Layer-by-Layer Coated Intralipid® Nanoparticles for Treatment of Influenza

Christopher Russo¹; Hilliard Kutscher²;³; Lisa Eagler²; Bruce Davidson⁴; Paras N. Prasad²; Paul Knight*¹

Department of Pharmacology and Toxicology;² Institute for Lasers, Photonics, and Biophotonics;³ Translational Pharmacology Research Core; NYS Center of Excellence in Bioinformatics and Life Sciences;*Department of Anesthesiology

Abstract

Layer-by-layer (LbL) coating is a technique used in development of nanomedicines to alter drug release, and biodistribution. Our ultimate goal is to demonstrate that using a layer-by-layer coating method for isoflurane loaded Intralipid® nanoparticles will increase isoflurane delivery to macrophages in the spleen; reduce flu pathology and its complications; and limit narcosis, an isoflurane side effect. We hypothesized that performing layer-by-layer coating on isoflurane loaded Intralipid® nanoparticles would result in decreased narcosis in mice. Isoflurane, an inhaled anesthetic, and a hydrophobic liquid at room temperature, readily partitions itself into nanoparticles upon vortexing for 15 seconds in a sealed container. After loading isoflurane, alternating layers of positively and negatively charged polysaccharides (chitosan and alginate, respectively) are electrostatically bound to Intralipid® nanoparticles and excess reagents were removed via centrifugation (10°C, 15 minutes @18,500 RCF). Zeta potential and size was measured both pre- and post-centrifugation and confirmed layer-by-layer coating with little change to nanoparticle size.

Methods

A. 80ul of isoflurane added
B. Isoflurane incorporated into Intralipid® via vortexing (15 seconds).
C. 3ml Isoflurane saturated water (isowater), and 1ml Chitosan added.
D. Sample centrifuged for 15 minutes. Pellet is formed.
E. Supernatant is removed and pellet is resuspended in 3ml iso-water and 1ml alginate.

Results

• Intralipid® nanoparticles are 270 nm in diameter and -39mV.
• Surface charge of chitosan coated Intralipid® nanoparticles becomes positive.
• Surface charge of alginate coated Chitosan-Intralipid® nanoparticles becomes negative.
• Size increased with each additional layer.
• Very low viscosity alginate caused the least increase in nanoparticle size.

Conclusions

• The results gathered show that the Layer-by-Layer coating method can be done using chitosan and alginate, and can be applied to Intralipid® nanoparticles.
• Addition of alternating layers of chitosan and alginate causes the overall surface charge of the nanoparticles to switch from positive (with addition of chitosan) to negative (with addition of alginate).
• Viscosity of alginate has influence on nanoparticle size, and that aggregation of nanoparticles becomes a problem after the second chitosan layer.

Future Directions

• To continue further addition of subsequent chitosan and alginate layers, while trying to minimize increase in nanoparticle size, and reduce aggregation of nanoparticles.
• Quantify amount of material lost after filtering nanoparticles through a syringe filter.
• Measure loss of isoflurane from nanoparticles through Raman spectroscopy and gas chromatography
• Injection into mice to see effect of additional layers on length of narcosis caused by isoflurane.

References

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