Development of novel nanodelivery systems of hydrophilic biomacromolecules for improved cells uptake and therapy

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Proteins, peptides, oligonucleotides, and small interfering RNA duplex (siRNAs) typically exhibit poor membrane permeability and high sensitivity to heat, pH, and enzymatic degradation. Furthermore, these hydrophilic biomacromolecules suffer from short biological half-lives and rapid clearance limiting their clinical applications. Most of the drawbacks can be overcome by incorporating such active macromolecules in adequate nanocarriers which can prolong their blood circulation and intracellular delivery in targeted pathological tissues. Two types of hydrophilic biomacromolecules were used in this research: (1) an siRNA (19-30 bp) known to produce gene silencing via a well-defined mechanism. (2) An antisense oligonucleotide (ASO) with great potential for the treatment of Duchenne muscular dystrophy (DMD) caused by mutated pre-mRNA, and currently is the most promising therapy for DMD patients. Although, both macromolecules are currently in clinical trials (naked or loaded in different delivery systems including: liposomes, lipid or polymeric nanoparticles), there is no approved delivery system yet for them in the market. The issue of limited dosage (mostly due to nephrotoxicity and hepatotoxicity) hampers their potential activity and still needs to be addressed. The objective of the present study was to develop novel nanodelivery systems that will double-encapsulate such biomacromolecules and provide protection, sustained release, and improved cells uptake and therapy, compared to their parenteral administration in solution following administration of much lower dosage of siRNA/ASO, avoiding or minimizing possible side effects and renal toxicities. The first line of protection is achieved by loading the siRNA (or ASO) into primary nanoparticles (PNPs ~100 nm) made from crosslinked human serum albumin (HSA), containing the cationic lipid DOTAP (1,2-dioleoyl-3-trimethylammonium-propane), added to increase loading of the negatively charged RNA molecules and to further facilitate endosomal escape following cell internalization. The second line of stability is obtained by further encapsulating the PNPs into sub-micron capsules (i.e. nanocapsules or DNCs <1µm), made from PLGA (Poly D,L-lactic-co-glycolic acid), with or without PEG moieties, using a novel technique of nano spray drying (launched by Buchi in 2009). The main findings up to now are that a weekly administration of PNPs up to 2 mg/mouse (loaded with 100µg of active ASO) was well tolerated by the mice (n=3). Pathology of the spleen, liver, kidney, lungs, heart and brain found all examined tissues to be within normal range. New formation of dystrophin was observed in quadriceps muscle's, already at the low dosage (1 mg/mouse), with significantly more positive dystrophin fibers formation in the mice treated with 2 mg/mouse. It should be emphasized that a weekly administration of up to 3 mg/mouse, was well tolerated for Blank DNCs but not for active DNCs (loaded with 60µg ASO) whereas a weekly administration of active DNCs 1.5mg/mouse (30µg ASO), produced modest positive dystrophin fibers.

To the best of our knowledge, this work is the first attempt to produce double-nanocarriers using the nano spray drying technique. The main advantage of the platform developed in this project is the preparation of the final product in the form of dry powder easily dispersed prior to injection. Encouraging results were achieved in terms of loading capacity, drug content, extended *in vitro* drug release without a burst effect, cellular uptake, and preliminary efficacy results in animal models.