Simufilam's primary mechanism of action confirmed by time-resolved FRET

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INTRODUCTION

Simufilam is a novel drug candidate in Phase 3 clinical trials for Alzheimer's Disease (AD) dementia. This oral small molecule targets an altered form of filamin A (FLNA) found in AD. The drug disrupts FLNA's aberrant linkage to the α7 nicotinic acetylcholine receptor (α7nAChR), thereby blocking soluble amyloid beta$_{1-42}$ (Aβ_{42})'s signaling via α7nAChR that hyperphosphorylates tau.[1] Simufilam has reduced levels of Aβ_{42}–α7nAChR complexes in brains of transgenic AD mice and lymphocytes of AD patients (oral treatment) and in postmortem human AD brain (ex vivo incubation).[2,3] We also previously showed that simufilam reduced binding affinity of Aβ_{42} for α7nAChR by 1000- to 10,000-fold using direct binding of labelled simufilam.[2] The current work measured simufilam's effect on the Aβ_{42}–α7nAChR interaction using time-resolved fluorescence resonance energy transfer (TR-FRET),[4] a robust technology to detect highly sensitive molecular interactions.

OBJECTIVE

To replicate similar findings using other methods, we tested inhibition by simufilam of the tight binding of Aβ_{42} to α7nAChR using an established cell-based assay relying on TR-FRET.[4]

METHODS

To monitor Aβ_{42} binding to α7nAChR by a TR-FRET assay, HEK293T cells were transfected to express SNAP-α7nAChR and the chaperone protein NACHO. Surface SNAP-α7nAChR was labelled with the long-lived fluorophore Terbium cryptate (Tb) 48 h post-transfection by incubation with the Tb-conjugated SNAP substrate in Tag-lite labelling medium (100 nM, 1h, 4°C). After 3 PBS washes, cells were distributed into a 384-well plate with assay buffer. Varying concentrations of simufilam or unlabelled Aβ_{42} were added, followed by 10 nM Aβ_{42}-FAM (5-carboxyfluorescein-labelled Aβ_{42}). Plates were incubated 2–4 h at RT and read in a Tecan F500 plate reader with these settings: donor excitation at 340 nm; 1st emission detection at 520 nm (acceptor) and 2nd emission at 620 nm (donor); delay: 150 µs; integration time: 500 µs. Data are expressed as the acceptor/donor ratio normalized as % of maximal Aβ_{42}-FAM binding (maximal TR-FRET ratio = 100%). Specific binding is defined as the difference between total binding and non-specific binding (in the presence of an excess of unlabelled Aβ_{42} (1 µM).

RESULTS

Simufilam reduced Aβ_{42} binding to α7nAChR with a pIC$_{50}$ of 10.9 compared to 11.9 for unlabeled Aβ_{42} (direct competition) and similar to previously published pIC$_{50}$s of several agonists, partial agonists or competitive antagonists of α7nAChR (range: 8.4 to 12.7 pIC$_{50}$). The full inhibition by simufilam was 92% ± 16% of that of unlabelled Aβ_{42} (Fig. 1).

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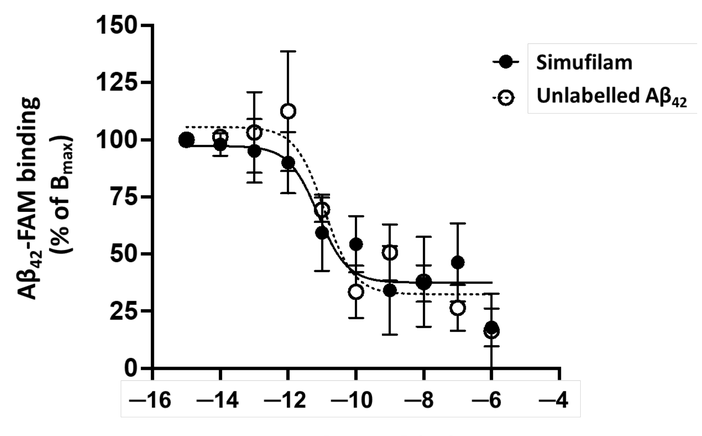

The pIC$_{50}$ of simufilam is similar to previously published[4] pIC$_{50}$s of agonists, partial agonists and antagonists of α7nAChR in the table below. The Aβ_{42} oligomer preparation (24-h incubation of monomers at 4°C) used previously as the reference competitor is slightly and non-significantly more effective than a monomer solution used immediately.[4] The present work used the monomer solution as the reference and for I_{max} calculation.

α7nAChR Ligand	Classification	pIC$_{50}$	I_{max} (% of Aβ_{42})
Aβ_{42} oligomers	Reference competitor	10.6 ± 0.2	100%
Aβ_{42} monomers	Reference competitor	11.9 ± 0.5	100%
Simufilam	FLNA-binding compound	10.9 ± 0.5	92% ± 16
Epibatidine	Agonist	9.5 ± 0.5	69% ± 12
PNU-282987	Agonist	9.3 ± 0.8	66% ± 10
S24795	Partial agonist	9.1 ± 0.5	83% ± 14
EVP-6124	Partial agonist	8.4 ± 0.5	72% ± 12
α-bungarotoxin	Competitive antagonist	12.7 ± 0.4	81% ± 8
Methylylcaconitine (MLA)	Non-competitive antagonist	9.5 ± 0.4	100% ± 20
Mecamylamine	Non-competitive antagonist	Could not be determined	Could not be determined
NS1738	PAM (Type 1)	Could not be determined	Could not be determined
PNU-120596	PAM (Type 2)	8.2 ± 0.6	85% ± 13

DISCUSSION

Using a robust technology designed to detect highly sensitive molecular interactions, we confirmed simufilam's primary mechanism of potently reducing Aβ_{42} binding to α7nAChR. Simufilam's low picomolar IC$_{50}$ and magnitude of inhibition very close to that of unlabelled Aβ_{42} are comparable to compounds acting by direct competition and unprecedented for binding a receptor-associated protein. These new data, now published,[5] confirm previous demonstrations of simufilam's disruption of Aβ_{42}–α7nAChR complexes in brains of treated mice and in postmortem human AD brain following ex vivo incubation.

CONCLUSIONS

Simufilam's high potency in reducing Aβ_{42}–α7nAChR binding, measured by TR-FRET, is unprecedented for its mechanism of binding a receptor-associated protein. Simufilam's picomolar IC$_{50}$ in reducing this interaction corroborates previous data using other techniques that show picomolar IC$_{50}$s for inhibiting the Aβ_{42}–α7nAChR interaction, tau hyperphosphorylation, and FLNA linkages to α7nAChR and TLR4.

REFERENCES

1. Burns, L.H., Pei, Z. and Wang, H-Y. (2023). Targeting α7 nicotinic acetylcholine receptors and their protein interactions in Alzheimer's disease drug development. *Drug Dev Res* DOI: 10.1002/ddr.22085.
2. Wang H-Y, Lee K-C, Pei Z, Khan A, Bakshi K, Burns L. PTI-125 binds and reverses an altered conformation of filamin A to reduce Alzheimer's disease pathogenesis. Neurobiol Aging 2017;55:99-114.
3. Wang H-Y, Pei Z, K.-C. Lee K-C, et al. PTI-125 reduces biomarkers of Alzheimer's disease in patients. J Prevent Alzheimer's Disease 2020:1-9.
4. Cecon E, Dam J, Luka M, et al. Quantitative assessment of amyloid β peptide binding to α7 nicotinic receptor. Br J Pharmacol. 2019;176:3475–3488.
5. Wang H-Y, Cecon E, Dam J, et al. Simufilam reverses aberrant receptor interactions of filamin A in Alzheimer's disease. 2023;24:13927.

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