Biocompatible Nanoparticles as Antisense ODN Delivery Systems for Exon Skipping-Mediated Dystrophin Restoration

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A most promising therapeutic approach for Duchenne Muscular Dystrophy (DMD) is the exon-skipping, based on the use of antisense oligonucleotides (AONs) to induce the exclusion of target exon from the mRNA and the restoration of the frame. The main difficulty associated with this approach is related to the fast degradation of AONs during transport to the target tissues.
Within this frame, we recently developed[1] a novel class of specifically designed biocompatible poly(methylmethacrylate) core−shell nanoparticles. These nanoparticles present a core of PMMA surrounded by an hydrophilic functional shell bearing cationic groups. These groups are able to anchor and release AONs with phosphorothioate backbone (2'-O-methyl-phosphorothioate 2'OMePs) through electrostatic interactions with the negatively charged internucleosidic phosphate groups of the AON. The major concern regarding the use of NPs in medicine is related to their slow biodegradability, the poor knowledge of their pharmacokinetic and safety, especially considering chronic treatments. It is therefore imperative to determine the pharmacodynamic of the NPs−AONs compounds, their diffusion way(s), half−life and clearance. In this context, fluorescent imaging is revealing as a fast, sensitive and cost effective technique to image and track molecules and particles in small animals. Among fluorescent dyes, near-infrared dyes are specially suited for high-performance optical imaging. They exploit the spectral region where light absorption and scatter properties of tissue are minimal. This enhances penetration depth (access of excitation light to the fluorophore) and escape of emitted fluorescence from the animal to reach the detector. Furthermore, endogenous cellular components produce little autofluorescence across the NIR spectral region, diminishing background interference and enhancing signal-to-noise ratios. This results in a very low detection limit.
It will be shown that the novel NPs-AON compounds greatly enhances dystrophin restoration in skeletal muscles and slightly enhances it in the cardiac muscles of *mdx* mice, thereby confirming that these NPs represent a very promising vehicle for systemic delivery of AONs. The observed dystrophin rescue was due to a high skipping percentage (10-20%), remarkable when one considers the low AON dose delivered, corresponding to $1/20^{th}$ of the routine dosage described in the literature for systemic treatments of mdx. The restored protein expression persists up to 12 weeks after treatment as highlighted by immunofluorescence and western blot analysis. Furthermore, the use of cationic nanoparticles to deliver AON into cultured myogenic cells significantly improve specific exon skipping 5 days post-transfection without any toxic effect. In addition, we will describe the biodistribution and pharmacokinetic characteristics of nanoparticle samples presenting at the surface an hydrophilic functional shell bearing cationic groups, able to anchor and release AONs, and primary amino groups binding reactive near NIR Dyes.

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[1] P. Rimessi, et al. Mol. Therapy, 2009, 17, 820-827.
A.Ferlini et al., Gene Ther. 2010, 17, 432-8.