Making blood safe

A filtration technology for removing infectious prions from red-cell concentrates

Prion diseases (TSEs, transmissible spongiform encephalopathies) are fatal neurodegenerative diseases that affect both humans and animals, including scrapie in sheep, BSE (bovine spongiform encephalopathy) in cattle and CJD (Creutzfeldt–Jakob disease) and its variant (vCJD) in humans. The recent occurrences of probable cases of transmission of vCJD through blood transfusion raises concerns about the safety of the blood supply and the possibility of transmission of the causative agent by blood transfusion from asymptomatic infected individuals.

Since the first case of vCJD in 1995, 164 people worldwide have died from the disease, which is believed to have emerged as a result of the consumption of meat from cattle infected with BSE. A new filtration technology that removes prions from red cell concentrates (RCCs), the most widely transfused blood component, has been developed and is available commercially in Europe this summer. The technical approaches used in developing this filtration technology, along with the proposed mechanisms for removal of prions by the device, are discussed in this article.

Prion diseases are believed to be caused by proteinaceous infectious agents called prions. Unlike viruses, bacteria, fungi and parasites, prions do not contain DNA or RNA and are therefore not destroyed by chemicals that target nucleic acids. In contrast with viruses, prions are non-immunogenic; that is, they do not elicit an immune response, because the host is tolerant to normal prions that are produced constitutively by the host. CJD is the most common form of human TSE, and although usually sporadic (sCJD), it has been transmitted from person to person through medical instruments and transplant of tissues or organs. In the latter instances, it has been referred to as iatrogenic (iCJD). A variant of CJD (vCJD) appeared in the UK in 1995, as a result of the consumption of tissue or meat products from cattle infected with BSE. This variant form of CJD has been shown to have significant involvement of the lymphoid organs, as demonstrated by the presence of abnormal prion (PrPSc) and infectivity in lymphoreticular organs, such as spleen, tonsils and appendix, all of which are interactive with circulating blood. Recent animal data, together with the reported cases of probable transmission of vCJD in humans from transfused blood products, have raised the concerns about the transmission of the causative agent through transfusion of blood products. In addition, PrPSc has also been found to be present in the spleen and muscles of patients with classical CJD and in the blood of affected mice and hamsters, raising the possibility that this disease too may be transmitted through blood transfusion.

There are no diagnostic tests for the detection of infectious prions in the blood of potential blood donors who are asymptomatic for the disease, nor can infectious prions be inactivated or destroyed by currently available technologies without destroying the essential therapeutic product. To deal with these challenges, several precautionary measures...
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blood is removed by standard leucocyte-reduction filters. Several technical approaches were investigated to develop technology that will allow removal of both leucocytes and infectious prions (cell and non-cell associated) from blood and blood components. The scientific bases of these technical approaches rely on the distinct differences in the biochemical and biophysical properties between PrPC and PrPSc. These differences in biophysical and biochemical properties reflect the different secondary and tertiary structures of PrPC and PrPSc and are responsible for the observed clinico-pathology of TSE. Therefore, a description of some of the structural properties of PrPSc is important in understanding the mechanism of its removal by filtration.

Biophysical and biochemical properties of prions

According to the protein-only hypothesis, prions are composed mainly of abnormal isoforms of a host-derived glycoprotein. The disease-related isoform, designated PrPSc, is derived from its normal cellular precursor, PrPC, by a post-translational process that involves a conformational change. Therefore, prion propagation involves a simple change in the conformation of PrPC to PrPSc (Figure 1). The two con-
5. PrPSc is readily precipitated by ethanol, ammonium sulphate and poly(ethylene glycol).
6. PrPSc has an increased tendency to form aggregates and polymeric structures. The infectious particle is considered to be a dimer or a high-order oligomer.
7. PrPSc is attached to cell surfaces and leucocytes through a GPI (glycosylphosphatidylinositol) anchor.

Pall’s technical approach for removing PrPSc

In addition to using the various biophysical and biochemical properties as a guideline in developing the technical approach for removing PrPSc, we also used available information about the distribution of prion infectivity in blood to design the appropriate method for its removal. Several reports using experimental models of TSE suggest that the majority of infectious prions are associated with leucocytes with lower levels of non-cell-associated infectivity in plasma. Platelets were not found to contain any significant amount of infectivity. These early studies in rodents raised the possibility that the PrPSc may convert PrPC into an intermediate infectious form (Figure 1). This intermediate complex (PrP33–35–PrPSc) may not bind to specific ligands that are designed to remove PrPSc, because the required epitope for binding may not be available or easily accessible. Therefore, the technical approach taken at Pall has been to investigate the use of different chemistries capable of binding not only PrPSc, but also the intermediate forms that are produced during the conversion of PrPSc.

Selection of surface chemistry for binding PrPSc

Several reports have shown that certain surface chemistries have specific affinity for prions. The mechanisms by which these compounds bind to prions are not well understood and it may involve ionic and hydrophobic interaction or hydrogen bonding. For example, prions are known to bind to amine-containing compounds through hydrogen bonding and they contain several octapeptide-repeat domains that interact specifically with Cu2+ ions. Therefore, in order to identify the appropriate surface chemistry with broad spectrum activity, capable of interacting with all the different forms of prions, we screened several proprietary surface chemistries along with those that have been reported in the literature as having specificity for PrPs, including Congo Red, polymeric polyanions, dextran sulphate, plasminogen, the bivalent cations copper, zinc, and nickel, heparin sulphate, hyaluronic acid, and stainless steel. During the screening tests, we were able to identify several proprietary surface chemistries that are able to bind to the PrPSc. In addition, we were able to confirm previous reports that show that copper ions are very effective in binding prions in buffered saline at pH 7.4, with significantly reduced efficacy in human plasma.

Attachment of selected surface chemistry to leucocyte reduction fibres

Since prion infectivity is associated with leucocytes, non-woven polyester fibres are specially constructed with proprietary technology for optimum binding of leucocytes (Figure 2). The main mechanisms utilized in removing leucocytes involve mechanical trapping and sieving of leucocytes and heterotypic aggregates by the polyester fibres. Surfaces of these fibres are also chemically modified to enhance the binding of the various populations of leucocytes to the fibres, through a specific activation-dependent mechanism. Multiple layers of these fibres are also coated with the selected prion-removal chemistry. The polyester fibres with properties for both efficient prion and leucocyte removal are then loaded into a filter housing that is specially designed to enhance the consistency and efficiency of filtration (Figure 3).

Demonstration of prion removal

In order to demonstrate the efficacy of the selected surface
chemistry in removing PrPSc and leucocytes from RCCs, we added 10 mL of 10% scrapie (263K strain)-infected hamster brain homogenate to a unit (about 270–300 ml) of RCC in citrate/phosphate/dextrose (CPDA-1) anticoagulant. The RCC was filtered at room temperature at a filtration height of 76.2 cm (30 in) corresponding to a flow rate of about 15 ml/min. The concentrations of PrPSc in the RCC before and after filtration were measured with a Western blot assay using 3F4 as the primary anti-PrP monoclonal antibody and goat anti-mouse IgG conjugated to horseradish peroxidase with a chemiluminescent substrate (Figure 4).

**Conclusion**

The Pall Leucotrap Affinity Prion Reduction Filter was developed specifically in response to the specificity of the current leucocyte reduction filters. This new filter concurrently removes leucocytes and all types of prions, both cell-associated and non-cell-associated, in a single step. It is based on a proprietary surface modification technology that removes all types of prions: aggregated, denatured and normal. The filter is expected to be commercialized this summer in Europe. The use of this type of filter will improve the safety of the blood supply by reducing the risk of human vCJD transmission through blood transfusion. Summaries of the mechanisms and technical approaches used in developing the filter are shown below.

**Pall’s technical approach to improving the safety of the blood supply: prion removal strategy.**

- Multi-targeted approach
- Removes prions that are associated with leucocytes
- Removes prions that are non-cell-associated but are present in plasma
- Removes all prions (normal/abnormal/intermediates)

Pall’s prion removal technology is not a monoclonal antibody or ligand that is specific to only a particular form of prion, but it is based on proprietary surface chemistry and a filter fibre matrix that removes all the different types of prions that are present in blood.

**References**

15. FDA final guidance: revised preventive measures to reduce the possible risk of transmission of Creutzfeldt–Jakob disease (CJD) and variant Creutzfeldt–Jakob disease (vCJD) by blood and blood products (January 2002). US Department of Health and Human Services Washington

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