Blocking the Neurotoxic Activities of Mutant Prion Proteins by a Novel Class of Therapeutic Agents

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One Sequence, Two Proteins: PrP\textsuperscript{C} & PrP\textsuperscript{Sc}

- self-propagates
- Aggregates (forms fibrils and amyloid structures)
- Accumulates in the brain
- induce toxic signals

Modified from Kraus A. et al. Mol Cell. 2021, 81, 4540-4551
PrPSc is an infectious protein (PRION), capable of multiplying by directly getting in contact with PrP\textsuperscript{C} and imposing its conformational rearrangement into new PrP\textsuperscript{Sc} molecules.

Prusiner S. B. PNAS USA, 1998, 95, 13363–13383
A Dual Role for PrP<sup>C</sup> in Prion Diseases

2. Mediator of toxic signals

PrP<sup>C</sup> → neurotoxic signals

A. gain of function
B. loss of function
C. subversion of normal signaling function

Modified from Westergard L. et al. Biochim Biophys Acta. 2007, 1772, 629-644

PrP<sup>c</sup>-directed Drug Discovery Strategies in Prion Diseases

- Lowering PrP gene expression
- Promoting PrP degradation
- Relocalization of PrP<sup>c</sup>
- Binding and stabilization of PrP<sup>c</sup>
- Blocking PrP<sup>c</sup>-mediated neurotoxic pathway

PrP^c-directed Drug Discovery Strategies in Prion Diseases

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- Relocalization of PrP^c
- Binding and stabilization of PrP^c
- Blocking PrP^c-mediated neurotoxic pathway

Transgenic mice expressing PrP with a deletion of residues 105–125 in the central region of the protein (referred to as ΔCR PrP) developed dramatic neurodegeneration.

The DBCA can be used to evaluate the anti-ΔCR PrP effects of compounds.

When expressed in cells, ΔCR PrP induces 1) spontaneous large ionic currents and 2) hypersensitivity to the toxic effects of two cationic antibiotics.

Quick cellular assay to identify small molecules able to suppress mutant PrP<sup>c</sup>-related toxicity

The active compounds should be able to restore cell viability.

Spontaneous ionic currents

DBCA


Modified from Le N T T et al. Brain Pathol. 2019, 29, 263-277

DBCA-based Drug Discovery

Identification of Anti-prion Compounds using a Novel Cellular Assay

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A Small-Molecule Inhibitor of Prion Replication and Mutant Prion Protein Toxicity


Selection of a DBCA-based Compound

- *Dibenzothiazine scaffold suitable for chemical modification and structure-activity relationship (SAR) studies.*
- *Synthetic procedures developed in our laboratory*

**DBCA**
- $RD_{50} = 0.4 \, \mu M$
- $LD_{50} > 100 \, \mu M$

**Prion infection**
- $EC_{50} \ (22L \ and \ RML) \sim 50 \, \mu M$

*Imberdis I H et al. J. Biol. Chem. 2016, 291, 26164-26176*
More than 80 compounds tested

compounds more active than LD24
First round of Chemical Optimization

Potency improvement in the DBCA

LD24
RD$_{50}$ = 1.1 µM
LD$_{50}$ > 50 µM
SI > 48

SM231
RD$_{50}$ = 0.32 µM
LD$_{50}$ = 22.4 µM
SI = 70

>3-folds increase in the cell protection potency
Similar toxicity
SM321 Does Not Target PrP^C Directly

SM231 does not act by directly binding PrP^C, or by altering its expression or localization.

SM231 does not directly bind PrP^C

SM231 does not alter the expression of PrP^C

SM231 does not alter the cell-surface localization of PrP^C
SM231 is a Weak Inhibitor of Prion Replication

SM231 fails to suppress prion replication in vitro, in cells and in brain slices.

SM231 does not inhibit prion replication in a cell-free system.

SM231 does not reduce PrP\(^{SC}\) formation in infected cells (reduction only at highest compound concentrations).

Slightly reduction of PrP\(^{SC}\) at 50 µM only with RML strain.
SM231 Suppresses the channel activity of Mutant PrP

SM231 abrogates inward ionic currents generated by ΔCR PrP

- ΔCR PrP induces spontaneous large ionic currents in a variety of cultured cells and neurons
- Several point mutations in PrP that cause genetic prion diseases also induce spontaneous currents
- The current-inducing activity of mutant PrPs observed in vitro is mechanistically related to their neurotoxicity in vivo


SM231 showed low metabolic stability and poor solubility in aqueous medium, two properties which hampers its use in pre-clinical settings.
Second Round of Chemical Optimization

Improvement of potency and selectivity

25-61-folds potency increase in cell protection

No toxicity

SM231

RD<sub>50</sub> = 0.32 µM
LD<sub>50</sub> = 22.4 µM
SI = 77

SM882

RD<sub>50</sub> = 0.044 µM
LD<sub>50</sub> > 100 µM
SI > 2273

SM884

RD<sub>50</sub> = 0.018 µM
LD<sub>50</sub> > 100 µM
SI > 5555

SM881

RD<sub>50</sub> = 0.032 µM
LD<sub>50</sub> > 100 µM
SI > 3333
SM884 rescues the synaptotoxic effects of prions in mouse brain slices.

SM884 (at 0.03 µM concentration) restores normal synaptic plasticity and excitatory post-synaptic potential in ex-vivo model of prion toxicity.

SM884 rescues the synaptotoxic effects of prions in mouse brain slices. Long-Term Potentiation (LTP) as an index of synaptic plasticity. Excitatory-Post-Synaptic Potential (EPSP) as an index of synaptic functionality.

Synthetic procedures developed in our laboratory at UNIPG

Maria Giulia Nizi
CJD Foundation bursary

➢ First task
Synthesis of the required amounts of compounds for the in vitro and in vivo experiments
Second task

Identification of the possible molecular target(s) for our bioactive compounds, given that we are aware that these small molecules do not act on PrP$_C$ directly.

Which is the molecular target of SM-derived compounds?

Output: short list of potential targets that we need to validate
Third task

Information obtained from *in vitro* and *in vivo* pharmacokinetic studies regarding how the body interacts with our compounds.

**In vitro Adsorption, Distribution, Metabolism and Excretion (ADME)**

- permeability in MDCKII-MDR1 cells (with and without inhibitor)
- binding (mouse plasma protein and brain tissue)
- metabolic stability in liver microsomes and hepatocytes.

**In vivo Pharmacokinetic (PK)**

Both compounds were administered via intravenous bolus administration in CD-1 mice at 2 mg/kg. Plasma and tissue (brain, kidney and liver) were collected by terminal sampling from 3 animals per time point up to 24 h.
Third task

Information obtained from *in vitro* and *in vivo* pharmacokinetic studies regarding how the body interacts with our compounds.

*In vitro* Adsorption, Distribution, Metabolism and Excretion (ADME) studies in mouse

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Permeability</th>
<th>Plasma binding</th>
<th>Brain tissue homogenate binding</th>
<th>Metabolic stability in liver microsomes</th>
<th>Metabolic stability in hepatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM881</td>
<td>moderate</td>
<td>Moderate</td>
<td>Moderate (F_b = 94.1%)</td>
<td>Moderate predicted <em>in vivo</em> hepatic clearance</td>
<td>Moderate predicted <em>in vivo</em> hepatic clearance</td>
</tr>
<tr>
<td></td>
<td>P-gp substrate</td>
<td></td>
<td>(F_b = 95.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM884</td>
<td>high</td>
<td>very high</td>
<td>high (F_b = 99.8%)</td>
<td>High predicted <em>in vivo</em> hepatic clearance</td>
<td>High predicted <em>in vivo</em> hepatic clearance</td>
</tr>
<tr>
<td></td>
<td>No P-gp substrate</td>
<td></td>
<td>(F_b = 98.6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules.

The compounds do not directly target PrP$^C$ and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication.
Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules.
- The compounds do not directly target PrPC and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication.

Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s).
Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules
- The compounds do not directly target PrP\textsuperscript{C} and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication

Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s)
- Analysis of \textit{in vivo} pharmacokinetic profiling results
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Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s)
- Analysis of \textit{in vivo} pharmacokinetic profiling results
- Testing \textit{in vivo} the therapeutic potential of the most promising compound(s). The goal is to directly test the hypothesis that blocking PrP-mediated toxicity could provide therapeutic benefits in prion diseases
Summary

- Dibenzothiazine derivatives potently inhibit electrophysiological abnormalities induced by mutant PrP molecules.
- The compounds do not directly target PrP\textsuperscript{c} and are weak inhibitors of prion propagation in cell cultures or brain slices, suggesting that their pharmacological target is mainly involved in prion toxicity but not replication.

Ongoing and Future Work

- Additional experimental studies to validate the postulated molecular target(s).
- Analysis of \textit{in vivo} pharmacokinetic profiling results.
- Testing \textit{in vivo} the therapeutic potential of the most promising compound(s). The goal is to directly test the hypothesis that blocking PrP-mediated toxicity could provide therapeutic benefits in prion diseases.
- Our dibenzothiazine derivatives may serve as chemical biological tools to unravel poorly understood aspects of prion biology.
**SUMMARY**

The following tasks have been achieved:

- Synthesis of the required amounts of compounds for the *in vitro* and *in vivo* experiments;
- Identification of the possible molecular target(s) for our bioactive compounds, given that we are aware that these small molecules do not bind the cellular prion protein;
- Information obtained from *in vitro* and *in vivo* pharmacokinetic studies regarding how the body interacts with our compounds.

**ONGOING AND FUTURE WORK**

- Additional experimental studies to definitively validate the postulated molecular target(s);
- Testing *in vivo* the therapeutic potential of the most promising compound by using mouse models of prion diseases. The goal is to directly test the hypothesis that blocking PrP-mediated toxicity could provide therapeutic benefits in prion diseases.