Poster 032

GAMMA-SECRETASE MODULATION RESULTS IN MULTIPLE ANTI-AMYLOIDOGENIC EFFECTS IN VIVO

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Background and objectives

Aggregation of the Aβ42 peptide results in amyloid plaque formation, a process that plays a pivotal role in early Alzheimer disease pathogenesis. To interfere with Aβ42 production and amyloid plaque formation is therefore a prioritized therapeutic strategy. Modulators of γ-secretase, the enzyme that generates Aβ42 and other Aβ peptides, do not affect total γ-secretase activity per se, but alters the production ratio of different Aβ peptides. Thus, production of the longer amyloidogenic peptides, Aβ40 and Aβ42, is decreased while production of shorter, less amyloidogenic peptides such as Aβ37 and 38, is increased. In this work, we set out to explore: 1) the impact of Aβ37 and Aβ38 on Aβ42 aggregation in vitro, and 2) the effect of the γ-secretase modulator (GSM) AZ4126 on CNS interstitial fluid level of different Aβ peptides and on Aβ-amyloidosis in vivo in the APP transgenic mouse brain.

Methods

In vitro: HEK APP/swe cells expressing the Alzheimer-associated presenilin 1 (PS1) mutant PS1ΔE9 were treated with different concentrations of AZ4126 and the effects on the formation of Aβ37, 38, 39, 40 and 42 were measured using MSD and Elisa. The effect of Aβ37 and Aβ38 on Aβ42 oligomer and fibril formation were explored using a THT assay.

In vivo microdialysis: Tg2576 mice were treated with AZ4126 (100 μmol/kg, p.o.), or vehicle and the levels of Aβ37, 40, and 42 in interstitial fluid from hippocampus were measured using MSD and Elisa from aliquots collected from *in vivo* microdialysis for a duration of 48 h. As comparison, the levels of Aβx-40 in interstitial fluid from the hippocampus of APPswe/PS1ΔE9 mice after treatment with AZ4126 (100 μmol/kg, p.o.) were determined for a period of 24 h.

2-photon imaging: The effect of chronic AZ4126 treatment for 28 days (100 μmol/kg p.o., o.d.) on amyloid plaque appearance and growth was examined using serial intravital 2-photon imaging in APPswe/PS1ΔE9 mice (6 months old). Methoxy-X04 (i.p.) was injected 24 h prior to imaging. A closed window (skull thinned but remains intact) was used. Pial vasculature provided stable landmarks for subsequent imaging. A 20-40x water immersion objective was used (720 nm excitation, z/step 1-5 μm, depth -200 μm).

Results

In vitro: AZ4126 caused marked Aβ–modulation in HEK APP/swe cells expressing PS1ΔE9. The compound induced a large increase in Aβ37 and Aβ38 as well as a reduction of Aβ42, Aβ40 and Aβ39 (Fig 1). In the THT assay, Aβ42 but not Aβ37 and Aβ38 showed potent aggregation. When mixed together at equimolar levels, both Aβ37 and 38 inhibited Aβ42 aggregation (Fig 2).

In vivo microdialysis: AZ4126 caused a pronounced modulation of interstitial fluid Aβ levels: Aβ42 and Aβ40 levels were decreased (Fig 3A) while Aβ37 levels were increased (Fig 3B) in the Tg2576 mice. AZ4126 was not able to reduce the levels of Aβx-40 to the same extent in interstitial fluid in APPswe/PS1ΔE9 mice as in Tg2576 mice (Fig 3C).

2-photon imaging: 28-days treatment of APPswe/PS1ΔE9 mice with AZ4126 (100 μmol/kg, p.o., once daily (o.d.) resulted in inhibition of plaque formation, plaque growth and, in some cases, even stimulated plaque regression (Fig 4).

Conclusion

GSMs are a novel class of compounds that alters the cleavage of APP. Our data show that the GSM AZ4126 significantly increases Aβ37 *in vitro* and *in vivo,* while reducing Aβ40 and 42. The presence of Aβ37 appear to inhibit the aggregation of Aβ42 in vitro. This modulatory effect on the Aβ peptide profile by AZ4126 also mediates several anti-amyloidogenic activities: attenuating plaque appearance, growth and in some cases clearing pre-existing amyloid pathology. In summary, our data suggest that GSMs are a highly promising anti-amyloidogenic therapy for the treatment of early Alzheimer's disease.

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Figure 1. HEK APP/swe cells expressing PS1ΔE9 were treated with different concentrations of AZ4126. The amount of different Aβ species formed were analyzed using ELISA.

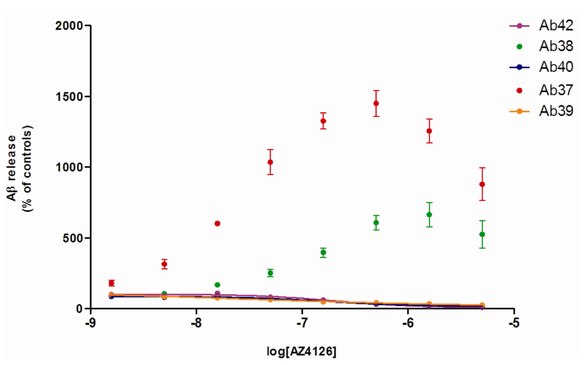

Figure 2. Aβ37 & Aβ38 inhibit Aβ42 aggregation in a THT assay

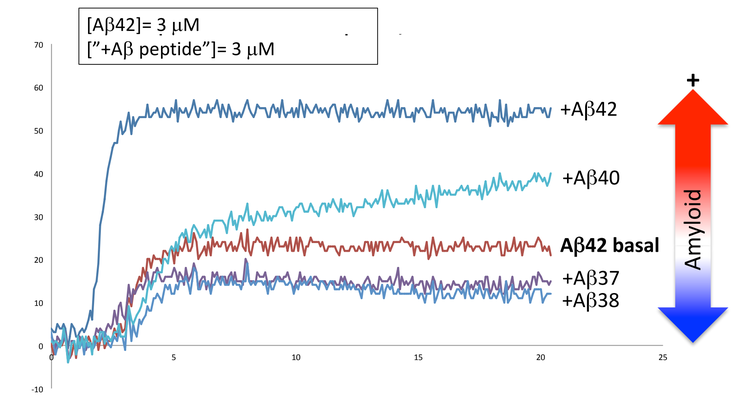

Figure 3. In vivo microdialysis A) Tg2576 mice (6 months) treated with 100 μmol/kg AZ4126 (p.o.) show significant lowering of Aβ42 and Aβ40 in interstitial fluid as measured by in vivo microdialysis B) Tg2576 mice (15 months) treated with 100 μmol/kg AZ4126 show significant increase in Aβ37 in interstitial fluid C) Reduction of Aβx-40 in Tg2576 compared to APPswe/PS1ΔE9 mice after treatment with 100 μmol/kg AZ4126 (p.o.).

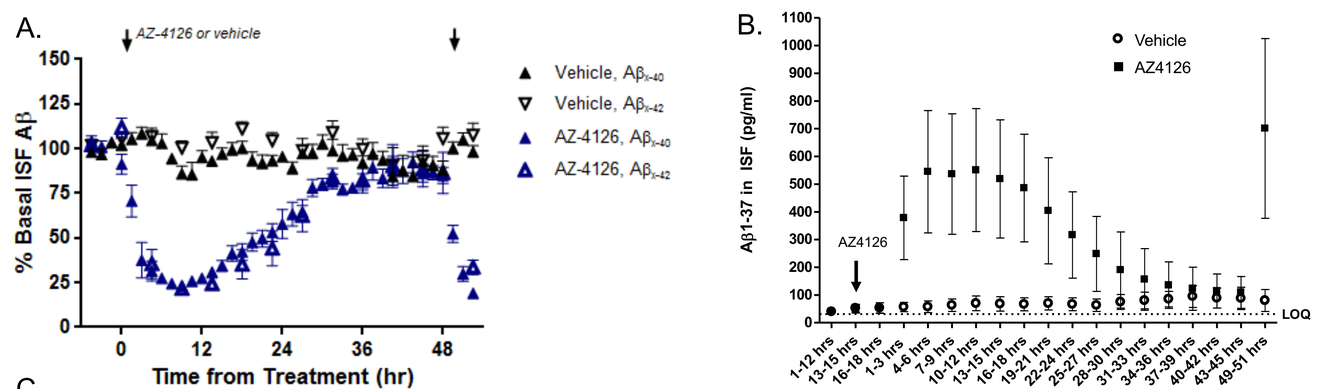

Figure 4. Representative image (A) from two-photon study in APP/PS1ΔE9 mice showing control animals and animals treated with AZ4126 (100 μmol/kg, p.o., o.d. for 28 days) at day 0 and day 28. Plaques that appeared (birth), grew, were unchanged or regressed during this time period are labeled with arrows (see legend). Analysis two-photon study showed that at day 28 the treated group showed (B) significant reduced appearance of plaques and (C) a regression of the plaque size.