Expert Opinion

Cyclodextrin-based siRNA delivery nanocarriers: a state-of-the-art review

Kiran Chaturvedi, Kuntal Ganguly, Anandrao R Kulkarni, Venkatarao H Kulkarni, Mallikarjuna N Nadagouda, Walter E Rudzinski & Tejraj M Aminabhavi[†]

[†]*SET's College of Pharmacy, Department of Pharmacology, Dharwad, India*

Introduction: The discovery of synthetic small interfering RNA (siRNA) has led to a surge of interest in harnessing RNA interference (RNAi) technology for biomedical applications and drug development. Even though siRNA can be a powerful therapeutic drug, its delivery remains a major challenge, due to the difficulty in its cellular uptake. Naked siRNA has a biological half-life of less than an hour in human plasma. To increase the lifetime and improve its therapeutic efficacy, non-viral vectors have been developed. As a natural evolution, cyclodextrins (CDs), which are natural cyclic oligosaccharides, have recently been applied as delivery vehicles for siRNA, and this in turn, has led to a surge of interest in this area.

Areas covered: This review discusses the recent advances made in the design of delivery strategies for siRNA, focusing on CD-based delivery vectors, because these have demonstrated clinical success. The methods of preparation of CD-based vectors, their characterization, transfection efficiencies, cellular toxicity, preclinical and clinical trials are also addressed, as well as future therapeutic applications.

Expert opinion: siRNA-mediated RNAi therapeutics is beginning to transform healthcare, particularly, for the treatment of solid tumors. For example, CALAA01, a targeted, self-assembling nanoparticle system based on CD complexed with siRNA has been effective in phase I clinical trials. Although siRNA therapeutics suffers from problems related to off-target effects and non-specific gene silencing, these problems can be overcome by reducing the nanoparticle size, improving the targeting efficiency and by modifying the primary sequence of the siRNA.

Keywords: cyclodextrin, nanocarriers, non-viral vector, siRNA, targeted delivery

Expert Opin. Drug Deliv. (2011) ***8****(11):1455-1468*

1. Introduction

Gene silencing by RNA interference (RNAi) technology has recently gained widespread attention in pharmaceutical biotechnology in view of its ability to mitigate diseases such as cancer, viral diseases and genetic disorders [1]. RNAi is triggered by siRNA, which is a double-stranded RNA (dsRNA) consisting of two 21 – 22 base pair complementary strands with special recognizing two-base 3′ overhangs [2]. These are generated from a long dsRNA of either exogenous or endogenous origin that is recognized by a dsRNA-specific endonuclease, called Dicer, an enzyme, which can cleave long dsRNAs into siRNAs. Once inside the cytoplasm of a given cell, siRNA gets integrated into a nuclease complex, called RNA-induced silencing complex (RISC). One of the strands of the siRNA duplex (the passenger strand) is cleaved and discarded, while the other, the antisense strand

informa
healthcare

10.1517/17425247.2011.610790 © 2011 Informa UK, Ltd. ISSN 1742-5247

remains bound to the RISC complex and serves as a 'guide strand' to direct the sequence specificity of RISC. The single-stranded RNA–RISC complex then detects messenger RNA (mRNA) sequences inside the cytoplasm and locates mRNA sequences with a homologous sequence to induce the cleavage of mRNA, thus inhibiting its translation into protein [3,4].

siRNAs are more stable to nuclease degradation than the unmodified antisense oligonucleotides and, therefore, they can show a more sustained therapeutic effect than that obtained with antisense therapy and are thus a potent tool for the therapeutic study of diseases both *in vitro* and *in vivo* [3,5-6]. The therapy can be achieved by specific delivery of siRNAs using suitable vectors [7]. Naked siRNAs have a biological half-life of less than an hour and are rapidly excreted by the kidney without penetrating through the cellular phospholipid membrane [8,9]. Since siRNAs do not readily cross the cellular membrane because of their negative charge and size [1], the simple addition of naked, unmodified siRNAs to the culture medium that overlies a mammalian cell does not result in an effective knockdown of the target gene [10]. However, suitable vectors have been developed for the specific delivery of siRNAs [7] in which the vector encapsulates siRNAs so that they can reach the cytoplasm of target cells and induce silencing.

Barriers for siRNA delivery have been elegantly summarized by Li and Szoka [11], but a number of approaches have been developed to circumvent these barriers [5]. One hurdle associated with intravenous administration involves the need to avoid potential interactions with plasma proteins and uptake by macrophages of the monocyte phagocytic system (MPS). In order to stabilize siRNA in the serum, chemical modification of the ribose backbone or diester linkage on siRNA by substituting a phosphorothioate linkage has been attempted to enhance the siRNA resistance to nucleases and increase the plasma half-life [12]. Interactions with plasma proteins are particularly problematic for cationic delivery systems because these interact to form aggregates that are either entrapped in the lung endothelial capillary bed or taken up by the MPS [13]. Another hurdle involves crossing the permeable endothelium such as in neovascularized tumors or inflammation. Here, size is crucial, since only small particles of 50 – 200 nm are able to pass through the fenestrated barriers through an enhanced permeability and retention (EPR) effect [1-3]. Before reaching the cellular level, the delivery system also needs to cross the tight network junctions of the extracellular matrix that contains a variety of polysaccharides and proteins enveloping the surface of the cells. Finally, having reached the surface of the tumor cells, siRNAs then need to overcome the problems of poor intracellular penetration and sub-cellular localization. These five barriers need to be considered when developing new delivery systems.

Despite continuous efforts in the development of novel delivery vehicles for targeting siRNA to a specific site, a number of critical parameters have yet to be superceded in order to attain the optimal activity, improved stability, increased plasma half-life, tissue or cell specificity and improved cellular penetration. Several research groups [1-5] have been working on designing suitable polymeric nanoparticles that can address these critical requirements in order to achieve optimal delivery of siRNA.

Polymeric nanoparticles often used as siRNA delivery vectors are normally positively charged at physiological pH such that they can electrostatically interact with anionic charges (e.g., RNA) to form nanocomplexes. As the complexes possess a global net positive surface charge, these can be easily attached to the negatively charged cell surface with subsequent endocytosis. However, for full therapeutic availability of siRNA for gene silencing, such delivery vehicles must also protect siRNA from degradation and exhibit site specificity for efficient cellular uptake. Polymer and/or lipid encapsulation strategies have been attempted to shield the vector against degradation, clearance and immune response [14].

Polymeric carriers have great versatility, since these can be modified by changing the molecular weight, the geometry (linear vs. branched) and introducing a binding ligand through a covalent linkage. Polymers also have an advantage over lipid-based systems because they are relatively small, offer protection against nucleases, can be prepared in a narrow size distribution (50 – 200 nm) and with elegant control over a number of physical factors (e.g., hydrophilicity and charge). Nevertheless, polymers still have limitations and elicit questions about their potential toxicity and non-biodegradability [15,16]. In addition, siRNA-encapsulated nanoparticles, because of their net positive charge, tend to aggregate and in effect, increase their hydrodynamic radius and decrease their ability to penetrate into cells [16]. Polyethylene glycol (PEG) or sugar molecules such as cyclodextrin (CD) may be used to reduce the surface charge and nanoparticle aggregation [17]. In this regard, cyclodextrin-based polycations (CDPs) developed by the Davis group are an excellent choices as a delivery system as these have almost reached the clinical success [18-21]. For instance, one of their products based on a CD has already been tested on primates and has entered clinical trials. The present review will address the latest developments in CDP-based carriers for site-specific delivery of siRNA.

2. Nanocarriers for siRNA delivery

The approaches adopted for plasmid DNA (pDNA) delivery may also be used to enhance siRNA targeting and intracellular trafficking. However, the selection of a suitable delivery vehicle should be carefully chosen, since an inappropriate choice of delivery vector can decrease the gene silencing activity and increase its off-target effects [22,23]. Parameters controlling the efficient delivery of siRNA into the cytoplasm of the target cell have been investigated in order to prevent non-specific silencing toxicity during systemic treatment of multiple diseases [24]. Even though many polymers have been considered as ideal candidates in gene therapy [14], yet more recently,

CDPs have generated a new interest because of their versatility, ease of manipulation, low toxicity and clinical success [9].

From a perusal of the published literature, one finds conflicting reports on the appropriate size of an ideal vector. However, as stated before, the ideal systemic delivery vector should be approximately 50 – 200 nm in size, non-toxic, non-immunogenic, stable and capable of efficient intracellular delivery with specific targeting ability. Researchers are now beginning to understand the mechanisms of siRNA transport and attempting the rational design of effective vectors. In any of these studies, the factors to consider include: choice of material, toxicity, systemic immune or cellular response, dynamic pH or oxidative environment, RNA–protein or RNA–RNA interaction, covalent versus non-covalent bonding and passive versus active internalization mechanisms. Already, combinations of PEG, fusogenic and non-fusogenic lipids, dendrimers [25], liposomes, polycations, nucleic acid modifications and targeting moieties have been developed to improve the pharmacology of RNA-based therapeutics [26]. Synthetic vectors such as cationic lipids and/or liposomes, cationic polymers, cationic dendrimers and cationic cell-penetrating peptides have been employed as delivery devices [27-33].

A list of typical non-viral polymeric vectors used for siRNA delivery is given in **Table 1**. It appears that almost 100% knockdown efficiency of siRNA has been achieved by CDPs [20]. Of the other devices, even though polyamidoamine (PAMAM) dendrimer and polyethylenimine (PEI) can condense siRNA into nanoscale particles and protect them from enzymatic degradation to achieve substantial release over an extended time for efficient gene silencing [34], yet clinical studies have shown lower values of transfection efficiency compared with CDP-based vectors. One of the additional advantages of CDP-based delivery systems is that these are well tolerated, since even repeat doses fail to elicit a significant delivery system-specific antibody response [31]. For instance, the LD_{50} (median lethal dose) of linear PEI (molecular weight, 22 kDa) is around 4 mg/kg in mice, which significantly limits its *in vivo* delivery [35]. On the other hand, CDPs have an LD_{50} of ~ 200 mg/kg in mice [36]. The present review will address siRNA delivery technologies based mainly on CDP and chemically modified CDPs.

3. Cyclodextrin-based polymeric nanocarriers

CDs are enzymatically modified starch derivatives with a donut shape or a truncated cone and are made of D-glucopyranose units connected through α-(1-4) linkages and each are designated by a Greek letter according to the number of glucose units. For instance, α-, β- and γ-CDs are produced commercially and consist of 6, 7 and 8 glucose units, respectively; all these exhibit low toxicity [37]. The three-dimensional structure of a CD is such that the inner cavity surface is hydrophobic, but the exterior of the cavity is hydrophilic, giving a unique structure that enables the CDs to include a wide variety of compounds to form host–guest inclusion complexes. Among the three natural CDs, the β-CD is currently the most widely used, and also the most thoroughly studied. Numerous toxicological tests have been conducted on β-CD in accordance with the guidelines of the US Food and Drug Administration (FDA) and the use of CDs in controlled release applications is well known [38].

Compared with other cationic non-viral vectors employed for siRNA delivery, CDPs are excellent alternatives as these can be prepared in the size range of 50 – 200 nm and can serve as adapter molecules; wherein, different molecules, for example, modified adamantanes can be easily 'plugged' into the cone of the CD to offer additional functionality. Thus, for both short- and long-term administration of CDP–siRNA nanoplexes, the CDP protects siRNA from serum avoiding the use of modified siRNA [39]. The CDP vectors have been functionalized with adamantane-transferrin (AD-Tf) and adamantane-PEG (AD-PEG) conjugates and the resulting PEGylated and Tf-targeting CDPs delivered siRNA to animals at dosages that are likely to be amenable to therapeutic use in humans. Perhaps the most favorable feature associated with the CDP–siRNA nanoplexes is that when siRNA contains an immune stimulatory sequence, the nanoplex does not produce an immune stimulation like that seen with other siRNA–lipid complexes. These advantages have triggered active research in developing CDP-based delivery devices of siRNA for multiple disease treatment. For instance, a CDP-based device of about 50-nm size shown in **Figure 1** was targeted to metastatic cancer cells in a murine model of the Ewing's family of tumors (EFT) [39]. The nanocarrier self-assembled with siRNA was stable in physiological fluid and protected siRNA from nuclease degradation up to 72 h, and facilitated the cellular uptake of the functional siRNA.

In a novel approach for the delivery of macromolecular therapeutics, the Davis group [40] has synthesized, for the first time, linear cationic β-CD-containing polymers. They copolymerized difunctionalized β-CD monomers (AA type) with other difunctionalized comonomers (BB type) to produce AABBAABB type polymers. Since CDs can form inclusion complexes with hydrophobic moieties, derivatives of adamantane with β-CD with association constants on the order of 10^4 – 10^5 M^{-1} were prepared. Inclusion complexes of adamantane end-capped polyethylene oxide (PEO) with CD copolymers have also been developed [41].

In a simple and novel approach, Pun and Davis [42] reported a method of modifying β-CDP-based polyplexes in which adamantane was conjugated to a desired polyplex modifier such as PEG. The resulting AD-PEG was exposed to β-CDP for self-assembly in order to prepare the β-CDP–AD-PEG polyplexes. By this method, nanoplexes that are stable in salt solution were produced for targeting hepatoma cells. The synthetic steps involved in this protocol are simple as displayed in **Figure 2**, wherein PEG or PEG-ligand conjugated with adamantane form complexes with β-CD and provide steric stabilization.

Table 1. Comparison of some typical non-viral polymeric vectors used to target siRNA *in vivo*.

Polymer vector	Size (nm)	Molecular weight of polymer/dendrimer generation	*In vivo* delivery	Targeting site	Transfection efficiency	Remarks	Ref.
CDPs	70 – 140	< 10 kDa	Intravenous injection	Ewing's sarcoma, solid tumors	70 – 100%	No abnormalities in IL-12 and IFN-α, liver and kidney function tests	[21,39,68]
Chitosan nanoparticles	40 – 600	10 – 1000 kDa	Intranasal	Systemic and mucosal diseases	78 – 89%	Low toxicity, low immunogenicity, excellent biodegradability and biocompatibility	[69]
PEG	< 10	3400 Da	Intravenous injection	Liver hepatocytes	80%	Non-toxic, well tolerated	[70,71]
PEI	100 – 200	22 – 25 kDa	Intraperitoneal	Lungs (influenza virus), systemic effect	~ 70%	Cytotoxic	[72-75]
PEI-*g*-PEG	200 – 500	PEI (25 kDa)-PEG (2 kDa)	Intravenous injection	Local application preferred as breaks down on liver passage	70%	Less cellular uptake in liver	[76]
PLGA nanoparticles (75/25)	< 200	14.4 kDa	Topical	Intravaginal therapy	Significant gene knockdown *in vivo*	Less irritation and inflammation	[77,78]
Polycationic dendrimer conjugated with targeted lipid moieties	< 200	G3, ethylenediamine core, 6909 Da	Intravenous	Colon cancer	Potent RNAi activity	Low cytotoxicity	[79,80]

CDP: Cyclodextrin-based polycation; PEG: Polyethylene glycol; PEI: Polyethylenimine; PEI-g-PEG: Polyethylenimine-*grafted*-polyethylene glycol; PLGA: Poly (d,l-lactic-co-glycolic acid; RNAi: RNA interference; siRNA: Small interfering RNA.

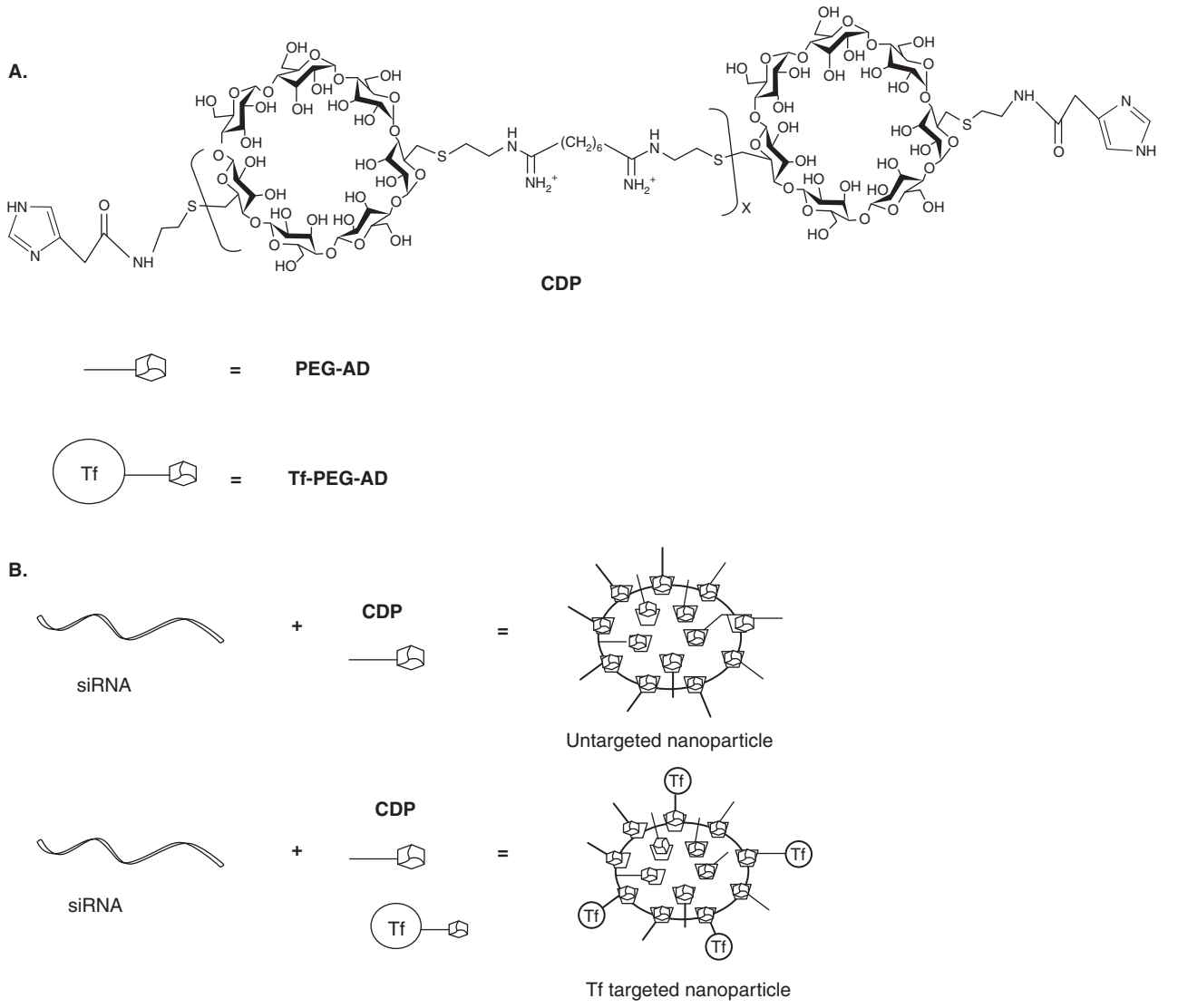

Figure 1. Illustration of the delivery system: (A) components of the delivery systems and **(B)** assembly of non-targeted and targeted nanoparticles.

For the inclusion complexes of CD-adamantane discussed above [39], a multi-dosing study of siRNA in non-human primates (cynomolgus monkeys) with a targeted, systemic delivery system was performed [43]. It was found that the administered siRNA targeting the M2 subunit of ribonucleotide reductase was well tolerated at doses of 3 and 9 mg siRNA/kg, but induced kidney toxicity at a dose of 27 mg siRNA/kg. The higher dose affected the liver to some extent, as indicated by a mild elevation in the alanine amino transferase and aspartate transaminase levels. A mild immune response was also reported at this dose with no clinical signs of toxicity.

The Davis group [18] further enhanced the siRNA release capability of CDP by developing imidazole inclusion complexes with CDP in the form of a polyplex. They investigated the difference between gene delivery behavior of CDP and its imidazole-containing variant, CDPim (cyclodextrin-based polymer-imidazole) to understand the mechanism (s) by which related materials show differences in gene delivery. Their study also emphasized the need to evaluate the behavior of non-viral gene delivery vectors within cellular environments. From the study, it was surmised that CDPim does provide pH buffering, and enhanced transfection efficiency due to an increased endosomal escape; however, the whole sequence was not well understood by the authors because CDPim produced a greater unpacked intracellular nucleic acids than CDP [18].

Bartlett and Davis [19] prepared nanoparticles of CDPs-containing siRNA and inclusion complexes formed between AD and β-CD attached to PEG to form AD-PEG conjugates

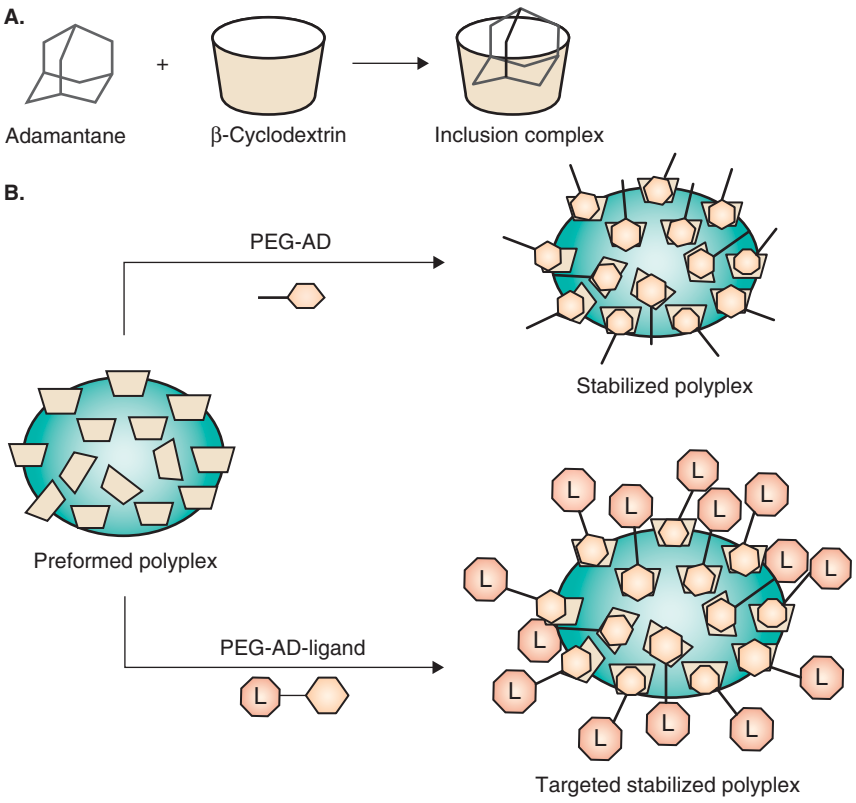

Figure 2. (A) Formation of inclusion complex and **(B)** post-ligand complexation PEGylation by inclusion complex formation by AD-PEG and AD-PEG ligand.
AD-PEG: Adamantane-PEG conjugate.

along with targeting ligand (adamantane-polyethylene glycol-transferrin, AD-PEG-Tf) for cell-specific targeting. These were tuned to obtain a size distribution of 60 – 150 nm, a zeta potential of 10 – 30 mV and a molecular weight of 7×10^7 to 1×10^9 g/mol. Simultaneously, Bartlett *et al.* [44] used the positron emission tomography (PET)/computer tomography (CT) to monitor the whole body biodistribution kinetics and tumor localization of these nanoparticles. Bioluminescent imaging (BLI) was used to measure the luciferase knockdown by the delivered siRNA. The nanoparticles with or without Tf targeting ligands were used to study the effect of cell-specific targeting on both biodistribution and function simultaneously within the same animal. The nanoparticle uptake was measured by HeLa cells using flow cytometry and visualized by confocal microscopy to demonstrate that targeting ligand to payload ratio is important to consider when designing siRNA delivery vehicles for *in vivo* applications. The Tf-targeted nanoparticles showed enhanced affinity for the transferrin receptor (TfR) through avidity effects (multi-ligand binding), which was confirmed by the competitive uptake studies [44]. In the above study, HeLa cells were used, since these overexpress TfRs [45]. **Figure 3** shows the probable mechanism of gene silencing activity of CD-based siRNA nanoplex after intravenous injection.

The possibility of combining CDP that contained siRNA to modulate gene expression in a light-directed manner through photochemical internalization (PCI) technology was recently reported, for the first time, by Bøe *et al.* [46]. The authors used Human S100A4 as a model gene to evaluate gene transfer efficacy of gene silencing. The carrier:siRNA ratio and illumination dose were optimized, and a real-time reverse transcriptase–polymerase chain reaction was carried out. The data indicated that siRNA samples treated with PCI showed 80 and 90% silencing effect when compared with the untreated control. From 0 to 10%, the silencing effect was achieved with siRNA samples that did not receive PCI treatment, thus demonstrating the effectiveness of light-specific delivery of siRNA. However, the time-lapse results showed a maximum gene silencing effect at 5 h after the endosomal release. The authors suggested a strategy to combine β-6CDP (β-cyclodextrin polymer with 6 methylene groups separating the charges)-based nanoparticles with PCI technology for systemic tumor targeting.

Brahmamdam *et al.* [47] have recently reported a potential application of siRNA-based therapy for the treatment of infectious disease. The authors used a novel CDP-based TfR targeted delivery vehicle to administer siRNA to Bim and PUMA, the two key cell death proteins that are markedly

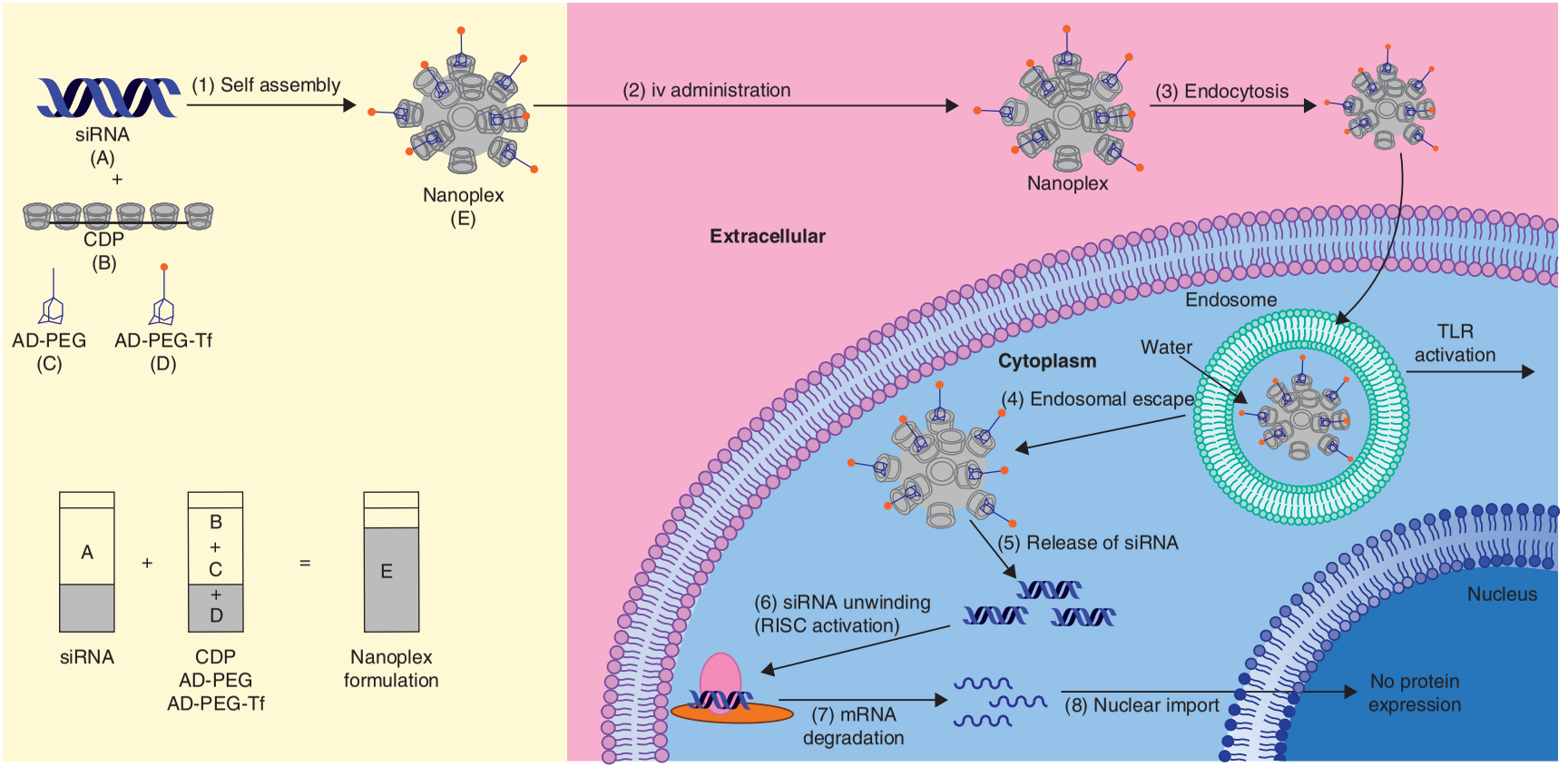

Figure 3. Probable gene silencing activity of CDP-based siRNA nanoplexes.

AD-PEG: Adamantane-PEG conjugate; AD-PEG-Tf: Adamantane-PEG-transferrin conjugate; CDP: Cyclodextrin-containing polycation; siRNA: Small interfering RNA; TLR: Toll-like receptors.

upregulated during sepsis. The study was conducted on mice immediately after cecal ligation and puncture to show that antiapoptotic therapy could markedly decrease lymphocyte apoptosis and prevent the loss of splenic CD4 T and B cells as confirmed by flow cytometry. The study also demonstrated the possibility of simultaneous targeting of two critical mediators of sepsis-induced apoptosis. The system effectively delivered the siRNA to immune effector cells with no noticeable off-target effects, suggesting a major advancement for the treatment of infectious disease.

4. Dendrimer-based CDP nanocarriers for siRNA

Dendrimers are highly branched tree-like polymers that can be used in masking the charge of siRNA long enough for *in vivo* delivery. As an example of this approach, Arima *et al.* [48] synthesized a series of PAMAM dendrimer–CD conjugates by grafting CD (α, β and γ) to create different generations of PAMAM dendrimers (generations G2, G3 and G4). The end result was that α-CD conjugation to PAMAM dendrimers boosted *in vitro* and *in vivo* gene transfer without significant changes in cytotoxicity. Tsutsumi *et al.* [49] also compared PAMAM dendrimer (G3) conjugate with α-CD (α-CDE conjugate) for delivering siRNA with transfection reagents like lipofectamine 2000 (L2), TransFast (TF) and lipofection (LF) containing siRNA. The nanoplexes were evaluated for cytotoxicity, physicochemical properties and intracellular distribution to demonstrate the efficacy of α-CD as a novel carrier for siRNA.

Tsutsumi *et al.* [50] also evaluated the potential of starburst PAMAM dendrimer (G3) conjugate with α-CD with an average degree of substitution of 2.4 for siRNA delivery. NIH3T3 cells were transfected with binary pDNA/carrier or ternary pDNA/siRNA/carrier and the cell viability was determined by cell counting. The cell viability was not lowered by the complexes up to 1.0 μg of siRNA, but a sequence-specific siRNA-mediated gene silencing effect was observed. The study revealed that ternary complexes with α-CDE conjugate did not show any cytotoxicity. The sequence-specific gene silencing effect of the complex was evaluated by measuring the luciferase activity after transfection with ternary complexes of pDNA/siRNA/α-CDE conjugate. The α-CDE conjugate showed the highest gene transfer efficiency in these cells as was evident from the luciferase activity compared with the binary complexes of pDNA/carriers in the absence of siRNA.

Of all the carriers used, α-CDE conjugate showed the highest inhibition ratio, indicating the sequence-specific effects of siRNA. Similar results were observed in A549 cells, suggesting that α-CDE conjugate could be considered as a useful new carrier for delivering siRNA. Another study indicates that the problems associated with off-target effects can be ameliorated if the siRNA itself is conjugated [51]. However, an ideal example of safe and effective conjugation is that of CDP–siRNA system, which is now under clinical trials [21]. In the literature, cell-penetrating peptide (CPP) and PEG have also been successfully conjugated with siRNA, boosting its gene transfection ability *in vivo* [52,53].

Several studies on CDP associations using nanostructured multi-layers of polylysine-CD [54], polyelectrolyte [55], linear polyethylenimine-CDPs [56,57] or amphiphilic cationic CDPs [58] have been effectively tested *in vitro* using non-viral gene delivery. Menuel and coworkers [59] synthesized the novel *bis*-(guanidinium)-tetrakis-(β-CD) dendrimeric tetrapod as a potential gene delivery system, whose structure is shown in **Figure 4**. Affinity and size exclusion chromatography were used to characterize the self-assembly behavior with siRNA oligonucleotide and single-stranded DNA as well as to determine the binding parameter. The capacity of tetrapod to form a molecular association with siRNA was investigated by affinity capillary electrophoresis. *In vitro* cellular viability was measured by MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay to understand the toxicity limits in living cellular systems. The siRNA labeled with CDP was investigated for intracellular delivery and trafficking in MRC-5 cells. *In vitro* transfection results demonstrated that the CD tetrapod, shown in **Figure 4**, delivers the siRNA to the nuclear periphery. A detailed scheme of the synthesis of novel *bis*-(guanidinium)-tetrakis-(β-CD) dendrimeric tetrapod is available elsewhere [59].

Effective biomimetic catalysts may be attached to CD derivatives, though it is not an easy task if a short spacer is used, to connect a CD to the catalytic group. On the other hand, if a longer spacer is used, then conformational freedom of the resulting CD derivative should be suppressed in order to freeze the molecule in a productive conformation. In this pursuit, Suh *et al.* [60] constructed artificial enzymes using dendrimers as a molecular skeleton wherein, β-CD was attached to dendritic poly(ethylenimine)s. The possible conformation of poly(ethylenimine) linked to β-CD is displayed in **Figure 5** (A, B and C). Of these, the most probable conformation as per the authors' [60] suggestion is **Figure 5B**.

The conformation of CD-I and CD-II in water and kinetic data for deacylation of carboxyl esters containing *t*-butylphenyl residues in the presence of CD-I and CD-II have been investigated. The kinetics data obtained with several ester substrates reveals that two amino groups located in the vicinity of each CD cavity of CD-I or CD-II participate as nucleophiles. Optimum reactivity is attained when the spacer connected *t*-butylphenyl with the ester groups. On the basis of kinetics data, the authors [60] elucidated the structures of active sites for accelerated deacylation of esters.

Other siRNA conjugates with ligands like biotin, folate or cholesterol, etc. [25,61-64] have also been investigated, but these are beyond the scope of the present review. In addition, stable nucleic acid lipid particles (SNALPs) in the size range of 77 – 83 nm have been investigated to show a rapid silencing of apoB (apolipoprotein B) gene in the liver of cynomolgus monkey [65].

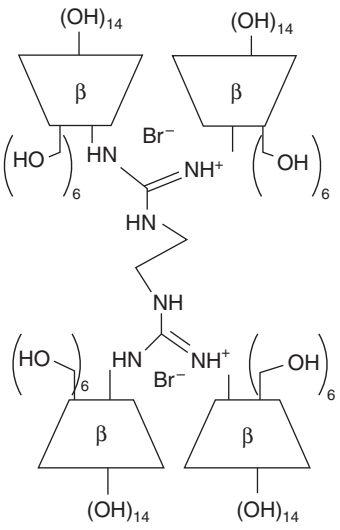

Figure 4. Structure of cyclodextrin tetrapod.

5. Commercial products

Calando Pharmaceuticals (Pasadena, CA) has been successful in advancing siRNA/oligonucleotide nanoparticle delivery (RONDEL™) technology toward clinical applications. A summary of the RONDEL technology has been discussed before in **Figure 1**, which is based on the original concepts proposed by Hu-Lieskovan *et al.* [39]. This targeted nanoplex of siRNA, that is, CALAA-01 consists of a CDP, PEG as a steric stabilizing agent and human Tf as the targeting ligand for binding to TfRs that are typically upregulated on cancer cells. This multi-component delivery system provides low toxicity when administered intravenously to patients. These systems were evaluated for safety in patients having solid tumors.

The product under clinical trial by the Davis group is a targeted nanoparticle formulation, which is a combination of RONDEL, the self-assembling nanoparticle formulation (United States Patent (USP) # 7807198, 5 October 2010) and siRNA (USP # 7427605, 23 September 2008) that targets the M2 subunit of ribonucleotide reductase. It is a clinically proven cancer targeting system containing siRNA, CDP, PEG as a steric stabilization agent and human Tf as a targeting ligand for binding to TfR. This was developed into a two-vial formulation where the delivery components are contained in one vial and siRNA in the second vial. After mixing the contents of the two vials, they self-assemble into 70-nm size nanoparticles that can be delivered intravenously.

Characterization of the above-mentioned nanoplexes was done by a variety of techniques, such as electrophoresis shift assay to find siRNA serum stability. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) were also used to estimate the particle size and morphology. Isothermal titration calorimetry (ITC) and percentage of AD-PEG and AD-PEG-Tf bound after formulation were carried out to find the binding of AD-PEG conjugates to the surface of nanoparticles through inclusion complex formation to show that β-CD forms an inclusion complex with AD-PEG conjugate.

Dynamic light scattering, salt stability and erythrocyte aggregation studies were used to estimate the steric stabilization to nanoparticles and reduction in non-specific interactions due to PEGylation. Flow cytometry and confocal microscopy were used to demonstrate the intracellular delivery of siRNA into HeLa cells *in vitro*. Competitive uptake studies revealed that targeting ligands like Tf enhanced the cellular uptake of PEGylated nanoparticles. Luciferase knockdown after siRNA transfection was performed to determine the functional efficacy of delivered siRNA and to observe the successful delivery of functional siRNA to cells *in vitro* [19].

Table 2 compares different CDP-based nanocarriers for siRNA delivery wherein, one can observe 100% transfection efficiency for CDP-based carriers. Even though still in its infancy with many challenges to overcome, RNAi is poised to take its place as a major therapeutic modality. There are many companies with transfection reagents that describe their product listings other than CDP [66,67].

6. Conclusions

The discovery of RNAi less than a decade ago was a major breakthrough in molecular and cellular biology, since it is a powerful tool for studies involving the gene function in mammals. However, the lack of efficient targeted delivery, low transfection efficiency, instability to nucleases, poor tissue penetration and non-specific immune stimulation by many cationic delivery vectors has hindered the translation of RNAi into full and satisfactory clinical applications. This article has reviewed the latest developments in nanoparticle-assisted delivery of siRNA using CDP-based carriers.

7. Expert opinion

It is believed that RNAi has emerged as a major breakthrough technology in the life sciences area, since it has tremendous potential in other multiple areas, such as drug discovery, drug development, therapeutics and research in basic metabolic mechanisms. RNAi should find wide applications in various sectors, such as functional genomics, disease diagnostics and agriculture, among others. The usage of RNAi technology in industries and academia should witness a substantial growth in the near future. According to a new research report 'Global RNAi Market Analysis', the global RNAi market is anticipated to grow stupendously at a compound annual growth rate (CAGR) of around 28% in the next 5 years.

Within the gene delivery field, CD-based vectors are emerging as promising novel candidates for the safe and effective delivery of siRNA to target sites. The presence of several different variants with a wide range of physicochemical and biological properties is a major asset of these complexes. Among all the CDP-based vectors developed to date, the

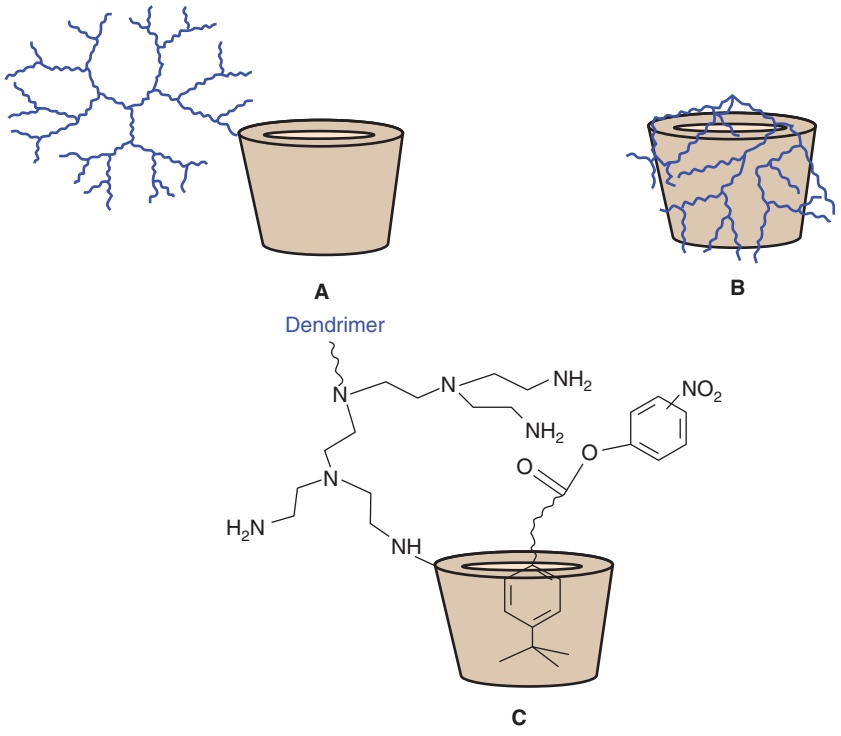

Figure 5. Possible conformations of β-cyclodextrin-linked poly(ethylenimine) dendrimer.

Calando pharmaceutical product is the most advanced in its development and has been successful in a clinical Phase I study.

Different strategies have been employed for preparing nanoplexes of siRNA such as using CDPs or dendrimer-based CDPs. Researchers have also focused on conjugation of other polymers with CDs that allows safe siRNA delivery. However, functionalization of CDP-based nanoparticles still remains as an important challenge for the future and is considered to be the most promising, particularly for tumor-targeted delivery.

Various CD manufacturers and research groups working on CDs worldwide provide a strong platform for CDP-based research and development. Consequently, there are increasing number of studies in the literature on CDP nanoparticles for therapeutic and/or diagnostic applications because the US FDA has approved the way for CDP nanoparticles to be commercialized and used for the treatment of major diseases like cancer.

Current research on nanoparticles prepared from CDP still lacks some important refinements that need to be addressed. Primarily, CDPs reported in the literature fall within the size range of 50 – 200 nm, in which case, they will not circulate for a long time in the bloodstream before they are recognized by the macrophages and taken up by the reticuloendothelial system (RES). Therefore, future research should focus on methods to prolong the circulation time of CDP nanoparticles by maintaining the particle size below 100 nm or by attaching hydrophilic molecules such as PEG or PEO. However, reducing the size drastically would also result in toxicity issues and accumulation in the lungs or kidneys; thus, chemical modification of CD to alter the surface charge and render a protein-repellant effect is believed to be a better promising alternative.

Besides the challenges of specific delivery, other challenges of siRNA therapy are the problems related to non-specific gene silencing, immune response and cytotoxicity. Currently, these areas are under active investigation and feasible answers to these questions are emerging. The non-specific gene silencing and off-target effects can be reduced by altering the siRNA size, targeting location and modifying the primary sequence. The sense strand is more tolerable to sequence modification without losing the gene-silencing potential.

The ideal systemic delivery vector should, therefore, be 50 – 200 nm in size, non-toxic, non-immunogenic, stable, capable of efficient intracellular delivery and possessing specific targeting ability. In order to make the application of therapeutic siRNA a reality, future research efforts must continue to focus on achieving efficient delivery to the desired cells, minimizing off-target effects, increasing the resistance to nuclease degradation, avoiding immune responses such as IFN-α, IFN-β, Toll-like immunity and trapping of polymerized particles by Kupffer cell and lung macrophage.

The almost ideal specificity of siRNA is not entirely possible and off-target effects remain a critical issue for therapeutic applications of siRNA. Hopefully, novel protein array technology will provide a better picture of siRNA effects on cellular protein expression profiles, which provides a better way of screening siRNA. Therefore, developing and identifying hyperfunctional siRNA will help to resolve the

Table 2. Comparison of different CDP-based nanocarriers for siRNA delivery.

Type	Modification	Size (nm)	*In vivo* delivery	Targeting site	Transfection efficiency	Remarks	Ref.
β-CD	Polymerization of difunctionalized β-CD monomers (AA type) with difunctionalized comonomers (BB type) to produce AABBAABB type copolymer	100 – 150	Intravenous and intraperitonial	*In vivo* toxicological mice experiments	100%	No mortality observed up to 200 mg/kg dose Cell line-dependent toxicity observed for serum-free transfection. No toxicity was observed at charge ratio 70+/- in transfection in the presence of serum	[40]
β-CD	CDPim complexed with AD-PEG conjugate and transferrin ligand	50 – 70	Intravenous	Metastatic Ewing's sarcoma in mice, *In vivo* toxicity studies in female cynomolgus monkeys, solid tumors in human	70 – 100%	No abnormalities in IL-12 and IFN-α, liver and kidney function tests Mild immune response seen in monkeys, but no toxicity Phase I clinical trials (CALAA-01) in human has shown dose-dependent accumulation of nanoparticles in tumor and evidence of specific gene silencing activity of siRNA targeted nanoparticles	[21,39,43]
β-6CDP	Unmodified	100 – 150	*In vitro* light-directed PCI	Osteosarcoma and melanoma cells	80 – 90%	PCI produced eight- to ninefold increase in transfection efficiency	[46]
α-CD	PAMAM starburst dendrimer/CD conjugate (G3)	160	-	*In vitro* studies on NIH3T3 cells	Superior to commercial transfection agents	Negligible toxicity	[50,80]
β-CD	*bis*-(Guanidinium)-tetrakis-(β-CD) dendrimeric tetrapod	–	*In vitro* cell lines transfection and toxicity studies	MRC5 cell line	Superior transfection efficiency	Less cytotoxic than PEI	[59]

AD-PEG: Adamantane-polyethylene glycol; β-6CDP: β-cyclodextrin polymer with 6 methylene groups separating the charges; CD: Cyclodextrin; CDP: Cyclodextrin-based polycation; CDPim: Cyclodextrin-based polymer-imidazole; PAMAM: Polyamidoamine; PCI: Photochemical internalization; PEG: Polyethylene glycol; PEI: Polyethylenimine; siRNA: Small interfering RNA.

unwanted off-targeting, since these siRNAs work at sub-nanomolar concentrations. Likewise, intelligent and effective designing of siRNA will improve the selectivity possible to significantly avoid off-target effects.

Declaration of interest

Kiran Chaturvedi (research fellow) and Tejraj M Aminabhavi (Emeritus Scientist) were financially supported by the Council of Scientific and Industrial Research (Grant no. 21/(0760)/09/EMR-II), New Delhi, India. Kuntal Ganguly (research fellow) was financially supported by the Council of Scientific and Industrial Research (Grant no. 08/558(0001)/2010-EMRI), New Delhi, India. Walter E Rudzinski received a support for the development of a gene delivery program from a Welch Foundation Departmental Grant awarded to Texas State University. All other authors state no conflict of interest and have received no payment for the preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Aagaarda L, Rossi JJ. RNAi therapeutics: principles, prospects and challenges. Adv Drug Deliv Rev 2007;59:75-86
2. Sunshine JC, Bishop CJ, Green JJ. Advances in polymeric and inorganic vectors for nonviral nucleic acid delivery. Ther Deliv 2011;2(4):493-521
3. Zhang S, Zhao B, Jiang H, et al. Cationic lipids and polymers mediated vectors for delivery of siRNA. J Control Release 2007;123(1):1-10
4. Behlke MA. Progress towards in vivo use of siRNAs. Mol Ther 2006;13(4):644-70
5. Wang J, Lu Z, Wientjes MG, Au JLS. Delivery of siRNA therapeutics: barriers and carriers. AAPS J 2010;12(4):492-503
6. Alekseev OM, Richardson RT, Alekseev O, O'Rand MG. Analysis of gene expression profiles in HeLa cells in response to overexpression or siRNA-mediated depletion of NASP. Reprod Biol Endocrinol 2009;7(45):1-17
7. Davidson BL, Paulson HL. Molecular medicine for the brain: silencing of disease genes with RNA interference. Lancet Neurol 2004;3(3):145-9
8. Li CX, Parker A, Menocal E, et al. Delivery of RNA interference. Cell Cycle 2006;5(18):2103-9
9. Gary DJ, Puri N, Won Y. Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions from polymer-based DNA delivery. J Control Release 2007;121(1-2):64-73

• **This paper elegantly describes the fundamentals of polymer-based delivery devices for siRNA. Some recent studies have been discussed critically.**

10. Dykxhoorn DM, Lieberman J. Knocking down disease with siRNAs. Cell 2006;126:231-5
11. Li W, Szoka FC Jr. Lipid-based nanoparticles for nucleic acid delivery. Pharm Res 2007;24(3):438-49
12. Daniela R, Andreas Z. Drug delivery of siRNA therapeutics: potentials and limits of nanosystems. Nanomed Nanotechnol Biol Med 2009;5(1):8-20
13. Opanasopit P, Nishikawa M, Hashida M. Factors affecting drug and gene delivery: effects of interaction with blood components. Crit Rev Ther Drug Carrier Syst 2002;19(3):191-233
14. Rudzinski WE, Aminabhavi TM. Chitosan as a carrier for targeted delivery of small interfering RNA. Int J Pharm 2010;399:1-11

•• **In this review, the authors have compared different chitosan-based devices for siRNA delivery for cancer treatment.**

15. Fischer D, Li Y, Ahlemeyer B, et al. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 2003;24(7):1121-31
16. Yuan X, Naguib S, Wu Z. Recent advances of siRNA delivery by nanoparticles. Expert Opin Drug Deliv 2011;8(4):521-36
17. Tokatlian T, Segura T. siRNA applications in nanomedicine. WIREs Nanomed Nanobiotechnol 2010;2:305-15
18. Mishra S, Heidel JD, Webster P, Davis ME. Imidazole groups on a linear, cyclodextrin-containing polycation produce enhanced gene delivery via multiple processes. J Control Release 2006;116:179-91
19. Bartlett DW, Davis ME. Physicochemical and biological characterization of targeted, nucleic acid-containing nanoparticles. Bioconjug Chem 2007;18(2):456-68
20. Davis ME. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. Mol Pharm 2009;6(3):659-68

•• **This paper describes the first targeted delivery of siRNA in humans and the devices are mainly based on CDP.**

21. Davis ME, Zuckerman JE, Choi CH, et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 2010;464(7291):1067-70

•• **The paper is a continuing study by the authors that gives evidence of RNAi in humans from systemically administered siRNA using nanoparticles of CDP-based carriers.**

22. Akhtar S, Benter I. Toxicogenomics of non-viral drug delivery systems for RNAi: potential impact on siRNA-mediated gene silencing activity and specificity. Adv Drug Deliv Rev 2007;59:164-82
23. Hollins AJ, Omidi Y, Benter IF, Akhtar S. Toxicogenomics of drug delivery systems: exploiting delivery system-induced changes in target gene expression to enhance siRNA potency. J Drug Target 2007;15(1):83-8
24. Foged C, Nielsen HM, Frokjaer S. Liposomes for phospholipase A2 triggered siRNA release: preparation and in vitro test. Int J Pharm 2007;331:160-6
25. Guo P, Coban O, Snead NM, et al. Engineering RNA for targeted siRNA delivery and medical application. Adv Drug Deliv Rev 2010;62(6):650-66
26. Liu H, Guo S, Roll R, et al. Phi29 pRNA vector for efficient escort of hammerhead ribozyme targeting surviving in multiple cancer cells. Cancer Biol Ther 2007;6(5):697-704

27. Kim TH, Kim SI, Akaike T, Cho CS. Synergistic effect of poly(ethylenimine) on the transfection efficiency of galactosylated chitosan/DNA complexes. J Control Release 2005;105:354-66

28. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. J Control Release 2006;115:216-25

29. Akhtar S, Benter IF. Nonviral delivery of synthetic siRNAs in vivo. J Clin Invest 2007;117(12):3623-32

30. Couzin J. Small RNAs make big splash. Science 2002;298:2296-7

31. Gao K, Huang L. Non-viral methods for siRNA delivery. Mol Pharm 2009;6(3):651-8

32. Wang X, Yu B, Wu Y, et al. Efficient Down-regulation of CDK4 by novel lipid nanoparticle-mediated siRNA delivery. Anticancer Res 2011;31(5):1619-26

33. Hatakeyama H, Akita H, Ito E, et al. Systemic delivery of siRNA to tumors using a lipid nanoparticle containing a tumor-specific cleavable PEG-lipid. Biomaterials 2011;32:4306-16

34. Zhou J, Li H, Zaia J, Rossi JJ. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. Mol Ther 2008;16(8):1481-9

35. Chollet P, Favrot MC, Hurbin A, Coll JL. Side effects of a systemic injection of linear polyethylenimine–DNA complexes. J Gene Med 2002;4:84-91

36. Hwang SJ, Bellocq NC, Davis ME. Effects of structure of beta-cyclodextrin-containing polymers on gene delivery. Bioconjug Chem 2001;12(2):280-90

37. Szejtli J, Osa T. editors. Cyclodextrins, Comput Supramol Chem 3. Pergamon; Oxford: 1996

38. Li J, Loh XJ. Cyclodextrin based supramolecular architecture: synthesis, structures and applications for drug and gene delivery. Adv Drug Deliv Rev 2008;60:1000-17

39. Hu-Lieskovan S, Heidel JD, Bartlett DW, et al. Sequence specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. Cancer Res 2005;65:8984-92

40. Gonzalez H, Hwang SJ, Davis ME. New class of polymers for the delivery of macromolecular therapeutics. Bioconjug Chem 1999;10(6):1068-74

41. Sandier A, Brown W, Mays H, et al. Interaction between an adamantane end-capped poly(ethylene oxide) and a beta-cyclodextrin polymer. Langmuir 2000;16:1634-42

42. Pun SH, Davis ME. Development of a nonviral gene delivery vehicle for systemic application. Bioconjug Chem 2002;13(3):630-9

43. Heidel JD, Yu Z, Liu JY, et al. Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proc Natl Acad Sci USA 2007;104(14):5715-21

44. Bartlett DW, Su H, Hildebrandt IJ, et al. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. Proc Natl Acad Sci USA 2007;104(39):15549-54

45. Hermanson GT. Bioconjugate techniques. Academic Press; San Diego: 1996

46. Boe SL, Longva AS, Hovig E. Cyclodextrin-containing polymer delivery system for light-directed siRNA gene silencing. Oligonucleotides 2010;20(4):175-82

47. Brahmamdam P, Watanabe E, Unsinger J, et al. Targeted delivery of siRNA to cell death proteins in sepsis. Shock 2009;32(2):131-9

48. Arima H, Kihara F, Hirayama F, Uekama K. Enhancement of Gene Expression by polyamidoamine dendrimer conjugates with alpha-, beta-, and gamma-cyclodextrins. Bioconjug Chem 2001;12(4):476-84

49. Tsutsumi T, Hirayama F, Uekama K, Arima H. Evaluation of polyamidoamine dendrimer/alpha-cyclodextrin conjugate (generation 3, G3) as a novel carrier for small interfering RNA (siRNA). J Control Release 2007;119(3):349-59

50. Tsutsumi T, Arima H, Hirayama F, Uekama K. Potential use of dendrimer/alpha-cyclodextrin conjugate as a novel carrier for small interfering RNA (siRNA). J Incl Phenom Macrocycl Chem 2006;56:81-4

51. Jeong JH, Mok H, Oh YK, Park TG. siRNA conjugate delivery systems. Bioconjug Chem 2009;20:5-14

52. Moschos SA, Jones SW, Perry MM, et al. Lung delivery studies using siRNA conjugated to TAT(48–60) and penetratin reveal peptide induced reduction in gene expression and induction of innate immunity. Bioconjug Chem 2007;18:1450-9

53. De Paula D, Bentley MV, Mahato RI. Hydrophobization and bioconjugation for enhanced siRNA delivery and targeting. RNA 2007;13:431-56

54. Zhang X, Sharma KK, Boeglin M, et al. Transfection ability and intracellular DNA pathway of nanostructured gene-delivery systems. Nano Lett 2008;8(8):2432-6

55. Jessel N, Oulad-Abdelghani M, Meyer F, et al. Multiple and time-scheduled in situ DNA delivery mediated by beta-cyclodextrin embedded in a polyelectrolyte multilayer. Proc Natl Acad Sci USA 2006;103(23):8618-21

56. Huang H, Tang G, Wang Q, et al. Two novel non-viral gene delivery vectors: low molecular weight polyethylenimine cross-linked by (2-hydroxypropyl)-beta-cyclodextrin or (2-hydroxypropyl)-gamma-cyclodextrin. Chem Commun 2006;14(22):2382-4

57. Yamashita A, Choi HS, Ooya T, et al. Improved cell viability of linear polyethylenimine through gamma-cyclodextrin inclusion for effective gene delivery. Chembiochem 2006;7(2):297-302

58. Cryan SA, Donohue R, Ravoo B, et al. Cationic cyclodextrin amphiphiles as gene delivery vectors. J Drug Deliv Sci Technol 2004;14(1):57-62

59. Menuel S, Fontanay S, Clarot I, et al. Synthesis and Complexation Ability of a novel bis-(guanidinium)-tetrakis-(beta-cyclodextrin) dendrimeric tetrapod as a potential gene delivery (DNA and siRNA) system. Study of cellular siRNA transfection. Bioconjug Chem 2008;19:2357-62

60. Suh J, Hah SS, Lee SH. Dendrimer poly (ethylenimine)s linked to beta-cyclodextrin. Bioorg Chem 1997;25:63-75

61. Guo S, Huang F, Guo P. Construction of folate-conjugated pRNA of bacteriophage phi29 DNA packaging

motor for delivery of chimeric siRNA to nasopharyngeal carcinoma cells. Gene Ther 2006;13:814-20

62. Zhang K, Wang Q, Xie Y, et al. Receptor-mediated delivery of siRNAs by tethered nucleic acid base-paired interactions. RNA 2008;14:577-83

63. Lorenz C, Hadwiger P, John M, et al. Steroid and lipid conjugates of siRNAs to enhance cellular uptake and gene silencing in liver cells. Bioorg Med Chem Lett 2004;14:4975-7

64. Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 2004;432:173-8

65. Zimmermann TS, Lee ACH, Akinc A, et al. RNAi-mediated gene silencing in non-human primates. Nature 2006;441:111-14

66. Liszewski K. Conquering RNAi Delivery Challenges. Genetic engineering and biotechnology News 2008 Jul 1; 28(13): [1 screen]. Available from: URL:http://genengnews.com/gen-articles/conquering-rnaideliverychalleng es/2520/

67. Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. Nat Rev Drug Discov 2005;4:581-93

68. Davis ME, Pun SH, Bellocq NC, et al. Self-assembling nucleic acid delivery vehicles via linear, water-soluble, cyclodextrin-containing polymers. Curr Med Chem 2004;11:179-97

69. Schaffert D, Wagner E. Gene therapy progress and prospects: synthetic polymer-based systems. Gene Ther 2008;15:1131-8

70. Rozema DB, Lewis DL, Wakefield DH, et al. Dynamic polyconjugates for targeted in vivo delivery of siRNA to hepatocytes. Proc Natl Acad Sci USA 2007;104:12982-7

71. Oishi M, Nagasaki Y, Itaka K, et al. Lactosylated poly(ethylene glycol)-siRNA conjugate through acid-labile beta-thiopropionate linkage to construct pH-sensitive polyion complex micelles achieving enhanced gene silencing in hepatoma cells. J Am Chem Soc 2005;127:1624-5

72. Urban-Klein B, Werth S, Abuharbeid S, et al. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther 2005;12:461-6

73. Thomas M, Lu JJ, Ge Q, et al. Full deacetylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung. Proc Natl Acad Sci USA 2005;102:5679-84

74. Xie FY, Woodle MC, Lu PY. Harnessing in vivo siRNA delivery for drug discovery and therapeutic development. Drug Discov Today 2006;11:67-73

75. Richards Grayson AC, Doody AM, Putnam D. Biophysical and structural characterization of polyethylenimine-mediated siRNA delivery in vitro. Pharm Res 2006;23(8):1868-76

76. Merkel OM, Librizzi D, Pfestroff A, et al. Stability of siRNA polyplexes from poly (ethylenimine) and poly (ethylenimine)-g poly (ethylene glycol) under in vivo conditions: effects on pharmacokinetics and biodistribution measured by fluorescence fluctuation spectroscopy and single photon emission computed tomography (SPECT) imaging. J Control Release 2009;138:148-59

77. Woodrow KA, Cu Y, Booth CJ, et al. Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. Nat Mater 2009;8:526-33

78. Murata N, Takashima Y, Toyoshima K, et al. Anti-tumor effects of anti-VEGF siRNA encapsulated with PLGA microspheres in mice. J Control Release 2008;126:246-54

79. Arima H, Yoshimatsu A, Ikeda H, et al. Folate-PEG-appended-PAMAM dendrimer (G3) conjugate with alpha-cyclodextrin as a tumor selective siRNA carrier. AAPS J 2010;12(S2):M1047

80. Tsutsumi T, Hirayama F, Uekama K, Arima H. Potential use of polyamidoamine dendrimer/alpha-cyclodextrin conjugate (Generation 3, G3) as a novel carrier for short hairpin RNA-expressing plasmid DNA. J Pharm Sci 2008;97(8):3022-34

Affiliation

Kiran Chaturvedi[1], Kuntal Ganguly[1], Anandrao R Kulkarni[1], Venkatarao H Kulkarni[1], Mallikarjuna N Nadagouda[1,2], Walter E Rudzinski[3] & Tejraj M Aminabhavi[†1]
[†]Author for correspondence
[1]SET's College of Pharmacy, Department of Pharmacology, Dharwad 580 002, India
Tel: +91 836 2778279; Fax: +91 836 2467190; E-mail: aminabhavi@yahoo.com
[2]Present address: The US Environmental Protection Agency, ORD, NRMRL, WSWRD, 26 West MLK Drive, Cincinnati, OH 45268, USA
[3]Texas State University, Department of Chemistry, San Marcos, TX 78666, USA