Prion Removal Strategies: Challenges and Solutions

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Pathogen Removal and Diagnostic Technologies Inc. (PRDT)
Origin of vCJD

Late 1970’s
- Changes to preparation of meat and bone meal used in animal feed

1986
- First cases of BSE identified; feed ban introduced 1988

1990
- Government assures the public British beef safe for human consumption

1995
- First case of vCJD identified

1996
- Link between BSE and vCJD made; 30 month cull initiated

2003
- First vCJD transfusion transmission case identified
Distribution of TSE infectivity in blood

Blood Composition:
- Plasma: 20% WBC, 60% platelets, 14% plasma, 7-10% RBC
- Buffy Coat
- RBC

Percent Infectivity:
- 80% Recovered
- Normalized
  - Plasma: 30%
  - Buffy Coat: 25%
  - RBC: 45%
To date 4 cases of vCJD associated with blood transfusion identified (U.K.).

2 further possible cases identified (U.K.) but transmission route cannot be verified (Chohan, G. et al., Transfusion, 50, 1003-1006, 2010).

1 incubating case of vCJD linked with a contaminated plasma product (FVIII).
vCJD Prevalence

- To date 213 people globally have died from vCJD (171 in the UK);
- 3 vCJD positives found in 11,247 appendix samples indicating a UK prevalence of 292 per million - approx. 20,000 incubating cases for the UK population (*Hilton, DA et al., J. Pathol.*, **203**, 733-739, 2004);
- A recent study of tonsil samples in the 1961-1985 birth cohort found 1 positive in 9,160 tonsil samples suggesting a UK prevalence of 109 per million (*Fernandez de Marco, M et al., J. Pathol.*, 2010);
- UK vCJD epidemic likely to continue at a low incidence level for decades (*Garske, T. & Ghani, A., PLoS One*, **5**, 1-12, 2010);
- First non-MM genotype case of vCJD (MV genotype) discovered 2008 (37% of the population has MM genotype, 51% MV and 12% VV ) which raises the possibility of second and third “waves” of vCJD cases;
- Iatrogenic CJD case in Holland developed the disease 38 years after receiving a low dose of contaminated human growth hormone (*Croes, E.A., J. Neurology*, **72**, 792-793, 2002).
UK Incidence of CJD

Data from University of Edinburgh, National Creutzfeldt-Jakob Disease Research & Surveillance Unit; May 2nd 2011
Approaches to increasing the prion safety of blood and plasma products

- **Prion detection**
  - No blood screening assay exists for vCJD; issues surrounding false-positives.

- **Prion inactivation**
  - PrPres is significantly more stable than cellular components and plasma proteins

- **Donor deferral**
  - Option for countries with low incidence of BSE/vCJD; not viable where large % of population has potentially been exposed to TSE’s.

- **Prion removal**
  - Viable approach with appropriate affinity ligands; protection against known and future TSE’s.
Prion testing

Blood-based prion assays need to be:
- Approaching 100% accurate (to avoid false positives);
- Specific for PrPres (vCJD);
- Capable of detecting prion infectivity in pre-clinical cases (donors incubating vCJD) where blood may contain $\leq 1$ ID/ml.

Most sensitive blood assay announced to date (Edgeworth, J. A. et al., The Lancet, Published Online Feb 3rd 2011, DOI: 10.1016/s0140-6736(10)62308-2) is an experimental procedure based on prion capture by stainless steel particles followed by immuno-chemiluminescent detection. 15 out of 21 clinical vCJD blood samples (71%) were detected from a blinded panel of 190 blood samples with no false positives.
Prion filtration

• Effective at very low concentrations:
• Still active below Limits of Detection (most of the disease incubation period);
• Discrimination of normal vs abnormal PrP is irrelevant;
• There are no false positive issues:
  • No notification, counseling, retesting, or trace-backs,
  • No notification, testing, counseling of trace-backs themselves,
  • No product withdrawals;
• No alienation of donor population from fear of positive result;
• Protection against a broad spectrum of TSEs (eg. new prion diseases).
• Synergistic with prion testing for robust protection.
Prion removal with affinity ligands

- Ligands specific to prion proteins (ideally different types of prion protein);
- Minimal interaction/binding of biological product (low impact on product yield);
- Inexpensive ligands/adsorbents (single-use disposable);
- Synthetic ligands (Mimetic ligands™, peptide ligands);
- Successfully applied to whole blood, RBC, Plasma and various plasma proteins.
Discovery of prion binding ligands

Selection

1°

2° ~200

3° 7

4° 5

1

Method

Bead Blot™/CCL (PrP<sub>c</sub>)

Western Blot (PrP<sub>sc</sub>)

Spiked TSE

Endogenous infectivity (ligand selected for P-CAPT)
Removal of TSE Infectivity by PRDT Ligands

- Challenge titer = 1 million ID/ml
- Blood titer is 10 ID/ml
- R1, R3, R7, R8 and R10 are selected PRDT resins
- R5 and R4 are negative controls

[Gregori et al., Transfusion, 46, 1152-1161, 2006]
P-Capt® prion reduction filter

- Sterile, single-use prion-reduction device.

- Incorporates selected PRDT prion-binding affinity resin trapped between supporting membrane layers.

- Dockable filter - designed for use with human leuko-reduced rbcc.

- Device is CE Marked in Europe since Sept. 2006.

- Efficacy conforms to UK NBS requirements:
  - $\geq 3 \log_{10}$ reduction by brain spike study
  - $\geq 1 \log_{10}$ reduction by endogenous study
Hemocompatibility

- Hemocompatibility of resin with whole blood showed no negative effects:
  - No hemolysis
  - No platelet activation
  - No complement activation
  - No factor VII activation

- RBC yields within acceptable limits;

- No significant differences in red cell recoveries at 24h for P-Capt filtered cells compared to normal controls;

- No evidence of neoantigenicity following transfusion of P-Capt filtered RBC.
P-Capt Clinical Experience (Ireland)

• Transfusion of 20 patients with 1 unit P-Capt filtered RCC (Cork Hospital)
  • No adverse reactions reported
  • 6 week antibody follow-up data
    » No antibodies produced

• Transfusion of 100 patients
  • 100 patients transfused with 180 units P-Capt filtered RCC
    • No adverse reactions reported
    • On-going routine use at Cavan General Hospital (target 1000+ patients transfused)
P-Capt Clinical Experience (UK)

- **PRISM** (Prion-filtered vs Standard Red cells in Surgical and Multi-transfused Patients) - NBS/SNBTS
  
  **Study A:** Non-randomised controlled trial in surgical patients
  - Transfusion of P-Capt filtered RCC (single transfusion episode).
  - 270 control patients + 270 prion filtered patients.
  - 8 UK hospitals involved;
    - 329 patients transfused with prion-filtered RCC (March 2011);
    - 942 units of prion-filtered red cells transfused;
  - PRISM A study follow-ups anticipated to be complete late 2011.

- **SaBTO** recommendation to UK Ministry of Health to implement P-Capt in the UK for paediatric red cell transfusions (Nov 2009):
  - Filtered red cells be provided to those born since 1 January 1996;
  - Extended to 16 year olds and adults with haemoglobinopathies;
  - Subject to final decision by Minister of Health.
Reasons to implement prion filtration for RBC:

• High rates of TSE transmission by blood transfusion (30-40%);
• Leucodepletion is not sufficient to prevent TSE transfusion transmission;
• Unknown prevalence and long incubation times of the prion agent in an asymptomatic population that donate blood;
• Unknown impact of genotype:
  - Will only MM genotype be affected?
  - Will MV/VV have longer incubation times and eventually become sick?
  - Will most MV/VV never become sick but infect MM by blood transfusion?
  - Will MV/VV show the same symptoms as MM?
• No screening test available.
Prion reduction of S/D treated plasma

- Octaplas® is a virally inactivated (solvent-detergent treated) plasma product manufactured by Octapharma from pooled donor plasma.

- Existing manufacturing procedures provide a total of 2.5 log reduction of prion protein.

- PRDT prion-reduction technology implemented as a means of providing additional safety with respect to reducing the risk of vCJD transmission.

- Studies conducted by PRDT and Octapharma to demonstrate the performance of PRDT prion-binding resin and the biochemical properties and stability profile of the treated plasma.
Prion capture from 25% w/v HSA solution: PRDT resin performance

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<tr>
<th>challenge</th>
<th>Resin 1</th>
<th>Resin 2</th>
<th>Resin 3</th>
<th>Resin 4</th>
<th>Resin 5</th>
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Western blot of PrP\textsuperscript{res} binding to different PRDT affinity resins in the presence of 25% w/v HSA.
Prion binding in the presence of 3% w/v IgG

- PRDT resins are able to bind and concentrate prions in the presence of 3% w/v IgG
Prion binding performance

Prion capture from fresh frozen plasma (FFP) with different prion binding ligands

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<th>Challenge</th>
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- Prion binding performance may be subject to interferences depending upon the nature of the sample material;
- Need to verify prion binding performance for each application.
Where to implement prion filtration?

- Plasma
  - Intermediate fraction
    - Intermediate fraction
      - Intermediate fraction
        - Product 1
        - Product 2
        - Product 3
Choice of prion assay

**Spiked infectivity**
- Western Blot
- PMCA
- Bioassay
  - 301v Mouse (lifetime 18 months)
  - 263k Hamster (lifetime 2 years)
  - Sheep (lifetime 12 years)
  - Primate (squirrel monkey, macaque) (lifetime 20+ years)

**Endogenous (blood) infectivity**
- Bioassay (Hamster, Sheep, Primate)
Infectivity spike preparation

• Various preparations of infectious material used in spiking studies:
  • Crude brain homogenates
  • Microsomal fractions
  • Soluble brain extracts (centrifugation + detergent)

• PRDT brain spike preparation:

  10% (w/v) hamster brain extensively sonicated in PBS buffer, pH 7.2 then incubated with 0.5% (w/v) sarkosyl for 30 mins on ice, centrifuged at 12,000 x g for 15 mins and the supernatant fraction carefully removed. This fraction is diluted 100:1 with the sample to provide a final brain concentration of 0.1% (w/v).
Validation of infectivity spike

- Prion binding by PrioClear™ A affinity resin before (1) and after (2) chemical modification of the ligand to extinguish prion binding.

- Absence of bound prion for the chemically modified resin indicates the brain spike preparation is free of particulate infectivity which might become trapped by the packed resin bed.
Infectivity spike dilution

ID$_{50}$/ml
(Hamster 263k)

10$^{10}$
10$^{9}$
10$^{8}$
10$^{7}$
10$^{6}$
10$^{5}$
10$^{4}$
10$^{3}$
10$^{2}$
10$^{1}$
1
0.1

10% Brain homogenate

Measurable range

Western Blot

Incubation time bioassay (50 µl inoculation)

End-point bioassay (50 µl inoculation)

Limiting dilution bioassay (50 µl inoculation)

Endogenous blood infectivity
Western blot dynamic range

• 2 – 3 log reduction in prion measurable by Western blot starting with 0.1% SBH;

• 1 log reduction = removal of $10^7$ ID (about 1 million times more infectivity than present in 1 unit of infected blood or plasma);

• Log removal vs log safety.

1 2 3 4 5 6 7

(1) 0.1% 263k scrapie brain homogenate
(2) 1:2 dilution
(3) 1:4 dilution
(4) 1:8 dilution
(5) 1:16 dilution
(6) 1:32 dilution
(7) 1:64 dilution
**PrP^sc** binding to P-Capt™ membrane layers

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<thead>
<tr>
<th>Filter 1</th>
<th>Filter 2</th>
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<td>S</td>
<td>L1</td>
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**0.02% Spike** = 60,000,000 ID$_{50}$ or 7.8 log$_{10}$ ID$_{50}$/Filter 1

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**0.01% Spike** = 30,000,000 ID$_{50}$ or 7.5 log$_{10}$ ID$_{50}$/Filter 1

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**0.005% Spike** = 15,000,000 ID$_{50}$ or 7.2 log$_{10}$ ID$_{50}$/Filter 1

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Stock hamster scrapie brain homogenate titer = 10$^8$ ID$_{50}$/ml
Measurement of prion infectivity by incubation time bioassay

Dose response curve for SBH in RBCC

(Data courtesy of R. Rohwer, VA Hospital, University of Maryland)
Incubation time bioassay for control and prion-reduced RBC

Dose Response Standard Curve

Test Group

Dilution relative to whole brain

Challenge
R10
R8
R7
R5
R4
R3
R1

Days post inoculation

4.33 log_{10}

PRDT BioSciences
0.05 ml injected intracranially per animal

23 infected of 100 inoculated

= 23 infections per 5.0 ml

= 23/5.0 = 4.6 ID/ml

Poisson Correction

5.2 ± 1.0 ID/ml
Reduction of endogenous blood infectivity

<table>
<thead>
<tr>
<th></th>
<th>Whole Blood</th>
<th>Leukoreduced whole blood challenge</th>
<th>Treated blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. infections</td>
<td>21/47</td>
<td>15/99</td>
<td>0/100</td>
</tr>
<tr>
<td>Poisson Titre (ID/mL)</td>
<td>11.8 ± 2.2</td>
<td>3.3 ± 0.8</td>
<td>&lt;0.2 ± 0.2</td>
</tr>
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Endogenous infectivity reduced to below limit of detection \((\geq 1.2 \log_{10})\)

[Gregori et al., Lancet 2006, 368, 2226-30]
Transmission of BSE in sheep by transfusion of blood components

<table>
<thead>
<tr>
<th>Component</th>
<th>Number +ve transmissions</th>
<th>Total number transfused</th>
<th>Transmission efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>3</td>
<td>8</td>
<td>37.50%</td>
</tr>
<tr>
<td>Red cells</td>
<td>3</td>
<td>37</td>
<td>8.10%</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>9</td>
<td>37</td>
<td>24.30%</td>
</tr>
<tr>
<td>Plasma</td>
<td>4</td>
<td>38</td>
<td>10.50%</td>
</tr>
<tr>
<td>Platelets</td>
<td>6</td>
<td>37</td>
<td>16%</td>
</tr>
<tr>
<td>Leuco-reduced red cells</td>
<td>1</td>
<td>29</td>
<td>3.40%</td>
</tr>
<tr>
<td>Leuco-reduced plasma</td>
<td>1</td>
<td>29</td>
<td>3.40%</td>
</tr>
<tr>
<td>Leuco-reduced platelets</td>
<td>0</td>
<td>29</td>
<td>0</td>
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(From S. McCutcheon, Transmissible Spongiform Encephalopathies, Cologne, June 2010)
Summary

• vCJD is a real and on-going threat to human health;
• Care required in selection of infectivity assay and infectious material for spiking experiments;
• Need to mimic as far as possible endogenous blood infectivity;
• Caution required in interpreting the results of prion removal studies which use particulate/aggregated infectivity spikes;
• Need to validate each application of a prion reduction step;
• Prion affinity-filtration is an established and validated means of increasing the prion safety of blood/plasma products;
• Continued assay development may one day enable the accurate detection of endogenous blood infectivity opening the way to scale-down validation models which do not rely on brain infectivity spikes or animal bioassays.
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