INFECTIVITY OF PRION PROTEIN BOUND TO STAINLESS STEEL WIRES: A MODEL FOR TESTING DECONTAMINATION PROCEDURES FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Zheng-xin Yan, PhD; Lothar Stitz, MD; Peter Heeg, MD; Eberhard Pfaff, PhD; Klaus Roth

ABSTRACT

OBJECTIVES: To establish an animal model to study transmissible spongiform encephalopathy using hamsters and steel wires contaminated with infectious brain materials as transfer vehicles, and, based on this model, to test decontamination procedures against the infectious prion proteins on the steel wires as a near real situation bioassay.

DESIGN: Infectious brain materials were given to healthy hamsters intracerebrally either as a suspension or as dried materials on the surface of steel wires. The animals were observed for 18 months. During this period, animals showing definitive clinical signs were euthanized. Decontamination studies were performed by reprocessing contaminated steel wires with different disinfection agents and procedures before implantation.

RESULTS: Pathological prion proteins were able to bind to the steel wires and caused disease after the contaminated wires were implanted in the brains of hamsters. When the contaminated wires were treated with different reprocessing procedures before implantation, infectivity was reduced, which was manifested directly by prolonged survival time of the test animals. These results show that this model can be used as a bioassay to validate reprocessing procedures for surgical instruments.

CONCLUSIONS: At the time of submission of this article, only the group of hamsters incubated with wires reprocessed with an alkaline detergent, followed by sterilization with a modified cycle in a hydrogen peroxide gas plasma sterilizer (4 injections), showed no clinical signs of disease and remained alive. Two animals from the group receiving sodium hydroxide followed by autoclaving (at 134°C for 18 minutes) died. Furthermore, the tested enzymatic cleaning agent seemed to have no positive effect (*Infect Control Hosp Epidemiol* 2004;25:280-283).

Studies have shown that pathological prion protein causes transmissible spongiform encephalopathies.[1-3] The infectious prion proteins are found in and outside of the central nervous system. They can be found in lymphatic as well as non-lymphatic tissues, such as the appendix, Peyer's patches, and the eyes.[4,5] Several studies have shown that prion proteins resist many conventional chemical and physical procedures used in hospitals for the cleaning, disinfection, or sterilization of surgical instruments and medical devices.[6,7] Currently, there is concern among clinicians and healthcare professionals, especially those involved in surgical procedures, about the possibility of transmission of prion disease by surgical procedures, which is merited by a recent report that the infectivity can be detected also in extraneural tissues such as spleen and tonsil.[8]

There is an obvious need to develop new chemical and physical decontamination procedures to prevent such transmission. Novel procedures have to be developed especially for thermolabile instruments because they cannot be sterilized in an autoclave. An alternative non-autoclaving method, such as the low-temperature hydrogen peroxide gas plasma sterilizer (Sterrad, Advanced Sterilization Products, Irvine, CA), has been assumed to be ineffective against infectious prion proteins.

We present an infectivity model using hamsters and steel wires contaminated with prion protein to demonstrate transmission and the possibility of decontamination of infectious prion proteins. The hamster-adapted pathological prion proteins tightly bind to the steel wires and cause disease after the contaminated wires are implanted or inserted for a short time in the brains of healthy hamsters. When the contaminated wires were treated with different cleaning, disinfection, or sterilization procedures before implantation, infectivity was reduced, which was manifested directly by prolonged survival time of the test animals. These results show that this model can be used as a bioassay to validate reprocessing procedures for surgical instruments. In the current study, the most effective procedures to date are sequential treatments with an alkaline detergent at a pH of 11 followed by either disinfection or sterilization. After 13 months, all hamsters in the group

Dr. Yan and Mr. Roth are from SMP GmbH Pruefen Validieren Forschen; Drs. Stitz and Pfaff are from the Institute of Immunology, Federal Research Centre for Virus Diseases of Animals; and Dr. Heeg is from the Department of Medical Microbiology and Hospital Infection Control, Eberhard-Karls University, Tuebingen, Germany.

Supported by Advanced Sterilization Products, Irvine, California, and in part by grants of the TSE-Programm, Baden-Württemberg, Germany.

Address reprint requests to Klaus Roth, SMP GmbH Pruefen Validieren Forschen, Paul-Ehrlich-Strasse 40, 72076 Tuebingen, Germany.

The authors thank Professor Dr. Martin Mielke and Dr. Michael Beekes (Robert-Koch-Institut, Berlin, Germany) for providing materials and scientific support.

receiving alkaline cleaning followed by sterilization with a modified Sterrad sterilizer cycle were still alive.

METHODS

Scrapie Material

A hamster brain that was infected with the 263K strain of scrapie was used. Ten percent brain homogenates were intracerebrally inoculated into healthy hamsters; brains were harvested after 80 days when the animals showed typical signs of scrapie and were kept frozen at -70°C and used for further experiments. Brain samples were examined by digestion with protease K and by Western blot test for protease-resistant infectious prion proteins.

Inocula Preparation

Ten percent brain homogenates were prepared fresh from thawed brain tissues. Tissues taken ranged from 0.04 to 0.1 g. Brain tissues were homogenized with Lysing Matrix D tube (Q-Biogene, Inc., Carlsbad, CA) in 1× phosphate-buffered saline (PBS) (Gibco, Invitrogen, Carlsbad, CA) in a FastPrep 120 Tissue Homogenizer (Q-Biogene, Inc.) on a speed scale of 6.0 for 40 seconds.

Western Blot Test

Brain tissues taken from healthy or diseased animals were prepared as 10% brain homogenates, and were examined for the presence of protease-resistant pathological prion proteins after digestion with protease K (50 µg/mL; DakoCytomation GmbH, Hamburg, Germany) followed by the Western blot test.

Protein separation and transfer were performed with the NuPAGE Novex Pre-Cast Gel System (Invitrogen). Prion protein bands were visualized by using the WesternBreeze Chemiluminescent Kit (Invitrogen), according to the manufacturer's instructions. For detecting prion protein, the monoclonal antibody 3F4 was used (concentration, 1:5,000; DakoCytomation).

Steel Wire Preparation

Soft stainless steel wire (1.4301; diameter, 0.25 mm; Forestadent, Pforzheim, Germany) was placed in the Sterrad 100S GMP sterilizer, a hydrogen peroxide gas plasma sterilizer. The steel wire was then aseptically cut into fragments of 30 mm in length.

The wires were contaminated by immersion into freshly prepared 10% brain homogenates from hamsters with scrapie in PBS for 16 hours at room temperature; spiked wires were then air dried for at least 1 hour before further treatment.

The dried steel wires were treated with different agents according to the manufacturer's instructions for each agent (Table). For cleaning, an enzymatic detergent containing protease was used in 2% and 100% solutions at 20°C. For alkaline cleaning, a potassium hydroxide–based detergent was used at 70°C for 10 minutes.

Cleaning was performed in a custom-made test washing machine, a closed two-container system. The lower container served as an open reservoir; a working volume of 10 L was pumped into the upper container and then drawn out freely back into the lower container through an opening on the bottom of the upper container. A circular rack with wires fixed in the edge was placed within the upper container and the lid of the upper container was tightly closed during the washing process.

Bathing of the wires was performed by submerging them into the test solutions. Disinfection was performed in a bath with either 0.55% ortho-phthalaldehyde solution (Cidex OPA, Advanced Sterilization Products) (30 minutes) or 0.35% peracetic acid (NU-Cidex, Johnson & Johnson Medical, Ltd., High Wycombe, United Kingdom) (5 minutes) at room temperature.

Sterilization, when used, was performed either by steam (134°C for 18 minutes) or in the Sterrad 100S sterilizer, with either the normal cycle with 2 injections or special software for 4 injections.

After each treatment, wires were rinsed once with 1× PBS followed by washing three times with double distilled water.

Wire Implantation

The processed wires were then implanted into hamster thalami with the assistance of a stereotaxic apparatus for small animals (coordinates: bregma, -2.0 mm; mediolateral, 2.0 mm; and dorsoventral, 6.0 mm). The same location was chosen for the intracerebral injection of scrapie brain homogenates into the hamster brain by a syringe and for the controls.

Animals were deeply anesthetized with 10% ketamine (SANOfi-CEVA GmbH, Düsseldorf, Germany) during the operation.

Animal Surveillance

Hamsters implanted with the wires were kept three to four per cage in a biological safety level-3 facility, with free access to standard diet and water. Test animals were initially observed twice a week, and daily after 60 days postimplantation. Hamsters with definite signs of scrapie (Dr. Michael Beekes, PhD, personal communication, February 6, 2003) were designated as terminally sick and euthanized.

Brain Tissue Examination

Whole brains were taken from euthanized hamsters. Half of the brain was cryopreserved at -70°C and later examined by protease digestion and Western blot test. The hemisphere with the implanted wire was fixed in 4% paraformaldehyde in PBS (pH, 7.2) for further histopathologic examination.

RESULTS

Infectivity of Pathological Prion Protein Bound to Steel Wire

After incubation of the steel wires in 10% infected brain homogenate, pathological prion proteins from ham-

TABLE
INFECTIVITY OF THE STEEL WIRES BEFORE AND AFTER TREATMENTS*

Inoculation	No. of Sick Hamsters/ Total No.	Incubation Time ± SD (d)
Control experiment		
Wire exposed to 10% normal brain homogenate	0/8	> 592
Wire exposed to 10% infected brain homogenate	5/5	81
1% infected brain homogenate, 0.02 mL	10/10	80
Wire exposed to 10% infected brain homogenate, but only transiently inserted for 5 minutes	9/9	101 ± 5
Wire decontamination experiment		
Group A		
Hydrogen peroxide gas plasma sterilizer (Sterrad),† standard cycle (without cleaning)	9/9	97 ± 4
134°C for 18 minutes autoclave (without cleaning)	1/10	> 174
1 M sodium hydroxide bath for 24 hours plus 134°C for 18 minutes autoclave	2/10	197 ± 199‡
Group B		
59% hydrogen peroxide bath, 10 minutes	3/10	> 264 ± 35
59% hydrogen peroxide bath, 20 minutes	4/10	> 308 ± 35
Group C		
Enzymatic detergent (1:50) wash	10/10	95 ± 0.4
Enzymatic detergent (1:50) wash plus 0.55% ortho-phthalaldehyde solution	10/10	107 ± 4
Enzymatic detergent (1:50) wash plus 134°C for 18 minutes autoclave	10/10	145 ± 17
Enzymatic detergent (1:50) wash plus Sterrad, standard cycle	10/10	111 ± 12
Enzymatic detergent (1:1) bath, 24 hours	10/10	93 ± 1
Enzymatic detergent (1:1) bath, 30 minutes	10/10	94 ± 2
Enzymatic detergent (1:1) bath, 30 minutes, plus 0.55% ortho-phthalaldehyde solution	10/10	118 ± 9
Enzymatic detergent (1:1) bath, 30 minutes, plus Sterrad, 4 injections	7/8	190 ± 61
Group D		
0.35% peracetic acid bath, 5 minutes	10/10	95 ± 3
Group E		
Alkaline detergent (pH, 11) wash 70°C		
plus 0.55% ortho-phthalaldehyde solution	2/10	> 318 ± 88
plus 134°C for 18 minutes autoclave	2/9	> 263
plus Sterrad, 4 injections	0/9	> 397

SD = standard deviation.
*Animals reported as sick tested positive for prions by Western blot test (unpublished data).
†Advanced Sterilization Products, Irvine, CA.
‡One animal died on day 151 and another on day 432; all other animals in this group were still clinically unsuspicious after 579 days.
Note. Enzymatic detergent is mainly composed of subtilisins (1% to 5%).

ster brains infected with scrapie were found to be able to bind to the surface of the wires, rendering them infective because hamsters with implanted contaminated wires became sick and moribund at the same time (80 to 81 days) as did hamsters infected intracerebrally with a 0.02-mL suspension of 1% brain homogenate (Table). Contaminated steel wires also caused disease after insertion into the brain as brief as 5 minutes (Table).

Effects of Different Reprocessing Methods on Infectivity of Contaminated Wires

Results showed that pathological prion protein bound to a steel surface is resistant to most of the conventional reprocessing procedures, including enzymatic, fixative, acidic treatments, and autoclaving. However, greater reductions of infectivity were achieved when strong oxidizing and alkaline agents were used; the latter was shown to be the most effective treatment. The contact time between the wire and the brain had a minor influence on the survival time of the hamsters (101 ± 5 days) (Table).

Procedures selected for group A provided an overview of the effect of different sterilization procedures. The hamsters treated with wires prepared in a 1 M sodium hydroxide bath for 24 hours followed by steam sterilization at 134°C for 18 minutes were selected as a reference group, as this procedure is recommended by several official bodies.[9]

Group B provided information about the effect of highly concentrated hydrogen peroxide. Submerging the wires in hydrogen peroxide prolonged the survival time of the hamsters significantly, and the longer contact time of the hydrogen peroxide also had a positive effect.

Group C provided information about enzymatic cleaning at a low temperature to develop a process for thermolabile devices. There was no significant difference in the survival time between the use of the 2% enzymatic detergent in the washing machine and the use in pure concentration in a beaker and different contact times (10 minutes, 30 minutes, and 24 hours). Following disinfection or sterilization by steam or Sterrad sterilizer increased the survival time. The best results in group C were delivered by enzymatic cleaning followed by a modified Sterrad sterilizer cycle. The survival time in comparison with the positive control was twice as long, and one hamster is still alive.

Group D was using a disinfection agent based on peracetic acid, which showed only little effect.

In group E, an alkaline detergent was used due to the experience we had from group A with the sodium hydroxide bath. The alkaline cleaning in the machine for 10 minutes at 70°C followed by chemical disinfection with 0.55% ortho-phthalaldehyde solution or with steam (134°C for 18 minutes), or the modified Sterrad sterilizer cycles delivered the best results in the study. The animals of group E were incubated much later than the animals of the other groups and most of them are still alive. The modified Sterrad sterilizer cycle showed an efficacy as high as that in group C.

DISCUSSION

With the development of sensitive postmortem detection methods, pathological prion proteins are being found more frequently in different body tissues.[4] Because pathological prion proteins are highly resistant to most of the routine hospital sterilization procedures, reusable surgical instruments exposed to tissues with high prion protein content need to be regarded as a potential source of transmission.[5,8] Possible contamination can be excluded only after diagnostic methods have been made available that allow the identification of patients with transmissible spongiform encephalopathy. Novel and nondestructive reprocessing methods are therefore urgently needed to prevent the risk of transmission, especially in the fields of surgery and endoscopy.

In this study, we found that steel wires exposed to brain homogenates infected with pathological prion proteins were highly infectious after implantation into hamsters. In general, the duration of the contaminated steel wire in the brain has a minor influence on the survival time. We regard this procedure, originally described by Zobeley et al.[10] and Flechsig et al.,[11] as a valid model for iatrogenic transmission and decontamination studies. With this model, wire made of materials other than steel can be studied as well.

We tested cleaning and disinfection agents as well as sterilization procedures currently being used in hospitals for their effects on the infectivity of contaminated wires. Using this method for the evaluation of the efficacy of decontamination, reflected by the mean survival time of the test animals, we can clearly demonstrate a direct correlation between successful reprocessing and survival time.

In this study, successful reprocessing was defined as a total survival time of 18 months after implantation of the reprocessed wires. During this study, titration of the infectious brain homogenate was performed by comparing direct intracerebral injection of the brain homogenates with implantation of the dried steel wires that were first incubated in the brain homogenate of the same concentration used for injection. The results of these titration groups may provide a basis for calculation of the log reduction of infectivity and for verification about the sensitivity of the wire model.[10,11]

At the time of submission of this article, only the group of hamsters incubated with wires reprocessed with an alkaline detergent, followed by sterilization with a modified Sterrad 100S sterilizer cycle (4 injections), showed no clinical signs and the hamsters were still alive. Even 2 animals from the group treated with sodium hydroxide followed by autoclaving (134°C for 18 minutes) died of the prion disease. Furthermore, the tested enzymatic cleaning agent seemed to have no positive effect.

The current study provides us with the following information: (1) Treatment of wires with an alkaline detergent at a pH of 11 shows significant reduction of infectivity, independent of the procedure (disinfection or sterilization) that follows. (2) Steam sterilization at 134°C for 18 minutes in combination with initial enzymatic cleaning does not result in the inactivation of the prion proteins. (3) Steam sterilization at 134°C for 18 minutes without initial enzymatic treatment results in much longer survival times of the animals. (4) Sterilization with the Sterrad system seems to have an effect similar to that of steam sterilization. (5) Highly concentrated (59%) hydrogen peroxide shows high efficacy in the inactivation of prion proteins.

REFERENCES

1. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982;216:136-144.
2. Prusiner SB. Prion diseases and the BSE crisis. *Science* 1997;278:245-251.
3. Aguzzi A, Weissmann C. Prion research: the next frontiers. *Nature* 1997;389:795-798.
4. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001;358:171-180.
5. Hilton DA, Ghani AC, Conyers L, et al. Accumulation of prion protein in tonsil and appendix: review of tissue samples. *BMJ* 2002;325:633-634.
6. Taylor DM, Fraser H, McConnell I, et al. Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Arch Virol* 1994;139:313-326.
7. Taylor DM. Inactivation of transmissible degenerative encephalopathy agents: a review. *Vet J* 2000;159:10-17.
8. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. *Lancet* 2001;358:208-209.
9. La Ministre de l'Emploi et de la Solidarité. *Circular Letter No. DGS/5C/DHOS/E2/2001/138 from March 14, 2001.* Paris: La Ministre de l'Emploi et de la Solidarité; 2001. Available at: www.hosmat.com/circulaires/textes01/138.htm. Accessed January 2003.
10. Zobeley E, Flechsig E, Cozzio A, Enari M, Weissmann C. Infectivity of scrapie prions bound to a stainless steel surface. *Mol Med* 1999;5:240-243.
11. Flechsig E, Hegyi I, Enari M, Schwarz P, Collinge J, Weissmann C. Transmission of scrapie by steel-surface-bound prions. *Mol Med* 2001;7:679-684.