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

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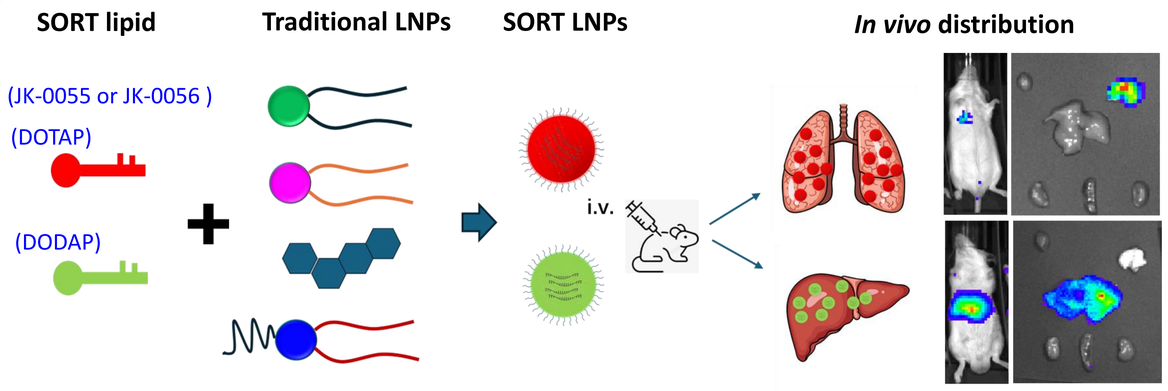
Abstract

Lipid nanoparticles (LNPs) typically consist of four components—ionizable lipids, phospholipids, cholesterol, and PEGylated lipids. Approved mRNA COVID-19 vaccines benefit from this delivery system. However, traditional LNPs accumulate in the liver and are internalized by liver hepatocytes after intravenous injection, greatly limiting their therapeutic applications. 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. 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. This differs from the *in vivo* distribution of traditional 4-component LNPs, which only accumulated significantly in the liver of mice after intravenous injection. In summary, this study developed two SORT LNPs that can be taken up by cells *in vitro* and specifically accumulated to lungs *in vivo*. These SORT LNPs provide a promising and innovative method for the lung-specific delivery of nucleic acid drugs, with the potential to significantly improve the treatment of lung diseases.

Preparation

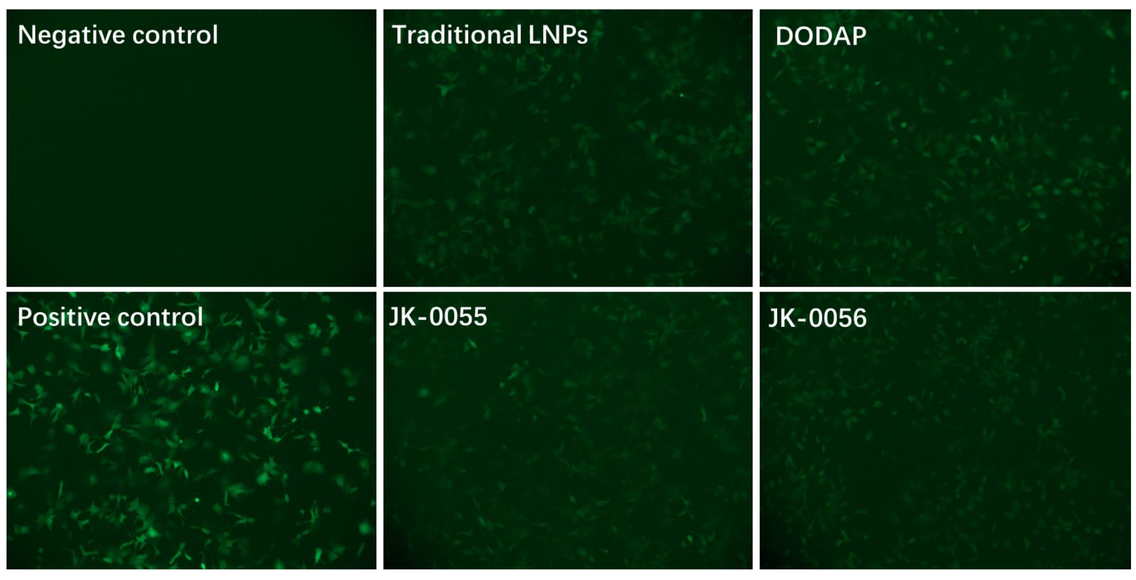


JK-0055 or JK-0056 was added to traditional LNPs to form stable SORT LNPs, which can specifically deliver mRNA to lung tissues ***in vivo***.

Physical and Chemical Characteristics of JK-0055 or JK-0056 based SORT LNPs

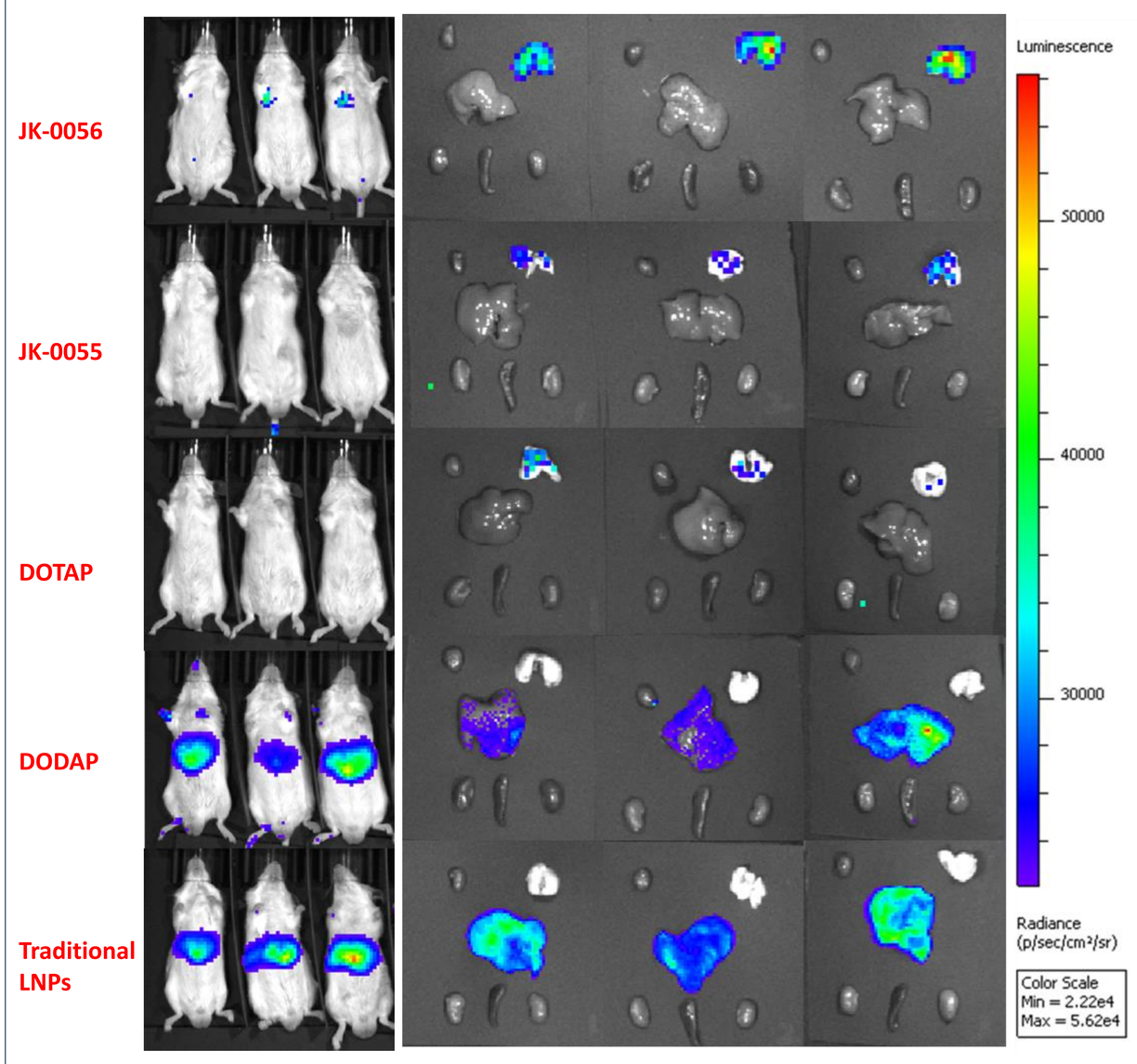
LNP formulation	Particle size	PDI	Zeta-potential	mRNA encapsulation efficiency
DODAP based SORT LNPs	71.483nm	0.091	-1.163mV	98.66%
DOTAP based SORT LNPs	88.263nm	0.087	2.670mV	96.66%
JK-0055 based SORT LNPs	79.503nm	0.112	0.2447mV	97.56%
JK-0056 based SORT LNPs	81.297nm	0.072	0.143mV	97.06%
Traditional LNPs	80.997nm	0.060	-0.737mV	98.74%

In vitro transfection



SORT LNPs carrying GFP mRNA can stably express green fluorescent protein at 12h after being transfected into Hep3b cells.

Biodistribution



6-8 weeks old female Balb/c mice were administrated with 5 ug mRNA-LNP through i.v. injection. *In vivo* bioimaging were taken at post 24 hours. *Ex-vivo* bioimaging was taken at post 24 h including liver, heart, spleen, kidney, and lung.

1. After i.v. administration of Luc-mRNA encapsulated within traditional LNPs or DODAP based SORT LNPs, fluorescence was detected only in the liver tissue of mice.
2. After i.v. administration of Luc-mRNA encapsulated in DOTAP based SORT LNPs, fluorescence was concentrated in the lungs of mice.
3. 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.
4. The bioimaging results indicated that both JK-0055 and JK-0056 based SORT LNPs were specifically concentrated in the lungs of mice after intravenous injection.

Conclusion

1. Two types of permanently cationic lipids (JK-0055 & JK-0056) were synthesized to form SORT LNPs.
2. Both JK-0055 and JK-0056 based SORT LNPs carrying GFP mRNA can be transfected into Hep3b cells and stably express green fluorescent protein *in vitro*.
3. Luc-mRNA encapsulated in JK-0055 or JK-0056 based SORT LNPs can be specifically delivered to the lungs of mice and stably express luciferase after intravenous injection.
4. These SORT LNPs provide a promising and innovative method for the lung-specific delivery of nucleic acid drugs, with the potential to significantly improve the treatment of lung diseases.