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Orally bioavailable small molecule drug protects memory in Alzheimer's disease models

Eugene O'Hare [a,*], David I.C. Scopes [b], Eun-Mee Kim [c], Philip Palmer [a], Martyn Jones [d], Andrew D. Whyment [d], David Spanswick [d,e], Hozefa Amijee [b], Edmund Nerou [b], Bridgeen McMahon [a], J. Mark Treherne [b], Ross Jeggo [d]

[a] School of Psychology, Queen's University, Belfast BT7 1NN, UK
[b] Senexis Limited, Babraham Research Campus, Cambridge CB22 3AT, UK
[c] School of Psychology, University of Ulster, Coleraine BT52 1SA, UK
[d] NeuroSolutions Limited, Coventry CV4 7ZS, UK
[e] Warwick Medical School, University of Warwick, Coventry CV4 7CL, UK

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ABSTRACT

Oligomers of beta-amyloid (Aβ) are implicated in the early memory impairment seen in Alzheimer's disease before to the onset of discernable neurodegeneration. Here, the capacity of a novel orally bioavailable, central nervous system-penetrating small molecule 5-aryloxypyrimidine, SEN1500, to prevent cell-derived (7PA2 [conditioned medium] CM) Aβ-induced deficits in synaptic plasticity and learned behavior was assessed. Biochemically, SEN1500 bound to Aβ monomer and oligomers, produced a reduction in thioflavin-T fluorescence, and protected a neuronal cell line and primary cortical neurons exposed to synthetic soluble oligomeric $A\beta_{1-42}$. Electrophysiologically, SEN1500 alleviated the in vitro depression of long-term potentiation induced by both synthetic $A\beta_{1-42}$ and 7PA2 CM, and alleviated the in vivo depression of long-term potentiation induced by 7PA2 CM, after systemic administration. Behaviorally, oral administration of SEN1500 significantly reduced memory-related deficits in operant responding induced after intracerebroventricular injection of 7PA2 CM. SEN1500 reduced cytotoxicity, acute synaptotoxicity, and behavioral deterioration after in vitro and in vivo exposure to synthetic Aβ and 7PA2 CM, and shows promise for development as a clinically viable disease-modifying Alzheimer's disease treatment.

1. Introduction

One of the major diseases of the nervous system associated with age is Alzheimer's disease (AD). Recent theories of the development of neuropathology and behavioral decline in AD provide a persuasive argument that an early stage involves the formation of extracellular soluble β-amyloid peptide (Aβ) into oligomers (Verdile et al., 2004; Walsh and Selkoe, 2004a, 2004b). As a consequence of this approach, memory loss, 1 of the major early symptoms of AD, has been proposed to be the result of synaptic dysfunction related to extracellular Aβ oligomer formation (Haass and Selkoe, 2007; Shankar et al., 2008), which might precede the development of discernible neuropathology. Support for the contention that initial accumulation of extracellular oligomeric Aβ detrimentally affects memory before the onset of quantifiable histopathology has come from several sources. Cultured cells that express human Aβ precursor protein (7PA2 [conditioned medium] CM) secrete oligomers of human Aβ (Podlisny et al., 1995) and these Aβ oligomers are similar to those found in human cerebrospinal fluid (Walsh et al., 2002). Secretions from the cultured cells contain natural dimers, trimers, and tetramers of Aβ that are composed of N- and C-terminally heterogeneous human Aβ peptides, including the $A\beta_{1-40}$ and $A\beta_{1-42}$ species that occur in human brain and extracellular fluids (Podlisny et al., 1995, 1998; Walsh et al., 2002, 2005). Using long-term potentiation (LTP), a well established model of synaptic plasticity which provides an experimental representation of memory processes at the cellular level, it has been shown that LTP induction in the hippocampus is detrimentally affected by exposure to these Aβ oligomers in normal rats and mice. Aβ oligomers have been found to significantly inhibit hippocampal LTP in vitro (Walsh et al., 2000) and in vivo (Walsh et al., 2002), and it has been suggested that this inhibition effect might mimic an early manifestation of the memory loss seen in AD (Ondrejcak et al., 2010). Moreover, using the alternating-lever cyclic-ratio (ALCR) schedule of behavioral analysis, it has been shown that

* Corresponding author at: School of Psychology, Queen's University, University Road, Belfast BT7 1NN, UK. Tel.: +44 2890 975445; fax: +44 2890 975446.
E-mail address: e.ohare@qub.ac.uk (E. O'Hare).

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intracerebroventricular (i.c.v.) injection of Aβ oligomers in the rat produces acute cognitive deficits, and that Aβ oligomers are necessary and sufficient to disrupt memory-related behavior in freely moving intact animals (Cleary et al., 2005; Poling et al., 2008; Townsend et al., 2006).

Consequently, contemporary research findings suggest that extracellular formation of Aβ oligomers at an early stage of disease development warrants consideration relative to processes underlying AD. From a therapeutic perspective, the progressive accumulation of Aβ assemblies has long been considered fundamental to development of the neurodegenerative pathology seen in AD (Tanzi et al., 2004), and preventing the aggregation of Aβ has been an appealing therapeutic approach. Targeting the effects of Aβ oligomers, and the oligomer assembly mechanism, is of interest because it is likely to offer a better safety profile than other pharmacotherapies (Amijee and Scopes, 2009). Unfortunately, nonpeptide small molecule inhibitors of Aβ aggregation have been extremely scarce, and the majority of compounds claimed to be Aβ aggregation inhibitors are unsuitable from a pharmacotherapeutic perspective because of their biologic profile, poor oral bioavailability, and short half-life (Rishton, 2008). However, 1 nonpeptide small molecule, RS-0406, inhibits Aβ toxicity and $A\beta_{1-42}$ aggregation (Nakagami et al., 2002) and has been shown to protect primary hippocampal neurons against $A\beta_{1-42}$-induced cytotoxicity, to rescue $A\beta_{1-42}$-impaired LTP (Walsh et al., 2005), and to arrest Aβ oligomer-induced behavioral deterioration in the rat when administered intracerebroventricularly (O'Hare et al., 2010). Taking these findings into account, our group has modified the RS-0406 chemical structure to improve its potency, specificity, and oral bioavailability. In the current series of experiments a novel derivative of RS-0406, the 5-aryloxypyrimidine SEN1500 (Fig. 1), was investigated relative to $A\beta_{1-42}$ aggregation and $A\beta_{1-42}$-induced deficits in cell viability, depression of LTP in vitro and in vivo after exposure to aggregated $A\beta_{1-42}$ and Aβ oligomers, and the effects of oral administration on cognitive impairment after i.c.v. injections of 7PA2 CM-derived Aβ in normal freely moving rats.

2. Methods

2.1. Compound

SEN1500 (2-Fluoro-5-[5-(3-morpholin-4-ylphenoxy)-pyrimidin-2-ylamino]benzonitrile, 98.9% purity) was synthesised by Oxygen Healthcare Limited (Ahmedabad, India).

2.2. SEN1500 effects on Aβ binding, aggregation, and cell viability

Recombinant $A\beta_{1-42}$ (rPeptide, Bogart, GA, USA) was prepared for amyloid aggregation and toxicity assays by dissolving $A\beta_{1-42}$ HCl salt in hexafluoroisopropanol with brief sonication and vortexing. This solution of the $A\beta_{1-42}$ peptide in hexafluoroisopropanol was stored at 4 °C at 2 mM. When required, an aliquot of the stock solution was freeze-dried and dissolved in dimethyl sulfoxide (DMSO) to 200 times the required final assay concentration. For thioflavin-T and cell viability assays, a 20 mM stock solution was prepared in DMSO and aliquots of this solution were used to prepare further stock solutions in DMSO, ranging in concentration from 3 μM to 10 mM. These stock solutions were prepared for use as required and stored at −20 °C (maximum of 3 freeze-thaw cycles).

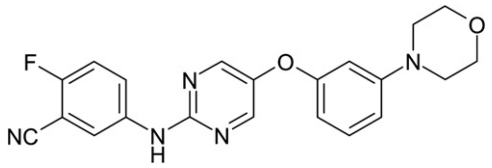

Fig. 1. Chemical structure of SEN1500 (2-Fluoro-5-[5-(3-morpholin-4-ylphenoxy)-pyrmidin-2-ylamino]benzonnitrile). Purity 98.9%.

2.3. Surface plasmon resonance studies with SEN1500

A Biacore T-100 protein interaction analysis system (GE Healthcare) equipped with 4 flow cells on a sensor chip was used for real-time binding studies. HBS-EP buffer, which contained 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.15 M NaCl, 3 mM ethylenediaminetetraacetic acid and 0.5% of Tween was used as the assay running buffer and also for sample preparation. A streptavidin (SA) chip (GE Healthcare) was used to immobilize biotinylated $A\beta_{1-42}$ monomer or oligomers on different flow cells. N-terminus biotinylated $A\beta_{1-42}$ was freshly prepared in 50% DMSO and pulse injected onto the SA surface until the required immobilization level of approximately 1300 response units was reached to give a monomer surface. $A\beta_{1-42}$ oligomers were prepared using the method described by Kayed et al. (2003) to facilitate more reproducible biotinylation than with alternative oligomeric preparations, but modified in that a 1:10 ratio of biotinylated $A\beta_{1-42}$ to non-biotinylated $A\beta_{1-42}$ was used. When prepared, these Aβ oligomers were injected over a flow cell at 2 μL/min for 15 minutes to achieve full saturation of the flow cell surface. As a control, 1 mM biotin was injected onto another flow cell to allow saturation of the SA chip with biotin. The binding of SEN1500 in the flow phase, onto immobilized $A\beta_{1-42}$ monomer or oligomer, was measured by response units. The response units elicited by SEN1500 injected onto the biotin control flow cell was set as the reference response, which represented the refractive index signals caused by solvent in the injected samples and was subtracted from the response units elicited by the same compounds injected onto the $A\beta_{1-42}$ flow cells. To perform the binding studies, 120 μL of SEN1500 ranging from 0.5 μM to 30 μM prepared in HBS-EP buffer containing 5% DMSO were injected and passed onto the sensor chip for 4 minutes at 30 μL/min, and responses were recorded. After each injection, HBS-EP with 5% DMSO buffer was passed over the chip for 10 minutes at 30 μL/min, to allow the bound compound to dissociate from the immobilized $A\beta_{1-42}$ monomer, and the dissociation curves were obtained. The response elicited by injecting HBS-EP buffer alone with 5% DMSO was used as the blank. To establish the negative control condition, an analogue of SEN1500 which was inactive in the fibrillogenesis and cell viability assays was used, and was found not to bind to immobilized $A\beta_{1-42}$. After the dissociation phase, 15 μL of the regeneration solution, 1 M NaCl in 20 mM NaOH was injected and passed over the chip for 1 minute at 30 μL/min to remove residual bound compound from immobilized $A\beta_{1-42}$. Biacore T-100 control and evaluation software (version 2.0.2) was employed to record the changing responses, plot the binding curves, analyze the data, and calculate the K_D of SEN1500 according to a 1:1 binding model for binding to $A\beta_{1-42}$ monomer.

2.4. Thioflavin-T assay

Activity in inhibiting 10 μM $A\beta_{1-42}$ aggregation was assessed using a thioflavin-T fluorimetric assay. SEN1500 was incubated in 50 mM NaPi, 150 mM NaCl with 20 μM $A\beta_{1-42}$ at a final DMSO concentration of 2% for 24 hours at 37 °C, pH 7.4. A 50 μL aliquot was taken and dispensed into a black 96-well plate. An equal volume (50 μL) of thioflavin-T (40 μM) (in Glycine buffer [50 μM] and NaOH pH 8.5) was added to each well. The plate was shaken and fluorescence and recorded using the top reader (Biotek Synergy) setting