

Putative gamma secretase modulators lower A β_{42} in multiple in vitro and in vivo models.

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INTRODUCTION

Inhibition of gamma secretase (GS) has been the focus of multiple drug discovery efforts over the last decade. However, the successful identification of potent gamma secretase inhibitors (GSIs) also led to the discovery of unacceptable side effects associated with this mechanism, i.e., the concomitant inhibition of Notch processing by GS. The observation that some non-steroidal anti-inflammatory drugs (NSAIDs) selectively inhibit the production of toxic A β_{42} while sparing A β and Notch has prompted the development of a class of compounds commonly referred to as gamma secretase modulators (GSMs).

METHODS

Brain A β Extraction

Mouse and/or rat brain sections were resuspended in 1 ml of 0.4% Diethylamine/50mM NaCl pH 10 + protease inhibitors. All samples were sonicated for 20-30 seconds on ice at 30% power. Homogenates were centrifuged at 355,000 x g for 30 min and resulting supernatants transferred to a fresh tube.

Brain A β Purification

Oasis HLB columns (Waters) were used to remove non-specific antibody reactive material from brain lysates according to Lanz & Schachter, 2006 J Neurosci Methods 157:71-81). Columns were conditioned with 1 ml of 100% methanol, and equilibrated with 1 ml of H₂O. Non-neutralized brain lysates were loaded through the columns and washed twice with 1 ml of 5% methanol, and (2) with 1 ml of 30% methanol. A β was eluted from the columns using 90% MeOH/2% NH₄OH. The eluate was transferred into 1.5 ml tubes and concentrated in a speed-vac concentrator on high heat for 2 hours. The concentrated A β was resuspended in 80 ml PBS-T containing 1% BSA plus protease inhibitors.

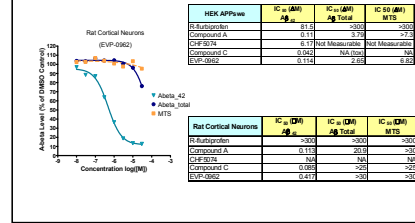
Brain A β Quantitation

For A β measurements, Nunc MaxiSorp assay plates were coated with either anti A β_{42} or rA β_{42} (P. Mehta) diluted to ~1 mg/ml in 0.05 M carbonate-bicarbonate buffer pH 9.6 overnight at 4°C and then assayed using QuantaBlu (Pierce). Briefly, plates were rinsed with 150 ml Wash Buffer (PBS-T) and blocked for 4 hrs at room temp with 100 ml of 1% BSA in PBS-T. 60 ml of samples were applied to the blocked plate in duplicate and incubated overnight at 4°C. A β was detected with biotinylated-4G8 (Signet) in diluted 1:1000 in PBS-T + 0.67% BSA for at least 1 hr. After washing (4X) wells were exposed to HRP-linked Streptavidin 1:10,000 dilution from 0.5 mg/ml stock and incubated for 30-60 minutes. Wells were then washed 5X with Wash Buffer before the addition of 100 ml per well of QuantaBlu Solution prepared according to the manufacturers instructions. Relative fluorescence units (RFUs) were measured using the manufacturer Devices M5e Reader.

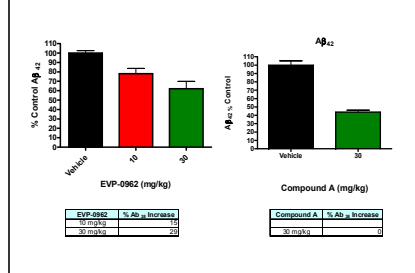
A β_{38} Peptide Quantification

Frozen rat brain pieces were homogenized for 30 seconds in 2% of total weight with 6M GuHCl using a PT 10/35 Polytron. Homogenates were subjected to solid-phase extraction using Oasis 60 mg HLB cartridges. Each column was equilibrated with 1.0 ml methanol and then 1.0 ml 0.1% TFA. After addition of 2 ml of homogenate, samples were washed with 1.5 ml 0.1% TFA. Samples were eluted with 1.0 ml of 70% ACN / 0.1% TFA and transferred into 2.0 ml Eppendorf DNA LoBind tubes. Peptides were concentrated and pellets were resuspended in 10% acetic acid and fractionated by RP-HPLC on a C18 column. HPLC fractions containing A β_{38} were resuspended in 150 μ l of UltraCulture Serum-free Medium with protease inhibitors and quantified by ELISA assay as described above.

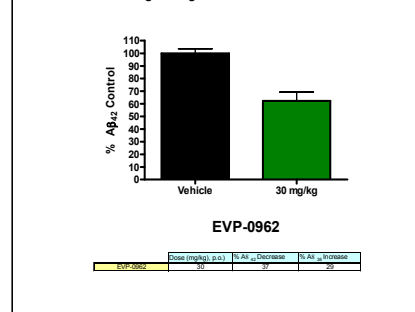
1 GSMs Lower A β_{42} in Cell-Based Assays Without Affecting Total A β



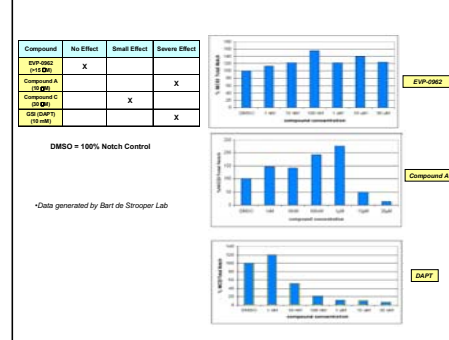
2 EVP-0962 decreases A β_{42} and increases A β_{38} in normal mouse brain following a single acute dose



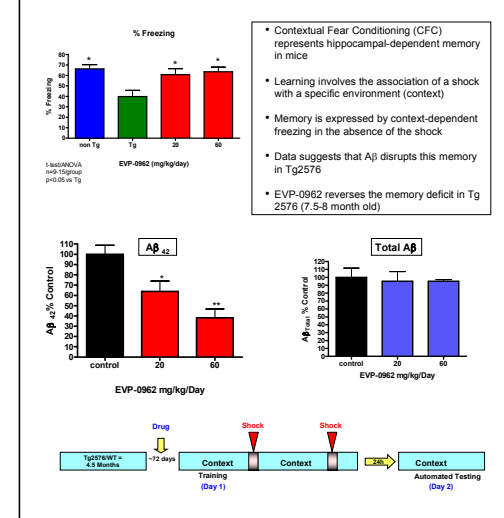
3 EVP-0962 decreases A β_{42} and increases A β_{38} in normal rat brain following a single acute dose



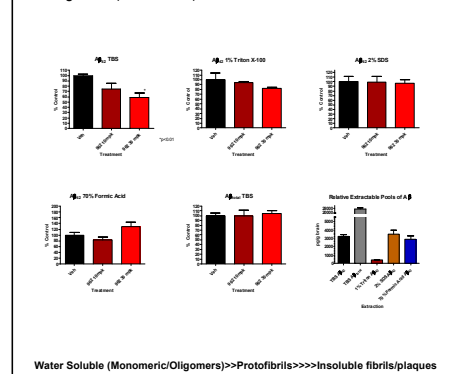
4 EVP-0962 Has No Effect on Notch Processing



6 Chronic dosing of EVP-0962 Selectively Lowers A β_{42} and Improves Memory in Tg2576 Mice



5 Single Administration of EVP-0962 Lowers Soluble A β_{42} in Tg 2576 (6month old)



SUMMARY

- EVP-0962
- Demonstrates acute A β_{42} lowering in mice, rats in a manner consistent with gamma secretase modulation
 - Decreases A β_{42}
 - No effects on Total A β
 - Increases A β_{38}
 - No effects on Notch Processing
 - Lowers human A β in Tg2576 following an acute dose
 - Improves hippocampal dependent memory in Tg2576 mice in a chronic dosing paradigm and while concomitantly lowering A β_{42}

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