 DOI: 10.1002/masy.200750903

Effective Drug Therapies from Functional, Macromolecular Building Blocks with a Biomimetic Design

*Kui Huang, Teresa A. Croce, Sharon K. Hamilton, Chinessa T. Adkins, Billie L. Evans III, Eva Harth**

Summary: The development of suitable delivery systems for intracellular delivery of proteins, peptides and other bioactive materials opens the possibility to establish refined strategies for small drug delivery, gene delivery and vaccination. We present the assembly of advanced drug delivery systems from tailored building blocks to scaffolds and bioactive cargos to afford targeting and transport across biological barriers. In particular, the utilization of novel molecular transporter will advance the bioavailability of small and macromolecular drugs that show targeted intracellular delivery.

Keywords: bioactivity; biomimetic; carriers; drug delivery

Introduction

So far, the limited uptake of bioactive cargos has been a challenge of great significance in chemistry, biology and medicine.[1,2] In order to address critical factors such as targeting, molecular transport and drug load, the combinations of biocompatible scaffolds, suitable targeting units as well as vectors of cellular uptake are of fundamental importance. The sophistication of biological systems gives means to vectors in which each function is segmented and emerges from architectural features such as antennas for recognition and cell penetration.[3] Consequently, drug delivery strategies that accommodate many functions are predestinated to emerge from macromolecules comprising partitioned segments, each one achieving a different level of specification. In order to reach this high level of specification we differentiated the macromolecular delivery vector into building blocks such as the carrier molecule, targeting unit and an intracellular transporter entity. In particular, more effective intracellular delivery can revitalize therapeutics with previously unrealized potential due to poor pharmacokinetic profiles. Many different technologies have been developed to deliver drugs intracellularly but often evidence of clinical effectiveness has been limited. One of the challenges is not only cellular uptake, but also maintaining targeting of intracellular sites such as the nucleus or the cytosol, in order to limit unwanted intracellular probe metabolism and transport. Technologies that enable the delivery to specific subcellular locations are in high demand to reduce non-specific effects, toxicity and dosage levels with the goal to overcome current limitations in drug delivery, gene therapy and vaccination. We sought to develop a molecular transporter from branched dendritic architectures that provide means to target and control intracellular delivery and mimic features of branched polyarginines and linear protein transduction domains (PTD), also called cell penetrating peptides (CPP).[5–7] Dendritic organic structures overcome difficulties of PTDs in that they are easily scalable, show no immune response and functionalities

Department of Chemistry, Vanderbilt University, 7619 Stevenson Center, Nashville, Tennessee 37235
E-mail: eva.m.harth@Vanderbilt.Edu

can be easily introduced. The straightforward variation of the chemistry enables the conjugation to a multitude of different macromolecular therapeutics including nanoparticle carriers.[8]

Discussion

We designed a compact dendritic backbone to implement highly symmetric macromolecular peripheries in which the critical presentation of the guanidine groups to biological systems is controlled by the length of the alkyl spacer (Figure 1).

In this work we utilized the classic Newkome-type dendrimer as a structural building block in which nine end-functionalities can be achieved in just one generation of dendritic growth. The cell penetration capabilities of two Newkome-type dendritic transporters (FD-1 and FD-2) differentiated over a varied alkyl spacer to the scaffold, with a fluorophore conjugated to the focal point as the sample cargo molecule were initially demonstrated in NIH-3T3 fibroblasts and human microvascular endothelial cells (HMEC). It was shown that these molecules feature similar, high levels of cellular uptake, but differ considerably in their subcellular localization (cytoplasm vs. nucleus).[4]

In particular, FD-1 appeared to concentrate in the nucleus and FD-2 appeared to concentrate in the cytosol. These data suggest that the length of the alkyl spacer at the terminal generation of the dendrimer not only controls the rate of uptake but also regulates the subcellular localization. After the two novel transporters were identified we sought to develop simple instituted linker chemistries at the focal point of the scaffold in order to deliver bioactive cargos such as peptides and proteins in their native form (Figure 2).

Depending on the application, this building block is also available to be conjugated to more complex delivery systems such as nanoparticles in size dimensions of 5–15 nm. These nanoparticle carriers are suitable to be conjugated to many potent drugs that have unrealized potential

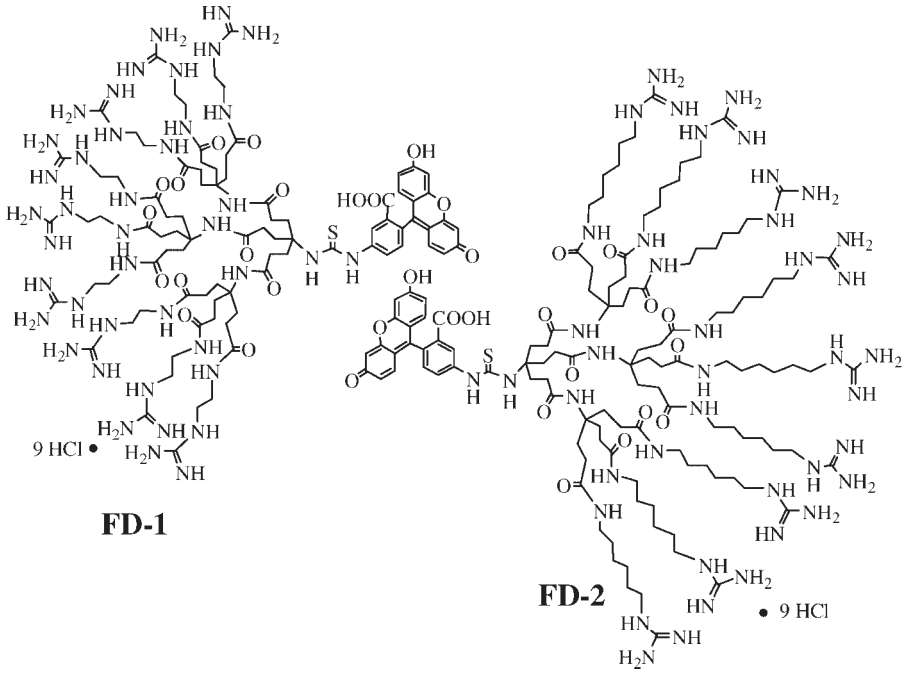

Figure 1.
Dendritic Molecular Transporter FD-1 and FD-2.

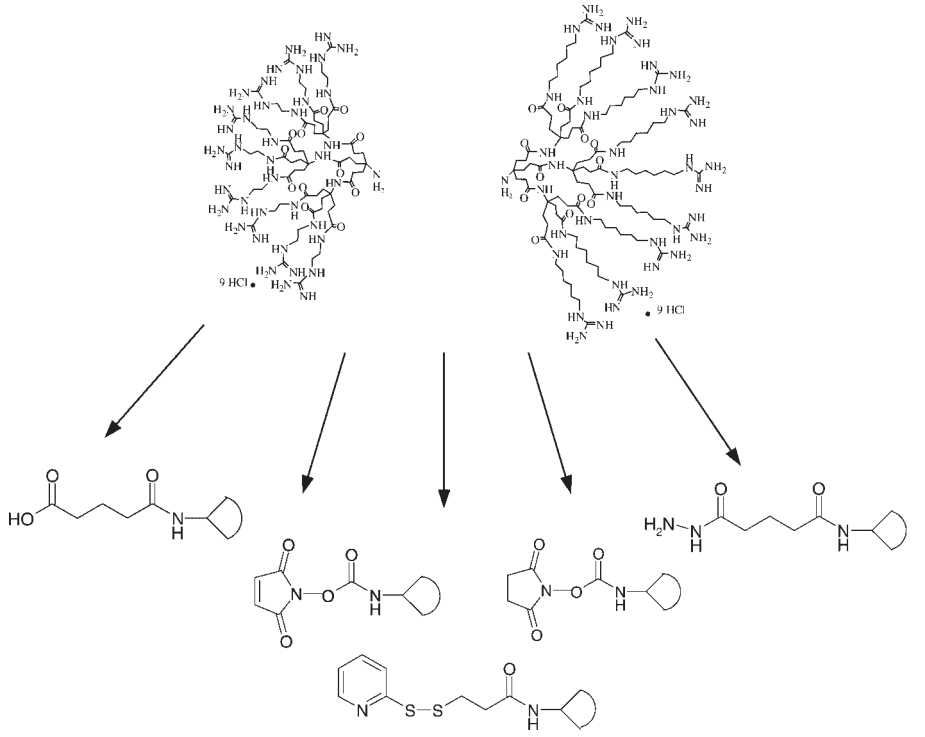

Figure 2.
Dendritic transporter allows for attachment chemistries depending on required application.

because of poor pharmacokinetic profiles. Efficient intracellular delivery can also avoid non-specific effect and reduce toxicity, allowing a reduction in dosage levels and leading to a better patient compliance. In particular, we started to investigate the uptake of exogenous molecules for the study of cellular functions, which could be both less cytotoxic and could afford cytosolic-targeted cargos with greater accuracy in delivery. The peptidic cargo can be conjugated to the dendritic transporter via a sulfide exchange reaction, in which a cysteine modified peptide forms a disulfide bond to the transporter. Cytosolic environments can cleave this bond reductively and we are currently investigating the biochemical effects of this approach. Another focus is the development of gene delivery tools and non-viral vectors, which suffer so far from low efficiency in comparison with viral vectors. Although, viral vectors are currently by far the most efficient means of DNA delivery,[9] limitations are associated with immunogenicity, toxicity, restricted targeting of specific cells types and cost of production.[10] The ability to attach the carrier after protein cloning, expression and purification would afford methods to use recombinant proteins and peptides. Furthermore, tremendous opportunities are envisioned in the development of methodologies for immunogenic agents for viral diseases.

The outlined conjugation chemistries are key in the utilization of these bioactive cargos and will determine their future biochemical function.(Figure 2) The influence of intracellular targeting depends on the size and shape of cargo, its density and arrangement on the linear or globular scaffold has been the aim of current investigations and efforts.

Conclusion

In summary, we described the development of drug delivery vectors comprised of a

novel developed dendritic molecular transporter and biologically active cargo molecules. The ability to target cargo to different subcellular locations is a valuable asset and will be further investigated in a variety of different cell types. The presented linker chemistries allow the covalent attachment to functional groups in natural or artificial systems such as proteins, peptides and nanoparticle carrier. The aim of these efforts will be to establish the utility of theses vectors in terms of vaccination, gene and drug delivery systems.

Acknowledgements: E.H. gratefully acknowledges Vanderbilt University of a start-up fund and thanks NSF for the support (NSF-CAREER, CHE-0645737).

[1] V. P. Torchilin, Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annual Review of Biomedical Engineering* **2006**, *8*, 343–375.

[2] P. A. Wender, D. J. Mitchell, K. Pattabiraman, E. T. Pelkey, L. Steinman, J. B. Rothbard, The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters. *Proceedings of the National Academy of Sciences of the United States of America* **2000**, *97*(24), 13003–13008.

[3] R. L. Garcea, L. Gissmann, Virus-like particles as vaccines and vessels for the delivery of small molecules. *Current Opinion in Biotechnology* **2004**, *15*(6), 513–517.

[4] K. Huang, B. Voss, D. Kumar, H. E. Hamm, E. Harth, Dendritic molecular transporter provide control of delivery to intracellular compartments. *Bioconjugate Chemistry* **2007**, *17*, 140–146.

[5] J. B. Rothbard, E. Kreider, C. L. Vandeusen, L. Wright, B. L. Wylie, P. A. Wender, Arginine-rich molecular transporters for drug delivery: Role of backbone spacing in cellular uptake. *Journal of Medicinal Chemistry* **2002**, *45*(17), 3612–3618.

[6] H. M. Kang, R. DeLong, M. H. Fisher, R. L. Juliano, Tat-conjugated PAMAM dendrimers as delivery agents for antisense and siRNA oligonucleotides. *Pharmaceutical Research* **2005**, *22*(12), 2099–2106.

[7] H. Y. Jia, M. Lohr, S. Jezequel, D. Davis, S. Shaikh, D. Selwood, I. Zachary, Cysteine-rich and basic domain HIV-1 Tat peptides inhibit angiogenesis and induce endothelial cell apoptosis (vol. 283, pg. 469, 2001). *Biochemical and Biophysical Research Communications* **2001**, *284*(1), 245–245.

[8] E. Harth, B. Van Horn, V. Y. Lee, D. S. Germack, C. P. Gonzales, R. D. Miller, C. J. Hawker, A facile approach to architecturally defined nanoparticles via intramolecular chain collapse. *Journal of the American Chemical Society* **2002**, *124*(29), 8653–8660.

[9] S. R. Schwarze, S. F. Dowdy, In vivo protein transduction: intracellular delivery of biologically active proteins, compounds and DNA. *Trends in Pharmacological Sciences* **2000**, *21*(2), 45–48.

[10] P. Vaillancourt, K. A. Felts, Retroviral delivery of the ecdysone-regulatable gene expression system. *Biotechnology Progress* **2003**, *19*(6), 1750–1755.

