Prion Removal Capacity of Plasma Protein Manufacturing Processes

A data collection from PPTA member companies

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Member Companies
The Drivers for Studying Prion Removal Capacity

>200 prion removal studies by PPTA member companies

Modified from Ludlam and Turner, 2006
<table>
<thead>
<tr>
<th><strong>Prion</strong></th>
<th><strong>Plasma Protein</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Misfolded</td>
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<tr>
<td>Hydrophobic</td>
<td>Hydrophilic</td>
</tr>
<tr>
<td>Polymerized</td>
<td>Monomeric</td>
</tr>
<tr>
<td>Associated with lipids</td>
<td>Devoid of lipids</td>
</tr>
<tr>
<td>Insoluble</td>
<td>Soluble</td>
</tr>
</tbody>
</table>
Scale Down Characterization

Starting Material

Scale Down Process Step(s)

Output

Critical Process Parameters
- pH
- temperature
- ionic strength
- precipitation rates
- contact time
- filter load ratio

Waste

Biochemical Characterization
- activity/content
- total protein
- sp. activity
- impurities
- SEC
- yield

Demonstrate that Scale Down Process is a Valid Representation of Full Scale Manufacturing Process
**Prion Clearance Studies**

- **Prion Spike**
- **Starting Material**
  - Scale Down Process Step(s)
  - **Output**
  - **Waste**

Reduction (LRV) = Input - Output

**Biochemical**

Half log dilutions

- PrPRES

**BioAssay**

Log dilutions

- PrPRES

Western blot of pathogenic prion protein, Lee et al. 2001

[Image: Western blot showing PrPRES at different dilutions]
Spikes for Studying Prion Reduction

**Blood Spike**
- Direct model
- Not readily available
- Low titer
- Spike may alter composition of intermediate: invalid scaledown model
- Biochemical assays for prion are not sensitive enough

Very limited studies

**Brain Spike**
- Indirect model
- Readily available
- High titer
- Nonetheless it shares feature of:
  - misfolding
  - hydrophobicity
  - polymerization
  - lipid association
  - insolubility
  - infectivity

Large amount of studies that may reveal trends
## Prion Brain Spike Preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Abbrev</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude brain homogenate</td>
<td>cr</td>
<td>Mechanically generated brain homogenate, typically 10% w/v</td>
</tr>
<tr>
<td>Clarified brain homogenate</td>
<td>cl</td>
<td>Centrifuged at low speed to remove large debris</td>
</tr>
<tr>
<td>Microsomes</td>
<td>mi</td>
<td>Centrifuged multiple times to selectively concentrate microsomal membrane structures</td>
</tr>
<tr>
<td>Caveolae-like domains (CLDs)</td>
<td>ca</td>
<td>Centrifuged using gradient to enrich CLD structures</td>
</tr>
<tr>
<td>Detergent treated</td>
<td>de</td>
<td>Treated with detergent and/or nuclease to generate membrane-poor prion</td>
</tr>
<tr>
<td>Purified</td>
<td>pu</td>
<td>Purified with multiple steps including detergent and nuclease treatments, purity &gt; 90%</td>
</tr>
</tbody>
</table>

All prion spike preparations were derived from brains of syrian hamsters infected with 263K strain of TSE, unless otherwise noted.
Purification Steps Studied for Prion Reduction

- Pooled Plasma
  - Cryo Precipitate
    - Adsorption
      - Precipitation
      - Depth Filtration
      - Chromatography
      - Nanofiltration
    - FVIII/vWF, Fibrinogen
  - Adsorption
    - Precipitation
    - Depth Filtration
    - Chromatography
    - Nanofiltration
    - FVII, FIX, PCC, Fibrinogen, Protein C, Thrombin
    - FXIII, ATIII
    - C1-INH

- Cryopoor Plasma
  - Adsorption
    - Precipitation
    - Depth Filtration
    - Chromatography
    - Nanofiltration
  - Fraction (I)+II+III
    - Adsorption
      - Precipitation
      - Depth Filtration
      - Chromatography
      - Nanofiltration
  - IVIG, SCig, IGIM, Hyperimmunes

- Effluent II+III
  - Fraction IV
    - Adsorption
      - Precipitation
      - Depth Filtration
      - Chromatography
      - Nanofiltration
  - A1PI, AT III, C1-INH
  - Albumin

- Effluent IV
  - Fraction V
  - Albumin
PEG Precipitation

PEG precipitation consistently removes prion – complete removal obtained at higher PEG concentrations

Spikes used: mi = microsomes; pu = purified; cr = crude brain homogenate; de = detergent treated; cl = clarified brain homogenate
Cold Ethanol Precipitation

Increasing [EtOH] and/or low pH drives precipitation and increases prion removal.

Spikes used: mi = microsomes; pu = purified; cr = crude brain homogenate; de = detergent treated; cl = clarified brain homogenate; ca = caveolae-like domains
### Salt/Other Precipitation

<table>
<thead>
<tr>
<th>Precipitation</th>
<th>Gly/NaCl</th>
<th>Gly supe</th>
<th>Gly supe</th>
<th>AMS</th>
<th>Caprylate</th>
<th>Low pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>BC=6, BA=1</td>
<td>BC=3, BA=0</td>
<td>BC=3, BA=0</td>
<td>BC=7, BA=1</td>
<td>BC=5, BA=1</td>
<td>BC=4, BA=0</td>
</tr>
<tr>
<td>Spike</td>
<td>mi, pu, de</td>
<td>microsomal CJD</td>
<td>purified CJD</td>
<td>mi, cr, pu</td>
<td>cl</td>
<td>mi, cr</td>
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- **Reduction (log₁₀)**

**Same process conditions but purified spike led to higher removal in this case**

Spikes used:
- **mi** = microsomes
- **pu** = purified
- **cr** = crude brain homogenate
- **de** = detergent treated
- **cl** = clarified brain homogenate

/> biochemical assays (BC)

/> bioassays (BA)
Nanofiltration

15 to 20 nm nanofiltration steps were evaluated

Complete clearance attained

Spikes used: mi = microsomes; pu = purified; cr = crude brain homogenate;
 de = detergent treated; cl = clarified brain homogenate

biochemical assays (BC)

bioassays (BA)
Prion Reduction of Coupled Steps Only

Example #1

Coupled (BC)
- Pooled plasma ➔ Suspended cryo ➔ Al(OH)₃ ➔ Al(OH)₃ adsorbed ➔ Gly ppt ➔ Gly ppt ➔ Al(OH)₃ ➔ Fibrinogen intermediate ➔ Pasteurization ➔ Gly/NaCl ppt ➔ Depth filtration ➔ UF, Sterile filtration ➔ Fibrinogen
- Total (BC)

Example #2

Coupled (BA)
- Pooled ppt ➔ Cryopoor plasma ➔ Frac I ppt ➔ Super I ➔ Frac II+III ppt ➔ Frac II+III washed ➔ Washed Frac II+III ➔ Frac III ppt ➔ Supe III ➔ Additional purification ➔ IgG
- Total (BA)

Substantial overall TSE removal can be obtained by coupling multiple manufacturing steps
Prion Reduction of Single vs Coupled Steps

Example #1

- Pooled plasma
- Suspended cryo
- PEG ppt
- Clarified supernatant
- SD
- Heparin affinity
- Heparin column eluate
- Additional purification
- FVIII/vWF

Single (BA) 3.2 log 6.7 log 3.5 log
Total (BA) 2.9 log 3.0 log
Coupled (BC) ≥ 5.9 log
Total Coupled (BC) ≥ 3.0 log

Example #2

- Pooled plasma
- Suspended cryo
- Al(OH)3 Heparin ppt
- Al(OH)3 adsorbed
- Gly/NaCl ppt depth
- Filtrate
- SD treatment Chromatography
- Eluate
- 0.1 μm filtration
- FVIII/vWF

Single (BC) 0.8 log 2.9 log 1.1 log ≥ 3.0 log
Total Single (BC) > 7.8 log
Coupled (BC) 1.1 log 1.2 log 0.3 log 1.4 log
Total Coupled (BC) 4.0 log

Additive

Non-Additive
Prion Reduction of Single vs Coupled Steps

Example 3

Assessment of overall removal capacity (coupled vs additive single step) should consider the potential influence of prion conditioning effects from upstream steps.

Evaluation of TSE clearance across coupled process steps is a powerful method for demonstrating TSE clearance capacity of manufacturing processes.
Conclusions

Intrinsic Prion reduction capacity of manufacturing purification processes should be considered as a major risk reduction factor with respect to the safety of plasma derived therapeutic proteins.

>200 studies
vCJD Risk Over Time

Prion removal capacity manufacturing processes adds additional safety margins to donor exclusion measures

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Biochemical Differences b/w Prions and Plasma Proteins

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