SANDIA BIOSCIENCE

Technology for Antiviral Delivery to Inhibit Encephalitic Alphavirus Infection

SAND2019-7147C

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Background

- Several New World alphaviruses, including Venezuelan equine encephalitis virus (VEEV), are classified as a category B bioterrorism agents by the CDC and NIAID due their amenability to aerosolization while remaining highly infectious and the lack of controlled vaccines and antivirals[1]
- Lipid coated silica nanoparticle (LC-MSN) technology is a flexible, modular platform for delivery of small molecule antivirals to treat VEEV infection[2]
- LC-MSNs possess advantages of both MSNs and liposomes, including high loading capacity, controlled release, targeting specificity, colloidal stability, and biocompatibility[2]
- Recently, a small molecule called ML336 was developed and shown to inhibit VEEV[3]
- However, ML336 has a limited pharmacological profile, poor solubility in aqueous solutions, and requires significant improvements for clinical translation

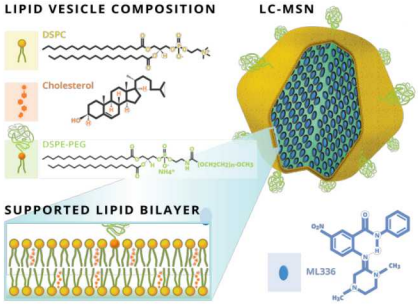

Figure 1. Schematic of the lipid-coated mesoporous silica nanoparticle therapeutic cargo delivery platform.

Antiviral ML336

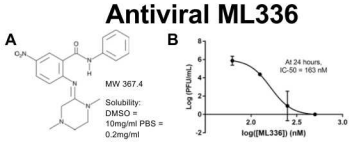

Figure 2. (A) ML336 chemical structure (B) Dose response curve in HeLa cells infected with VEEV TC-83 90.1MOI)

[1]Sidwell R. et al. *Antiviral Research* 2003; [2]Butler K. et al. *Small* 2016; [3]Schroeder C. et al. *Journal of Medicinal Chemistry*

LC-MSN Synthesis, Assembly and Characterization

- Hexagonal MSNs were fabricated using ammonium-base catalyzed reaction [1]
- Liposome composition:
  - 77.5% (DSPC) 1,2-distearoyl-sn-glycero-3-phosphocholine
  - 2.5% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N [methoxy-(polyethylene glycol)-2000]
  - 20% cholesterol
- 1 mg of MSN cores were loaded with 100 µL of 1 mg/mL ML336 in DMSO at 4° C overnight
- LC-MSNs were formed by combining liposomes and nanoparticles under sonication at a 5:1 mass ratio of liposomes: nanoparticles. Lipid bilayer visualized in figure 3 C and D.

Figure 3. TEM/SEM/CryoEM Characterization

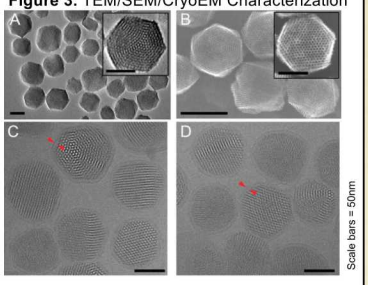

Figure 4. LC-MSN formation

Methods: After nanoparticle loading and LC-MSN formation, the concentration of ML336 in the LC-MSN supernatant was used to determine particle loading. For release data, samples were centrifuged at indicated timepoints and ML336 concentration was determined using absorption (320 nm).

Table 1. Dynamic light scattering (size), polydispersity index (PDI) and zeta potential for LC-MSNs.

Particle Type	Medium	Z-average Diameter (nm)	PDI	Zeta Potential (mV)
MSN	Water	95.9 ± 2.1	0.072 ± 0.005	-25.0 ± 0.42
LC-MSN	PBS	149.5 ± 1.5	0.116 ± 0.01	-0.263 ± 0.41
Loaded LC-MSN	PBS	164 ± 1.5	0.144 ± 0.02	-1.76 ± 0.26

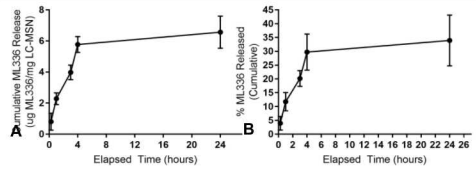

Figure 4. (A) Cumulative mass release from LC-MSNs and (B) cumulative percent release, normalized to loading, from LC-MSNs. (N=3).

Table 2. Loading and release summary for LC-MSNs.

Total ML336 Loaded (µg ML336/mg LC-MSN)	% ML336 Loaded	Total ML336 Released (µg ML336/mg LC-MSN)	% ML336 Released
20 ± 3.4	20 ± 3.4	6.6 ± 1.3	33.5 ± 6.6

Monosized LC-MSNs were formed and ML336 was released in a burst fashion during initial four hours.

[1]Lin Y. et al. *J Am Chem Soc*. 2010;

ML336 Loaded LC-MSN Viral Inhibition

Methods: HeLa cells were pretreated with LC-MSNs at 25 µg/well in a 12-well plate for 1 hour. Cells were infected at 0.1 MOI with TC-83 for 30 minutes, rinsed, and treatment groups were replaced. Viral loads were measured using plaque assays on VERO cells and reported as plaque forming units (PFU)/mL.

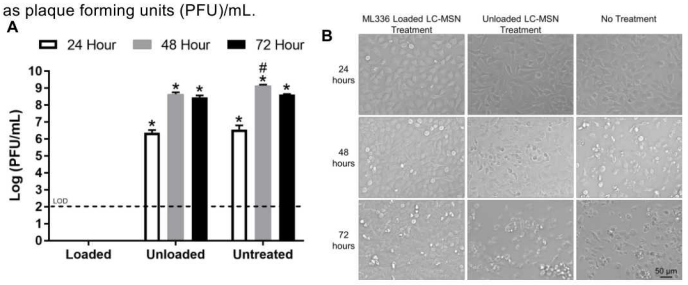

Figure 5. (A) Viral titers after cells treated with loaded, unloaded, and untreated conditions were infected for 24-72 hrs. (B) Phase microscopy images of cells post infection. (*= significantly different than loaded group at same timepoint, # = significantly different than unloaded group at the same timepoint, p<0.05).

Methods: LC-MSNs were incubated in OptiMEM for 4 hours, centrifuged, and supernatant was collected. HeLa cells were pretreated with LC-MSN at 25 µg/well or the equivalent amount of LC-MSN supernatant in a 12-well plate for 1 hour. Cells were infected at 0.1 MOI with TC-83 for 30 minutes, rinsed, and treatment groups were replaced. Viral titers were measured using plaque assays.

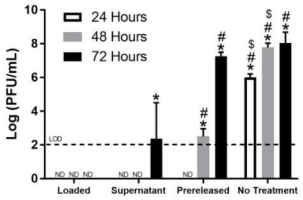

Figure 6. Viral titer reduction assay for loaded, supernatant, prereleased, and untreated groups. (*= significantly different than loaded group at same timepoint, # = significantly different than supernatant group at the same timepoint, $ = significantly different than prereleased group at the same timepoint, p<0.05).

ML336 loaded LC-MSNs inhibit virus significantly more than unloaded LC-MSNs and retain antiviral activity after initial burst release.

In Vivo Safety and Efficacy Studies

Methods: Five CeH/HeN mice were injected with 1 mg unloaded LC-MSNs diluted in 200 µL PBS and three mice were injected with 200 µL PBS only twice a day for four days.

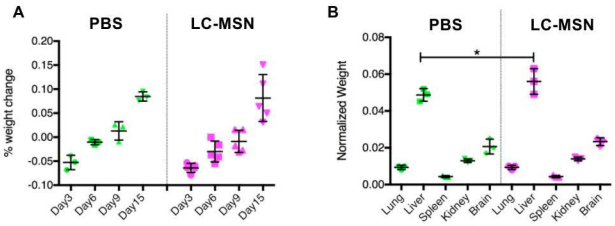

Figure 7. (A) Average percent weight change for animals with PBS and LC-MSN treatments. (B) Organ weight normalized to total mouse weight. (*= significantly different indicated group, p<0.05).

Methods: (A) Ten CeH/HeN mice were injected with 1 mg loaded LC-MSNs, 1 mg unloaded LC-MSNs, 20 µg free ML336 in 200 µL 1% carboxymethylcellulose in PBS, or 200 µL PBS twice a day for four days. Mice were challenged intranasally with 10^8 pfu of VEEV TC-83 four hours after the first treatment dose. (B) Five mice were sacrificed 4 days post infection and organs were homogenized to measure viral l

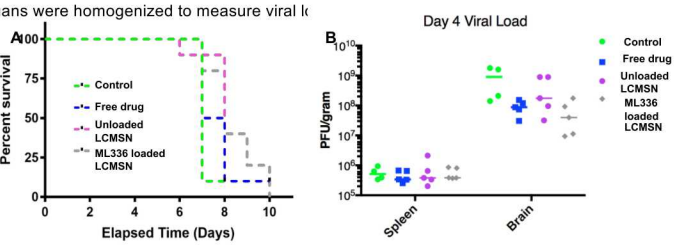

Figure 8. (A) Percent survival of ten animals for each group over the course of ten days. (B) Viral titers in the brain and spleen at four days post intranasal challenge with VEEV TC-83 for each group.

LC-MSNs did not affect animal weight and ML336 loaded LC-MSNs showed slightly higher survival with decreased viral titers in the brain compared to other experimental groups.

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