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The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery☆

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ABSTRACT

Lipid nanoparticles (LNPs) have shown promise as delivery vehicles for therapeutic oligonucleotides, including antisense oligos (ONs), siRNA, and microRNA mimics and inhibitors. In addition to a cationic lipid, LNPs are typically composed of helper lipids that contribute to their stability and delivery efficiency. Helper lipids with cone-shape geometry favoring the formation hexagonal II phase, such as dioleoylphosphatidylethanolamine (DOPE), can promote endosomal release of ONs. Meanwhile, cylindrical-shaped lipid phosphatidylcholine can provide greater bilayer stability, which is important for in vivo application of LNPs. Cholesterol is often included as a helper that improves intracellular delivery as well as LNP stability in vivo. Inclusion of a PEGylating lipid can enhance LNP colloidal stability in vitro and circulation time in vivo but may reduce uptake and inhibit endosomal release at the cellular level. This problem can be addressed by choosing reversible PEGylation in which the PEG moiety is gradually released in blood circulation. pH-sensitive anionic helper lipids, such as fatty acids and cholesteryl hemisuccinate (CHEMS), can trigger low-pH-induced changes in LNP surface charge and destabilization that can facilitate endosomal release of ONs. Generally speaking, there is no correlation between LNP activity in vitro and in vivo because of differences in factors limiting the efficiency of delivery. Designing LNPs requires the striking of a proper balance between the need for particle stability, long systemic circulation time, and the need for LNP destabilization inside the target cell to release the oligonucleotide cargo, which requires the proper selection of both the cationic and helper lipids. Customized design and empirical optimization is needed for specific applications.

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1. Introduction

1.1. Oligonucleotide (ON) therapeutics

ONs are an emerging therapeutic modality with potential applications in many human diseases, such as metabolic diseases, infectious diseases, cancer and regenerative medicine. Therapeutic ONs can be classified based on their mechanisms of action. Antisense ONs targeting mRNAs are usually DNA-based and can down-regulate gene expression by mechanisms such as RNaseH activation, translational inhibition, and exon skipping [1]. MicroRNAs (miRNAs) are naturally occurring non-coding RNAs that regulate gene expression through RNA interference (RNAi). Anti-microRNAs (antimiRs), usually RNA-based, are ONs that can bind tightly to their corresponding miRNA targets and indirectly up-regulate gene expression by inhibiting the activity of the miRNAs [2,3]. Both antisense ONs and antimiRs are single-stranded molecules. Meanwhile, small interfering RNAs (siRNAs) and miRNA mimics are typically RNA ON duplexes, which is a form that can be efficiently loaded into RNA-induced silencing complexes (RISCs) once inside the cytoplasm [4]. Other types of ONs with potential therapeutic application include aptamers [5], ribozymes [6], "CpG" immunostimulatory ONs [7], etc. Antisense ONs and siRNA constitute a majority of therapeutic ONs that have been studied in the clinic. They are relatively straight forward to design and synthesize following established rules. However, their site of action is in the cellular cytoplasm and they often require the use of a transfection agent for delivery in vitro. In vivo therapeutic delivery of ONs faces numerous challenges. First, ONs generally have high molecular weights and are polyanionic, therefore, have very limited cellular membrane permeability on their own [8]. Secondly, ONs can be rapidly cleared from circulation by renal excretion and by the reticuloendothelial system [9]. Finally, ONs are sensitive to degradation by serum exo- and endonucleases while in circulation and following cellular internalization [8]. To adequately address these problems are likely to require a combination of chemical modifications on the ONs and encapsulation into appropriately designed nanoparticles.

1.2. Chemical modifications on ONs

The problem of poor nuclease stability can be partially addressed by introducing chemical modifications to ONs, such as 2′-O–Me, 2′-F, 2′-O-(2-methoxyethyl) (2'MOE), morpholino, and locked-nucleic acid (LNA) nucleoside substitutions, and phosphorothioate, peptide nucleic acid (PNA), and phosphorodiamidate backbone substitutions [10].

It has often been assumed that while antisense ONs typically require the use of a transfection agent for in vitro delivery, they can be delivered in vivo without the use of a delivery vehicle [12]. This argument is supported by the FDA-approval of mipomersen. Mipomersen, trade name Kynamro, is an antisense ON that targets apolipoprotein B. It is given to patients by once a week subcutaneous injection at 200 mg without the use of a delivery system [11]. It is a "gapmer" that contains 2'MOE modified nucleotides at 5′ and 3′ ends and phosphorothioate linkages in the middle [11]. It is possible that the delivery of mipomersen is facilitated by its ability to bind to plasma proteins and by the fact that the liver is the target organ, which is highly accessible from circulation.

However, the overall performance of "naked" antisense ONs in clinical trials has been mixed [13]. There is substantial evidence in preclinical studies that delivery vehicles such as lipid nanoparticles (LNPs) can greatly enhance the therapeutic efficacy of antisense ONs in vivo [12]. For therapeutic siRNAs, the consensus in the field seems to be that development of an efficient delivery system is the key to their successful clinical translation [14]. End-modification of siRNA with cholesterol or N-acetylgalatosamine (GalNAc) moiety was effective in delivery of these agents into hepatocytes in the liver, facilitated by the low density lipoprotein (LDL) [15] and asialoglycoprotein receptor (ASGR) [15], respectively. It is important to know that liver is a particularly easily accessible organ due to the presence of fenestrated sinusoids allowing easy extravasation of macromolecules and nanoparticles [14]. For delivery to tissues other than the liver, various types of lipid nanoparticles (LNPs) seem to have the greatest success in ON therapeutic delivery [16].

1.3. Lipid nanoparticles (LNPs) for ON delivery

Naturally occurring vesicles, such as enveloped viruses and exosomes, are efficient vehicles of shuttling nucleic acids between different cells. LNPs can be viewed as synthetic versions of these carriers that can be custom-engineered to do the same with therapeutic ONs. Optimized LNPs can simultaneously protect ONs from serum nucleases, extend the systemic circulation time of ONs by preventing renal excretion and reticuloendothelial system (RES) clearance, enhance tumor uptake via the enhanced permeability and retention (EPR) effect, and, at the cellular level, facilitate internalization and endosomal escape of ONs [16]. LNPs for ON delivery typically contain a cationic lipid and other components that are commonly called "helper lipids". LNPs comprise lipid bilayers that encapsulate ONs inside their aqueous core and between bilayers, typically in a multilamellar structure [16]. Sometimes an additional targeting ligand attached to a lipophilic anchor is also incorporated into the LNPs to enable selective delivery to targeted cells [17]. Non-lipid components, such as polycations (e.g., protamine and cationic polymers), calcium phosphate and membrane lytic peptides can be incorporated into LNPs to generate "hybrid" nanovehicles.

1.4. Cationic lipids in LNP formulations

Cationic lipids can facilitate electrostatic interactions with anionic ONs [16]. This is needed to efficiently incorporate ONs into LNPs during their synthesis. In addition, these lipids can mediate electrostatic interaction between LNPs and the cellular plasma or endosomal membrane and facilitate cellular uptake and endosomal release of ONs [16]. Many cationic lipids have been synthesized for nucleic acid delivery since the initial report by Felgner et al. [18]reported the gene transfer activity of 1-(2, 3-dioleyloxy) propyl]-N,N,N-trimethyl-ammonium (DOTMA). These include lipids with various types of headgroups (tertiary amine-based, quaternary amine based, univalent and multivalent cationic) and lipophilic moieties (typically consisting of unsaturated alkyl or acyl chains or cholesterol) [19]. A few examples of cationic lipids are show in Fig. 1.

Cationic lipids when used alone carry a high density of positive charge, can be cytotoxic, and are not optimal for synthesis of LNPs designed for ON delivery in vivo. A number of factors can affect the delivery efficiency of ON-carrying LNPs, including the scheme of chemical modifications on the ON [10], the structure of the cationic lipids, and the choice of helper lipids and their percentages in the formulation [19]. Other important factors include lipid-to-ON ratio and the resulting positive–negative charge ratio and the resulting LNP zeta potential, pH-responsiveness of the zeta potential, degree and reversibility of PEGylation, and the LNP synthetic protocol, which can exert an

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