Effect of Escitalopram on Aβ levels and plaque load in an Alzheimer mouse model

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The Article Processing Charge was funded by NIH/NINDS R01 NS094692 (JRC).

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Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
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Word count:
Title: 79 characters
Abstract: 203 words
Manuscript: 3,834 words
References: 36
Tables: 0; Figures: 4

Study Funding:
This work was supported by NIH/NINDS R01 NS094692 (JRC), NIH/NIA P50 AG00568 (JRC), NIH/NINDS P01 NS074969 (JRC), NIH/NIA R01 AG064902 (JRC), R01 NS094692 (JML), R21 AG05533301 (JML) R01 AG041502 (YIS); and by the Knight Alzheimer’s Disease Research Center at Washington University. The Article Processing Charge was funded by NIH/NINDS R01 NS094692 (JRC).

Disclosures:
The authors report no disclosures relevant to the manuscript.
**Rationale:** Several neurotransmitter receptors activate signaling pathways that alter processing of the amyloid precursor protein (APP) into amyloid-β (Aβ). Serotonin signaling through a subset of serotonin receptors suppresses Aβ generation. We proposed that escitalopram, the most specific selective serotonin reuptake inhibitor (SSRI) that inhibits the serotonin transporter, SERT, would suppress Aβ levels in mice.

**Objectives:** We hypothesized that acute treatment with ESC would reduce Aβ generation which would be reflected chronically with a significant reduction in Aβ plaque load.

**Methods:** We performed in vivo microdialysis and in vivo two-photon imaging to assess changes in brain interstitial fluid (ISF) Aβ and Aβ plaque size over time, respectively, in the APP/PS1 mouse model of Alzheimer’s disease treated with vehicle or ESC. We also chronically treated mice with ESC to determine the effect on plaques histologically.

**Results:** ESC acutely reduced ISF Aβ by 25% by increasing α-secretase cleavage of APP. Chronic administration of ESC significantly reduced plaque load by 28% and 34% at 2.5 mg/day and 5 mg/day, respectively. ESC at 5mg/kg did not remove existing plaques, but completely arrested individual plaque growth over time.

**Conclusions:** ESC significantly reduced Aβ in mice similar to previous findings in humans treated with acute dosing of an SSRI.
Introduction

Alzheimer’s disease (AD) is the most common cause of dementia, with an incidence that doubles every 5 years after 65 years of age and typically leads to death within 7-8 years of diagnosis. The prevalence across all world regions is projected to increase dramatically in the next decades to 130 million patients by mid-century, unless preventive measures are developed. AD is characterized by the accumulation of Aβ plaques and oligomers as well as tau neurofibrillary tangles. Aggregation of the Aβ peptide is concentration-dependent with high levels being much more likely to form higher-ordered, toxic species. Amyloid plaques and soluble Aβ oligomers exist within the brain extracellular space. While the initial aggregation seed may form intracellularly or extracellularly, it appears that soluble Aβ within the brain extracellular space, or interstitial fluid (ISF), is one source that contributes to these toxic species. Consequently, mechanisms that regulate Aβ levels could be therapeutic targets to maintain low levels of the peptide to reduce or eliminate toxic Aβ species from the brain.

Aβ levels are regulated by multiple mechanisms. Synaptic transmission leads to Aβ generation at or near the presynaptic terminal which is then released into the ISF. Additionally, activation of certain neurotransmitter receptors, such as M1 muscarinic acetylcholine receptors, NMDA receptors, and AMPA receptors can alter APP processing which impacts Aβ generation. Direct infusion of serotonin into the brain, or treatment with a selective serotonin reuptake inhibitor (SSRI) antidepressant, also decreases Aβ acutely in brain by reducing Aβ production with no obvious effect on Aβ clearance. Our group and others have found in mice and humans that NMDA and Gαs-coupled serotonin receptors 5-HT4, 5-HT6 and 5-HT7 activate the ERK signaling cascade which increases α-secretase enzymatic activity to reduce Aβ levels.

Long-term reductions in Aβ levels in many cases reduces the aggregation of peptide into plaques mice and humans. Chronic SSRI treatment over the course of four months in APP/PS1
mice reduced Aβ plaque load in the hippocampus and cortex by 50% \(^{15}\). Similar reductions in Aβ were demonstrated by SSRI treatment in the 3xTg AD mouse model \(^{20}\). In a retrospective study, humans with a history of SSRI use to treat depression had less PIB binding in the brain \(^{15}\) and acute studies in humans demonstrated a significant reduction in CSF Aβ in young, cognitively-normal following a single dose of an SSRI. Similarly, in this issue, Sheline and colleagues demonstrate that subchronic dosing of ESC for 2 weeks or 8 weeks also suppresses CSF Aβ levels significantly \(^{21}\).

SSRI antidepressants are commonly used FDA-approved drugs with a reasonable safety record with long-term use. Given the serotonin signaling is one synaptic mechanism that regulates Aβ, we proposed that the SSRI escitalopram, the most selective drug in this class for blocking serotonin reuptake as opposed to norepinephrine \(^{22}\), would suppress brain Aβ levels and reduce plaque load in mice. In the current study we determined the acute effects of escitalopram on APP processing and Aβ metabolism in APP transgenic mice using in vivo microdialysis. In addition, we prospectively determined the chronic effects of ESC on plaques burden and individual plaque growth over time.

**Methods**

**Mouse studies:**

We bred \(APP/PS1^{\Delta E9}\) hemizygous mice \(^{23}\) to wild type C3H/B6 mice (Jackson Labs, Bar Harbor, Maine). These mice harbored the PS1\(\Delta E9\) deletion and the human APP Swedish mutation, inserted into a single locus. Male and female littermate mice were equally distributed between all experimental groups. For multiphoton studies, 6 month-old mice were randomly entered into 3 study arms: 1) escitalopram (ESC) 5 mg/kg, 2) ESC 2.5 mg/kg, or 3) vehicle (2% DMSO in normal saline), injected intraperitoneally, qD for 28 days. Multiphoton imaging was performed on the day before the first injection, and after the last injection (see below).

**Standard Protocol Approvals**
All experimental procedures involving animals were performed in accordance with guidelines established by the Animal Studies Committee at Washington University.

**In Vivo Microdialysis**

In vivo microdialysis to assess brain ISF Aβ in the hippocampus of awake, freely moving \textit{APP/PS1}^{+/−} mice was performed similar to previously described \textsuperscript{15, 24}. This technique samples soluble molecules within the extracellular fluid that are smaller than 38-kilodaltons, the molecular-weight cut off of the probe membrane. Aβ capable of entering the probe has been termed “exchangeable Aβ or eAβ”.

Under isoflurane volatile anesthetic, guide cannula (BR-style, Bioanalytical Systems, Indianapolis, IN) were cemented above the left hippocampus (3.1 mm behind bregma, 2.5 mm lateral to midline, and 1.2 mm below dura at a 12° angle). Two millimeter microdialysis probes were inserted through the guides so the membrane was contained entirely within the hippocampus (BR-2, 30-kilodalton MWCO membrane, Bioanalytical Systems). Microdialysis perfusion buffer was artificial CSF (aCSF) (perfusion buffer in mM: 1.3 CaCl$_2$, 1.2 MgSO$_4$, 3 KCl, 0.4 KH$_2$PO$_4$, 25 NaHCO$_3$, and 122 NaCl, pH 7.35) containing 15% bovine serum albumin (Sigma, St. Louis, MO) that was filtered through a 0.22 µM membrane. Flow rate was a constant 1.0 µl/min. Samples were collected every 60-90 minutes with a refrigerated fraction collector into polypropylene tubes and assessed for Aβ$_{40}$ by ELISA at the completion of each experiment. Basal concentrations of ISF Aβ were defined as the mean concentration of Aβ over the 9 hours preceding drug treatment. Once basal ISF Aβ concentrations were established, \textit{APP/PS1} mice were administered either vehicle (PBS) or CIT (R,S mixture), ESC 2.5 mg/kg; ESC 5 mg/kg or R-CIT. After drug treatment, ISF Aβ concentrations were sampled every 60 minutes for an additional 24 hours, then all samples were assayed for Aβ concentration by sandwich ELISA. All ISF Aβ concentrations were normalized to the basal Aβ concentration in each mouse.

**Secretase assays:**
Enzymatic activity of α-secretase and β-secretase was measured using FRET-based cleavage assays (R&D Systems, Minneapolis, MN). Hippocampal tissue was isolated from 3 months old APP/PS1 hemizygous mice treated with ESC (5mg/kg) or vehicle (PBS) and sacrificed 8 hours later. Tissue was lysed in Cell Extraction Buffer (Novagen, Madison, WI) by mechanical homogenization. Cell extracts were incubated with secretase-specific peptides conjugated to the reporter molecules EDANS and DABCYL for 15-30 minutes. EDANS fluorescence was read on a Cytation 5 microtiter plate reader (BioTek, Winooski, VT).

Aβ Sandwich ELISA:

ISF Aβ concentrations were assessed using sandwich ELISAs as described. Briefly, a mouse-anti-Aβ40 antibody (mHJ2) or mouse-anti-Aβ42 antibody (mHJ7.4) was used to capture and a biotinylated central domain antibody (mHJ5.1) was used to detect, followed by streptavidin-poly-HRP-40 (Fitzgerald Industries, Concord, MA). All ELISA assays were developed using Super Slow ELISA TMB (Sigma, St. Louis, MO) and absorbance read on a Bio-Tek Epoch plate reader (Winooski, Vermont) at 650nm. The standard curve for each assay utilized synthetic human Aβ1-40 peptide (American Peptide, Sunnyvale, CA).

Chronic escitalopram administration

Beginning at 4 months of age, APP-PS1 hemizygous female mice were administered normal drinking water (vehicle) or 5mg/kg/day ESC or 2.5 mg/kg/day ESC in drinking water for a total of 4 months. Littermate mice were divided equally between the treatment groups. Mice were housed 3-5 animals per cage. Volume of water drunk per cage and animal body weight were tracked throughout the study and did not differ between cages or treatment groups. At 8 months of age, mice were sacrificed and CSF drawn from the cisterna magna followed by transcardial perfusion of chilled PBS with 0.3% heparin. One hemisphere of the brain was post-fixed overnight in 4% paraformaldehyde followed by processing for histological analysis of Aβ plaque burden. The other hemisphere had the hippocampus and cortex micro-dissected, then snap frozen on dry ice for future biochemical analysis of brain Aβ levels.
Tissue extraction of Aβ

To evaluate various pools of brain Aβ we performed a sequential extraction of tissue with PBS, 1% triton X-100 in PBS, then 5M guanidine to grossly assess the extracellular-enriched fraction, the membrane-bound and intracellular fraction, and the insoluble fraction, respectively. All lysis buffers were chilled to 4°C and contained protease inhibitors without EDTA. Tissue was lysed at a 1:10 wet weight/volume ratio. PBS and Triton X-100 extractions were performed by mechanical dounce homogenization while the guanidine extraction was performed with sonication to maximally solubilize remaining Aβ within the tissue. Tissue was spun in a microcentrifuge at 21,000 g for 15 minutes at 4°C following each extraction. Aβ1-40 and Aβ1-42 were measured by sandwich ELISA and normalized to total protein in each sample, as determined by a micro-BCA assay (Peirce, Rockford, IL).

Quantitative analyses of Aβ deposition

Brain hemispheres were placed in 30% sucrose before freezing and cutting on a freezing sliding microtome. Serial coronal sections of the brain at 50 μm intervals were collected from the rostral anterior commissure to caudal hippocampus as landmarks. Sections were stained with biotinylated human-specific anti-Aβ antibody, mHJ3.4 (a kind gift from Dr. David Holtzman, Washington University, St. Louis). Stained brain sections were scanned with a NanoZoomer slide scanner (Hamamatsu Photonics). For quantitative analyses of mHJ3.4-biotin staining, scanned images were exported with NDP viewer software (Hamamatsu Photonics) and converted to 8 bit grayscale using ACDSee Pro 3 software (ACD Systems). Converted images were thresholded to highlight plaques and then analyzed by “Analyze Particles” function in the ImageJ software (National Institutes of Health). Identified objects after thresholding were individually inspected to confirm the object as a plaque or not. Three brain sections per mouse, each separated by 300 μm, were used for quantification. These sections correspond roughly to sections at Bregma –1.7, –2.0, and –2.3 mm in the mouse brain atlas. The average of three sections was used to represent a plaque load for each mouse. For analysis of Aβ plaque in the
cortex, the cortex immediately dorsal to the hippocampus was assessed. All analyses were performed in a blinded manner by two independent researchers. The data that were statistically analyzed and plotted represents the average plaque load per section between the two researchers.

**Cranial window surgery:**

Thinned-skull cranial windows were prepared on the day of the first multiphoton imaging session as described previously. Briefly, mice were anesthetized under volatile isoflurane and the skin and periosteum were removed to expose the skull. A high-speed drill and microsurgical blade (Surgistar) were used to thin the skull until transparent and flexible. Two thinned-skull windows (each 0.8–1.0 mm in diameter) over each hemisphere were prepared in each animal.

**In vivo multiphoton microscopy:**

Six month-old APP/PS1 mice were treated with ESC (n=4 per group) or vehicle (n=5) for 28d. To quantify growth of individual amyloid plaques, longitudinally intravital multiphoton imaging was used, as described previously. Briefly, mice were injected intraperitoneally with the fluorescent amyloid-binding compound methoxy-X04 (5 mg/ml) 24 hours prior to each imaging session. Animals were mounted on a custom-built stereotaxic apparatus and a small ring of molten bone wax was applied to the skull surrounding the perimeter of the window to create a water immersion chamber. The cranial window was centered under the objective lens on a two-photon microscope (LSM 510 META NLO system (Carl Zeiss Inc.) with a Cameleon Ti: Sapphire laser (Coherent Inc.)). Two-photon fluorescence was generated with 750 nm excitation and fluorescence emission was detected at 435–485 nm. A 10X water-immersion objective [numerical aperture (NA) = 0.33, Zeiss] was used to create a site map during initial imaging and a 40X water-immersion objective (NA = 0.75, Zeiss) was used for high-resolution quantification of individual amyloid plaques. Incremental z-stack image series (step distance = 10 and 5 μm under 10X and 40X objectives, respectively) were acquired from the skull surface to approximately 200 μm into cortex.
To determine the effect of ESC on amyloid plaque formation and growth, the same sites for each animal were imaged on day 0 and day 28. Collapsed z-stack images for each individual plaque were captured. The plaque radius and intensity was determined using SigmaScan Pro Image Analysis Software (Systat Software) with a preset threshold (threshold = mean + 4 * SD). Plaques were excluded from analysis if they were located at the edge of the window, exhibited fluorescence intensity less than the mean intensity of an adjacent background region or if the image acquisition was affected by motion artifacts from heartbeat or respiration.

Statistical Analyses for Mouse Studies

Statistically significant differences were determined using Prism statistical software (Graph Prism 8, San Diego, CA, USA). All data were presented as mean ± SEM. For data analysis, one-way ANOVA with Dunnett's Multiple Comparison post test was applied. All p values resulted from two-sided statistical tests and statistical significance was set at *p < 0.05, **p < 0.01, and ***p < 0.001.

Data Availability

Raw and analyzed data will be shared upon written request to the corresponding author from any qualified investigator.

Results

Comparison of Citalopram, Escitalopram and R-Citalopram in brain ISF Aβ levels in mice

Citalopram (CIT) is a mixture of two enantiomers; S-citalopram, the active molecule, and R-citalopram, (R-CIT) the inactive molecule. Escitalopram (ESC) consists only of the active enantiomer, whereas R-CIT should be an inactive component of CIT. In theory, CIT should have comparable efficacies as half the dose of ESC. We sought to determine the effect of each of these compounds on ISF Aβ42 levels at comparable “active doses”.

Mice treated with CIT (R,S mixture) and ESC had a gradual reduction in ISF Aβ40 and Aβ42 whereas R-CIT and vehicle produced no change (Fig. 1A,B). At the end of the 24 hour
sampling, 10mg/kg CIT significantly reduced ISF Aβ_{40} by 25.6 ± 6.1%, (mean ± SEM; n=4; p=0.006; 95% CI [7.9%, 41.6%]) while 5mg/kg ESC, a comparable dose of the active enantiomer, reduced ISF Aβ_{40} by 20.2 ± 4.1% (n=6; p=0.014; 95% CI [4.1%, 34.7%]). CIT and ESC were not significantly different from each other, however (p=0.668; 95% CI [-21.6%, 11.0%]). R-citalopram, the inactive enantiomer of citalopram, had no effect on change in ISF Aβ levels (n=5, p=0.923; 95% CI [-24.9%, 16.8%]) compared to vehicle (Fig. 1B). Aβ_{42} was altered similar to Aβ_{40} (Fig 1B). CIT reduced Aβ_{42} by 21.8 ± 2.8% (n=5, p < 0.0001; 95% CI [12.1%, 29.7%]) while ESC reduced Aβ_{42} by 26.8 ± 2.5% (n=5, p <0.0001; 95% CI [17.1%, 34.8%]). Each drug that a similar effect on both Aβ_{40} and Aβ_{42} levels.

**Escitalopram increases α-secretase activity**

3 month old APP/PS1 were administered 5mg/kg ESC or vehicle then sacrificed 8 hours later and brains processed for biochemistry. The hippocampus was lysed and then measured by enzymatic activity of α-secretase and β-secretase using a FRET-based cleavage assay. Following ESC treatment, α-secretase activity significantly increased by 51.1 ± 3.3% (n=8, p < 0.001, 95% CI [19.8%, 42.1%]) whereas β-secretase activity only changed by 10.8 ± 3.5% (n=8, p = 0.212; 95% CI [-4.0%, 16.6%]) compared to vehicle-treated mice (Fig. 2).

**Chronic escitalopram effect on brain plaque load in mice**

*APP/PS1* mice, starting at 4 months of age prior to plaque pathology, were administered plain water, or ESC at 2.5 mg/kg and 5mg/kg in drinking water for 4 months. At 8 months of age mice were sacrificed to assess Aβ aggregation. As assessed histologically, ESC at both doses significantly reduced plaque burden within the brains of these mice compared to littermate controls that drank only water (Fig. 3A with representative histology images). Hippocampal plaque load was significantly reduced by 28.7 ± 0.05% (p=0.029; 95% CI [0.022%, 0.43%]) and 34.4 ± 0.05% (p=0.009; 95% CI [0.067%, 0.48%]) for ESC 2.5 mg/day and 5 mg/day, respectively (Fig. 3A, n=8 per group). The observed reduction in the ESC 2.5 mg/kg and 5 mg/kg groups did not differ significantly from each other (p=0.849; 95% CI [-0.16%, 0.25%]).
The contralateral brain regions were processed for biochemical analysis of Aβ protein levels. PBS-soluble and Triton X-100-soluble Aβ levels did not change significantly in escitalopram-treated mice (data not shown); however levels of insoluble Aβ40 and Aβ42 extracted with 5M guanidine were significantly reduced by 42.7 ± 5.6% (p=0.005; 95% CI [40.4%, 241.5%]) and 56.3 ± 3.4% (p=0.041; 95% CI [11.4%, 588.1%]) in the 2.5 mg/kg ESC group respectively, and by 43.8 ± 6.9% (p<0.001; 95% CI [85.3%, 286.4%]) and 69.0 ± 3.0% (p<0.001; 95% CI [322.7%, 899.4%]) in the 5mg/kg ESC group, respectively (Fig. 3B; n=8 per group). Though plaque load histologically and insoluble Aβ biochemically measure overlapping pools of Aβ, we propose that the discrepancy in significance between the analyses is that by its nature quantification by histology is less precise than biochemically using an ELISA. There was no change in CSF Aβ40 and Aβ42 levels at 5mg/kg ESC (Aβ40 p=0.357; 95% CI [-15.6%, 65.5%]; Aβ42 p=0.215; 95% CI [-28.0%, 30.4%]).

Escitalopram arrests individual plaque growth assessed by in vivo two-photon microscopy

During a 28-day interval, plaques in vehicle-treated mice grew 116.8 µm², while plaques in mice treated with 2.5 mg/kg ESC grew an average 65.5 µm². In both vehicle and 2.5 mg groups, the average size of plaques was significantly larger at day 28 compared to day 0 (p<0.001; 0d vehicle 341 µm² vs 28day vehicle 458 µm² [382, 534]; and p<0.05; 2.5mg/kg ESC 0d 387 µm² vs. 2.5mg/kg ESC 28d 452 µm² [348, 556]; pair t-test), respectively. However, in mice treated with 5mg/kg ESC, mean plaque size at day 28 was not significantly different from that at day 0 (p=0.83; 0d 404 µm² vs. 28d 399 µm² [343, 456], paired t-test), suggesting that plaques did not grow. The rate of plaque growth in the vehicle group was significantly higher than that in the 5 mg/kg ESC group (p=0.002; vehicle 190% [140, 241] vs 5mg/kg ESC 119% [101, 137]), but not that of the 2.5mg/kg ESC group (p=0.391; vehicle 190% [140, 241] vs 2.5mg/kg ESC 140% [110,170]; Fig. 4A, B). Furthermore, treatment with 5mg/kg ESC reduced the number of newly appearing plaques compared with vehicle (p=0.03; vehicle 9.6 plaques [8.2, 10.9] vs 5mg/kg ESC 3 plaques [1.34, 4.68]), but had no effect on the number of
disappearing plaques (p=0.47; vehicle 1.1 [0.224, 2.04] vs 5mg/kg ESC 2.2 [1.29, 3.09]), whereas 2.5mg/kg ESC treatment had no effect on either the number of appearing plaques compared with vehicle (p=0.07; vehicle 9.6 [8.2, 10.9] vs 2.5mg/kg ESC 7.6 [4.26, 11]) or the number of disappearing plaques (p= 0.47; vehicle 1.1 [0.224, 2.04] vs 2.5mg/kg ESC1.0 [0.144, 1.89]; Fig. 4C). There was not sufficient CAA in the image captures to analyze vascular Aβ changes in response to ESC.

Discussion

Rodent studies showed that the chronic administration of ESC 5 mg/kg/day blocked the growth of existing amyloid plaques and significantly reduced the appearance of new plaques, compared to vehicle-treated animals. This dose is roughly comparable to a 24 mg dose in a 60 kg human ²⁹, similar to the 10mg and 20 mg doses often prescribed to depressed individuals. Importantly, the rates that a bolus dose of drug is absorbed when given intraperitoneally in mice versus when given orally in humans will differ, so direct comparison of dosages can be difficult. Further, ESC had a rapid and sustained acute effect on ISF Aβ₄₀ and Aβ₄₂ levels. Importantly, studies showed that there was no effect of R-CIT, the inactive enantiomer; only an effect of S-CIT (also known as ESC). This complements a study in cognitively-normal, elderly individuals whereby 8 weeks of ESC reduced CSF Aβ levels by almost 10% ²¹.

ESC had similar effects on both ISF Aβ₄₀ and Aβ₄₂ in terms of both magnitude of change as well as kinetics. Interestingly, in humans ESC reduced CSF Aβ₄₂ but not Aβ₄₀ (Sheline et al. 2020). Treatment with the SSRI increased enzymatic activity of α-secretase without a change in β-secretase activity. This is similar to previous findings demonstrating that serotonin receptors activate the ERK signaling pathway to increase non-amyloidogenic processing of APP ¹⁵. Interestingly, there was a dose-dependent change in Aβ in only select measures; overall plaque load, as assessed histologically, did not differ between the 2.5 and 5.0mg/kg treatment groups,
whereas guanidine-extractable $\text{A}\beta_{42}$ levels were different with a trend for a difference in $\text{A}\beta_{40}$.

While plaque load and an insoluble extraction of tissue measure similar pools of $\text{A}\beta$, guanidine-extractable $\text{A}\beta$ in tissue consists of more than just plaque (e.g. $\text{A}\beta$ inside small vesicles that are not easily lysed in detergent) which appear to be more mobile and reduced in escitalopram-treated mice.

Chronic reductions in $\text{A}\beta$ levels, either by suppressing production or enhancing clearance, can have profound influences on the amount of $\text{A}\beta$ that accumulates as plaques, as well as associated synaptic and neuritic pathologies. Formation of these toxic conformations from the normal soluble $\text{A}\beta$ species is concentration-dependent; with high $\text{A}\beta$ concentrations much more likely to aggregate than lower $\text{A}\beta$ concentrations. Even small decreases in $\text{A}\beta$ concentration have been associated with a substantial lowering of plaque burden. A 12-25% decrease in soluble $\text{A}\beta$ concentration in animal models produced a substantial plaque reduction.

In humans, $\text{A}\beta$ levels that are 20-50% higher, such as in familial AD or Down's Syndrome, produce pathology and symptoms decades before sporadic AD patients. In our prior studies with the SSRI CIT, soluble $\text{A}\beta$ concentration was reduced by 25% with a concurrent 78% reduction in new plaque formation in mice whereas in our current rodent studies we found a reduction in new plaque formation of 34%. So while reducing $\text{A}\beta$ will likely limit plaques within the brain, a complete reduction may not be required.

Independent of medications, Depression appears to increase the risk of developing AD. Several retrospective studies have found that SSRIs reduce the risk of AD symptoms in depressed individuals while depressed individuals taking SSRIs are at lower risk of AD compared to untreated depressed individuals, however still at greater risk compared to non-depressed controls. Though acute studies in humans have demonstrated that SSRIs can reduce $\text{A}\beta$ levels, prospective trials are still necessary to determine whether SSRIs, or other serotonin modulators, are directly responsible for the reduction in amyloid plaque load in human brain.
Numerous clinical trials using γ-secretase inhibitors, β-secretase inhibitors, and anti-Aβ vaccinations have been conducted to suppress Aβ levels. The initial trials treated individuals who already exhibited mild to moderate behavioral symptoms of AD and failed to produce a measureable improvement in cognition. Later studies treated individuals that were cognitively normal, but at risk of developing AD within a few years. Unfortunately, these trials also failed to show cognitive benefit. Given the failure of numerous Aβ targeted therapeutics, it remains possible that targeting Aβ may not be beneficial to AD. Alternatively however, these studies may have failed because they were started too late in the course of the disease: Aβ and tau had already caused substantial cell death which could not be reversed. As such, if targeting Aβ as a therapeutic intervention, treatment may need to be started much earlier, meaning that individuals may need to be on a drug for a decade or more before changes could be detected. A drug will need to be safe and can be tolerated for that period of time. While SSRIs certainly are not innocuous, millions of individuals take them for decades with generally manageable side-effects. Of the 15 serotonin receptor subtypes, only 5-HT₄, 5-HT₆, and 5-HT₇ receptors are responsible for suppressing Aβ levels following SSRI administration; thus it may be feasible to develop more targeted agents to lower Aβ levels.
Tables and Figures

Figure 1: ESC acutely reduce brain ISF Aβ levels over a 24 hour period.

(A) Mice treated with CIT (R,S mixture) and ESC had a gradual reduction in ISF Aβ whereas R-CIT and vehicle produced no change. At the end of 24 hour sampling, CIT significantly reduced ISF Aβ<sub>40</sub> by 25.3 +/- 6.1%, (mean +/- SEM; n=4; p<0.01 and ESC reduced Aβ by 20.2 +/- 4.1% (n=6; p<0.01). R-citalopram had no effect on ISF Aβ levels (n=5) compared to vehicle.  (B) CIT reduced Aβ<sub>42</sub> by 21.8 ± 2.8% (n=5, p < 0.0001) while ESC reduced ISF Aβ<sub>42</sub> by 26.8 ± 2.5% (n=5, p <0.0001). R-CIT had no effect on Aβ<sub>42</sub> levels. Data presented as mean ± SEM. All p values resulted from two-sided statistical tests and statistical significance was set at *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure 2: ESC increased α-secretase enzymatic activity.

APP/PS1 mice were administered 5mg/kg ESC then sacrificed 8 hours later. Enzymatic activity of α-secretase and β-secretase were measured in the hippocampus. α-secretase activity significantly increased by 51.1 ± 3.3% (n=8, p < 0.001) while β-secretase activity changed insignificantly by 10.8 ± 3.5% (n=8, p = 0.212) compared to vehicle-treated mice. Data presented as mean ± SEM.

![Graph showing α-secretase and β-secretase activity]
Figure 3: Chronic administration of ESC reduced Aβ plaque load.

(A) Hippocampal plaque load for both Aβ40 and Aβ42 were significantly reduced for ESC 2.5 mg/day and 5 mg/day, respectively (n=8 per group). Representative images at 5X magnification.

(B) Levels of insoluble Aβ40 and Aβ42 were significantly reduced in the 2.5mg/kg ESC group and in the 5mg/kg ESC group, respectively (n=8 per group). All p values resulted from two-sided statistical tests and statistical significance was set at *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure 4: ESC over 4 weeks completely arrested individual plaque growth.

Six month-old APP/PS1 mice were treated with ESC at doses of 2.5 or 5 mg/kg per day or vehicle i.p. for 28d (Vehicle: n=11 mice, 104 plaques; ESC 2.5mg/kg: n=5 mice, 40 plaques; ESC 5mg/kg: n=4 mice, 66 plaques) and imaged using 2 photon microscopy. (A) Representative multiphoton micrographs (20X magnification) of individual amyloid plaques in the cortex of APP/PS1 mice (before (0d) and 28 days after treatment. P, plaque; New P, new plaque. (B) 5mg/kg ESC and 10 mg/kg ESC attenuated the growth of pre-existing plaques. (C) ESC at 5 mg/kg and ESC 10mg/kg reduced the appearance of new plaques. All p values resulted from one-way ANOVA with Dunnett’s Multiple Comparison post statistical tests and statistical significance was set at *p < 0.05, **p < 0.01, and ***p < 0.001.
Appendix 1: Authors

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<tr>
<th>Author</th>
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<td>Designed and conceptualized study; analyzed the data; drafted the manuscript</td>
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References


Effect of Escitalopram on Aβ levels and plaque load in an Alzheimer mouse model
John R. Cirrito, Clare E. Wallace, Ping Yan, et al.
Neurology published online September 10, 2020
DOI 10.1212/WNL.0000000000010733

This information is current as of September 10, 2020

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