



**Karolinska
Institutet**

Department of Laboratory Medicine

Optimization of the formulation and design of oligonucleotide-based pharmaceuticals for the purpose of gene therapy

AKADEMISK AVHANDLING

som för avläggande av medicine doktorexamen vid Karolinska
Institutet offentligen försvaras i Föreläsningssal C1.87, Karolinska
Universitetssjukhuset, Huddinge.

Tisdagen den 7 Februari, 2012, kl 09.00

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Stockholm 2012

ABSTRACT

Oligonucleotides (ONs) are short sequences of nucleic acids which may be used in a therapeutic context to modulate gene expression. According to their target, ONs can be classified into two main classes: antisense ONs which target mRNA and antigene ONs that target chromosomal DNA. In order to be pharmaceutically efficient, both kinds of ONs have to possess enough stability against degrading enzymes and rapid clearance. They must pass the cell membrane, and in some cases the nuclear membrane, and bind with enough specificity and high affinity to their target site to successfully exert their desired effect. In fact, the use of natural nucleic acids as drugs is hindered by both their inherent instability in biological fluids and their highly charged nature, which hampers their cellular uptake. Therefore, research in the field of ON-based pharmaceuticals focuses on two main strategies: chemical modification of nucleic acids to produce analogues with better stability and binding properties, and development of delivery systems to further stabilize the ONs and enhance their cellular uptake.

Splice-switching antisense ONs (SSOs) made of phosphorothioate 2'-*O*-methyl RNA are promising therapeutics for several disorders caused by aberrant splicing. However, as other ONs, their usefulness is hindered by the lack of efficient delivery. In the first study of this thesis, four amino acid modified versions of the well-known polycation polyethylenimine (PEI) were evaluated for the formulation and delivery of SSOs. The formulations were physically characterized via assessment of their particle size and stability and this characterization was then correlated to their splice-correction efficiency after transfection into mammalian cells. Tyrosine-modified PEI (PEIY) was identified as a successful delivery system for SSOs as shown by splice-correction efficiency of 80% measured in HeLa705; a cell-model containing a mutated β -globin intron sequence found in β -thalassemia splicing disorder.

In the second study, a new cell penetrating peptide (PepFect 14) was developed and investigated for the formulation and delivery of SSOs using cell-models for two splicing disorders; β -thalassemia and Duchenne muscular dystrophy. The feasibility of incorporating this delivery system into solid formulations via solid dispersion technique was also demonstrated. The formed solid formulations were as active as the freshly prepared nanocomplexes in solution even when stored at elevated temperatures for several weeks.

In the third study, PepFect 14 was evaluated for the formulation and delivery of another kind of ONs: short interfering RNA (siRNA) in different cell lines. RNA interference effect was obtained at low siRNA doses with a unique kinetic profile. Solid formulations were then prepared and assessed for their stability in gastric conditions. PF14/siRNA solid formulations showed marked stability after incubation with simulated gastric fluid, which is extremely acidic and contains proteolytic enzymes.

The fourth study of this thesis addressed design optimization of the newly developed antigene ON, Zorro-LNA (Zorro). Here, double-strand invasion was proven as the mechanism by which Zorro binds to duplex DNA. The original Zorro, targeting both strands of the DNA duplex, was made of two ONs connected via a 7-nucleotide linker. In this report, the possibility to synthesize Zorro as a bi-directional single-stranded ON was investigated, thus reducing the size, facilitating the design and improving Zorro efficiency.

In conclusion, this thesis has dealt with developing formulation strategies for two different types of ON-based pharmaceuticals; SSOs and siRNA. Optimizing the design of Zorro LNA as an antigene ON has been also investigated. These findings may represent a step in the development of ON-based drug products as a new class of therapeutics.