Evaluation of triazine-based lipid nanoparticle (LNP) formulations for mRNA delivery in a mouse model

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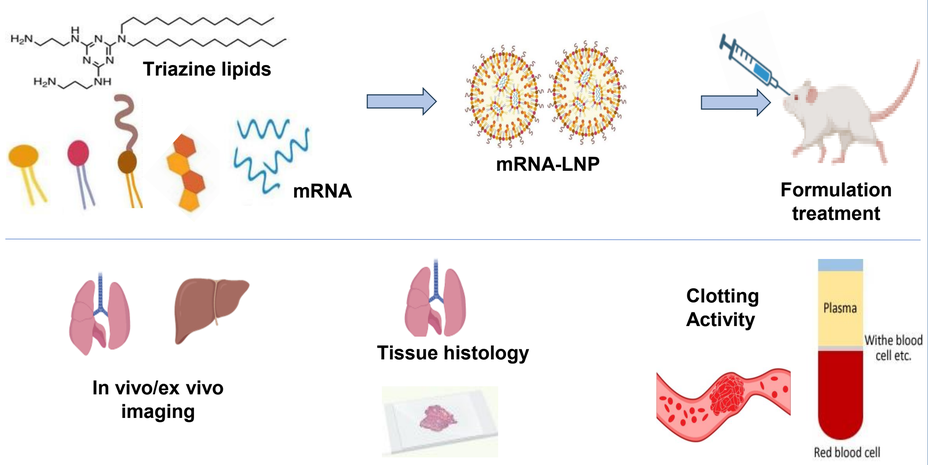
ABSTRACT

Lipid nanoparticles (LNPs) are the most advanced delivery systems for messenger RNA (mRNA). Recent clinical trials of mRNA-LNP therapeutics have focused on vaccines, liver diseases, and cancer. However, safe and effective delivery of mRNA to the lungs is still a formidable challenge [1]. To meet the critical need, the primary component of LNP, called ionizable lipids, can be engineered to target specific tissues. In this study, we designed a library of triazine-based (TZ) ionizable lipids with cyanuric chloride and dialkyl amines [2] that are capable of systemic mRNA delivery to the lung tissues. The TZ-based LNPs specifically delivered Fluc mRNA to the lungs following intravenous injections in mice. Furthermore, evaluation for blood toxicity or clotting [3,4] in the tissue is still under investigation. Our findings highlight the potential of TZ lipids for safe pulmonary delivery of mRNA, opening therapeutic avenues for lung-related diseases.

EXPERIMENTAL DESIGN



OBJECTIVE

To evaluate mRNA transfection efficiency of Triazine (TZ) lipids both in vitro and in vivo for fine-tuning its biocompatibility in the blood and tissue of a mouse model.

CONCLUSION AND FUTURE DIRECTIONS

Triazine (TZ) lipids demonstrated lung-specific mRNA expression in mice with minimal liver expression, unlike ALC-0315. Also, compared to DOTAP cationic lipid, TZ lipids demonstrate reduced blood clotting and tissue damage in mouse lungs. For the future, the planned experiments are to test

- Types of pulmonary cells transfected by TZ lipid formulations
- Assess TZ transfection efficiency in a mouse disease model
- Continue tissue staining to further evaluate blot clotting in the mouse lung.

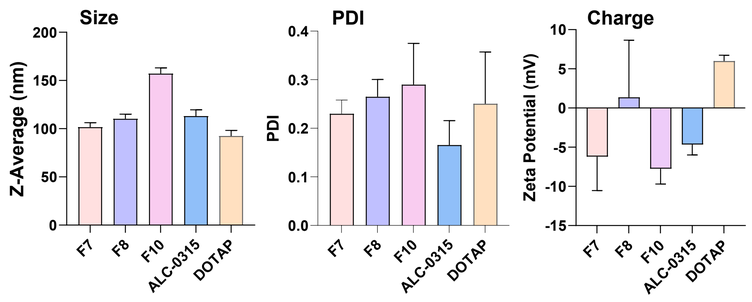
RESULTS

Physico-chemical characterization

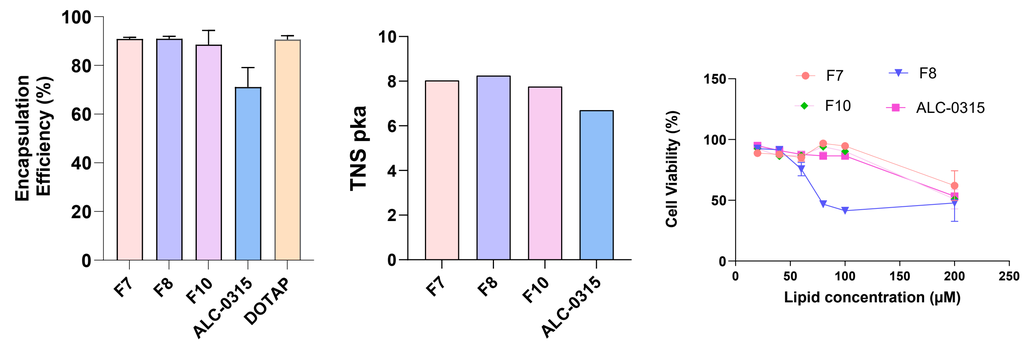
Table 1: Composition of lipids tested

	Composition	Mol ratio
F7	Lipid 7 : DOPE : DSPE-PEG2000 : Chol	50 : 10 : 1.5 : 38.5
F8	Lipid 8 : DOPE : DSPE-PEG2000 : Chol	50 : 10 : 1.5 : 38.5
F10	Lipid 10 : DOPE : DSPE-PEG2000 : Chol	50 : 10 : 1.5 : 38.5
DOTAP	DOTAP : CKK-E12 : DOPE : DMG-PEG2000 : Chol	50 : 25 : 5 : 1.5 : 18.5
ALC	ALC-0315 : DSPC : DMA-PEG2000 : Chol	46 : 9.4 : 1.7 : 42.9

TZ lipid formulations (F7, F8, F10) optimized by design of experiment (DOE) approach. DOTAP and ALC formulations based on literature reports.

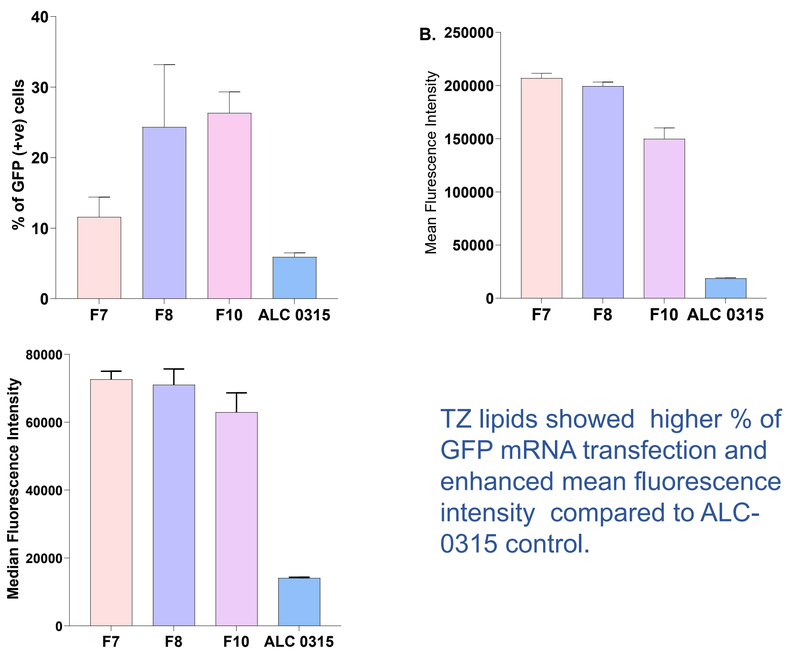


TZ lipids exhibited similar formulation characteristics compared to the industry standard (ALC-0315, Pfizer).



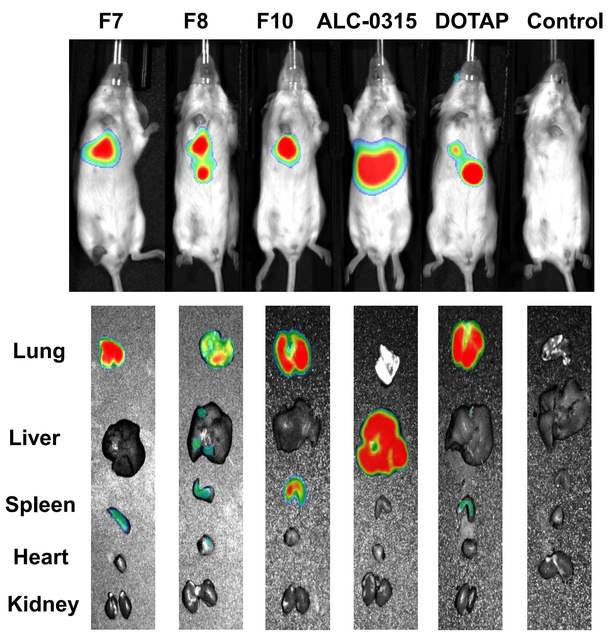
TZ lipids have improved EE%, similar toxicity, and pKa values greater than ALC-0315.

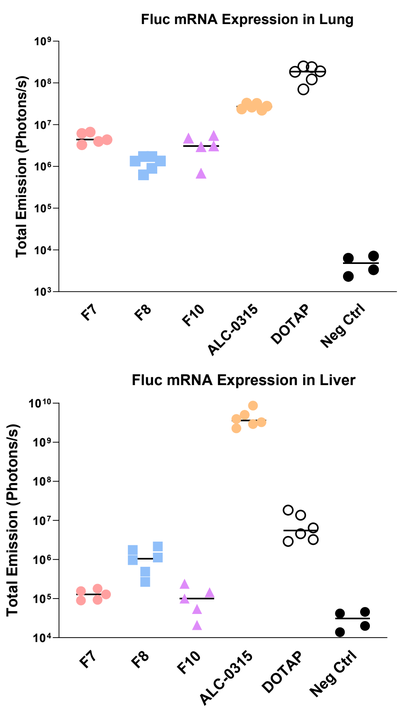
In vitro HEK-293 transfection efficiency

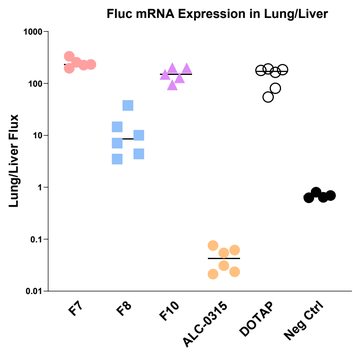


TZ lipids showed higher % of GFP mRNA transfection and enhanced mean fluorescence intensity compared to ALC-0315 control.

In vivo transfection efficiency

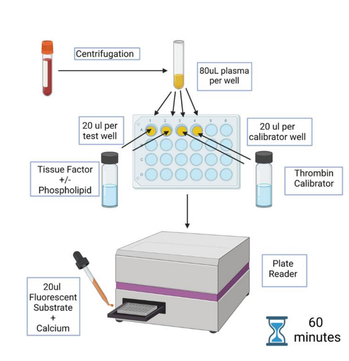


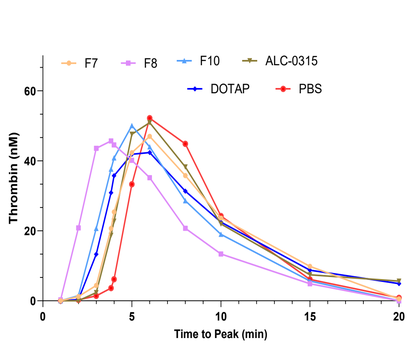


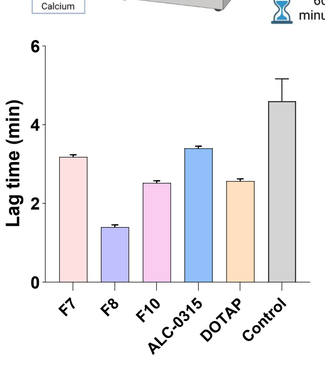


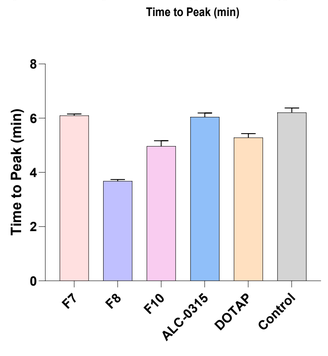
TZ lipids demonstrated lung-specific expression of Fluc mRNA in mouse; with 100-fold enhanced lung/liver in comparison to ALC-0315, and similar ratio as DOTAP lipid.

Calibrated Automated Thrombin (CAT) Assay in ex vivo plasma from naïve mice



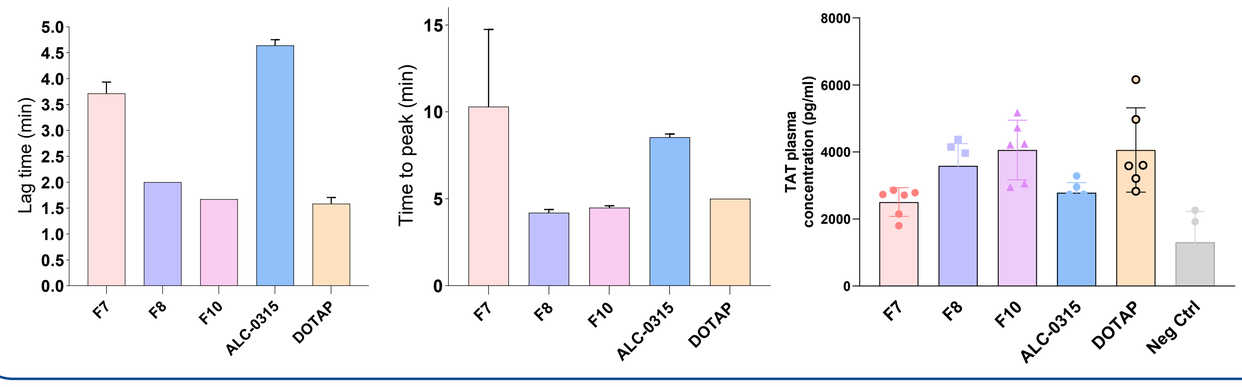






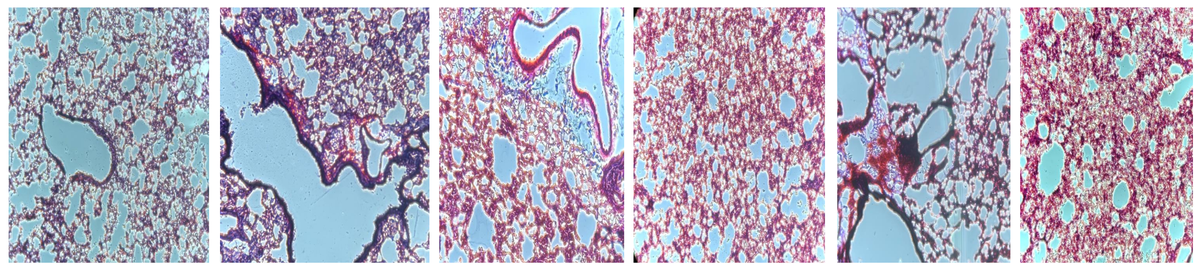
TZ lipid, F7 exhibited similar lag time and time to peak as the ALC-0315 control for thrombin generation in the CAT assay.

Blood clotting assessment in plasma collected from mRNA-LNP-treated mice



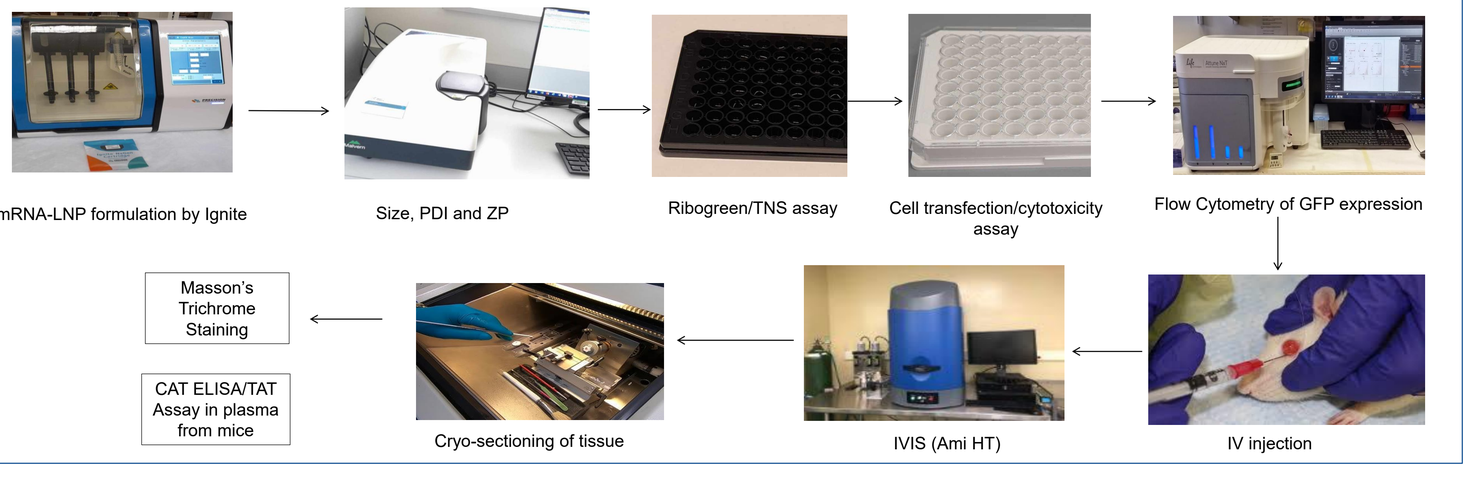
TZ lipid F7, showed similar lag time and Thrombin-antithrombin plasma (TAT) concentration as ALC-0315 control.

Evaluation of tissue damage and blood clotting in lung tissues by Masson's Trichrome staining



TZ lipids and DOTAP display tissue damage in the lung of mice compared to ALC-0315 and control untreated mice.

METHODS



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