Bioorganic Chemistry 40 (2012) 10–18

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Reviews

Non-viral vectors for the mediation of RNAi

Shubiao Zhang [a,*], Yinan Zhao [a,b], Defu Zhi [a,b], Shufen Zhang [b,*]

[a] SEAC-ME Key Laboratory of Biochemistry Engineering, Dalian Nationalities University, Dalian 116600, Liaoning, China
[b] State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, Liaoning, China

ARTICLE INFO

Article history:
Received 23 May 2011
Available online 5 August 2011

Keywords:
RNA interference
Gene delivery
Non-viral vectors
Clinical trials
Conjugates of siRNA

ABSTRACT

Though the delivery of siRNA into cells, tissues or organs remains to be a big obstacle for its applications, recently siRNA therapeutics has developed rapidly and already there are clinical trials ongoing or planned. Some non-viral vectors have attracted much more attention and shown the great potential for combating the delivery obstacle. As a novel class of lipid like materials lipidoids have the advantages of easy synthesis and large library of compounds. Cell penetrating peptides and chitosans have been used for the delivery of bioactive molecules for many years, but they are showing great promise for the delivery of siRNA. The hybrids of inorganic particles and the conjugates of siRNA have indicated the complex utilization different materials may provide another solution to the delivery problem. The most exciting thing is some clinical trials are undergoing, which provokes the hope of real curing method by using RNAi mediated by some non-viral vectors.

Contents

1. Introduction

RNA interference (RNAi), a naturally occurring process that mediates sequence specific inhibition of gene expression through the activation of RNA-induced silencing complex (RISC) by interaction with the small duplex RNA molecules termed siRNA [1], has been recognized "one of the most exciting discoveries in biology in the last couple of years" [2], since Fire et al. [3] have demonstrated that double-stranded RNA is much more effective at producing interference than either strand individually and that interference occurs at the posttranscriptional level, after successful par-1 suppression by injecting single stranded sense and antisense RNA [4]. The process of RNAi is related to a normal defense against viruses and the mobilisation of transposable genetic elements [5]. RNAi is triggered by small interfering RNA (siRNA), short RNA duplexes with a common length of 21–23 nt being generated from long dsRNAs of exogenous or endogenous origin [6–10], in

* Corresponding authors.
E-mail addresses: zsb@dlnu.edu.cn (S. Zhang), zhangshf@dlut.edu.cn (S. Zhang).

0045-2068/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2011.07.005

this process long dsRNAs are recognized by a dsRNA-specific endonuclease (a cytoplasmic ribonuclease III (RNase III)-like protein) called Dicer, an enzyme which can cleave long dsRNA into siRNA of 21–23 nt in length.

RNAi technology is being evaluated as a potentially useful method to cure diseases including cancer, infection, respiratory disease, neuronal disease, and autoimmune disease [2,11]. RNAi shows many advantages over other therapeutic methods as very specific, without the toxic effects often observed during chemotherapy and the sequence-independent toxic effects of antisense therapy, and siRNA are more resistant to nuclease degradation than antisense oligonucleotides and, therefore, exhibiting longer therapeutic effects than antisense therapy [12].

However, the delivery of siRNA remains to be the biggest challenge, because it is imperative for siRNA to reach the cytoplasm of the targeted cells to become effective and induce silencing. As naked RNAs cannot penetrate cellular lipid membranes by themselves [13], siRNA must be enclosed in carriers such as viral vectors and non-viral vectors to be transported to the targeted cells *in vitro* or *in vivo*. The successful application of siRNA, is largely dependent on the development of a delivery vehicle which should be administered efficiently, safely, and repeatedly. Viral systems usually give high transfection efficiencies, safety concerns from potential mutation, recombination, oncogenic effect, and high cost, however, greatly limit their therapeutic applications. In contrast, non-viral vectors are believed to cause less safety problems due to their relative simplicity, though nonspecific cytotoxicity associated with cationic liposomes has been observed [14–16].

We could see many non-viral systems for successful delivery of siRNA both *in vitro* and *in vivo* including in nonhuman primates and humans [11,17–24] have been developed in recent years, though RNAi therapy has met great challenge during the past decade. Many reviews [25–29] have shown us the promising future of siRNA delivery by using lipid-based systems, chitosans, polymeric micelles, siRNA conjugates and peptide delivery systems. Herein we show the readers the recent development of siRNA delivery by using lipidoids, chitosans, cell penetrating peptides, inorganic particles and siRNA conjugates, and new progress in clinical trials. Based on these we could realize that RNAi is approaching the real need through the non-viral delivery method.

2. Lipidoids

Lipids have long been used for the delivery of genes and siRNAs, since the first cationic lipid (*N*-[1-(2,3,-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride) (DOTMA) was introduced by Felgner et al. in 1987. Many studies also show that cationic liposomes hold great promise as vehicles for effective human gene therapy. Many high quality reviews [30–32] with respect to cationic liposomes for gene therapy are available; we ever reviewed cationic compounds used for gene therapy [33]. In our article, many lipids have been described based on the chemical structure nature of these compounds; here we only introduce lipidoids to show the prosperous future of lipids for the delivery of siRNA.

Recently, a new approach to the synthesis (Fig. 1) lipid-like materials for siRNA delivery vectors using combinatorial methods was reported [34]. This one-step synthetic scheme enabled the straightforward parallel generation of large libraries of delivery materials. In this study, a library of over 1200 lipid-like materials, termed lipidoids, were generated through the conjugate addition of an amine to an α,β-unsaturated carbonyl and evaluated for siRNA delivery performance. The safety and efficacy of lipidoids were evaluated in three animal models: mice, rats and nonhuman primates. Therapeutic efficacy was observed *in vivo* in liver, lung and peritoneal macrophages. The studies suggest that these materials may have broad utility for both local and systemic delivery of RNA therapeutics.

Huang et al's results [35] suggest that lipidoid-formulated CLDN3 siRNA has the potential as a therapeutic for ovarian cancer, in which they have proved the efficacy of lipidoid-formulated CLDN3 siRNA in three different ovarian cancer models, by intratumoral injection to result in dramatic silencing of CLDN3, significant reduction in cell proliferation, reduction in tumor

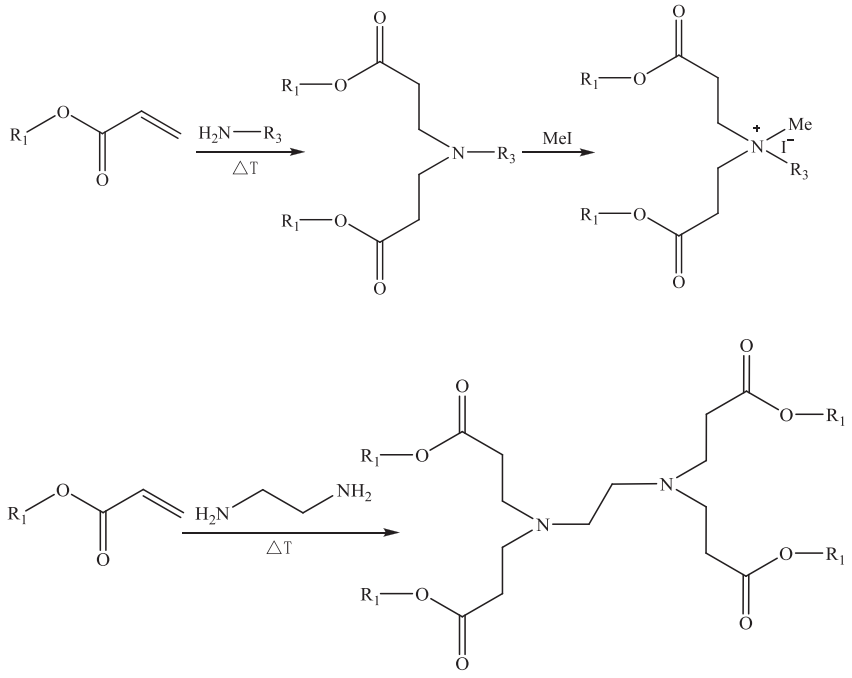

Fig. 1. Synthesis schematic of lipidoids by the conjugate addition of amine to α,β-unsaturated carbonyl compounds.