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Lipopeptide Delivery of siRNA to the Central Nervous System

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Summary/Abstract

i.
RNA interference is a relatively new tool used to silence specific genes in diverse biological systems. The development of this promising new technique for research and therapeutic use in studying and treating neurological diseases has been hampered by the lack of an efficient way to deliver siRNA transvascularily across the blood-brain barrier (BBB) to the central nervous system (CNS). Here we describe the generation of three different liposomal siRNA delivery vehicles to the CNS using the thin film hydration method. Utilizing cationic or anionic liposomes protects the siRNA from serum nucleases and proteases en route. To deliver the siRNA specifically to the CNS, the liposomes are complexed to a peptide that acts as a neuronal address by binding to nicotinic acetylcholine receptors (nAChRs). When injected intravenously, these liposome-siRNA-peptide complexes (LSPCs) or peptide addressed liposome encapsulated therapeutic siRNA (PALETS) resist serum degradation, effectively cross the BBB and deliver siRNA to AChR-expressing cells to suppress protein expression in the CNS.

Keywords

RNAi; siRNA; delivery; central nervous system; blood-brain barrier; liposomes; peptides; complexes; prions

1. Introduction

RNA interference (RNAi) is a biological process that diverse organisms use to help regulate the amount of messenger RNA (mRNA) available for translation. Dicer, an endogenous mammalian protein complex that mediates RNAi, cleaves double-stranded or short hairpin RNA (shRNA) into functional, small interfering RNA (siRNA). The RNA-induced silencing complex (RISC) guides siRNA to its complementary mRNA target, which is then digested by argonaute, an endonuclease present in the RISC. A concomitant reduction in protein expression ensues. Researchers have begun exploiting RNAi as a tool for silencing specific genes in biological systems(1–4). RNAi has shown potential efficacy in treating several diseases including hepatitis(5, 6), cancer(7), ocular disorders(8), chronic pain(9) and neuropathologies induced by viruses(10) and prions(11). Initial attempts to use RNAi in the

central nervous system (CNS) primarily used intracranial injections of lentiviral vectors encoding shRNA. Lentivirus encoding shRNA against mRNA transcripts have demonstrated decreased expression of the cognate protein(12, 13), but several aspects of this method limit its efficacy. Inadequate neuroinvasion by lentiviral vectors across the blood-brain barrier (BBB) necessitates their direct delivery to the CNS via stereotactic intracranial injection of shRNA lentivirus directly into the brain. Even then, lentiviral infection and shRNA expression was limited to a small area around the injection site, resulting in spatially limited knockdown of protein expression. This method also lacks desired temporal control of protein expression, since once brain cells are infected with lentivirus, it will likely irreversibly suppress protein expression in these cells. Moreover, despite improvements in lentiviral vector technology and construction(14–16), concerns over the oncogenic capacity of lentiviral delivery systems remain(17–19).

Non-viral strategies to deliver siRNA molecules to cells include using cationic peptides capable of crossing plasma membranes(20–22). Conjugating siRNA to these cell-penetrating peptides via thiol linkages allows for efficient dissociation of siRNA-peptide complexes by reduction of the disulfide bond in the cytoplasm(21, 23). While this delivery method holds therapeutic promise, it still does not solve two important problems of drug delivery to the brain: cell specificity and transport of its cargo across the blood-brain barrier (BBB). Kumar et al developed a transvascular method to deliver siRNA across the BBB specifically to neuronal cells in the brain via intravenous (iv) injection(10). This method involves complexing siRNA to a short peptide derived from the rabies virus glycoprotein that binds specifically to acetylcholine receptors (AChRs) on neuronal cells(24, 25). Adding nine d-Arginines to the carboxyl terminus of this peptide (RVG-9r) enabled it to electrostatically interact with siRNA and specifically deliver siRNA to neurons in mouse brains to suppress protein expression and protect against fatal viral encephalitis. This significant advance in siRNA delivery to the CNS was tempered by the lack of direct detection of siRNA in the brains of a majority of treated mice, suggesting that these naked complexes may have degraded significantly in the blood during transport. Efficient peptide-mediated delivery of siRNA to the brain after iv injection relies on protecting the complex from serum nuclease and protease degradation en route.

Liposomes are widely used as delivery vehicles for gene therapy products in *in vitro* assays. They are also being investigated for use in animal and human models as delivery vehicles for siRNA. Complexing or encapsulating siRNA with liposomes has been shown to protect siRNA from degradation and improve delivery through the vasculature(26–31). Cationic liposomes are most commonly used because they electrostatically interact with the negatively charged phosphate backbone of RNA. Cationic liposomes also readily interact with the anionic phosphate head groups of cell membranes(32–34). Liposomes will transfer siRNA into cells either through direct fusion to the plasma membrane or through endocytosis(35–37). Anionic liposomes are not commonly used as the anionic lipid is thought to be incapable of passing the plasma membrane and repels the negative charge of the siRNA backbone. Still, anionic liposomes are less immunogenic than cationic ones as they bind to less of the negatively charged serum proteins than cationic liposomes. Also, there are reports that anionic liposomes can effectively package and deliver DNA by using cations to condense the DNA and shield its negative charge(38–43). Liposomes can be

targeted to specific cell types using peptides that either electrostatically interact with or are covalently bound to the liposomes(7, 10, 21, 44). Thus, siRNA is protected from nucleases by liposomes and is targeted to specific cell types to reduce off-target effects by cell-specific peptides. Adding polyethylene glycol (PEG) to lipids increases bioavailability and circulation time in blood(45, 46) by decreasing immune clearance(47). PEGylated liposomes increased delivery of doxorubicin and cisplatin in experimental tumor models(48) and has been used in clinical trials to treat a variety of cancers(49), including AIDS-related Kaposi's sarcoma(50, 51) and advanced malignant solid tumors(52), including those found in ovarian(53) and breast(54) cancer. Also, adding PEG groups reduces recognition by serum proteins and uptake of the liposomes by cells of the mononuclear phagocyte system(47).

While protecting therapeutic agents from serum degradation represents a crucial step in transvascular delivery to target sites in the brain, liposomes alone do not provide cell specific delivery, nor do they ensure transport across the BBB. Coupling a monoclonal antibody against glial fibrillary acidic protein (GFAP) to PEGylated liposomes successfully targeted them to astrocytes in cell culture, but still failed to deliver them across the BBB to astrocytes in mouse brains(55). An effective, efficient delivery system must do all three: (1) provide a molecular address to the agent for delivery to neuronal cells, (2) gain access to the brain by crossing the BBB and (3) protect the agent from serum degradation en route. Using siRNA as a therapeutic agent and prion diseases as models, we have developed three therapeutic delivery systems that address all of these issues. Liposome-siRNA-peptide complexes (LSPCs) are composed of cationic liposomes with siRNA and a targeting peptide (RVG-9r) electrostatically coating the outer surface of the liposome. RVG-9r is a small modified peptide from the rabies virus glycoprotein that targets nicotinic acetylcholine receptors in the CNS to reduce siRNA off-target effects. Peptide addressed liposome encapsulated therapeutic siRNA (PALETS) are composed of either cationic or anionic liposomes with siRNA encapsulated inside the liposome, and RVG-9r either electrostatically or covalently bonded to lipid PEG groups. For anionic PALETS, we use the cation protamine sulfate to condense the siRNA so that it becomes encapsulated within the anionic liposomes. We show that LSPCs decrease surface PrP^C 40–50% in neuronal cells, and PALETS result in a decrease of the total number of PrP^C expressing cells.

2. Materials

2.1 LSPC and PALETS reagents

2.1.1 siRNA: designed using siRNA scales(56) and synthesized, pre-annealed and lyophilized by Qiagen (*see* Note 1). Re-suspend in sterile RNase-free water (supplied by Qiagen) to 40 μ M according to manufacturer's directions. Store in single-use aliquots at -70°C (*see* Note 2).

2.1.2. Peptides: RVG-9r and control RVM-9r peptides (table 1) were synthesized and purified by high performance liquid chromatography by ChemPeptide. Re-suspend in sterile phosphate-buffered saline (PBS), pH 7.4 to 400 μ M, or approximately 2 mg/mL). For example, re-suspend 100 mg of peptide in 50 mL PBS. Store at -70°C in single-use aliquots (typically 400 μ L, *see* Note 2).

2.1.3. 10% sterile sucrose: weigh 1 g of sucrose and mix into 9 mL of sterile deionized water.

2.1.4. Liposomes:

A. DOTAP LSPCs: Prepare cationic LSPCs by mixing N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTAP, Avanti Lipids) and cholesterol (Avanti Lipids) at 1:1 molar ratio in a 1:1 chloroform:methanol (C:M) solution. Weigh and mix 20 μ moles of cholesterol and 20 μ moles of DOTAP to a final concentration of 8 mM (a total of 40 μ mole) C:M solution in a round-bottom, 15-mL glass flask. Evaporate the C:M solvent off using N₂ gas. Remove any excess solvent by placing the tube in a vacuum desiccator and drying overnight to a thin film (*see* Note 3). Rehydrate liposomes to a final stock concentration of 8 μ M in sterile 10% sucrose. Keep the thin lipid film and the 10% sucrose at 55°C throughout the rehydration procedure. Add 1 ml of 10% sucrose every 10 minutes and swirl the lipid solution every 3 minutes for a total of 5 ml across 50 minutes. Size either through an extruder (Avanti lipids) using 1, 0.45, and 0.2 μ m pore filters (Avanti Lipids) or sonication using a Qsonica S-4000 sonicator (Qsonica). Store at 4°C for several months.

B. DOTAP PALETS: Prepare cationic PALETS by mixing DOTAP, cholesterol, and DSPE-PEG (Avanti Lipids) at a 55:40:5 molar ratio in a 1:1 C:M solution. Weigh out 22 μ moles of DOTAP, 16 μ moles of cholesterol, and 2 μ moles of DSPE-PEG to a final concentration of 8 mM (total of 40 μ mole) C:M solution in a round bottom, 15ml glass flask. Evaporate the solvent off using the same above procedure as DOTAP LSPCs. Rehydrate DOTAP PALETS using 5 ml of sterile (*see* Note 4) 1X PBS. Keep both the PBS and the lipid solutions above 65°C (*see* Note 5) while rehydrating. Add 1 ml of 1X PBS every 10 minutes for a total of 5 ml across 50 minutes. Swirl the solution every 3 minutes. Size using above procedure. Store at 4°C for several months.

C. DSPE PALETS: To prepare DSPE PALETS, use the same above procedure as DOTAP PALETS. Use a 55:40:5 molar ratio (*see* Note 6) of DSPE:cholesterol:DSPE-PEG lipids in a 1:1 C:M solution by weighing out 22 μ moles of DSPE, 16 μ moles of cholesterol, and 2 μ moles of DSPE-PEG to a final concentration of 8 mM (total of 40 μ moles). The solvent evaporation, rehydration, and sizing steps are the exact same as the DOTAP PALETS procedure.

2.1.5. Before use, dilute liposomes (LSPCs and PALETS) 1:100 in sterile 1X PBS. For example, briefly mix stock liposomes by inverting the tube and add 2 μ L of liposomes to 198 μ L of sterile 1X PBS. Mix by pipetting and put 80 μ L of diluted liposome solution into screw cap tubes (VWR). Place into the tube holder of S-4000 horn sonicator (Qsonica). Sonicate liposomes for forty seconds at a power setting of 20 (*see* Note 7)

2.1.6. The liposomes can be used immediately, or stored for several weeks at 4°C (*see* Note 8).

2.2 LSPC delivery supplies

- 2.2.1. Mice: Choose mouse lines and strains appropriate for the experimental design. Various strains of wild type mice can be purchased from Charles River or Jackson labs (*see* Note 9).
- 2.2.2. 26–30 Gauge insulin syringes.
- 2.2.3. Heat lamp.
- 2.2.4. Rodent anesthesia machine dispensing Isoflurane/Oxygen (Parkland Scientific, *see* Note 10).

3. Methods

3.1 Preparing LSPCs and PALETS

Complex siRNA, liposomes and peptides using filter barrier tips and sterile technique in a cell culture hood. The following preparation will produce enough LSPCs/PALETS to treat one mouse.

3.1.1. LSPCs

- A. Thaw 100 μ L of stock siRNA and 80 μ L stock peptide on ice.
- B. Add 50 μ L of the diluted and sonicated liposomes (sonicate first if necessary, *see* Note 7) to 100 μ L of 20 μ M siRNA and mix gently by pipetting.
- C. Incubate at 4°C for ten minutes (figure 1).
- D. Add 80 μ L of peptide, mix gently by pipetting and incubate at 4°C for ten minutes. (*see* Note 11).

3.1.2. DOTAP PALETS

- A. Thaw 100 μ L of stock siRNA and 80 μ L stock peptide on ice.
- B. Lyophilize 50 μ L of diluted DOTAP PALETS liposomes in a glass vial using a benchtop lyophilizer (LabConCo) for 15 minutes.
- C. Add 100 μ L of stock siRNA to the lyophilized single use aliquot of liposome to encapsulate the siRNA within the liposome. Incubate for 5 minutes.
- D. Optional: Covalently link RVG-9r to DSPE-PEG groups. (*see* Note 12)
 - 1. Add 10 μ L of a 60 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) solution to the liposome/siRNA solution.
 - 2. Add 10 μ L of a 150 mM N-hydroxysulfosuccinimide (sulfo-NHS) solution to the liposome/siRNA solution.
 - 3. Incubate the EDC/sulfo-NHS liposome/siRNA solution for 10 minutes.
- E. Add 80 μ L of stock RVG-9r and incubate for either 10 minutes if not covalently modifying or for 2 hours if covalently linking to PEG groups.

3.1.3 DSPE PALETS

- A. Thaw 100 μ L of stock siRNA and 80 μ L of stock peptide on ice.
- B. Lyophilize 50 μ L of diluted DSPE PALETS liposomes in a glass vial using a benchtop lyophilizer for 15 minutes.
- C. Incubate 100 μ L of stock siRNA solution with 1.4 μ L of a 26.6 μ M solution of protamine sulfate (*see* Note 13) for 10 minutes at room temperature.
- D. Add the siRNA/protamine to the single use aliquot of lyophilized DSPE PALETS liposomes. Incubate for 10 minutes at 4°C.
- E. Add 80 μ L of stock RVG-9r and incubate for 10 minutes on ice.
- F. Optional: Covalently link the RVG-9r to the DSPE PEG liposomes using the above protocol in 3.1.2 (D).(*see* Note 12)

3.2 Injecting LSPCs or PALETS

1. Warm mice in their cage by placing it four to six inches below a heat lamp for five to ten minutes (*see* Note 14).
2. While warming the mice, turn on the anesthesia machine such that oxygen flows at a rate of 3–4 L/min. Turn on the isoflurane at 3 L/min.
3. When the tail vasculature is clearly visible, place a single mouse in the anesthesia chamber until its respiration visibly slows to 1–2 breaths per second (*see* Note 15).
4. Meanwhile, load ~250 μ L of LSPCs into a sterile insulin syringe (*see* Note 16).
5. Place the mouse on a flat surface with its face in the nose cone attached to the anesthesia machine (*see* Note 17).
6. Starting with the mouse laying on its ventral side (dorsal side up), locate one of the two lateral veins. Starting as far posterior as possible, rotate the tail slightly and insert the needle with the bevel up at a shallow 10–15° angle 1–2 mm into the vein. Slowly press the plunger to inject the LSPC or PALETS solution (*see* Note 18).

4. Notes

1. We have used siRNA prepared for us by several biotechnology companies, but have observed optimal knockdown using siRNA prepared from Qiagen. Additionally, Qiagen can label siRNA with Alexa fluorochromes to monitor LSPC trafficking for determining delivery kinetics (*see* Note 15).
2. We typically treat cages of five mice at a time and therefore aliquot 1 mL of 200 μ M stock siRNA, enough to treat five mice. Aliquots should be adjusted to meet researchers' experimental requirements.
3. Desiccation time may vary. Most importantly, the lipids must be completely dry. For faster desiccation, the solution can be split into several tubes.

4. Rehydration medium depends on lipid composition and experimental requirements. Lipid mixtures with anionic lipids, such as DSPE-PEG, have improved rehydration with the addition of a salt buffer, such as PBS. If injecting either LSPCs or PALETS *in vivo*, a biologically safe hydration solution needs to be chosen.
5. It may be possible to use different cationic and anionic lipids in these formulations depending on researchers' requirements. If other lipids are used, other than what is listed above, rehydration should always be performed above the gel-liquid phase transition (T_m) temperature to ensure proper mixing of lipids.
6. At increasing molar concentrations, DSPE lipid molecules become more attracted to itself than to other types of lipids. The result is insoluble DSPE complexes that cannot be rehydrated or formed into liposomes. Therefore, DSPE should never be used above a 55% molar ratio.
7. Do not sonicate more than 100 μ L per tube using this sonicator. Other sonicators may be used, but volumes may have to be increased significantly if a probe sonicator is used. Horn sonicators produce the best, most consistent results with the small volumes used here.
8. If used later, sonicate liposomes again prior to complexation
9. White mice (FVB, C3H, CD-1, for example) tend to be much easier to inject intravenously in the tail than brown or black mice (129sv, C57/B16).
10. The machine should have an incubating chamber and a nose cone. We recommend isoflurane, but alternative anesthesia, such as ketamine, can be used. While tail vein injections can be done using physical restraint, we strongly recommend using chemical restraint to avoid sudden movements from the mouse that can compromise the injection and waste valuable time and reagents.
11. LSPCs are ready to use in ten minutes, but are stable for several hours at room temperature.
12. The covalent linkage of the peptide to the PEG groups is optional. If using DOTAP PALETS, the positive charge of RVG-9r may be able to bind to the negatively charged COO^- groups of the PEG lipids. If using DSPE PALETS, the positively charged RVG-9r can bind to the negatively charged DSPE lipids.
13. This protocol uses protamine sulfate to condense the siRNA so that it can be encapsulated within DSPE PALETS. However, other small positively charged cations can also be used, such as Ca^{2+} , depending on the application.
14. This step is crucial for successful tail vein injections, especially in dark-colored mice. The cage should reach an ambient temperature between 32 and 35°C. The lateral tail veins and/or the ventral artery should be clearly visible before proceeding. This usually coincides with salivation, so look for moisture around the mouth and nose. Monitor mice under the heat lamp every two minutes for

signs of distress or hyperthermia. Do not leave a mouse cage under the heat lamp for more than fifteen consecutive minutes.

15. This should take one to three minutes. Isoflurane is very safe and accidental overdoses are very rare. Distressed mice can be given pure oxygen to help revive them if necessary.
16. If bubbles appear, hold the syringe vertically with the needle pointing up and flick the syringe until the bubbles dislodge and float to the top of the syringe, toward the needle. Carefully keeping the needle tip above the syringe barrel, place it on the inside of the tube containing the LSPCs or PALETS and gently push the syringe plunger until the bubbles are evacuated (i.e., when a small amount of liquid emerges from the needle). Load additional LSPC/PALETS mixture into the syringe to 250 μ L if necessary.
17. Mice can be placed in physical restraints at this time if desired, but we recommend simultaneous chemical restraint.
18. Curling the tail over a finger can help orient the tail in a position that facilitates inserting the needle at the shallow 10–15° angle required for entering the vein. You should, but not always, feel the needle entering the vein lumen. You should feel little or no resistance on the plunger if you are in the lumen, and you will often see the vein blanche as the solution displaces the blood. If you feel resistance or see the tail swell just anterior to the injection site, stop immediately. You are most likely not in the vein. Apply direct pressure to the injection site for a few seconds then attempt the injection again further anterior to the previous site. If this fails after multiple attempts, or if the vasculature is no longer visible, stop. Allow the mouse to recover, proceed to the next mouse, and try injecting the former mouse after attempting the others.
19. To monitor LSPC trafficking and determine delivery kinetics, label peptides with DyLight 649 or similar fluorochrome (Alexa dyes, for example) according to manufacturer's directions (Pierce). Use a 3 Kd molecular weight cutoff Microcon centrifugal filter (Millipore) or similar device to exchange labeling buffer for storage buffer (10 mg/mL bovine serum albumin in sterile PBS).
20. LSPCs differ from PALETS in that the siRNA is electrostatically bound to the outside of the liposome with RVG-9r in LPSCs. With PALETS, the siRNA is usually encapsulated within the liposome with the RVG-9r bound on the outside; however, depending on the researcher's preferences, the siRNA can also be electrostatically bound on the outside of the liposome (Figure 1).
21. LSPCs can be monitored in live mice by injecting them with fluorochrome-labeled LSPCs, which can be visualized using a Xenogen (Ivis) or similar live animal imager. We observed LSPC delivery in as little as two minutes using this technique (Figure 2) and detected LSPCs up to ten days after injection.
22. LSPC localization can be observed in the CNS by fluorescent microscopy. Euthanize injected mice and remove and snap-freeze their brains in OCT (Tissue Tek) in liquid nitrogen. Using a cryostat cooled to –20°C (Leica), cut 8 μ m-thick

brain sections and mount them on glass slides. Fix in ice-cold acetone for ten minutes and air dry overnight. Slides can be stained with additional markers (the protein knockdown target, for example) or DAPI before mounting in ProLong Gold (Invitrogen) or similar anti-fade mounting media. Visualize LSPCs in brain sections using a microscope capable of detecting the fluorochromes used (Figure 3a–t).

23. Quantitative analysis of LSPC delivery can be performed on brain and kidney cells isolated from injected mice by flow cytometry and digital droplet PCR. Sacrifice mice and remove their brains and kidneys. Strain the organs through a 45 μ m nylon mesh (Fisher Scientific) to produce a single cell suspension for flow cytometry. Cells can be stained with additional cell marker of interest (the protein knockdown target) and analyzed using a Cyan (BD Pharmingen) or similar flow cytometer. RNA can be isolated from the cells using an RNeasy kit (Qiagen). cDNA obtained from the RNA can be analyzed using the Bio-Rad digital droplet PCR system. Flow cytometric analysis of brain cells reveals that DOTAP LSPCs decrease cell surface PrP^C levels compared to untreated controls (Figure 4A), and DOTAP PALETS decrease the number of PrP^C-positive cells (Figure 4B). Protein analysis in the kidneys shows that DSPE PALETS reduce the amount of cell surface PrP^C (Figure 4C), with no other statistical differences seen in the kidneys (Figure 4D). mRNA analysis of mice treated with DOTAP LSPCs, DOTAP PALETS, or DSPE PALETS reveal no statistical difference in PrP^C mRNA levels in the brains of mice (Figure 5A). PrP^C mRNA levels in the kidney are decreased about 2-fold in mice treated with DOTAP LSPCs and DOTAP PALETS, and increased 2-fold in mice treated with DSPE PALETS (Figure 5B).

5. References

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LSPCs **PALETS**

Figure 1. Schematic representation of LSPCs and PALETS.

Figure 2. LSPC delivery to the CNS in live animals.

Mice were injected i.v. into the tail vein with PBS alone (middle mouse in each panel), PrP siRNA complexed to the RVG-9r peptide (first mouse in each panel) or PrP LSPCs (third mouse in each panel). siRNA was detected in the brains of mice in as little as two minutes after injection. LSPCs targeted siRNA more specifically to the brain and persisted to ten days post injection than siRNA complexed with peptide alone.

Figure 3. LSPC delivery to PrP^C-expressing cells in vivo (11).

FVB mice were intravenously injected with liposomes alone (panels A-E) or LSPCs containing DyLight 488-labeled peptides and Alexa 488-labeled siRNAs as follows: PrP siRNA-control RVM-9r (F-J), Control siRNA -RVG-9r (K-O), or PrP siRNA-RVG-9r (P-T). Mice were sacrificed 24 hours later, dissected, and their brains snap frozen and 8 μ m sections thereof stained with DyLight 649-labeled anti-PrP antibody (A, F, K and P) and DAPI (C, H, M, and R) and visualized by fluorescence microscopy. Panels E, J, O, and T depict magnifications of the boxed areas in panels D, I, N, and S. Inset in panel P depicts a serial section stained with an irrelevant isotype control antibody. Scale bars, 50 μ m.

Figure 4. Analysis of PrP^C protein levels in the brain after LSPCs or PALETs treatment. FVB mice were intravenously injected with either 1X PBS (untreated control), DOTAP LSPCs, DOTAP PALETs, or DSPE PALETs. Mice were sacrificed four days after treatment and their brains and kidneys were harvested. Of all mice treated, only DOTAP LSPCs showed a decrease in median fluorescent intensity in the brain (A), indicating that DOTAP LSPCs decrease the amount of cell surface PrP^C in the brain. DOTAP PALETs were shown to decrease the number of PrP^C-positive cells in the brain (B). While none of the treatments resulted in a decrease in the number of PrP^C-positive cells in the kidney (D), DSPE PALETs decreased the amount of cell surface PrP^C (C).

Figure 5. mRNA analysis of brains and kidneys from mice treated with LSPCs or PALETS. Treated mice exhibited a decrease in PrP^C mRNA levels in the brain, although more mice need to be treated to establish statistical significance (A). Mice treated with DOTAP LSPCs and DOTAP PALETS showed a significant decrease in PrP^C mRNA levels in the kidney, whereas DSPE PALETS showed a 2-fold increase (B), likely due to immune activation of PrP^C transcription in response to siRNA

Table 1.

Delivery peptide sequences

RVG-9r ¹	YTIWMPENPRPGTPCDIFTNSRGKRASNGGGGrrrrrrrr
RVM-9r ²	MNLLRKIVKNRRDEDTQKSSPASAPLDGGGGrrrrrrrr

¹ lower case r denotes d-Arginine racemer

² control