nanospheres. Although the dispersion of $^{14}C$-albumin was affected at extremely high and low osmolarities (Fig. 4), there was a wide range near physiological osmolarity over which the $V_d$ remained unchanged. Correspondingly, particles 20, 40, 100, and 200 nm in diameter, which were suspended in 145 mOsm PBS solutions with and without albumin coating (four animals in each group), had the same distribution as their isotonic counterparts (Fig. 4). To deter-

and of adenoviral GFP expression (*p < 0.05). Following the convective delivery of 4 μl $^{14}C$-phenanthrene–labeled uncoated and albumin-coated nanospheres, CY5-labeled AAV and adenovirus capsids (10$^{12}$ particles/ml), and adenovirus encoding GFP (10$^{12}$ particles/ml), the $V_d$ was quantified by autoradiography (albumin and nanospheres) or fluoroscopy (capsids and GFP expression). *p < 0.005. Lower: Graph showing the correlation between particle size and distribution of nanospheres with and without albumin pretreatment. The $V_d$ of nanospheres in rat striata was inversely proportional to the size of the particle. The $V_d$ of albumin-coated particles is significantly (asterisks) larger than their uncoated counterparts for 40-, 100-, and 200-nm beads (p < 0.05).

J. Neurosurg. / Volume 103 / August, 2005

315
mine the safety of altering the osmolarity, four rats that received striatal infusions of 145 and 450 mOsm 4.5% albumin were examined. They exhibited no behavioral or neurological deficits during the 3 days they were allowed to remain alive. Hematoxylin and eosin and glial fibrillary acidic protein staining revealed only minimal gliosis around the needle track and edema that was only slightly more pronounced in the 145-mOsm group.

**Distribution of Virus and Viral Gene Expression**

Viruses and polystyrene nanospheres have different surface characteristics and thus the CED of AAV (diameter 23 nm) and adenoviruses (diameter 80–90 nm) with fluorochromes attached to their capsids were compared with the distribution of similarly sized nanospheres. Fluorescent CY5–labeled AAV capsids (CY5-AAV) were stereotactically convected (4 μl) into rat striata and the animals were immediately killed. The distribution of CY5-AAV capsids (23 nm in diameter) was 12.86 ± 1.54 mm² (mean ± standard deviation) when the excitation intensity was 100% (Figs. 2 upper and 3), which was less than the spread of 4C-albumin but in the same range as the 20-nm-diameter polystyrene beads. Retardation of movement of CY5-AAV particles could have resulted in the formation of a concentration gradient in which the concentration of particles at the center of the injection site is greater than the concentration of particles at the periphery. To detect this gradient, the CY5-AAV distribution was quantified using an excitation intensity of 50%; the apparent Vₐ would decrease if the concentration of particles at the periphery was less because fluorescence is almost linearly proportional to concentration). The Vₐ of CY5-AAV was 47% less when the excitation intensity was 50%.

The capacity of CED to distribute adenovirus (80–90 nm in diameter) was used to examine the distribution of a virus larger than AAV (23 nm in diameter). The distribution of CY3-labeled adenovirus capsids immediately after microinjection was greater than that of albumin and substantially greater than the distribution of the AAV capsids (Figs. 2 upper and 3). This distribution did not change significantly with a 50% decrease in excitation intensity (data not shown).

It is not known how well the distribution of viral particles correlates with the amount of tissue expressing the exogenous gene after regional infusion. To determine this correlation, four rat striata were infused with Ad-CMV-GFP. The distribution of gene expression 3 days after infusion was large, with transduction of most of the striatum (Figs. 2 upper and 3).

Coinfusion with BSA increased the Vₐ of adeno viral gene expression (Figs. 2 upper and 3). This increase, however, was also associated with more edema, which could cause an apparent increase in the volume of gene expression without increasing the amount of cells transfected. To account for this source of error, total fluorescence within the outlined region was quantified; there was 56% more total fluorescence in the group in which adenovirus had been coinfused with albumin. To determine whether albumin increased the spread of the virus or merely enhanced transfection efficiency, U2OS cells were exposed in vitro to Ad-GFP-βGal with or without 4.5% albumin. The addition of albumin had no effect on transfection efficiency.

**Discussion**

Limited gene delivery and vector distribution remain the principle obstacles to successful gene therapy of solid tissues, including the CNS. Convection-enhanced delivery was discovered and developed with the goal of achieving the distribution of large molecules, including proteins and genetic vectors, into the interstitial space of solid tissues, particularly the CNS. Findings of several experiments have shown that CED can be used successfully to deliver and distribute small and large molecules in the CNS while circumnavigating the BBB. Furthermore, Bankiewicz, et al., have shown previously that, compared with simple injection, CED can be used to enhance the distribution of gene expression by AAV in the brain, and Kordower and colleagues have successfully used CED for transfection of the striatum with lentivirus expressing β-Gal and glial cell–derived neurotropic factor for the treatment of a primate model of Parkinson disease. Nevertheless, given that neuronal components of the CNS have processes that may extend millimeters or centimeters from the cell body and, thus, gene expression may distribute widely from a locus of tissue transduction, it remains unknown whether widespread distribution of the virus itself is enhanced by CED and whether larger viruses can be distributed in the CNS by CED. Kroll and colleagues achieved widespread distribution of a 20-nm-diameter iron particle coated with dextran in the extracellular space of rats. Enhanced intracerebral distribution of neurotrophins that bind heparan sulfate and of Type 2 AAV, which also binds heparan sulfate, occurs when these agents are mixed with homeopathically concentrated heparin before convective delivery, indicating the importance of tissue binding in the convective distribution of proteins and small viral vectors, as predicted by Morrison, et al. Variables influencing the success of distribution of large particles, such as their sizes, surface characteristics, and others, however, have not been previously examined. It was uncertain whether CED could deliver gene therapy vectors, which are much larger than proteins, to clinically relevant volumes of brain parenchyma. A critical determinant would be whether nano-sized gene therapy vectors such as nanospheres and viruses could fit through the channels of the extracellular space. If these particles could not be widely distributed using bulk flow, then alternative, more complex, strategies would need to be developed.

**Striatal Distribution of Nanospheres**

Use of nanospheres permitted isolation of certain features, such as size and surface charge, to examine influence on convective distribution in the extracellular space. If CED could distribute nanospheres, then, based on size, high-flow microinjection of viruses would seem possible. In the current study dynamic light scattering experiments revealed that the nanospheres were monodispersed during experimental conditions and that in a solution of 4.5% albumin the nanospheres became coated with a monolayer of albumin, a phenomenon that has been previously described and attributed to the hydrophobic nature of polystyrene, the major constituent of nanospheres. Hydrophobic regions on the nanosphere surface avidly bind to hydrophobic portions of proteins such as albumin. The formation of an albumin
monolayer increases the effective particle diameter (a 13-nm increase), alters the surface charge by masking carboxylate surface groups, and increases hydrophilicity by covering the hydrophobic regions of the nanosphere.

With the assurance of monodispersion, nanospheres were infused via CED into the striatum. Under model circumstances in CED, the Vᵢ should be five times larger than the Vₑ because the fraction of extracellular space is approximately 20% (Vₑ/Vᵢ = 5).\(^{10}\) The \(^{14}\)C-BSA is distributed (Vᵢ = 20.7 ± 2.2 mm\(^3\), Vₑ = 4 μL) in this manner. Thus, the achievement of a Vₑ/Vᵢ of 5 further implies unrestricted mobility, which would be critical for scaling up CED for larger volumes. Although all the nanospheres displayed particle retardation (Vₑ/Vᵢ < 5) with albumin, convective distribution of 20-nm-diameter nanospheres in the extracellular space of gray matter was achieved. The Vᵢ of nanospheres in rat striata was inversely proportional to the size of the particle (Fig. 2 lower). This inverse relationship reveals that with all other particle properties being equal, particle size determines the Vᵢ achieved with CED. Our initial results indicated that there was a threshold in particle diameter between the 40- and 100-nm diameter beyond which the space available for movement in the extracellular space of the gray matter would completely restrain distribution. Nevertheless, when the particle surface was coated with albumin, all the larger diameter beads showed significantly more mobility. This distribution indicates that some channels are at least 200 nm in diameter (the largest nanosphere) and that virus-sized particles can traverse the extracellular space if unhindered by binding. Albumin treatment may enhance the distribution of particles by partially masking hydrophobic regions of the nanosphere that can bind to proteins in the extracellular space. It may also enhance the distribution by retarding any aggregation of particles that might occur in the extreme chemical potential of the in vivo environment. Such retardation would be most observable for the largest particles because even limited aggregation of these conceivably could produce poorly mobile clusters and could explain why albumin treatment had the least effect on the Vᵢ of the 20-nm particles. Regardless of the mechanism responsible for the significant spread of the uncoated 20-nm nanospheres, the albumin-coated spheres with larger diameters demonstrated an increased Vᵢ compared with noncoated microspheres of the same size, illustrating the critical importance of surface properties for distribution in the extracellular space with CED.

Effects of Solution Osmolarity

Having determined which particle characteristics influence distribution, it was important to examine the effect of the solutions in which the nanospheres were suspended because the influence of solution osmolarity on the distribution of materials delivered via high-flow microinfusion has not been previously assessed. The electrolyte osmotic balances associated with these infusions and maintained between the extracellular and other fluid spaces could be primarily achieved either by transport of water across the microvasculature, transport of water across the cell membranes of the parenchyma, or both. In the former case, infusion of hypertonic solutions would be expected to draw water from the plasma, effectively increasing the Vₑ and, under the assumption of constant extracellular volume fraction, the Vᵢ. The converse would be expected with hypotonic solutions. In the case of transport dominance across parenchymal cell membranes, the necessary water adjustments would be expected to be achieved by shrinking (hypertonic) or swelling (hypotonic) cell volumes with nearly compensating changes in the extracellular volume and, hence, little effect on the Vᵢ. The results of the current study revealed no dramatic effects on the Vᵢ when the infusate osmolarities were varied over a broad range. The results are thus highly indicative of a delivery mechanism in which the cells, acting as osmometers, serve as a major control element to maintain a constant Vᵢ in the face of significant electrolyte variability in the infusate. In turn, this finding indicates that solution osmolarity can be varied widely to accommodate optimal buffering conditions for CED of large particles. Extremely hypertonic solutions may even enhance distribution.

Distribution of Viruses Using Convection

Although the results of experiments with nanospheres indicate the importance of size and surface properties, they also lead to the inference that scaling up CED for viral vector distribution to human proportions may be problematic. The difficulty is indicated by the inability of any modification of the nanoparticles to achieve a Vₑ/Vᵢ of 5. Nevertheless, the Vᵢ of the larger albumin-coated nanospheres was much larger than the spread expected from a simple tissue displacement due to total particle volume. This distribution indicates that some channels at least 200 nm in diameter (the largest nanosphere) exist in the interstitial space of gray matter and that virus-sized particles can traverse the extracellular space if unhindered by binding.

Adeno-associated virus capsids were less mobile (Vᵢ = 12.9 ± 1.5 mm\(^3\)) than \(^{14}\)C-albumin (Vᵢ = 20.7 ± 2.2 mm\(^3\)) and just slightly more mobile than comparably sized albumin-coated 20-nm nanospheres (9.3 ± 2.5 mm\(^3\)). If sufficiently sized channels exist, then the difference in distribution between AAV capsids and \(^{14}\)C-albumin is likely due to binding. The binding can be specific (cell receptor) or nonspecific (ECM). Similarly, the small (and nearly insignificant) difference in distributions between the CY5-AAV and the albumin-free or coated classes of 20-nm nanospheres might reflect small binding differences between the two types of particles. There may also be some contribution from the size difference between the AAV particles (23 nm) and the albumin-coated nanospheres (33 nm = 20 nm + 13 nm monolayer thickness).

The dispersions of CY5-AAV and 20-nm nanospheres were reasonably similar. The distribution of CY5-AAV appears to have been influenced by tissue binding, indicated both by the enhanced distribution after pretreatment with albumin and a viral particle concentration gradient caused by particle retardation. The presence of a gradient is indicated by the decrease in Vᵢ as the excitation intensity was decreased. This binding effect may also be used to explain previous observations that concentration is important in convective distribution,\(^{11,12}\) because a sufficient dose would overcome the gradient when binding sites are saturated.

Adenovirus capsids distribute at least as far as \(^{14}\)C-albumin. This distribution shows that a particle as large as adenovirus (diameter 80–90 nm) can move through substantial portions of the interstitial space unhindered (Vₑ/Vᵢ > 5). This finding, more than any previous result, indicates that CED can be scaled up to deliver gene transfer vectors. That CY3-Ad spread somewhat farther in tissue than \(^{14}\)C-albumin may be due to partial channel blockage by the virus, an
associated reduction in accessible local extracellular volume fraction, and subsequent broader tissue spread to accommodate the infused volume. Although this mechanism, if operative, might also be expected to affect the larger nanosphere particles as well, its effect may have been obscured by their relatively greater binding. The capability of adenovirus to move widely is also supported by the V0 of Ad-CMV-GFP expression, which was comparable to the Vd of albumin.

Effects of Coinfusion of Albumin With Virus

Coinfusion of albumin with adenovirus increased the volume of gene expression significantly. We saw from the in vitro data that albumin had no effect on the efficiency of transfection, indicating that the increased volume of gene expression confered by albumin was due to a greater distribution of adenovirus and not because of enhanced transfection. Although this phenomenon is not fully understood, it is known that albumin binds to adenovirus and changes its solubility, indicating the possibility that albumin has the capacity to alter surface properties (although apparently less than with polystyrene nanospheres) and, therefore, enhance interstitial spread. The more intense edema in the adenovirus–albumin group, which may have been an exaggerated inflammatory response commonly associated with adenovirus, cannot be easily explained. It is not solely attributed to albumin because infusion of albumin alone did not provoke a similar response in our osmolarity experiments and has not previously been associated with CED in the rat striatum.

Conclusions

The size of the channels in the extracellular space of brain gray matter is sufficient to accommodate convective distribution of virus-sized particles and viruses. Surface properties, more than size, are the limiting factors for distribution of viral vectors in the interstitial space. Particles that do not extensively bind to the ECM behave similarly to the model macromolecule albumin. Techniques to alter surface properties should enhance distribution. With albumin pretreatment, the V0 of adenovirus remains linearly related to the Vd, resulting in the capability for large volumes of distribution. Other approaches to alter surface properties may have similar effects for adenovirus and for other genetic vectors. These observations also establish that CED can be used for widespread distribution of virus-sized gene therapy vectors in the CNS for genetic therapy, which should enhance the opportunity for laboratory studies to be translated to clinical applications.

Acknowledgments

We thank Krys Bankiewicz, Edward Muszuka, and Howard Fine for providing the virus; Peter Schuck for the dynamic light scattering; John Harvey for spectroscopy; Christine Piggott for capillary zone electrophoresis; Poomam Mannam and Barbara Ikejiri for histological analysis; and Stuart Walbridge, Eric Curry, and Andrew Bennett for animal procedures and tissue processing.

References


Convective distribution of viral vectors in the rat brain


Manuscript received September 29, 2004. Address reprint requests to: Edward H. Oldfield, M.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10, Room 5D37-1414, Bethesda, Maryland 20892-1414. email: eo10d@nih.gov.