Cationic phosphorus-containing dendrimers reduce prion replication both in cell culture and in mice infected with scrapie

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Over the last 30 years, many drugs have been tested both in cell culture and in vivo for their ability to prevent the generation of prions and the development of transmissible spongiform encephalopathies. Among the compounds tested, dendrimers are defined by their branched and repeating molecular structure. The anti-prion activity of new cationic phosphorus-containing dendrimers (P-dendrimers) with tertiary amine end-groups was tested. These molecules had a strong anti-prion activity, decreasing both PrPSc and infectivity in scrapie-infected cells at non-cytotoxic doses. They can bind PrP and decrease the amount of pre-existing PrPSc from several prion strains, including the BSE strain. More importantly, when tested in a murine scrapie model, the dendrimers were able to decrease PrPSc accumulation in the spleen by more than 80%. These molecules have a high bio-availability and therefore exhibit relevant potential for prion therapeutics for at least post-exposure prophylaxis.

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases that include Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Scheinker syndrome and fatal familial insomnia in humans, scrapie in sheep and goats and bovine spongiform encephalopathy (BSE) (Collinge, 2001). They are characterized by the accumulation of the abnormal scrapie isoform of the prion protein (PrPSc) in the brain. PrPSc corresponds to a conformational variant of a normal protein, the cellular isoform of PrP (PrPC) (Prusiner, 1982). Both PrP isoforms share the same amino acid sequence, but, unlike PrPC, PrPSc is relatively resistant to digestion by proteases and is insoluble in non-denaturing detergents (Meyer et al., 1986). Concerns about CJD have been raised following the identification of more than 130 patients who have developed new variant CJD in Europe after exposure to the BSE agent (Collinge, 2001; Will et al., 1996). These patients develop progressive fatal neurological dysfunctions and death frequently occurs less than 1 year after the first symptoms appear. Currently, no effective therapy exists for prion diseases at the symptomatic phase for either humans or animals. However, several molecules have been studied in animal models for their capacity to delay the appearance of the disease when administered either at the moment of inoculation or during incubation (Aguzzi et al., 2001; Brown, 2002). Importantly, as animal models are expensive and time consuming, scrapie-infected neuroblastoma N2a cells (ScN2a) have been widely used for screening anti-prion agents as well as to better understand their mechanism of action (Béranger et al., 2001). Among the drugs tested, molecules like Congo red, amphotericin B, porphyryins, phthalocyanine, branched polyamines, suramin and quinacrine were reported to inhibit PrPSc formation significantly in these cells (Caughey et al., 1998; Gilch et al., 2001; Mangé et al., 2000; May et al., 2003; Priola & Caughey, 1994; Supattapone et al., 1999).

In the present work, we tested cationic phosphorus-containing dendrimers (P-dendrimers) for anti-prion

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activity. These new classes of branched-polyamines differ from the molecules described previously by Supattapone et al. (1999), principally by their protonated tertiary amine end-groups (Loup et al., 1999). They have a hydrophilic surface and a hydrophobic backbone which allows very efficient membrane penetration (Loup et al., 1999). Here we show that P-dendrimers were able to clear PrPSc rapidly in ScN2a cells with an IgG5 in the nM range. They can interact with PrP, and are effective against pre-existing PrPSc, as was observed when incubated with brain homogenates infected with different prion strains. Using a rapid in vivo screening model we show that these drugs are able to inhibit PrPSc accumulation in the spleen of mice intraperitoneally inoculated with scrapie. P-dendrimers represent, therefore, new molecules for TSE therapy, or at least for post-exposure prophylaxis, since no pre-clinical diagnosis is available to date.

METHODS

Reagents and antibodies. Proteinase K (PK) and Pefabloc were purchased from Roche Diagnostics. Opti-MEM, MEM, trypsin and genetin were from Life Technologies, and fetal calf serum from BioWhittaker. Secondary antibodies were from Jackson ImmunoResearch. All other reagents were from Sigma. Rabbit polyclonal antibody P45–66, raised against a synthetic peptide encompassing mouse PrP residues 45–66, was described previously (Lehmann & Harris, 1995). SAF60, 69 and 70 are three mAbs produced by the group of J. Grassi (CEA-Saclay, France) and recognize peptide epitope 142–160 of mouse PrP (Demart et al., 1999). A mixture consisting of equal volumes of ascites of these three antibodies was used to improve PrPSc detection (SAF mix). The mAb against gyceraldehyde-3-phosphate dehydrogenase (GAPDH) used in the study was purchased from Interchim.

P-dendrimers. P-dendrimers were synthesized by the Laboratoire de Chimie de Coordination du CNRS and were selected following the study by Supattapone et al. (1999) with the aim of obtaining new anti-prion agents that could be useful in vivo. P-dendrimers are characterized by the presence in their backbone of aminothiophosphates at each branching point which may enhance biocompatibility. The repetitive controlled divergent growth technique which forms the P-dendrimer backbone used these phosphorus atoms at each branching point. Phosphorus-containing dendrimers pd-G3, C26H514N119O40P4S42 (generation 3); pd-G4, C1296H2256N136O51P4S42 (generation 4) and pd-G5, C780H1500N759Cl192O186-P185S42 (generation 5) were synthesized and purified as described previously (Loup et al., 1999). They have protonated terminal tertiary amines (Fig. 1a). P-dendrimers were dissolved in sterile distilled water at a stock concentration of 10 mg ml−1 and filtered through a 0.22 μm Millipore filter before use. A plot of the effect of the pH of the medium on the charge carried by P-dendrimers was obtained by polyelectrolyte titration (Fig. 1b), which consisted of a stoichiometric reaction of a polycation with a polyanion. The titration end point was determined by means of a particle charge detection apparatus from Müttek, München, Germany.

We observed the same efficiency for P-dendrimers G4 and G5 and performed most experiments with both of them without seeing any variability in the results.

Incubation of brain homogenates with P-dendrimers. Brain homogenates of 263K, Chandler and 22L strains at 10% (w/v) were kindly provided by R. Carp (New York State Institute for Basic Research, USA) and BSE homogenates by T. Baron (AFSSA, Lyon, France). Eighty micrograms of brain homogenate in 40 μl PBS (2% w/v) was incubated with 200 μg P-dendrimers ml−1 or PBS for 2 h at 37°C with constant shaking. After incubation, samples were made up to 0.5% NP-40, 0.5% sodium deoxycholate, and were digested with PK as described for cell lysates (see below). The samples were then mixed with an equal volume of 2× SDS sample buffer and analysed by Western blotting for the presence of PrPSc.

Cell culture and detection of PrP. The ScN2a cells used in this study correspond to clone N2a#858 infected with the mouse-adapted scrapie strain 22L (Nishida et al., 1999). Cells were cultured in MEM containing 10% fetal calf serum, 300 μg geneticin ml−1, 100 U penicillin ml−1 and 100 μg streptomycin ml−1 in an atmosphere of 5% CO2. After incubation in the presence of P-dendrimers for the indicated time, cells were collected in PBS and lysed for 20 min at 4°C in PBS containing 0.5% NP-40, 0.5% sodium deoxycholate, 1 μg pepstatin and leupeptin ml−1 and 2 mM EDTA. After 1 min of centrifugation at 10,000 g, the supernatant was collected and the total protein concentration was measured using the BCA protein assay kit (Pierce). Samples were adjusted for protein concentration and were treated with 16 μg PK (mg total protein)−1 for 30 min at 37°C. Digestion was stopped by the addition of 1 mM Pefabloc and incubation for 5 min on ice. After centrifugation (20,000 g for 45 min, 4°C), the supernatants were discarded and the pellets were resuspended in reducing SDS sample buffer. Proteins were electroblotted onto Immobilon membranes. PrPSc was detected using SAF-mix antibodies and a peroxidase-conjugated goat anti-mouse secondary antibody. PrPSc was detected in the cell lysate before PK digestion with rabbit polyclonal antibody P45–66 and a peroxidase-conjugated goat anti-rabbit secondary antibody. Blots were developed using enhanced chemiluminescence (ECL). Films were analysed using Sigma Scan image analysis software.

Cell-to-cell ex vivo transmission. Cells were collected from a confluent 175 cm2 flask under sterile conditions and resuspended in 100 μl cold PBS with 5% glucose. Cell suspensions were submitted to four cycles of freeze-thawing in liquid nitrogen to prepare the inoculum. The extracts were then passed through a 27-gauge needle several times. Twenty microlitres of the extracts diluted in 1 ml Opti-MEM were added to uninfected N2a#858 cells for 2 days. Cells were passaged every 3–4 days at confluence and tested for the presence of PrPSc.

Interaction of P-dendrimers with PrP. To study the possible interaction between PrP and P-dendrimers, 15 ml Amberlite IR-120 (plus) ion exchange resin (sodium form) was immersed in pentane and mechanically stirred for 1 h, and then filtered and rinsed with distilled tetrahydrofuran and water (until the filtrate remained colourless). Ten millilitres of this wet resin was introduced into a flask containing a solution of 342.5 mg pd-G4 in 15 ml distilled water. The mixture was mechanically stirred for 96 h. The resin was then filtered and washed with distilled water. The filtrate was evaporated, weighed and analysed by 31P NMR in D2O to control for the absence of P-dendrimer (Loup et al., 1999). For ‘pull-down’ experiments, beads were rinsed several times in a buffer containing 150 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 50 mM Tris/HCl (pH 7.5) and protease inhibitors (1 μg pepstatin and leupeptin ml−1, 0.5 mM PMSF, 2 mM EDTA). Twenty micrograms of beads coated, or not, with pd-G4 were added to 50 μl N2a#858 cell lysate at 0.5 mg protein ml−1 for 2 h at 4°C under constant agitation. After a rapid centrifugation (10,000 g for 30 s), the beads were collected, washed several times with the same buffer and boiled for 5 min with 30 μl SDS loading buffer. After centrifugation, the supernatant was collected, subjected to SDS-PAGE and Western blotted as described above.
Infectivity assay. Experiments were performed in C57BL/6 mice acquired from R. Janvier (Charles Rivers, L’Arbresle, France). Three groups of eight 3-week old female mice were inoculated with strain C506M3 by the intraperitoneal (i.p.) route with 100 \( \mu l \) 2 % brain homogenate in 5 % glucose. The murine strain C506M3 is derived from a natural case of sheep scrapie (a kind gift from Corinne Lasmezas, CEA, Fontenay aux Roses, France). Strain C506M3 has been characterized in intracerebral and i.p. infection models (Lasmezas et al., 1996).

For the treatment with pd-G4, mice were intraperitoneally injected every 2 days, from day 2 after the inoculation to day 30, with either 50 or 100 \( \mu g \) per mouse. The untreated group were injected with physiological serum. The mice were sacrificed 30 days after infection, the time when spleen PrP\(^{Sc}\) reaches a plateau in untreated animals.

PrP\(^{Sc}\) detection in the spleen of C57BL/6 mice by Western blot. Tissues were homogenized at 10 % (w/v) in the same lysis buffer used for PrP\(^{Sc}\) detection in cell culture. PrP\(^{Sc}\) was purified by centrifugation in the presence of detergents, after adapted PK digestion (10 \( \mu g \) PK for 100 mg tissue). Samples were loaded on a 12 % polyacrylamide SDS gel and then transferred onto a nitrocellulose membrane. Immunoblotting was performed as described for the cell culture detection of PrP\(^{Sc}\). For each experiment, the brain of a C57BL/6 mouse at the terminal stage of the disease was used as a positive control.

RESULTS

Inhibition of PrP\(^{Sc}\) formation in ScN2a cells

The ability of P-dendrimers pd-G3, pd-G4 and pd-G5 (Fig. 1) to inhibit PrP\(^{Sc}\) formation was investigated in ScN2a cells using increasing concentrations of the compounds (Fig. 2). One day after the cells were plated, the drugs were added directly to the culture medium and left for 3 days. Cells were then lysed and the presence of PrP\(^{Sc}\) was detected by Western blot after PK digestion. The three P-dendrimers revealed different anti-prion activities. Based on band quantification, the concentrations at which 50 % of PrP\(^{Sc}\) replication was inhibited (IC\(_{50}\)) were estimated at 600 nM (10 \( \mu g \) ml\(^{-1}\)) for pd-G3, 45 nM (1.5 \( \mu g \) ml\(^{-1}\)) for pd-G4 and 75 nM (5 \( \mu g \) ml\(^{-1}\)) for pd-G5 (Fig. 2b). No obvious effect on the morphology or growth rate of the cells was observed at the concentrations used for the
dose–response. In fact, a significant toxic effect was only observed with concentrations above 25 µg P-dendrimer ml⁻¹.

Time-course of P-dendrimer action

Cells were incubated for 0.5, 1, 2, 4, 8, 17, 24 and 36 h with 10 µg pd-G4 ml⁻¹, and the level of PrPSc was determined in order to evaluate the time-course of action of the P-dendrimers (Fig. 3). Duration of the treatment influenced the ability of P-dendrimers to remove PrPSc from ScN2a. A significant reduction in PrPSc was observed as early as 4 h, showing that such molecules act rapidly to reduce PrPSc in cells.

Curing of ScN2a and effect of P-dendrimers on PrPSc

To determine whether P-dendrimers could totally remove PrPSc from ScN2a cells, the cultures were first treated with 10 µg P-dendrimers ml⁻¹ for 3 weeks to check whether residual PrPSc, if present, could facilitate further PrPSc replication (Fig. 4a, lane 2). Cells were then cultured for 1, 2 and 3 additional weeks in the absence of the drug to check whether residual PrPSc could enhance PrPSc replication. We did not observe any PrPSc reappearance in the cells after these 3 weeks (Fig. 4a, lanes 3–5) or even after an additional 2 weeks of culture without P-dendrimers (data not shown).

The amount of PrPSc in these cells was evaluated by Western blot analysis using the P45–66 antibody (Fig. 4a, PK panel). No differences in PrPSc levels were observed between the samples. In addition, the levels of GAPDH remained unchanged in the cell lysates collected at the different time points (Fig. 4a, lower panel).

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Fig. 2. Dose–response curves of PrPSc inhibition in ScN2a cells. ScN2a cells were plated at 10⁵ cells per well and cultured for 3 days in the absence (lane C) or presence of pd-G3, pd-G4 and pd-G5 at the indicated concentrations. (a) PrPSc was detected by immunoblotting after PK digestion. Molecular mass markers in kDa are indicated to the left of the immunoblot. (b) Densitometry data (PrPSc level as a % of untreated controls) from (a) and from two additional independent experiments were plotted for each P-dendrimer. Values represent the mean ± SD. IC₅₀ for pd-G3 (■), pd-G4 (▲) and pd-G5 (●) were 600 nM (10 µg ml⁻¹), 45 nM (1.5 µg ml⁻¹) and 75 nM (5 µg ml⁻¹), respectively.

Fig. 3. Time-course of action of P-dendrimer in ScN2a cells. ScN2a cells were plated and incubated for 0.5, 1, 2, 4, 8, 17, 24 and 36 h with 10 µg pd-G4 ml⁻¹, rinsed three times with PBS and cultured further with fresh medium. Three days after plating, levels of PrPSc were evaluated by Western blotting (a) and by densitometry (b). An incubation of 4 h with pd-G4 was sufficient to observe a significant effect on PrPSc formation after 3 days.
A cell-to-cell transmission experiment was performed to evaluate the possibility of infectivity remaining following P-dendrimer treatment of ScN2a. This alternative approach to animal inoculation is now used by several laboratories and illustrates the possibility of detecting infectivity by means of highly susceptible cell lines (Bosque & Prusiner, 2000; Nishida et al., 2000; Klohn et al., 2003). We have previously demonstrated that prion transmission to N2a#58 was possible over a four logs range of infectivity (Lehmann et al., 2001). Cell extracts from ScN2a treated with 10 μg P-dendrimer ml⁻¹ for 3 weeks, and from untreated cells were therefore prepared and used to infect N2a#58. The presence of PrPSc was subsequently assayed after several passages (Fig. 4b). Even after up to eight passages (lanes 2–6), PrPSc could not be detected in the N2a#58 cells inoculated with treated cells, while cells inoculated with control ScN2a accumulated significant levels of PrPSc after only three passages (lanes 7–11).

P-dendrimers modified protease resistance of PrPSc from brain homogenates at neutral pH

The effect of P-dendrimers on PrPSc from 263K, BSE, Chandler and 22L brain homogenates from different species was tested (Fig. 5a). An average decrease of 50% in the PrPSc level from infected 22L brain was observed following incubation of the homogenate with pd-G4 at neutral pH. pd-G4 was also able to induce a significant decrease of PrPSc in Chandler, BSE and even a disappearance in 263K samples.

Binding of PrP by P-dendrimers

To determine if P-dendrimers could bind to PrP, ‘pull-down’ experiments with pd-G4-coated amberlite beads were performed (Fig. 5b). PrP C could be recovered only from coated beads, suggesting a specific binding to the P-dendrimer (lane 3). The three glycosylated isoforms of PrP C could be recovered with the same apparent ratio as in the original lysate, suggesting that N-glycans did not contribute to the binding. By Coomassie staining, we could not detect significant amounts of protein eluted from the beads after the pull-down experiment (data not shown).

Inhibition of prion replication in C57BL/6 mice

To confirm the potential of P-dendrimers as anti-prion agents in vivo, we applied a protocol allowing for a rapid estimation of the peripheral accumulation of PrPSc during the incubation period. C57BL/6 mice were challenged intraperitoneally (i.p.) with C506M3 scrapie brain homogenate derived from terminally ill mice. Two different groups of eight mice were treated with 50 or 100 μg of P-dendrimer per mouse, every 2 days by i.p. injection from day 2 to day 30 post-inoculation. Control animals were injected with the vehicle only (physiological saline). The mice were sacrificed 30 days after infection, the time when spleen PrPSc reaches a plateau in untreated animals. Western blot analysis of mouse spleens revealed that treatment with 50 or 100 μg P-dendrimers inhibited PrPSc accumulation significantly by up to 66 or 88%, respectively (P<0.05; Mann–Whitney U-test) (Fig. 6 b–c).

DISCUSSION

Currently, there is no effective treatment to cure prion disease. Several candidates have been tested and proposed...
as anti-prion agents based on observations in cell models. However, in vivo studies do not always corroborate with in vitro results, as has been shown recently by Barret et al. (2003) for quinacrine. Here we have investigated the effect of a new class of dendrimers using well-established experimental models. Recently, Supattapone et al. (1999) showed that branched polyamines were able to clear PrP<sub>Sc</sub> from ScN2a cells. Based on these data, we have selected and produced related molecules, which could possess high in vivo bio-availability and minor toxic effects, in order to assess their therapeutic potential. The dendrimers used contain phosphorus atoms in their backbone and are thus called phosphorus-containing dendrimers or P-dendrimers. These phosphorus groups render the molecule more stable against both nucleophilic attack and acid-catalysed hydrolysis, thus avoiding rapid and premature degradation (Loup et al., 1999). Moreover, the presence of tertiary amine end-groups renders them highly hydrophilic. This property decreases the likelihood of rapid elimination as observed with regular cationic systems, due to electrostatic interactions with negatively-charged cellular membranes and extracellular matrices (Padilla De Jesus et al., 2002). In addition, the amphipathic structure of these molecules allows for their association with a lipid matrix and with hydrophobic proteins such as PrP. This was confirmed by the observation that P-dendrimer-coated amberlite beads can bind PrP molecules.

In this study, P-dendrimers were able to remove PrP<sub>Sc</sub> from ScN2a cells infected with the 22L strain, and inhibited PrP<sub>Sc</sub> replication in the spleen of C57BL/6 mice that had been intraperitoneally infected. Therefore, P-dendrimers can be considered relevant candidates for prion therapy. Indeed, cell culture studies showed that P-dendrimers were able to inhibit PrP<sup>Sc</sup> replication in a dose-dependent manner (Fig. 2). Dendrimers were also able to clear prion infectivity, since PrP<sup>Sc</sup> did not reappear 5 weeks after treatment was stopped and no infectivity was detected in cell-to-cell transmission assays (Fig. 4b). When the different P-dendrimer generations were compared, the generation 4 (pd-G4) and generation 5 (pd-G5) species, which have higher surface densities than pd-G3, were the most efficient. These data also confirmed the potency of branched polyamines for denaturing PrP<sup>Sc</sup> as observed by Supattapone et al. (1999), and showed that branching architecture and high surface density of terminal tertiary amines were required for dendrimers to denature PrP<sup>Sc</sup> in vitro. This suggested that an optimum balance exists between the size of the compounds and the number of end-groups presented on their surface.

The possible mechanism by which P-dendrimers could interfere with prion replication was also investigated. In particular, P-dendrimers can bind to PrP molecules as shown by the retention of PrP on dendrimer-coated amberlite beads. These molecules also reduced PrP<sup>Sc</sup> PK resistance in different animal brain tissues from several sources (experimentally infected rodents and BSE-infected cow). This suggests that preformed PrP<sup>Sc</sup> aggregates are disrupted by P-dendrimers. Moreover, time-course experiments demonstrated that P-dendrimers are rapid in action, as PrP<sup>Sc</sup> clearance occurred within 4 h of treatment. The low IC<sub>50</sub> of these molecules allows them to be well tolerated, with no apparent cytotoxicity. This was confirmed by the fact that the levels of total protein, PrP<sub>C</sub> and GAPDH remained unchanged in the cultures during treatment (Fig. 4). Toxic concentrations of P-dendrimers in culture were above 25 µg ml<sup>−1</sup>, which is much lower than those observed for other dendrimers such as PAMAM (Malik et al., 2000). In our study, in vivo experiments with C57BL/6 mice showed that P-dendrimers were well tolerated at the concentration used during the 1 month of treatment. No significant changes in behaviour or body weight of the animals were observed. Significant haemolysis, granuloma or abscess reaction were also absent from the injection site and histopathology analysis of several organs was unaltered.

Importantly, dendrimers appear to have a wide biodistribution throughout the body, as they can be detected after peripheral injection in various organs such as liver, intestine, stomach, lung muscle, kidney and bone (Malik et al., 2000). In our experiments, P-dendrimers were most likely able to reach the spleen where they inhibited PrP<sup>Sc</sup> replication. With regard to the pathogenesis of TSEs, the role

![Fig. 5. P-dendrimers reduce PrP<sup>Sc</sup> level in scrapie brain homogenate and bind to PrP. (a) 22L, Chandler, BSE and 263K brain homogenates were incubated at pH 7 with (+ lanes) or without (− lanes) P-dendrimer G4 as described in Methods. Samples were then digested with PK and the amount of PrP<sup>Sc</sup> evaluated by Western blotting. Molecular mass markers in kDa are indicated to the left of the immunoblot. Results are representative of three independent experiments. (b) Lysate from N2a#58 cells (lane 1) was mixed with control amberlite beads (lane 2) or with beads coated with pd-G4 (lane 3) for 2 h at 4 °C. After several washings, the amount of PrP<sup>Sc</sup> retained by the beads was evaluated by Western blot using antibody 45–66. Molecular mass markers in kDa are indicated to the left of the immunoblot. Results are representative of three independent experiments.](image-url)
of the lympho-reticular system organs in the early stage of infection has been demonstrated in a number of studies (Bruce et al., 2000). This prompted us to develop a protocol based on a rapid estimation of peripheral accumulation of PrPSc. In particular, the spleen of C57BL/6 mice is infectious and exhibits PrPSc accumulation long before the agent reaches the central nervous system after intraperitoneal or oral inoculation of strain C506M3, thus more probably mimicking the natural route of contamination by prions (Lasmezas et al., 1996). Since therapies to control brain pathology are very challenging, it is essential to develop strategies that can inhibit prion replication before or during neuroinvasion. It has been demonstrated that blocking replication in the spleen with different compounds increased the incubation time (Beringue et al., 2000; Heppner et al., 2001; Mabbott et al., 2003). In our in vivo experiments, we observed a significant but variable inhibition of PrPSc generation in the spleen of infected mice treated with P-dendrimers (Fig. 6). It is possible that higher doses of P-dendrimers would have completely blocked PrPSc accumulation in the spleen. Recent data obtained following the same protocol using quinacrine and MS-8209, on C57BL/6 animal models with the identical strain C506M3, have shown that quinacrine was not effective on peripheral PrPSc accumulation, while MS-8209, which is an analogue of amphotericin B, was able to inhibit PrPSc replication with efficacies between 70 % and 90 %. Since we observed an average efficacy of the P-dendrimer close to the one observed for MS8209, and considering the fact that this latter molecule was described in the past as an anti-prion agent delaying the incubation time by a factor of two in hamsters (Barret et al., 2003), we can confidently predict that P-dendrimers will delay the incubation time. Although dendrimers are well biodistributed, there are no data reporting their entry into the central nervous system through the blood–brain barrier. In this regard, it might be possible to consider P-dendrimer treatment at least as a post-exposure prion prophylaxis as has been proposed for other molecules (Sethi et al., 2002). Whether treatment with P-dendrimers would also be effective when given later after exposure is not yet known. We will investigate this in the future and are optimistic for the outcome, since P-dendrimer also induced the degradation of pre-existing PrPSc aggregates in vitro. Indeed, as stated above, once aggregates exist in brain homogenates, dendrimers are effective and can reduce PrPSc PK resistance.

Interestingly, high molecular mass polymers, like dendrimers, have been widely used as soluble drug carriers to improve drug targeting and therapeutic efficacy (Padilla De Jesus et al., 2002). They could thus be designed to possess cavities which can accommodate guest molecules. In this

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**Fig. 6.** P-dendrimers reduce splenic PrPSc levels. (a) Western blot of PK-digested PrPSc from spleens of mice 30 days post infection with scrapie strain C506M3, treated or not with P-dendrimers as described in Methods. Upper panel, spleens of untreated mice; middle panel, mice treated with 50 μg P-dendrimer at each injection (lanes 1–5); lower panel, mice treated with 100 μg at each injection (lanes 6–10). For each experiment, a dilution scale of the positive control (lane C), the brain of a C57BL/6 mouse at the terminal stage of the disease (scrapie strain C506M3), was subjected to the same protocol for PrPSc detection. The same untreated mouse spleen was used as an internal control. Molecular mass markers in kDa are indicated to the left of the immunoblot. (b) Densitometry of PrPSc levels in Western blots. The amount of PrPSc in mice treated with 50 or 100 μg P-dendrimer per injection was compared with that obtained in untreated scrapie-infected mice. P-dendrimers induced substantial dose-dependent reduction of PrPSc; a mean of 52 % ± 14 % for mice treated with 50 μg per injection and 82 % ± 11 % for mice treated with 100 μg per injection (asterisk, P<0.05 compared with untreated spleens, Mann–Whitney U-test.)
context, it might be possible to synthesize hybrid molecule to combine the 'natural' anti-prion activity of P-dendrimers with that of other molecules such as porphyrins or tricyclic derivatives (Korth et al., 2001; Priola et al., 2000). Targeting the dendrimers to particular tissues might also be possible by having different types of end-groups, as demonstrated by several projects aiming to treat infectious diseases (Bourne et al., 2000; Nishikawa et al., 2002) or cancer (Vincent et al., 2003).

In summary, the results reported here indicate that P-dendrimers exert an inhibitory effect on PrP\textsuperscript{Sc} generation and an anti-infectivity action as observed in both cell and mice models, thus offering a therapeutic approach to prion diseases. In particular, P-dendrimers were effective on different prion strains including that responsible for BSE. This is of a particular interest, given the unique properties of the latter strain and its involvement in the appearance of variant CJD (Will et al., 1996).

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