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Research report

Distribution in brain of liposomes after convection enhanced delivery; modulation by particle charge, particle diameter, and presence of steric coating

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Abstract

We have investigated the role of diameter, charge, and steric shielding on the brain distribution of liposomes infused by convection enhanced delivery (CED) using both radiolabeled and fluorescent-labeled particles. Liposomes of 40 and 80-nm diameter traveled the same distance but penetrated significantly less than a 10-kDa dextran; whereas 200-nm-diameter liposomes penetrated less than 80 nm liposomes. A neutral liposome shielded by polyethylene glycol (PEG; 2 kDa; 10% by mole) penetrated significantly farther than an unshielded liposome. Even when shielded with PEG, positive surface charge (10% by mole) significantly reduced the penetration radius compared to a neutral or negative charged liposome (10% by mole). A mathematical CED model including a term for liposome cell binding was applied to analyze the radius of particle penetration. Neutral liposomes had a binding constant of $k = 0.0010 \pm 0.0002$ min^{-1}, whereas for positive charged liposomes k increased 50-fold. The binding constant was independently verified using a degradable lipid radiolabel that eliminated from the brain with a 9.9 ± 2.0 h half-life, equivalent to the calculated elimination constant $k = 0.0012 \pm 0.0002$ min^{-1}. During CED, liposomes accumulated in a subpopulation of perivascular cells within the brain. A non-degradable lipid radiolabel showed that lipid components remained within these perivascular brain cells for at least 2 days. To reduce this uptake, 100-fold molar excess of non-labeled liposomes were co-infused with labeled liposomes, which significantly increased liposome penetration. These studies suggest that optimization of therapeutic CED using particles such as drug-loaded liposomes, polymeric nanoparticles, non-viral DNA complexes, and viruses will require a strategy to overcome particle binding and clearance by cells within the CNS.

Theme: Other systems of the CNS
Topic: Brain metabolism and blood flow

Keywords: Cationic lipid; DNA; PEG; Rodent; Tumor; Rat

1. Introduction

For many compounds, the blood–brain barrier impedes drug delivery to the brain parenchyma so that systemic drug administration is ineffective for treating diseases of the CNS. For life-threatening or highly debilitating diseases, a drug infusion directly into the brain (intra cranial) may be the only feasible route of administration. However, agents directly infused into the brain in a small volume do not readily disperse from their infusion site. Diffusion coefficients are typically too low to allow even small molecule drugs to move more than a few millimeters. Additionally, diffusion requires a high concentration gradient to drive effective drug concentrations over a large distance [37]. Such high drug concentrations often lead to dose limiting

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neuro-toxicity. A straightforward solution is to provide bulk convective flow at the site of infusion, a technique called convection-enhanced delivery (CED) [5,13]. Small molecule agents have been administered using CED for many years [8,14,15,21,24,26,29,45]. Convective flow is also effective for distributing large macromolecules (M_w > 500 Da) [5,6,9,17,25,27,38,39] and nanoparticles, including: viruses [2,4,11,19,31], magnetic nanoparticles [22], liposomes [28,34,36,40], and non-viral DNA complexes [7,19,20,23,32,35,41]. Research into CED has progressed such that clinical trials have been initiated for treatment of brain tumors with conventional chemotherapeutic drugs including taxol [29] and with DNA lipoplex [43,44].

A phase I/II clinical trial using CED of cationic DNA lipoplexes was completed with the recommendation that basic research needs to address the interaction between nanoparticle composition and brain/tumor tissue [43,44]. This paper is a response, in part, to such recommendations [43,46]. Here we show that the ability of liposomes to flow by bulk fluid through living brain tissue is highly dependent upon their physical–chemical characteristics including size, charge, and steric coating. Additionally, a subset of cells in the brain accumulate neutral liposomes and necessitate a strategy to saturate liposome binding if CED is to reach its full potential for disseminating nanoparticle drug carriers through the brain.

2. Materials and methods

2.1. Materials

1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-3-trimethylammonium-propane (DSTAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-distearoyl-*sn*-glycero-3-phosphoglycerol (DSPG), and cholesterol were obtained from Avanti Polar Lipids (Alabaster, AL). 10-kDa FITC-dextran-lysine and fluorescent lipid tracers DiO (D-275), DiI (D-282), and DiD (D-307) were purchased from Molecular Probes (Eugene, OR). 1,2-Distearoyl-*sn*-glycero-3-monomethoxy-ether-poly-ethylene glycol (mPEGDSG, Sunbright DSG-20H) was a generous gift from A. Suginaka (NOF, Tokyo, Japan). POD was synthesized in our lab as described elsewhere [16]. Alzet™ osmotic pumps (200 μl/1 day) and the Brain Infusion Kit II were purchased from Durect (Cupertino, CA). Green fluorescent protein (GFP) encoding plasmid was a generous gift from Valentis (Burlingame, CA).

2.2. Preparation of liposomes

For the preparation of liposomes, 10 μmol total lipid was mixed in chloroform and dried in a glass test tube by rotary evaporation under reduced pressure. The lipid film was placed under high vacuum for 4 h to remove residual solvent. Liposomes were formed in pH 8.5 Tris-acetate buffered saline (150 mM NaCl, 5 mM Tris base/acetate). The lipid (10 mM) was hydrated at 70 °C with intermittent vortexing. ~80-nm liposomes were formed by extrusion (11×) through 80-nm pores in polycarbonate membranes at 60 °C. ~200-nm-diameter liposomes were formed by three freeze (−20 °C)/thaw (70 °C) cycles followed by extrusion three times through a 400-nm polycarbonate membrane at 60 °C. To prepare ~40-nm small unilamellar vesicles (SUV), hydrated liposomes were sonicated for 1 h at 65 °C in the dark under argon. SUV were separated from larger particles by centrifugation at 20 °C for 1 h at 50,000 rpm in a Beckman Rotor TLA100.3. The supernatant was collected and sterile filtered through 0.45 μm to further remove larger aggregates. After particle formation, the concentration of lipid phosphate was determined by the method of Bartlett [3]. Particle size and zeta potential were determined using a Malvern (Southborough, MA) Zeta3000 Dynamic Light Scattering Instrument and are presented in Table 1.

Table 1
Compositions and characteristics of polymers and particles used in CED experiments

Particle	Color	Composition	Formation	Size[a] (nm)	Zeta potential[b] (mV)
Dextran	green	10-kDa dextran, FITC labeled, lysine fixable	polymer	–	nd[c]
40 nm	red	DSPC:Chol:mPEGDSG:DiD [50:40:10:0.1]	sonicated liposome	43.2	nd[c]
80 nm	green	DSPC:Chol:mPEGDSG:DiO [50:40:10:0.1]	80-nm extruded liposome	84.0 ± 2.1	2.6 ± 1.8
80 nm	red	DSPC:Chol:mPEGDSG:DiD [50:40:10:0.1]	80-nm extruded liposome	85.8 ± 0.8	5.1 ± 1.3
200 nm	red	DSPC:Chol:mPEGDSG:DiD [50:40:10:0.1]	400-nm extruded liposome	197.3 ± 0.6	nd[c]
10% negative	red	DSPG:DSPC:Chol:mPEGDSG:DiD [10:40:40:10:0.1]	80-nm extruded liposome	83.3 ± 9.6	−8.6 ± 2.4
10% positive	red	DSTAP:DSPC:Chol:mPEGDSG:DiD [10:40:40:10:0.1]	80-nm extruded liposome	89.3 ± 1.1	70.0 ± 2.4
No PEG	red	DPSC:Chol:DiD [60:40:0.1]	80-nm extruded liposome	91.0 ± 2.8	−10.3 ± 2.7
Excess liposomes	none	DSPG:DSPC:Chol:mPEGDSG:DiD [10:40:40:10:0.1]	80-nm extruded; high pressure	108.3 ± 0.8	nd[c]
^{125}I-BPE liposomes	none	DSPC:Chol:mPEGDSG [50:40:10] < 0.01% ^{125}I-BPE	80-nm extruded liposome	89.9 ± 1.9	nd[c]
^{3}H-Chol liposomes	none	DSPC:Chol:mPEGDSG [50:40:10] < 0.01% ^{3}H-Chol	80-nm extruded liposome	82.5 ± 0.4	nd[c]
GFP–NLP	red	DOTAP:DOPE:POD:DiI [50:40:10:0.1]	detergent dialysis 2.5 μmol lipid + 200 μg plasmid	110.6 ± 5.5	nd[c]

[a] Particle diameter measured by light scattering zeta average size, ± indicates standard deviation.
[b] Zeta potentials all at low salt 1 mM NaCl, 1 mM Hepes, pH 7.5, ± indicates 95% confidence interval.
[c] Not determined.