

RESEARCH PAPER

A β oligomer toxicity inhibitor protects memory in models of synaptic toxicity

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BACKGROUND AND PURPOSE

Amyloid- β (A β) aggregation into synaptotoxic, prefibrillar oligomers is a major pathogenic event underlying the neuropathology of Alzheimer's disease (AD). The pharmacological and neuroprotective properties of a novel A β aggregation inhibitor, SEN1269, were investigated on aggregation and cell viability and in test systems relevant to synaptic function and memory, using both synthetic A β_{1-42} and cell-derived A β oligomers.

EXPERIMENTAL APPROACH

Surface plasmon resonance studies measured binding of SEN1269 to A β_{1-42} . Thioflavin-T fluorescence and MTT assays were used to measure its ability to block A β_{1-42} -induced aggregation and reduction in cell viability. *In vitro* and *in vivo* long-term potentiation (LTP) experiments measured the effect of SEN1269 on deficits induced by synthetic A β_{1-42} and cell-derived A β oligomers. Following i.c.v. administration of the latter, a complex (alternating-lever cyclic ratio) schedule of operant responding measured effects on memory in freely moving rats.

KEY RESULTS

SEN1269 demonstrated direct binding to monomeric A β_{1-42} , produced a concentration-related blockade of A β_{1-42} aggregation and protected neuronal cell lines exposed to A β_{1-42} . *In vitro*, SEN1269 alleviated deficits in hippocampal LTP induced by A β_{1-42} and cell-derived A β oligomers. *In vivo*, SEN1269 reduced the deficits in LTP and memory induced by i.c.v. administration of cell-derived A β oligomers.

CONCLUSIONS AND IMPLICATIONS

SEN1269 protected cells exposed to A β_{1-42} , displayed central activity with respect to reducing A β -induced neurotoxicity and was neuroprotective in electrophysiological and behavioural models of memory relevant to A β -induced neurodegeneration. It represents a promising lead for designing inhibitors of A β -mediated synaptic toxicity as potential neuroprotective agents for treating AD.

Abbreviations

ALCR, alternating-lever cyclic-ratio; AD, Alzheimer's disease; APP, amyloid precursor protein; A β , amyloid- β ; CM, conditioned medium; DMSO, dimethyl sulfoxide; fEPSP, extracellular field excitatory post-synaptic potential; HFS, high-frequency stimulation; LTP, long-term potentiation; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium; SA, streptavidin

Introduction

Progressive accumulation of amyloid- β (A β) assemblies is considered fundamental to the development of neurodegenera-

tive pathology and inflammation that contribute to Alzheimer's disease (AD) (Tanzi *et al.*, 2004). The key pathogenic event in the onset of AD appears to be the formation of extracellular soluble A β peptide into synaptotoxic, prefibrillar

oligomers (Verdile *et al.*, 2004; Walsh and Selkoe, 2004a,b), and accumulating evidence supports a cogent argument that compounds preventing the generation of toxic A β assemblies could provide successful new treatments for AD (Estrada and Soto, 2007). Several studies indicate that AD severity correlates closely with accumulation of soluble oligomers of A β (Lue *et al.*, 1999; McLean *et al.*, 1999; Wang *et al.*, 1999). Consequently, pharmacotherapies that block initial A β assembly prior to the stage of oligomerization (Selkoe and Schenk, 2003; Walsh and Selkoe, 2004b), so as to prevent oligomerization and allow natural neurophysiological clearance mechanisms to effect the removal of A β (Golde, 2006; Walsh and Selkoe, 2007) and preserve normal synaptic function, might provide an effective treatment strategy.

AD is considered a product of initial synaptic failure that precedes neuronal degeneration, and A β -induced dysfunction of synaptic plasticity appears to contribute to early memory loss (Haass and Selkoe, 2007; Shankar *et al.*, 2008). Long-term potentiation (LTP), a well-established model of synaptic plasticity related to memory, involves a sustained increase in excitatory synaptic transmission; and inhibition of LTP by A β may mimic an early manifestation of the memory loss seen in AD (Ondrejcek *et al.*, 2010). Cultured cells that express human A β precursor protein (APP) generate and secrete soluble oligomers of human A β , which contain the A β ₁₋₄₀ and A β ₁₋₄₂ species (Podlisny *et al.*, 1995), and LTP induction in the hippocampus *in vivo* is inhibited by their conditioned medium (7PA2 cell conditioned medium, 7PA2 CM) that contains naturally secreted human A β oligomers (Walsh *et al.*, 2002; Townsend *et al.*, 2006a). In normal adult rats and mice, synaptotoxic A β oligomers have been found to potently inhibit hippocampal LTP *in vitro* (Walsh *et al.*, 2000) and *in vivo* (Walsh *et al.*, 2002). Support for the role of low-n A β oligomers as a key toxic species has come from Cleary *et al.* (2005), who demonstrated that defined molecular species of the A β protein cause acute cognitive deficits in the rat. Specifically, Cleary *et al.* and others have shown that soluble oligomeric forms of A β are both necessary and sufficient to disrupt learned behaviour (Cleary *et al.*, 2005; Townsend *et al.*, 2006b; Poling *et al.*, 2008).

Preventing A β aggregation is a therapeutically attractive neuroprotective approach to preserve synaptic function because this process is believed to be an exclusively pathological event, and drugs targeting this mechanism are likely to have a better safety profile than other pharmacotherapeutic approaches (Amijee and Scopes, 2009). Small molecule starting points for inhibitors of A β aggregation are scarce, and the vast majority of compounds claimed as inhibitors of A β aggregation are unsuitable in terms of their biological profile (Rishton, 2008). RS-0406 (Figure 1) is a non-peptide small molecule that has been shown to be an effective inhibitor of A β toxicity and is also capable of inhibiting A β ₁₋₄₂ aggregation (Nakagami *et al.*, 2002). Our group has used the RS-0406 structure to engineer modifications that improve potency, specificity and bioavailability to provide inhibitors of A β toxicity as improved pharmacological tools to better understand the neuroprotection afforded by such compounds. The novel 5-aryloxy pyrimidine, SEN1269 (Figure 1), shows greater cell penetration and an improved *in vitro* ADME profile (Scopes *et al.*, 2008). The greater cell penetration may additionally allow SEN1269 to

act on intracellular A β oligomer-producing pathways (LaFerla *et al.*, 2007). Here, we demonstrate for the first time that a pharmacologically active, non-peptide, small molecule was protective against A β -induced cell death, synaptotoxicity *in vitro* and *in vivo*, and, consequently, prevented the deficit in cognition induced by 7PA2 CM in a model of memory in freely moving intact animals.

Methods

A β ₁₋₄₂ binding, aggregation and cell viability assays

Surface plasmon resonance was used to measure binding of SEN1269 to A β ₁₋₄₂ (see Item S1). Biotinylated A β ₁₋₄₂ was prepared in 50% dimethyl sulfoxide (DMSO) and pulse injected into a streptavidin (SA) chip surface until the immobilization level was established (Biocore T-100 biosensor); 1 mM biotin was injected into a second flow cell to allow saturation of the SA chip as control. Binding of SEN1269 in the flow phase onto immobilized monomeric A β ₁₋₄₂ was established, and K_D data relative to SEN1269 according to a 1:1 model were determined. A thioflavin-T assay (LeVine, 1999) was used to quantify A β fibrillogenesis and to measure SEN1269 inhibition of A β ₁₋₄₂ aggregation (see Item S1). A β ₁₋₄₂ HCl salt was dissolved in hexafluoroisopropanol, with brief sonification and vortexing. This solution was freeze-dried then dissolved in DMSO. The ability of SEN1269 (or RS-0406 for comparison) to inhibit aggregation induced by 10 μ M A β ₁₋₄₂ was assessed by incubation with A β ₁₋₄₂ at 37°C for 24 h in PBS. At 24 h, a 50 μ L aliquot was dispensed into a black 96-well plate, and 50 μ L thioflavin T (40 μ M in 50 mM glycine buffer) was added to each well. The plate was shaken, and fluorescence was recorded using excitation and emission filters of 440 ± 15 and 485 ± 10 nm, respectively. An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium) assay (Shearman *et al.*, 1995) was used to determine the effect of SEN1269 on the deficit in SH-SY5Y cell viability caused by A β ₁₋₄₂ (see Item S1). Cell wells were prepared with 3 μ L of SEN1269 (or 3 μ L RS-0406 for comparison) in DMSO (mixed with 294 μ L Opti-MEM), then 3 μ L of A β ₁₋₄₂ (2 mM) was added and mixed thoroughly. Fifty microlitres of this solution was aspirated and dispensed into wells containing 50 μ L of media opti-MEM + SH-SY5Y cells (A β ₁₋₄₂ final concentration 10 μ M), and the wells were then incubated for 24 h. Fifteen microlitres of MTT was added to the cell wells, which were then incubated in 5% CO₂ at 37° for 4 h. One hundred microlitres of stop/solubilization solution was added, and the wells were placed in a humidified box at room temperature overnight before reading.

In vitro LTP

Extracellular field excitatory post-synaptic potential (fEPSP) recordings were made from 400 μ m thick transverse hippocampal slices prepared from male Sprague-Dawley rats humanely killed by cervical dislocation. After a minimum of 1 h recovery period in aCSF at room temperature, slices were transferred to an interface chamber, warmed to $30 \pm 1^\circ\text{C}$ and perfused with aCSF. Schaffer collaterals were stimulated (7–28 V, 0.1 ms pulse width, 0.05 Hz) every 20 s with a con-

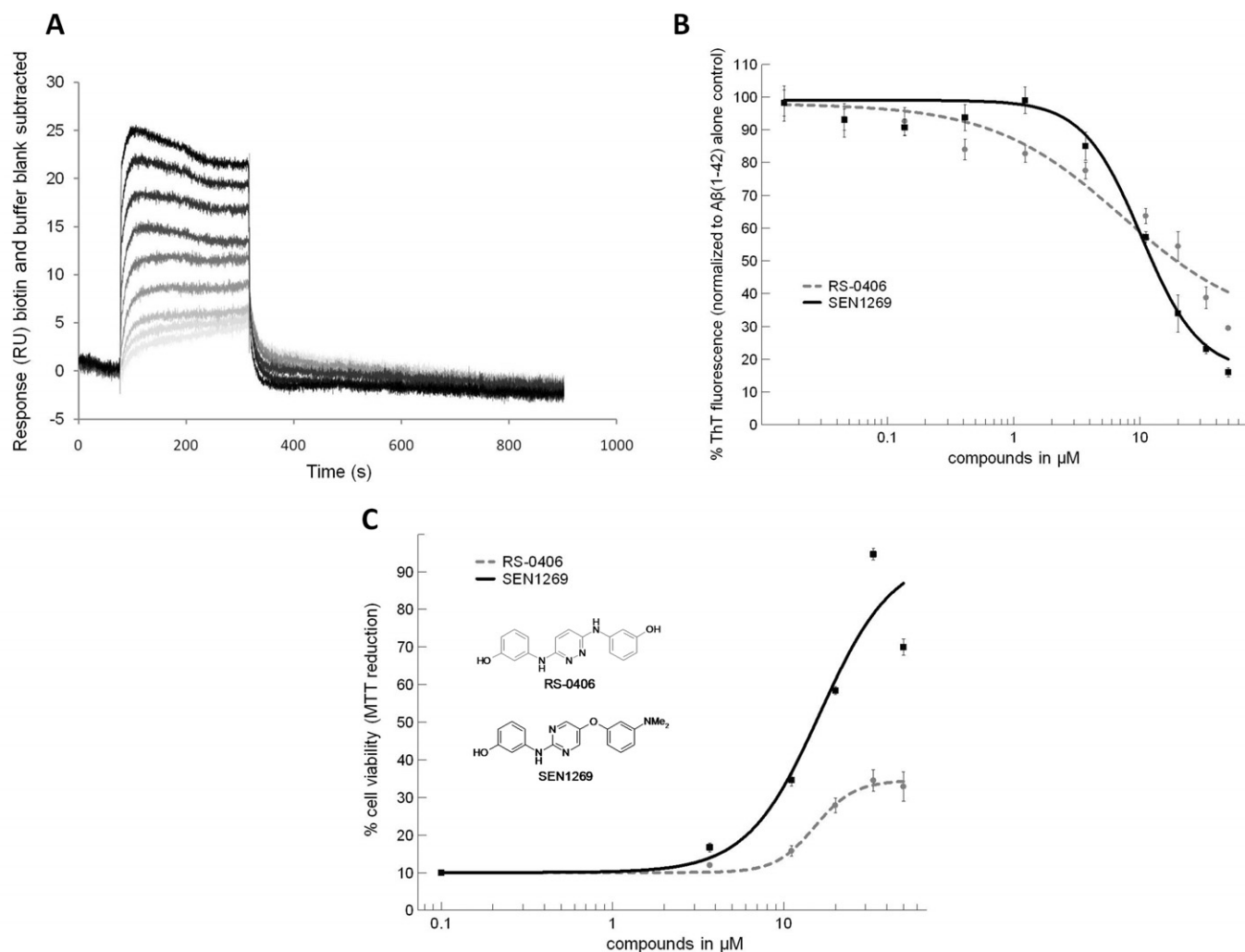


Figure 1

SEN1269 inhibits A β aggregation, protects cell viability against A β _{1–42}-induced insults and binds to A β _{1–42}. (A) Surface plasmon resonance response curves elicited by binding of SEN1269 (0.5, 1, 3, 5, 10, 15, 20 and 30 μ M) to monomeric A β _{1–42}. (B) Thioflavin-T fluorescence concentration–response curves of compounds incubated with 10 μ M A β _{1–42} for 24 h. (C) Concentration–response curves of compounds incubated with 10 μ M A β _{1–42} for 24 h on SHSY5Y cells using MTT colorimetric assay (cell viability level for 10 μ M A β _{1–42} was 10%).

centric bipolar electrode, and fEPSPs were recorded from the stratum radiatum of the CA1 region (Axoclamp 2A amplifier and software; pClamp 8.2, Axon, MDS Analytical Technologies, Sunnyvale, CA, USA) using a glass microelectrode. Stimulation intensity was set to evoke fEPSPs of approximately 50% of the maximum amplitude. A minimum 10 min stable baseline period was recorded before the administration of test substances in aCSF (30 min application period), 10 min before three periods of high frequency stimulation (HFS; 1 s of 100 Hz) at 10 min intervals. A β _{1–42} experimental manipulations (see Item S1) consisted of exposure of the slices to aggregated 1 μ M A β _{1–42} [33 μ M in Tris–HCl + 20 μ L DMSO (stock solution) agitated at 37°C for 24 h using an orbital shaker], or 20 μ L of 5 mM SEN1269 added to the A β _{1–42} stock solution and agitated. The control consisted of 20 μ L DMSO in 1 mL Tris–HCl agitated. These agitated solutions were then diluted to 33 mL in aCSF immediately before they

were applied to the hippocampal slices. 7PA2 CM experimental manipulations (see Item S1) consisted of slice exposure to 7PA2 CM or CHO CM (control) with DMSO; or 60 μ M SEN1269 added to 7PA2 CM or CHO CM, equilibrated for 1 h at room temperature and diluted to 20 mL with aCSF prior to slice application (giving a final SEN1269 concentration of 3 μ M). The fEPSPs were recorded for 80 min after the final HFS stimulation, and the final 10 min of recording (30 sweeps) were selected and averaged for group comparisons using the unpaired *t*-test. 7PA2 CM contains a total human A β concentration of 3223 ± 1217 pg·mL^{–1}, which is similar to that found in human CSF (Walsh *et al.*, 2002), and the oligomeric A β species comprises approximately 10–20% of the total immunoprecipitate of 7PA2 CM (Podlisy *et al.*, 1995). Using ELISA, it was found that the concentration of total A β in the 7PA2 CM used in the LTP and behavioural studies was in the range of 2–5 nM.

In vivo LTP

Animals were kept on a 12 h light/dark cycle with food and water available *ad libitum*. Extracellular fEPSPs were recorded from the hippocampi of 36 anaesthetized (urethane i.p. 1 mL 100 g⁻¹ assessed by cardiovascular responses to paw-pinch and the stability of measured cardiovascular variables with supplements i.v. of 0.2 mL 100 g⁻¹, 12% solution) adult male Sprague–Dawley rats. Core body temperature was maintained at 37°C using a homeothermic blanket, and polyethylene catheters were inserted into the right femoral artery and vein for monitoring arterial blood pressure and for anaesthetic drug administration, respectively. The head was mounted in a stereotaxic frame before lowering a concentric bipolar stimulating electrode and carbon fibre recording electrode vertically into the CA1 area of the hippocampus. Stimulating electrode coordinates (Paxinos and Watson, 1998): bregma -3.5 mm, lateral 2.6 mm and 2.0–2.5 mm below the pial surface, recording electrode coordinates; bregma -4.0 mm, lateral 3.0 mm and 2.0–2.5 mm below the pial surface. Subsequently, a preloaded 30 gauge stainless steel i.c.v. cannula was lowered into the lateral ventricle: bregma +0.5 mm, lateral 1.5 mm and 3.6 mm below the pial surface with a 15–17° rostro-caudal angle. Electrical stimulation (0.1 ms pulse width, 10–100 V, 0.14 Hz) was used to identify and optimise fEPSPs, and an input/output curve was created to determine maximal fEPSP amplitude and the voltage required to generate fEPSPs of 30–40% of maximum amplitude. Stimulation parameters were maintained at 0.03 Hz, and after a stable fEPSP had been recorded for 8 min, 7PA2 or CHO CM with/without SEN1269 (5 µL of 10 or 100 µM SEN1269 added to 495 µL 7PA2/CHO CM, making final concentrations of 100 nM or 1 µM SEN1269) was administered i.c.v., followed by further baseline recording (28 min) before HFS of the Schaffer–collateral pathway. This comprised of three periods (inter-period interval 30 s) of HFS (10 trains of 10 stimuli; 200 Hz; inter-train interval 50 ms). The fEPSPs were recorded for 90 min after HFS and the final 10 min of recording (20 sweeps) were selected and averaged for group comparisons using the unpaired *t*-test.

Behavioural test

Behavioural data were collected using an alternating-lever cyclic ratio (ALCR) schedule of food reinforced operant behaviour, which is recognized as an appropriate and sensitive experimental analysis of behaviour technique (Cleary *et al.*, 2005). Eighty-four male Sprague–Dawley rats (Harlan, Oxford, UK), weighing 220–250 g at the beginning of the experiment, were maintained at 90% of their free-feeding body weights and housed individually with water available *ad libitum* in the home cage. The temperature in the vivarium was maintained at 23°C under a 12 h light/12 h dark cycle (lights on 0800 h). The rats were trained and tested in two-lever rat test chambers (Med Associates Inc., St. Albans, NJ, USA) enclosed in sound-proof compartments. Reinforcers were 45 mg sucrose pellets (BioServ, Frenchtown, NJ, USA), which were delivered into a tray situated midway between the two operant levers, and ALCR training was according to the procedure previously reported (Cleary *et al.*, 2005). Briefly, rats were initially trained to press both levers, in a two-lever operant chamber, according to an

alternating-lever fixed ratio 1 (FR-1) schedule for reinforcement. During the training, the active lever was extended into the chamber and illuminated by a light, while the inactive lever was retracted (forced choice). When the rats were able to obtain 100 reinforcers in 30 min, they were reinforced according to a hierarchical series of alternating-lever FR schedules (FR-2, FR-4, FR-6 and FR-8). They were then trained under a forced choice arithmetic ALCR schedule, comprising of an ascending followed by a descending sequence of the ratio values 3, 6, 9, 12, 15 and 18, over six cycles, until performance showed no change in trends. They were then trained under a forced choice ALCR schedule comprising of an ascending followed by a descending sequence of the ratio values 2, 6, 12, 20, 30, 42 and 56, presented over six cycles. When the rats were capable of completing this forced choice ALCR schedule in 50 min, the forced choice conditions were removed, both levers were extended into the chamber and the light cues were extinguished. When the rats were capable of completing the terminal ALCR schedule in 50 min, they were anaesthetized with fentanyl citrate (0.4 mL·kg⁻¹), placed in a stereotaxic frame and fitted with a permanent indwelling cannula (23 gauge) aimed at the lateral ventricle of the brain. Half of the rats received left lateral ventricle cannula implants, and half received right cannula implants. With the incisor bar set 3.0 mm below the interaural line, stereotaxic coordinates (Paxinos and Watson, 1998) were -1.5 mm lateral, 1.0 mm posterior to bregma and 3.0 mm below the pial surface. The animals were allowed 10 days for recovery before experimental testing, and cannula placement was verified by the observation of vigorous drinking (>5.0 mL in 20 min) following i.c.v. injection of 5.0 µL (0.5 µg·mL⁻¹) angiotensin II (Johnson and Epstein, 1974). Following recovery from surgery, the animals were randomly assigned to one of six groups. Group 1 received an i.c.v. injection of 10 µL 7PA2 CM, followed 2 min later by i.c.v. injection of 10 µL DMSO (the vehicle SEN1269 was dissolved in), to establish the untreated Aβ oligomer behavioural deficit. Group 2 received i.c.v. injection of 10 µL CHO CM, followed 2 min later by i.c.v. injection of 10 µL DMSO, to establish response parameters in the absence induction of a behavioural deficit or treatment. Dose-response parameters were then established in groups 3, 4 and 5, where i.c.v. injection of 10 µL 7PA2 CM was followed 2 min later by i.c.v. injection of (group 3) 10 µL of 100 nM SEN1269, (group 4) 1.0 µM SEN1269 or (group 5) 10 µM SEN1269. In order to determine any behavioural effects specific to SEN1269, group 6 was injected i.c.v. with 10 µL CHO CM, followed 2 min later by 10 µL of 10 µM SEN1269. ALCR data were collected 2 h after i.c.v. injections (Cleary *et al.*, 2005), mean baseline error rates (lever switching errors and incorrect lever perseverations) were established and data were analysed as % of overall baseline performance. To test for motivational or peripheral motor effects induced by the experimental manipulations, the slopes of functions were obtained by plotting response rates against reinforcement rates (motivation) and running response rates against ratio values (motor retardation) were compared. Data were analysed by one-way ANOVA followed by Fisher's *post hoc* least significant difference tests.

All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involv-

ing animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). All studies using animals were conducted under Home Office License (UK), the UK Animals (Scientific Procedures) Act, 1986 and with Ethics Committee approval from the contributing institutions.

Results

A β ₁₋₄₂ binding, aggregation and cell viability assays

Using surface plasmon resonance (see Item S1), immobilized A β ₁₋₄₂ was characterized using A11, OC, 6E10 and 4G8 antibodies: A11 and OC (A β oligomer and A β fibril specific, respectively) showed no binding; whereas 6E10 and 4G8 showed strong binding responses. SEN1269 bound to monomeric A β ₁₋₄₂ in a concentration-related manner (Figure 1A) with a K_D of 4.4 μ M, which suggests that its mode of action involves direct interaction with A β ₁₋₄₂. SEN1269 also bound to A β ₁₋₄₂ oligomers (data not shown). Incubation of SEN1269 with A β ₁₋₄₂ reduced the fluorescence exhibited by thioflavin-T (LeVine, 1999) (see Item S1) in a concentration-related manner (IC_{50} = 11 μ M), indicating blockade of the A β aggregation process, and SEN1269 was more effective in this regard than the parent compound RS-0406 (Figure 1B). In the MTT assay (Shearman *et al.*, 1995) (see Item S1), SEN1269 was effective in protecting neuronal cell lines against an A β ₁₋₄₂ insult (IC_{50} = 15 μ M) and was considerably more effective than the parent compound (Figure 1C). As a positive comparator in the thioflavin-T and MTT assays, the Praecis pentapeptide aggregation inhibitor PPI-1019 (Findeis, 2002) displayed IC_{50} values of 1.5 and 7 μ M, respectively. The cell permeability of SEN1269 was 20.7 and 17.8 $\times 10^{-6}$ cm $\cdot$ s $^{-1}$ for apical-basolateral and basolateral-apical permeability, respectively. RS-0406 showed corresponding values of 0.9 and 1.8 $\times 10^{-6}$ cm $\cdot$ s $^{-1}$, indicating that biological membranes are highly permeable to SEN1269.

In vitro LTP

The LTP of fEPSPs in the CA1 region of hippocampal slices was reduced following application of A β ₁₋₄₂, from a control increase of 66.8 $\pm$ 10.9% to an increase of 17.5 $\pm$ 8.9% after A β ₁₋₄₂ application (P < 0.05 vs. control). This effect was completely reversed by co-incubation of A β ₁₋₄₂ with SEN1269, with LTP under these conditions amounting to 77.2 $\pm$ 9.6% (P < 0.05 vs. A β ₁₋₄₂ alone; Figure 2A–C). SEN1269 (3 μ M) was also effective at reducing the deficit in LTP caused by application of cell-derived A β oligomers (7PA2 CM) to hippocampal slices (Figure 2D–F). Following HFS of the Schaffer collaterals, the LTP of fEPSPs in control amounted to an increase of 66.3 $\pm$ 6.3% of baseline fEPSP amplitude. In the presence of 7PA2 CM, the amplitude of fEPSPs following HFS was lower than that observed prior to HFS, being reduced to 93.7 $\pm$ 9.7% (P < 0.05 vs. control) of baseline fEPSPs amplitude (100%). This inhibition of LTP was almost completely reversed following co-incubation of 7PA2 CM with SEN1269, the magnitude of LTP being restored to 56.9% $\pm$ 11.9% (P < 0.05 vs. 7PA2 CM alone).

In vivo LTP

In anaesthetized rats, SEN1269 was also effective at preventing deficits in LTP induced by 7PA2 CM. After i.c.v. administration of 7PA2 CM, 30 min before HFS, only a modest LTP of 19.9 $\pm$ 8.9% was observed, significantly less than that produced after administration of control CHO CM; 97.2 $\pm$ 12.3% (P < 0.05 vs. control; Figure 3A–C). This depression of LTP induced by 7PA2 CM was partly prevented when it was co-administered with 100 nM SEN1269; the magnitude of LTP recorded under these conditions amounting to 75.5 $\pm$ 13.3% of baseline fEPSPs (P < 0.05 vs. 7PA2 CM alone; Figure 3A–C). In a separate set of experiments, i.c.v. co-administration of 1 μ M SEN1269 with 7PA2 CM 30 min before HFS also significantly prevented the depression of LTP induced by administration of 7PA2 CM alone. LTP of fEPSPs observed after 7PA2 CM administration was depressed, amounting to an increase in fEPSP amplitude of 21.3 $\pm$ 7.7% prevented by co-administration with 1 μ M SEN1269, where LTP amounted to an increase of 98.6 $\pm$ 16.0% (P < 0.05 vs. 7PA2 CM alone; Figure 3D–F). In vehicle experiments, after i.c.v. administration of CHO CM, HFS evoked LTP of 111.2 $\pm$ 14.2%. These data show that SEN1269 protects against the synaptotoxicity induced by 7PA2 CM.

Behaviour

Following i.c.v. injection of 7PA2 CM in rats trained under the ALCR schedule, there was a significant overall treatment effect on lever switching errors 120 min post injection ($F_{5,78}$ = 3.460, P = 0.0071). The 7PA2 CM injected rats exhibited significantly more lever switching errors as compared with the vehicle-injected group (P < 0.05). The 7PA2 CM-injected groups treated with SEN1269 (100 nM, P = 0.532; 1 μ M, P = 0.046; and 10 μ M, P < 0.029) demonstrated significantly fewer lever switching errors than the 7PA2 CM-injected subjects that received vehicle alone (Figure 4A). With respect to incorrect lever perseverations, there was a significant overall treatment effect on incorrect lever perseverations 120 min following 7PA2 CM injections ($F_{5,78}$ = 8.213, P < 0.0001). The 7PA2 CM-injected rats exhibited significantly more incorrect lever perseverations as compared with the vehicle-injected group (P < 0.0001). The 7PA2 CM-injected groups treated with SEN1269 (100 nM, P = 0.019; 1 μ M, P < 0.009; and 10 μ M, P < 0.0001) demonstrated significantly fewer incorrect lever perseverations than the 7PA2 CM-injected subjects that received vehicle alone, and this effect was dose-dependent (Figure 4B). These findings indicate that SEN1269, which inhibited the toxic effects of 7PA2 CM on LTP *in vitro* and *in vivo*, was also protective against 7PA2 CM-induced cognitive deficits *in vivo*. The ALCR schedule also provides data enabling evaluation of the effects of experimental manipulations on the ability to track the changing parameters of the operant schedule, changes in the motivational state of the animal and peripheral effects on motor ability. Analysis of the data indicated no significant effect of 7PA2 CM or SEN1269 (P -values < 0.05); consequently, the data reported are strongly indicative of authentic central effects of the experimental manipulations. These data show that SEN1269 also pharmacologically protects against synaptotoxicity induced by 7PA2 CM to alleviate A β oligomer-induced memory deficits *in vivo*.

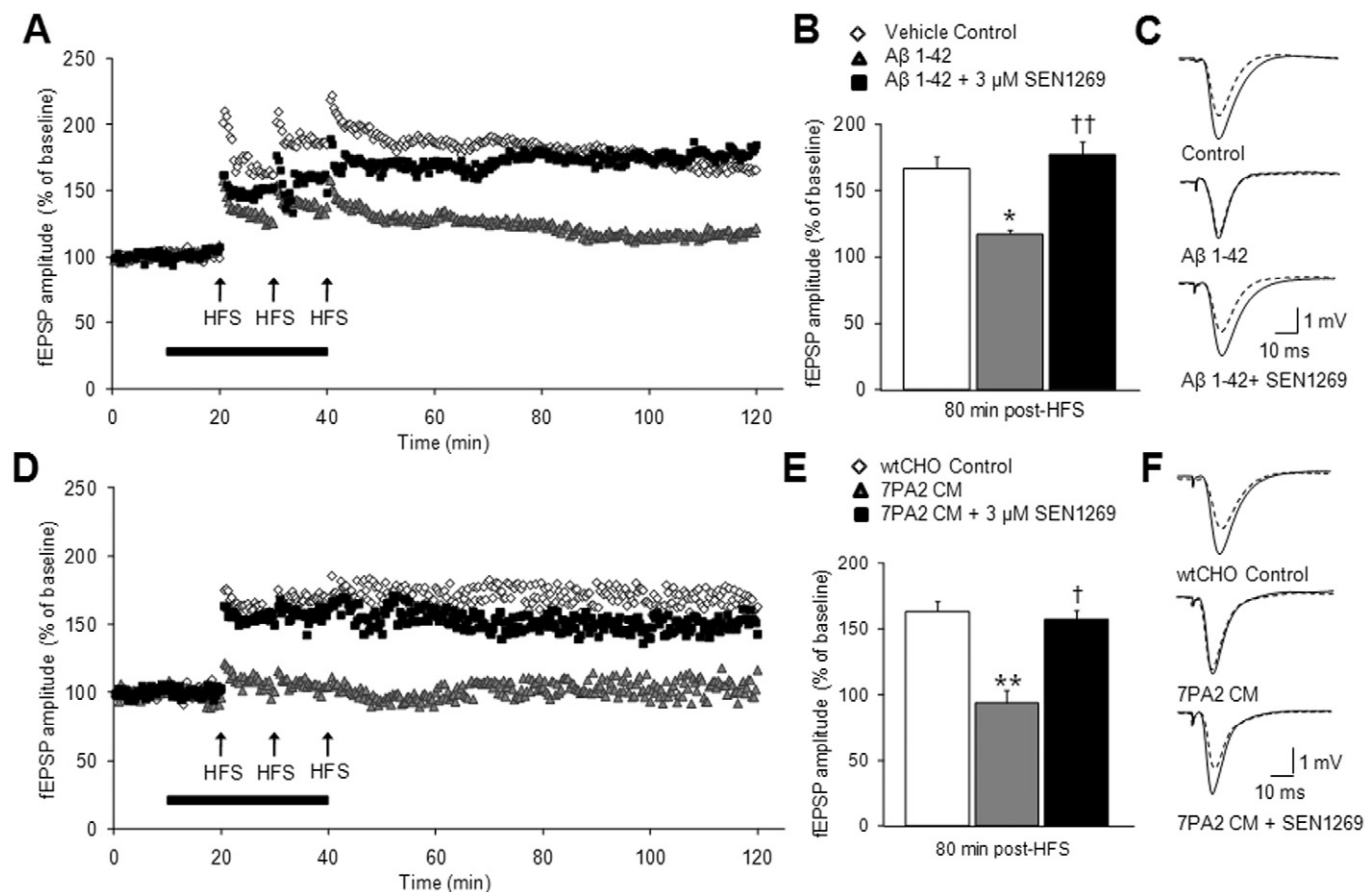


Figure 2

Inhibition of Aβ aggregation and fibrillogenesis by SEN1269 protects against synthetic Aβ₁₋₄₂ and 7PA2 CM-induced deficits in LTP *in vitro*. (A) Scatter plot showing the effect of co-administration of SEN1269 (3 μM) + synthetic Aβ₁₋₄₂ ($n = 4$), Aβ₁₋₄₂ alone ($n = 7$), vehicle control ($n = 4$) on the enhancement of fEPSP amplitude evoked by HFS on hippocampal slices. (B) Histograms showing the level of LTP recorded 80–90 min post-HFS. ** $P < 0.01$ compared to control, †† $P < 0.01$ compared to Aβ₁₋₄₂, † $P < 0.05$ compared to Aβ₁₋₄₂. (C) Typical fEPSPs before and 80 min after HFS for the three treatment groups. Traces shown are the average of 10 sweeps. (D) Scatter plot showing the effect of co-administration of SEN1269 (3 μM) + 7PA2 CM ($n = 4$), 7PA2 CM alone ($n = 4$) CHO CM alone ($n = 4$) on the enhancement of fEPSP amplitude evoked by HFS on hippocampal slices. (E) Histograms showing the level of LTP recorded 80 min post-HFS. ** $P < 0.01$ compared to CHO CM, †† $P < 0.01$ compared to 7PA2 CM, † $P < 0.05$ compared to 7PA2 CM. (F) Typical fEPSPs before and 80 min after HFS for the three treatment groups. Traces shown are the average of 10 sweeps.

Discussion and conclusions

Available therapies only treat the symptoms of AD (Melnikova, 2007; Shah *et al.*, 2008; Wilcock, 2008); however, disease-modifying pharmacological interventions preventing the generation of toxic Aβ assemblies may have the potential to slow, or even halt AD progression. RS-0406, a non-peptide small molecule, has been shown to be an inhibitor of Aβ (Nakagami *et al.*, 2002), to protect primary hippocampal neurons against Aβ₁₋₄₂-induced cytotoxicity, to rescue synthetic Aβ₁₋₄₂-induced impairment of LTP in hippocampal slices, to prevent Aβ-mediated block of LTP by specifically inhibiting oligomer formation (Walsh *et al.*, 2005) and to arrest Aβ oligomer-induced behavioural deterioration in the rat (O'Hare *et al.*, 2010). The current study demonstrates that a modified version of RS-0406, SEN1269, is pharmacologically active and is an improved research tool for *in vitro* and

in vivo use to better understand the relative acute synaptotoxicity caused by Aβ. SEN1269 is a novel, non-peptide small molecular weight compound that binds to Aβ and offers significant neuroprotection biochemically and in electrophysiological and behavioural models of memory relevant to synaptic dysfunction caused by Aβ. Thus, surface plasmon resonance studies illustrated that SEN1269 binds directly to Aβ₁₋₄₂. The thioflavin-T assay indicated that SEN1269 produced a concentration-related blockade of Aβ₁₋₄₂ aggregation, and the MTT assay demonstrated that SEN1269 protected neuronal cell lines exposed to Aβ₁₋₄₂. These results suggest that mechanistically SEN1269 acts by binding to Aβ to prevent its assembly and fibrillogenesis, properties of considerable interest given that the key pathogenic event in AD is believed to be aggregation of the Aβ peptide into synaptotoxic, prefibrillar oligomers (Verdile *et al.*, 2004; Walsh and Selkoe, 2004a,b).

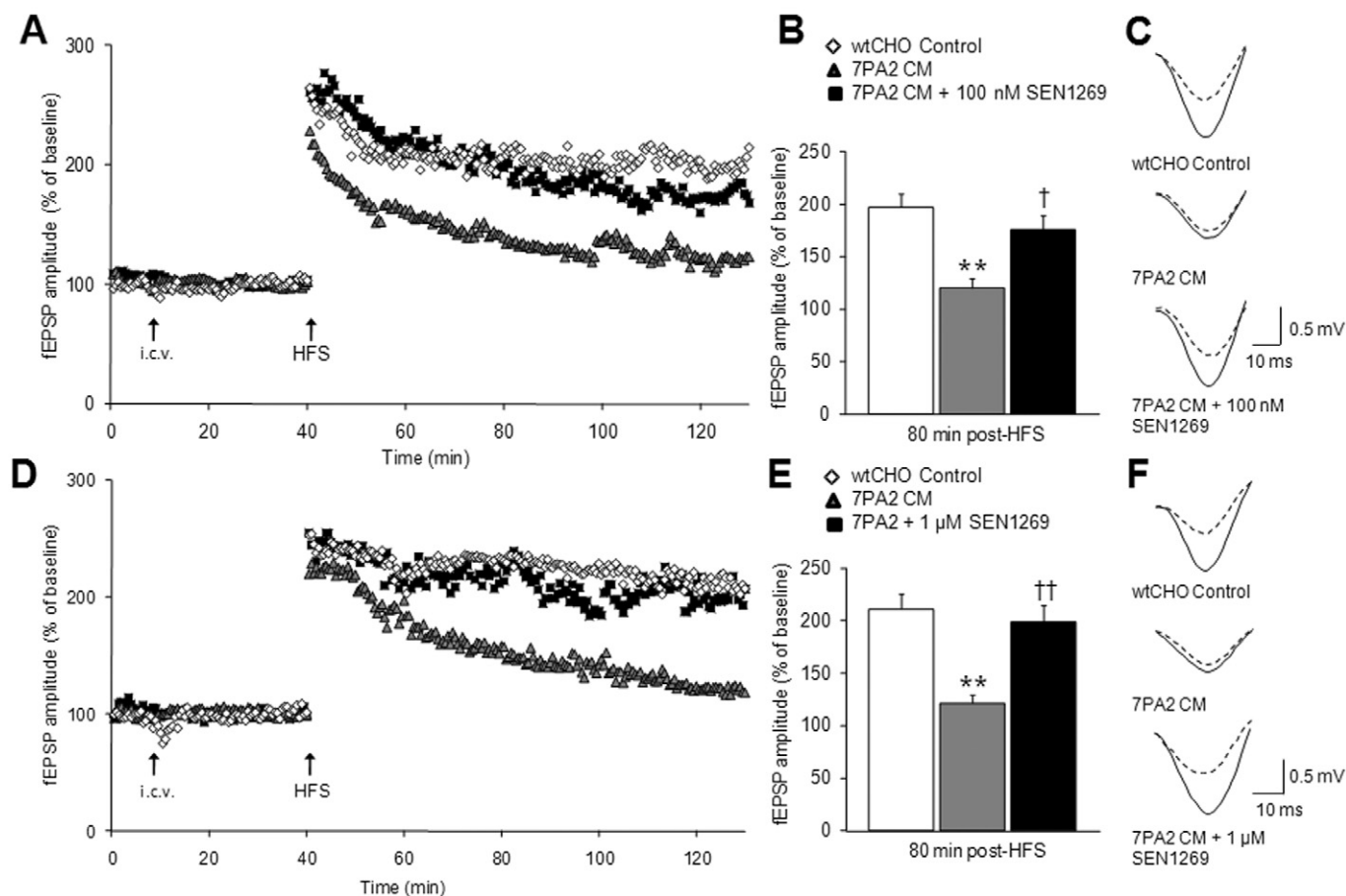


Figure 3

Inhibition of A β aggregation and fibrillogenesis by SEN1269 protects against 7PA2 CM-induced deficits in LTP *in vivo*. (A) Scatter plot showing the effect of co-administration of SEN1269 (100 nM) + 7PA2 CM ($n = 6$), 7PA2 CM alone ($n = 4$) and CHO CM ($n = 4$) on the enhancement of fEPSP amplitude evoked by HFS in the CA1 region of the hippocampus in an anaesthetized rat. (B) Histograms showing the level of LTP recorded 90 min post-HFS. ** $P < 0.01$ compared to CHO CM, † $P < 0.05$ compared to 7PA2 CM. (C) Typical fEPSPs before and 80 min after HFS for the three treatment groups. Traces shown are the average of 10 sweeps. (D) Scatter plot showing the effect of co-administration of SEN1269 (1 μ M) + 7PA2 CM ($n = 6$), 7PA2 CM alone ($n = 10$) and CHO CM ($n = 5$) on the enhancement of fEPSP amplitude evoked by HFS in the CA1 region of the hippocampus in an anaesthetized rat. (E) Histograms showing the level of LTP recorded 90 min post-HFS. ** $P < 0.01$ compared to CHO CM, †† $P < 0.01$ compared to 7PA2 CM. (F) Typical fEPSPs before and 80 min after HFS for the three treatment groups. Traces shown are the average of 10 sweeps.

Electrophysiological studies confirmed that *in vitro* and *in vivo* LTP of synaptic transmission, considered to underlie synaptic plasticity associated with learning and memory, was compromised by synthetic A β_{1-42} or cell-derived A β oligomers (7PA2 CM); and that these effects were reversed by incubation of SEN1269 with A β_{1-42} or 7PA2 CM. Thus, SEN1269 protects against A β -induced synaptotoxicity. Low-n oligomers are present in 7PA2 CM at low- to sub-nanomolar concentrations, similar to those of A β in human CSF (Walsh *et al.*, 2002), and it has been established that these cell-derived A β oligomers are composed of N- and C-terminally heterogeneous human A β peptides, including the A β_{1-40} and A β_{1-42} species that occur in the human brain and extracellular fluids (Podlisny *et al.*, 1995; 1998; Walsh *et al.*, 2002; 2005). Furthermore, in freely moving intact animals trained to respond under a complex schedule of food reinforced behaviour, SEN1269 significantly reduced numbers of lever switching

errors and incorrect lever perseverations induced by i.c.v. 7PA2 CM injections. There were no significant differences in reinforcement rates or running response rates between the 7PA2 CM-injected groups treated with SEN1269 and the 7PA2 CM-injected group that received vehicle, demonstrating that SEN1269 neither increased nor decreased the motivational state or peripheral motor output of the animals, and indicating that the behavioural effect of SEN1269 was central and rescued cognitive processes.

A number of studies have established that A β_{1-42} is the more neurotoxic form of A β (McGowan *et al.*, 2005), A β_{1-40} and A β_{1-42} possess different biochemical properties, and A β_{1-42} is considered to be the major aetiological agent in the pathogenesis of AD due to its more aggressive aggregation or oligomerization properties (Burdick *et al.*, 1992; Jarrett *et al.*, 1993). Compelling evidence indicates that the toxic amyloid species underlying the cognitive deficit in AD are soluble

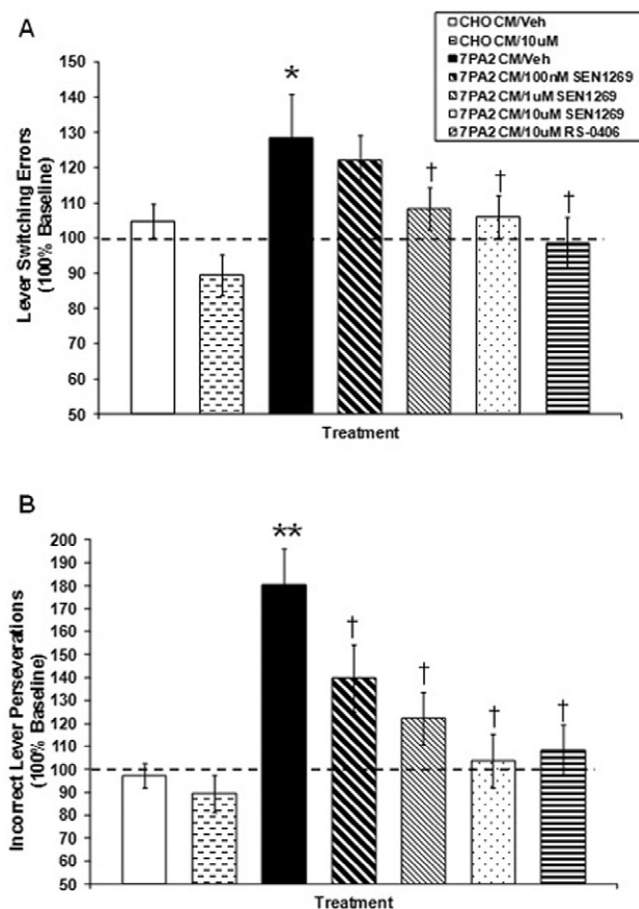


Figure 4

Effect of SEN1269 on 7PA2 CM-induced memory deficits ($n = 14$ for each study arm). (A) Effect of SEN1269 treatment on 7PA2 CM-induced lever switching errors. * $P < 0.05$ compared to CHO-CM, † $P < 0.05$ compared to 7PA2 CM. (B) Effect of SEN1269 treatment on 7PA2 CM-induced incorrect lever perseverations. ** $P < 0.0001$ compared to CHO-CM, † $P < 0.05$ compared to 7PA2 CM. RS-0406 data obtained using the identical behavioural protocol included for comparison (from O'Hare *et al.*, 2010).

low- n oligomers of the A β peptide, which subsequently form insoluble amyloid fibrils via association of the peptide β -strands (Walsh and Selkoe, 2007; Klyubin *et al.*, 2008), and A β dimers have been identified as the smallest synaptotoxic species in Alzheimer's brains (Shankar *et al.*, 2008). Oligomeric A β is able to permeate biological membranes (Glabe, 2006) and the current study used conditioned medium from 7PA2 cells, which contains low- n A β oligomers, to induce electrophysiological and behavioural deficits. Our pharmacological observations lend support to the biological hypothesis that inhibiting the process of A β peptide aggregation centrally may be a viable neuroprotective strategy to potentially treat AD. Moreover, SEN1269 is the first non-peptide small molecule (molecular weight 321) that binds to A β monomer and oligomers, inhibits A β fibrillogenesis, maintains cell viability against a toxic insult of A β_{1-42} , prevents the deficit in LTP caused by synthetic A β_{1-42} or cell-derived oligomers of A β and is effective in protecting against the deficit in cognition

induced by cell-derived oligomers of A β in a behavioural model of memory relevant to synaptic dysfunction caused by A β . As synaptic loss underlies the neuropathology of AD, these new acute data on SEN1269 provide support for the design of further molecules in this structural class, which are orally bioavailable, CNS penetrating and that have therapeutically useful half-lives suitable for testing in chronic animal models that are thought to be relevant to the neuropathology of AD. In the future, development of an orally bioavailable analogue of SEN1269 would be of interest as a potential disease-modifying treatment of AD.

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Conflicts of interest

D S, H A and M T are employees of Senexis Limited and hold share options in the Company. E O'H holds share options in Senexis Limited.

References

- Amijee H, Scopes DIC (2009). The quest for small molecules as amyloid inhibiting therapies for Alzheimer's disease. *J Alzheimers Dis* 17: 33–47.
- Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A *et al.* (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *J Biol Chem* 267: 546–554.
- Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ *et al.* (2005). Natural oligomers of the amyloid- β protein specifically disrupt cognitive function. *Nat Neurosci* 8: 79–84.
- Estrada LD, Soto C (2007). Disrupting β -amyloid aggregation for Alzheimer's disease treatment. *Curr Top Med Chem* 7: 115–126.
- Findeis MA (2002). Peptide inhibitors of beta amyloid aggregation. *Curr Top Med Chem* 2: 417–423.
- Glabe CG (2006). Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. *Neurobiol Aging* 27: 570–575.
- Golde TE (2006). Disease modifying therapy for AD. *J Neurochem* 99: 689–707.
- Haass C, Selkoe DJ (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8: 101–112.
- Jarrett JT, Berger EP, Lansbury PT Jr (1993). The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 32: 4693–4697.

- Johnson AK, Epstein AN (1974). The cerebral ventricles as the avenue for the dipsogenic action of intracranial angiotensin. *Brain Res* 86: 399–418.
- Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). NC3Rs Reporting Guidelines Working Group. *Br J Pharmacol* 160: 1577–1579.
- Klyubin I, Betts V, Welzel AT, Blennow K, Zetterberg H, Wallin A *et al.* (2008). Amyloid beta protein dimer-containing human CSF disrupts synaptic plasticity: prevention by systemic passive immunization. *J Neurosci* 28: 4231–4237.
- LaFerla FM, Green KN, Oddo S (2007). Intracellular amyloid- β in Alzheimer's disease. *Nat Rev Neurosci* 8: 499–509.
- LeVine H III (1999). Quantification of beta-sheet amyloid fibril structures with thioflavin T. *Methods Enzymol* 309: 274–284.
- Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L *et al.* (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol* 155: 853–862.
- McGowan E, Pickford F, Kim J, Onstead L, Eriksen J, Yu C *et al.* (2005). Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron* 47: 191–199.
- McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *Br J Pharmacol* 160: 1573–1576.
- McLean CA, Cherny RA, Fraser FW, Fuler SJ, Smith MJ, Beyreuther K *et al.* (1999). Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 46: 860–866.
- Melnikova I (2007). Therapies for Alzheimer's disease. *Nat Rev Drug Discov* 6: 341–342.
- Nakagami Y, Nishimura S, Murasugi T, Kaneko I, Meguro M, Marumoto S *et al.* (2002). A novel β -sheet breaker, RS-0406, reverses amyloid β -induced cytotoxicity and impairment of long-term potentiation in vitro. *Br J Pharmacol* 137: 676–682.
- O'Hare E, Scopes DI, Treherne JM, Norwood K, Spanswick D, Kim EM (2010). RS-0406 arrests amyloid-beta oligomer-induced behavioural deterioration in vivo. *Behav Brain Res* 210: 32–37.
- Ondrejcek T, Klyubin I, Hu NW, Barry AE, Cullen WK, Rowan MJ (2010). Alzheimer's disease amyloid beta-protein and synaptic function. *Neuromolecular Med* 12: 13–26.
- Paxinos G, Watson C (1998). *The Rat Brain in Stereotaxic Coordinates*. Academic Press: New York.
- Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB *et al.* (1995). Aggregation of secreted amyloid beta-protein into sodium dodecyl sulphate-stable oligomers in cell culture. *J Biol Chem* 270: 9564–9570.
- Podlisny MB, Walsh DM, Amorante P, Ostaszewski BL, Stimson ER, Maggio JE *et al.* (1998). Oligomerization of endogenous and synthetic amyloid beta-protein at nanomolar levels in cell culture and stabilization of monomer by Congo red. *Biochemistry* 37: 3602–3611.
- Poling A, Morgan-Paisley K, Panos JJ, Kim EM, O'Hare E, Cleary JP *et al.* (2008). Oligomers of the amyloid-beta protein disrupt working memory: confirmation with two behavioural procedures. *Behav Brain Res* 193: 230–234.
- Rishton GM (2008). Aggregator compounds confound amyloid fibrillization assay. *Nat Chem Biol* 4: 159–160.
- Scopes DI, Amijee H, Nerou E, Treherne JM, O'Hare E, Jeggo R *et al.* (2008). SEN1269: a novel inhibitor of amyloid beta toxicity. *Alzheimers Dement* 4: P2–358.
- Selkoe DJ, Schenk D (2003). Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol* 43: 545–548.
- Shah RS, Lee HG, Xiongwei Z, Perry G, Smith MA, Castellani RJ (2008). Current approaches in the treatment of Alzheimer's disease. *Biomed Pharmacother* 62: 199–207.
- Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I *et al.* (2008). Amyloid- β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14: 837–842.
- Shearman MS, Hawtin SR, Taylor VJ (1995). The intracellular component of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction is specifically inhibited by beta-amyloid peptides. *J Neurochem* 65: 218–227.
- Tanzi RE, Moir RD, Wagner SL (2004). Clearance of Alzheimer's Abeta peptide: the many roads to perdition. *Neuron* 43: 605–608.
- Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006a). Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers. *J Physiol* 572: 477–492.
- Townsend M, Cleary JP, Mehta T, Hofmeister J, Lesné S, O'Hare E *et al.* (2006b). Orally available compound prevents deficits in memory caused by the Alzheimer amyloid-beta oligomers. *Ann Neurol* 60: 668–676.
- Verdile G, Fuller S, Atwood CS, Laws SM, Gandy SE, Martins RN (2004). The role of beta amyloid in Alzheimer's disease: still a cause of everything or the only one who got caught? *Pharmacol Res* 50: 397–409.
- Walsh DM, Selkoe DJ (2004a). Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* 44: 181–193.
- Walsh DM, Selkoe DJ (2004b). Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration. *Protein Pept Lett* 11: 213–228.
- Walsh DM, Selkoe DJ (2007). A β oligomers – a decade of discovery. *J Neurochem* 101: 1172–1184.
- Walsh DM, Tseng BP, Rydell RE, Podlisny MB, Selkoe DJ (2000). The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry* 39: 10831–10839.
- Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS *et al.* (2002). Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416: 535–539.
- Walsh DM, Townsend M, Podlisny MB, Shankar GM, Fadeeva JV, El Agnaf O *et al.* (2005). Certain inhibitors of synthetic amyloid β -peptide (A β) fibrillogenesis block oligomerisation of natural A β and thereby rescue long-term potentiation. *J Neurosci* 25: 2455–2462.
- Wang J, Dickson DW, Trojanowski JQ, Lee VM (1999). The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Exp Neurol* 158: 328–337.

Wilcock GK (2008). The pharmacological treatment of Alzheimer's disease with cholinesterase inhibitors and memantine. In: Cuello AC (ed.). *Pharmacological Mechanisms in Alzheimer's Therapeutics*. Springer: New York, pp. 36–49.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Item S1 Preparation of stock solutions for biological assays. Full procedures for thioflavin-T and cell viability assays and surface plasmon resonance studies. Compound sample preparation and storage for LTP experiments.

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