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ScienceDirect
Advanced Drug Delivery Reviews 60 (2008) 559–571
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Multifunctional envelope-type nano device (MEND) as a non-viral gene delivery system ☆

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Received 24 August 2007; accepted 9 October 2007
Available online 25 October 2007

Abstract

In this review, we describe a key role of octaarginine (R8) in developing our new concept of "Programmed Packaging", by which we succeeded in creating a multifunctional envelope-type nano device (MEND) as a non-viral gene-delivery system. This concept can be applied not only to nuclear targeting of plasmid DNA (pDNA) but also to cytosolic delivery of functional nucleic acids such as oligonucleotides or siRNA. This concept has been extended to other organelles such as mitochondria as a foundation for innovative nanomedicine. Finally, we discuss the rate-limiting step in gene delivery by comparing non-viral and viral gene delivery systems, which clearly indicates the importance of nuclear disposition of pDNA for efficient transfection.

Keywords: Octaarginine; Gene delivery; Nanoparticle; Membrane fusion; Nuclear deposition; Multifunctional; Nano device; Programmed packaging; MEND; siRNA

Contents

Abbreviations: Bmpr1a, bone morphogenetic protein receptor type 1 A; CAR, coxsackie and adenovirus receptor; CHEMS, cholesteryl hemisuccinate; Chol, cholesterol; CIDIQ, Confocal image-assisted 3-dimensionally integrative quantification; CPP, cell-penetrating peptide; CW, cell wall; DCs, dendritic cells; DOPE, dioleoylphosphatidylethanolamine; EPC, egg phosphatidylcholine; GFP, green fluorescent protein; HIV-1, human immunodeficiency virus; HSPGs, heparan sulfate proteoglycans; IL-12, interleukin-12; LA2000, LipofectAmine2000; LFN, lipofectamine PLUS; MEND, multifunctional envelope-type nano device; mtDNA, mitochondrial DNA; MTS, mitochondrial targeting signal peptide; N/P, nitrogen/phosphate; NBD, 4-nitrobenzo-2-oxa-1,3-diazolyl; NLS, nuclear localization signal; ODN, oligodeoxynucleotide; ODN-MEND, ODN-encapsulated R8-MEND; pDNA, plasmid DNA; PLL, poly-L-lysine; PTD, protein transduction domain; R7W, R7 modified with a tryptophan residue in the C-terminus; R8, octaarginine; R8-Lipo, R8-modified liposomes; R8-Lipo-HD, liposomes modified with high density R8; R8-Lipo-HD-E, empty R8-Lipo-HD; R8-Lipo-LD, liposomes modified with low density R8; R8-Lipo-LD-R, R8-Lipo-LD containing a rhodamine aqueous phase; R8-MEND, MEND modified with high-density R8 peptide; siRNA, short interfering RNA; siRNA-MEND, siRNA-encapsulated R8-MEND; S-Rho, Sulfo-Rhodamine; STR-R8, stearylated R8; TAT-mMDH-GFP, TAT fusion protein that consisted of an MTS derived from mitochondrial malate dehydrogenase and green fluorescent protein.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Membrane Permeable Peptide Vectors: Chemistry and Functional Design for the Therapeutic Applications".

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doi:10.1016/j.addr.2007.10.007

1. Introduction

A successful gene delivery system requires a rational strategy for overcoming many biological barriers such as membrane and enzymatic barriers. When plasmid DNA (pDNA) packaged in a delivery system is internalized via endocytosis, it must escape from endosomes before lysosomal degradation. The pDNA also must pass through the nuclear membrane before it can be transcribed in the nucleus. Therefore, the ideal gene delivery system should be equipped with a variety of functional devices to overcome these barriers, such as ligands for specific targeting, pH-sensitive fusogenic peptides, and a nuclear localization signal (NLS) [1–5]. Most non-viral vectors developed to date contain some of these devices [1–5]; however, it is difficult to integrate all of them into a single delivery system and to have each function exerted according to a specific program to overcome the barriers. Simple mixing of these nano devices is not sufficient for developing such an "artificially intelligent" nano device. Therefore, a new concept was required to develop a non-viral gene delivery system which can compete with viral ones in terms of efficiency. We succeeded in developing a multifunctional envelope-type nano device (MEND) as a non-viral gene delivery system based on a new concept, "Programmed Packaging". In this review, we describe how we developed this concept and how we construct the MEND by focusing on the function of octaarginine as a key molecular component of the MEND.

2. Uptake mechanism of octaarginine (R8)-liposomes

2.1. Importance of topology control

Since the R8 peptide was found to be taken up via a nonclassical endocytic pathway [6], which can circumvent lysosomal degradation, R8 was proposed as a functional device for efficient intracellular trafficking of nanoparticles, such as liposomes. Thus, the mechanism of uptake of nanoparticles tagged with R8 peptide was confirmed using nano-size complexes of pDNA encoding luciferase and complexed with either R8 peptide or stearylated R8 (STR-R8) [7]. The transfection activity of the STR-R8/DNA nanoparticle was significantly higher than that of the R8/DNA nanoparticle. Atomic force microscopic images of an STR-R8/DNA nanoparticle differed from those of an R8/DNA nanoparticle — almost all of the pDNA complexed with STR-R8 was well condensed in the nanoparticle, whereas most of the pDNA complexed with R8 was in a free state and not condensed. However, the cellular internalization of both nanoparticles was significantly reduced at 4 °C or in the presence of a hypertonic medium (0.4 M sucrose), which is known as an inhibitor of clathrin-mediated endocytosis. Thus, this strongly suggested that the STR-R8/DNA nanoparticle and the R8/DNA nanoparticle were taken up into the cells via the same entrance mechanism, while the transfection activities of the two nanoparticles differed. Therefore, contrary to expectation, the cellular internalization mechanism of R8-modified nanoparticles was not "nonclassical endocytosis", but clathrin-mediated endocytosis [7].

However, cellular internalization of the R8 peptide was not prevented by inhibitors of classical endocytosis, while complexes of R8 and pDNA were internalized via classical endocytosis [7]. From these results, the flexibility of the R8 peptide was suggested to be important for induction of nonclassical endocytosis. Therefore, STR-R8 peptides were adopted for use with liposomes — the stearyl moiety firmly anchored the peptide to the surface of the liposome while allowing the flexibility of the peptide to be maintained [8]. The addition of heparin to the cell culture medium prior to the addition of R8-modified liposomes (R8-Lipo) dramatically inhibited the cellular binding and internalization of R8-Lipo, especially in the case of liposomes modified with low density (0.86 mol%) R8 (R8-Lipo-LD). These data indicated the involvement of negatively charged cell-surface components, such as heparan