Novel Ionizable Cationic Lipids and the Corresponding LNPs for Selective Delivery of Nucleic Acids

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Abstract:

INTRODUCTION: Lipid nanoparticles (LNPs) typically consist of four components—ionizable lipids, phospholipids, cholesterol, and PEGylated lipids. However, traditional LNPs accumulate in liver after intravenous injection or intramuscular administration, greatly limiting their therapeutic applications. The risk of RNA delivery to off-target tissues highlights the necessity for LNPs with enhanced tissue selectivity.
Research has shown that the addition of different types of selective organ targeting (SORT) lipids to traditional LNPs can alter their *in vivo* delivery profile, achieving targeted delivery of mRNA to various non-liver tissues.

RESULTS: Our laboratory has developed two types of permanently cationic lipids (JK-0055 & JK-0056), which can form stable SORT LNPs with traditional LNPs. *In vitro* experiments have shown that two types of SORT LNPs carrying GFP mRNA can stably express green fluorescent protein after being transfected into Hep3b cells. *In vivo* experiments have shown that two types of SORT LNPs can specifically concentrate in the lungs of mice after intravenous injection.
We also designed a novel ionizable steryl lipids (ISLs) based three components LNP (3C-LNP), which exhibited high efficiency of mRNA encapsulation and excellent stability during storage. *In vitro* assays indicated that C3-JK-0039 LNPs achieved targeted delivery specifically to keratinocytes, without affecting breast cancer cells. Additionally, the C3-LNPs demonstrated no cytotoxicity in CCK-8 assays and did not induce skin allergies *in vivo*. What is more, the 3C-LNPs were identified as local mRNA delivery systems through intramuscular administration.

CONCLUSION: Both JK-0055 and JK-0056 based SORT LNPs can be taken up by cells *in vitro* and specifically accumulated to lungs *in vivo*. C3-JK-0039 LNPs is a potential muscle-targeted delivery system with high efficiency of mRNA encapsulation, excellent stability and a good safety profile.

Part 1: Novel Permanently Cationic Lipids (PCL) Based SORT Lipid Nanoparticles (SORT LNPs) for Specific Delivery of mRNA to Lungs in Mice.

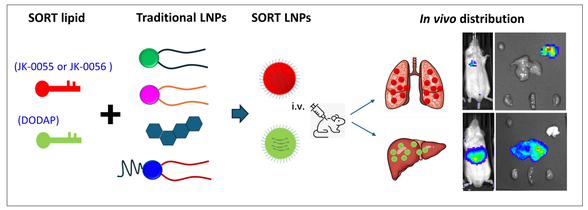


Figure 1: Design and preparation of JK-0055 and JK-0056 based SORT LNPs. JK-0055 or JK-0056 was added to traditional LNPs to form stable SORT LNPs, which can specifically deliver mRNA to lung tissues *in vivo*.

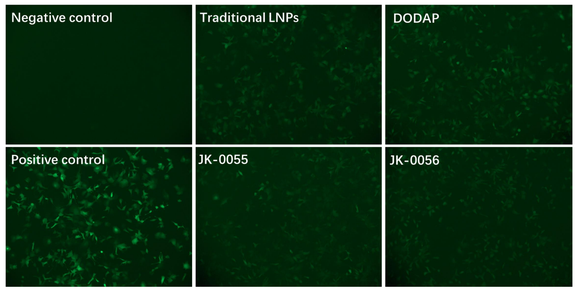


Figure 2: Transfection efficiency of different SORT LNPs *in vitro*. SORT LNPs carrying GFP mRNA can stably express green fluorescent protein at 12h after being transfected into Hep3b cells.

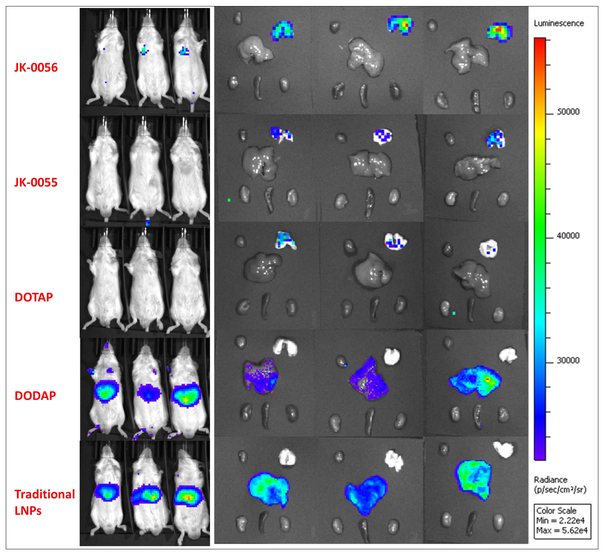
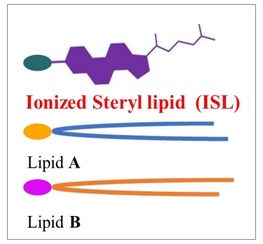
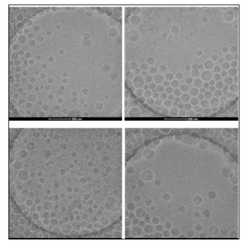


Figure 3: The tissue distribution of different SORT LNPs in mice. After i.v. injection of Luc-mRNA encapsulated within traditional LNPs or DODAP based SORT LNPs, fluorescence was detected only in the liver tissue of mice. After i.v. administration of Luc-mRNA encapsulated in DOTAP based SORT LNPs, fluorescence was concentrated in the lungs of mice. After i.v. administration of Luc-mRNA encapsulated in JK-0055 or JK-0056 based SORT LNPs, fluorescence was concentrated in the lungs of mice.

Part 2: Novel Ionizable Steryl Lipid (ISL) Based 3-Component Lipid Nanoparticles (3C-LNPs) For Selective Delivery of siRNA in Muscle.





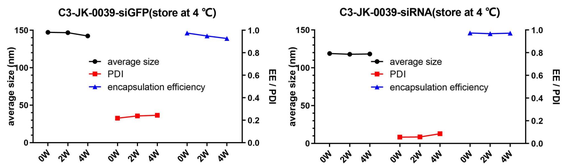
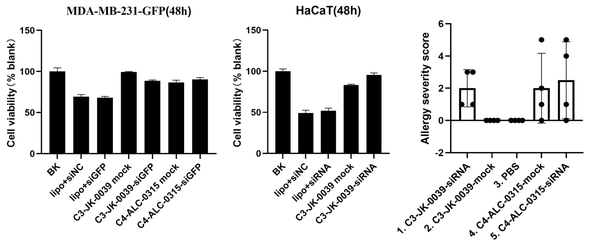
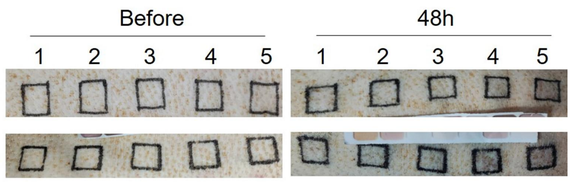


Figure 1: Preparation, characterization and stability of 3-Component Lipid Nanoparticles (3C-LNPs). LNPs with different cationic lipids were prepared. The physical and chemistry quality attributes were characterized as above. C3-JK-0039 LNPs were stable at 2-8℃ for at least 4 weeks.





G1: C3-JK-0039-siRNA G2: C3-JK-0039-mock G3: PBS
G4: C4-ALC-0315-mock G5: C4-ALC-0315-siRNA

Figure 3: Safety evaluation of C3-JK-0039 LNPs. The *in vitro* cytotoxicity study was analyzed by CCK-8 assay. C3-JK-0039 LNPs showed no toxicity to both MDA-MB-231-GFP and HaCaT cells at 48 hours after transfection.
LNPs contained different ionizable lipid were administrated to the back of a Bama Xiang pig by subcutaneous injection. After 48 hours, no obvious allergic response was observed at the site of PBS and C3-JK-0039 LNPs mock while C4-ALC-0315 based LNPs did.

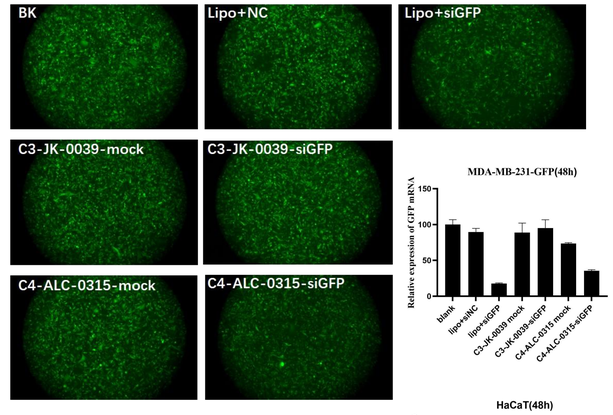


Figure 2: Transfection efficiency of C3-JK-0039 LNPs *in vitro*. Three components-(C3-JK-0039-siRNA) and four components-LNP (C4-ALC-0315) were prepared separately and transfected into MDA-MB-231-GFP cell. After 48h, relative mRNA expression level and average fluorescence intensity were assayed. It indicated C3-JK-0039 did not deliver siRNA into breast cancer (MDA-MB-231-GFP) cells.
Three components-(C3-JK-0039-siRNA) was transfected into HaCaT cell. The relative mRNA expression level was assayed by qPCR. It showed C3-JK-0039 could deliver siRNA into keratinocytes (HaCaT) and downregulate gene expression. Therefore, LNP has potential for applications in cosmetic medicine.

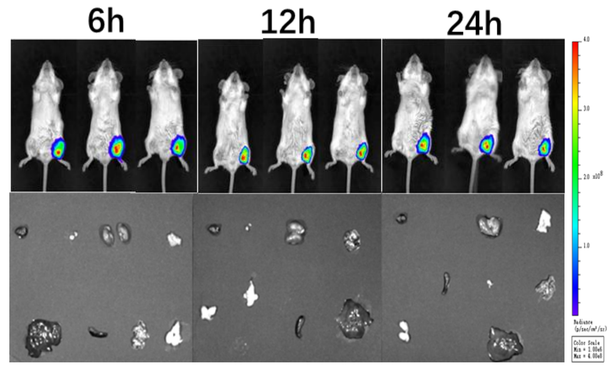


Figure 4: The tissue distribution of C3-JK-0039 LNPs in mice: Balb/c mice were administrated with 5 ug mRNA-LNP through i.m. injection and *in vivo* bioimaging were taken at post 6\12\24 hours. ex-vivo bioimaging was taken at post 24 hours including liver, heart, spleen, kidney, urogenital system, and lung. The bioimaging results indicated the 3C-LNPs identified as local mRNA delivery systems via intramuscular administration without systemic exposure.