Iterative Design of Novel Ionizable Steryl Lipid (ISL) Based 3-Component Lipid Nanoparticles (3C-LNPs) for Intramuscular Delivery of mRNA Vaccines

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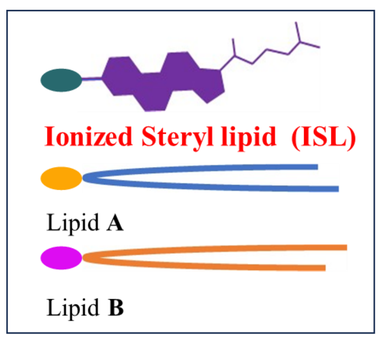
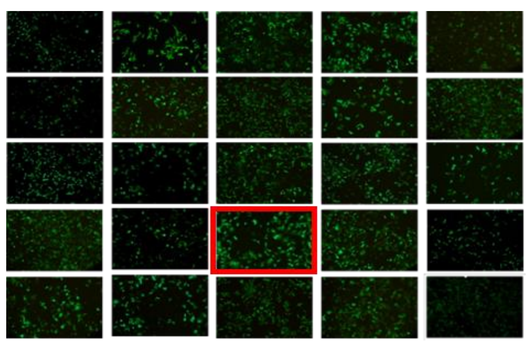
Abstract

RNA-based therapies including mRNA, siRNA, and ASO, have shown great promise in treating a broad spectrum of diseases such as infections, tumors, and rare diseases. Most recently, two vaccines of lipid nanoparticles (LNPs) encapsulating mRNA, mRNA-1273 and BNT162b2, have achieved great success in the prevention and control of COVID-19 pandemic. Conventional LNPs are formulated with four lipid components including ionizable lipid, cholesterol, PEG-lipid, and helper lipid. The functional delivery of mRNA by LNP greatly depends on the inclusion of ionizable lipids. The risk of mRNA delivery to off-target tissues highlights the necessity for LNPs with enhanced tissue selectivity. mRNA delivered by conventional LNPs after intramuscular administration partly reaches the liver and results in substantial expression of the target proteins in the liver. Herein, we showed that the iterative design of novel ionizable steryl lipids (ISLs) based on three-components LNP (3C-LNP) exhibited high efficiency of mRNA encapsulation and delivery, good safety profile, and excellent stability during storage. Furthermore, the 3C-LNPs were identified as local mRNA delivery systems through intramuscular administration. It manifested high transfection efficiencies at local sites without systemic exposure which minimized systemic side effects. This study indicated that the ISL-3C-LNPs have great potential for mRNA vaccine delivery, which is prioritized for the CD8+T cell activation such as mRNA tumor vaccine. Meanwhile, the local delivery feature of the ISL-3C-LNPs introduces a promising approach for safe and effective gene therapy targeting muscle tissue.

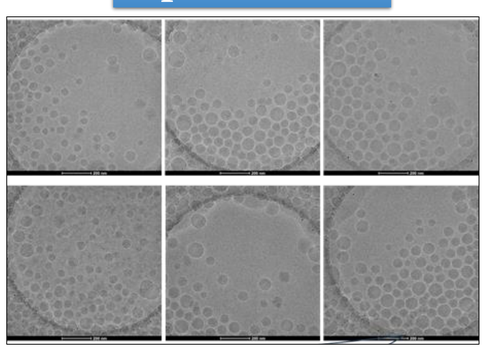
Optimization



More than 30 ISLs were synthesized and evaluated



EGFP expression in Hep3B cell



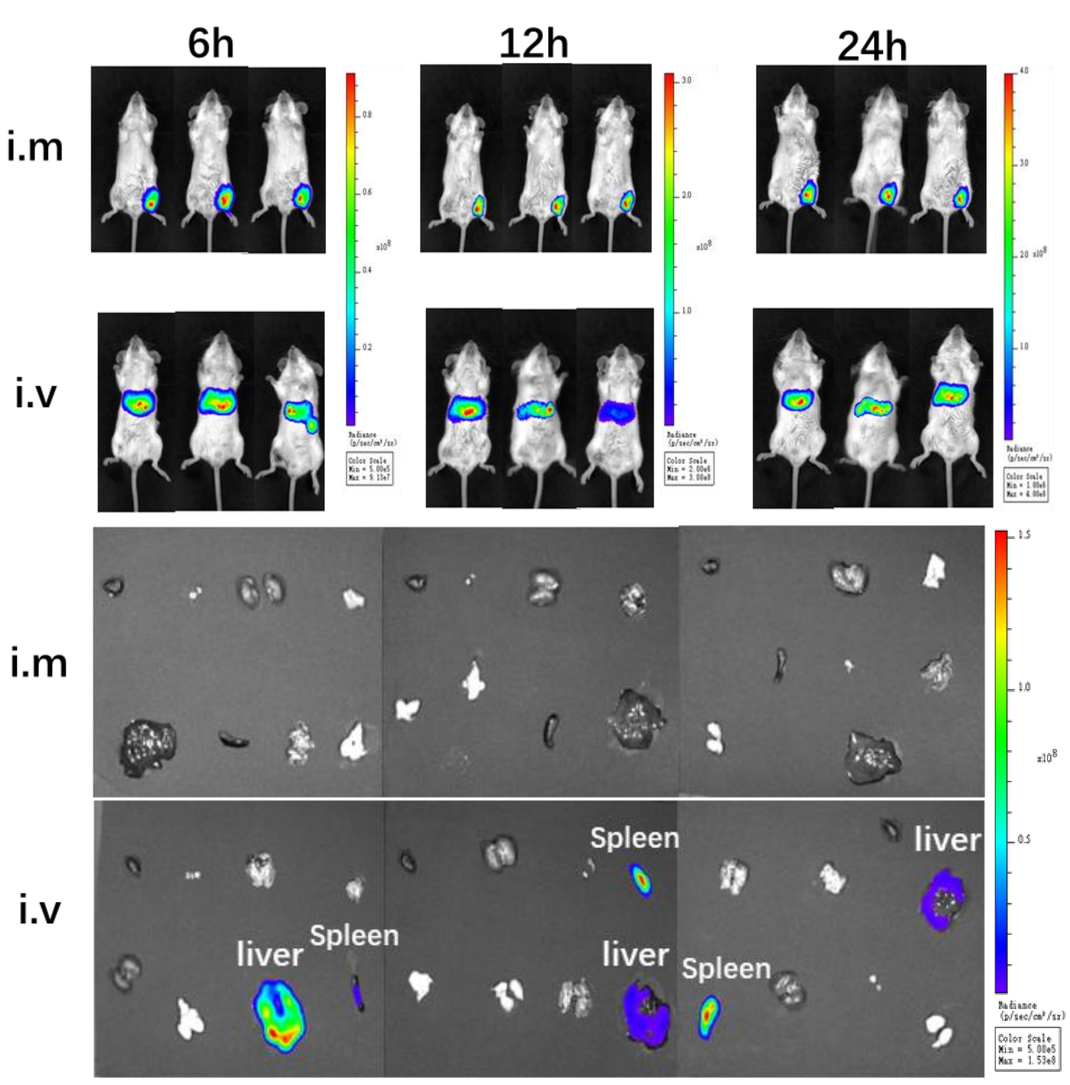
Cryo-EM imaging of 3C-LNP

LNP formulation	Particle size	PDI	Zeta-potential	mRNA encapsulation efficiency	pKa (TNS)
JK-0039	90.33 nm	0.07837	-4.895 mV	91.59 %	5.61
SM-102 (Moderna)	85.23 nm	0.06328	-3.736 mV	93.19 %	6.57
ALC-0315 (BioNTech)	89.29 nm	0.04496	-1.160 mV	92.41 %	6.01

The physical and chemistry quality attributes of JK-0039 formed 3C-LNP and SM-102 and ALC-0315 formed 4C-LNPs.

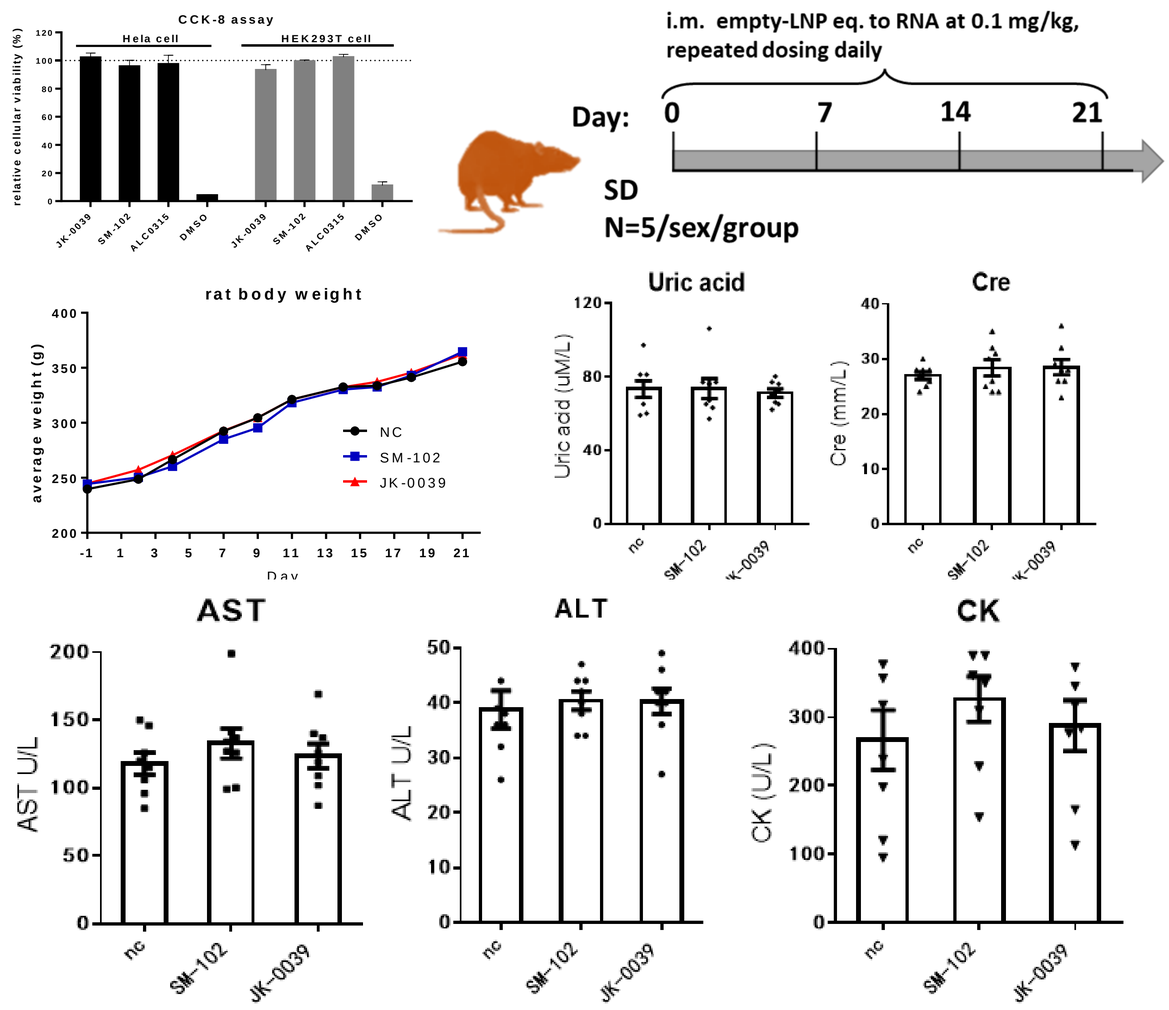
After optimization of the chemistry and the components of LNP formulations, the ISL-based 3C-LNP showed efficient encapsulation of mRNA. Safety and bioactivity were also evaluated both in vitro and in vivo. The ISL compound JK-0039 was screened as a candidate with great potential.

Biodistribution



6-8 weeks old female Balb/c mice were administrated with 5 ug mRNA-LNP through i.m. or i.v. injection and *in vivo* bioimaging were taken at post 6\12\24 hours. *ex-vivo* bioimaging was taken at post 24 h including liver, heart, spleen, kidney, urogenital system, and lung. The Bioimaging results indicated the 3C-LNPs identified as local mRNA delivery systems *via* intramuscular administration without systemic exposure.

Safety



The *in vitro* toxicity study were obtained including CCK-8 assay, AMES test and hERG test. At the concentration of 200 μM JK-0039 showed no toxicity to both HEK293T and Hela cells. AMES results showed no genotoxicity. hERG showed JK-0039 low potential cardiotoxicity.

The repeated dosing toxicity study in SD rats showed no obvious adverse effects after administrated with JK-0039 based 3C-LNP.

Conclusion

1. A novel series of ionizable steryl lipids based on three-component LNPs (3C-LNPs) were successfully developed which showed high efficiency of mRNA encapsulation and delivery.
2. The novel ISL-3C-LNP were identified as local mRNA delivery systems through intramuscular administration without systemic exposure which minimized systemic side effects.
3. JK-0039 formed 3C-LNP showed low toxicity both *in vitro* and *in vivo*.