

A novel compound, SEN606 recovers Amyloid β 1-42 induced depression of LTP *in vitro*.

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❶ INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterised by a progressive loss of cognitive abilities. AD is the most common cause of dementia among people aged over 65 and it is conservatively estimated that 4.5 million people currently have the disease, with the prevalence doubling for every 5-year age group beyond age 65.

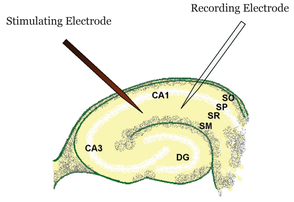
AD is pathophysiologically characterised by neuropathological amyloid deposits, neurofibrillary tangles and neuronal loss in a number of brain regions. The principal constituents of amyloid deposits are amyloid beta proteins (Aβ). Aβ proteins are derived from amyloid precursor protein (APP). Advancements in molecular, cellular and animal model studies have revealed that the formation of Aβ and other derivatives of APP are key factors in cellular changes in the AD brain, including the generation of free radicals, oxidative damage, and inflammation.

It is widely accepted that application of Aβ results in an impairment of long-term potentiation (LTP), a model of synaptic plasticity. Here, we show, a novel compound; SEN606 can reverse the depression of LTP caused by 1μM rat Aβ (1-42) in the CA1 region of rat hippocampal slices.

❷ METHODS

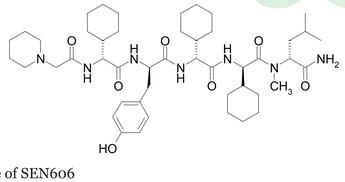
Female Sprague Dawley rats (6 - 8 weeks, 150-250g) were humanely killed by cervical dislocation. Transverse hippocampal slices of 400μm thickness were cut in chilled (< 4°C) aerated artificial cerebrospinal fluid (aCSF) using a Leica VT1000S microtome. Slices were subsequently maintained in oxygenated (95% O2 / 5% CO2) aCSF at room temperature for at least 1 hr prior to electrophysiological recording; aCSF had the following composition (mM): NaCl 127, KCl 1.6, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26 and Glucose 10. After the recovery period, slices were transferred to a modified 'Haas' interface chamber and constantly perfused with warmed (~30°C) aerated aCSF at a rate of 1.5-3.0 ml min^{-1}. Schaffer collaterals were stimulated (3-22 V, 0.1 ms pulse width, 0.05 Hz) with a concentric bipolar electrode and the evoked field excitatory postsynaptic potentials (fEPSPs) extracellularly recorded using an Axoclamp 2A amplifier from the stratum radiatum of the CA1 region of the hippocampus with a glass capillary microelectrode filled with 4 M NaCl (resistance 2-4 MΩ). Stimulation parameters were set so as to produce a fEPSP of approximately 50% of the maximum amplitude. A 10 minute stable baseline period (control) was recorded using Axon software (pClamp suite 8.2), followed by administration of appropriate concentration of Aβ1-42/SEN606 (see below) or vehicle control solution (Tris-HCl, pH 7.33) for 10 minutes prior to high frequency stimulation (HFS; 1 sec 100Hz train) applied three times at 10 minute intervals and subsequent wash of Aβ1-42 after the final HFS protocol. Experiments with unstable baselines were discarded. A minimum of 3 animals were used for each concentration of compound.

Aβ1-42 was prepared as a 120μM Stock solution in Tris-HCl. SEN606 were prepared as a 12mM stock solution in DMSO. All solutions were stored at -20°C until use. Prior to the day of experimentation, SEN606 was applied to the stock Aβ1-42 at the appropriate concentration (immediately upon defrosting) and agitated at 37°C for 24hr using a heated orbital shaker. On the experimental day it was dissolved at the appropriate concentration in aCSF immediately prior to use. Aβ1-42 solutions containing 1% DMSO (aggregated as described above) were ran as a control.



All data are presented as means ± S.E.M. Significant differences of data were calculated using the Students t-test. Probability values of $p < 0.05$ were considered to represent significant differences.

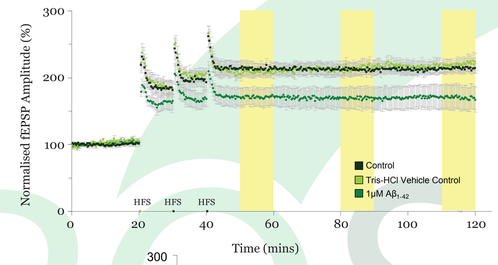
❸ SEN606



Molecular Structure of SEN606

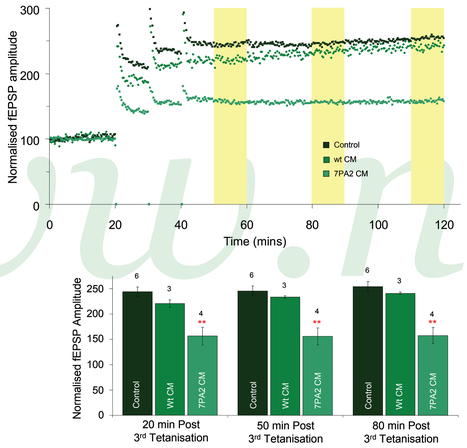
❹ 1μM Aβ1-42 depresses LTP

1 μM Aβ1-42 had a significant effect on the induction of LTP, depressing LTP from control values of 222.1 ± 8.5%, 228.3 ± 9.8% and 232.3 ± 8.7% to values of 158.5 ± 10.6%, 156.8 ± 10.7% and 156.9 ± 11.3%, at 20, 50 and 80 minutes post HFS respectively (n=8, $p < 0.001$). Vehicle controls (Tris-HCl) were without significant effect on LTP induction.



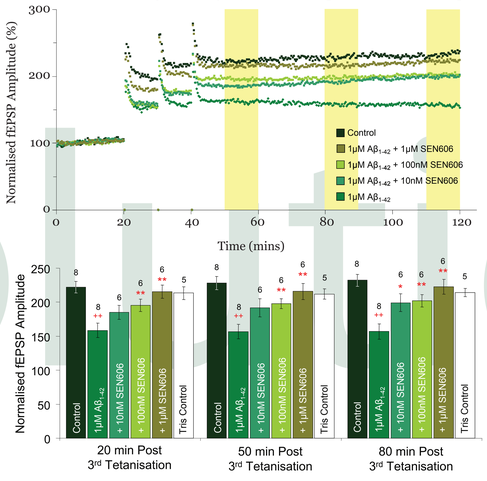
❺ 7PA2 Conditioned Media depresses LTP

7PA2 CM had a significant effect on the induction of LTP, depressing LTP from control values of 244.3 ± 9.1 %, 245.9 ± 9.6 % and 254.2 ± 10.0 % to values of 156.5 ± 17.7 %, 156.0 ± 16.6 % and 157.7 ± 15.8 %, at 20, 50 and 80 minutes post final tetanisation respectively (n=4, $p < 0.01$). wtCHO CM had no significant effect on LTP induction, slightly reducing control values to 221.0 ± 7.6 %, 233.6 ± 2.6 % and 241.3 ± 2.5 %



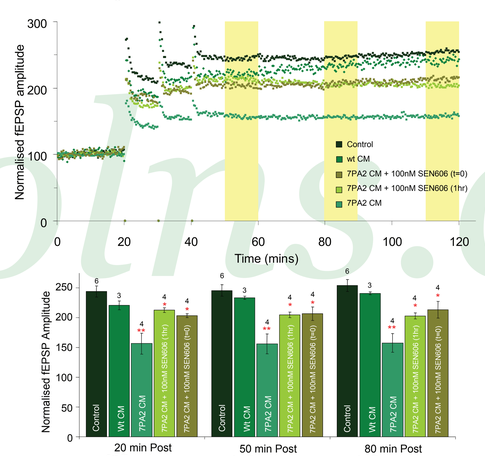
❻ SEN606 recovers Aβ1-42 induced depression of LTP

100 nM SEN606 resulted in a significant reversal of the Aβ1-42 induced depression of LTP, recovering the depression to mean normalised values of 195.4 ± 11.8%, 198.1 ± 8.1% and 202.2 ± 7.0%, at 20, 50 and 80 minutes post HFS respectively (n=6, $p < 0.05$ for 20 mins, $p < 0.01$ for 50 and 80 mins), a 60.1% recovery of the Aβ1-42 induced depression at 80 mins post HFS



❼ SEN606 recovers 7PA2 CM induced depression of LTP

100nM SEN606 resulted in a significant reversal of the 7PA2 CM induced depression of LTP, recovering the depression to mean normalised values of 213.1 ± 4.1 %, 204.7 ± 4.5 % and 203.1 ± 5.0 %, at 20, 50 and 80 minutes post final tetanisation respectively (n=4), a 47.0 % recovery of the 7PA2 CM induced depression



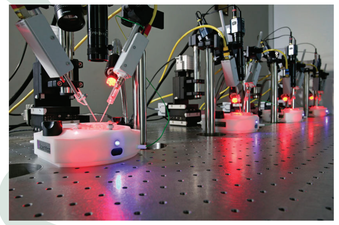
❽ CONCLUSIONS

Our data support the idea that novel compounds can directly neutralize potentially synaptotoxic Aβ and may lead to a reversal of Alzheimer's disease.

❾ Scientifica Slicemaster

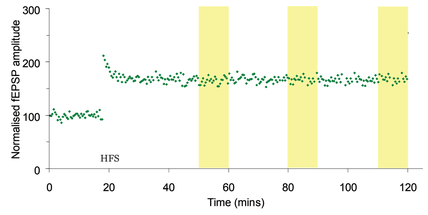
NeuroSolutions Ltd. have recently aquired a Scientifica Slicemaster semi-automated novel high throughput screening system allowing concurrent and independent recordings from up to four brain slices thus improving throughput, statistical design, and reducing animal useage.





❿ *In Vivo* Electrophysiology

NeuroSolutions Ltd. also offer *in vivo* LTP recording capabilities allowing our clients to test their compounds from the level of the brain slice through to the level of whole animal physiology.



NeuroSolutions Ltd.

NeuroSolutions Ltd. are a UK based leading provider of specialised *in vitro* and *in vivo* electrophysiological services to the biopharmaceutical industry. *In vivo* services employ extracellular recordings from many areas of the CNS, PNS and spinal cord, while *in vitro* services include patch clamp recordings from stable cell lines, neuronal cultures and a diverse range of brain and spinal cord slice preparations. NeuroSolutions Ltd. pride themselves on our ability to work with clients to provide individually tailored protocols which they require.

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