Published in final edited form as:

J Alzheimers Dis. 2012 ; 32(1): 147–156. doi:10.3233/JAD-2012-120763.

The Effects of Ramipril in Individuals at Risk for Alzheimer's Disease: Results of a Pilot Clinical Trial

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Abstract

Research shows that certain antihypertensives taken during midlife confer Alzheimer's disease (AD) related benefits in later life. We conducted a clinical trial to evaluate the extent to which the angiotensin converting enzyme inhibitor (ACE-I), ramipril, affects AD biomarkers including CSF amyloid β levels ($A\beta$) and ACE activity, arterial function and cognition in participants with a parental history of AD.

This four month randomized, double-blind, placebo-controlled, pilot clinical trial evaluated the effects of ramipril, a blood-brain-barrier (BBB) crossing ACE-I, in cognitively healthy individuals with mild, or Stage I hypertension. Fourteen participants were stratified by gender and apolipoprotein E $\epsilon 4$ (*APOE* $\epsilon 4$) status and randomized to receive 5mg of ramipril or matching placebo daily. Participants were assessed at baseline and month 4 on measures of CSF $A\beta 1-42$ and ACE activity, arterial function and cognition.

Participants were middle-aged (mean 54yrs) highly educated (mean 15.4yrs), and included 50% men and 50% *APOE* $\epsilon 4$ carriers. While results did not show a treatment effect on CSF $A\beta 1-42$ ($p=0.836$), data revealed that ramipril can inhibit CSF ACE activity ($p=0.009$) and improve blood pressure (BP), however there were no differences between groups in arterial function or cognition.

In this study, ramipril therapy inhibited CSF ACE activity and improved BP, but did not influence CSF A β 1–42. While larger trials are needed to confirm our CSF A β results, it is possible that prior research reporting benefits of ACE-I during midlife may be attributed to alternative mechanisms including improvements in cerebral blood flow or the prevention of Angiotensin II-mediated inhibition of acetylcholine.

Keywords

Alzheimer's disease; Hypertension; Blood Pressure; Clinical Trial; Vascular Risk; Cognition; Angiotensin Converting Enzyme; Antihypertensive; Arterial Function; Prevention

Introduction

Increasing research reporting hypertension's contribution to AD has led investigators to explore the use of antihypertensive medications as a potential therapy for persons with AD [1–7]. Large cohort and observational studies and a limited number of clinical trials report a beneficial effect of antihypertensives on AD incidence, disease progression and improved cognitive function, however not all studies support these associations [8–11]. Discrepant findings are likely attributed to methodological inconsistencies such dementia type and severity, length of the study, concomitant vascular dysfunction, cognitive assessment employed and the type of antihypertensive medication under investigation.

Some antihypertensive medications have been shown to decrease the incidence of AD and progression of cognitive decline among AD patients [11–14]. Recent studies show that AD-related cognitive benefits attributed to antihypertensive therapy is most promising in patients taking angiotensin II receptor blockers (A2RB) or angiotensin converting enzyme inhibitors (ACE-I [15, 16]; For a comprehensive review see [17]). Both classes of medications treat hypertension by inhibition of the production or signaling of angiotensin II (Ang II), a potent vasoconstrictor. An additional benefit of these medications is their reported ability to reduce inflammation within the peripheral rennin-angiotensin system (RAS) via inhibiting production of Ang II.

Within the class of ACE-I, those medications that are reported to be centrally acting (i.e. cross the blood brain barrier (BBB)) have shown the most benefits [12, 13, 18–20]. For instance, results from the Cardiovascular Health Study show that centrally acting ACE-I were associated with a 65% decrease in cognitive decline per year of exposure compared to participants assigned to a non BBB crossing ACE-I or a calcium channel blocker [21]. These results are consistent with prior research showing reduced AD progression among participants assigned to a centrally acting ACE-I compared to non-centrally acting ACE-I [12]. The mechanistic underpinnings explaining the reported benefits of BBB crossing ACE-I in observational and clinical AD research is complicated. There is conflicting evidence surrounding the ability of centrally acting ACE-I to cross the BBB, as well as the effects of ACE on A β activity and levels. The current randomized, double-blind, placebo-controlled, pilot clinical trial aimed to evaluate the extent to which ACE-Is affect A β levels and ACE activity in CSF, measures of peripheral arterial function and cognition in non-demented participants with a parental history of AD.

Methods

Participants

Participants were recruited from the Wisconsin Alzheimer's Disease Research Center (ADRC) registry and from the community for the clinical trial (Studying the Effects of

Antihypertensives in Individuals at Risk for Alzheimer's (SEAIRA). Individuals who had a parent with AD were eligible to participate. Our target enrollment was twenty participants, but funding ran out prior to meeting this goal. Twenty-six cognitively normal, middle-aged (40–65 yrs) adult children of persons with AD completed the screening visit for this four month trial. Of these, 14 met enrollment criteria. Inclusion / exclusion criteria are listed in Table 1. All participants had at least one parent with either autopsy-confirmed or probable AD as defined by NINDS-ADRDA criteria. All participants' parents were diagnosed with late onset AD with no known autosomal mutations. Individuals were enrolled only if they met the American Heart Association's definition of prehypertension or Stage I hypertension (systolic 121–159 mmHg or diastolic 81–99 mmHg). We enrolled only mildly hypertensive participants because they did not clinically require assignment to treatment. The most common preclusion for enrollment was a BP reading below the targeted range at screening or the baseline visit (N = 12). Participants were free of major vascular, neurologic or psychological conditions.

Procedures

Participants attended five visits over the course of the four month trial. At the screening visit, individuals were consented and underwent clinical BP assessment and a blood draw for *APOE* genotyping. BP assessment to determine study inclusion was conducted by a trained research nurse. Participants remained seated for ten minutes before BP was measured. BP was taken on the left arm resting on a table at chest level while the participant was seated with their legs uncrossed. BP was taken with the same monitor at each study visit on all participants (GE DINAMAP PRO 400 V2) and the average of three readings at least five minutes apart was calculated. Individuals then completed medical and medication history questionnaires and were advised to follow the Dietary Approaches to Stop Hypertension (DASH) lifestyle intervention over the following four weeks. The purpose of the four week run-in period was to ensure that all participants had similar education regarding potential causes of high BP and had at least 4 weeks to apply this education. After the run-in period, participants returned to the research center and BP levels were reassessed. Individuals were fasting at this visit because those who were able to be included in the study went on to undergo baseline visit procedures, which required a 12 hour fast. Participants whose BP remained in the targeted range were randomized in a 1:1 ratio by *APOE* ε4 status and gender by the UW Pharmaceutical Research Center (PRC) to receive a 5mg dose of ramipril or a matching placebo to be taken once daily. Individuals whose BP was outside of the inclusion range were not randomized.

At the baseline and month 4 visits participants underwent a cardiovascular research assessment to ascertain comprehensive, preclinical measures of arterial function. Participants fasted and abstained from smoking for 12 hours for the cardiovascular assessment. Subjects then underwent phlebotomy, CSF collection, and 1.5 hours of cognitive testing. After the baseline visit, the participant, study coordinator, and the PI were blind to BP reads at all visits. Participants returned to the UW ADRC during the 2nd and 4th weeks of treatment for a one half hour safety visit to test for elevated potassium and creatinine and to ensure BP levels were within a clinically safe range. The study coordinator then collected information on potential side effects, obtained an update on medication and medical history since the last study visit and reviewed potential medication side effects with the participant.

Study drug compliance was assessed via pill count by an ADRC study coordinator not directly involved with the SEAIRA trial. Premenopausal women were given a pregnancy test at each visit. All procedures and safety labs were overseen by Data Safety and Monitoring Boards at the UW ADRC and the UW Clinical Research Unit. The SEAIRA

study (NCT00980785) was approved by the UW Institutional Review Board (IRB) and all participants provided informed consent.

CSF Biomarkers—CSF samples were collected using a 25 gauge Sprotte needle and a gentle extraction technique widely used in CSF biomarker research in AD. Twenty-two mLs of CSF were collected according to guidelines put forth in the “*NIA Biospecimens Best Practice Guidelines for the Alzheimer’s Disease Centers*” [22]. Samples were gently mixed, centrifuged, pellet removed, aliquoted and stored in polypropylene tubes at -80°C until analysis. CSF T-tau concentration was determined using a sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Ghent, Belgium) specifically constructed to measure all tau isoforms irrespective of phosphorylation status, as previously described [23]. Tau phosphorylated at threonine 181 (P-tau) was measured using a sandwich ELISA method (INNOTEST® PHOSPHO-TAU (181P), Innogenetics, Ghent, Belgium), as previously described [24]. A β 1–42 levels were determined using a sandwich ELISA (INNOTEST® β -AMYLOID(1–42), Innogenetics, Gent, Belgium), specifically constructed to measure A β containing both the first and 42nd amino acid, as previously described [25]. Samples were assayed in one batch after study completion by experienced and board-certified laboratory technicians. Intra-assay coefficients of variation were below 10% for all three analytes. CSF ACE activity was analyzed by ARUP® laboratories by spectrophotometric enzymatic assay.

Ultrasound Measurement of Brachial Artery Reactivity—Endothelial function was evaluated by measuring flow-mediated dilation (FMD) of the brachial artery (BA) in an ultrasound laboratory using a standardized protocol [26, 27]. Subjects were placed in a supine position in a temperature-controlled room for 10 minutes before imaging. A BP cuff was placed on the widest part of the proximal right forearm. Using a 10 MHz linear array vascular ultrasound transducer and a Siemens Medical Solutions (Issaquah, WA) Acuson Sequoia C512 ultrasound system, the BA was located above the elbow and scanned in longitudinal sections. Extravascular landmarks were identified and labeled to assure reproducibility within and between studies. After recording baseline B-mode images of the BA and spectral Doppler images of flow, the cuff was inflated to 250 mmHg for 5 minutes to induce reactive hyperemia. Immediately after deflation, spectral Doppler images were obtained to verify hyperemia. BA images were obtained 60 and 90 seconds later. BA diameters were measured in triplicate with a digital border tracing tool by a single readers blinded to subject information.

Measurement of Pulse Wave Velocity (PWV) and Central Aortic Pressures—PWV was measured by arterial tonometry using the AtCor SphygmoCor PX system. Participants rested in the supine position for at least 10 minutes in a quiet, darkened, temperature-controlled room before data collection started. Tonometry recordings of carotid and femoral arteries were taken when a reproducible signal with a clear upstroke was obtained. The PWV was determined by the intersecting tangents method [28]. PWV (m/s) was calculated as the distance to transit time ratio of the pulse wave. Transit time was calculated as follows: the mean and standard deviation time delay (seconds) from the electrocardiogram R-wave to the foot of the pulse waveform measured at the proximal (carotid) and distal (femoral) sites, based on an analysis of 10 seconds of stable tonometry tracings. Central aortic pressures were derived from radial tonometry using a generalized, validated, transfer function [29, 30]. Time to P1 (initial systolic inflection generated by the ventricular ejection prior to secondary systolic wave) and P2 (additional pressure secondary to arrival of wave reflection to the Aorta) was determined and augmentation pressure (P2-P1) and Augmentation Index ((P2-P1)/PP) were calculated using the SphygmoCor system. Radial tonometry tracing was calibrated by inputting the mean and diastolic brachial arterial pressures, measured non-invasively using a high-fidelity oscillometric BP monitor.

Tonometry tracings were calibrated using the mean and diastolic brachial BP values as input pressures to avoid variability in systolic pressures. Three brachial BP readings were obtained after 10 minutes of rest. Tonometry signals were insured by requiring an operator index higher than 80% for all analyzed tracings.

Laboratory Evaluation—Blood and CSF samples were collected after a 12-hour overnight fast. Potassium and creatinine levels were measured using enzymatic precipitation techniques on a Hitachi 747 analyzer with standard reagents.

Active study drug (ramipril) and matching placebo were purchased from University of Iowa Pharmaceuticals (UIP). Study drug label was wiped off active medication and the placebo was color-matched to ensure the blind. All procedures were conducted in accordance with current Good Manufacturing Practices as set forth in the Code of Federal Regulations. Drug randomization, dispensing and maintenance of the blind were overseen by the UW Pharmaceutical Research Center. Potential side effects evaluating mild cough, dizziness upon standing and fatigue were addressed at each visit in a symptom questionnaire.

Cognitive Testing—Cognitive ability was evaluated by a 1.5 hour battery of nine neuropsychological tests in cognitive domains reportedly affected in early AD [31]. Different but comparable test versions were administered at baseline and month 4 visits to avoid practice effects. The PI or the study coordinator administered the battery and each participant was tested by the same person at baseline and month 4. The battery included measures of working memory and executive function (Trail-Making test B and the Stroop Interference test [32, 33]), language (verbal fluency [34]), verbal memory (list learning task [35] and paragraph recall [36, 37]), and visuospatial ability (Mental Rotation Test [38]). Similar batteries have been utilized in our previous studies and are used to evaluate aspects of cognition selectively impaired in AD [31]. The Beck Depression Inventory (BDI) was administered to control for the effects of mood on cognitive task performance [39]. The Physical Activity History questionnaire was administered to ensure that any potential treatment affects were not due to changes in cardiovascular activity [40].

Data Analyses—The primary study objective was to evaluate the effects of 4 months of ramipril therapy vs. placebo on changes in CSF A β . Additional outcomes included CSF ACE activity, peripheral arterial function and cognition. Due to our small sample size, the analyses focused on patterns and trends in the data using unadjusted analyses in addition to a statistical significance. There were no differences at baseline on any of the outcome measures. Means and standard deviations were ascertained for all variables of interest. Difference scores in outcome parameters between baseline and month 4 were computed to test for between group differences using Mann-Whitney tests. All tests were two-tailed tests using a significance level of 0.05.

Results

Table 2 lists demographic characteristics of the SEAIRA participants by treatment group. Participants were randomized by sex and *APOE* ϵ 4 status. Retention rate was 100%. Both groups were highly educated, cognitively normal and were not depressed. Medication compliance assessed via pill count was over 96%.

Table 3 shows the results of the BP and peripheral arterial function measures by treatment group. Values listed include baseline and month 4 measures, in addition to the difference scores. Blood pressure results are highlighted in the black box. Results confirm that both groups were mildly hypertensive and had similar systolic and diastolic BP values at baseline. Mann-Whitney analyses show that four months of ramipril therapy produced

significant reductions in systolic ($p = 0.029$) and diastolic ($p = 0.008$) BP in the treatment group.

Results of peripheral arterial function analyses confirm that all participants were mildly hypertensive but very healthy in regard to overall vascular function (Refer to Table 3). There were no significant differences between groups on any measure at baseline. While results showed a treatment effect on both systolic and diastolic BP over the four month trial, results did not reveal a treatment effect on additional measures of arterial function.

Table 4 shows the results of the CSF analyses. There were no significant differences between groups on any CSF measure at baseline. Results of the Mann-Whitney analysis did not reveal a treatment effect on CSF A β 1–42 levels ($p = 0.836$). In both groups, A β levels dropped over the four month trial. There were also no between group differences in P-tau levels ($p = 0.366$). Conversely, T-tau levels showed some between group differences before controlling for multiple comparisons, such that T-tau remained stable in the treatment group but levels trended toward a decline in the placebo group ($p = 0.051$). Results also show a treatment effect in CSF ACE activity ($p = 0.009$). Participants in the treatment arm exhibited decreases in CSF ACE activity, while ACE activity in the placebo group remained constant.

Discussion

While larger, more definitive clinical trials in both at-risk populations and in AD patients are needed to further assess the effects of ramipril on CSF A β levels, our findings indicate that four months of ramipril therapy at 5mg daily has the ability to affect brain CSF ACE activity. Taken together, these results suggests that the AD-related benefits associated with BBB crossing ACE-Is could be attributed, at least in part, to an alternative mechanism such as improved CBF. That the total tau results remained stable in the treatment group but declined in the placebo group should be noted, though neither groups' levels were suggestive of AD-related pathology (total tau <400 pg/ml [41]). It is clear from our data that ACE-Is improve BP in mildly hypertensive, middle-aged adults. These improvements were not due to increases in cardiovascular exercise or changes in mood, diet or medications (c.f. study drug). While there were no between-group differences on cognitive task performance in middle-aged, at risk individuals, these results are not surprising considering our young sample was cognitively normal.

There has been conflicting evidence concerning the ability of ACE-I to inhibit ACE activity in the human brain [4, 7]. Our data indicate that ramipril is able to cross the BBB and inhibit brain ACE activity. This effect may be exacerbated in participants with advanced AD or hypertension, as both have been linked to increased permeability of the BBB [42, 43]. These data are important due to reports demonstrating increased ACE activity in CSF and postmortem tissue of AD patients [6, 44]. It is possible that increased ACE activity and subsequent increases in Ang II play a central role in AD neuropathology and decreasing Ang II production via ACE-I therapy could explain reports of AD related benefits by BBB crossing ACE-I, particularly during midlife.

While elevated ACE activity reportedly correlates with AD severity and with parenchymal A β load [6], the exact relationship between ACE activity and A β remains unclear and warrants further clinical investigation [45]. Our data did not reveal a treatment effect of four months of ramipril on A β levels. From this pilot clinical trial, we cannot definitively conclude whether ACE-I affect A β because CSF A β levels declined in both groups. However, prior reports of AD-related benefits such as cognitive performance among AD patients and decreased disease incidence do not appear to be mediated by a direct effect of ACE-I on A β from our data.

The lack of a treatment effect in the present study could be due to the short duration of the trial, too few participants, or possibly an effect of dose. Additionally, our middle aged sample demonstrated CSF A β levels found in normal control populations (A β > 450 pg/ml; [41]), suggestive of efficient A β clearance mechanisms and lower brain A β levels compared AD patients. Thus the apparent lack of A β neuropathology may lessen the potential treatment effect in our sample more so than an AD sample.

While some basic science studies have reported that Ang II stimulation promotes A β production, it is also possible that prior studies reporting AD-related cognitive benefits by ACE-I may be attributed to an alternative mechanism, such as improved CBF [46]. Recent neuroimaging studies show that patients with MCI and early AD exhibit elevated CSF ACE and hypoperfusion in the parietal cortex [47, 48]. This region is also co-localized by ACE, Ang I and Ang II receptors and is therefore a potential site for increased vasoconstriction in early AD [48, 49]. Thus, ACE-I may have the ability to improve CBF via inhibiting ACE activity in regions selectively affected in early AD. While the current study did not employ a measure of CBF, prior research has shown that ACE-I increase CBF in patients with heart failure [50]. Also, a large randomized trial reported that treatment with perindopril, a BBB crossing ACE-I, prevented development of new white matter hyperintensities (WMH) and delayed the progression of existing WMH in patients with cerebrovascular disease [51].

Further evidence for the potential of ACE-I to improve CBF is supported by ACE-I mechanism of action. The ACE-I mechanism of action involves preventing the conversion of Ang I to Ang II, thereby dilating the peripheral arteries and improving vascular function. In the brain, Ang II can exacerbate A β production and is known to increase inflammation, vasoconstriction and mitochondrial dysfunction, decrease G-protein signaling and increasing cerebral endothelial dysfunction (For a comprehensive review see [52]). Thus, if ACE-I have the ability to reduce ACE activity in the CNS, as supported by our data, subsequent reductions in Ang II, a potent vasoconstrictor may improve CBF in the brain as it does in the peripheral vasculature.

Another mechanism by which centrally acting ACE-I may produce favorable AD-related effects is by increasing the release of acetylcholine [52]. Ang II has been implicated in inhibiting the release of acetylcholine, the neurotransmitter widely recognized in AD [53]. Thus, ACE-I mediated increases in acetylcholine release via reduction in Ang II may contribute to AD related cognitive benefits [44, 53, 54].

A BBB crossing ACE-I was selected over alternative antihypertensive medications based on prior basic science, observational and clinical studies reporting cognitive benefits and reduced AD incidence and progression compared to other medications [12, 13]. Ramipril is among the most effective agents for lowering BP and the most commonly prescribed ACE-I, comprising 50% of ACE-I prescriptions and 38% of the cost according to the United Kingdom's NICE guidelines [55]. The ability of ramipril to cross the BBB is significant, as we were interested in ramipril's direct action on A β levels and ACE activity, and meta-analyses have shown that BBB crossing antihypertensives elicit more pronounced cognitive benefits than non-centrally acting medications [12, 21].

The central limitation of the SEAIRA pilot trial is the small sample size. While only 14 well characterized participants were enrolled, this pilot clinical trial has successfully demonstrated the feasibility of conducting randomized clinical trials to investigate the effects of antihypertensive medications on AD biomarkers in humans. While it was not appropriate to control for multiple comparisons in our analyses, it is important to note that post-hoc analyses revealed that the significant effects of ACE activity and BP measures did not survive such analyses, which was likely also due to our small sample size. This is the

first randomized, placebo controlled clinical trial examining the effects of a BBB crossing ACE-I on AD biomarkers in a preclinical sample. Initial results from the SEAIRA trial provide support for larger clinical trials which are urgently needed considering the increasing incidence of AD, vascular disease and the number of individuals currently taking antihypertensive medications. Similar clinical trials in AD cohorts are also particularly important, as AD-related cognitive deficits and A β neuropathology are more pronounced in patient populations and therefore may be more sensitive to treatment effects.

Further clarification of RAS-acting antihypertensive medications on AD and cognition is crucial. If hypoperfusion and the presence of A β lead to neuronal damage in part through deregulation of CNS ACE activity and elevated BP, then modifying CBF and A β accumulation through the use of ACE-Is may potentially reduce the risk of developing AD in high-risk individuals. Larger clinical trials with ACE-I and A2RB, particularly those that incorporate CSF A β and CBF measures, are highly warranted. Future trial designs would also benefit by including a non RAS-acting treatment arm in order to differentiate between BP mediated effects vs. Ang II related effects in relation to AD pathology.

Acknowledgments

The study was supported by a grants from the State of Wisconsin and the Wisconsin Alzheimer's Institute (WAI) R01AG027161 and from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources of the NIH 1UL1RR025011. We utilized the resources of the School of Medicine and Public Health at the University of Wisconsin (UW), the Wisconsin Alzheimer's Disease Research Center (UW ADRC) NIH P50 AG033514, UW Department of Medicine Division of Geriatrics and Gerontology, the Geriatric Research, Education and Clinical Center (GRECC) of the William S. Middleton Memorial Veterans Hospital, Madison WI and the UW Atherosclerosis Imaging Research Program.

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TABLE 1**SEAIRA Inclusion / Exclusion Criteria****INCLUSION CRITERIA:**

Parent with diagnosed AD

> 40 and < 65 years of ages

systolic 120–159 mmHg and diastolic 80–99 mmHg

EXCLUSION CRITERIA:

Diagnosis of, or Treatment for a Memory Disorder

MMSE < 27

Severe Untreated, Obstructive Sleep Apnea

Current or Recent (<1yr) major psychiatric condition

Major Neurologic Disorder other than Dementia

Psychotic Illness (Schizophrenia, Bipolar Disorder)

Potassium > 5.5 mg/dL

Creatinine >1.8 mg/dL at Baseline

Diabetes Type I or II

History of Major Stroke

Pregnancy or Planning to Become Pregnant

Severe Congestive Heart Failure

Contraindications to LP

History of an adverse reaction to an ACE-I

Use of an antihypertensive within the last 3 months

Current involvement in an investigational drug trial

Use of a weight loss medication

Table 2

Participant Demographics by Treatment Group

Variable	Treatment (N = 8)	Placebo (N = 6)
Systolic blood pressure (baseline; mmHg)	145.63	132.83
Gender (Male %)	50%	50%
ApoE4 positive (%)	50%	50%
Age (years)	56.3 (6.1)	52.0 (6.7)
Education (years)	15.0 (3.0)	15.8 (1.6)
BDI (baseline)	6.0	2.8
MMSE (baseline)	29.5	29.8

BDI = Beck Depression Inventory,

MMSE = Mini Mental State Exam

Note: No significant between-group differences at baseline.

Table 3**Blood Pressure and Arterial Function Measures by Treatment Group**

Variable	Treatment (N = 8)	Placebo (N = 6)	Mann-Whitney p Value
Systolic (mmHg)			
Baseline	145.63	132.83	
Month 4	129.13	135.83	
Difference	-16.5	3.0	p = 0.029
Diastolic (mmHg)			
Baseline	83.0	81.3	
Month 4	76.1	86.7	
Difference	-6.9	5.3	p = 0.008
Central Mean Pressure (mmHg)			
Baseline	103.1	100.0	
Month 4	95.9	100.8	
Difference	-7.3	0.8	p = 0.228
Systemic Vascular Resistance (dyne*sec)/cm5)			
Baseline	1648.3	1429.1	
Month 4	1496.5	1519.1	
Difference	-151.8	90.0	p = 0.345
Augmentation Index (HR corrected; %)			
Baseline	23.8	16.7	
Month 4	22.0	18.3	
Difference	-1.8	1.7	p = 0.491
Pulse Wave Velocity (meters/second)			
Baseline	7.79	7.78	
Month 4	7.66	7.73	
Difference	-.1	-.05	p = 0.755
Relative Flow-Mediated Vasodilation (%)			
Baseline	4.94	6.69	
Month 4	4.65	5.99	
Difference	-.29	-.70	p = 0.755

Note: No significant between-group differences at baseline.

Table 4

CSF Analyses by Treatment Group

Variable	Treatment (N = 8)	Placebo (N = 6)	Mann-Whitney p Value
A β 1-42 (pg/ml)			
Baseline	672.5	613.5	
Month 4	641.1	591.0	
Difference	-31.71	-22.5	p = 0.836
T-tau (pg/ml)			
Baseline	235.25	196.5	
Month 4	240.29	178.83	
Difference	3.43	-17.67	p = 0.051
P-tau (pg/ml)			
Baseline	47.25	40.83	
Month 4	48.57	39.17	
Difference	1.43	-1.67	p = 0.366
ACE activity (U/L)			
Baseline	1.53	1.55	
Month 4	.800	1.45	
Difference	-.63	-.10	p = 0.009

Note: No significant between-group differences at baseline.