Biochemical and Biophysical Research Communications 377 (2008) 373–378

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Reduction of prion infectivity in packed red blood cells

Rodrigo Morales [a,b], Kimberley A. Buytaert-Hoefen [c], Dennisse Gonzalez-Romero [a], Joaquin Castilla [a], Eric T. Hansen [c], Dennis Hlavinka [c], Raymond P. Goodrich [c], Claudio Soto [a,*]

[a] Department of Neurology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555, USA
[b] Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[c] CaridianBCT Biotechnologies, L.L.C., Lakewood, CO, USA

ARTICLE INFO

Article history:
Received 25 September 2008
Available online 11 October 2008

Keywords:
Prions
Blood transfusion
Blood safety
Diagnosis
Prion diseases
Transmissible spongiform encephalopathy

ABSTRACT

The link between a new variant form of Creutzfeldt–Jakob disease (vCJD) and the consumption of prion contaminated cattle meat as well as recent findings showing that vCJD can be transmitted by blood transfusion have raised public health concerns. Currently, a reliable test to identify prions in blood samples is not available. The purpose of this study was to evaluate the possibility to remove scrapie prion protein (PrP^{Sc}) and infectivity from red blood cell (RBC) suspensions by a simple washing procedure using a cell separation and washing device. The extent of prion removal was assessed by Western blot, PMCA and infectivity bioassays. Our results revealed a substantial removal of infectious prions ($\geqslant$3 logs of infectivity) by all techniques used. These data suggest that a significant amount of infectivity present in RBC preparations can be removed by a simple washing procedure. This technology may lead to increased safety of blood products and reduce the risk of further propagation of prion diseases.

Transmissible spongiform encephalopaties (TSEs), also known as prion diseases, are a group of fatal neurodegenerative disorders affecting several mammalian species [1]. The main features of these diseases include the spongiform degeneration of the brain and the accumulation of an abnormal isoform of the prion protein, termed PrP^{Sc} [2]. PrP^{Sc} appear to be the main (or sole) component of the infectious agent [2,3]. Although prion diseases are rare in humans, the established link between the new variant form of Creutzfeldt–Jakob disease (vCJD) and the consumption of cattle meat contaminated by bovine spongiform encephalopathy (BSE) have raised concern about a possible outbreak of a large epidemic in the human population. Over the past few years, BSE has become a significant health and economic problem affecting many countries, principally in Europe. In addition to BSE epidemics, the incidence of other prionopaties in economically relevant species such as sheep, goats, and cervids presents an alarming scenario to the animal farming industry. Moreover, the possible spread of BSE in other species (i.e., sheep, goats, and porcine) has raised a concern about the emergence of multiple new sources of prions that may possibly affect human population [4].

The identification of PrP^{Sc} and infectivity in blood opens a new source of public health concern [5–9]. This alarming information urge the development of methodologies able to remove prions from organs and fluids designated for transplant or transfusion. Exacerbating this state of affairs is the lack of a reliable test to identify individuals incubating the disease during the long and silent period from the infection to the appearance of clinical symptoms [10]. Recent studies had demonstrated that vCJD can be iatrogenically transmitted from human to human by blood transfusion [11,12]. Transmission of prion disease through blood transfusion has also been described in sheep and experimental rodents [8,9]. Since blood used for transfusion was taken from animals and humans months or years before the onset of clinical disease, and because millions of people have been exposed to BSE infected material, there is a large concern that a portion of donated blood units might be contaminated with prions. The lack of routine methodologies approved to detect infectious prions in blood creates a need for prion elimination devices, which must be suitable for high throughput and effectiveness without affecting the quality of blood components.

The dynamic and distribution of PrP^{Sc} and infectivity in different blood fractions is unknown, but recent studies suggest that at pre-symptomatic stages of the disease PrP^{Sc} may be mostly attached to white blood cells (WBCs) originating from early peripheral prion replication in lymphoid tissues [6]. Later in the disease, a substantial amount of PrP^{Sc} is present in plasma and red blood cells (RBCs) where it seems to be mostly not cell associated and coming from brain leakage [6,13] (Marcelo Barria and CS, unpublished data). These findings suggest that there are two pools of infectious PrP^{Sc} in blood; one that is cell associated that can be mostly eliminated by efficient leukoreduction and a second pool freely circulating in plasma and contaminating RBC preparations. Indeed, it was reported that leucofiltration removed 42% of the

* Corresponding author. Fax: +1 409 7470020.
E-mail address: clsoto@utmb.edu (C. Soto).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.141

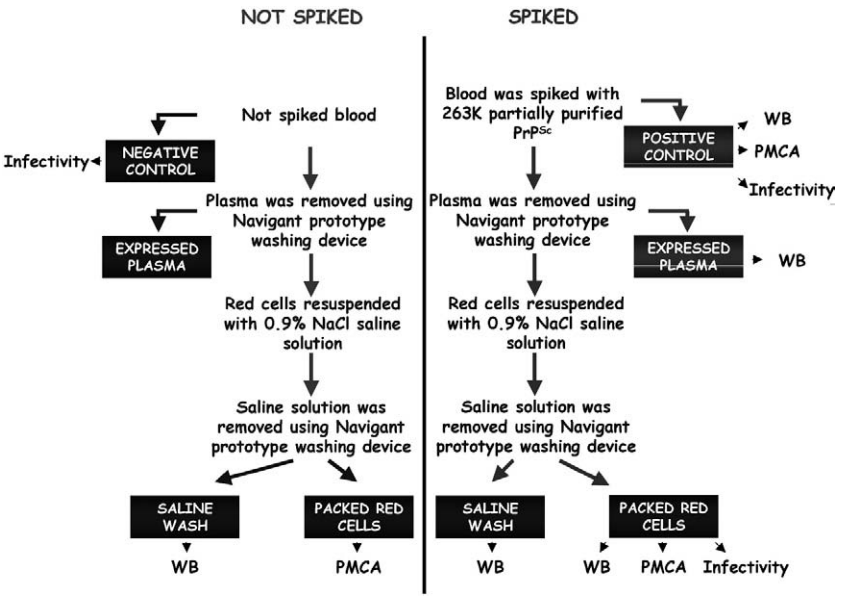

Fig. 1. Schematic representation of the RBC washing procedure. Different units of human whole blood were spiked with partially purified 263K PrP^{Sc} and samples were treated using the prototype device to separate and wash RBCs. From this purification protocol complete spiked blood (positive control), expressed plasma, saline wash, and pRBCs suspensions were obtained. PrP^{Sc} levels in each fraction were evaluated by Western blot, PMCA, and/or infectivity studies. Determinations were done by triplicate in independent experiments.

total TSE infectivity in endogenously infected blood [14]. Leucoreduction is efficient for the removal of WBC-associated TSE infectivity from blood; however, it is not, by itself, sufficient to remove all blood-borne TSE infectivity [14].

Every year, about 75 million units of blood are collected worldwide [15]. RBC transfusion is one of the treatments most extensively used in clinical practice. To reduce the risk for infection, RBCs can be filtered, washed, frozen, or irradiated for specific indications [15]. It has been suggested that PrP^{Sc} in RBCs can be depleted with specific adsorptive ligand resins. Indeed, a recent study reported that PrP-binding resins were able to reduce infectivity titer by 3 to more than 4 ID_{50} units [16]. We hypothesize that PrP^{Sc} in RBCs preparations can be removed by a simple washing of this fraction, since the infectious agent appears not to be tightly bound to RBCs. Various procedures to wash RBC preparations are currently being used to purify the cells before use for blood transfusion [15]. In order to assess PrP^{Sc} removal from the final pRBC fraction we used a hamster experimental model of prion diseases. We assessed the removal of prions by infectivity bioassays as well as by studying the levels of PrP^{Sc} using Western blot and protein misfolding cyclic amplification (PMCA) technology.

Materials and methods

Preparation of PrPSc samples. Symptomatic 263K infected hamsters were sacrificed by CO_2 inhalation and brains were collected. Ten percent brain homogenates (w/v) were prepared in phosphate-buffered saline (PBS) plus Complete™ cocktail of protease inhibitors (PI) (Boehringer Mannheim, Mannheim, Germany). The samples were clarified by a brief, low-speed centrifugation (1500 rpm for 30 s). Brain homogenates were mixed with 1 volume of 20% sarkosyl and the mixture was homogenized and sonicated using a Bandelin Sonoplus sonicator. This sample was centrifuged at 100,000*g* for 1 h at 4 °C. Supernatant was discarded and 2 volumes of PBS plus PI were added to pellets. Ultracentrifugation step was repeated. Supernatants were discarded and pellets were resuspended in 1 volume of PBS by pipetting and sonication. The sample was stored at −80 °C until use.

Treatment of spiked blood samples with the prototype cell separation and washing device. The cell separation and washing device was capable of processing two partial units of whole blood at one time. Three units of whole blood from Interstate Blood Bank (in Tennessee) were used in this study. Each unit was leukoreduced using a Pall™ Leukotrap Filter. Two hundred and thirty-five milliliters of each unit was transferred into a centrifugation bag. Five milliliters of the remaining whole blood from each unit was transferred into a 15 mL conical tube to serve as a negative control. Each of the three centrifugation bags was spiked with 5×10^6 LD_{50}/mL of PrP^{Sc}. Five milliliters of the mixture were removed from each bag and transferred to a 15 mL conical tube to serve as a positive control. Two units of prion spiked whole blood were loaded into the centrifuge (Fig. 1). Plasma was expressed by centrifugation of the whole blood for approximately 15 min at 3000 rpm bringing the pRBC hematocrit to $\geqslant$95%. Each pRBC unit was washed with 120 mL of normal saline solution. Saline was expressed by centrifugation of the RBCs for approximately 15 min at 3000 rpm bringing the pRBC hematocrit to $\geqslant$95%. Five milliliters of pRBCs final fraction were removed from each bag and transferred to a 15 mL conical tube to serve as a post-treatment sample (Fig. 1). The entire process can be performed in approximately 30 min.

Analysis of the quality of packed red blood cells. To assess RBC quality after washing, whole blood was washed as previously described without a PrP^{Sc} spike. After, the saline was expressed and discarded, and a full volume bag of AS-3 (GNI AS-3 Storage Solution, Larne, UK), approximately 107 mL, was added for storage at 4 °C. Stored RBCs were monitored on days 35 and 42 of storage to assess blood gases (pO_2, pCO_2), CBC (Becton–Dickinson Coulter AcT Diff, Fullerton, CA), and biochemistry; pH with Accumet research AR20 meter, lactate, glucose, Na+, K+, methemoglobin, plasma free hemoglobin, and hemolysis (calculated: % Hemolysis = [(hemoglobin$_{free}$ mg/dL) × (1 − Hct)/(hemoglobin$_{Total}$ g/dL)] × 100).

PrPSc concentration by sarkosyl precipitation. For detection of PrP^{Sc} in blood fractions, it was first necessary to concentrate diluted samples, which cannot be loaded directly into a gel or that interfere with electrophoresis and blotting. The concentration procedure consists in adding 1 volume of 20% sarkosyl, incubate the mixture for 10 min at room temperature and centrifuge it at 100,000*g* for