Oral Delivery of Anti-inflammatory Small Oligonucleotides with Lipid Nanocrystals (LNCs)

Hui Liu, Mariam Mikhael, Amra Tabakovic, Shan Liu, Vinod Ramgolam, Partha Samadder, TJ Sheu,
Eugene Dank, Thomas Hoover, Jeffrey Bender, James Ferguson
Matinas Biopharma, Bedminster, NJ

MATINAS BIOPHARMA

Background

Recent years have seen remarkable advances in the treatment of inflammatory diseases, but effectively delivering RNAi therapeutics orally still poses a significant challenge.

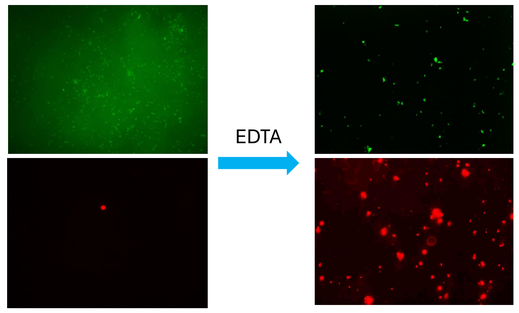
Lipid nanocrystals (LNCs) are highly stable nanoparticles that self-assemble when phosphatidylserine (PS) liposomes and calcium are combined, and their unique anhydrous multilayered structure permits incorporation of cargo molecules between the lipid bilayers. LNCs have been successfully used for the oral delivery of amphotericin in patients with cryptococcal meningitis.

We sought to assess whether the LNC platform could be similarly successful in orally delivering mall oligo therapeutics for inflammatory disease.

Methods and Results:

LNCs successfully encapsulate/protect RNAi (Fluorescence microscopy)

Recognizing the difficulties of encapsulating small oligos into lipid structures due to their charge and hydrophilic nature, we adopted formulation strategies that enabled both single-stranded ASOs and double stranded siRNA to be embedded in the LNC lipid layer, with excellent encapsulation efficiency (>90% by HPLC). Protection of the cargo within the crystalline LNC structure was further confirmed through fluorescence imaging with dual-labeled LNCs (lipid labeled with FITC; small oligo labeled with Cy5). Under fluorescent microscopy, only intact LNCs (green FITC) were visible. However, upon the addition of EDTA, both green FITC and red Cy5 were evident, confirming protection with intact LNCs, and release of cargo when calcium concentrations are lowered.

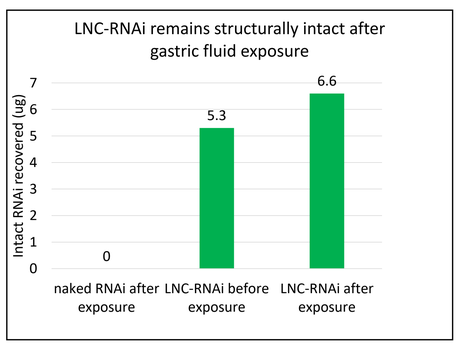


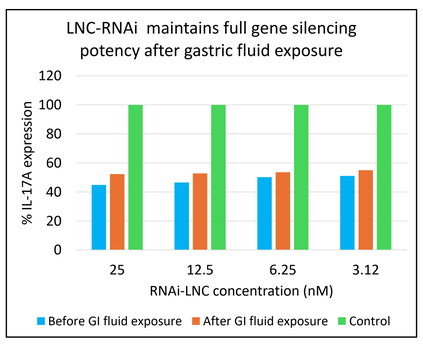
Before adding EDTA:
RNAi cargo (Cy5) is encapsulated in LNC and not visible

After adding EDTA:
Cargo release, both cargo (Cy5) and lipids (FITC) are visible

LNCs preserve the structural integrity and biological potency of oligonucleotides after simulated gastric fluid exposure (HPLC & gastrointestinal stability assay)

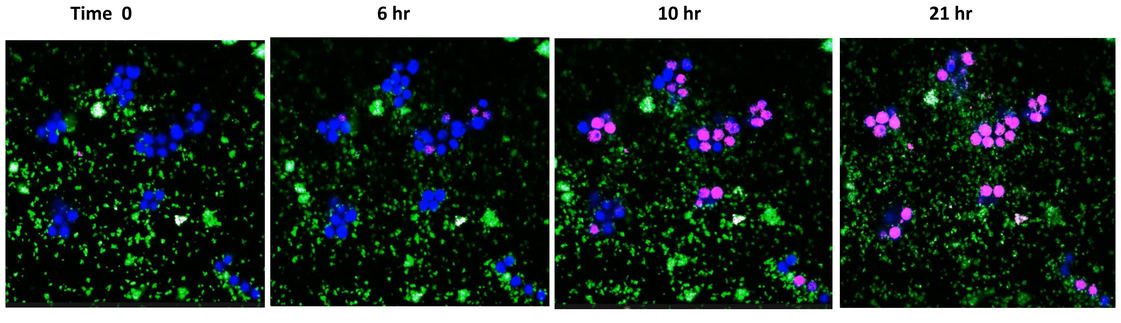
The LNC structure also provided robust protection for small oligonucleotides targeting specific cytokines in a simulated gastrointestinal (GI) stability test, where unprotected small oligonucleotides were rapidly degraded upon exposure to simulated gastric fluid (pH 1.2, with pepsin, exposed for 1 hour), while LNC-encapsulated small oligos remained intact, with no change in their cytokine knockdown capabilities after GI fluid exposure.





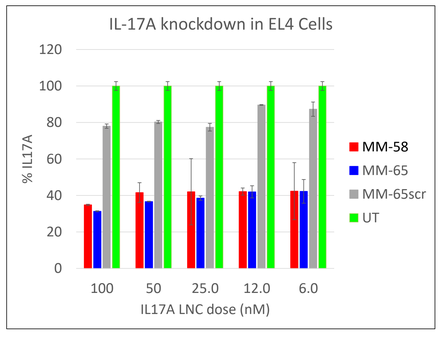
LNCs release oligonucleotides in response to the intracellular ultra-low calcium levels (Confocal microscopy)

We also conducted live cell imaging studies in HEK293 cells. Dual-labeled LNCs (256 ng/mL scrambled siRNA) were added, and cells imaged every 30 minutes for a period of 24 hours to examine the dynamics of LNC uptake in these cells. For higher-resolution mechanistic imaging, HEK293 cells were treated with LNCs and harvested at key time points. Over a 24-hour time course, only green FITC-LNC was observed for the initial 5 hours, strongly suggesting that all siRNA was encapsulated, and no free nucleotides were present. From the 6th hour onward, more and more red Cy5 signals appeared inside the cells, indicating that LNCs had been taken up by the cells and that the oligo cargo was released only in the ultra-low calcium intracellular environments.

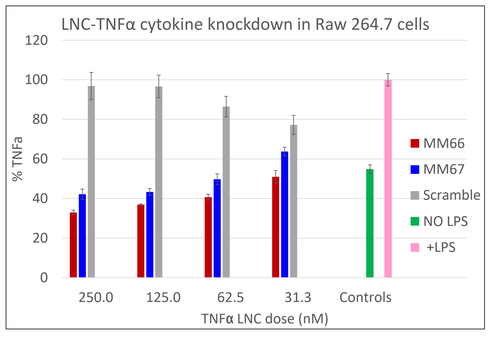


LNCs encapsulated therapeutic oligonucleotides showed robust knockdown potency in vitro

We then went on to perform *in vitro* cell culture studies with LNC formulations of different cytokine-targeted small oligos. Up to 70% of IL-17A cytokine knockdown was observed in EL4 cell lines, and similar potency of TNFα knockdown (around 70%) was observed in RAW264.7 murine macrophages using LPS stimulation to activate TNFα production, while scrambled small oligo formulation did not show much potency.



IL-17A cytokine knockdown in EL4 cells



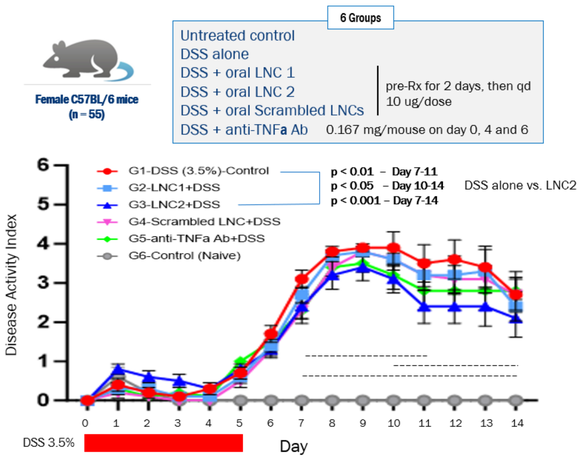
TNFα cytokine knockdown in RAW 264.7 cells

In vivo study: Oral LNC-TNFα in a Murine DSS acute colitis model

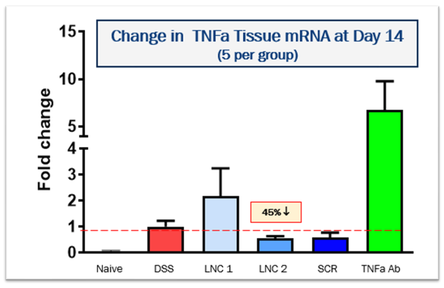
Study design:

- Female C57BL/6 mice were evaluated in this study. Acute colitis was induced by administering 3.5% DSS in drinking water for 5 days.
- Disease group (no treatment) and LNC with scrambled RNAi served as negative controls, and anti- TNFα antibody was administered intraperitoneally on Day 0,2, 4, and 6 as a positive control.
- Two formulations, LNC1 and LNC2, were orally administered at 10 ug/dose from Day -2 to 14
- Mice body weight, disease activity index (DAI) and serum cytokine were primary readouts.

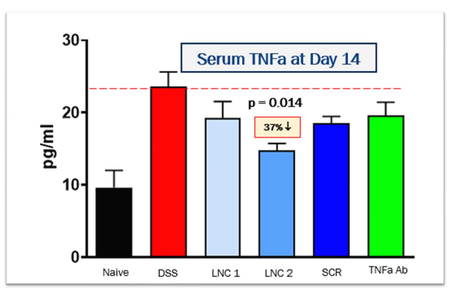
Results:



clinical disease activity scores at key time points in the studies were significantly improved with an active LNC formulation



Colon tissue TNFα mRNA levels, as assessed by quantitative real-time PCR analysis, were 46% lower following orally administered active LNCs, in comparison to diseased animals without treatment



Serum TNFa levels, as assessed by ELISA, were 46% lower following orally administered active LNCs, in comparison to diseased animals without treatment

Conclusions

The LNC delivery platform can successfully encapsulate and protect small oligonucleotides. This capability enables oral delivery of biologically active oligonucleotides targeting key cytokines in inflammatory disease models. Oral delivery of an anti-TNFα small oligo improved disease activity scores, reduced colon TNFα mRNA and serum TNFα levels. While these initial results are promising, future work will be focused on optimizing the LNC formulations to improve delivery efficiency, increase their potency, and extend the application of oral cytokine-targeting oligo therapeutics in cancer and other inflammatory disease models.