Titanium dioxide photocatalytic inactivation of prions

Ioannis Paspaltsis,[1] Konstantia Kotta,[1] Roza Lagoudaki,[2] Nikolaos Grigoriadis,[2] Ioannis Poulios[3] and Theodoros Sklaviadis[1,4]

Correspondence
Theodoros Sklaviadis
sklaviad@auth.gr

[1]Prion Disease Research Group, Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

[2]B′ Neurological Clinic, AHEPA University Hospital, 54124 Thessaloniki, Greece

[3]Laboratory of Physical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

[4]Centre for Research and Technology-Hellas, Institute of Agrobiotechnology, 57001 Thessaloniki, Greece

Prions are postulated to be the infectious agents of a family of transmissible, fatal, neurodegenerative disorders affecting both humans and animals. The possibility of prion transmission constitutes a public-health risk that confronts regulatory authorities everywhere. The main problem in handling prions is the fact that they are extremely resistant to standard decontamination methods. Thus, the use of harsh and expensive practices to destroy prions is inevitable. The development of applicable and efficient prion-inactivation practices is still highly important for the prevention of accidental transmission. In the search for effective and environmentally friendly methods to eliminate organic compounds and bacteria, much attention has been focused on the so-called advanced oxidation processes. These are based on the formation of hydroxyl radicals, which are known to possess a high reductive potential. This study tested the potential of titanium dioxide, an inexpensive and completely inert reagent, to inactivate prions in a heterogeneous photocatalytic process. Initial *in vitro* experiments were followed by a bioassay with the scrapie strain 263K in Syrian hamsters. The results obtained from this study indicate that titanium dioxide photocatalytic treatment of scrapie-infected brain homogenates reduces infectivity titres significantly.

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INTRODUCTION

Prions are proteinaceous infectious particles postulated to be the causative agents of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs), including Creutzfeldt–Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats and chronic wasting disease in wild ruminants (Prusiner, 1998). They are composed mainly, if not solely, of an abnormal isoform, designated PrP^{Sc}, of the normal host-encoded cellular PrP protein (PrP^{C}).

Prion transmission constitutes a public-health risk, especially for surgical patients, health-care workers and hospital laboratory personnel. In the acquired prion diseases, the infectious agent enters the body either through ingestion of contaminated food or during medical treatments with contaminated biological materials or surgical tools. It is noteworthy that all forms of the human TSEs are transmissible, including the sporadic and inherited ones (Dormont, 2002). Infectivity can also be harboured in tissues of humans or animals that are either subclinically infected or in the preclinical stage of the disease. Such individuals, although carriers of the infectious agent (Hill & Collinge, 2003), display no symptoms.

Prions are unusually resistant to the conventional physical and chemical methods of decontamination commonly used to inactivate other infectious agents. Standard methods for the inactivation of TSE infectious agents used in everyday practice include treatment with bleach containing 20 g active chlorine l^{-1}, exposure to formic acid, and boiling with 1 % SDS or 1 M sodium hydroxide (Taylor, 1999). Unfortunately, in many cases these methods have proven to be inefficient (Rutala & Weber, 2001).

Advanced oxidation processes (AOPs) comprise a group of alternative chemical treatments used for the decontamination of water and air polluted with organic compounds. The different AOPs [e.g. titanium dioxide (TiO_2)/UVA photocatalysis, Fe^{2+}/H_2O_2 (the Fenton system), O_3/UVB] share the same chemical feature, which is the generation of free hydroxyl radicals, $^{\cdot}OH$ (Agustina *et al.*, 2006). $^{\cdot}OH$ radicals

are strong oxidizing agents that attack organic compounds non-selectively, leading ultimately to their mineralization. TiO_2 is a non-toxic compound used widely as a pigment in food and cosmetics. It is inexpensive, non-corrosive and displays catalytic activity upon exposure to natural or artificial light (UVA). When illuminated, the TiO_2 particles act as semiconductors, generating $^{\cdot}OH$ radicals and other reactive oxygen species by electrochemical reactions conducted at the surface of the photocatalyst (Mills & Le Hunte, 1997; Agustina *et al.*, 2006).

Here, we report results concerning the heterogeneous photocatalytic degradation of PrP and PrP^{Sc}, as well as the inactivation of an experimental scrapie infectious agent under artificial UVA illumination using TiO_2 as a catalyst. One of the potential objectives of the study was the development of a scaled-up method for the elimination of TSE pathogens before their environmental release from facilities that produce large amounts of possibly contaminated waste, such as hospitals, laboratories, abattoirs or even farms. The method outlined could also be adapted for decontamination of surgical instruments and smaller medical devices. The present work does not describe an inactivation protocol for any specific application, but should be seen as a first approach, demonstrating the effectiveness of TiO_2-based photocatalytic treatment in the field of prion inactivation.

METHODS

Substrates for TiO_2 digestion treatments. BSA was obtained commercially (A-3059; Sigma). Recombinant proteins MBP–rhuPrP (maltose-binding protein fused to human PrP aa 23–230), rhuPrP (human PrP aa 23–230) (Sachsamanoglou *et al.*, 2004) and bovine and ovine PrP were expressed in *Escherichia coli* cells. All of the recombinant proteins were used at a final concentration of 0·15 g l^{-1} in the photocatalytic treatments. PrP^{Sc}-infected brain homogenates (CJD, BSE, sheep scrapie, hamster scrapie, mouse scrapie and mouse BSE) were also treated photocatalytically with TiO_2. Infectious material was used either as crude brain homogenate or after PrP^{Sc} biochemical enrichment, as described below.

Photocatalytic experiments. TiO_2 P-25 (cat. no. 23.8595.0000.26; Degussa) was used for all photocatalytic experiments. In the P-25 formulation, the TiO_2 particles are non-porous; their anatase : rutile ratio is 3·6 : 1 and their mean surface area is 50 m^2 g^{-1}. Experiments were performed in disposable 1·5 ml plastic tubes. The reaction mixture in each tube was maintained in suspension by stirring. Irradiation was carried out by using two parallel 8 W black light/blue fluorescent tubes, mounted in standard 8 W fluorescent tube holders (TLD 8W/08; Philips). Light intensity in the region of 340–400 nm was measured by using a photometer/radiometer (PMA 2100; Solar Light). The initial light intensity used in the photocatalytic experiments was evaluated to be 6·1 mW cm^{-2}.

In all photocatalytic treatments except those with BSA and recombinant proteins, a protease inhibitor cocktail (P2714; Sigma) was included to ensure that no protein degradation by endogenous proteases occurred. The final volume of each reaction was 50 µl, containing TiO_2 diluted from an 8 g l^{-1} stock solution in distilled water, the indicated concentrations of H_2O_2 from a freshly prepared 3 % solution and the appropriate amounts of the organic load and distilled water. Samples were exposed to UVA light for 2 h (recombinant proteins, BSA) or 12 h (brain homogenates and enriched PrP^{Sc} preparations).

BSA and the recombinant prion proteins were illuminated in the presence of TiO_2 (2 g l^{-1}) and H_2O_2 (0·5 g l^{-1}). After exposure to UVA light, aliquots of each sample containing the equivalent of 3 µg of each protein were electrophoresed through polyacrylamide gels and stained with Coomassie brilliant blue.

Brain homogenates (10 %, w/v) were diluted 10-fold in 50 µl of a suspension containing 4 g TiO_2 l^{-1} and 4 g H_2O_2 l^{-1}. Control homogenates without the reactants were run in parallel. After UVA exposure, the remaining proteins, if any, were precipitated with 10 vols methanol overnight at −80 °C. Precipitated proteins from each 0·5 mg brain equivalent starting sample were separated on 12 % polyacrylamide gel and stained with silver as described previously (Polymenidou *et al.*, 2002).

Enriched PrP^{Sc} preparations were treated similarly with 4 g TiO_2 l^{-1} and 4 g H_2O_2 l^{-1} for 12 h and proteins were precipitated with 10 vols methanol overnight at −80 °C. Pellets were resuspended in 100 µl 2·5 × O'Farrell sample buffer, containing 125 mM Tris/HCl (pH 6·8), 5 % (w/v) SDS, 25 % (v/v) glycerol and 7·5 % (v/v) 2-mercaptoethanol (O'Farrell, 1975). Aliquots of 30 µl of the suspension, corresponding to 3 mg brain equivalent, were used for PrP^{Sc} detection on Western blots.

PrP^{Sc} enrichment. PrP^{Sc} was isolated quantitatively as described previously (Polymenidou *et al.*, 2002). Sheep, hamster and mouse scrapie samples, as well as BSE samples, were treated with proteinase K (P6556; Sigma) at a concentration of 30 µg ml^{-1}, whilst the sporadic CJD samples were treated with 50 µg ml^{-1}. Proteinase K digestion was performed for 1 h at 37 °C in a thermomixer with shaking at 500 r.p.m. The PrP^{Sc}-enriched material was treated photocatalytically as described above and afterwards diluted to 100 µl with 5 × O'Farrell sample buffer. The equivalent of 3 mg brain was used for PrP^{Sc} detection on immunoblots.

Electrophoresis and immunoblotting. SDS-PAGE and immunodetection of PrP^{Sc} were performed as described previously (Polymenidou *et al.*, 2002; Sachsamanoglou *et al.*, 2004). Blots were probed with the monoclonal anti-PrP antibody 6H4 (a generous gift from Prionics). Proteins were visualized with a CDP-Phototope Star chemiluminescence kit (New England Biolabs) following the manufacturer's instructions.

Bioassays. Brain from hamsters in the final state of disease after inoculation with scrapie strain 263K (Kimberlin & Walker, 1977) and normal hamster brain were kindly provided by Dr Ruth Gabizon (Hadassah University, Jerusalem, Israel). Homogenates (10 %, w/v) were prepared from both scrapie and control hamster brain in cold PBS containing 10 mM Tris/HCl (pH 7·4) and 300 mM sucrose using a Polytron apparatus (Kinematica). After centrifugation at 1000 ***g*** for 5 min at 4 °C, the supernatant was aliquotted and stored at −80 °C for future use.

For preparation of treated inocula, brain homogenate was diluted fourfold prior to photocatalysis. After the photocatalytic treatment, the mixture was diluted a further 2·5 times with sterile PBS containing 1 % BSA. Aliquots of 50 µl of this material were used for each inoculation.

Forty male golden Syrian hamsters, 30–40 days old, were obtained from Charles River Laboratories. They were handled according to the regulations of the local ethics committee (reference number 13/985) in a Biosafety Level 3 containment facility.

Fifteen animals were each injected intracerebrally with 50 µl treated material, which initially contained the infectivity of 1 % (w/v) brain homogenate.

Ten matched control hamsters were also injected intracerebrally with 50 µl scrapie-infected hamster brain homogenate [1 % (w/v) homogenate prepared from a 10-fold dilution of a 10 % (w/v) homogenate with PBS plus 1 % (w/v) BSA]. Fifteen negative-control animals were injected with 1 % (w/v) normal hamster brain homogenate.

Clinical evaluation. Two observers, blind to the identity of the inoculated homogenate, performed the clinical evaluation. After day 50 post-inoculation, hamsters were examined daily for clinical symptoms characteristic of scrapie, as described previously (Prusiner *et al.*, 1984). The onset date of the disease was considered to be the day when at least two typical symptoms were observed. Animals were sacrificed when they were terminally ill or as otherwise stated.

Statistical analysis was carried out with a χ^2 test using PRISM 4.0 software (GraphPad).

RESULTS AND DISCUSSION

In vitro experiments

Initially, we tested the effect of TiO_2 photocatalysis on purified homogeneous proteins. This was monitored by using Coomassie brilliant blue staining of photocatalysis products following SDS-PAGE. Treatment with TiO_2/H_2O_2 resulted in the complete degradation of all of the proteins that were used as test substrates, i.e. BSA and recombinant PrPs from different species (Fig. 1a).

The necessity for illumination was seen in control experiments, where reaction mixtures containing the test substrates were treated in the dark in the presence of TiO_2 and H_2O_2. Without illumination, BSA and purified prion proteins remained unaffected in the presence of both TiO_2 and H_2O_2 (Fig. 1b, lanes 1–5).

The addition of a powerful oxidizing reagent such as H_2O_2 or potassium peroxydisulfate ($K_2S_2O_8$) to TiO_2 suspensions is a well-known procedure and in many cases leads to an increased rate of photooxidation (Poulios *et al.*, 2003; Agustina *et al.*, 2006), especially when the initial organic load is high. H_2O_2 acts as an electron scavenger, which reacts with electrons at the conduction band of the TiO_2 molecule to generate additional $^{\cdot}OH$ radicals (Parra *et al.*, 2001), thus potentiating the effect of TiO_2. However, protein oxidation promoted solely by H_2O_2, at the concentrations used, could not account for the observed levels of protein oxidation. Reaction mixtures containing purified recombinant proteins and H_2O_2 were illuminated as above. As shown in Fig. 1(b, lanes 6–10) proteins remained unaffected by this treatment.

Non-specific protein oxidation mediated by TiO_2 in the presence of UVA light was confirmed in experiments in which the complex protein content of infected brain homogenates from various species was present in the photocatalytic reaction. After treatment with TiO_2/H_2O_2, complete degradation of the proteins was achieved (Fig. 2a), as assessed by silver staining. In control reactions, the proteins appeared not to be degraded after similar exposure to UVA in the absence of the photocatalyst and H_2O_2. These

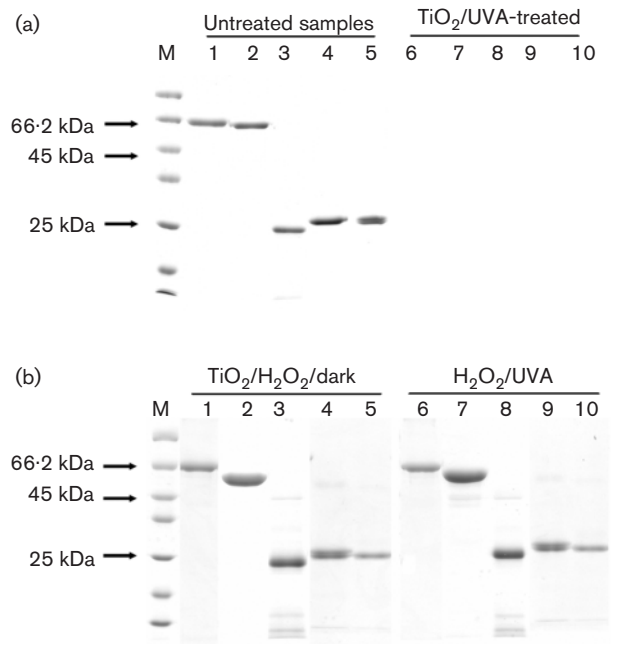

Fig. 1. Effect of TiO_2/H_2O_2/UVA on purified proteins. (a) Each marker protein (7·5 µg) in a final reaction volume of 50 µl was exposed to UVA light for 2 h with stirring. Lanes 1–5 contained untreated control reactions. In lanes 6–10, 2 g TiO_2 l^{-1} and 0·5 g H_2O_2 l^{-1} were added to the reaction. (b) Each marker protein (7·5 µg) in a final reaction volume of 50 µl was kept in the dark in the presence of 2 g TiO_2 l^{-1} and 0·5 g H_2O_2 l^{-1} (control lanes 1–5) or samples were illuminated as in (a) in the presence of 0·5 g l^{-1} H_2O_2 only (lanes 6–10). Proteins were analysed by SDS-PAGE (12 % gel) and stained with Coomassie brilliant blue. Each lane contained 3 µg protein. Lanes: 1 and 6, BSA; 2 and 7, MBP–rhuPrP; 3 and 8, rhuPrP; 4 and 9, ovine rPrP; 5 and 10, bovine rPrP. M, Molecular mass standards.

results suggested that photocatalytic treatment can be used not only for elimination of the prion protein, but for the entire protein load.

Quantitatively purified PrP^{Sc} preparations from clinical TSE cases and experimental animal TSE models were treated photocatalytically with TiO_2 in the presence of H_2O_2. This treatment resulted in the apparent degradation of the pathological prion protein. After 12 h illumination, PrP^{Sc} was no longer detectable on Western blots (Fig. 2b) developed with high-sensitivity enhanced chemiluminescence reagent.

Bioassay

The effectiveness of the photocatalytic treatment was evaluated further by an *in vivo* study. TiO_2-treated and untreated brain homogenates from hamsters terminally ill with scrapie strain 263K were analysed on a Western blot before the initiation of the bioassays. As expected, PrP^{Sc}

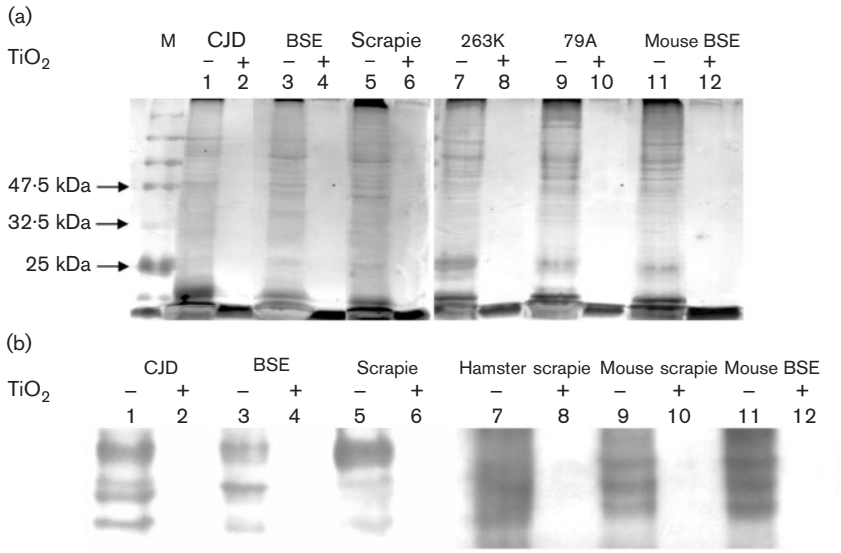

Fig. 2. Effect of TiO_2/UVA on brain homogenates and purified PrP^{Sc} from TSE-infected individuals of various species. Lanes: 1 and 2, sporadic CJD; 3 and 4, BSE; 5 and 6, sheep scrapie; 7 and 8, hamster scrapie strain 263K; 9 and 10, mouse scrapie strain 79A; 11 and 12, mouse BSE strain 301V. Samples were treated with TiO_2/H_2O_2/UVA as described in Methods (+) or left untreated (–). M, Molecular mass standards. (a) The equivalent of 0·5 mg crude brain homogenate was loaded per lane. The 12% polyacrylamide gel was stained with silver. (b) The equivalent of 3 mg brain homogenate from PrP^{Sc}-enriched preparations was loaded per lane. Proteins were transferred to PVDF membrane and the immunoblot was probed with monoclonal anti-PrP antibody 6H4.

could only be detected in the untreated sample. In the TiO_2/H_2O_2-treated inoculum, no PrP^{Sc} could be detected, even after extended film-exposure times (Fig. 3a).

Whilst improvements in the sensitivity of PrP Western blot analysis make it possible to detect quantities of the protein at the nanogram level, the technique is still not adequate for infectivity determination. Based on our 10-fold serial-dilution experiments (Fig. 3b), the PrP signal arising in the untreated inoculum (500 μg brain equivalent) disappeared completely after a 1000-fold dilution containing 5 μg brain tissue.

Inoculated hamsters were examined daily for symptoms of the disease after day 50 post-inoculation. The scrapie-inoculated animals entered the clinical disease phase $69{\cdot}4 \pm 4{\cdot}85$ days post-inoculation and the terminally ill animals were sacrificed. Twelve out of 15 hamsters inoculated with TiO_2/H_2O_2-treated material presented some clinical symptoms about 50 days later than the positive controls (mean incubation period, $119{\cdot}6 \pm 9{\cdot}73$ days). Interestingly, the clinical phase in those animals was significantly extended, with disease symptoms lasting for over 2 months compared with a 2 week disease course seen with the positive controls. Three hamsters injected with the TiO_2/H_2O_2-treated inoculum were sacrificed asymptomatically at 380 days post-inoculation. Fig. 4 shows the survival curves of all three hamster groups tested. Variations were statistically significant, as assessed by a χ^2 test ($P<0{\cdot}0001$).

All scrapie control animals developed the disease in the shortest time frame that has been reported previously for this particular model. Therefore, it was not feasible to determine precise measurements of the infectivity titre. However, the effectiveness of TiO_2 treatment in more concentrated samples was prominent and in favour of our proposition.

Brain tissues from all animals were examined for the presence of proteinase K-resistant PrP on Western blots in order to confirm the presence of the disease. Traces of PrP^{Sc} were detectable in the brains of the three asymptomatic animals, suggesting either a preclinical state of the disease or a subclinical infection due to the low infectivity titre of the inoculum (Hill & Collinge, 2003). Hamsters belonging to the negative-control group remained asymptomatic for the time span of the experiment.

Conclusions

TiO_2 photocatalytic treatment has been shown to be effective *in vitro* for the destruction of nucleic acid molecules (Ashikaga *et al.*, 2000) as well as proteins. Experiments performed in our laboratory showed degradation of nucleic acids (data not shown) and purified recombinant PrPs and PrP^{Sc}. Although there is no direct evidence for nucleic acid involvement in the pathogenesis of TSEs, there are indications of interactions between prion protein and nucleic acids (Murdoch *et al.*, 1990; Akowitz *et al.*, 1994; Cordeiro *et al.*, 2001; Adler *et al.*, 2003). The fact that TiO_2, in the presence of UVA light, non-specifically oxidizes and destroys organic substances including proteins and nucleic acids suggests that it would cause the indistinguishable inactivation of any kind of organic component that might also be involved in TSE pathogenesis. Additionally, TiO_2 has been found to be effective against both bacteria (Bekbölet & Araz, 1996; Maness *et al.*, 1999) and viruses (Sjogren & Sierka, 1994; Watts *et al.*, 1995). This property can be seen as a very important secondary benefit in parallel with its use in the field of prion inactivation.

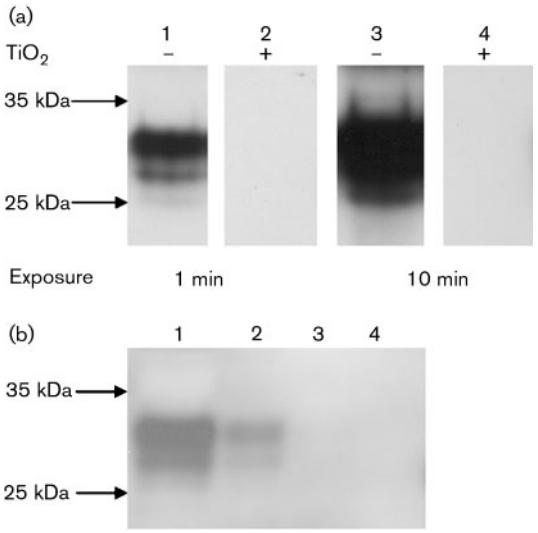

Fig. 3. Western blot of the inoculum used for the bioassay. (a) Hamster brain homogenate containing strain 263K PrP^{Sc} was treated photocatalytically with 4 g TiO_2 l^{-1} and 4 g H_2O_2 l^{-1} for 12 h in the presence of protease inhibitors before inoculation. Golden Syrian hamsters received residual infectivity contained in 50 µl 1 % brain homogenate. The film was exposed for 1 min (lanes 1 and 2) and overexposed for 10 min (lanes 3 and 4) to ensure that no PrP^{Sc} could be detected in the treated material. Lanes: 1 and 3, untreated control; 2 and 4, photocatalytically treated inoculum. (b) Proteins in the untreated inoculum (lane 1) and three 10-fold serial dilutions (lane 2, 1 : 10; lane 3, 1 : 100; lane 4, 1 : 1000) were probed with the monoclonal anti-PrP antibody 6H4. Immunoblotting was performed as described in Methods.

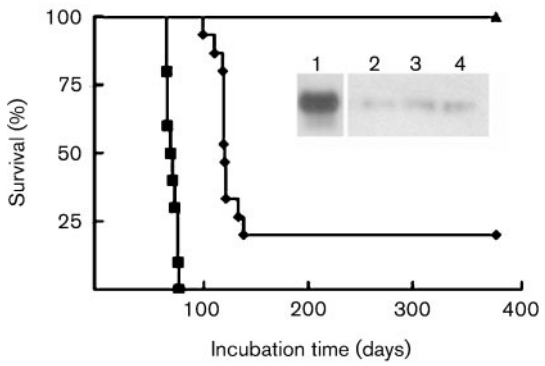

Fig. 4. Bioassay survival plot. The percentage of surviving hamsters was plotted against time post-inoculation to appearance of scrapie onset for all three groups tested. Compared with the positive-control group (■; $n=10$), hamsters inoculated with the titanium-treated material clearly showed a prolonged incubation time (◆; $n=15$), with three hamsters showing only one symptom even at 380 days after inoculation when they were sacrificed. As expected, hamsters in the negative-control group (▲; $n=15$), which were inoculated with normal hamster brain homogenate, remained healthy for the duration of the experiment (380 days). The inset blot shows the presence of proteinase K-resistant PrP in a hamster with early development of symptoms that received the TiO_2-treated inoculum (lane 1) compared with the three asymptomatic animals from the same group (lanes 2–4). Immunoblotting was performed after PrP^{Sc} enrichment as described in Methods.

Taking into account our experimental results for the photocatalytic inactivation of the TSE pathogen, it is clear that the photocatalytic treatment of liquid waste should be evaluated further as a powerful tool for disinfection of the TSE infectious agent. The method described could also be adapted for decontamination of surgical instruments. The non-specific activity of TiO_2 does not exclude inactivation of unknown organic substances that may be involved in TSE pathogenesis. Of course, protocols for specific applications will have to be optimized and validated. However, the use of a low-cost and biologically inactive catalyst and the possibility of activating it with solar light, combined with the simple equipment required for this method, can offer economically reasonable, user- and environmentally friendly solutions to the processing of prion-contaminated liquid waste.

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REFERENCES

Adler, V., Zeiler, B., Kryukov, V., Kascsak, R., Rubenstein, R. & Grossman, A. (2003). Small, highly structured RNAs participate in the conversion of human recombinant PrP^{Sen} to PrP^{Res} *in vitro*. *J Mol Biol* **332**, 47–57.

Agustina, T. E., Ang, H. M. & Vareek, V. K. (2006). A review of synergistic effect of photocatalysis and ozonation on wastewater treatment. *J Photochem Photobiol C: Photochem Rev* **6**, 264–273.

Akowitz, A., Sklaviadis, T. & Manuelidis, L. (1994). Endogenous viral complexes with long RNA cosediment with the agent of Creutzfeldt-Jakob disease. *Nucleic Acids Res* **22**, 1101–1107.

Ashikaga, T., Wada, M., Kobayashi, H., Mori, M., Katsumura, Y., Fukui, H., Kato, S., Yamaguchi, M. & Takamatsu, T. (2000). Effect of the photocatalytic activity of TiO_2 on plasmid DNA. *Mutat Res* **466**, 1–7.

Bekbölet, M. & Araz, C. V. (1996). Inactivation of *Escherichia coli* by photocatalytic oxidation. *Chemosphere* **32**, 959–965.

Cordeiro, Y., Machado, F., Juliano, L., Juliano, M. A., Brentani, R. R., Foguel, D. & Silva, J. L. (2001). DNA converts cellular prion protein

into the β-sheet conformation and inhibits prion peptide aggregation. *J Biol Chem* **276**, 49400–49409.

Dormont, D. (2002). Prion diseases: pathogenesis and public health concerns. *FEBS Lett* **529**, 17–21.

Hill, A. F. & Collinge, J. (2003). Subclinical prion infection in humans and animals. *Br Med Bull* **66**, 161–170.

Kimberlin, R. H. & Walker, C. A. (1977). Characteristics of a short incubation model of scrapie in the golden hamster. *J Gen Virol* **34**, 295–304.

Maness, P.-C., Smolinski, S., Blake, D. M., Huang, Z., Wolfrum, E. J. & Jacoby, W. A. (1999). Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. *Appl Environ Microbiol* **65**, 4094–4098.

Mills, A. & Le Hunte, S. (1997). An overview of semiconductor photocatalysis. *J Photochem Photobiol A: Chem* **108**, 1–35.

Murdoch, G. H., Sklaviadis, T., Manuelidis, E. E. & Manuelidis, L. (1990). Potential retroviral RNAs in Creutzfeldt-Jakob disease. *J Virol* **64**, 1477–1486.

O'Farrell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* **250**, 4007–4021.

Parra, S., Malato, S., Blanco, J., Péringer, P. & Pulgarin, C. (2001). Concentrating versus non-concentrating reactors for solar photocatalytic degradation of *p*-nitrotoluene-*o*-sulfonic acid. *Water Sci Technol* **44**, 219–227.

Polymenidou, M., Verghese-Nikolakaki, S., Groschup, M., Chaplin, M. J., Stack, M. J., Plaitakis, A. & Sklaviadis, T. (2002). A short purification process for quantitative isolation of PrP^{Sc} from naturally occurring and experimental transmissible spongiform encephalopathies. *BMC Infect Dis* **2**, 23.

Poulios, I., Micropoulou, E., Panou, R. & Kostopoulou, E. (2003). Photooxidation of eosin Y in the presence of semiconducting oxides. *Appl Catal B: Environ* **41**, 345–355.

Prusiner, S. B. (1998). Prions. *Proc Natl Acad Sci U S A* **95**, 13363–13383.

Prusiner, S. B., McKinley, M. P., Bolton, D. C. & 7 other authors (1984). Prions: methods for assay, purification and characterization. *Methods Virol* **8**, 293–345.

Rutala, W. A. & Weber, D. J. (2001). Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. *Clin Infect Dis* **32**, 1348–1356.

Sachsamanoglou, M., Paspaltsis, I., Petrakis, S., Verghese-Nikolakaki, S., Panagiotidis, C. H., Voigtlander, T., Budka, H., Langeveld, J. P. M. & Sklaviadis, T. (2004). Antigenic profile of human recombinant PrP: generation and characterization of a versatile polyclonal antiserum. *J Neuroimmunol* **146**, 22–32.

Sjogren, J. C. & Sierka, R. A. (1994). Inactivation of phage MS2 by iron-aided titanium dioxide photocatalysis. *Appl Environ Microbiol* **60**, 344–347.

Taylor, D. M. (1999). Inactivation of prions by physical and chemical means. *J Hosp Infect* **43**, S69–S76.

Watts, R. J., Kong, S., Orr, M. P., Miller, G. C. & Henry, B. E. (1995). Photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent. *Water Res* **29**, 95–100.