

HEMOCOMPATIBILITY OF P-CAPT™ PRION REMOVAL FILTERS

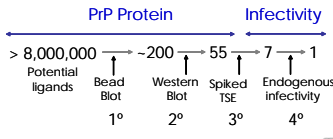
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Background

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases that are transmissible by blood transfusion and it is several years before symptoms arise. As there is not yet a diagnostic test for variant Creutzfeldt-Jakob Disease (vCJD) for screening blood donors selective reduction of infectious prion from donations is the most practical method of risk reduction.

Pathogen Removal and Diagnostic Technologies (PRDT), a joint venture between the American Red Cross and ProMetic Life Sciences, in partnership with MacoPharma, are developing a prion removal filter, P-Capt™. From millions of affinity ligands and polymers tested, one high affinity ligand has been incorporated into the device (Figure 1). This filter removes spiked TSE infectivity from red blood cell concentrates, as well as endogenous infectivity from whole blood¹. Here we describe initial studies investigating the effect of affinity resins and the P-Capt™ filter on RBC hemocompatibility parameters.

Figure 1. PRDT ligand selection process. Several different methods were used over 4 rounds of selection to identify and characterize the ligands



Aims

The aim of these studies was to investigate if exposure of whole blood, red blood cell concentrates (RBC), and plasma to the affinity ligand and the P-Capt™ filter has any deleterious effect on the quality of RBCs or activation and capture of plasma proteins.

Results

Figure 3. Effect of Prion Removal Resins on Hemolysis of RBC

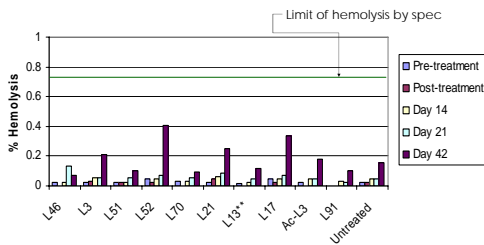


Figure 3. RBC concentrates were contacted with columns of various prion affinity ligands. The flow through fractions were stored at 4°C with weekly mixing. Samples were taken at the indicated time points and hemolysis measured with HemoCue and Cell-Dyne instruments. Results: All hemolysis was < 0.4%, well below the specification of <0.8% after 42-days storage.** Resin L13 is a form of the resin that was incorporated into the P-Capt™ filter.

Table 1. Effect of Selected Prion Removal Resins on Plasma Protein Activation

Assay	Results					
	Resin L13		Resin L13A*		Untreated	
Volume of plasma/ 0.5 ml resin	5 ml	10 ml	5 ml	10 ml	NA	
Platelet activation (%)	CD61/CD62P	0.11	ND	ND	ND	0.11
Complement activation (ng/ml)	Complement C3a-desArg ELISA	193.20	ND	ND	ND	147.20
Factor II activity (mg/ml)	Coagulation	0.19	1.30	0.49	0.92	1.03
Factor VII activity (U/ml)	Coagulation	0.61	ND	ND	ND	0.87
Factor VIII activity (U/ml)	Coagulation	0.77	1.33	0.98	1.11	1.08
	Chromogenic	0.69	0.76	0.63	0.76	0.75
Factor IX activity (U/ml)	Coagulation	ND	ND	0.75	0.80	ND
	Chromogenic	<0.1	0.75	0.29	0.54	0.83
Factor XI activity (mg/ml)	Coagulation	0.87	0.97	0.88	0.97	0.87

Table 1. Two prion affinity resins were contacted with plasma and the activity of various plasma proteins was examined. Results: No activation of platelets, complement or Factor VII was observed. No depletion of Factor VIII or Factor IX was observed. Factor IX activity was decreased in the plasma after exposure to these resins. *Resin L13A was incorporated into the P-Capt™ filter.

References

- Gregori et al (2006) Transfusion 46(7):1152-1161; Gregori et al (2006), accepted for publication.
- Guidelines for the Blood Transfusion Services in the United Kingdom (2005) 7th edition, V. James, ed. The Stationary Office, London, UK.

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Table 2. Plasma Protein Activation by P-Capt™ Filter

Platelet Activation (n=2)	# platelets (K/ul)	% Activation
Non LR, Untreated	312.5 ±31.82	6.23 ±4.52
Non LR, treated	1.60 ±0.99	9.64 ±1.49
LR Positive control	37.60	86.96

Complement Activation (n=3/trial)	C3-desArg (ng/ml)		
	Trial 1	Trial 2	Trial 3
Non LR, Untreated	135 ±2.34	318.19 ±25.13	698.82 ±51.66
Non LR, treated	88.08 ±1.09	306.50 ±7.72	160.13 ±1.41
LR Positive control	153.54 ±4.13	340.35 ±8.38	167.26 ±1.23

Factor VII Activation (n=3)	U/ml
LR, Untreated	0.94 ±0.17
LR, Treated	0.94 ±0.17

Table 2. Platelet-poor plasma (for complement and Factor VII activation) or platelet-rich plasma was applied to P-Capt™ filters. Flow through was collected and assayed as indicated. Results: No activation of complement or Factor VII was detected. >90% of the platelets were removed by the device; no substantial activation of those remaining was detected.

Table 3. Stability of Red Blood Cell Concentrates After Exposure to P-Capt™ Filter

	Control		Treatment		UKBTS parameters ²
	Day 2	Day 41	Day 2	Day 41	
Leukocyte Count (10 ⁹ /U)	0.05*	0.03*	0.01*	0.02*	<5
RBC Count (10 ¹² /U)	5.73	5.88	5.89	5.73	-
Hemoglobin (g/U)	61.13	61.75	62.02	59.96	> 40
Hematocrit (L/L)	51.75	54.23	53.32	52.17	-
Platelet count (10 ⁹ /U)	2.48*	1.40*	1.11*	2.37*	-
Hemolysis (%)	0.05	0.32	0.05	0.32	< 0.8
K ⁺ (mmol/L)	3.44	44.65	3.57	38.26	-
Glucose (mmol/L)	464.83	212.42	477.20	271.40	-
Lactate (mmol/L)	6.76	17.51	6.76	12.38	-
pCO ₂ (kPa)	56.10	14.85	62.78	54.50	-
pO ₂ (kPa)	60.00	275.39	44.60	150.25	-
pH	6.87	6.31	6.85	6.40	-

* below limit of quantitation of Cell-Dyne

Table 3. Units of red blood cell concentrates were leukoreduced and a 50 ml aliquot of each reserved as untreated control. The remainder of the units were treated with the P-Capt™ device. The indicated parameters were measured on a Sysmex XT 1800i hematology analyzer, Cell-Dyne 3700, Rapid-Lab 860 and HemoCue. Analysis was conducted before and after treatment, and on day 2, 7, 14, 21, and 41 days storage at 4°C with weekly mixing. Results: There were no substantial differences in the values obtained comparing treated versus untreated samples. Parameters that are defined by the UKBTS guidelines² are indicated; all results are within specifications.



Figure 4. P-Capt™ prion removal filter in use.

Conclusion

These studies found that the P-Capt™ filter and its component affinity resin have no adverse effects on the stability of red blood cell concentrates treated with the device. Furthermore, there was no detectable activation of Factor VII or complement. The filter removed approximately 90% of the platelets present in platelet-rich plasma, and did not activate the remaining platelets. The ability of the filter to remove endogenous and spiked infectivity is presented in posters #P-089 (Gregori et al) and #P-092 (Rohwer et al).