Nanoparticle-mediated brain delivery of a tetracationic porphyrin with potent anti-prion activities

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Conversion of PrP\textsuperscript{C} into PrP\textsuperscript{Sc} is the key pathogenic event in prion diseases.

- Cellular protein
- Soluble
- Protease-sensitive
- 43% α-helix, 3% β-sheet
- NMR structure

- Disease-specific protein
- Insoluble/aggregated
- Partially protease-resistant
- 30% α-helix, 43% β-sheet
- 3D structure unknown
Possible therapeutic strategies for prion diseases

- Down-regulate PrP<sub>C</sub>
- Stabilize PrP<sub>C</sub> conformation
- Inhibit PrP<sub>C</sub>-PrP<sub>Sc</sub> interaction
- Inhibit PrP<sub>Sc</sub> polymerization
- Inhibit PrP<sub>Sc</sub> polymer fragmentation
- Enhance PrP<sub>Sc</sub> degradation
- Block toxic signaling downstream of PrP<sub>Sc</sub> replication

Vorberg and Chiesa, 2019
VA01: a porphyrin with potent anti-prion activity

VA01

Fe(III)TM-PyP

Caughey et al., 1998
Priola et al., 2000
Pharmacological chaperone for the structured domain of human prion protein

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A cell-free PrP$^{Sc}$ conversion assay  
(Protein Misfolding Cyclic Amplification, PMCA)
VA01 inhibits PrP$^{Sc}$ replication in PMCA

**A**

<table>
<thead>
<tr>
<th>PrP$^{Sc}$ seed</th>
<th>PMCA</th>
<th>VA01 (10 μM)</th>
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<td>1:1</td>
<td>CT</td>
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**B**

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**C**

Amplification factor

- CT
- VA01
- Fe(III)TM-PyP
A cell assay for analysis of anti-prion activity

Cultures of prion-infected N2a cells (ScN2a-22L)

- PK, 5 µg/ml for 30 min

- Non-infected cells
- Prion-infected cells

PK

1 2 3 4

PrP
VA01 is more potent than Fe(III)TMPyP in clearing prions from cells.
The prion-infected cerebellar organotypic cultures (COCS)

Wolf et al., 2015
VA01 is more potent than Fe(III)TM-PyP in the prion-infected COCS assay.

22L-infected COCS, treated for 1 week
Does VA01 reach the brain?
VA01 pharmacokinetics

10 mg/kg, i.p., single dose

10 mg/kg, i.p., chronic treatment
How can we boost the brain delivery of VA01?
PLGA

Poly(lactic-co-glycolic acid) (PLGA) nanoparticles

- Safe (FDA approved)
- Relatively inexpensive
- Amenable to chemical modification
- Forms nanoparticles
  - 100-200 nm
  - -20/-40 mv charge
  - can be loaded with various molecules
The g7 peptide improves brain delivery of PLGA nanoparticles

The g7 peptide

- Synthetic opioid-like glycopeptide modified to avoid opioid effects
- Crosses the BBB through receptor-mediated endocytosis
- Can be linked to PLGA
- g7-PLGA forms nanoparticles like PLGA
g7-PLGA NPs have been successfully used in mouse models of brain diseases
VA01-loaded g7-NPs reduce PrP^{Sc} levels in prion-infected COCS

22L-infected COCS, single treatment, analyzed after 48h
Summary

• VA01 inhibits PrP$^{Sc}$ replication in PMCA, N2a cells and COCS

• VA01 is more potent than Fe(III)TM-PyP

• A fraction of VA01 reaches the brain after systemic administration but its biological activity in the brain is variable

• Functionalized nanoparticles (g7-NPs) improve brain delivery of drugs

• VA01 can be efficiently loaded in g7-NPs maintaining its anti-prion activity \textit{in vitro}
Conclusions

• VA01 is a promising therapeutic molecule for prion diseases

• Before testing the therapeutic efficacy of VA01 in preclinical models, we need to improve its brain penetration

• VA01-loaded g7-NPs are active *in vitro*

• We are now testing the brain delivery of VA01 loaded in g7-NPs
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