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Nucleic acid delivery: reports from the search of the Magic Bullet

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av

Joana Viola

Huvudhandledare:
Prof. C.I. Edvard Smith
Karolinska Institutet
Institutionen för laboratoriemedicin
Enheten för Molekylär Cellbiologi och Genterapivetenskap

Bihandledare:
Prof. Roger Strömberg
Karolinska Institutet
Institutionen för Biovetenskaper och Näringslära
Enheten för Bioorganisk Kemi

Fakultetsopponent:
Prof. Gilles Divita
Montpellier University
Research Center for Macromolecular Biochemistry

Betygsnämnd:
Prof. Magnus Essand
Uppsala Universitet
Institutionen för Immunologi, Genetik och Patologi

Prof. Bengt Fadeel
Karolinska Institutet
Institutionen för Miljömedicin, Molekylär Toxikologi

Prof. Jorma Hinkula
Linköpings Universitet
Institutionen för Molekylär och Klinisk Medicin

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ABSTRACT

Gene therapy is regarded as one of the most promising therapeutic approaches, since it has the potential to treat disorders by correcting malformations at the nucleic acids level rather than proteins, as opposed to conventional medicine. However, for non-viral gene therapy to successfully fulfill the requirements associated to the "magic bullet" has proven difficult.
This thesis aimed at the development of non-viral nanovectors of different nature to transport plasmid DNA and single-stranded splice-switching oligonucleotides while shedding some light on the following aspects: (1) the interaction carrier–nucleic acid; (2) relevant assets of the vector for efficient delivery; and (3) relatable features of the nucleic acid particles for *in vivo* delivery.

In our first study we compared a series of systematically modified spermines to evaluate the contribution of the lipophilic component of the carrier to the nanocomplexes formulation. We have observed that all alkylated spermines protected DNA against DNase I degradation, and that this protection directly correlated with the length of the aliphatic component. Also toxicity directly correlated to the length of the fatty acid. Further characterization studies suggested the shortest lipospermines (butanoyl- and decanoylspermine) as the two most suitable candidates for *in vivo* delivery (intramuscular and intradermal).

More than 90% of human genome undergoes alternative splice-switching, and numerous disorders impart from malfunctions at this level. These can be corrected by splice-switching (SSO) or antisense oligonucleotides (ASO). In an optimization study for *in vitro* ASO delivery, we evaluated different amino acid-modified polyethylenimine (PEI). We found that PEI modified with amino acids of equal hydrophobic nature (and to an equal extent) still resulted in carriers with very different properties. One amino acid modification in particular (tyrosine-modified PEI) showed significant improvement of ASO activity, in a splice-correction context. Also the extent of this modification was proven to significantly decrease the vehicle's delivery efficiency. These findings suggested the existence of parameters, other than the hydrophilic/hydrophobic balance of the carrier, relevant for the interaction with the nucleic acids and also for the activity of the ensuing particles.

In our third study, by stearylation of the cell-penetrating peptide (CPP) $(RxR)_4$ we were able to successfully deliver DNA and ASO. We showed that the stearyl-$(RxR)_4$ was significantly more effective than its parental form for DNA and ASO delivery. Our results suggest that the stearic acid-modification contributes to enhanced endosomal escape. Importantly, stearylation of another commonly used CPP – Arginine 9 – did not result in increased activity, supporting the earlier findings that properties such as length, composition and three-dimensional structure of the carrier are all determinant factors for the activity of the nucleic acid-containing particles.

Finally, we evaluated a novel class of carriers that share similarities with arginine and histidine-based peptides – D-diaminopropionic acid-based peptides ($Dapa_8$). Dapa have several attractive properties for gene delivery, with the advantage that they permit tailored-design and facilitate the manipulation of the delivery properties of the peptide. All peptides interacted with plasmid DNA and provided protection against DNase I degradation, at concentrations that did not decrease cell viability. Interestingly, all fatty acid-conjugates, including the palmitoyl-$Dapa_8$, formed stable and well defined nanoparticles, with an average diameter between 120 and 160 nm. However, further modifications or *de novo* peptide design are required to achieve efficient DNA delivery.

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