Soluble Aβ aggregates can inhibit prion propagation

Citation
Sarell, Claire J., Emma Quarterman, Daniel C.-M. Yip, Cassandra Terry, Andrew J. Nicoll, Jonathan D. F. Wadsworth, Mark A. Farrow, Dominic M. Walsh, and John Collinge. 2017. “Soluble Aβ aggregates can inhibit prion propagation.” Open Biology 7 (11): 170158. doi:10.1098/rsob.170158. http://dx.doi.org/10.1098/rsob.170158.

Published Version
doi:10.1098/rsob.170158

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:34651723

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Mammalian prions cause lethal neurodegenerative diseases such as Creutzfeldt–Jakob disease (CJD) and consist of multi-chain assemblies of misfolded cellular prion protein (PrP<sup>C</sup>). Ligands that bind to PrP<sup>C</sup> can inhibit prion propagation and neurotoxicity. Extensive prior work established that certain soluble assemblies of the Alzheimer’s disease (AD)-associated amyloid β-protein (Aβ) can tightly bind to PrP<sup>C</sup>, and that this interaction may be relevant to their toxicity in AD. Here, we investigated whether such soluble Aβ assemblies might, conversely, have an inhibitory effect on prion propagation. Using cellular models of prion infection and propagation and distinct Aβ preparations, we found that the form of Aβ assemblies which most avidly bound to PrP<sup>C</sup> in vitro also inhibited prion infection and propagation. By contrast, forms of Aβ which exhibit little or no binding to PrP were unable to attenuate prion propagation. These data suggest that soluble Aβ aggregates can compete with prions for binding to PrPC and emphasize the bidirectional nature of the interplay between Aβ and PrP<sup>C</sup> in Alzheimer’s and prion diseases. Such inhibitory effects of Aβ on prion propagation may contribute to the apparent fall-off in the incidence of sporadic CJD at advanced age where cerebral Aβ deposition is common.

1. Introduction

Prion diseases are fatal neurodegenerative disorders associated with propagation of multi-chain assemblies of misfolded cellular prion protein (PrP<sup>C</sup>) [1,2]. Prions propagate by recruitment of α-helical-rich PrP<sup>C</sup> into β-sheet-rich infectious rod-like structures [3,4]. In addition to serving as the precursor of infectious prions, expression of PrP<sup>C</sup> is also required for the neurotoxicity in prion infection [5–8].

Numerous studies suggest PrP<sup>C</sup> may play a role in Alzheimer’s disease (AD), and there is evidence that PrP can modulate the production, aggregation and toxicity of the amyloid β-protein (Aβ) [9–11]. In 2009, Lauren et al. [11] reported that a preparation of aggregated synthetic Aβ1-42 known as Aβ-derived diffusible ligands (ADDLs), which contained a mixture of globular oligomers and protofibrils, bound to PrP<sup>C</sup>. Using a series of deletion constructs and anti-PrP antibodies, it was shown that PrP residues 95–110 were required for ADDL binding. In accord with this finding, the authors demonstrated that knock-out of the mouse PrP gene (Prnp) or pre-treatment of hippocampal slices with an antibody directed to PrP<sub>93-109</sub> protected against ADDL-induced synaptotoxicity. These provocative findings were followed by multiple in vivo and in vitro studies, most of which supported a role for PrP<sup>C</sup> in aspects of Aβ-mediated toxicity [10,12–21]. However, others have reported deleterious effects of Aβ that do not require PrP<sup>C</sup> expression [22–25]. Recently, we reported that
only certain Aβ assemblies exert toxicity in a PrP-dependent fashion which may explain some of these apparently discrepant findings [26].

However, all published studies that have examined binding of Aβ to PrP\textsuperscript{C} agree that there is high affinity and specific binding for soluble aggregates [11,13,21–23,26–29], and high-resolution analysis suggests that binding of Aβ occurs at two sites: centred around residues approximately 23–33 and approximately 88–113 [29]. Although initially surprising, the finding that PrP\textsuperscript{C} can serve as an acceptor for soluble aggregates of Aβ [11] is consistent with the hypothesis that the unstructured N-terminus (encompassing residues approx. 23–128) of PrPC acts as a molecular sensor that the unstructured N-terminus (encompassing residues approx. 23–128) of PrPC can serve as an acceptor for soluble aggregates of Aβ [11] is consistent with the hypothesis that the unstructured N-terminus (encompassing residues approx. 23–128) of PrPC acts as a molecular sensor which can interact with a broad range of ligands [30], including other β-sheet-rich oligomeric proteins [28]. Moreover, the same binding sites for soluble aggregates of Aβ have previously been shown to also be important for binding of prions to PrP\textsuperscript{C} [31–36].

While many studies have investigated the interaction between Aβ and PrP\textsuperscript{C}, and how it might contribute to AD pathogenesis, there has been little research on whether Aβ binding to PrP\textsuperscript{C} can affect prion propagation. Here, we used the well-established cell-based prion bioassay (the scrapie cell assay using PK1/2 neuroblastoma-derived cells) [37] and a chronically prion-infected cell line (iPK1/2 cells) [38,39] used the well-established cell-based prion bioassay (the scrapie cell assay using PK1/2 neuroblastoma-derived cells) [37] and a chronically prion-infected cell line (iPK1/2 cells) [38,39] to address these critical issues. We found that soluble Aβ aggregates (ADDLs), but not Aβ monomers or fibrils, could prevent infection of PK1/2 cells when ADDLs were co-administered with the prion inoculum. Strikingly, when added to iPK1/2 cells already chronically infected with prions, ADDLs had a marked cell curing effect. This protective effect appears to be mediated by ADDL binding to PrP\textsuperscript{C}. While diverse studies have linked PrP to AD [9,11,40–43], our data raise the possibility that soluble Aβ aggregates may actually protect against prion disease. Thus, whether Aβ binding to PrP\textsuperscript{C} has pathogenic or protective effects may depend on the relative concentrations of relevant Aβ and PrP assemblies.

2. Material and methods

2.1. Reagents

All chemicals and reagents were purchased from Sigma-Aldrich unless otherwise noted. Synthetic Aβ\textsubscript{1–42} and Aβ\textsubscript{1–40} were synthesized and purified using reversed-phase HPLC by Dr James I. Elliott at the ERI Amyloid Laboratory (Oxford, CT, USA). Peptide mass and purity (greater than 99%) were confirmed by reversed-phase HPLC and electrospray/ion trap mass spectrometry. All tissue culture reagents were obtained from Invitrogen.

2.2. Aβ preparations

Aβ is prone to aggregate and can form an array of different assemblies. In this study, we used conditions to yield preparations highly enriched in: (i) monomers, (ii) pre-fibrillar aggregates, known as ADDLs and (iii) amyloid fibrils. Monomeric Aβ was prepared by dissolving dry Aβ\textsubscript{1–40} peptide at 2 mg ml\textsuperscript{−1} in 6 M guanidine hydrochloride (GuHCl) and then subjecting this preparation to asymmetric flow field-flow fractionation (AFFF). The AFFF channel was eluted in 50 mM ammonium bicarbonate pH 8.5, and fractions containing monomeric Aβ, as judged by molar mass (approx. 4000 g mol\textsuperscript{−1}), were collected and immediately frozen at −80°C. ADDLs were prepared essentially as described previously [19], approximately 25 mg of Aβ\textsubscript{1–42} peptide was dissolved in anhydrous DMSO, gently rocked for approximately 5 min and then diluted to 0.5 mg ml\textsuperscript{−1} in phenol red-free Ham’s F12 medium without L-glutamine (Caisson Labs) and incubated quiescently at room temperature (RT). At approximately 6 h intervals, aliquots were removed, briefly centrifuged at 16 100 g and analysed using AFFF. Typically, at 24–36 h, less than 20% of the injected mass eluted as monomer, as judged by the area under the curve of both monomer and oligomer peaks. Thereafter, the material was aliquoted and stored frozen at −80°C. To form fibrils, Aβ was solubilized and incubated as for ADDLs, but the incubation continued for 30 days. For cell culture experiments, Aβ preparations were buffer exchanged into Opti-MEM using a centrifugal concentrator (Amicon, Ultra 0.5 ml, 5 K cut-off).

2.3. Asymmetric flow field-flow fractionation and multi-angle light scattering

Experiments were conducted using a 24.6 cm long channel fitted with a 350 μm spacer and a 5 kDa MWCO polyethersulfone membrane. Aliquots of Aβ preparations (190 μl) were injected onto an Eclipse DualTec AFFF (Wyatt Technology, Santa Barbara, CA, USA) and eluted with 50 mM ammonium acetate pH 8.5. The sample was injected at 0.2 ml min\textsuperscript{−1}, followed by a 1 min focusing period, and then eluted with a 1.5 ml min\textsuperscript{−1} cross-flow for 45 min. Light scattering was performed using a Wyatt Dawn Hefei II multi-angle light scattering module to calculate the molar mass.

2.4. Electron microscopy

Negative stain electron microscopy (EM) was performed as described previously [26]. Peptide solutions (5 μl) were loaded onto negatively charged glow-discharged copper grids coated with a continuous carbon film. Samples were left to adhere for 120 s and excess solution blotted with grade 4 Whatman paper. Thereafter, grids were stained with 2% uranyl acetate for 40 s, blotted and air-dried. Images were acquired on an FEI Tecnai T10 electron microscope operating at 100 kV and recorded on a 1 k × 1 k charge-coupled device camera (Gatan) at a typical magnification of 34 000 with a pixel size of 5 Å.

Prion rods were purified as described previously [4,44] concentrated to 100× (relative to starting 10% brain homogenate) and mixed with 10 μM ADDLs and incubated at 21°C for 1 h. Prion rods were pelleted by centrifugation at 16 100 g and 25°C for 30 min. The pellet was washed once with Opti-MEM and centrifuged a final time. The pellet was resuspended in Opti-MEM to one half the volume of the starting prion/ADDL solution and stained for EM as described above. Images were analysed for evidence of ADDLs binding to prion rods. First, the number of protofibrillar and spherical Aβ species in an area containing a rod cluster were counted, then the number of Aβ species in an equivalent sized area that did not include prion rods were counted. This was repeated for three rod clusters and three
2.5. Automated scrapie cell assay

An automated version of the standard scrapie cell assay (SCA) using PK1/2 cells [45] was used as described previously [37,46]. Briefly, PK1/2 cells were grown in Opti-MEM, containing 10% fetal calf serum; 100 U ml\(^{-1}\) penicillin and 100 µg ml\(^{-1}\) streptomycin at 37°C, 5% CO\(_2\). Twenty-four hours before infection with Rocky Mountain Laboratory (RML), PK1/2 cells were seeded in 96-well plates at 18 000 cells per well and grown in Opti-MEM. ADDLs or bovine serum albumin (BSA) were incubated with RML prion-infected brain homogenate (I-BH) (designated I8700; [44]) for 1 h at RT and then added to cells and incubated for 72 h. Thereafter, cells were split 1:8 into fresh cell culture media containing fetal calf serum and grown to confluence. Two further passages were conducted, removing initial inoculum, before transferring a sample of the cells to enzyme-linked immunospot (ELISPOT) plates for measurement of the number of prion-infected cells (identified by detection of protease K-resistant PrP\(_{\text{Rm}}\), PrP\(_{\text{nSc}}\), the ‘spot count’ [45]). The viability of the cells was monitored using Trypan Blue. Prion titre in the experimental samples was determined by reference to a calibration curve in each experiment derived from a serial dilution of an RML brain homogenate of known prion titre (10\(^{8.3}\) intracerebral LD\(_{50}\) units g\(^{-1}\) brain) determined by prior mouse bioassay [37,44].

The ability of ADDLs to retard prion propagation was calculated relative to the number of infected cells ‘spot count’ of cells incubated with the equivalent RML concentration alone (positive control) and the ‘spot count’ (background noise) of cells incubated without RML present.

2.6. Curing assay of chronically Rocky Mountain Laboratory prion-infected cells

Chronically RML prion-infected PK1/2 (designated iPK1/2) cells were used to assay curing activity. As described previously, these cells are able to maintain a robust prion infection long term in culture [38,39,47]. Briefly, iPK1/2 cells were produced by incubating cells with 1 x 10\(^{-3}\) RML-I-BH for 72 h [44]. Thereafter, cells were passaged every 2–3 days for 2 weeks to remove any remaining inoculum. A portion of infected cells was analysed for RML prion infectivity by ELISPOT and the remainder stored in liquid nitrogen. For experiments, cells were thawed and cultured as described above. In order to maintain a consistent level of prion infection, cells were never passaged more than 15 times.

iPK1/2 cells were seeded at 6000 cells per well, in 384-well plates. The cells were grown in Opti-MEM for 3 days at 37°C and 5% CO\(_2\) ± Aβ. Additionally, positive (2 µM 5000 Da dextran sulfate) and negative (cells only) controls were included on each plate. Infected cells produce PrP\(_{\text{Sc}}\). On day 4, cells were analysed for both viability and PrP\(_{\text{Sc}}\) content. Cell viability was assessed using the CellTiter-Glo Luminescent assay (Promega) and PrP\(_{\text{Sc}}\) levels were measured by dot blot. For PrP\(_{\text{Sc}}\) analysis, the media was removed from the cells, lysis buffer added (Tris buffer pH 7.5 with NaCl, Triton X-100 and sodium deoxycholate) and the cells lysed on ice for 20 min. The lysates were mixed and bound to nitrocellulose membrane, using a 96-well biotin-dotted microfiltration apparatus (BioRad). The samples were treated with proteinase K (5 µg ml\(^{-1}\)) for 1 h at 37°C and then denatured using 3 M guanidine thiocyanate. PrP\(_{\text{Sc}}\) was detected using the anti-PrP antibody ICSM18 (D-Gen Ltd, London) and goat anti-mouse IgG-IRDye 800CW (LI-COR Biosciences, Santa Clara, CA, USA). Spots were visualized using an Odyssey infrared imaging system (LI-COR Biosciences) and the relative intensity of the infrared signal was determined using the systems software.

2.7. Immunofluorescence

PK1/2 cells were seeded at 18 000 cells per coverslip in 24-well plates. The cells were grown in serum-free media for 3 days at 37°C and 5% CO\(_2\) ± Aβ. On day 4, coverslips were washed three times with PBS and then fixed in 4% PFA at RT for 15 min. After fixation, coverslips were washed twice with PBS and then blocked with 5% BSA/PBS (1 h at RT) and stained. Cells were incubated for 1 h at RT with the anti-PrP antibody ICSM18 (1.25 µg ml\(^{-1}\)) and/or the anti-Aβ rabbit antisera #2454 at 1:2000 dilution (Cell Signalling, Danvers, MA, USA). Thereafter, cells were washed with PBS and then incubated with Alexa Fluor 488 tagged donkey anti-mouse IgG (H + L) (#A-21202) at 2 µg ml\(^{-1}\) and/or Alexa Fluor 546-tagged donkey anti-rabbit IgG (H + L) (#A10040) at 3.3 µg ml\(^{-1}\) (Invitrogen Life Technologies). Nuclei were stained with 4,6-diamidino-2-phenylindole, 1 µg ml\(^{-1}\) for 1 h at RT. Cells were washed with PBS and then mounted using fluorescence mounting medium (DAKO). Images were captured using a Zeiss LSM710 confocal laser scanning microscope and co-localization quantified using VOLocity 3D imaging software (Perkin Elmer).

3. Results

3.1. Aβ-derived diffusible ligands inhibit prion propagation and cure prion infection

ADDLs are a polydisperse solution of soluble Aβ aggregates which include globular oligomers, protobifils and monomer [11,48] and bind to the PrP\(_{\text{Sc}}\) specifically and with high affinity [11,13,21,26,27,29]. Two regions of PrP\(_{\text{Sc}}\) (one centred around residues 23–33 and the other around 88–113) are particularly important for Aβ binding [11,13,27,29], and these are the same sites thought to be important for PrP\(_{\text{Sc}}\) binding to PrP\(_{\text{Sc}}\) [31–36]. Thus, we sought to determine if ADDLs could compete with prions for binding to PrP\(_{\text{Sc}}\) and attenuate prion propagation.

As ADDLs are known to bind with high affinity to PrP\(_{\text{Sc}}\), whereas Aβ monomers show little or no binding and fibrils exhibit only weak binding [26], we generated ADDLs from Aβ42 and relatively homogeneous preparations of Aβ40 monomers and Aβ42 fibrils, and characterized each using AFFF and EM (figure 1). AFFF is a flow-based method in which separation takes place in a channel where sample retention is caused by the action of a cross-flow that is generated by a second independent stream that runs across the channel at right angles to the primary channel flow [49]. Unlike more commonly used size exclusion chromatography,
in AFFFF, small particles elute earlier than larger particles. AFFFF of ADDLs confirmed the presence of a small amount of monomer and a range of Aβ assemblies with molar masses from 300 000 to 3 000 000 g mol⁻¹ (figure 1b). EM also indicated that ADDLs contained a mixture of structures, including imperfect spheres of approximately 5–10 nm
diameter and abundant protofibrils (flexible fibrils) of approximately 5–10 nm diameter and less than 100 nm in length. By contrast, our monomer and fibril preparations were relatively homogeneous. Monomer preparations had a molar mass of 4000–5000 g mol⁻¹ (figure 1a) and contained no structures detectable by EM, whereas fibrils had a molar mass of greater than 10⁷ g mol⁻¹ and formed complex latticeworks of long fibrils with diameters of approximately 10 nm (figure 1c).

To test if ADDLs could attenuate prion infectivity, we used an automated high-throughput prion bioassay referred to as the automated scrapie cell assay (ASCA) [37,45,50,51]. PK1/2 cells were incubated with an RML prion-I-BH [44]. Cells were grown to confluence, split 1:8 and grown to confluence again. The cycle of growth and passage was repeated a further two times to remove initial infecting inoculum, and confluent cells from the third passage were used to measure the proportion of infected cells [37]. As expected, the extent of prion infection is strongly influenced by the dilution of I-BH, with lower dilutions resulting in more infected cells over the course of the SCA (figure 2a). To determine if ADDLs could attenuate prion infection, three dilutions of I-BH (3 × 10⁻⁶, 1 × 10⁻⁵, 1 × 10⁻⁴) were incubated with a range of ADDL concentrations (1–10 μM) for 1 h and then added to cells (figure 2b). The dilutions of I-BH were chosen to yield optimal spot counts within the linear dynamic range of the ELISPOT reader (see Material and methods) [37]. Importantly, addition of ADDLs to the inoculum caused a dose-dependent decrease in the extent of prion propagation, an effect that was directly related to the prion titre in the starting inoculum (figure 2b). For instance, when I-BH was used at a dilution of 3 × 10⁻⁶ (blue curve), maximal inhibition of infectivity was achieved with a dose of 5 μM ADDLs, whereas when more concentrated I-BH (1 × 10⁻⁴, green curve) was used, higher concentrations of ADDLs were required to significantly attenuate prion propagation (figure 2b). In comparison, addition of BSA had no effect on prion propagation (black curve, figure 2c).

Next, we investigated if ADDLs could cure cells with an established chronic prion infection. Chronically infected PK1/2 cells (iPK1/2 cells) accumulate PrPSc yet remain viable (see Material and methods) and have been successfully used in drug screening to identify anti-prion compounds [38,39,47]. Dextran sulfate is effective at curing prion infection in this assay and has been used as a positive control in drug screening to identify anti-prion compounds [38,39,47]. As expected, dextran sulfate caused a dose-dependent decrease in the proportion of infected cells [37]. As expected, dextran sulfate caused a dose-dependent decrease in the levels of PrPSc, whereas fibrils had a molar mass of greater than 10⁷ g mol⁻¹ and formed complex latticeworks of long fibrils with diameters of approximately 10 nm (figure 1c).

To allow comparison between experiments, we expressed the curing activity of ADDLs relative to the levels of PrPSc-treated cells plus and minus 2 × 10⁻⁶ M dextran sulfate (figure 3c). As in the ASCA assay (figure 2), ADDLs caused a dose-dependent decrease in the levels of PrPSc, whereas BSA did not (figure 3c). The ability of ADDLs to cure chronic prion infection was consistent across experiments when the same preparation of ADDLs was used (figure 3d, preparation C10) and when two other ADDL preparations were tested.

Figure 2. ADDLs inhibit prion propagation in the scrapie cell assay (SCA). ADDLs were incubated with RML prions for 1 h before addition to PK1/2 cells. Every 2–3 days, the cells were split 1:8 and passaging was repeated three times. After each passage, the viability and amount of infection of the cells was assessed by trypan blue and ELISPOT revelation, respectively. (a) The spot count of prion-infected PK1/2 cells increases with increasing concentration of prion containing brain homogenate. (b) PK1/2 cells incubated with a serial dilution of ADDLs and either 3 × 10⁻⁶ (blue curve), 1 × 10⁻⁵ (red curve) or 1 × 10⁻⁴ (green curve) diluted RML-I-BH. (c) About 3 × 10⁻⁶ RML homogenate incubated with a serial dilution of either ADDLs (blue curve) or BSA (black curve). ADDL concentration is based on the monomer equivalent concentration. Data shown are the mean and standard deviation of six replicates.
The IC$_{50}$ for ADDLs used in six different experiments ranged from 10.4 to 22.2 μM (figure 3d).

### 3.2. Aβ-derived diffusible ligands, but not Aβ monomers or fibrils inhibit prion propagation

Previous studies found that Aβ monomers do not bind to PrP$^\text{Sc}$ and that Aβ fibrils bind to PrP$^\text{Sc}$ less well than pre-fibrillar intermediates [11,26,27], hence we sought to determine if there was a relationship between the ability of different Aβ structures to bind to PrPC and their ability to reduce prion propagation (figure 4). First, we examined if Aβ40 monomer could influence prion propagation in the ASCA. As expected, ADDLs caused a dose-dependent decrease in prion propagation, whereas Aβ monomers had no effect (figure 4a). To determine if this prion-curing activity was similarly specific for pre-fibrillar Aβ species, we compared the effects of ADDLs versus Aβ40 monomers and Aβ42 fibrils using the chronic prion-infected cell assay. As before (figure 4b,c), ADDLs caused a dose-dependent decrease in prion infection (blue solid circles), whereas Aβ monomers and fibrils had no effect (figure 4b). Given that reduction in detectable PrP$^\text{Sc}$ could occur due to cell loss, and that Aβ is known to be toxic to certain cells, we were careful to measure cell viability in all of the cultures treated with Aβ. The number of metabolically active cells (as assessed by the CellTiter-Glo Luminescent Cell Viability assay) did not change over the concentration range at which ADDLs inhibited prion infectivity (less than or equal to 2 × 10$^{-5}$ M; figure 4c). Therefore, the reduction in PrP$^\text{Sc}$ levels mediated by ADDLs is not a consequence of cell compromise, but rather a specific effect comparable to that seen with other prion-curing agents [39,52,53].

### 3.3. Aβ-derived diffusible ligands bind to the surface of PrP$^\text{Sc}$-expressing cells, but not to purified prion rods

As both PrP$^\text{C}$ and PrP$^\text{Sc}$ share the same primary structure, including the amino acids that comprise the Aβ binding sites [27,29], we investigated whether the prion inhibition we observed was due to Aβ acting on PrP$^\text{C}$ or prions, or both. If ADDLs inhibited infectivity by binding to prions, then it should be possible to detect ADDLs bound to PrP$^\text{Sc}$. To address this issue, we incubated highly purified infectious prion rods [4,44] with ADDLs under the same conditions used in the ASCA, and then searched for binding of ADDLs to prions using negative stain EM (figure 5). Both prion rods and ADDLs were readily detected, but we saw no evidence of co-localization. This rather rudimentary assay provides the first evidence that Aβ does not bind to PrP$^\text{Sc}$.
As ADDLs did not seem to interact with prion rods, we looked for evidence of ADDL binding to PrP\(^{\text{C}}\) on the surface of PK1/2 cells. Immunostaining of non-permeabilized cells detected A\(\beta\) on the surface of PK1/2 cells and partial co-localization with PrP\(^{\text{C}}\) (figure 6a–c). Interestingly, we also observed that treating PK1/2 cells with ADDLs increased cell surface levels of PrP\(^{\text{C}}\) (figure 6b,d). In accord with earlier reports, ADDLs appear capable of binding to PrP\(^{\text{C}}\) [11,15] and retaining PrP\(^{\text{C}}\) at the plasma membrane [54]. Thus, it seems likely that ADDLs inhibit prion levels and propagation by competing with prions for binding to PrP\(^{\text{C}}\), and may also retard internalization of PrP\(^{\text{C}}\).

4. Discussion

Persuasive evidence from multiple investigators argues that certain soluble assemblies of A\(\beta\) can bind tightly to PrP\(^{\text{C}}\) [11,13,21–23,26–29]. The interaction between PrP and soluble A\(\beta\) aggregates is highly specific [11,13,26,27,29] and involves sites previously implicated in binding of PrP\(^{\text{Sc}}\) [31–36]. Attention has focused on how this interaction may contribute to AD pathogenesis, but A\(\beta\) binding to PrP\(^{\text{C}}\) also has implications for prion diseases. PrP\(^{\text{C}}\) is the obligate substrate for prion propagation and is essential for neurotoxicity [2,6,8,55] and agents that bind to PrP\(^{\text{C}}\) have the potential to modulate infectivity and toxicity [39,56].

Here, we show that ADDLs inhibit prion infectivity in a dose-dependent manner and reduced the levels of proteinase K-resistant PrP\(^{\text{C}}\) in chronically prion-infected cells. As both PrP\(^{\text{C}}\) and PrP\(^{\text{Sc}}\) have the same primary structure, including the sites involved in ADDL binding, the ability of ADDLs to attenuate prion propagation could result from interactions involving either PrP\(^{\text{C}}\) or PrP\(^{\text{Sc}}\). When ADDLs were mixed with highly purified prions, we found no evidence of
binding, whereas when ADDLs were added to PrPC-expressing cells ADDLs partially co-localized with cell surface PrPC. Moreover, as we and others have shown previously [11,13,21,26,27], and we confirmed for preparations used in this study (data not shown), recombinant monomeric PrP (rPrP) readily binds ADDLs. Taken together, these data suggest that ADDLs can attenuate prion infectivity by directly binding to PrPC and acting as a competitive inhibitor (electronic supplementary material, figure S1). Such a mechanism would allow the cells’ natural prion clearance rate [57] to outpace any residual propagation, resulting in the low to absent levels of PrPSc observed when ADDLs were used in our experiments. Consistent with this mechanism, we also found that Aβ species (monomer and fibrils) which show little or no affinity for monomeric PrP lack the ability to attenuate prion propagation.

The binding response between rPrP and ADDLs indicates an apparent dissociation constant of approximately 100 nM,
whereas the IC\textsubscript{50} of ADDLs in the chronic prion-infected cell assay was approximately 15 \(\mu\)M. The difference between binding to rPrP and the ability to inhibit prion propagation probably results because (i) ADDLs are known to bind non-PrP membrane components [11], (ii) our assays use mitotic cells which have a doubling time of approximately 24 h and (iii) ADDLs are competing with prions for binding to PrP\textsuperscript{C}. In terms of ADDLs, the concentration that might be needed to attenuate prion formation \textit{in vivo}, it is worth considering that the amount of ADDLs used in our experiments are expressed as monomer equivalents, yet we know that the component of ADDLs that binds to PrP has a relatively high molecular weight and only contributes a fraction of the total A\(\beta\) present [26]. Therefore, the actual \(K_d\) for the binding component of ADDLs must be significantly lower, and may be in the picomolar range. As to how much ADDLs would be required to inhibit prion propagation \textit{in vivo}, that will depend on the amount of infectious prions.

Our findings are in apparent conflict with a prior study that reported prion inoculation of Tg2576 APP transgenic mice accelerated both A\(\beta\) deposition and prion disease [58]. A possible explanation for the divergence in results seen with Tg2576 mice and those we detected in PK1 cells relates to the forms of A\(\beta\) tested in our study and those produced by Tg2576 mice. We and others have previously shown that only certain forms of A\(\beta\) bind to PrP [11,13] and that only particular effects of A\(\beta\) are mediated by PrP [26]. Similarly, it is known that certain APP transgenic mice exhibit cognitive phenotypes that depend on the expression of PrP, whereas others do not [16,25]. In terms of the acceleration of prion disease in Tg2576 mice, it is interesting to note that deleting PrP\textsuperscript{C} expression in Tg2576 results in only a partial rescue of cognitive performance as opposed to the complete recovery seen in other APP transgenic lines [59]. Further, Tg2576 mice have been shown to produce little or no A\(\beta\) species capable of binding to PrP [59]. Given that Tg2576 mice show minimal PrP-dependent deficits and produce little A\(\beta\) that binds PrP, it is perhaps not surprising that Tg2576 mice are unable to attenuate prion infectivity and propagation.

Clearly, high concentrations of ADDLs should completely inhibit prion propagation, but they are also expected to cause neuronal dysfunction. Thus, high levels of soluble A\(\beta\) assemblies may provide relative protection from human prion disease, but cause AD. The lack of co-localization of disease-associated PrP and A\(\beta\) deposits seen in a recent study is of interest in this regard [60]. These observations support the notion that soluble aggregates of A\(\beta\) and PrP may compete for binding to PrP\textsuperscript{C} \textit{in vivo} and that the balance between the levels of these aggregates is a critical determinant of whether and what form of neurodegenerative disease will result. The most common human prion disease, sporadic Creutzfeldt–Jakob disease, which has a relatively uniform incidence worldwide and apparently random population distribution, is thought to represent the spontaneous production of prions as a rare stochastic event [61,62]. In this regard, it has always been intriguing why its apparent incidence falls at advanced age (greater than 80 years) [63,64]. While this may in part be due to lower diagnosis rates in the elderly, it is conceivable that this could also be related to the common occurrence of A\(\beta\) deposition in this age group.

Data accessibility. All relevant data are presented in figures in the manuscript and electronic supplementary material.

Acknowledgements. We are extremely grateful to Prof. Helen Saibil and Natasha Lukoyanova at Birkbeck College London for providing electron microscope access and support. We thank Silvia Purro and Michael Farmer for helpful discussion, Christian Schmidt and Parvin Ahmed for assistance with scrapie cell assays, and Jessica Sells, Elizabeth Noble and Emmanuel Risse for assistance in characterizing ADDL preparations.

References

1. Prusiner SB. 1998 Prions. Proc. Natl Acad. Sci. USA 95, 13 363 – 13 383. (doi:10.1073/pnas.95.23.13363)
2. Collinge J, Clarke AR. 2007 A general model of prion strains and their pathogenicity. Science 318, 930 – 936. (doi:10.1126/science.1138718)
3. Pan KM et al. 1993 Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion protein. Proc. Natl Acad. Sci. USA 90, 10 962 – 10 966. (doi:10.1073/pnas.90.23.10962)
4. Terry C et al. 2016 Ex vivo mammalian prions are formed of paired double helical prion protein fibrils. Open Biol. 6, 160035. (doi:10.1098/rsob.160035)
5. Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M. 1993 Mice devoid of PrP are resistant to scrapie. Cell 73, 1339 – 1347. (doi:10.1016/0092-8674(93)90360-3)
6. Brandner S, Isemann S, Raebel A, Fischer M, Sailer A, Kobayashi Y. 1996 Normal host prion protein necessary for scrapie-induced neurotoxicity. Nature 379, 339 – 343. (doi:10.1038/379339a0)
7. Mallucci G, Dickinson A, Linehan J, Krohn PC, Brandner S, Collinge J. 2003 Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. Science 302, 871 – 874. (doi:10.1126/science.1090187)
8. Sandberg MK, Al-Doujaily H, Sharps B, Clarke AR, Collinge J. 2011 Prion propagation and toxicity \textit{in vivo} occur in two distinct mechanistic phases. Nature 470, 540 – 542. (doi:10.1038/nature09768)
9. Parkin E, Watt NF, Hussain I, Eckman EA, Eckman CB, Manson JC. 2007 Cellular prion protein regulates beta-sectase cleavage of the Alzheimer’s amyloid precursor protein. Proc. Natl Acad. Sci. USA 104, 11 062 – 11 067. (doi:10.1073/pnas.0609621104)
10. Nieznanski K, Choi JK, Chen S, Surewicz W, Surewicz WK. 2012 Soluble prion protein inhibits amyloid-\(\beta\) (A\(\beta\)) fibrilization and toxicity. J. Biol. Chem. 287, 33 104 – 33 108. (doi:10.1074/jbc.C111.009614)
11. Lauren J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. 2009 Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. Nature 457, 1128 – 1132. (doi:10.1038/nature07761)
24. Kessels HW, Nguyen LN, Nabavi S, Malinow R. 2010 Alzheimer’s disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. J. Neurosci. 31, 7259–7263. (doi:10.1523/JNEUROSCI.6050-10.2011)

23. Chen S, Yadav SP, Surewicz WK. 2010 Interaction between human prion protein and amyloid-beta (Abeta) oligomers: role of N-terminal residues. J. Biol. Chem. 285, 26 377–26 383. (doi:10.1074/jbc.M110.145516)

22. Resenberger UK et al. 2011 The cellular prion protein mediates neurotoxic signalling of beta-sheet-rich conformers independent of prion replication. EMBO J. 30, 2057–2070. (doi:10.1038/embj.2011.86)

21. Fluharty BR et al. 2011 Amyloid-beta nanotubes are associated with prion protein-dependent synaptic toxicity. Nat. Commun. 4, 2416. (doi:10.1038/ncomms5416)

20. Bate C, Williams A. 2011 Amyloid-beta-induced synapse damage is mediated via cross-linkage of the prion protein binds to amyloid-beta oligomers and increases the localization of prion protein at the cell membrane. J. Biol. Chem. 286, 8935–8951. (doi:10.1074/jbc.M110.002558)

19. Hu NW et al. 2010 Pharmacological chaperone for prion therapeutics in experimental models. Proc. Natl Acad. Sci. USA 107, 17 610–17 615. (doi:10.1073/pnas.1009062107)

18. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. 2007 Systematic meta-analyses of Alzheimer disease genetic association studies: the Alzheimer database. Nat. Genet. 39, 17–23. (doi:10.1038/ng1934)

17. Fluharty BR et al. 2011 Amyloid-beta-induced synapse damage is mediated via cross-linkage of the prion protein binds to amyloid-beta oligomers and increases the localization of prion protein at the cell membrane. J. Biol. Chem. 286, 8935–8951. (doi:10.1074/jbc.M110.002558)

16. Gimbel DA, Nygaard HB, Coffey EE, Gunther EC, et al. 2010 Memory impairment in transgenic Alzheimer mice requires cellular prion protein. Neuropathol. Appl. Neurobiol. 36, 6367–6374. (doi:10.1039/pn.2011.3178)

15. Larson ME, Lesne SE. 2012 Soluble Abeta oligomer production and toxicity. J. Neurochem. 120(Suppl. 1), 125–139. (doi:10.1111/j.1471-4159.2011.07478.x)

14. Rushworth JV, Griffiths HH, Watt NT, Hooper NM. 2013 Prion protein-mediated toxicity of amyloid-beta oligomers requires lipid rafts and the transmembrane LRP1. J. Biol. Chem. 288, 9825–9835. (doi:10.1074/jbc.M113002588)

13. Freir DB et al. 2012 The prion protein as a molecular sensor with diverse and contrasting potential functions. J. Virol. 86, 10395–10381. (doi:10.1128/JVI.04239-12)

12. Barry AE, Klyubin I, McDonald JM, Mably AJ, Farrell M, Vortmeyer A. 2012 Alzheimer amyloid-beta transgenic Alzheimer mice requires cellular prion protein. FASEB J. 27, 1847–1858. (doi:10.1096/fj.12-222388)

11. Bate C, Williams A. 2011 Amyloid-beta-induced synapse damage is mediated via cross-linkage of the prion protein binds to amyloid-beta oligomers and increases the localization of prion protein at the cell membrane. J. Biol. Chem. 286, 8935–8951. (doi:10.1074/jbc.M110.002558)

10. Kuemmerle K, сторожков DJ, Bujarski DJ. 2011 Evaluation of quinacrine as a sensitive prions using pronase E and phosphotungstic acid. PLoS ONE 5, e150165. (doi:10.1371/journal.pone.0015679)

9. Wagner M, Holzschuh S, Fahr A, Schubert U. 2014 Asymmetric flow field-flow fractionation in the field of nanomedicine. Anal. Chem. 86, 5201–5210. (doi:10.1021/acs.analchem.4b01513)

8. Hepler RW et al. 2006 Solution state characterization of amyloid-beta-mediated fibrillization. Biochemistry 45, 15 157–15 167. (doi:10.1021/bi051850f)

7. Wagner M, Holzschuh S, Fahr A, Schubert U. 2014 Asymmetric flow field-flow fractionation in the field of nanomedicine. Anal. Chem. 86, 5201–5210. (doi:10.1021/acs.analchem.4b01513)

6. Goldfarb PA, Swenson JB, Prather PA, et al. 2011 Sensitive cell-based infectivity assay for mouse scrapie prions. PLoS ONE 6, e2241 – 2265. (doi:10.1371/journal.pone.0015679)
surface. *J. Neurochem.* **117**, 538–553. (doi:10.1111/j.1471-4159.2011.07225.x)

55. Sandberg MK et al. 2014 Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. *Nat. Commun.* **5**, 519. (doi:10.1038/ncomms5347)

56. Antonyuk SV et al. 2009 Crystal structure of human prion protein bound to a therapeutic antibody. *Proc. Natl Acad. Sci. USA* **106**, 2554–2558. (doi:10.1073/pnas.0809170106)

57. Raymond GJ et al. 2006 Inhibition of protease-resistant prion protein formation in a transformed deer cell line infected with chronic wasting disease. *J. Virol.* **80**, 596–604. (doi:10.1128/JVI.80.2.596-604.2006)

58. Morales R, Estrada LD, Diaz-Espinoza R, Morales-Scheihting D, Jara MC, Castilla J 2010 Molecular cross talk between misfolded proteins in animal models of Alzheimer’s and prion diseases. *J. Neurosci.* **30**, 4528–4535. (doi:10.1523/JNEUROSCI.5924-09.2010)

59. Kostylev MA, Kaufman AC, Nygaard HB, Patel P, Haas LT, Gunther EC. 2015 Prion-protein-interacting amyloid-beta oligomers of high molecular weight are tightly correlated with memory impairment in multiple Alzheimer mouse models. *J. Biol. Chem.* **290**, 17415–17438. (doi:10.1074/jbc.M115.63577)

60. Jaunmuktane Z et al. 2015 Evidence for human transmission of amyloid-beta pathology and cerebral amyloid angiopathy. *Nature* **525**, 247–250. (doi:10.1038/nature15369)

61. Appleby BS, Appleby KK, Rabins PV. 2007 Does the presentation of Creutzfeldt–Jakob disease vary by age or presumed etiology? A meta-analysis of the past 10 years. *J. Neuropsychiatry Clin. Neurosci.* **19**, 428–435. (doi:10.1176/jnp.2007.19.4.428)

62. Collinge J. 2005 Molecular neurology of prion disease. *J. Neurol. Neurosurg. Psychiatry.* **76**, 906–919. (doi:10.1136/jnnp.2004.048660)

63. CJD Surveillance in the UK 25th Annual Report. 2016. See http://www.cjdenceuk/sites/default/files/report25pdf.

64. de Silva R, Findlay C, Awad I, Harries-Jones R, Knight R, Will R. 1997 Creutzfeldt-Jakob disease in the elderly. *Postgrad. Med. J.* **73**, 557–559. (doi:10.1136/pgmj.73.863.557)