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Assessing Brain Targeting Efficiency of Ionisable Lipid Nanoparticles Encapsulating Cas9 mRNA/gGFP Following Different Routes of Administration in Mice

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Abstract : Background: Treatment of neurological disorders with modern medical and surgical approaches remains difficult. Gene therapy, allowing the delivery of genetic materials that encodes potential therapeutic molecules, represents an attractive option. The treatment of brain diseases with gene therapy requires the gene-editing tool to be delivered efficiently to the central nervous system. In this study, we explored the efficiency of different delivery routes, namely intravenous (i.v.), intra-cranial (i.c.), and intra-nasal (i.n.), to deliver stable nucleic acid-lipid particles (SNALPs) containing gene-editing tools namely Cas9 mRNA and sgRNA encoding for GFP as a reporter protein. We hypothesise that SNALPs can reach the brain and perform gene-editing to different extents depending on the administration route. Intranasal administration (i.n.) offers an attractive and non-invasive way to access the brain circumventing the blood–brain barrier. Successful delivery of gene-editing tools to the brain offers a great opportunity for therapeutic target validation and nucleic acids therapeutics delivery to improve treatment options for a range of neurodegenerative diseases. In this study, we utilised Rosa26-Cas9 knock-in mice, expressing GFP, to study brain distribution and gene-editing efficiency of SNALPs after i.v.; i.c. and i.n. routes of administration. Methods: Single guide RNA (sgRNA) against GFP has been designed and validated by in vitro nuclease assay. SNALPs were formulated and characterised using dynamic light scattering. The encapsulation efficiency of nucleic acids (NA) was measured by RiboGreen™ assay. SNALPs were incubated in serum to assess their ability to protect NA from degradation. Rosa26-Cas9 knock-in mice were i.v., i.n., or i.c. administered with SNALPs to test in vivo gene-editing (GFP knockout) efficiency. SNALPs were given as three doses of 0.64 mg/kg sgGFP following i.v. and i.n. or a single dose of 0.25 mg/kg sgGFP following i.c.. knockout efficiency was assessed after seven days using Sanger Sequencing and Inference of CRISPR Edits (ICE) analysis. In vivo, the biodistribution of DiR labelled SNALPs (SNALPs-DiR) was assessed at 24h post-administration using IVIS Lumina Series III. Results: Serum-stable SNALPs produced were 130-140 nm in diameter with ~90% nucleic acid loading efficiency. SNALPs could reach and stay in the brain for up to 24h following i.v.; i.n. and i.c. administration. Decreasing GFP expression (around 50% after i.v. and i.c. and 20% following i.n.) was confirmed by optical imaging. Despite the small number of mice used, ICE analysis confirmed GFP knockout in mice brains. Additional studies are currently taking place to increase mice numbers. Conclusion: Results confirmed efficient gene knockout achieved by SNALPs in Rosa26-Cas9 knock-in mice expressing GFP following different routes of administrations in the following order i.v.= i.c.> i.n. Each of the administration routes has its pros and cons. The next stages of the project involve assessing gene-editing efficiency in wild-type mice and replacing GFP as a model target with therapeutic target genes implicated in Motor Neuron Disease pathology.

Keywords : CRISPR, nanoparticles, brain diseases, administration routes
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