PALL Medical

Technical Report

Prion Retention Properties of PALL *Ultipor* 25 Breathing System Filters

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Introduction

Transmissible Spongiform Encephalopathies (TSE's) are a class of progressive neurodegenerative diseases, (prion diseases), which are found in humans (Creutzfeld-Jakob Disease, Gerstmann-Sträussler-Scheinker syndrome, kuru, fatal familial insomnia) as well as in animals (e.g. Bovine Spongiform Encephalopathy and scrapie).

Prion proteins (PrP) can exist in two forms: normal (PrP^{C}) and abnormal (PrP^{Sc}). It is the abnormal form that is the causative agent of TSE's. Bovine Spongiform Encephalopathy (BSE) was first diagnosed in 1985-1986 in cattle in the United Kingdom. In 1996 a new variant of Creutzfeld-Jakob Disease (vCJD) was reported in the human population in the UK [1].

Subsequent neuropathological and transmission studies have shown zoonotical links to BSE [2]. Abnormal prion proteins have been identified in the brain, central nervous system and lymphoid tissue of people diagnosed with vCJD.

Due to the long incubation time of prion diseases the potential transmission of infective material during medical treatments that involve the exposure to nervous tissue or blood is a concern.

Medical procedures, in which patients share a common resource or facility pose an especially high risk of cross infection by any infective agent. Previous studies have shown that during general anaesthesia up to 80% of endotracheal intubation procedures are traumatic and involve the presence of blood [3,4,5].

Potential cross infection with Hepatitis C has been reported via infected respiratory material and/or bloodstained secretions from anaesthetic equipment [6,7].

Anaesthetists, infection control workers and regulatory bodies have therefore raised their concerns about the use of respiratory equipment between patients in anaesthesia. Patient end breathing system filters are designed for use in protecting breathing circuits against patient cross contamination.

This investigation was performed to test **Pall** Breathing System Filters for their specific ability to retain prion proteins in a simulation of their clinical use in anaesthesia ventilation.

Materials and Methods

Prion material

Scrapie infected hamster strain 263K brain homogenate was used as the challenge material.

A 1% infective hamster brain homogenate was prepared in phosphate buffered saline (PBS) pH 7.4 (approximately 1.5 – 4.5 x 10^{7} LD50 [8])

Immunoblot

Protein separation and transfer were performed with NuPAGE pre-cast gel systems (Invitrogen GmbH, Karlsruhe Germany). Prion protein bands were visualised by using a Western Breeze chemiluminescent kit (Invitrogen). For the detection of prion protein, monoclonal antibody 3F4 (DakoCytomation GmbH, Hamburg, Germany) was used at a dilution of 1:2000.

Filter pre-conditioning

Experiments were run with two *Ultipor* 25 Breathing System Filters (BB25) from each of three different lots (lot numbers: 00-1076, 00-1064, 326802).

The *Ultipor* 25 test filters were pre-conditioned for 23.5 hr as shown in Fig. 1.

Homecare ventilator: PLV-100 (Respironix Deutschland Herrsching, Germany)

Humidifier: Cascade II (Puritan Bennett, Pleasanton California)

Tidal volume: 700 mL

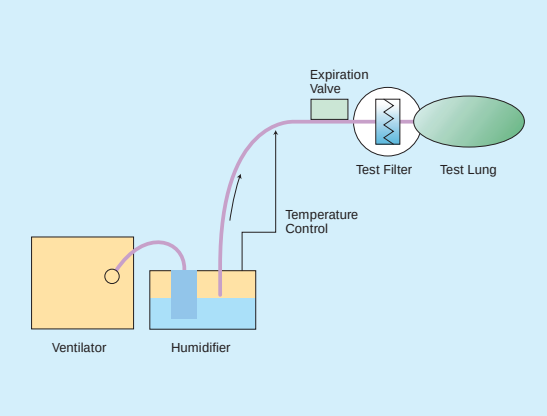

Figure 1

Breaths per minute: 10

Inspiration: Expiration ratio 1:2

Minute volume: 7 L

An active humidifier was connected into the inspiratory line to humidify the inspiratory air. This was controlled by a temperature sensor in the inspiratory line, giving 100 % relative humidity at 30 °C and resulting in condensation in the filter housing on each side.

After the preconditioning was complete (23.5 hr), the test filters were disconnected from the rig and the liquid that had accumulated during preconditioning discarded.

Materials and Methods cont'd.

Filter challenge with PrP^{Sc}

1.5 mL of 1% infective brain homogenate/PBS was added into the ventilator side of the filters. 2.0 mL PBS was added to the patient side to collect any prion protein which may have passed through the filter media. Different amounts of liquids were used to reflect different amounts of residual condensed water on each filter side after the pre-conditioning phase.

Ventilation was manually continued for another 0.5 hr by using a resuscitation bag (in order to prevent potential contamination of the ventilator).

The patient side solutions were collected (at least 1 mL) and subjected to total protein precipitation with phosphotungstic acid as outlined in figure 2 and as described by Wadsworth *et al* [9].

The pellet was suspended in a 50 µL volume of PBS buffer and subjected to Western blot analysis.

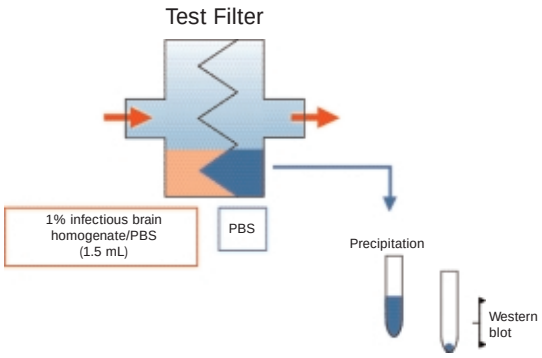

Figure 2: Processing of downstream solution of challenged filters

Western blot analysis

20 µL of each resuspended pellet was applied to a well of NuPAGE 10% Bis-Tris gel, 1.0 mm x 10 well, (Invitrogen, Cat. No.: NP0301); Protein gel electrophoresis was run in an Xcell SureLock Mini-Cell (Invitrogen, Cat. No.: EI0001) with MES running buffer (Invitrogen Cat. No.: NP0060), in accordance with manufacturer's instructions.

After the gel electrophoresis, protein transfer (Western Blot) was performed using a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BIO-RAD, Cat. No.: 170-3940), with transfer onto a 0.45 µm pore size nitrocellulose membrane (Invitrogen, Cat. No.: LC2001) in NuPAGE transfer buffer (Invitrogen, Cat. No.: NP0006), in accordance with manufacturer's instructions.

PowerEase 500 (Invitrogen, Cat. No.: EI8700) with pre-programmed settings was used as power supply throughout the experiment.

Proteins transferred onto the nitrocellulose membrane were detected with a monoclonal prion specific mouse antibody 3F4 (DakoCytomation Cat. No.: M7216) and Western Breeze Chemiluminescent Kit –Anti-Mouse (Invitrogen, Cat. No.: WB7104), in accordance with manufacturer's instructions. Detection sensitivity is in the order of 10 µg protein.

Results

The chemiluminescence stain of the Western blot with prion-specific antibody did not show prion protein in any of the samples drawn from the downstream side of the test filters (Lanes 1 – 6, Figure 3).

Lane number 7 represents 50 µL of 1% infective brain homogenate (as used on the ventilator side of the filter) and shows the typical chemiluminescence pattern of prion proteins.

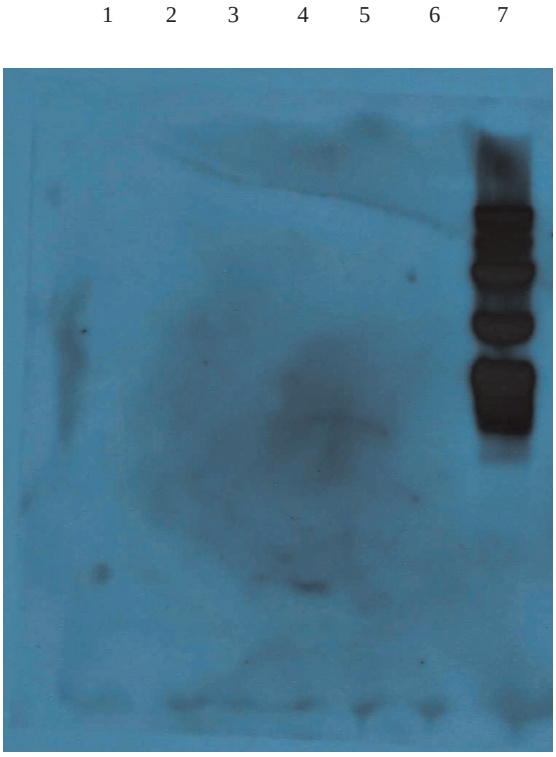

Figure 3

Discussion

TSE's remain a concern in the treatment of patients in surgery, anaesthesia and in blood transfusion. The surgical treatment of patients, who are known or suspected to have vCJD is performed using special precautions, especially when the procedure involves nervous or lymphatic tissue. In addition to this, many European countries have adopted a general policy of leucocyte depletion of blood donations to minimise vCJD risks through blood transfusion.

Inhalation of prion proteins has so far not been established as a transmission route for TSE's. However it has been proven that vCJD may be transmitted through blood transfusion in an animal model [10] . In December 2003 a statement was issued by the UK Health Secretary, John Reid, announcing that a patient who had received blood donated from a donor who subsequently died from vCJD, had also developed the disease and died. The statement concluded that *"…the possibility of this being transfusion-related cannot be discounted"* [11].

Llewelyn *et al* [12] detail the background behind this statement and interpret their findings as follows: *'Our findings raise the possibility that this infection was transfusion transmitted. Infection in the recipient could have been due to past dietary exposure to the BSE agent. However, the age of the patient was well beyond that of most vCJD cases, and the chance of observing a case of vCJD in a recipient in the absence of transfusion is about 1 in 15000 to 1 in 30000'.*

Another source of significant concentrations of prion proteins may be found in the tonsils or in nervous tissue [13], which may become dispersed in the course of specific surgical interventions. Tonsillectomy has been identified as a risk of transmission for vCJD [14] and recommendations have been made by the Association of Anaesthetists of Great Britain and Ireland to reduce this risk [15].

It is current practice in adult anaesthesia to re-use the breathing circuit between patients, when a filter is used at patient end. This may add to the uncertainties if the filter in use is not validated against specific patient contaminants from entering the breathing system.

In this report, simulated clinical experiments with the **Pall** *Ultipor* 25 Breathing System Filters have clearly shown that the filter retains infectious abnormal prion (PrP^{Sc}) proteins, according to this experimental protocol and the current detection limit of the method. The **Pall** *Ultipor* 25 has previously been shown to retain bacterial and viral contaminants [16] and, if used in accordance with the product instructions for use, enables the clinician to consider the re-use of the same breathing circuit between adult patients.

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16 Data available upon request

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Printed in England. PMED/1M/DBD/3.2004