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Research paper

Applications of lipid nanoparticles in gene therapy

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ABSTRACT

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have been recognized, among the large number of non-viral vectors for gene transfection, as an effective and safety alternative to potentially treat both genetic and not genetic diseases. A key feature is the possibility to be designed to overcome the numerous challenges for successful gene delivery. Lipid nanoparticles (LNs) are able to overcome the main biological barriers for cell transfection, including degradation by nucleases, cell internalization intracellular trafficking, and selectively targeting to a specific cell type. Additionally, they present important advantages: from a safety point of view LNs are prepared with well tolerated components, and from a technological point of view, they can be easily produced at large-scale, can be subjected to sterilization and lyophilization, and have shown good storage stability. This review focuses on the potential of SLNs and NLCs for gene therapy, including the main advances in their application for the treatment of ocular diseases, infectious diseases, lysosomal storage disorders and cancer, and current research for their future clinical application.

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1. Introduction

Delivery of nucleic acids is a rapidly advancing field which has been attracted more and more interest as potential treatment of a wide variety of diseases, from genetic disorders (such as haemophilia, human severe combined immunodeficiency, and cystic fibrosis) to acquired (such as viral infections, neurodegenerative diseases, and cancer), as well as for vaccination. The European Medicines Agency (EMA) defines gene therapy product as a therapy in which the medicinal product generally consists of a vector or delivery formulation/system containing a genetic construct engineered to express a specific therapeutic sequence or protein responsible for the regulation, repair, addition or deletion of a genetic sequence [1]. The genetic construct traditionally used is a plasmid DNA; in fact the first gene therapy treatment approved in Western nations by the EMA contains a human lipoprotein lipase (LPL) cDNA. The product, called Glybera®, is indicated for adult patients diagnosed with familial LPL deficiency and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions. Newer tools include directly acting nucleic acid sequences such as microRNA, RNA interference (RNAi) via short hairpin RNAs (shRNA) or molecular scissor approaches in order to repair, add or delete a genetic sequence via gene silencing, exon skipping, gene regulation or gene knock-down [1]. Alternatively, RNA delivery is also especially attractive for developing gene therapy treatments to cure pathological processes, in form of protein-encoding messenger RNA (mRNA) or short interfering RNA (siRNA). mRNA may lead to higher transfection efficiencies in non-dividing cells, since nuclear entry, which is an important limiting step in gene delivery, is not required. Other advantages of mRNA therapy should be the rapid expression, predictable kinetics, and safer profile [2]. With mRNA short-term transient gene expression is achieved due to its rapid onset and short duration of expression [3], which limits its therapeutic applications; however, it may be useful as nucleic acid vaccine [4,5].

Regarding RNAi, it is a naturally occurring process of gene regulation present in plants and mammalian cells. From a therapeutic point of view, RNAi offers the possibility to gene silencing in a sequence-specific manner. This process is based on siRNAs, which are short double-stranded RNAs (dsRNAs) of 21 nucleotides in length, and are able to mediate site specific cleavage and destruction of the targeted mRNA [6]. siRNAs may be directly introduced into the cell cytoplasm, or may be synthesized within the target cell from a shRNA expression DNA vector [7]. Other possibility is the use of microRNAs (miRNA) which are also transcribed from DNA and need to reach the nucleus before mRNA cleavage [8]. shRNAs and miRNAs have a greater durability and higher silencing

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capacity than siRNAs [9], but need to be delivered into the cell nucleus.

The advancements in the field of gene therapy have developed into two different strategies: *ex vivo* and *in vivo* gene delivery [10]. In the *ex vivo* strategy cells are removed from a patient, transducted *ex vivo* in a laboratory with the therapeutic gene, and then returned to the patient. Alternatively, *in vivo* gene therapy involves the injection of the therapeutic gene directly into a patient's body. A key challenge to exploit the potential of nucleic acid-based therapy both *ex vivo* and *in vivo* is the efficient delivery of the active molecule to the cells. Nucleic acids are rapidly removed from circulation and cross cell membranes with difficulty, resulting in limited or no therapeutic effect when administered as naked forms [11]. Therefore, it is necessary to use safe, non-toxic and efficient nucleic acid delivery systems, which are broadly classified as viral and non-viral vectors [12]. Most of gene delivery therapeutics in clinical trials are based on viral vectors [13], due to their efficacy, but safety and manufacturing concerns are still considered a limitation [14]. On the contrary, non-viral vectors are safer platforms, and their production is simpler, cheaper and more reproducible than viral vectors. Moreover, there is not a limitation concerning the size of the genetic material they can transport. Efficacy of transfection of non-viral vectors is their major limitation, although it has been improved by several strategies, resulting in an increased number of products entering into clinical trials [15]. Non-viral vectors comprise physical methods such as electroporation [16] or hydrodynamic injection [17], and synthetic systems composed of peptides [18], polymers [19] and/or lipids [20]. Among lipid-based systems, Lipid Nanoparticles (LNs) have shown promising efficacy as gene and RNAi delivery systems *in vitro* [21–23] and *in vivo* [24,25]. In addition, LNs show several advantages with respect to other non-viral vectors, such as low or absence of *in vivo* toxicity, related to their composition by well tolerated physiological lipids, often approved in pharmaceutical preparation for human use, good long-term stability, production by economic and solvent-free techniques or, possibility to be autoclaved or sterilized [26].

This review is focused on the use of LNs as nucleic acids-delivery systems. The advantages and disadvantages of LNs in the field of gene therapy will be summarized, as well as the strategies to overcome the barriers found to achieve an effective gene delivery system. Finally, an overview of the main applications of LNs in gene therapy is reported, as well as current research for their future clinical application.

2. LNs in gene therapy

Lipid-based systems are extensively recognized as drug/gene delivery systems, since they are composed of well tolerated physiological lipids, often approved in pharmaceutical preparations for human use. In the early nineties [27] Solid Lipid Nanoparticles (SLNs) were developed as alternative to liposomes and nanoemulsions owing to their higher capacity to protect the active ingredient from degradation and to the possibility of modulating the release profile [28]. Moreover, SLNs obtained with different techniques successfully implemented in pharmaceutical industry (i.e., high-pressure homogenization) show long-term stability and the possibility to be subjected to commercial sterilization and lyophilized procedures [29–31]. Initially, SLNs were designed as drug carriers, but since their introduction as gene delivery systems by Olbrich et al. [32,33] they have attracted increasing attention in this field [34,35].

SLNs consist of a solid lipid core surrounded by a layer of surfactants in an aqueous dispersion. SLNs should be smaller than 500 nm in diameter, and the ideal size for *in vivo* use is considered of 120 nm or less [36]. For gene therapy applications the surfactants are often positively charged in order to obtain cationic SLNs that bind electrostatically nucleic acids. SLNs usually show a zeta potential higher than +30 mV, as indirect measurement of the surface charge, which decreases upon addition of increasing concentrations of nucleic acids [37]. In some cases, anionic SLNs are also able to induce transfection, if the nucleic acid is previously bound to a cationic ingredient [38,39]. The electrostatic interactions with the SLNs lead to condensation of the nucleic acid, which facilitates its mobility and protects it from environmental enzymes. The features of the final vector depend on the ratio between particle and nucleic acid; there must be equilibrium between the condensation capacity to achieve protection and the later release in the site of action. Actually, release of nucleic acids from non-viral vectors is considered a major concern for transfection [40]. As an alternative to SLNs in terms of improved drug loading capacity and release properties, Nanostructured Lipid Carriers (NLCs) were developed [41]. NLCs differ from SLNs in that the core of NLCs is composed of mixtures of solid and liquid lipids (oils). NLCs have been also studied as gene delivery systems [42], and their structure opens up the possibility to bind electrostatically nucleic acids while encapsulating a lipophilic drug in the core region [43–45]; this approach has been also documented in the case of SLNs [46]. Tables 1 and 2 summarize the studies in which SLNs and NLCs have shown their potential as nucleic acid-delivery systems.

3. Barriers to be overcome by LNs in gene therapy

Nucleic acid-carrier systems need to beat several barriers before reaching cell cytoplasm, in the case of RNAs, or nuclear machinery, in the case of DNAs: attacks from extracellular environment, cell membrane, intracellular trafficking and nuclear envelope (Fig. 1). An advantage of LNs is the possibility of being functionalized in order to overcome all these hurdles in the transfection process.

3.1. Extracellular environment

Intravenously or intramuscularly delivered naked nucleic acids show very short half-life as result of nuclease degradation [79]. Cationic SLNs bind electrostatically nucleic acids, and upon condensation protect them from environmental degradation. This assessment has been extensively demonstrated *in vitro* by electrophoresis in agarose gel [37,66,80]. The binding capacity of the SLNs depends not only on the cationic lipid, but also on the solid lipid-cationic lipid combination: the use of solid lipids that mix well with cationic lipids while the cationic one maintains the crystalline state induces higher stability than the use of cationic lipids that dissolve in the solid lipid. However, the surfactants employed for SLNs production do not seem to influence the nucleic acid binding capacity [81]. It is important to note that excessive condensation of nucleic acids is undesirable because the release of the active molecule would be limited resulting in poor transfection efficacy [37]. As mentioned above, a balance between condensation, protection and release of the nucleic acid is mandatory to achieve good transfection levels. *In vivo* the combination of SLNs with components that display high condensation has been related to a longer stay of the plasmid in the organism. Our research group designed a non-viral vector composed of cationic SLNs, dextran, protamine and the plasmid that encodes the enhanced green fluorescent protein (EGFP). After intravenous administration to wild-type mice, EGFP was detected in liver, spleen and lungs from day 3 after administration and still remained 7 days post-administration [59]. Protamine is a peptide considered an excellent condenser of nucleic acids that contributes to the nuclease resistance, resulting in an extended stay of plasmid in the organism.

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