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Quantitative analysis of plasma proteins in whole blood-derived fresh frozen plasma prepared with three pathogen reduction technologies

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ABSTRACT

Several plasma pathogen reduction technologies (PRT) are currently available. We evaluated three plasma PRT processes: Cerus Amotosalen (AM), Terumo BCT riboflavin (RB) and Macopharma methylene blue (MB). RB treatment resulted in the shortest overall processing time and in the smallest volume loss (1%) and MB treatment in the largest volume loss (8%). MB treatment retained the highest concentrations of factors II, VII, X, IX, Protein C, and Antithrombin and the AM products of factor V and XI. Each PRT process evaluated offered distinct advantages such as procedural simplicity and volume retention (RB) and overall plasma protein retention (MB).

1. Introduction

Although mandatory testing for infectious agents, including HIV, Hepatitis B and C, and syphilis, has decreased safety concerns for these particular pathogens, the potential risk of emerging pathogens remains significant. Variables such as global air travel increase the risk of both human-to-human and even animal-to-human pathogen transmission [1]. A mathematical model constructed by the Canadian Blood Service (CBS) estimated that in a 5-year time frame, approximately 3500 recipient infections, in Canada alone, could occur from an emerging pathogen that establishes a chronic infection in donors [2]. Risk of infection from emerging pathogens not only presents a risk to blood recipients, but also limits the potential donor population and therefore the available blood supply. A continuous addition of screening tests as new pathogens emerge may not be the ultimate solution for many different reasons, including time to develop tests to limit the spread of infections, increase of donor deferrals and even the seasonal aspect of some outbreaks [3].

PRT processes provide a solution to the potential threat of emerging pathogens, improving the safety margin of the blood supply [4]. This PRT comparison was performed not only to evaluate the haemostatic potential of each PRT-treated plasma product, but also to compare the implementation and processing differences offered by each PRT process. Each PRT system offers a unique safety profile that must be considered prior to implementation. All three PRT systems tested in this study, are broadly effective against many relevant transfusion transmitted pathogens including viruses, bacteria, and parasites [5–7]. The AM and RB systems also inactivate white blood cells; the MB system involves a pre-illumination plasma filtration for leukoreduction and removal of aggregates, red blood cells and residual platelets prior to the addition of MB to inactivate white blood cells to remove the intra-cellular pathogens such as CMV.

Along with safety and effectiveness, procedural differences must also be considered prior to PRT implementation to ensure the system fits the needs of the individual blood bank. Illumination volume ranges, illumination and filtration times, as well as total processing time must be

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evaluated. This study assessed the procedural impact of each PRT process.

The toxicology profile varies between each individual PRT system. The RB process utilizes riboflavin, a water soluble, rapidly excreted vitamin with a safe toxicology profile. Consequently, the RB process does not require removal prior to transfusion [6,8]. On the other hand, an additional filtration and/or adsorption step is required to remove both methylene blue (optional and not routinely done in most Spanish blood banks) and amotosalen, as well as their respective photoproducts, from treated products prior to patient transfusion [5,7]. MB products have demonstrated limited toxicity; however, anaphylactic reactions have been recently reported resulting from methylene blue transfusion in France. Further investigation concluded that a higher rate of reaction was observed with MB plasma. As a result, MB products have been removed from the market in France, but have been retained in other European markets. Importantly, haemovigilance data in countries such as Spain and the United Kingdom have not corroborated an increase in allergic reactions. Further monitoring of plasma transfused patients developing severe allergic reaction will determine the statistical relevance of this French report [9,10]. In addition, a French haemovigilance retrospective study investigating four types of therapeutic plasma transfused over a decade, reported no increase in adverse reactions following MB plasma transfusions; this suggests that when the period of the study is extended, differences in allergic reactions rates between the different types of plasma become apparent [11]. Modest information on the AM photoproducts and on the long-term toxicity of the AM and its residual photoproducts is currently available [12].

Although PRT provides a distinct advantage in improving the safety profile of blood products, the haemostatic potential of the blood products is also impacted by PRT treatment and should be considered. To account for the balance between blood safety and product viability, many regional guidelines, including the Council of Europe (CoE) [13] have established protein quality specifications for pathogen reduced fresh frozen plasma. The CoE guidelines specify that, on average, PRT-treated plasma must demonstrate ≥0.50 IU/mL of FVIIIC and ≥ 60% of the Fibrinogen potency of fresh plasma.

This study was performed to directly compare the procedural simplicity and *in vitro* haemostatic potential of plasma products following treatment with three PRT processes currently available on the European market: Cerus Intercept Amotosalen and UVA light (AM) [5], Terumo BCT Mirasol riboflavin and UV light (RB) [6], and Macopharma methylene blue and white (visible) light (MB) [7]. The haemostatic potential of each product was evaluated through the analysis of plasma protein activity; loss of protein resulting from each PRT process was compared with a paired untreated plasma control.

2. Materials and methods

2.1. Blood collection and pooling

This study was performed over 3 days at the Centro de Transfusión de la Comunidad Valenciana in Valencia, Spain, during which 80 whole-blood derived fresh plasma units were used to perform 20 replicate experiments (Blood types: AB (1), B (2), O (8), and A (9)). Whole blood units were collected in CPD anticoagulant and held as whole blood for up to 18 hours at 22 ± 2 °C prior to plasma separation. Following separation, four ABO matched units (average 280 mL/unit) were combined to prepare plasma pools; plasma samples were frozen from each plasma pool to serve as the plasma control samples. All cryogenic vials were initially frozen in liquid nitrogen and later stored at –80 °C until coagulation analysis could commence.

2.2. PRT treatment

To prepare each sample for pathogen reduction, each plasma pool was divided into three paired plasma units containing unequal volumes of pooled plasma (AM: 569 ± 14 mL, RB: 309 ± 11 mL, MB: 287 ± 3 mL); the volume distributed to each technology was determined based on the product volume specification (Table 1). The weight of plasma aliquoted for each PRT treatment was recorded.

Pathogen reduction was performed with the following technologies: Cerus Intercept Blood System (AM), Terumo BCT Mirasol System (RB), and Macopharma Theraflex-MB System (MB).

Table 1 reports the processing specifications for each PRT technology. The AM system involves the addition of 15 mL of amotosalen hydrochloride to plasma and UV-A illumination followed by filtration with a compound adsorption device (CAD) to reduce the level of amotosalen in the final AM-plasma product requiring the pooling of two to three whole blood derived plasma units. The RB system involves the addition of 35 mL of riboflavin to the plasma product, followed by UV-A and UV-B illumination; a filtration step is not required for the RB-plasma product. Finally, the MB system requires plasma filtration with the Plasmaflex (PLAS4) filter to remove leucocytes, red cells, platelets, and aggregates. A dry capsule of methylene blue chloride is then dissolved in the filtered plasma and the product is illuminated with visible (white) light. In many regions, the Blueflex filter is then used to remove residual MB from the plasma product; the Blueflex filter was not used during this evaluation.

Product weights were recorded before (plasma weight only) and after illumination to assess the volume loss resulting from each inactivation process. Illumination times were also recorded for each PRT process. An assumed plasma density of 1.028 g/mL was used to calculate volume from each recorded weight. Following successful inactivation, samples were aliquoted from each pathogen reduced unit into cryogenic vials and immediately frozen in liquid nitrogen and transferred to a –80 °C freezer until protein analysis could commence.

2.3. Coagulation analysis (assay methods)

Paired plasma samples, frozen in cryogenic vials, were thawed in a 37 °C water bath to proceed with plasma protein analysis. Paired samples (control and PRT treated) were evaluated in St Luc Hospital, Brussels, Belgium for the following plasma protein factors and inhibitors on the