Route and Age-Dependent LNP Delivery to Cardiopulmonary Tissues

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Background: Cardiovascular and respiratory diseases remain the leading global causes of morbidity and mortality, imposing a substantial unmet need for regenerative and gene-modifying therapies. Although mRNA-LNP therapeutics offer transformative potential, their clinical utility is limited by the strong liver tropism of current LNPs and the difficulty of achieving extrahepatic delivery to cardiac cells in the heart and epithelial cells in the lungs. Robust cell-type-specific transfection and direct visual confirmation of transfection in these tissues remain limited. Neonatal delivery offers a unique therapeutic opportunity for congenital diseases, yet systematic comparisons of LNP performance across developmental stages and delivery routes are lacking. To address these gaps, we evaluated three LNP formulations with distinct ionizable and helper lipids to assess multi-route mRNA delivery to the heart and lungs in neonates and adults. Method: Neonatal and adult Rosa26-mTmG reporter mice received Cre-mRNA via intravenous (IV), intramyocardial (IC), or intratracheal (IT) administration. Transfection was quantified by fluorescence immunostaining and imaging across the heart, lung, trachea, and liver. Results: IV delivery revealed distinct chemistry-dependent tropism. Neutral-helper-lipid formulations showed expected hepatic uptake but achieved robust cardiomyocyte and pulmonary endothelial transfection in both neonates and adults. Cationic-helper-lipid formulations markedly reduced liver tropism and selectively targeted pulmonary and cardiac endothelium, with cardiomyocyte transfection in neonates and valve-cell transfection in adults. IC administration produced charge-independent, localized cardiomyocyte and endothelial transfection in the heart. In contrast, IT delivery resulted in strong epithelial transfection including ciliated, secretory, AT1, AT2, and basal stem cells exclusively with cationic formulations, while neutral formulations showed minimal epithelial activity. Conclusion: These results expand the transfection profile of LNP formulations, revealing how lipid composition and delivery route govern mRNA access to cardiac and pulmonary cell types across developmental stages. These insights provide a foundation for advancing mRNA therapeutics for congenital and acquired cardiovascular and pulmonary diseases.