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DRUG DELIVERY

siRNA: brain delivery breakthrough

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A key challenge in the development of drugs for CNS diseases is their transport across the blood–brain barrier (BBB). Reporting in *Nature*, Kumar and colleagues have now developed a method to target small-interfering RNA (siRNA) to the CNS, providing a safe and non-invasive approach for the delivery of this new therapeutic modality, and possibly other therapeutic molecules, across the BBB.

To facilitate the transport of siRNA across the BBB, the authors took advantage of a strategy that enables the rabies virus to enter neuronal cells and spread throughout the brain. A short (29 amino acid) peptide derived from the rabies virus glycoprotein (RVG) was shown to act as a 'key' to the CNS, by specifically binding to the nicotinic acetylcholine receptor (AchR) present on neurons and the vascular endothelium of the BBB, allowing transvascular delivery, probably by receptor-mediated transcytosis.

The RVG peptide was identified by its ability to competitively inhibit AchR binding of the snake-venom toxin α-bungarotoxin. To couple the peptide to the siRNA, a positively charged nonapeptide composed of D-arginine (9R) was fused to the RVG peptide, allowing the negatively charged siRNA to be bound by charge interactions. *In vitro*, this construct was shown to facilitate siRNA-mediated gene knockdown in neuronal cells, in contrast to a control construct engineered with a peptide derived from the rabies virus matrix protein.

For the *in vivo* examination of siRNA delivery, mice were intravenously injected with a fluorescent siRNA–RVG–9R peptide complex. The uptake of the siRNA was strictly brain specific, and no fluorescence was detected in the liver or spleen. Microscopic examination of brain sections confirmed fluorescence throughout the brain. Gene-specific silencing was demonstrated in green fluorescent protein (GFP)-transgenic mice, which were injected with GFP-specific siRNA bound to RVG–9R. After 3 days of treatment, a significant decrease (~50%) of GFP expression was observed in the brain, but not in other organs. Similar results were obtained when the endogenous gene superoxide dismutase 1 (*SOD1*), expressed in many cell types in the brain, was targeted. Potential clinical use was demonstrated by using the construct to deliver an antiviral siRNA, which had previously been shown to protect mice from fatal infection with the Japanese encephalitis virus (JEV) when injected intracranially. Immunocompromised mice were infected with JEV, and intravenous siRNA-RVG-9R treatment over 3 successive days resulted in 80% survival as opposed to 100% lethality in control mice. By assaying for the levels of type I interferon, the authors ruled out the involvement of immune mediators.

These results are the first demonstration of the safe and non-invasive transvascular delivery of siRNA to the CNS. The efficacy of gene silencing may still be improved, and the molecular details of the delivery mechanism remain to be defined. Nevertheless, given the potential of siRNA as a therapy, this approach could pave the way for a new generation of treatments for numerous neurological diseases. Moreover, RVG-9R might not only be a highly promising drug delivery system for siRNA and potentially other therapeutic molecules, but also a powerful tool to systematically analyse gene function in the brain under experimental settings.

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