Inactivation of Prions Using Electrical DC Discharges at Atmospheric Pressure and Ambient Temperature

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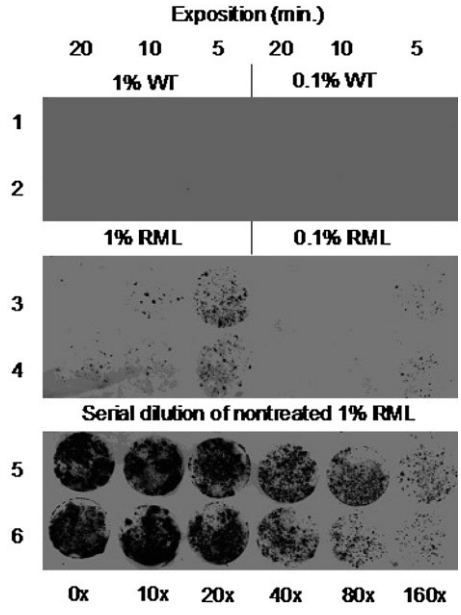
The ability of DC discharges to inactivate infectious prions was studied using prion cell infectivity assays and direct Western blot analysis of abnormal prion protein. The negative corona discharge of the point-to-plane type significantly decreased prion infectivity of mouse RML scrapie brain homogenates within 20 min of exposure. The positive discharge treated liquids display persistent cidal effect, which kills the cell culture and makes it impossible to assess the presence of prions by the cell infectivity assay. The point-to-point and 'cometary' discharges exhibit only a low ability to inactivate infectious prions. As a possible mechanism of prion inactivation, the formation of insoluble and non-infective high molecular weight complexes is suggested.



Introduction

The prion protein is a cellular membrane-bound glycosylphosphatidylinositol-anchored glycoprotein. The prevalent α-helical conformation, called PrP^{C}, is a common proteinaceous constituent of mammalian cells. However, if misfolded into the β-sheet conformation called PrP^{TSE}, it is capable of forming prions (infectious prion particles). Prions cause incurable and lethal neurodegenerative diseases (transmissible spongiform encephalopathies – TSE), referred to as scrapie, kuru, Creutzfeldt-Jakob disease, bovine spongiform encephalopathy, etc. (for details, see[1]). Preventing prion infection represents a serious problem both in veterinary and human medicine. There are various types (strains) of PrP^{TSE} with the same amino acid sequence but differing in phenotypic properties such as pathogenic potential or physicochemical characteristics.

In general, prions are resistant to proteases and common sterilisation procedures including UV and gamma irradiation. For inactivation of highly resistant prion strains, autoclaving at 134 °C for 60 min is recommended. However, in some cases, even this treatment is not considered reliable. Among chemical methods, treatment with formaldehyde, glutaraldehyde, hydrogen peroxide, iodine and others is ineffective; indeed, treatment with aldehydes has been shown to increase prion resistance. Good results were reported for treatment with 95% formic acid, chlorine, 1 N NaOH and/or sodium hypochlorite. However, due to the corrosivity of these agents,

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they are unsuitable for use with many materials. Immersion in 1 N NaOH or sodium hypochlorite for 1 h followed by autoclaving is the most recommended method and the most effective treatment for prion inactivation.[2] The incineration of contaminated materials, including carcasses, at a minimum temperature of 1 000 °C remains the only safe procedure for eliminating prions.

Non-thermal plasma, generated at ambient temperature, has displayed many effects on biological objects, which are mediated by the action of reactive and charged particles and UV light. The state-of-the-art of the technology in the generation of non-thermal plasma, its interactions with organic materials and its use in microbiology and other biological and medical disciplines have been thoroughly reviewed.[3–9] Three fundamental mechanisms causing inactivation of biological systems are thought to be (1) direct UV photon induced inactivation, (2) erosion of the micro-organisms through intrinsic photodesorption and (3) erosion through particle based etching.[10,11] To generate the plasma, the alternating current in the RF or microwave region has been used most frequently. The dielectric barrier discharge (DBD) seems to be a very promising tool, as well as the plasma needle (plasma jet), when working with plasma generated in a stream of gas.[12] The plasma generated by various methods seems to be a promising tool for decontamination or sterilisation of various materials, namely thermolabile ones. The bactericidal effect of plasma is well known and has been described by many authors (see the above-mentioned reviews), and its application in the inactivation of prions has been suggested many times, but often with no[13–17] or incomplete[18] experimental results. Recently, Baxter et al.[19] published study supporting the effective use of plasma to inactivate prions: a suspension of prions bound to stainless-steel spheres was treated with various reagents, and the efficiency of prion inactivation was determined by assessing the mortality of hamsters with intraperitoneally implanted spheres. In contrast to incomplete inactivation by autoclaving, complete inactivation of prions was observed after radio-frequency gas plasma treatment using the PE-200 Plasma System (Plasma Etch Inc.). Very recently, von Keudell et al.[11] reported the effectiveness of low pressure inductively coupled plasma (ICP) in inactivation of prions adherent on the surface of steel wires or silk sutures. Our present study complements the above studies by employing DC discharge generated plasma for inactivation of prions directly in water based suspension of infectious tissue. In our previous works,[20,21] we used direct high voltage to generate plasma by corona discharge in open air. The corona discharge appears around sharp coronising electrode with superposed high negative or positive DC voltage. Low electric current is passed to the second electrode realised by the grounded plane or a pinpoint. Depending on its polarity, voltage and geometry, various types of discharge are recognised: negative pulseless corona,[22] negative glow corona,[23] positive streamer corona and positive flashing corona[24] for the point-to-plane arrangement; the various types of discharge were previously described by Trinh and Jan[22] or Sigmond et al.[25] Recently, we described some properties of the point-to-point discharge.[26] We also described a new type of discharge designed as 'cometary',[27,28] which may be considered as the special case of the bipolar point-to-point corona. Corona discharges are known to generate UV radiation and a stream of charged particles, radicals and reactive molecules. It is likely that their composition depends on the superposed voltage, on the composition of the surrounding gas, on the composition of exposed liquids and other parameters. In air, atomic O and singlet $^1O_2$ oxygen, superoxide $O^{2\bullet-}$, hydroxyl OH^- and $OH^\bullet$, perhydroxyl $O_2H^\bullet$, ozone O_3, hydrogen peroxide H_2O_2, nitrogen oxides NO_x with corresponding acids and others were reported most frequently. However, the species generated in discharges used in this work were not yet analysed. Our positive and negative point-to-plane discharges are similar to the ones described and analysed in the work of Machala et al.: the emission spectroscopy identified the molecular (OH, NO, CN) and atomic (H, O, N) radicals and other reactive species. The discharges generated cold (300–550 K), strongly non-equilibrium plasma.[29] Despite the existing differences in experimental settings, it may be expected, that discharges used in our study have similar character. Among attempts to analyse the formation and composition of reactive particles in water, a thorough study was preliminary announced.[30]

In this communication, we attempted to apply various types of discharge to the inactivation of prions in water suspension. The effects of the treatments were evaluated by prion cell infectivity assays and by direct Western blot analysis of abnormal PrP^{TSE}.

Experimental Part

Plasma Generation

The low temperature plasma was generated using the previously described[31] simple apparatus of an open-air type. In all cases, a micrometer screw adjusted the position of the coronising electrode. The used source HT 2103 (Utes Brno, Czech Republic) made it possible to set a variable voltage up to 10 kV and current up to 0.5 mA. Electric characteristics of the discharges produced by our apparatus were verified and described previously.[32] The experimental arrangement is depicted schematically in Figure 1.

For the point-to-plane arrangement, the negative or positive discharges were generated on the point electrode represented by the tip of a syringe needle, stabilised by the serial resistance of 20 MΩ connected between the sources of DC high voltage and the plane electrode, realised by the surface of water suspension grounded with an immersed platinum wire.[20,21] Due to the serial resistance, the positive point-to-plane discharge burns in the regime of positive flashing corona for currents over 100 μA, as

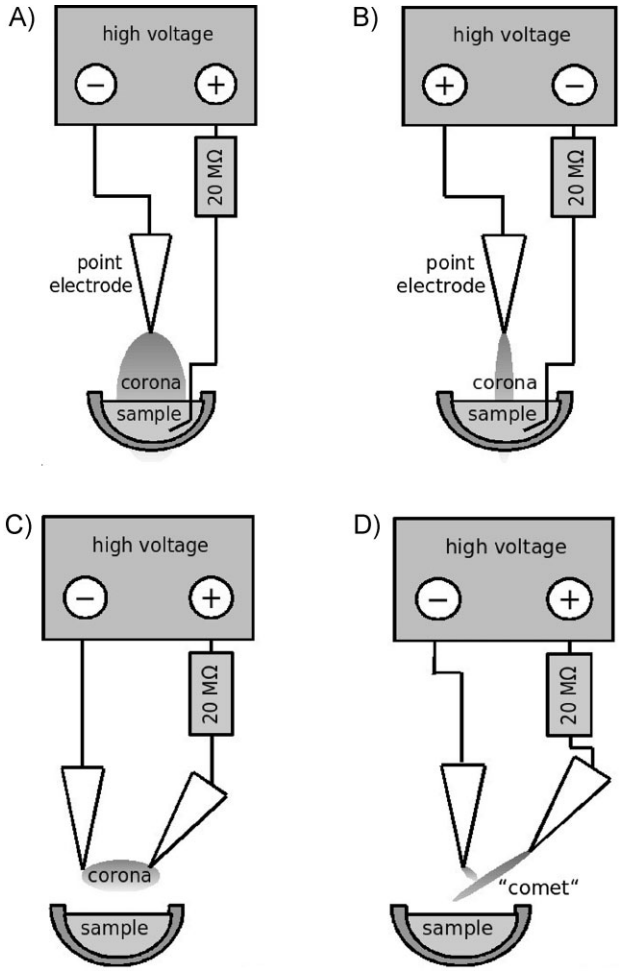

Figure 1. The configuration of used discharges. (A) Negative glow corona point-to-plane; (B) positive flashing corona point-to-plane; (C) ordinary point-to point discharge; (D) the 'cometary' point-to point discharge.

briefly described, e.g., in ref.[24] In the case of negative point-to-plane discharge, it burns in the regime of a corona glow discharge[23] for currents over 350 μA. For the point-to-point arrangement, the discharge burns between point electrodes arranged in parallel and tilted ca. 30° from vertical. The ordinary point-to-point discharge burns for the distance of electrodes tips approx. 4–6 mm apart and situated 4 mm over the sample.[26] The 'cometary' discharge is formed if the tip of the positive electrode was lifted 1–2 mm above the tip of negative one.[27,28]

Exposure

Diluted mouse brain homogenate (200 μl) was placed into the wells of a 96-well microtitration plate (Schoeller Pharma Praha, CR) and exposed to the following discharges:

1. negative glow corona of the point-to-plane type at the electrode distance of 3 mm, $U = 9.4$ kV and $I = 400$ μA, $P = 0.56$ W;
2. positive flashing corona of the point-to-plane type at the electrode distance of 3 mm, $U = 9.4$ kV and $I = 300$ μA, $P = 1.02$ W;
3. the point-to-point discharge at the electrode distance of 5 mm, $U = 10$ kV and $I = 200$ μA, $P = 1.2$ W;
4. the 'cometary' discharge at the electrode distance of 7 mm, $U = 8$ kV and $I = 300$ μA, $P = 0.6$ W.

Slight differences in the electrical parameters respect different regions of stability of particular discharges. Due to the geometry of discharges, the calculated power P is approximate, because only the positive corona forms a sharp streamer, which may be focused into the well. The plasma cloud formed by the point-to-point and 'cometary' discharges and in a lesser extent also the wide cloud of the negative corona, are diffuse and overhangs the edge of exposed well.

Exposition times of brain homogenates to corona discharges were 5, 10 and 20 min. After the exposure, the contents of the wells with treated 1% brain homogenates was diluted to 0.1% and, together with treated 0.1% homogenates, used for infection of prion-susceptible CAD5 cells or used undiluted for Western blot analysis, as described below.

Temperature Measurement

The temperature of exposed liquids was measured immediately after exposure using the contactless IR thermometer (Infrarot-Handthermometer Proscan 530, Dostmann electronics GmbH, Wertheim-Reicholzheim).

Prion Strain

The mouse adapted Rocky Mountain Laboratory strain of scrapie (RML5) was provided by Adriano Aguzzi (Institute of Neuropathology, University of Zurich) and propagated in CD1 mice (Charles River Laboratories, Wilmington, MA). Mice were inoculated intracerebrally with 30 μl of 1% RML5 brain homogenate and were killed when clinical signs became severe (147 ± 5 d post inoculation). The brains were removed and stored frozen at −80 °C.

Brain Homogenates

The frozen RML brains and the control uninfected brains (WT) of FVB mice were homogenised in 9 volumes of ice cold phosphate-buffered saline (PBS) using a Mini-Beadbeater-8 (Glen Mills, Inc., NJ). Homogenates were stored in aliquots at −80 °C. Thawed homogenates were further homogenised by passing through a 29-gauge needle and, before exposure to discharge, diluted with sterile PBS to concentrations of 1 and 0.1%. For the evaluation of the effectiveness of treatment, the untreated 1% RML homogenate was serially diluted 0–160 times with sterile PBS and analysed along with treated samples. As a negative control, the WT brain homogenate was used.

Cell Line

RML susceptible CAD5 cells derived from Cath.a-differentiated (CAD) cells[33] were provided by Charles Weissmann (Department

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of Infectology, Scripps, FL). Cells were grown in Opti-MEM medium (Invitrogen, Prague, CZ) supplemented with 10% bovine growth serum (Thermo Scientific HyClone, Logan, USA), 100 units · ml^{-1} of penicillin and 100 $\mu g \cdot ml^{-1}$ of streptomycin (PAA, Linz, Austria), as described previously.[34]

Cell Infectivity Assay

Confluent CAD5 cells were split at a 1:10 dilution into 24-well tissue culture plates (4×10^4 cells/well) and inoculated with brain homogenates at a 1:20 ratio. A 1% RML homogenate treated with discharge was also diluted to 0.1% prior to inoculation of cells. Cells were grown in the presence of inoculum for 24 h. Cells were washed twice with PBS and grown in the presence of fresh medium for another 24 h. Subsequently, cells were split at a 1:10 ratio every 3 d. After the third passage, prion infection of the cells was detected using cell blotting as described previously.[35] Briefly, cells were plated at a 1:10 dilution on round plastic coverslips and cultivated for 4 d. The coverslips were washed and cells (5×10^5 cells/coverslip) were blotted down onto the 0.45-μm nitrocellulose membrane. The membrane was air dried and treated with proteinase K (PK) to remove protease sensitive PrP^C. The blot was incubated in denaturing buffer (3 M guanidine isothiocyanate, 10^{-3} M Tris HCl pH 8.0), washed and incubated with the prion protein monoclonal antibody AH6 (Rosslin Institute, Scotland) followed by alkaline phosphatase conjugated secondary goat anti-mouse IgG antibody (Invitrogen, Prague, CZ). Antibodies were visualised with BCIP/NBT substrate (Millipore, Prague, CZ).

The cells infected with dilutions of untreated RML brain homogenate served as a positive control and for construction of the calibration curve. The cells inoculated with WT brain homogenate and uninoculated cells served as negative controls. Densitometry was performed using Gel quant software (DNR, Israel). All experiments were performed in duplicates and repeated independently three times ($n = 6$). Standard deviations were calculated.

Western Blot Analysis

WT and RML 1% brain homogenates were treated with discharge, adjusted to 150 μl with PBS and the pH was corrected to neutral using 1 M Tris HCl buffer. Homogenates were lysed by 0.5% Triton X-100 and 0.5% sodium deoxycholate. Aliquots of lysates were treated with 50 $\mu g \cdot ml^{-1}$ PK, which degrades all proteins except the resistant PrP^{TSE}. Subsequently, the PK activity was quenched by 2.5×10^{-3} M PMSF and samples were prepared for electrophoresis by boiling with Laemmli sample buffer. The electrophoresis was performed on 12.5% sodium dodecylsulphate (SDS)-polyacrylamide Tris-glycine gels. Separated proteins were electro-transferred to a 0.2-μm nitrocellulose membrane (Bio-Rad, Hercules, CA). Membranes were blocked with 5% milk in TBST and developed with the monoclonal antibody AH6 diluted 1:2 000 followed with alkaline phosphate conjugated secondary antibody and chromogen BCIP/NBT.

Dissolving of Corona Discharge-Denatured Proteins

To dissolve denatured proteins, aliquots of PK treated or untreated homogenate lysates were mixed with urea (4.7 M final concentration) in Laemmli sample buffer and incubated for 20 min prior boiling. Subsequently, the samples were analysed by Western blot and PrP^C/PrP^{TSE} was detected as described above.

To estimate the effect of negative corona discharge on the overall solubility of proteins in SDS, WT and RML brain homogenates were treated for 5, 10 and 20 min with negative corona. Samples were diluted 10× in 1% SDS, boiled 5 min and centrifuged (20 000 g, 30 min, RT) to sediment insoluble proteins. The protein content in the supernatants was estimated by the BCA assay (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's instructions.

Results

Effects of Positive Discharge

Exposure of brain homogenates to the positive flashing discharge led to rapid evaporation of the sample due to the ion wind, making it impossible to reach exposures longer than 15 min. The temperature of exposed liquid increased from initial 23.3 to 40.2, 41.5 and 42.1 °C after exposure for 5, 10 and 15 min, respectively. Simultaneously we observed a strong acidification of the well content, reaching pH 2 after 15 min. The addition of the homogenate to the indicator CAD5 cells led to cell death within 24–48 h. Therefore, the cell infectivity assay could not be used for the evaluation of prion infectivity in samples exposed to positive discharge. In order to explain this phenomenon, we also treated normal brain homogenates, PBS and distilled water with the same conditions. In order to exclude the possible effect of toxic pollutants eluted into water from the material (e.g., styrene from polystyrene), the same exposures were performed in wells made of polystyrene, polymethylmethacrylate, borosilicate glass and aluminium. Interestingly, in all cases, similar lethal effects of positive discharge-treated solutions on cells in culture were observed.

Effect of Other Discharges

Exposure of brain homogenates to point-to point and 'cometary' discharges yielded only poor inactivation of prions. In both cases, a significant reduction in CAD5 cell infection was observed only in RML homogenates diluted to 0.001% and exposed for 15 min. Only slight or negligible effects were found for higher concentrations of brain homogenates.

Effect of Negative Corona Discharge

In preliminary experiments, the effectiveness of negative corona discharge against prions was observed and further studied in the following representative set of experiments. The homogenates of RML brains in concentrations of 1 and 0.1% were exposed to negative corona for 5, 10 and 20 min each. As a control, the homogenates of WT brains were

identically diluted and exposed to the same conditions. The temperature of exposed liquid under this discharge elevated from 23.2 to 25.1, 27.2 and 29.8 °C after exposure for 5, 10 and 20 min, respectively. CAD5 cells were infected with both exposed and unexposed brain homogenates and prion infectivity was evaluated using cell blotting. The intensity of the staining of spots on cell blots represents the level of cell infection by prions. The higher level of staining of the spots correlates with a higher concentration of infectious prions. Infection of cells with WT brain homogenate produced no detectable staining (Figure 2A, lanes 1 and 2). A significant decrease in prion infectivity as a result of treatment with negative corona discharge was achieved in both 1 and 0.1% RML brain homogenates (Figure 2A, lanes 3 and 4). Infection with a serial dilution of untreated RML brain homogenate led to strong staining, which was clearly detectable even after a 160-fold sample dilution (Figure 2A, lanes 5 and 6). The effect was time-dependent and was more pronounced in more dilute RML homogenates, even though all homogenates were diluted to the same concentration before infection of the cells. Longer exposure times (10 and 20 min) of the 0.1% homogenate led to almost complete disappearance of the signal. Similar inhibitory effects of negative corona exposure on RML homogenates was observed in all three independent experiments. Densitometry analysis of the results from all three independent experiments is shown in Figure 2B, where the significant inactivation of prions is apparent with increasing exposure of brain homogenates to negative corona discharge.

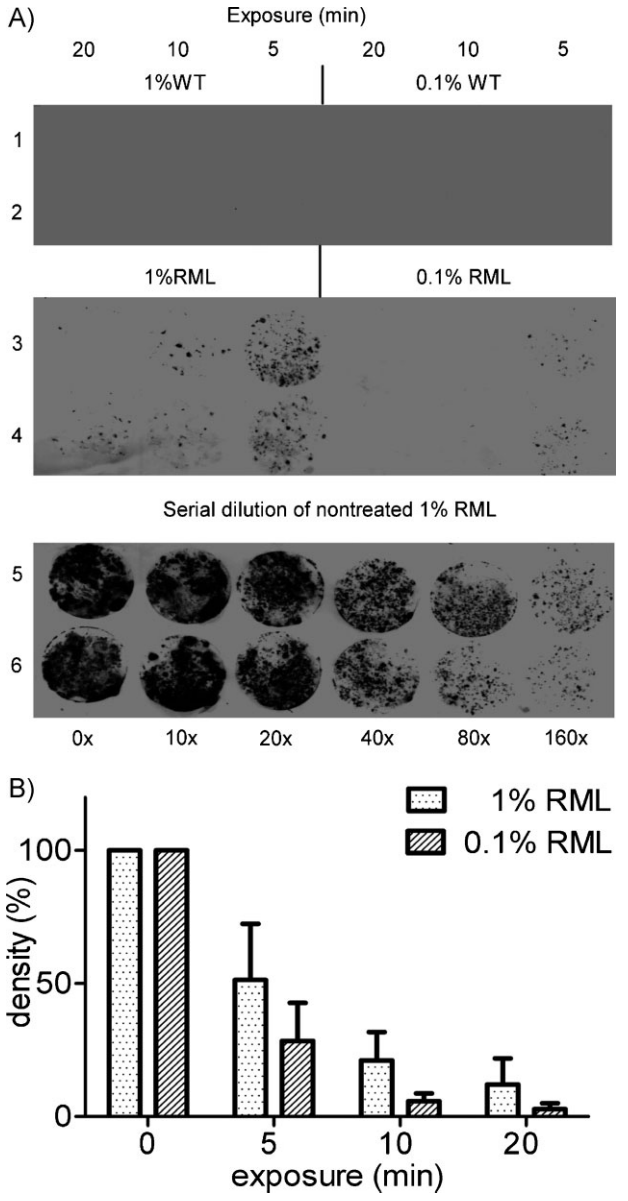

Figure 2. Cell infectivity assay of mouse brain homogenates exposed to negative corona discharge. (A) Normal (WT, lanes 1 and 2) and infective (RML, lanes 3 and 4) homogenates diluted to 1 and 0.1%, exposed for 5, 10 and 20 min. For comparison, lanes 5 and 6 show the serial dilution of non-exposed RML homogenate. (B) Densitometric evaluation of the intensities of cell blot spots after various exposure times.

Western Blot Analysis

The direct effect of treatment of brain homogenates on PrP^{C} and PrP^{TSE} was evaluated using Western blot. Digestion of normal brain homogenates with PK led to the disappearance of protease sensitive PrP^{C} (Figure 3, lane 16). In contrast, PK treatment of RML brain homogenates produced partially truncated forms of protease resistant PrP^{TSE} (Figure 3, lane 14). Exposure of normal homogenate (WT) to negative corona discharge led to the dose-dependent decrease of PrP^{C} (Figure 3, lanes 2, 4, 6), and no PrP^{C} was detected after treatment with PK (lanes 1, 3, 5). Similarly, the amount of PrP^{C} and PrP^{TSE} detected in treated 1% RML homogenates decreased over the indicated exposure times (Figure 3, lanes 8, 10, 12). Only trace amounts of PrP^{C} and PrP^{TSE} were detected after exposure to discharge for 20 min. However, PK treatment led to recovery of significant levels of PrP^{TSE} resistant bands after all exposure times. This effect could be explained by the production of insoluble protein complexes, which are excluded from entering the gel during electrophoresis. PK digestion may partly resolve these complexes, as documented by the higher intensity of the bands in PK-treated samples.

To confirm the effect of negative corona exposure on the production of SDS-insoluble protein complexes, we incubated treated RML homogenates with urea, as described in Experimental Part. The results of consecutive Western blots are shown in Figure 4A: The urea treatment led to recovery of significant amounts of PrP^{TSE}, as demonstrated by an increased intensity of bands in comparison with urea untreated samples (Figure 3, lanes 7–12). Formation

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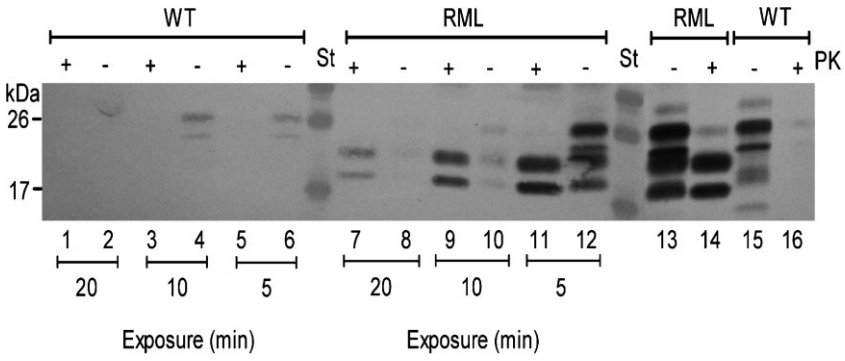

Figure 3. Detection of abnormal prion protein (PrP^{TSE}) in brain homogenates by Western blotting. In lanes 1–6 are normal (WT), in lanes 7–12 are RML-infected homogenates treated for 5, 10 and 20 min with negative corona discharge. Non-exposed RML and WT homogenates are in lanes 13–16. St denotes molecular weight standards, + and − denote PK digested and undigested samples, respectively.

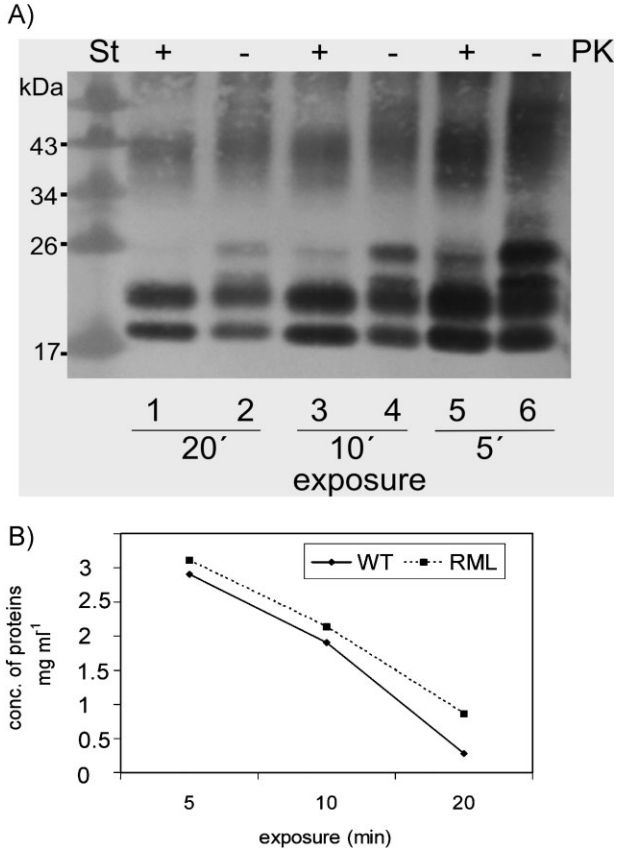

Figure 4. (A) Western blot of RML homogenates exposed to negative corona discharge for 5, 10 and 20 min, digested with PK and incubated with urea. St denotes molecular weight standards, + and − denote PK digested and undigested samples, respectively. (B) Estimation of SDS-soluble protein concentration in WT and RML homogenates after treatment with negative corona.

of SDS-insoluble complexes after exposure of samples to negative corona was verified by estimation of the protein content in the supernatants of negative corona-treated WT and RML homogenates after their boiling with SDS. The treatment led to a significant and time-dependent decrease of the protein content in the supernatants after 5, 10 and 20 min of exposure (Figure 4B).

Discussion

Cold plasma generated by electric discharges was shown to affect the composition and remove exposed proteins and amino acids from surfaces, as previously observed for ICP plasma discharge[17,36] and DBD plasma.[37] In our experiments with various arrangements of DC discharges presented here, we observed a differential influence of particular discharge types on the infectivity of prions in RML brain homogenates.

The positive streamer discharge appeared to be unsuitable for our study, not only because it caused rapid evaporation of liquid samples but especially due to the production of solutions that readily killed the indicator cells. The viability of cells is essential for the detection of prions, which was thus made impossible. The killing effect of solutions was not dependent on their protein content, as it was present also in treated PBS buffer or distilled water. Oehmigen et al.,[38] Ikawa et al.[39] and our preliminary publication[40] previously reported a plausible explanation of this phenomenon. The acidification of all water solutions and suspensions occurs due to the formation of HNO_3 and HNO_2, arising from nitrogen oxides formed in gas plasma produced by electric discharge. The presence of strong acids may contribute not only to prion inactivation, but also to the killing of cells in the acidic milieu. The last effect may be stronger in solutions treated by the positive streamer, but does not probably take place in solutions exposed to other discharges. We are currently studying this phenomenon in detail and the results will be presented in a separate report.

The point-to-point and 'cometary' discharges had only weak and limited effect on prion infectivity of exposed RML homogenates. This is probably caused by the lesser energy delivered to the sample due to the partial dissipation of the point-to-point and 'cometary' plasma out of the exposed well. Another reason may be different nature of plasma produced by these discharges, i.e., the presence of different reactive particles. This explanation however, seems to be less probable.

The negative corona discharge appeared to have significant effects on prions. The cell infectivity assay

suggested that the negative corona has the ability to reduce the titer of infective prion particles in treated samples by several orders of magnitude. This decrease depends on the experimental conditions, especially on the initial concentration of prions, the protein content of the samples and on the time of exposure. The observed inactivation was not caused by elevated temperature, which just marginally increased over the room temperature during the procedure. Prions are known to be quite thermo-resistant with temperature necessary for their inactivation exceeding substantially 100 °C. Similarly, due to known resistance of prions to UV irradiation it is unlikely that this component of generated plasma has significant role in their inactivation. We speculate that the effect of negative corona discharge on prions is probably mediated by generated reactive particles as was demonstrated for surface adsorbed prions.[11] However, in our settings the particles must penetrate into the water suspension. At present it is unknown which species of reactive particles play key role in inactivation of prions. Lower inactivation of prions in less diluted 1% brain homogenate could be probably attributed to the protective effect of higher level of brain proteins and lipids in the sample. Protective effect of tissue components, preventing effective sterilisation, is well known also for viruses and bacteria.

It should be noted that our cell infectivity assay is not able to precisely quantify prion content in the samples and serves as a tool to demonstrate relative differences between the infectivity of treated samples. In our hands, the cell infectivity assay can detect prions in up to 10^5 dilution of standard RML brain homogenate which translates approximately to the detection of 10^3 prion infectious doses. Despite this limitation, the results of the assay convincingly demonstrate the ability of the negative corona to impact the infectivity of RML prions in suspension at atmospheric pressure and ambient temperature. Importantly, the effect was confirmed by a decrease in the levels of abnormal PrP^{TSE} protein, which is widely recognised as the marker of prion infectivity. In addition, Western blot analysis of negative corona-treated brain homogenates also suggested the mechanism contributing to prion inactivation, which seems to be due to the formation of non-infective insoluble protein complexes. However, as stated above, the effect of negative corona discharge on prion particles is likely to be complex. Acidification of the suspension may contribute to destabilisation of prion protein conformation and make the protein vulnerable to the attack of reactive species present in the generated plasma. This may lead to covalent modification of the protein, its denaturation and formation of insoluble complexes either with itself or with other proteins present in the sample. Clearly, the above-mentioned remarks are rather speculative and more detailed experimentation is needed to elucidate the precise mechanism of prion inactivation by negative corona discharge. Our study does not exclude the effectiveness of positive streamer against prions. It is possible that its ability to inactivate prions may surpass the effect of negative corona discharge, as it did in its effect against bacteria.[41] However, to compare these two treatments, another system to detect prion infectivity must be employed. One option might be to inoculate groups of experimental mice and observe the attack rate and the length of incubation period. These experiments are very time consuming because of the long incubation period of prion diseases and due to ethical reasons should be avoided when possible. Another option would be to adapt the cell infectivity assay for the use with positive streamer-treated samples, which can be done by the use of cells resistant to the killing effect of positive streamer-treated samples or by the neutralisation of cell killing factors in the treated samples. Studies aimed on finding a solution of this experimental complication are under way.

Considering the possible hazards of prion infections, including the iatrogenic ones, the development of new reliable and rapid methods of inactivating infectious prion particles seems to be very advisable. Although our experiments were limited by a small volume of exposed liquids, the results offer a possibility of such a method, suitable for prion removal from thermolabile materials and liquids.

Conclusion

The negative corona discharge of the point-to-plane type significantly decreased the concentration of infectious prion particles in water suspension. The positive discharge caused a persistent killing of the cell culture, which made it impossible to assess the activity of prions by cell infectivity assay. The point-to-point and 'cometary' discharges exhibited only a low ability to inactivate infectious prions most likely due to lower energy delivery to the sample. The discharges cause negligible temperature increase in exposed samples, which cannot contribute to inactivation of prions. The inactivation is rather caused by action of reactive particles, but their nature remains uncertain. The acidification of samples together with the denaturation and/or formation of insoluble high molecular weight complexes of prion protein are suggested as the components of the complex mechanism of prion inactivation.

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