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Development of a multifunctional envelope-type nano device and its application to nanomedicine

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ABSTRACT

Successful nanomedicines should be based on sound drug delivery systems (DDS) the permit intracellular trafficking as well as the biodistribution of cargos to be controlled. We have been developing new types of DDS that are multifunctional envelope-type nano devices referred to as MENDs. First, we will focus the in vivo delivery of siRNA to hepatocytes using a YSK-MEND which is composed of pH-responsive cationic lipids. The YSK-MEND is capable of inducing efficient silencing activity in hepatocytes and can be used to cure mice that are infected with hepatitis C or B. The YSK-MEND can also be applied to cancer immunotherapy through the activation of immune cells by delivering different compounds such as cyclic-di-GMP, siRNA or alpha-galactosylceramide as a lipid antigen. The findings indicate that, as predicted, these compounds, when encapsulated in the YSK-MEND, can be delivered to the site of action and induced immune activation through different mechanisms. Finally, a MITO-Porter, a membrane fusion-based delivery system to mitochondria, is introduced as an organelle targeting DDS and a new strategy for cancer therapy is proposed by delivering gentamicin to mitochondria of cancer cells. These new technologies are expected to extend the therapeutic area of Nanomedicine by increasing the power of DDS, especially from the view point of controlled intracellular trafficking.

1. Introduction

The concept of drug delivery systems (DDS) was born in the 20th century and Doxil is now recognized as one of the most successful achievements in the history of DDS for delivering anticancer agents to tumor tissue with its distribution to normal tissue such as the heart, kidney, etc. decreased. This targeting strategy relies on the EPR-effect (enhanced permeability and retention effect) and is classified as passive targeting. In the case of doxorubicin (DOX), there is no need to control the cellular uptake and intracellular trafficking of DOX, since DOX efficiently enters cancer cells and reaches the nucleus where its pharmacological action is exerted. To expand the therapeutic scope of DDS from low molecular compounds such as DOX to peptides, proteins and nucleic acids, more sophisticated types of DDS are required to enhance their cellular uptake and intracellular trafficking. Nucleic acids are expected to be the next generation medicine, since their action is very selective due to very specific recognition of sequences of nucleic acid base pairs as well as the direct action against the causes of diseases at the DNA/RNA level.

We have been developing a multifunctional envelope-type nano device (MEND) for use as an intelligent DDS that will permit, not only the biodistribution but also the intracellular trafficking of cargos (nucleic acids, proteins, peptides, etc.) to be controlled [1]. An octaarginine peptide known as a cell penetrating peptide can be incorporated in the MEND for surface modification in the form of stearylated octaarginine (R8). The R8-MEND has the ability to enhance the cellular uptake and transfection activity of pDNA/short interfering RNA (siRNA) in most dividing cells, but its application was limited to cellular conditions, since cationically charged nanoparticles are taken up by the liver and spleen once they are introduced into the blood circulation. YSK lipids are new types of pH-responsive cationic lipids in which the cationic charge is eliminated at normal pH, but develops a cationic charge in the acidic conditions in endosomes after cellular internalization. Such types of environmentally responsive materials have the ability to efficiently escape from endosomes as well as being rapidly taken up by hepatocytes in in vivo conditions [2]. This family of YSK lipids has been used to stimulate immune action by delivering a variety of compounds such as a low molecular compound, siRNA or lipid antigens through different mechanisms.

We are also in the process of developing a MITO-Porter, a membrane fusion-type DDS that targets mitochondria. Mitochondrial dysfunction is known to be associated with many kinds of diseases, including diabetes, obesity, neurodegenerative diseases, cardiac infarction and cancer. In spite of this, developing a DDS that can reach the matrix of mitochondria where transcription/translation of the mitochondria genome occurs remains a formidable task. A MITO-Porter for achieving this was developed based on lipid compositions that were screened based on

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in vitro fusion assays using isolated mitochondria, and have been shown to fuse efficiently with mitochondrial membranes [3]. The MITO-Porter was shown to be capable of delivering peptides, proteins and nucleic acids to mitochondria via membrane fusion. A new strategy for cancer therapy is therefore now possible by using the MITO-Porter as an organelle targeting DDS.

2. Short interference RNA as a potential treatment of liver diseases

It is estimated that >4000 diseases are the result of genetic disorders in liver tissue [4]. Moreover, it is estimated that >200 million persons are infected with hepatotropic viruses, including the hepatitis B virus (HBV) and the hepatitis C virus (HCV) [5]. Short interfering RNA (siRNA) can induce the sequence-dependent specific silencing of gene expression through RNA interference (RNAi) [6]. siRNA theoretically can target all endogenous mRNAs and exogenous RNAs as well, including viral RNAs. Therefore, the realization of RNAi-based therapy for the liver-related diseases appears to be a realistic and achievable goal. Alnylam Pharmaceuticals, a leading RNAi therapeutics company, started a phase I clinical study for the treatment of transthyretin (TTR)-mediated amyloidosis (ATTR) in 2010 [7], and phase III clinical studies are now ongoing with investigational RNAi therapeutics, patisiran and revusiran, which target transthyretin. ALN-PSCsc, an investigational therapeutic targeting proprotein convertase subtilisin/kexin type 9 (PCSK9) for the treatment of hypercholesterolemia [8], is now in a phase II clinical study. Furthermore, ALN-AT3SC, ALN-CC5, ALN-AS1, ALN-AAT and ALN-GO1 are also in phase I clinical studies for the treatment of hemophilia, complement-related diseases, hepatic porphyrias alpha-1 antitrypsin deficiency and primary hyperoxaluria type 1, respectively. Moreover, Arbutus Biopharma (formerly Tekmira pharmaceuticals) focused on the treatment of HBV infections and advanced their RNAi product (ARB-1467) into phase I clinical studies in early 2015. In this way, several RNAi-based therapeutics for liver diseases have been tested in several clinical studies. However, no RNAi-based therapeutics have been approved as of this writing [9].

2.1. Current reports regarding delivery of siRNA to liver tissue

Because of the physicochemical properties the siRNA, which includes a high molecular weight, a polyanionic charge and hydrophilicity, the siRNA itself cannot pass through cell membrane and reach cytosol. Moreover, in the blood circulation, siRNA can be degraded by RNases and therefore, cannot be easily removed by renal filtration. Therefore, in order to overcome these severe limitations and to apply siRNA to in vivo situations an adequate delivery technology that permits the siRNA to be stabilized and reach the cytosol of target cells is needed. Among several tissues, the liver has a unique blood vessel structure, referred to as discontinuous sinusoidal capillaries, which has a number of pores with diameters of about 100 nm (fenestrae) [10]. Because of this structural characteristic, nanoparticles with diameters of 100 nm or less are able to reach parenchymal cells (hepatocytes), and thus attempts to develop siRNA delivery systems to hepatocytes have widely been advancing [11–16]. Zimmermann et al. first reported on RNAi-mediated specific gene silencing in the liver in non-human primates (non-rodent species) [17]. They administered an apolipoprotein B (APOB)-specific siRNA formulated in lipid-based nanoparticles, stable nucleic acid lipid particles (SNALP), and confirmed APOB gene silencing (>90%) and pharmacological effects including the reduction of total serum cholesterol concentrations and serum low-density lipoprotein (LDL) particle concentrations. The SNALP contains 1,2-dilinoleyloxy-*N*,*N*-dimethyl-3-aminopropane (DLinDMA) as an ionizable amino lipid, which is electrostatically neutral at physiological pH (e.g. in the blood circulation) and changes to a cationic form in weakly acidic conditions (e.g. endosome/lysosome) [18]. The DLinDMA is a key component to facilitate endosomal escape through membrane fusion between the SNALP and the endosomal membranes. In 2010, Semple and Akinc et al. rationally designed some amino lipids and developed DLin-KC2-DMA [19]. The DLin-KC2-DMA contains a ketal linker to emphasize the cone shape of the lipid for the disruption of endosomal membranes, and a lipid nanoparticle (LNP) containing the DLin-KC2-DMA achieved a 50% effective dose (ED_{50}) for coagulation factor 7 (F7) gene silencing at 0.02 mg/kg, which is approximately a 50-fold higher activity compared to the DLin-DMA benchmark. More recently, Jayaraman et al. identified an active amino lipid, DLin-MC3-DMA, which is currently in clinical trials [20]. On the other hand, lipid-like materials, termed lipidoids, have been developed through a combinatorial synthesis approach. In this approach, amine-containing head groups and nonpolar hydrocarbon tails are reacted via the ring-opening of epoxides or the addition of alkyl-acrylates or alkyl-acrylamides [21,22]. Through parallel synthesis and high-through put screening, Love and Mahon et al. identified the most potent lipidoid, C12-200, which achieved an ED_{50} of 0.01 mg/kg for F7 gene silencing [22]. More recently, the same research group developed lipopeptide nanoparticles (LPNs) containing cKK-E12 which achieved an ED_{50} of 0.002 mg/kg for F7 gene silencing [23].

For polymer-based siRNA delivery technology, Rozema et al. reported on the development of siRNA Dynamic PolyConjugates. This formulation contains PBAVE, an endosomolytic cationic polymer. In order to increase the stealth function and targeting ability to hepatocytes, a shielding agent, polyethylene glycol (PEG), and a hepatocyte-targeting agent, *N*-acetylgalactosamine (NAG), were conjugated through a maleamate linkage, a bond that is labile at an acidic pH [24]. The siRNAs are also attached to the PBAVE polymer through a disulfide linkage, which is labile under reducing conditions (e.g. the cytosol). The PBAVE formulation containing ApoB siRNA showed ApoB gene silencing with an ED_{50} of ~1 mg/kg [25]. More recently, Wooddell et al. reported on the development of a hepatocyte-targeted NAG-conjugated endosomolytic mellitin-like peptide (MLP) [26]. In this system, the NAG-MLPs were co-injected with hepatotropic cholesterol-conjugated siRNAs (chol-siRNAs). The system achieved an ED_{50} of 0.01 mg/kg for F7 gene silencing in mice, and showed therapeutic efficacy in transient and transgenic mouse models of HBV infections.

2.2. YSK-MEND, a lipid nanoparticles for the delivery of siRNA to the liver

We recently reported on the development of an original siRNA delivery system, a MEND, containing a pH-sensitive cationic lipid, referred to as a YSK-lipid (Fig. 1). As a first generation device, we developed YSK05, which contains a small pH-sensitive structure (*N*-methylpiperidine) and two long unsaturated carbon chains and have a tendency to form an inverted hexagonal phase with anionic lipids at an acidic pH because of its cationic properties and cone-shaped structure. It is well known that membrane fusion can be achieved by a phase transition from a lamellar to an inverted hexagonal phase. Therefore, a MEND that contained YSK05 would be expected to fuse with anionic lipid containing endosomal membranes, resulting in the efficient release of the siRNAs into the cytosol (Fig. 1). The YSK05 was compared with a conventional cationic lipid, 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and a conventional pH-sensitive cationic lipid, 1,2-dioleoyl-3-dimethylammonium propane (DODAP) in in vitro experiments [27]. The MENDs were composed of a (pH-sensitive) cationic lipid, DOPE, cholesterol and 1,2-dimyristoyl-*sn*-glycerol, methoxypolyethyleneglycol 2000 (PEG-DMG) at a molar ratio of 30/40/30/3. The DODAP-MEND and YSK05-MEND showed lower cellular uptake compared to the DOTAP-MEND because each pH-sensitive cationic MEND has an acid dissociation value (pKa) (5.8 for DODAP-MEND and 6.6 for YSK05-MEND) and are nearly neutrally charged in media. However, the YSK05-MEND showed the highest half maximal inhibitory concentration (IC_{50}) value (30 nM), which was approximately 10-fold higher than that for the DOTAP-MEND. Hemolytic activity, which is an indicator of fusogenic activity with endosomal membranes, was well correlated with gene silencing activity. After optimization of the lipid composition of theYSK05-MEND