REMOVAL OF PRION INFECTIVITY FROM PLASMA-DERIVED PRODUCTS

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Introduction

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, can be transmitted by blood and blood components. Blood leukoreduction provides only a partial protection against TSE transmission as 50% of endogenous blood infectivity remains in the leukoreduced blood.

Pathogen Removal and Diagnostic Technologies Inc. (PRDT), a joint venture between ProMetic and the American Red Cross, identified ligands with strong and selective binding to the TSE causative agent. A panel of resins was selected as the best prion binders and one of these resins was shown to reduce brain-derived scrapie infectivity by 3-4 $\log_{10}$ from RBC and to capture endogenous whole blood infectivity from scrapie-infected hamsters to the limit of detection of the bioassay. This resin was incorporated into a filter developed by MacoPharma as a prion capture device termed P-Capt™.

The same panel of resins was evaluated for their ability to remove prion from human plasma, 25% human serum albumin (HSA), and 3% IVIG solution. The materials differ substantially from whole blood and RBC in terms of protein concentration and potential competitors.

In the case of 25% albumin, the very high protein concentration makes chromatographic removal very challenging.

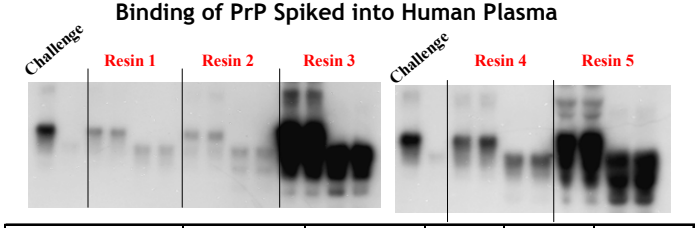
One of the resins in the panel is also currently being implemented into the manufacturing process of S/D plasma by a leading plasma fractionator.

Methodology

- Five resins containing ligands that displayed prion-binding were produced using a polymethacrylate-based resin.
- The base resin used had a pore size of 100 nm, and particle size ranging from 40 to 140 µm.
- Ligand resins were packed into 0.5-ml chromatographic columns and challenged with prion-spiked samples.
- Human plasma, albumin, and IVIG samples were spiked with 0.1% scrapie-infected hamster brain homogenate.
- The resin-bound proteins were eluted and analyzed by Western blot for the presence of the prion protein.

Results

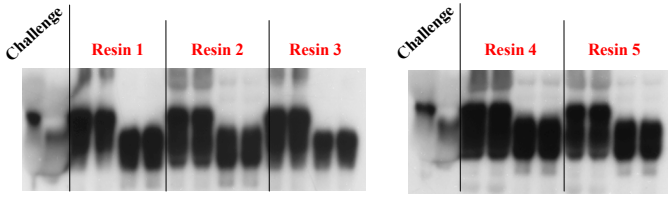
Binding of PrP Spiked into Human Plasma

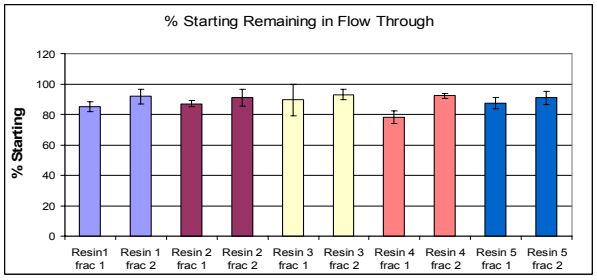


Sample	F II (Coag) (U/ml)	F VII (Coag) (U/ml)	F VIII (U/ml)	F IX (U/ml)	F XI (mg/ml)
Untreated	1.03	1.079	0.754	0.827	0.865
Resin 1	0.996	1.051	0.749	0.791	0.788
Resin 2	1.195	1.341	0.844	0.949	0.917
Resin 3	0.194	0.77	0.685	<0.1	0.866
Resin 4	0.984	1.049	0.771	0.861	0.833
P-Capt	0.49	0.977	0.631	0.293	0.878
P-Capt fraction 3	0.923	1.108	0.758	0.541	0.974

- Resin 3 was used in the manufacturing of the P-Capt device.
- Resins 3 and 5 showed strong binding of PrP when spiked into human plasma.
- Few of the resins bound some of the proteins of interest in plasma.
- When used in the filter format, Resin 3 initially bound some of the monitored proteins, but binding was reduced after some of the challenge solution passed through the device (P-Capt fraction 3 data).

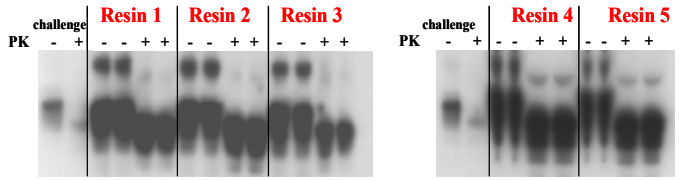
Binding of PrP Spiked into 3% IgG

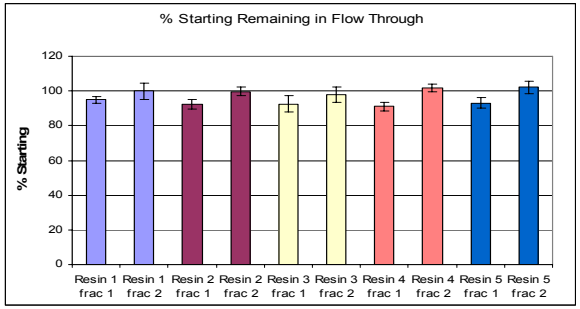




- All resins in the panel bound PrP in the presence of 3% IgG.
- Total loss of product was less than 10%.

Binding of PrP Spiked into 25% Albumin





- All resins were able to bind PrP in the presence of 25% albumin
- Product loss was negligible

Conclusions

- The five-resin panel of prion-binding resins display different behaviors when challenged with different challenge solutions
- The resins were able to bind the target protein even when the challenge containing a very high concentration of a competitor (25% albumin)
- In general, the results indicate that the panel of resins has the potential to remove prions from different plasma-derived materials
- Resin 3, currently used in the manufacturing of the P-Capt device, was able to remove prions from a complex solution (human plasma), and also from a solution containing a very high amount of a competing protein, without displaying significant binding of the product.

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