Arginine-rich cell-penetrating peptides with uncharged antisense oligomers

As reviewed by Jarver and Langel in *Drug Discovery Today* [1], cell-penetrating peptides (CPPs) can deliver various expression-regulating macromolecules into cells. CPPs range from 9–30 amino acid residues with some of the peptides cationic in nature, some amphipathic and rich in basic residues and others hydrophobic. Peptide-mediated delivery of common uncharged antisense oligomers, peptide nucleic acids (PNA) and phosphodiamidate morpholino oligomers (PMO), has been reviewed [2] and these are good candidates for delivery by arginine-rich CPP for the following reasons:

- Uncharged antisense oligomers do not interfere with the delivery efficacy of cationic peptides. The charges on the peptide are crucial for its delivery. Interaction of peptide polycations with oligonucleotide polyanions interferes with interaction of peptides and cell membranes, decreasing delivery efficacy. Uncharged antisense oligomers can be delivered effectively by arginine-rich peptides; conjugates of PNA or PMO with arginine-rich peptides have been used to inhibit γ-interferon [3], correct mispliced pre-mRNA [4] and inhibit viral proliferation [5].
- PNA or PMO are not degraded by cellular enzymes and cannot be denatured during delivery. Whether CPPs penetrate endosomal or plasma membranes, the antisense function of PNA or PMO is not affected, while proteins passing through endosomes might be denatured or degraded.
- PNA and some PMO rich in G content have poor aqueous solubility. Conjugation to arginine-rich CPP can increase their solubility.
- Previous delivery methods have been unsatisfactory. They consist of mechanical (scrape loading, electroporation, microinjection) and chemical (osmotic, cationic lipid) methods. These fail to deliver to all available cells, deliver into limited cell types, are complex and/or are tedious to use. CPP deliver antisense into a larger fraction of cells and many cell types by simply bathing cells in the peptide-antisense conjugate. Conjugation with peptide might assist *in vivo* delivery of antisense while mechanical and chemical methods have little *in vivo* relevance.

Toxicity of arginine-rich CPPs must be considered when interpreting gene inhibition data. Reported toxic concentrations of these peptides range from 4 μM to >100 μM. The degree of toxicity largely depends on arginine count, the hydrophobicity of the peptides, and the cell-line challenged [4]. Delivery efficacy and peptide toxicity increase with number of arginines and with peptide hydrophobicity. The peptide Arg_9Phe_2 delivered PMO more effectively than Arg_9 or $Tat_{48\text{-}58}$ but was also the most toxic (unpublished data). When down-regulating protein expression with peptide-antisense conjugates, it is important to control for peptide toxicity so that toxicity is not misinterpreted as antisense activity.

Whether arginine-rich CPPs usefully deliver therapeutic antisense *in vivo* is under investigation. Tat peptide has a short plasma half-life due to instability in plasma and rapid uptake into many different tissues [6]. Arginine-rich CPPs are toxic at high concentrations so large doses cannot be used to compensate for short plasma half-life. Toxicity at the site of administration might result, especially after repeated dosing. Peptide-antisense conjugates might need to be protected from tissue sequestration to be effective therapeutic drugs.

Enzymatic instability of the L-peptide could limit *in vivo* use. Replacing L- with D-amino acids circumvents instability but could cause nonspecific antisense effects due to electrostatic interaction between the peptide and RNA. Using a linker cleaved in the cell might solve this, but development of a linker that is both stable in blood and cleaved intracellularly remains challenging.

References

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