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Inactivation Methods for Prions

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Abstract

Incidences of iatrogenic Creutzfeldt–Jakob disease (iCJD) are caused by transplantation of prion-contaminated hormones, cornea and dura mater as well as contact with prion-contaminated medical devices, such as stereotactic electrodes, used in neurosurgery. Because prions are highly resistant and difficult to inactivate, prion contamination is a severe risk when medical instruments are reused after surgical procedures involving suspicious and confirmed cases of patients with prion diseases. Therefore, when high-risk procedures such as cerebral surgery, craniotomy surgery, orthopaedic spinal surgery and ophthalmic surgery are performed for high-risk patients or individuals with prion diseases, it is necessary to appropriately treat the medical devices using scientifically proven prion inactivation methods. In this chapter, we introduce fundamental aspects of prion inactivation methods, looking specifically at the practical issues involved in their implementation.

Introduction

Prions (proteinaceous infectious particles) are the causative agents of prion diseases, which are also known as transmissible spongiform encephalopathies (TSEs) (Prusiner, 1998). The prion agent of each prion disease is named after the disease itself [e.g. Creutzfeldt–Jakob disease (CJD) agent in CJD of human, chronic wasting disease (CWD) agent in CWD of cervids, scrapie agent in scrapie of sheep and goats, and bovine spongiform encephalopathy (BSE) agent in BSE of cattle].

Among CJD cases, about 85% represent sporadic CJD (sCJD), which is caused by an unknown mechanism, while the remaining 15% is familial CJD (fCJD), which is inherited and caused by mutation of the human prion protein gene. Less than 1% of cases constitute iatrogenic CJD (iCJD), which mainly results from transmission via contaminated tissue grafts (Brown *et al*., 2006).

Concerns have been raised about incidences of iCJD caused by prion-contaminated materials derived from human growth hormone (hGH), pituitary gonadotropin, dura mater and corneal transplants (Aguzzi and Heppner, 2000). It is hoped that the use of

recombinant hGH and a separation process for individual dura matter grafts will eradicate transmission by the two pathways through hGH and dura matter, respectively. Nonetheless, the risk of transmission of prion agent via the use of contaminated medical devices remains a concern (Aguzzi and Heppner, 2000; Thomas *et al.*, 2013). Six cases have been linked to prion contaminated equipment (CDC, 2018a). Four of these cases were linked to the use of neurological instruments, while the other two cases were associated with stereotactic electroencephalography (EEG) depth electrodes. However, these cases occurred before implementation of sterilization processes. Since 1976, no iCJD cases via medical devices have been reported (CDC, 2018a). However, the theoretical risk of transmission via prion-contaminated medical devices remains a serious concern (Hamaguchi *et al.*, 2009).

Prion agents are highly resistant to various physical and chemical treatments. Normal sterilization procedures are ineffective for the inactivation of prions. This resistance to inactivation is at least in part due to the absence of nucleic acid in prions (Fichet *et al.*, 2004.). Prions are mainly composed of protein and lack a genome, which gives them a high level of resistance to a range of physical and chemical treatments. Conventional sterilization procedures, such as exposure to ultraviolet (UV) or γ-ray irradiation as well as autoclaving at 121°C for 20 minutes, fail to inactivate prions (Fichet *et al.*, 2004.; Sakudo *et al.*, 2011). Likewise, treatment with alcohols, such as 70% ethanol, has no effect on the prion agent. Fixation using formaldehyde slightly reduces the infectivity of prions (Zobeley *et al.*, 1999), but this is insufficient to eliminate the risk of transmission. For the treatment of tissue sections from prion-infected animals, formic acid is recommended (Rutala *et al.*, 2010). In order to inactivate prions by autoclaving, severe conditions (134°C for 18 minutes) are required (Rutala *et al.*, 2010). In conclusion, appropriate procedures to inactivate prions are necessary to prevent prion-related iatrogenic diseases.

In this chapter, fundamental knowledge of prions related to the iatrogenic risks of surgical procedures and patients as well as inactivation methods of prions are introduced.

High-risk procedures and patients

The risk of transmitting prion diseases through medical intervention is not fully understood. The CJD incident panel highlight important factors for prion transmission including; (i) infectivity of contaminated tissue, (ii) residual infectivity on medical devices after sterilization, (iii) tissue of recipient coming into contact with contaminated medical devices, (iv) susceptibility of recipient (CJD Incident Panel, 2005). On the basis of the above background, tissue infectivity in patients with prion diseases is an important concern. Generally, high infectivity tissues for prions are considered to be brain, spinal cord, and eyes, while low infectivity tissues include cerebrospinal fluid, kidney, liver, lung, lymph node, spleen, and placenta (CDC, 2018a). This categorization of risk is based on the tissue distribution of abnormal prion protein (PrP^{Sc}) in sCJD patients. However, PrP^{Sc}, which is the main component of prion agent, is also found in peripheral lymphoid organs (e.g. tonsils and appendix) of variant CJD (vCJD) patients (de Marco *et al.*, 2010). Thus, precautions must be taken during surgical procedures and in the handling of tissues and body fluids of cases with vCJD. In addition, two probable transmissions of vCJD infection by blood transfusion have been reported, causing concerns about a possible blood route for transmission. Unfortunately, current technology aimed at prion removal by filtering blood is insufficient to prevent infection (Mccutcheon *et al.*, 2015).

Box 7.1 High-risk patients or individuals*

1 Patients showing rapid progressive dementia suspected of having a prion disease
2 Individuals with a family history of CJD, GSS, FFI
3 Individuals with mutations in a prion protein gene related to inherited prion diseases
4 Recipients of human dura matter grafts
5 Patients displaying signs of a prion disease by electroencephalography or elevated biomarkers such as 14-3-3 protein
6 Recipients of hormones derived from human pituitary glands such as growth hormone, gonadotropin

*This list is mainly based on WHO infection control guidelines for transmissible spongiform encephalopathies (WHO, 2000).

Therefore, stringent procedures must be introduced to monitor the management of blood or blood products and the handling of surgical instruments to prevent the potential spread of infectious prion agents.

In addition, prions can be transmitted via prion-contaminated medical devices. As such, medical devices used in high-risk procedures that may have come into contact with infected tissues should be subjected to appropriate prion inactivating treatments. In particular, countermeasures against prion contamination should be in place when performing cranial nerve surgery craniotomy, orthopaedic spinal surgery or ophthalmic surgery on high-risk patients. Individuals with confirmed or suspected prion diseases are classified as the highest-risk patients. Patients and individuals conforming to the criteria outlined in Box 7.1 are considered 'high-risk' for prion transmission. 'High-risk' includes asymptomatic but potentially 'at-risk' of developing disease. In short, high-risk patients and individuals include (i) dura mater recipients, (ii) recipients of human cadaver-derived pituitary hormones, especially cadaver derived hGH, (iii) cornea transplant recipients, (iv) individuals who have undergone neurosurgery, and (v) family members with inherited prion diseases. In addition, the route of exposure must also be considered along with the other factors. For example, inoculation of the eye or central nervous system (CNS) with any infectious material poses a very serious risk. Thus, appropriate precautions must be taken to avoid these kinds of exposure.

Inactivation methods of prions

It is difficult to inactivate prions on medical devices if the instrument is dried. Thus, the medical device should be washed with detergents immediately after use before it is allowed to dry out. Initially, medical devices are usually washed with alkaline detergents or enzyme detergents, which reduces microbial and protein contamination by 4 to 6 log (Merritt *et al.*, 2000). It is known that prions strongly adhere to surfaces, particularly metals (Lipscomb *et al.*, 2007). Nonetheless, alkaline detergents and enzyme detergents are effective in reducing the infectivity of surface associated prions (Fichet *et al.*, 2004, 2007; Jackson *et al.*, 2005; Dickinson *et al.*, 2009; United Kingdom Department of Health, Engineering and Science

Advisory Committee into the Decontamination of Surgical Instruments Including Prion Removal, 2008). However, care should be taken because some enzymatic detergents enhance resistance of prions to autoclaving (Yan *et al.*, 2008; Rogez-Kreuz *et al.*, 2009).

It is well known that treatment with sodium dodecyl sulfate (SDS) and sodium hydroxide (NaOH) have an inactivating effect on prions, but these procedures are generally impractical. However, various alkaline detergents and enzyme detergents that are suitable for prion inactivation are already available on the market. These include Klenzyme (Steris), Hamo-100 (Steris), Septo-clean (Septo-Clean), CIP100 (Steris), Prionzyme-M (Genencor), and Rely-On (Dupont) (Rutala *et al.*, 2010; Walker *et al.*, 2008; Sutton *et al.*, 2006).

Bioassay by intracerebral inoculation of animals is indispensable for analysing prion inactivation. However, this analysis is not perfect. The amount of protein remaining on surgical instruments following an operation is thought to be about 8 to 91 μg per instrument (Murdoch *et al.*, 2006). Inactivation assays are generally conducted using about 50 to 375 mg of tissue material (Fernie *et al.*, 2007). Thus, these assays are not compatible to the real-life situation. In addition, prions derived from animals may have a different resistance to treatment than the human prions. Even using human prion agent, resistance may vary depending on the precise nature of the prion i.e. vCJD or sCJD or whether it is derived from Gerstmann–Sträussler–Scheinker syndrome (GSS) or fatal familial insomnia (FFI). The extent to which treatment conditions can vary without significantly impacting on the inactivation efficiency also needs to be determined. For example, it is conceivable that various parameters, such as concentration and temperature, during processing may affect the inactivation process. Furthermore, the results will depend on the properties of the surface material to which the prion is attached. Nonetheless, despite these limitations, various treatments have been proposed that will overcome minor effects and give consistent prion inactivation (Table 7.1).

Providing the medical instrument is heat resistant, an autoclave cycle at 134°C for 18 minutes or more is generally effective for prion inactivation (Fichet *et al.*, 2004). For heat-sensitive instruments, such as endoscopes, hydrogen peroxide gas plasma sterilization using Sterrad NX is recommended (Rutala *et al.*, 2010). Ethylene oxide inactivates prions to log 3 or less, which is insufficient for medical applications. Treatments with formaldehyde and glutaraldehyde fix proteins. As such, any protein contamination on the instrument will decrease the efficiency of prion inactivation (Taylor *et al.*, 1988; Brown *et al.*, 1990a). Therefore, these fixation treatments should be performed after cleaning the instrument (Brown *et al.*, 1990b).

Taken together, the following procedures for prion inactivation, primarily based on guidelines for control of prion diseases 2008 by the Research Committee on prion disease and slow virus infection (Mizusawa and Kuroiwa, 2008), are recommended for practical use. These procedures include (i) treatment with alkaline detergents (90–93°C) using washer disinfector + autoclaving (134°C, 8–10 minutes) (ii) washing with appropriate detergents + autoclaving (134°C, 18 minutes) (iv) washing with alkaline detergents (at a concentration and temperature according to instructions) + vaporized hydrogen peroxide gas plasma sterilization. Although these methods can be used to inactivate prions on apparatuses or instruments, there is no corresponding treatment to inactivate prions in foods. Prion-contaminated foods should be promptly incinerated at 1000°C or greater (Brown *et al.*, 2000).

By contrast, there is no consensus on how to treat medical devices after coming into

Table 7.1 Effective inactivating treatment achieving > 3 log reduction of prions

Inactivation treatment	References
Sodium hypochlorite (NaOCl) (20000 ppm, 20°C, 1 hour)	Fichet *et al.* (2004)
Sodium hydroxide (NaOH) (1N, room temperature, 1 hour)	Fichet *et al.* (2004), Rutala *et al.* (2010)
Autoclave under soaked condition in water (134°C, 18 min)*	Fichet *et al.* (2004)
Alkaline detergent (1.6%, 43°C, 15 min)	Fichet *et al.* (2004)
Phenolic disinfectant (5%, 20°C, 30 min)	Fichet *et al.* (2004)
Sodium dodecyl sulfate (SDS), (3%, 100°C, 3 min)	Tateichi *et al.* (1991)
Guanidine hydrochloride (7M, room temperature, 2 h)	Tateichi *et al.* (1991)
Guanidine thiocyanate (3M, room temperature, 2 h)	Tateichi *et al.* (1991)
Trichloroacetic acid (3M, room temperature, 2 h)	Tateichi *et al.* (1991)
Formic acid (60%, room temperature, 2 h)	Tateichi *et al.* (1991)
Phenol (50%, room temperature, 2 h)	Tateichi *et al.* (1991)
Enzymatic detergent (0.8%, 43°C, 5 min) + Vaporized hydrogen peroxide gas (1.5 mg/L, 25°C, 3 h)†	Fichet *et al.* (2004)
Vaporized hydrogen peroxide gas (1.5 mg/L, 25°C, 3 h)	Fichet *et al.* (2004)
High frequency gas plasma	Rutala *et al.* (2010)
Hydrogen peroxide (59%, 1 h, room temperature)‡	Rutala *et al.* (2010)
Hydrogen peroxide gas plasma (Sterrad NX)	Rutala *et al.* (2010)
Chlorine (> 1000ppm, 1 h, room temperature)	Rutala *et al.* (2010)
Copper (0.5 mmol/L) + hydrogen peroxide (100 mmol/L) (1 h, room temperature)	Rutala *et al.* (2010)
Sodium metaperiodate (0.01 M, 1 h, room temperature)	Rutala *et al.* (2010)
Quaternary ammonium compound (1 h, room temperature)	Rutala *et al.* (2010)
Peracetic acid (1500 ppm, room temperature, 20 min; 0.2%, room temperature, 1 h)‡	Rutala *et al.* (2010); Vadrot and Darbord (2006)

*Autoclaving with no soaking in water is insufficient for prion inactivation (Dry conditions cause difficulty in inactivation; Fichet *et al*., 2004; Vadrot and Darbord, 2006).
†Enzymatic detergent (0.8%, 43°C, 5 min) + autoclave (121°C, 20 min), only enzymatic detergent (0.8%, 43°C, 5 min), only peroxyacetic acid (0.25%, 55°C, 12 min), only vaporized hydrogen peroxide gas (1.5 mg/l, 25°C, 3 h), or enzymatic detergent (0.8%, 43°C, 5 min) + vaporized hydrogen peroxide gas (1.5 mg/l, 25°C, 3 h) is insufficient for prion inactivation (Fichet *et al*., 2004).
‡Some of the guidelines in the reports state that hydrogen peroxide and peracetic acid are ineffective or suboptional at reducing infectivity for decontamination (WHO, 1997, 2000).

contact with low-risk tissues. Because the risk of infection by medical instruments contaminated with low-risk tissues is considered to be extremely low, conventional sterilization disinfection treatments are thought to be sufficient (Rutala *et al*., 2001; Favero, 1998; Favero and Bond, 2001).

Future perspectives

In this chapter, fundamental knowledge concerning prion inactivation methods is introduced. However, it should be noted that the nature of the sample material, for example brain

homogenate, cell lysate, or purified materials, can influence the resistance of the prions. For example, proteins in the local environment around the prions can reduce the inactivation efficiency of these treatments.

In addition, different prion strains vary in their resistance to physical and chemical inactivation procedures. In human prion diseases, two major types of PrP^{Sc} have been biochemically characterized i.e. type1 and type2 PrP^{Sc} in CJD (Head *et al.*, 2012). However, sCJD can occur with six genotype/PrP^{Sc} combinations as follows: MM1, MM2, MV1, MV2, VV1, and VV2. Based on more recent transmission studies and PrP genotypes, five major strains of sCJD including MM1/MV1, MV2/VV2. MM2 cortical (MM2c), MM2 thalamic (MM2t), and VV1 have been proposed (Parch *et al.*, 2010, 2012Bishop *et al.*, 2010; Moda *et al.*, 2012). It should be noted that all the sporadic and acquired human prion diseases have been transmitted to laboratory animals, although this is not the case for all of the inherited forms (Asante *et al.*, 2013). In BSE, there are different prion strains categorized as classical prion strain of C-type BSE (C-BSE) as well as atypical bovine prion strains including H-type BSE (H-BSE) or L-type BSE (L-BSE or bovine amyloidotic spongiform encephalopathy [BASE]) (CDC, 2018b). In the case of CWD, at least two and possibly three distinct CWD strains (e.g. CWD1 and CWD2; Sgha CWDmd-f and Sgha CWDmd-s; CWD-WI and CWD-CSU; WST and CKY) have been identified (EFSA Panel on Biological Hazards *et al.*, 2017; Bian *et al.*, 2018).

Currently, a washer disinfector is often used at hospitals for cleaning medical instruments, which utilizes alkaline detergents and hot water. However, future development of alkaline detergents and enzyme detergents are anticipated to achieve a 5–6 log reduction of prion infectivity.

Recent studies suggest that misfolded proteins responsible for neurodegenerative diseases may act as prions or share prion-like mechanisms (Prusiner, 2013). Examples include amyloid β and t in Alzheimer's disease, which form neurofibrillary tangles (Clavaguera *et al.*, 2009; Kane *et al.*, 2000), α-synuclein in Parkinson's disease, which form Lewy bodies (Luk *et al.*, 2009; Brundin *et al.*, 2008; Kordowev *et al.*, 2008; Masuda-Suzukake *et al.*, 2014), huntingtin in Huntington's disease, which form nuclear inclusion bodies, Cu/Zn superoxide dismutase (SOD) in amyotrophic lateral sclerosis (ALS), which form mitochondrial aggregates (Münch *et al.*, 2011; Grad *et al.*, 2011; Gunther *et al.*, 2004), as well as TDP-43 (transactive response DNA-binding protein 43) in frontotemporal dementia, corticobasal degeneration, progressive supranuclear palsy, and ALS (Prusiner, 2013). Recent studies have shown that these protein misfolding proteins do not only aggregate in brain or spinal cord but may also be transmissible. These proteins are referred to as Aβ prion, τ prion, α-synuclein prion, TDP-43 prion etc. to distinguish from TSE prions including BSE, CJD, CWD and scrapie prions etc. The implementation of medical countermeasures, similar to those for conventional prion agents, should be considered for these new prion agents. However, further studies on these new prion agents with regard to their transmissibility is required before assessing potential inactivation procedures.

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Guidelines for 'Disinfection and sterilization in healthcare facilities, 2008' by the Centers for Disease Control and Prevention (CDC) (Rutala and Weber, 2008) and Guideline for disinfection and sterilization of prion-contaminated medical instruments by Society for Healthcare Epidemiology of America (Rutala *et al.*, 2010) as well as guidelines for control of prion diseases, 2008 by the Research Committee on Prion Disease and Slow Virus Infection, The Ministry of Health, Labour and Welfare of Japan (Mizusawa and Kuroiwa, 2008) have been published. For clinical issues relating to disinfection and sterilization, these guidelines are appropriate for consultation.

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