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Trinity College Dublin

School of Medicine

Department of Medical Gerontology

An investigation of the safety, efficacy, and possible mechanisms of action, of the antihypertensive nilvadipine, in patients with mild moderate Alzheimer's disease

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Student ID: 07132981

Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

Submitted: January 2011
Declaration:

I declare that this work has not been submitted as an exercise for a degree at this or any other University. It is entirely my own work. I agree the library may lend or copy the thesis upon request.

Sean Kennelly

Date: 19/05/2011
Contents:

Introduction and summary

Chapter 1: How blood pressure influences our risk for developing dementia

Chapter 2: Antihypertensive therapy and the incidence of Alzheimer's disease

Chapter 3: Demonstration of safety in Alzheimer’s patients for intervention with an antihypertensive drug Nilvadipine: results from an eight week open label study

Chapter 4: APOE ε4 specific short-term cognitive benefits of treatment with the antihypertensive nilvadipine in Alzheimer’s patients- an open-label trial

Chapter 5: The relationship between cerebral haemodynamics and apolipoprotein E ε4 allele expression in patients with Alzheimer’s disease- a transcranial doppler study

Chapter 6: The effects of treatment with the antihypertensive nilvadipine on cerebral haemodynamics in patients with AD- a transcranial doppler study

Chapter 7: The effect of treatment with the antihypertensive nilvadipine on cerebrospinal fluid levels of Aβ-40 and Aβ-42 in patients with mild/moderate Alzheimer’s disease

Acknowledgements

References
Declaration of contribution: I would like to thank and acknowledge support for the Roskamp Neurosciences Institute in Sarasota, Florida who performed the apolipoprotein E genotyping, and the β-amyloid measurements in the cerebrospinal fluid. All other aspects of this study were performed by me.

Publications from thesis:


Presentations of results from thesis:

1. “APOE specific cognitive benefits of nilvadipine in Alzheimer’s patients”— Irish Association of internal Medicine Nov 2009, Wexford, Ireland
Introduction and summary

This doctoral investigation endeavoured to explore how patients with Alzheimer’s disease (AD) would respond to treatment with the antihypertensive nilvadipine.

Initially I reviewed the complex relationship between blood pressure (BP) and the incidence of AD. Mid-life hypertension appears to be a strong and consistent risk factor for AD, whereas it is less clear how high BP in later life influences one’s risk. Importantly it also appears that low BP; especially later in life is also associated with an increased risk of developing AD. Patients, who develop AD, also appear to have a reduction in their BP preceding the onset of clinical symptoms. I discuss how the risk for AD attributable to different BP’s may be as result of diminished cerebral perfusion.

Given this association between BP and AD we examined how treatment with antihypertensive medications affected one’s risk of developing AD. There is considerable evidence from observational, and some randomised placebo controlled studies, that treatment with certain antihypertensive medications is indeed associated with a lower risk of developing AD. However, the evidence is not consistent, and this effect on cognition is not specific to any one class of antihypertensive; rather it appears specific to certain medications within classes that have certain neuroprotective properties.

Nilvadipine is a lipophillic dihydropyridine calcium channel blocker (DHP-CCB) antihypertensive. Previous imaging studies have demonstrated its ability to increase cerebral perfusion in patients with mild cognitive impairment (MCI), and AD. In one small study, treatment of hypertensive MCI patients with nilvadipine reduced their risk of cognitive decline over several months.
In an eight-week open labelled study, I investigated the safety and tolerability of nilvadipine treatment in AD patients. Treatment was well tolerated, with no patient requesting or requiring discontinuation of the medication. Safety was investigated using regular automated BP measurements, and ambulatory BP monitoring, as well as active stand measurements using a finometer. Nilvadipine reduced BP in patients with elevated BP, while having little or no effect on non-hypertensive patients. There was no increase in the drop in BP on standing.

Results from cognitive tests performed suggest an APOE ε4 dependent effect, with APOE ε4 non-carriers having better cognitive scores at follow-up.

Using transcranial doppler (TCD), I found an association between APOE ε4 genotype and cerebral perfusion velocities, and resistance and pulsatility indices in patients with AD. APOE ε4 homozygous patients have higher velocities and lower pulsatility and resistance indices. I found no treatment effect of nilvadipine on cerebral artery velocities or pulsatility and resistance indices in patients with AD.

Finally I investigated if treatment with nilvadipine had any effect on cerebrospinal fluid (CSF) levels of Beta-amyloid (Aβ). There was a trend towards a proportional increase in CSF Aβ-42, although this did not reach statistical significance.

The findings of this study represent an original contribution to the understanding of how treatment with nilvadipine and antihypertensives in general may influence AD. Given the limitations attached to an open label study these findings warrant confirmation in a blinded randomised control study with a longer follow up period.
Chapter 1: How blood pressure influences the risk for Alzheimer’s disease

Abstract

Alzheimer’s disease (AD) and vascular dementia (VaD) are important causes of cognitive decline in the elderly. As a result of the aging population, the incidence of dementia is expected to increase substantially over the coming decades. Many studies have identified that vascular risk factors are implicated in the pathogenesis of both AD and VaD. Longitudinal studies have suggested that high blood pressure in midlife is associated with a higher incidence of both AD and VaD in later life. The association appears weaker for hypertension in later life. Some studies also suggest that hypotension; especially low diastolic blood pressure in late-life is also associated with an increased risk of AD. Long-standing hypertension may lead to severe atherosclerosis and impaired cerebrovascular autoregulation. A decline in blood pressure in later life may contribute to diminished cerebral perfusion. The subsequent ischaemic state may lead to increased cerebral β-amyloid accumulation.

Introduction

It is commonly accepted that the most frequently occurring causes of dementia in older people are Alzheimer’s (AD), Vascular Dementia (VaD) and mixed variants of the two [2]. Alzheimer’s dementia is classified as a progressive neurodegenerative disorder with the histopathological hallmarks of β-amyloid plaques, neurofibrillary tangles of hyperphosphorylated tau, and cerebral amyloid angiopathy [3].
Figure 1 Pathogenesis of AD: Aggregation and accumulation of β-amyloid (Aβ) in the brain may result from increased neuronal production of Aβ, decreased activity of Aβ-degrading enzymes, or alterations in transport processes that shuttle Aβ across the blood–brain barrier. Aβ oligomers impair synaptic functions, whereas fibrillar amyloid plaques displace and distort neuronal processes. Aβ oligomers interact with cell-surface membranes and receptors, altering signal-transduction cascades, changing neuronal activities and triggering the release of neurotoxic mediators by microglia (resident immune cells). Vascular abnormalities impair the supply of nutrients and removal of metabolic by-products, cause microinfarcts and promote the activation of astrocytes and microglia. The lipid-carrier protein apoE4 increases Aβ production and impairs Aβ clearance. When produced within stressed neurons, apoE4 is cleaved into neurotoxic fragments that destabilize the cytoskeleton and, like intracellular Aβ, impair mitochondrial functions. The proteins tau and α-synuclein can also self-assemble into pathogenic oligomers and can form larger intra-neuronal aggregates, displacing vital intracellular organelles [1]. (Mucke, Nature, 2009)

Hypertension is a major risk factor for cerebrovascular disease, and therefore VaD.

Vascular dementia occurs as a result of cerebrovascular insults in cortical and subcortical areas responsible for memory and executive function; however there are no objective
neuropathological criteria to indicate the exact vascular “threshold” for making a diagnosis of VaD (Table 1) [3][348]. In spite of the vast and continuing literature on the division between AD and VaD, emerging concepts highlight the role of cardiovascular risk factors in the pathogenesis of AD [4, 5]. Cerebrovascular ischaemic abnormalities are often found in conjunction with pathological changes of AD [3]. This co-occurrence of the two disorders is commonly referred to as mixed dementia, but often referred to as Alzheimer’s disease with cerebrovascular disease [6, 7].

Table 1: NINDS-ARIEN criteria for diagnosing vascular dementia

Cerebrovascular disease:

Focal central nervous system signs

Evidence of cerebrovascular disease by neuro-imaging

A relationship between the two manifest by one or more of the following:

Dementia onset within three months after having a stroke

Abrupt deterioration in cognition or fluctuating stepwise course

These ischaemic lesions appear to be important determinants of cognitive function, even in the presence of AD. The “Nun study” showed that of 61 participants meeting the neuropathological criteria for AD, those with brain infarcts had poorer cognitive performance and a higher prevalence of dementia than those without infarcts [8]. In the Oxford projects to investigate memory and aging, the authors demonstrated that
additional cerebrovascular disease significantly worsened cognitive performance, at least in the earliest stages of AD [9]. As a result of this interplay between the neurodegenerative processes of AD, and the presence of cerebrovascular disease, AD has been described as a vasculopathic disorder [10]. This theory proposes that the neurodegenerative process underlying AD is provoked by pre-morbid vascular-related events. Most of the risk factors that have been described in epidemiological studies of elderly people for AD are also risk factors for the development of VaD (Table 2). Ultimately these vascular pathologies lead to the reduction or impairment of optimal cerebral perfusion [11]. Brain hypoperfusion therefore may be a critical factor in the development of both VaD [12, 13] and AD [14].

The risk of hypertension increases with advancing age. The prevalence of hypertension in persons 60 years and older is double that of persons aged 49-50 years and, despite recent improvements, BP control rates in older persons remain suboptimal with only 50% of treated elderly patients achieving adequate control [15]. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) classified hypertension as a systolic BP ≥ 140mm Hg and/or a diastolic BP ≥ 90mmHg [16]. Numerous epidemiological studies have examined the relationship between blood pressure and the incidence of dementia.
Table 2: Risk Factors for AD as reported in epidemiological studies

<table>
<thead>
<tr>
<th>Ageing</th>
<th>Thrombogenic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis</td>
<td>APOE ε4</td>
</tr>
<tr>
<td>Stroke</td>
<td>High serum homocysteine</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Smoking</td>
<td>Hypotension</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>High fibrinogen levels</td>
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<tr>
<td>High HDL Cholesterol</td>
<td>Head Injury/Loss of consciousness</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>Menopause</td>
</tr>
<tr>
<td>Migraine</td>
<td>Lower education</td>
</tr>
<tr>
<td>High Serum viscosity</td>
<td>Transient ischaemic attacks</td>
</tr>
<tr>
<td>Depression</td>
<td>Microvessel pathology</td>
</tr>
<tr>
<td>Fat intake</td>
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</tbody>
</table>

Note that despite the discrete aetiologies, pathological course, and clinical outlook of each risk factor, all are linked by 2 activities: (1) all are vascular related and (2) all impair or reduce cerebral perfusion. It should be noted that most of the risk factors listed are also risk factors for VaD.

Given the temporal lag that may exist between blood pressure anomalies and the incidental development of AD, studies using a prospective longitudinal approach are the best equipped to determine a causal relationship between these two factors. Cross sectional studies, while of benefit in directing us to investigate possible relationships between two factors longitudinally, are not particularly helpful when trying to attribute causality. I will focus on population based epidemiological studies here, because of the selection bias that may be introduced when other populations are investigated. The age at which blood pressure is being measured also appears to influence ones risk for
developing dementia, so we will examine the evidence for mid-life (age 40-64 years) and late life (≥65 years) blood pressure separately. It is also worth noting that with the aging demographics of both developed and developing countries, a cut-off age of ≥65 years may reflect a population which by current standards is considered quite young. I will then explore some of the possible mechanisms by which changes in blood pressure over time might exert this influence on the incidence of dementia.

**Late-life hypertension and the risk of dementia**

Several cross sectional studies have attempted to assess the relationship between late-life BP and dementia (Table 3) [17-23]. Of the seven reviewed that examined this relationship in this fashion, only two reported no association between blood pressure and prevalent dementia and AD [17, 18]. No cross sectional study noted an association between late life hypertension (either systolic or diastolic) and the incidence of AD. One Japanese study demonstrated an increased risk for acquiring VaD [17]. However, in this study VaD accounted for 59% of the 50 patients diagnosed with dementia. This was 2.2 times greater than the number of patients diagnosed with AD, and would be contrary to rates reported in most other studies. The Kuopio study in Finland analysed blood pressure as a continuous variable, and that may have masked a non-linear relation between blood pressure and the risk of prevalent AD [18]. The other 5 cross sectional studies observed an inverse association between these parameters and this will be discussed further later.
<table>
<thead>
<tr>
<th>Settings</th>
<th>Participants</th>
<th>Outcomes</th>
<th>Covariates*</th>
<th>Main Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ueda et al 1992[17]</strong></td>
<td>Hisayama Study, Japan 887 people age ≥65 years</td>
<td>Dementia, AD, VaD DSM III AD, NINCDS-ADRDA</td>
<td>Alcohol use, smoking, BMI, cholesterol, glucose, ECG</td>
<td>Hypertension (≥160/95mmHg) related to VaD, but not AD</td>
</tr>
<tr>
<td><strong>Kujaisto et al 1997[18]</strong></td>
<td>Kuopio Study, Finland 980 people age 69-78 years</td>
<td>AD NINCDS-ADRDA APOE e4, total cholesterol, fasting glucose, insulin</td>
<td>BP not significantly related to AD</td>
<td></td>
</tr>
<tr>
<td><strong>Guo et al, 1996[19]</strong></td>
<td>Kungsholmen project, Sweden 1642 people age ≥75 years</td>
<td>Dementia, AD; DSM III-R</td>
<td>Low BP (≤140/75 mmHg) - high prevalence of dementia/ AD</td>
<td></td>
</tr>
<tr>
<td><strong>Petitti et al 2002[21]</strong></td>
<td>Women’s memory study, USA 3924 women age ≥75 years</td>
<td>Dementia; medical records</td>
<td>Self reported hypertension - low dementia prevalence</td>
<td></td>
</tr>
<tr>
<td><strong>Morris et al 2000[20]</strong></td>
<td>Chicago Health and Aging project, 709 people (54% black) age ≥65 years</td>
<td>AD; NINCDS-ADRDA Ethnic origin, use of antihypertensive drugs, BMI, vascular disorders</td>
<td>Low BP (≤130/75 mmHg) - high prevalence of AD</td>
<td></td>
</tr>
<tr>
<td><strong>Kokmen et al 1991[22]</strong></td>
<td>Rochester Epidemiology Project, USA 415 patients and 415 controls</td>
<td>AD; post mortem; residence</td>
<td>Documented hypertension: OR for AD 0.70</td>
<td></td>
</tr>
<tr>
<td><strong>Rockwood et al 1996[23]</strong></td>
<td>Canadian Study of Health and Aging 204 patients and 511 controls aged ≥65 years</td>
<td>AD; NINCDS-ADRDA Residence</td>
<td>Hypertension: OR 0.3 for AD (95% CI 0.25-0.58)</td>
<td></td>
</tr>
</tbody>
</table>

*Demographic variables (i.e. age, sex, education was included as covariates in all studies. DSM-III(R)= Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (revised); ECG= electrocardiogram; NINCDS-ADRDA= National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer’s Disease and related Disorders Association; APOE= apolipoprotein E genotype; BP=blood pressure; OR= odds ratio
Whereas no cross sectional study described an association between late life hypertension and dementia, several observed a relation between hypertension at this stage of life and impaired cognition [24-27]. These studies, including the large-scale Atherosclerosis Risk in Communities study, usually used cut-offs for systolic BP of ≥ 160 mmHg to define systolic hypertension. Cognitive impairment was measured using tests that examined several cognitive domains, and hypertension in late-life was associated with lower Mini mental state examination (MMSE) scores [25, 27], impaired performance on digit symbol subtest and word fluency [24], and lower CAMCOG (Cambridge examination for mental disorders of the elderly- cognitive section) scores. By contrast 4 other cross sectional studies with over 10,000 participants in total found no association between these two variables [28-31].

Longitudinal studies offer a better opportunity to explore the temporal relationship between hypertension, and the onset of dementia. Several studies (Table 4) have addressed the issue but only two Swedish studies identified a link between hypertension in late-life and dementia [32, 33]. In the Kungsholmen project, a community-based cohort of 1270 participants (aged ≥75 years) were followed up for a period of 6 years, and 339 subjects were diagnosed with dementia according to DSM IV criteria (256 developing AD) [33]. Subjects with very high SBP (>180mmHg) had an adjusted relative risk of 1.5 for AD (95% CI 1.0-2.3), and 1.6 (95% CI 1.1-2.2) for dementia in general. High DBP (90mmHg) by contrast was not associated with an increased risk. Low DBP (<65mmHg) on the other hand was associated with an increased risk of dementia (95% CI 1.1-2.1). Only one study described an association between both elevated systolic and diastolic blood
pressure and a subsequent diagnosis of AD or dementia [32]. This group of 382 subjects (aged 70 years) were followed up at intervals over a period of 15 years. Participants who developed dementia at age 79-85 had higher SBP at age 70, (mean 178 mmHg vs 164 mm Hg, p = 0.034) and higher DBP at ages 70, (101 mm Hg vs 92 mmHg, p = 0.004) and 75, (97 mmHg vs 90 mmHg, p = 0.022), than those who did not develop dementia. Higher DBP at age 70 and 75 years was associated with a higher incidence of both AD and VaD. Interestingly, this study also described that blood pressure declined in the years preceding the onset of dementia, and was then similar to, or lower than that in non-demented individuals. Recently, the Adult Changes in Thought Study assessed the variation in the association between BP and the risk for both AD and dementia across a spectrum of older ages, and examined BP changes before dementia onset [34]. The 2,356 participants were all dementia free and ≥65 years of age but for analyses were divided into 3 age categories at baseline (65-74 years, 75-84 years and ≥85 years) and followed up for eight years. Cognition was assessed using the Cognitive Abilities Screening Instrument (CASI) and those with scores <86 underwent a dementia diagnostic evaluation [35]. Diagnoses were assigned according to DSM-IV criteria for dementia and the NINCDS-ADRDA criteria for AD. BP was measured at enrolment and at each biennial examination. SBP was divided into 3 categories: high (≥160 mmHg), borderline high (140-159 mmHg), and normal (<140 mmHg). Similarly DBP was also categorised as high (≥90 mmHg), borderline high (80-89 mmHg), and normal (<80 mmHg). During the follow up, 380 of the 2,356 participants received a diagnosis of all cause dementia; 204 had probable AD. After adjustment for sex, race, years of education and the presence of APOE ε4 allele, the youngest age group showed a significant association between high SBP (≥160 mmHg) and
all cause dementia (HR 1.6; 95%CI 1.01-2.55). The risk estimates were similar although not statistically significant for this group and the development of AD (HR 1.38 95% CI 0.71-2.71). The risk estimates for both AD and dementia associated with SBP declined with advancing age. In fact, there was a trend towards a lower AD and dementia risk with higher SBP in the oldest age group (>85 years) (HR for AD 0.70; 95% CI 0.25-1.95) (HR for dementia 0.64; 95% CI 0.32-1.30). Results with DBP showed weaker but similar trends. Strengths of this study include its community based prospective design, with a relatively long follow up period.
<table>
<thead>
<tr>
<th>Study settings</th>
<th>Participants / follow up</th>
<th>Outcomes</th>
<th>Covariates*</th>
<th>Main Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiu et al, 2003[33]</td>
<td>Kungsholmen Project, Sweden</td>
<td>1270 people age ≥75 years, follow up 6 years</td>
<td>Dementia, AD; MMSE score</td>
<td>SBP&gt; 180 mmHg vs 141-180 mmHg: RR for AD 1.5</td>
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<td></td>
<td>SBP&gt; 140 mmHg vs 141-180 mmHg: RR for AD 1.5</td>
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<tr>
<td></td>
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<td></td>
<td>DBPs 65mmHg mmHg vs 66-90 mmHg: RR for AD 1.7</td>
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<tr>
<td>Skoog et al, 1996[32]</td>
<td>Gothenburg H-70 Study, Sweden</td>
<td>382 people, age 70 years followed up to 15 years</td>
<td>Dementia, BMI</td>
<td>BP&lt; 65 mmHg vs 66-90 mmHg: RR for AD 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SBP and DBP levels at baseline were higher in individuals who developed dementia than those who did not.</td>
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</tr>
<tr>
<td>Li et al, 2007[34]</td>
<td>Adult Changes in thought Study, HMO Cohort, U.S</td>
<td>2356 non-demented people aged ≥65 years, stratified into 3 age categories</td>
<td>AD, NINCDS-ADRDA; APOE, Ethnic origin</td>
<td>Greatest risk for dementia in “youngest” old age group with SBP ≥160 mmHg HR 1.6 or borderline-high DBP (80-89 mmHg) HR 1.59</td>
</tr>
<tr>
<td>Yoshitate et al, 1995[36]</td>
<td>Hisayama Study, Japan</td>
<td>828 non-demented people age 65-98 years, follow up 7 years</td>
<td>Dementia, Alcohol use, physical activity, diabetes, stroke, baseline cognition</td>
<td>BP≥160/95 mmHg was not related to AD, but was to VaD (OR =1.6 per 1 SD increase in SBP)</td>
</tr>
<tr>
<td>Brayne et al, 1998[37]</td>
<td>Cambridge cohort study, UK</td>
<td>376 people age ≥75 years, follow up 2.4 years</td>
<td>Dementia, AD; similar to ICD-10</td>
<td>History of hypertension was not related to dementia; OR 1.1</td>
</tr>
<tr>
<td>Study settings</td>
<td>Participants / follow up</td>
<td>Outcomes</td>
<td>Covariates*</td>
<td>Main Results</td>
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<tr>
<td>Posner et al, 2002[38]</td>
<td>Washington heights- Inwood Columbia Ageing Project, US</td>
<td>1259 Medicare recipients aged ≥65 yrs F/U over 7 years</td>
<td>AD, NINCDS-ADRDA; VaD, NINCDS-ARIENS</td>
<td>Ethnic origin, heart disease</td>
</tr>
<tr>
<td>Lindsay et al, 2002[39]</td>
<td>Canadian Study of Health and Ageing</td>
<td>3566 people aged ≥65 years, F/U 5 years</td>
<td>AD, DSM-IV</td>
<td></td>
</tr>
<tr>
<td>Kuller et al, 2003[40]</td>
<td>Cardiovascular health Study, US</td>
<td>3275 Medicare recipients in 4 clinical centres, aged ≥65 years, F/U 7 years</td>
<td>Dementia; AD diagnosed by an expert committee</td>
<td>Ethnic origin</td>
</tr>
<tr>
<td>Borenstein et al, 2005[41]</td>
<td>Kame project, US</td>
<td>1859 Japanese Americans aged ≥65, F/U 6 years</td>
<td>AD, NINCDS-ADRDA</td>
<td>APOE</td>
</tr>
<tr>
<td>Petitti et al, 2005[42]</td>
<td>Women’s Memory Study, US</td>
<td>1133 women from pre-paid health plan aged ≥75 years, F/U 10 years</td>
<td>Dementia-diagnosed telephone interview and medical record review</td>
<td></td>
</tr>
<tr>
<td>Tyas et al, 2001[43]</td>
<td>Population study in Manitoba, Canada</td>
<td>694 people aged ≥65 years, F/U 5 years</td>
<td>AD, NINCDS-ADRDA</td>
<td></td>
</tr>
</tbody>
</table>

* Demographic variables (i.e. age, sex, and education were included as covariates in all studies). NINCDS-ADRDA= National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer’s Disease and related Disorders Association, NINCDS-ARIEN= National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l’Enseignement en Neurosciences; APOE= apolipoprotein E genotype; BP=blood pressure; OR= odds ratio; ICD-10= International Classification of Diseases, 10th revision; DSM-IV= Diagnostic and Statistical Manual of Mental Disorders, 4th edition; RR=risk ratio; DSM-III(R)= Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (revised); BMI=Body Mass Index.
Several longitudinal studies failed to demonstrate any relationship between late life hypertension and the incidence of AD and dementia [36-43] (Table 4). Some of these studies did describe an association between hypertension and VaD but not AD [36, 38]. However, in many of these studies the diagnosis of hypertension was ascertained by subjects self reporting high blood pressure or a history of high BP as opposed to any objective measurements [37-41, 43].

Several longitudinal studies with a combined total of over 20,800 subjects have described an association between late life hypertension and diminished scores on various neuropsychological tests, [44-50] including the 10,963 subjects aged 47-70 years followed up for 6 years in the Atherosclerosis Risk in Communities cohort [50]. The presence of hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, or use of antihypertensive medications) at baseline was associated with greater decline on the digit symbol subtest of the Weschler Adult Intelligence Scale (WAIS) (p < 0.05) [50]. Other longitudinal studies failed to report this association [51-53]. In particular the Italian longitudinal Study on Aging [53] which followed up 1445 subjects aged 65-84 years for 3.5 years failed to detect an association between hypertension (i.e. having a history, on antihypertensive medication, or BP≥140/90 mmHg), and the onset of mild cognitive impairment (MCI) as defined by Petersen et al [54, 55]. The failure to identify a relationship in this study may have been related to the relatively short follow up period. Three studies described a U-shaped relationship between late life hypertension and impaired cognition [56-58], where high SBP (≥ 160 mmHg), and systolic blood pressures less than 130mmHg were associated with diminished performance on mental status questionnaires [57, 58], and the MMSE [56] when followed up over 3-9 years.
Could late life hypertension be protective against dementia?

Some authors suggest that higher blood pressure in later life may be associated with a lower incidence of dementia at this stage of life, however these assumptions should be considered with caution [59, 60]. The recent Hypertension in the Very Elderly Trial (HYVET) was terminated early because of a substantial reduction in all cause mortality and stroke rates in patients ≥80 years of age treated with a diuretic and/or an ACE inhibitor to a target BP of ≤ 150 mmHg compared to placebo [61]. The Cache County Study assessed the relationship between vascular risk factors and the subsequent risk of AD or VaD in a community based cohort of 3264 patients over the age of 65 years [59]. Hypertension was diagnosed as a result of self-reporting a prior history, or a current prescription for an antihypertensive. Dementia was diagnosed according to the DSM-III-R criteria, using information from cognitive tests, activities of daily living scales, physical examination and finally by consensus at conferences with a geriatrician, neurologist and neuropsychologist. AD cases met the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association (NINCDS-ADRDA) criteria. Cases of VaD were diagnosed according to the NINDS- Association Internationale pour la Recherché et l’Enseignement en Neurosciences (AIREN) criteria. 104 cases of AD and 37 cases of VaD were identified after a 3 year follow up period. Hypertension was associated with an increased risk of VaD in women alone (aHR 4.58, 95% CI 1.23-29.85), but not AD. In fact hypertension as reported in this fashion was associated with a reduced incidence of AD, although no statistically significant (aHR 0.66; 95% CI 0.43-1.02). A related study in the same group found that the use of antihypertensive medication protected against new incident AD [62]. Differential
survival may have influenced the results, and limited the study to persons with hypertension who were in better health than those who did not survive to the follow up evaluation. It is also possible that the risk of AD associated with late-onset hypertension would not have manifest until older ages, and may have been missed in a 3 year follow up. The diagnosis of hypertension in this fashion does not take into account any effect attributable to midlife hypertension. More recently another study monitored 339 non-demented subjects aged ≥85 years at baseline for a follow up period of 9 years [60]. A diagnosis of hypertension was based on self-reporting and a review of patient’s medical records. BP was measured once at baseline using a calibrated mercury sphygmomanometer. The diagnosis of dementia was made according to the DSM-III-R criteria, using patient performance on certain cognitive tests including the MMSE, performance on activities of daily living instruments, and a physical examination as a guide. The authors did not categorize different dementia subtypes. Of the 339 non-demented subjects at the baseline there were 100 new dementia cases identified during the follow up period. A history of hypertension was associated with a reduced risk of developing dementia (HR 0.59; 95% CI 0.34-1.00, p= 0.049). The strengths of this study include the recruitment of an entire population of individuals ≥85 years old, and the high participation rate which was >90% throughout. Also they allowed for a lengthy follow up period. Only cognitive decline severe enough to be classified as dementia was identified. A lack of availability of neuro-imaging meant that an exact aetiological diagnosis of dementia was lacking. The high age of the study cohort may also have proved a source of bias given the increased risk of mortality in subjects with vascular risk factors; many
patients with these risk factors have died before the detection of possible dementia. This limits the possibility of finding an association between these factors and dementia.

**Mid-life hypertension and the risk of dementia**

Several studies have examined the relationship between elevated blood pressure in midlife (age 40-64 years) and the onset of dementia and AD later in life [63-67] (Table 5). The Honolulu Asia Aging Study studied this relationship in 3703 Japanese-American men aged 45-68 years at their midlife examination, who were followed prospectively for 26 years in the Honolulu Heart programme [63]. 5.9% of the total sample had a midlife SBP of $\geq 160$ mm Hg. The Cognitive Abilities Screening Instrument (CASI) was used to evaluate their cognitive status. VaD and AD were diagnosed according to the DSM-III-R. Among men untreated for high blood pressure (57% of the sample), there was a strong association between midlife hypertension and both AD and VaD when 160/95 mmHg was used as the BP cut-off. A higher, although non-significant, risk was also associated with BP cut-offs of 140/90 mmHg. There was no identifiable association between elevated blood pressure and dementia in individuals receiving antihypertensives. This association was further confirmed when autopsy and neuro-imaging data from the same study was reviewed [68, 69]. The autopsy study of 243 participants demonstrated that along with vasculopathic changes, elevated SBP in midlife was associated with lower brain weights, and greater numbers of neuritic (β-amyloid) plaques in both the neocortex and the hippocampus; while elevated DBP was associated with greater numbers of neurofibrillary (tau protein) tangles in the hippocampus [68]. Neurofibrillary tangles and neuritic plaques, while they can occur as neuropathological features of aging, are classically associated with AD. The neuro-imaging study demonstrated an association between
midlife untreated hypertension and hippocampal atrophy (OR 1.98 95% CI 0.89-4.39) [69]. Although hippocampal atrophy can occur in the presence of either VaD or AD, it is considered a radiological hallmark of AD and correction for white matter lesions and lacunes did not alter the association between midlife hypertension and hippocampal volume (HV). This suggests that the mechanism of blood pressure on HV is probably different from the hypertensive effect on white matter lesions.

The Kuopio and Joensuu studies also found that both elevated systolic and/or diastolic BP in midlife was associated with an increased risk of both AD and VaD [64]. This increased risk was independent of APOE genotype, and furthermore when high SBP was combined with an elevated total cholesterol level, the risk for AD or VaD was greater than when either were present alone [70]. A similar synergistic relationship was identified between midlife obesity (Body mass index >30Kg/m2), midlife hypertension, and total cholesterol, whereby they were all significant risk factors in their own right with odds ratios of about 2.0 for each factor, but they also increased the risk additively for both AD and VaD [71].

In 2006, Kivipelto et al devised a risk score (Midlife Dementia Risk score) for the prediction of dementia, 20 years on in middle aged individuals [349]. Data was used from the population based CAIDE study, which included 1409 individuals who were studied in mid-life and re-examined 20 years later for signs of dementia. Several midlife vascular risk factors were studied to create a scoring tool, and the dementia risk score was the sum of these individual scores (0-15). Occurrence of dementia during the 20 year follow up was 4%. Future dementia was predicted by high age (≥ 47 years), low education (< 10 years), hypertension, hypercholesterolemia, and obesity. The dementia risk score
predicted dementia well (c statistic 0.77), and inclusion of APOE genotype improved the accuracy of the index (c statistic, 0.78). The risk of dementia was 1.0% for those with a score of 0-5, 1.9% for a score of 6-7, 4.2% for a score 8-9, 7.4% for a score of 10-11, and 16.4% for a score of 12-15. When the cut-off of 9 points or more was applied the sensitivity was 0.77, the specificity was 0.63, and the negative predictive value was 0.98. This risk score and the associated risk of dementia attributable to these midlife vascular co morbidities emphasises the strength of effect these vascular risks have on the later life incidence of dementia. Information on midlife vascular risk factors should be collected in all individuals, so that high-risk middle-aged adults can be targeted for primary prevention efforts.
<table>
<thead>
<tr>
<th>Study Setting</th>
<th>Participants, F/U</th>
<th>Outcomes</th>
<th>Covariates*</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Launer et al, Honolulu-Asia Ageing study, US 2000[63]</td>
<td>3703 Japanese-American men aged ≥65 years, BP measured at age 45-68 years</td>
<td>Dementia, Stroke, heart disease, Dementia, AD, NINCDS-ADRDA</td>
<td>APOE, ankle-brachial index, smoking, alcohol use, ankle-brachial index, smoking, alcohol use, ankle-brachial index, smoking, alcohol use</td>
<td>Midlife hypertension (≥160/95 mmHg) increased risk of dementia in later life in men not treated with AH medication</td>
</tr>
<tr>
<td>Kivipelto et al, Kupio and Joensuu Study, Finland 2001[64]</td>
<td>1449 people aged ≥65 years, F/U 21 years</td>
<td>AD, DSM-IV, BMI, stroke, myocardial infarct, smoking, alcohol use</td>
<td>SBP ≥160 mmHg vs &lt;140 mmHg: OR 2.8; DBP ≥95 mmHg vs &lt; 90 mmHg: OR 1.7</td>
<td></td>
</tr>
<tr>
<td>Wu et al, Linxian County, China 2003[65]</td>
<td>602 people aged ≥65 years, BP recorded 15 years prior</td>
<td>AD, DSM-IV Smoking, alcohol intake, dietary habits, anaemia</td>
<td>Hypertension (≥160/95 mmHg) in midlife related to a higher prevalent of AD in later life: OR 2.0</td>
<td></td>
</tr>
<tr>
<td>Yamada et al, Adult Health Study in Hiroshima, Japan 2003[66]</td>
<td>1774 people aged ≥60 years, Bp was recorded 25 years earlier</td>
<td>AD, VaD; DSM-IV Milk, salt, or soy sauce intake</td>
<td>High SBP related to VaD; OR per 10 mmHg increase: 1.33</td>
<td></td>
</tr>
<tr>
<td>Whitmer et al, Medicare Program of Northern California, US 2005[67]</td>
<td>8845 people, mean age 69 years; hypertension- age 42 years</td>
<td>Dementia; Ethnic origin</td>
<td>Hypertension (self reported use of drugs), or measured BP ≥140/95 mmHg related to dementia HR: 1.24</td>
<td></td>
</tr>
</tbody>
</table>

* Demographic variables (i.e. age, sex, and education were included as covariates in all studies. BP=blood pressure; DSM-III-R= Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (revised); NINCDS-ADRDA= National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer’s Disease and related Disorders Association, APOE= apolipoprotein E genotype; OR= odds ratio; ICD-9-CM= International Classification of Diseases, 9th revision clinical modification; DSM-IV= Diagnostic and Statistical Manual of Mental Disorders, 4th edition; RR=risk ratio; BMI=Body Mass Index; HR= Hazard Ratio
This cumulative risk of midlife hypertension and obesity was also recently noted in the Framingham Offspring sample when they were associated with poorer scores on tests of executive function and visuomotor skills [72]. The Adult Health Study in Japan was the only study that linked midlife systolic hypertension to late life VaD (OR per 10 mmHg 1.33; 95% CI 1.14-1.56), but not AD [66].

Many studies have also demonstrated a positive association between midlife hypertension and diminished cognition in later life [25, 73-78].

**Hypotension and the risk of dementia**

Several studies have identified lower BP as a risk factor for the development of both AD and VaD. Hypotension in most of these studies referred to a DBP of ≤70 mmHg, however several cut off values were used for SBP. Of seven studies examining the cross sectional association between blood pressure in older persons and the prevalent risk of AD or VaD [17-23], two reported an association between low BP and the incidence of AD and VaD [19, 20], while another 3 studies described a lower incidence of AD or VaD in patients with higher BP in later life [21-23]. Of the other 2 studies, one reported an association between hypertension (≥160/95 mmHg) and VaD, but not AD [17], while the other study analysed BP as a continuous variable and therefore may have missed non-linear associations. In the Kungsholmen Project the incidence of dementia in a cohort of 1642 patients (≥75 years) was defined according to DSM-III-R criteria, and their BP was checked using a mercury sphygmomanometer [19]. 202 subjects were diagnosed with dementia, and of that group 112 were diagnosed with AD. Lower BP (≤140/75 mmHg) was related to a higher prevalence of both AD, and dementia in general. A similar
relationship was described in the Chicago Health and Ageing Project where low BP (<130/70 mmHg) was related to a higher prevalence of AD [20].

<table>
<thead>
<tr>
<th>Study settings</th>
<th>Participants / follow up</th>
<th>Outcomes</th>
<th>Covariates*</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruitenberget al, 2001[79]</td>
<td>Gothenburg: H-70 and Rotterdam studies</td>
<td>382 people, aged 70 years, F/U for 15 years</td>
<td>Dementia, DSM-III-R; AD, NINCDS-ADRDA</td>
<td>BP (as a continuous variable) inversely related to dementia risk in patients taking antihypertensive medication</td>
</tr>
<tr>
<td>Morris et al, 2001[80]</td>
<td>East Boston study, US</td>
<td>378 people, aged ≥65 years, F/U 3 years</td>
<td>Dementia, AD; NINCDS-ADRDA</td>
<td>Follow up interval-stratified sampling SBP ≥160 mmHg vs 130-139 mmHg: OR 0.39, DBP &lt;70 mmHg vs &gt;90 mmHg: OR 1.8</td>
</tr>
<tr>
<td>Verghese et al, 2003[81]</td>
<td>Bronx Ageing Study, US</td>
<td>488 community volunteers, aged ≥75yrs, F/U 7 years</td>
<td>Dementia; AD; diagnosed at consensus conferences</td>
<td>Medical diagnosis (e.g. hypertension, diabetes) SBP 140-179mmHg vs 111-139 mmHg: RR for dementia 0.6 ; DBP &lt;70mmHg vs &gt;90 mmHg: RR for dementia 2.1</td>
</tr>
<tr>
<td>Nilsson et al, 2007[82]</td>
<td>OCTO-Twin Study, Sweden</td>
<td>599 people, randomly ≥80 years at baseline; F/U 4 years</td>
<td>Dementia; DSM-III-R; MMSE</td>
<td>Homocysteine levels, MMSE</td>
</tr>
</tbody>
</table>

* Demographic variables (i.e. age, sex, and education were included as covariates in all studies. BP=blood pressure; DSM-III(R)= Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (revised); NINCDS-ADRDA= National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer's Disease and related Disorders Association, MMSE= Mini Mental State Examination, RR=risk ratio; BMI=Body Mass Index; OR= Odds Ratio
Further evidence that low blood pressure may be a risk factor for AD and dementia in general can be found in several longitudinal studies (Table 6) [79-82]. The Bronx Aging Study followed an elderly non-demented cohort (≥75 years old), for up to 21 years [81]. Dementia was diagnosed following detailed neuropsychological testing, clinical examination, and neuro-imaging. Individual’s cases were then discussed at a consensus meeting and diagnosed according to DSM-III-R criteria. Over a median follow up of 6.7 years 122 subjects developed dementia, and of these 65 were diagnosed as AD. Individuals with a DBP of <70 mmHg were twice as likely to develop AD when compared to those with a DBP >90mmHg. The risk was higher in individuals with a persistently low DBP. An association was not described for SBP, or for a relationship between low DBP and VaD. The Kungsholmen Project’s six year longitudinal follow up of 1270 dementia free individuals reported similar results, with low DBP (≤65 mmHg) at baseline being associated with an adjusted relative risk for AD of 1.7 (95% CI 1.1-2.4), and 1.5 (95% CI 1.0-2.1) for dementia in general [83]. This relationship was especially pronounced in subjects who were on antihypertensives, or in APOE ε4 allele carriers [83]. Similarly, the pooled data from the Gothenburg-H (n=317 aged ≥85 years) study and the Rotterdam (n=6,668 aged ≥55 years) studies demonstrated that BP measured as a continuous variable was inversely related to dementia risk (both AD and VaD), in users of antihypertensive medication [79]. More recently a population based study of 599 individuals (mean age at baseline 82.8 years), low SBP and DBP were associated with a higher incidence of AD, whereas higher SBP was associated with better cognitive performance as measured on MMSE [82].
Blood pressure changes may evolve alongside the development and progression of cognitive symptoms in patients with AD [84]. In 327 participants in the French Research Programme on AD (REAL-FR), a significant decrease in both SBP and DBP was noted from baseline to follow up one year later. Cognition was assessed using the MMSE, ADAS-Cog, IADL, ADL, and clinical dementia rating scales, and demented subjects with the worst cognitive impairment at baseline showed larger decreases in BP at follow up [84]. The authors suggest that there is a dynamic relationship between BP and AD, and that although hypotension may increase the risk of developing AD, reductions in BP may also occur as a secondary phenomenon to disease progression.

**Methodological considerations**

Several issues need to be considered when analyzing the above data. Given the potentially lengthy lag period that may exist between the onset of hypertension/hypotension and the onset of cognitive deficits, longitudinal studies are superior when trying to identify a causal relationship between these variables. The length of the follow up period is also of great importance, in that trials with shorter follow up periods may miss the association. Population based studies in specific age categories assist our understanding with less potential for bias. Studies looking at older persons are particularly helpful given the higher incidence of dementia in this group. One must also consider how blood pressure was diagnosed and recorded within these trials. Trials that record hypertension on a self report basis depend on patients being aware that they have hypertension, or that cognitive restraints at baseline don’t impede their recall of this fact. Blood pressure was analysed as a continuous variable in some studies while it was categorised with different cut-off values in others. There are merits to both these
methods of analyses in that when it is measured as a continuous variable it enables general associations between blood pressure and dementia to be observed, whereas categorically analysing blood pressure enables us to draw conclusions on the relationship for specific BP cut-offs. The classification of cognitive disorders is also of importance. Studies offering a definite diagnosis of dementia, AD, and VaD according to internationally accepted guidelines are superior to trials that merely described altered performance on cognitive testing. Diminished scoring on cognitive tests, especially when performed at a single point may not represent a progressive deficit, and therefore multiple point measurements over a longer follow up period, with a definite diagnosis are preferred.

**Mechanisms by which blood pressure affects the risk for dementia**

Midlife hypertension increases the risk for VaD by the same pathological mechanism as it increases the risk for lacunar infarcts and stroke. Chronically elevated blood pressure leads to vessel wall thickening and reduced luminal diameter in microvessels. Within larger cerebral arteries, thickening of the media and atheromatous plaques also leads to narrowing of the vessel lumens. Rupture of these plaques can lead to complete occlusion of these arteries and infarction of the surrounding cerebral areas [85]. Chronically elevated blood pressure is also associated with heart failure, atrial fibrillation, and atheromatous disease in more distal arteries. These conditions can lead to the formation of thrombotic material locally, which when it becomes unstable can break down into circulating micro-emboli that get lodged in and occlude the smaller cerebral arteries also resulting in infarction of the surrounding cerebral tissue. Similar to other vascular risk factors such as diabetes mellitus, obesity and hyperlipidaemia;
hypertension increases the risk for both cortical and subcortical VaD, by ultimately leading to ischaemia and infarction of areas of the brain involved in memory and function.

As with hypertension in midlife, several studies have described an association between atherosclerosis [86], high cholesterol, diabetes mellitus, obesity [70, 87-89] and the incidence of AD. The exact mechanism underlying this relationship remains unclear.

An important pathological feature of Alzheimer’s disease (AD) is the formation of extracellular senile plaques in the brain, whose major components are small peptides called β-amyloid (Aβ) derived from β-amyloid precursor protein (APP) (Figure 1) [90].

Given the association between the development of β-amyloid plaques and AD progression, logic suggests that there must be interplay between these vascular risk factors and the increased accumulation of β-amyloid. Several studies have indicated that cerebral ischemia/stroke significantly increases not just the risk of VaD, but also that of AD [91-93]. Knowledge regarding the pathological basis of dementia has been advanced by incorporating epidemiological techniques into clinical-pathological studies. These studies highlight the interplay between vascular pathologies and age-related memory loss, MCI, and dementia. The recent Rush Religious Orders [350, 351], and Memory and Ageing Project [352] clinical-pathology studies demonstrate that the underlying pathology of clinically diagnosed dementia and probable AD is heterogeneous. Indeed, most persons with dementia and nearly half of those with probable AD have mixed pathologies, most commonly AD and infarcts. These infarcts are not benign but add to the likelihood of cognitive impairment and lower the threshold for clinical AD and dementia. These VaD and AD phenotypes overlap to such a degree that the pathological
basis for an individual's dementia is not easily extricated when there are mixed pathologies. Thus the use of cognitive profiles to distinguish AD from VaD is limited. I would therefore exercise caution when attributing a risk factor for clinical AD to amyloid or tangle accumulation alone. Because vascular pathology contributes to the AD phenotype, some risk factors for clinical AD are likely to be risk factors for vascular pathology. For instance diabetes, a risk factor for clinical AD, is related to increased infarct pathology, but not AD pathology [353]. Furthermore, the APOE ε4 genotype, which is strongly related to AD pathology, is also associated with increased infarcts, and therefore may contribute to dementia via an alternative mechanism. However, there are proposed pathological pathways which directly associate vascular events with increased amyloid burden. APP expression is elevated in post-ischaemic brain, and cleavage of APP leading to amyloidogenic Aβ peptides may hence be increased by ischemia [94-96]. Recently, evidence has emerged that hypoxia induced factors (HIF) may potentiate these amyloidogenic mechanisms, which may ultimately result in AD expression [97]. These hypoxic factors induce BACE (beta-site APP cleaving enzyme) a protein associated with the production of β-amyloid. It is possible that the cerebral ischaemia arising from both cortical and subcortical infarcts, as well as diminished cerebral perfusion as a result of luminal narrowing may potentiate the development of AD. In fact several studies using different modalities including Positron Emission Tomography (PET), Single Photon Emission Computer Tomography (SPECT), and Transcranial Doppler (TCD), have demonstrated diminished cerebral perfusion in patients with AD [14, 98-100]. This reduction in blood flow has also been described in the pre-clinical and early stages of AD, suggesting it's not simply as a consequence of reduced metabolic needs caused by
neuronal tissue loss [101, 102]. Also, one cross sectional study found an association between low cerebral blood flow and the development of hippocampal and amygdalar atrophy on magnetic resonance imagings (MRI), which are radiological hallmarks of AD [103]. This association was evident, even in subjects without overt dementia suggesting that diminished cerebral perfusion may precede and contribute to the development of AD [103]. However, microangiopathic changes associated with chronic vascular disease may not account for this diminished perfusion alone. In animal models for AD and in vitro experiments, a direct action of β-amyloid on cerebral arteries has been shown to produce vasoconstriction and diminished vasodilation, which may represent an early event in the development of the disease [104-106].

**How might low blood pressure increase the risk of dementia?**

As discussed earlier in the review lower BP in later life is associated with an increased risk of dementia, and in particular AD. Conversely, hypertension in later life does not appear associated with an increased risk. Ageing itself acts as a significant contributor to the presence of vascular disease. Vascular ageing is associated with changes in the mechanical and the structural properties of vessel walls, which leads to the loss of arterial elasticity and reduced arterial compliance [107]. These effects of aging ultimately lead to a dampening down of the auto regulatory capabilities of cerebral arteries, which within normal circumstances can maintain cerebral perfusion at a constant rate despite fluctuations in systemic BP. This diminished auto-regulatory capacity means the brain is more vulnerable to ischaemic insults when systemic blood pressure dips below a critical threshold for maintaining perfusion [10]. A recent study in 809 elderly men demonstrated that nocturnal dips in DBP were associated with
diminished cerebral perfusion, especially in temporal and infero-parietal areas [108]. A higher proportion of subjects with these nocturnal dips had increasing systolic BP during the 14 year follow up period, irrespective of baseline values and the prevalence of hypertension [108]. Subjects with AD also have a higher incidence of orthostatic hypotension than non-demented age matched controls [109]. Orthostatic hypotension is arbitrarily defined as a fall in systolic BP of ≥ 20 mmHg, and/or a fall in DBP of ≥ 10 mmHg on standing, but when associated with symptoms suggestive of cerebral hypoperfusion (e.g. dizziness), a smaller drop in BP may be of equal importance [110]. One study demonstrated that systolic blood pressure reduction during orthostasis is associated with cognitive decline as measured by performance on MMSE during a 5 year follow up [111]. It also appears that cerebrovascular autoregulation is severely impaired in patients with symptomatic orthostatic hypotension [112]. Therefore, these episodes of reduced blood pressure may be associated with frequent episodes of diminished cerebral perfusion, given the breakdown in cerebrovascular autoregulation that can occur alongside them. Further research is required to examine the relationship between low systemic BP and subsequent cerebral hypoperfusion. It is possible that transient ischemic periods may contribute to the hypoxic-driven amyloidogenesis referred to above [97].

Conclusion

There appears to be an age dependent relationship between the occurrence of hypertension or hypotension and the risk of developing dementia in later life. Midlife hypertension is particularly associated with an increased risk of developing both AD and/or VaD. Elevated BP occurring later in life does not appear associated with the same risk. Hypertension is a potentially reversible risk factor for the development of dementia.
The Systolic Hypertension in Europe study (Syst-Eur) found that treatment of hypertension with the dihydropyridine calcium channel blocker Nitrendipine reduced the incidence of AD by 55% [113, 114]. The Systolic Hypertension in the Elderly Programme (SHEP) [115], and more recently the Hypertension Treatment in the Very Elderly Trial (HYVET) [116] demonstrated significant benefits to antihypertensive treatment in older persons, with regards reductions in all cause mortality and cardiovascular events. When the results of 4 large randomised trials that have examined the effects of antihypertensive treatment on incident dementia (HYVET, Syst-Eur, SHEP, and PROGRESS studies), the pooled ratio is borderline significant: relative risk 0.87 (95% CI 0.76-1.00; p=0.045) [117]. However the results from this meta-analysis were based on the inclusion of trials that examined the efficacy of antihypertensives in both primary and secondary prevention roles, and as discussed earlier patients who have already had a vascular event may differ significantly from patients with little or no vascular burden in a primary prevention trial. Overall results from antihypertensive trials have not been consistent, with some trials failing to show any treatment effects on cognition, despite adequate blood pressure control. This suggests that there is more to certain antihypertensive treatment than simply blood pressure control *per se* and this will be discussed further in the next chapter. Potentially reversible risk factors for dementia are extremely uncommon, and the demonstration of this association between hypertension and the incidence of dementia is extremely important because effective therapies for elevated BP are available.

Hypotension in later life, especially diastolic blood pressures ≤70mmHg may be associated with a higher incidence of AD, however further studies are required to
investigate whether this occurs as a primary phenomenon triggering the development of AD, or secondary to the neurodegenerative process affecting blood pressure centres within the brain.

I have proposed a mechanism by which either high or low BP at different stages of life may contribute to the development of dementia. The accumulation of soluble and insoluble forms of β-amyloid is associated with the development of AD, so one would expect these vascular risk factors to contribute to this accumulation in some fashion. The subsequent reduction in cerebral blood flow which may occur in the setting of either high or low blood pressure may contribute to the development of dementia. In chapter 2 we will discuss how the properties of certain antihypertensive agents may counteract this risk, and subsequent chapters will give details on how treatment with the antihypertensive nilvadipine is tolerated in patients with AD.
Chapter 2: Antihypertensive therapy and the incidence of Alzheimer's disease

Abstract

Alzheimer's disease (AD) and vascular dementia (VaD) are important causes of cognitive decline in the elderly. As a result of an ageing population worldwide, the incidence of dementia is expected to rise exponentially over the coming decades. Vascular risk factors are implicated in the pathogenesis of both AD and VaD.

Hypertension in midlife is particularly associated with an increased risk of developing dementia. One might hope the treatment of high blood pressure in midlife would reduce the risk of developing dementia, as it does the risk of stroke. Divergent results have been reported in studies examining this effect, with the evidence suggesting that certain antihypertensives confer benefits beyond others. This implies that certain drugs may have neuroprotective properties separate to their blood pressure lowering capabilities. Recent trials have added to our understanding of these relationships. This chapter reviews the evidence for a treatment effect on the incidence of dementia with the use of different antihypertensive classes. I also propose some explanations for the variation in response.

Introduction

Given the association between hypertension and the development of dementia (discussed in Chapter 1), a reasonable hypothesis is that antihypertensive (AH) therapy may protect against the development of dementia. However, the association between hypotension in later life and an increased risk of AD, raises the possibility that overtreatment of BP may in fact contribute to the development of dementia. This chapter will examine the evidence from observational and randomised controlled trials that have
studied the effects of antihypertensive therapy on the incidence of dementia. I have primarily focused where possible on studies examining the effects of AH treatment on the incidence of AD. I will also examine the potential mechanisms of action for different classes of AH medication, and how they contribute to any potential treatment effect in patients with dementia.

**Antihypertensives and dementia- Evidence from observational studies**

Several observational studies have longitudinally examined the effect of AH treatment on cognitive function and dementia (Table 1). The Kungsholmen Project demonstrated that the use of diuretics in 1301 dementia free hypertensive patients (Aged ≥75 years) was related to a lower prevalence of dementia (RR 0.7 95% CI 0.5-1.0) [118]. Participants were followed up over 3 years and cognition was screened using the Mini Mental State Examination (MMSE) as well as additional neuropsychological testing in a random sample. All participants with MMSE scores of <24 points had detailed neuropsychological assessments. 987 subjects were still alive at the end of the follow up period. Dementia was diagnosed according to DSM-III-R criteria but no differentiation between AD and VaD cases were made. A slower rate of cognitive decline was also noted in patients with established dementia who received a diuretic, compared to patients with dementia who did not (regression coefficient=0.07, p= 0.04). This study was limited in that information on treatment was only available at baseline and subjects may have been commenced on other antihypertensives in the intervening period. Also, information on the duration of treatment and the indication was not available. The Rotterdam Study, a community cohort of 6416 non-demented people followed up for 2.2 years, reported a significant association between antihypertensive therapy and VaD but not AD [119]. The
Baltimore Longitudinal Study of Aging [120], and the Cache County study [62] also found that the use of AH therapy was associated with a reduced risk of developing AD. The majority of people included in the above trials were Caucasian. A five-year follow-up study on a community sample of 1617 African Americans demonstrated that the use of medications that mediate vascular risk factors (mainly AH drugs, and among others anti-hyperlipidaemic and anti-diabetic drugs) reduced the risk of incident dementia by 40% (odds ratio 0.60; 95% CI 0.45-0.81) [121].

The Honolulu-Asia Ageing Study (HAAS) was conducted on a sample of Asian American men between 1965 and 1995 [63]. The relationship between the use of AH drugs and hippocampal atrophy (a radiological feature of AD) was analysed in a random sample of 543 participants [69]. The risk of hippocampal atrophy was increased in patients who never received AH drugs (OR 1.7, 95% CI 1.12-2.65), compared to those who did receive AH therapy. A further report of 848 participants from the HAAS who had a history of midlife hypertension and were dementia free in 1991, compared 446 participants, who were normotensive throughout the study period until 1991 [122]. The study patients were assigned to one of four groups: never treated with AH, treated for <5 years, treated for 5-12 years, and treated for >12 years. Longer duration of AH treatment was associated with a reduced risk of dementia, AD and VaD. Each year of AH therapy was associated with a 6% reduction in the risk for dementia and subtypes (HR 0.94, 95% CI 0.89-0.99), and those treated for >12 years had the lowest risk (HR 0.40 95% CI 0.22-0.75), compared to those never treated.

Two population studies failed to show any association between AH therapy and dementia. The East Boston cohort (n=634 subjects ≥65 years of age) [80], and the
Canadian Study of Health and Aging (n=3238 subjects, ≥65 years of age) [39], with follow-up periods of 4 and 5 years respectively, demonstrated no benefit for AH treatment. Very few participants in the East Boston study had chronically elevated BP, and about one third of patients had BP values missing. Almost 20% of patients who were administered a baseline questionnaire in the Canadian study were lost to follow up over the follow up and this may have distorted results.

In summary four out of seven large observational studies reported that antihypertensive use was associated with a lower incidence of dementia. Two trials failed to diagnose dementia subtype. Two trials report an association between AH use and a reduced incidence of AD, while one trial found no effect on the incidence of AD, but did report an association with reduced VaD incidence (Table 1).

### Antihypertensives and dementia?- summary of trials

<table>
<thead>
<tr>
<th>Positive Observational studies</th>
<th>Negative Observational Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kungsholmen project (Guo et al, 1999)</td>
<td>Canadian study of Health and Ageing (Lindsay et al, 2002)</td>
</tr>
<tr>
<td>Rotterdam study (Int’ Veld et al, 2001)</td>
<td>East Boston Study (Morris et al, 2001)</td>
</tr>
<tr>
<td>BLSA – NS reduced AD risk with DHP-CCB (Yassar et al, 2005)</td>
<td></td>
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<tr>
<td>Cache County Study- reduced AD risk (Khachaturian et al, 2006)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive RCT’s</th>
<th>Negative RCT’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td>F/ up</td>
</tr>
<tr>
<td>------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Guo et al, 1999</td>
<td>1301</td>
</tr>
<tr>
<td>dementia free, hypertensive patients (Aged ≥75 years)</td>
<td>3</td>
</tr>
<tr>
<td>Kungsholmen, Sweden</td>
<td>1999</td>
</tr>
<tr>
<td>In’t Veld et al, 2001</td>
<td>6416</td>
</tr>
<tr>
<td>dementia free people (Aged ≥55 years), Rotterdam</td>
<td>2.2</td>
</tr>
<tr>
<td>Yasar et al, 2005</td>
<td>1092</td>
</tr>
<tr>
<td>community (Aged ≥60 years), Baltimore</td>
<td>19</td>
</tr>
<tr>
<td>Study of Aging</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Observational studies investigating the association of AH treatment and the incidence of dementia
<table>
<thead>
<tr>
<th>Study population</th>
<th>F/ up</th>
<th>Outcomes</th>
<th>Medications</th>
<th>Covariates*</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khachat et al, 2006[62]</td>
<td>patients (Aged ≥65 years), Cache County, Utah</td>
<td>dementia free</td>
<td>3 years</td>
<td>Dementia, Diuretics, K⁺ sparing high cholesterol, AD, diuretics, cholesterol, NINCDS-ADRDA inhibitors, CCB</td>
<td>Use of AH meds was associated with a reduced risk of AD, AHR 0.64 (95% CI 0.41-0.98); K⁺ sparing diuretics had greatest effect AHR 0.26 (95% CI: 0.08-0.64)</td>
</tr>
<tr>
<td>Murray et al, 2002[121]</td>
<td>1617 African Americans Age, 77.7 years, Indiana</td>
<td>Dementia, ICD-10, AH Baseline medication cognitive scores, BP</td>
<td>5 years</td>
<td>OR 0.62 (95% CI: 0.45-0.84)</td>
<td></td>
</tr>
<tr>
<td>Lindsay et al, 2002[39]</td>
<td>3238 Canadians Aged ≥65 years</td>
<td>AD; DSM-IV medication</td>
<td>5 years</td>
<td>RR 0.91 (NS)</td>
<td></td>
</tr>
<tr>
<td>Morris et al, 2001[80]</td>
<td>A random sample of 634 people (Aged ≥65 years), East Boston</td>
<td>AD, Diuretics, β-blockers</td>
<td>4 years</td>
<td>No association</td>
<td></td>
</tr>
</tbody>
</table>

* Demographic variables (i.e. age, sex, and education were included as covariates in all studies. CCB= Calcium Channels blockers; K⁺ = potassium; AH= antihypertensive; AD= Alzheimer’s dementia; VaD= vascular dementia; APOE= apolipoprotein E genotype; BP=blood pressure; OR= odds ratio; ICD-10= International Classification of Diseases, 10th revision; DSM-IV= Diagnostic and Statistical Manual of Mental Disorders, 4th edition; RR=risk ratio; DSM-III(R)= Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (revised); BMI=Body Mass Index; AHR= adjusted hazard ratio.
Antihypertensives and dementia- Evidence from randomised controlled trials

Several large randomised, placebo controlled trials on hypertension have evaluated the effects of antihypertensive drugs on cognition with divergent results (Table 2). Most trials were designed to assess the impact of AH medication on cardiovascular and stroke outcomes, and only referred to cognitive outcomes as a secondary measure. No trials have been conducted with a primary focus on the prevention of cognitive decline per se.

- **Systolic Hypertension in the Elderly Programme (SHEP) (diuretic +/- β blocker or Reserpine)**

  This double blind placebo controlled trial included 4736 patients with a mean age of 72 years [115]. Active treatment consisted of the diuretic chlorthalidone, with the possible addition of atenolol or reserpine. SHEP failed to demonstrate any significant effect of antihypertensive treatment on the incidence of dementia, despite between group blood pressure differences of >10 mmHg SBP, and >4 mmHg DBP. The average follow up BP was (SBP/DBP) 155/72 mmHg in the placebo group, versus 143/68 mmHg in the treatment group. The rates on placebo and active treatment were 4.2 and 3.6 dementia cases per 100 patient-years, (RRR: 14%; 95% CI: -26% to 54%; p=0.44).

  Cognition was assessed using the short-Comprehensive Assessment and Referral evaluation (short-CARE) test. A subsequent report noticed that although retention to the two clinical examinations was very high, SHEP patients who missed cognitive assessments were more likely to be older, less educated, non-white, randomly assigned to placebo, and to have a higher occurrence of nonfatal cardiovascular events before each follow up visit [123]. This may well have biased the analysis of cognitive effects toward the null
hypothesis of no differences between the treatment groups. Also the follow up period was only for five years, which may not have been sufficient to demonstrate a treatment effect.

- Medical Research Council Treatment Trial of Hypertension Study (diuretic and/or \(\beta\) blocker)

In this prospectively planned Medical Research Council (MRC) trial of treatment in 2584 patients (age 65-74) with hypertension, subjects were randomised to a diuretic, \(\beta\) blocker, or placebo [124]. There was a mean fall in SBP following treatment of 33.5 mmHg in the diuretic group, 30.9 mmHg in the \(\beta\) blocker group, and 16.4 mmHg in the placebo group (p<0.0001). Subjects were followed up for 54 months and no significant difference in neuropsychological testing was observed between the treatment and placebo groups. Although this study failed to demonstrate any positive effect of AH therapy, it did reassure doctors at that time, that treating hypertension in older patients did not adversely affect cognition. Numerous subjects crossed over from placebo to active treatment group, and confounded the "intention to treat" analysis. Only 2 cognitive tests, the paired associate learning test, and the trail making test (part A) were used to assess cognitive function, and more detailed testing may have revealed a treatment effect. Moreover the 54-month follow up period may have been too short to detect a difference between groups. A follow up study of 387 surviving MRC patients for 9-12 years revealed that less decline in systolic blood pressure, led to poorer cognitive outcomes on MMSE testing, even with adjustments applied for a family history of dementia, cognitive function at baseline, aging, and alcohol intake [125]. In the Framingham cohort, historical [74] rather than concurrent BP records were associated
with cognitive impairment [28]. The follow up findings are consistent with those from the Framingham cohort.

- **Systolic Hypertension in Europe study (Syst-Eur) (DHP calcium channel blocker +/- ACE inhibitor +/- diuretic)**

  The vascular dementia project included in the Systolic Hypertension in Europe study demonstrated for the first time, a reduction in the incidence of dementia following AH treatment [113]. Participants who were at least 60 years old (n=2418, mean age 70.0 years) with isolated systolic hypertension were randomised to placebo or initially treated with the dihydropyridine calcium channel blocker (DHP-CCB) nitrendipine. If necessary they received an ACE inhibitor (enalapril), or a diuretic (hydrochlorothiazide), or both drugs to achieve adequate BP control. Median follow-up was short at only 2 years. This was a primary prevention trial and it was stopped prematurely, because active treatment resulted in a 42% reduction in the primary endpoint of fatal and non-fatal stroke. Nitrendipine was the only antihypertensive used in 60% of patients in the active treatment group. Cognitive function was assessed using the mini mental state examination (MMSE). If the MMSE score was ≤23, diagnostic tests for dementia were performed, and dementia was diagnosed according to DSM-III-R criteria. The cause of dementia was established using the modified ischaemic score with brain imaging or the Hachinski score. The incidence of dementia was reduced by 50%, from 7.7 cases in the placebo group to 3.7 cases per 100 patient years in the active treatment group (p= 0.05). However these findings were based on only 32 incident cases. The incidence of AD, VaD, and mixed dementia was reduced.
Following the initial double blind, placebo-controlled period, all patients were invited to continue on, or commence the study medication for a further follow up period of 2 years (Syst-Eur 2) [114]. The incidence of dementia was updated in patients treated since randomisation (4 years) compared with patients actively treated since the end of the double blind period only (2 years). After 4 years there was still a significant difference in blood pressure between the two groups SBP/DBP was 7.0/3.2 mmHg higher in the 1417 former placebo than in the 1485 subjects initially allocated to active treatment. The risk of dementia increased with age and baseline DBP. Compared with the controls, long-term antihypertensive therapy reduced the incidence of dementia (using the same criteria as Syst-Eur 1) by 55% (CI: 24%-73%; p<0.001) from 7.4 to 3.3 cases per 1000 patient years (p < 0.001). Both the incidence of AD and VaD were reduced. After adjustment for sex, age, education and initial blood pressure, the relative hazard rate associated with the use of a calcium channel blocker was 0.38 (95% CI, 0.23- 0.64; p, 0.001). These results indicate that the treatment of 1000 patients for 5 years can prevent 20 cases of dementia (95% CI, 7- 33).

- **Perindopril Protection Against Recurrent Stroke Study (PROGRESS) (ACE inhibitor +/- diuretic)**

The PROGRESS study was a randomised, double blind, placebo controlled clinical trial on 6105 patients from 19 countries (mean age 64 years) [126]. This was a secondary prevention trial, and the main objective of the study was to examine whether a defined blood pressure lowering regimen could lower the risk of recurrent stroke and other vascular events in patients with a prior history of stroke or transient ischaemic attack (TIA). The active treatment group received the angiotensin converting enzyme (ACE)
inhibitor perindopril for all participants, and the diuretic indapamide for those with neither an indication for, nor a contraindication to, a diuretic. The main outcomes for the cognitive analyses were dementia (using DSM-IV criteria) and cognitive decline (a decline of 3 or more points on the Mini-Mental State Examination score). Following a mean follow up period of 3.9 years, active treatment reduced the risk of cognitive decline by 19% (95% CI, 4% to 32%, p=0.01) in the whole population, particularly in subjects with recurrent strokes (RR 45%; [95% CI, 21% to 61%], p=0.001). The risk of dementia in the whole population was reduced by 12% (95% CI, -8% to 28%) in the active treatment group, to 12 cases per thousand patient years compared to 19 cases per 1000 thousand patient years in the placebo group. Similar to the effect on cognitive decline, a significant reduction of 34% (95% CI 3% to 55%, p=0.03) was observed in the incidence of dementia in patients with recurrent strokes receiving active treatment. The effect was similar in hypertensive or non-hypertensive subjects. Combination therapy with perindopril and indapamide induced a mean reduction of blood pressure of 12/5 mmHg (SBP/DBP) and was more effective in reducing the risk of dementia (23% [95% CI, 0% to 41%], p=<0.05), than monotherapy with perindopril alone (-8% [95% CI, -48% to 21%], p=0.60) where the mean decrease in blood pressure was 5/3 mmHg (SBP/DBP). There was no apparent effect of active treatment among participants (n=1001 patients) with evidence of cognitive impairment at baseline (-5% [95% CI, -42% to 22%], p=0.70), whereas among patients without such impairment (84.2%), active treatment prevented post stroke dementia by 31% (95% CI, 6% to 49%; p=0.02). In effect PROGRESS demonstrated that if the risk of recurrent stroke was reduced then the risk of cognitive decline and dementia
was also reduced. However, if there was no prevention of recurrent stroke then there was no effect on cognition.

- **Study on Cognition and Prognosis in the Elderly (SCOPE) (Angiotensin receptor blocker +/- diuretic)**

The SCOPE study evaluated the effect of the angiotensin receptor blocker Candesartan, with or without diuretic in 4964 non-demented (MMSE score ≥24) elderly (mean age: 76 years), hypertensive patients [127]. Although it was a double blind, placebo controlled study; a considerably greater proportion of the patients randomly assigned to the placebo group received open label antihypertensive drugs, which mainly consisted of diuretics and β blockers. After 3.7 years of follow up, there was no significant difference between the two groups for cognitive function and dementia. Although originally designed as a trial, comparing the effects of an angiotensin receptor blocker with placebo, it evolved as a study comparing two active treatment groups. As a result, only small blood pressure differences were observed between the active treatment group and the placebo group (3.2/1.6 mmHg, SBP/DBP), which dramatically reduced the power to detect a difference. Cognitive evaluation was based on the MMSE, and the lack of sensitivity of this test to detect a cognitive decline in non-demented subjects could have biased the results toward the null effect, and made the comparison between the two groups difficult. Within the PROGRESS trial the greatest effect on the incidence of dementia was seen in the group on combination therapy, who also had the largest reduction in their BP. However, a later sub-study of 257 SCOPE participants (mean age 76 years) using more specific neuropsychological testing (Cognitive Drug Research computerized assessment battery, trail-making tests, and verbal fluency tests), described
a "small to moderate" effect on attention (0.004 vs -0.036, \( p=0.04 \)), episodic memory (0.14 vs -0.22, \( p=0.04 \)), but no significant effect on speed of cognition (-2.3 vs -17.4, \( p=0.15 \)), working memory (0.0014 vs 0.0010, \( p=0.90 \)) or executive function (-0.0031 vs -0.0023, \( p=0.95 \)) [128]. A further study of the SCOPE data by Skoog et al [354], reported that elderly patients with mild/moderate dementia (MMSE 24-28) were at greater risk for adverse cardiovascular outcomes, and that MMSE declined less in the candesartan group than in the placebo group (Mean difference 0.49, 95% CI: 0.02-0.97, \( p=0.04 \)). The incidence of non-fatal stroke was also reduced in the candesartan group, so similar to PROGRESS there was an association between the reduced incidence of stroke and better cognitive outcomes.

- **Hypertension in the Very Elderly Trial- cognitive function assessment (HYVET-COG)**

(Diuretic +/- ACE inhibitor)

The Hypertension in the Very elderly Trial (HYVET) was designed to assess the risks and benefits of treatment of hypertension in elderly patients and included a cognitive assessment, the HYVET-COG [117]. 3336 non-demented patients with hypertension (SBP 160-200 mmHg; DBP<110 mmHg) aged ≥80 years of age were enrolled. Participants were randomly assigned to receive 1.5 mg slow release indapamide, with the option of 2-4mg perindopril, or placebo. The target SBP and DBP was 150 mmHg and 80 mmHg respectively. The main trial was stopped early because a substantial reduction in mortality and stroke was established at the second pre-planned interim analysis. As a result of this the mean follow up was short at 2.2 years. Active treatment was associated with a 30% reduction in the in the rate of fatal or non-fatal stroke (95% CI: 0 to 51%; \( p=0.06 \)), although this failed to reach statistical significance. Cognitive function was
assessed at baseline and annual follow up using the MMSE, and patients with a 3 points decline on follow up, or whose score fell to <24 points had more detailed assessment. Patients were diagnosed with dementia according to DSM-IV criteria, clinical history, neuro-imaging, and a modified Hachinski ischaemic score (HIS) were used to differentiate subtypes. 1469 (44%) of patients attended a 2 year follow up visit. There were fewer adverse events reported in the treatment group than in the placebo group. The mean decrease in SBP at 2 years was 14.6 mmHg on placebo versus 29.6 mmHg on treatment (mean difference 15 mmHg; p<0.0001). The mean reduction in DBP was 7.2 mmHg in the placebo group versus 13.1 mmHg on treatment, (mean difference 5.9 mmHg; p<0.0001). No significant differences were found between treatment and placebo groups with regard to cognitive decline or dementia. In total 971 patients were categorised with cognitive decline, of which 263 cases of dementia were diagnosed (164 with AD, 84 with VaD, and 15 with unspecified cause of dementia). The rates of all incident dementia diagnosed were 38 per 1000 patient years in the placebo group versus 33 per 1000 patient years in the treatment group. The unadjusted hazard ratio (HR) for cognitive decline on AH treatment was 0.93 (95% CI 0.82-1.05), 0.85 for AD (95% CI 0.63-1.15), 0.87 for VaD (95% CI 0.57-1.34), and 0.86 for all cause dementia (95% CI 0.67-1.09). Given that previous studies have reported an association between a reduction in the incidence of stroke and subsequent better cognitive outcomes, the fact that HYVET failed to meet the primary endpoint of reducing the risk of stroke, may account for the failure to detect significant positive cognitive outcomes in the treatment group.

- Prevention Regimen for Effectively avoiding Second Strokes trial (PRoFESS)
  (Antiplatlet +/- ARB)
In this trial patients who had had an ischaemic stroke were randomly assigned in a two by two factorial design to receive either 25 mg aspirin (ASA) and 200 mg extended-release dipyridamole (ER-DP) twice a day or 75 mg clopidogrel once a day, and either 80 mg telmisartan or placebo once per day [388]. The predefined endpoints for this sub-study were disability after a recurrent stroke, assessed with the modified Rankin scale (mRS) and Barthel index at 3 months, and cognitive function, assessed with the mini-mental state examination (MMSE) score at 4 weeks after randomisation and at the penultimate visit. 20,332 patients (mean age 66 years) were randomised and followed-up for a median of 2.4 years. Recurrent strokes occurred in 916 (9%) patients. Modified Rankin scales scores were not statistically different in patients with recurrent stroke who were treated with ASA and ER-DP versus clopidogrel (p=0.38), or with telmisartan versus placebo (p=0.61). There was no significant difference in the proportion of patients with recurrent stroke with a good outcome, as measured with the Barthel index, across all treatment groups. Additionally, there was no significant difference in the median MMSE scores, the percentage of patients with an MMSE score of 24 points or less, the percentage of patients with a drop in MMSE score of 3 points or more between 1 month and the penultimate visit, and the number of patients with dementia among the treatment groups. There were no significant differences in the proportion of patients with cognitive impairment or dementia among the treatment groups.

Telmisartan therapy was not associated with a lower incidence of recurrent stroke (HR 0.95, 95% CI: 0.86-1.04, p=0.23). Similar to previous trials this may account for the failure to identify any significant effect of therapy on cognition.
<table>
<thead>
<tr>
<th>Study population</th>
<th>F/U</th>
<th>Outcomes</th>
<th>Medications</th>
<th>Covariates*</th>
<th>Main Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHEP Research Group, 1991[115]</td>
<td>4-5 years</td>
<td>Dementia, diagnosed through expert evaluation</td>
<td>Chlorthalidone +/or atenolol or reserpine</td>
<td>BMI, BP, smoking, alcohol use, stroke, diabetes</td>
<td>Reduction in dementia in treatment group by 16%, non significant</td>
</tr>
<tr>
<td>Prince et al, MRC trial, 1996[124]</td>
<td>4.5 years</td>
<td>Cognitive impairment as judged by the rate of change in the PALT and TMT tests</td>
<td>Diuretic or β-blocker</td>
<td>Entry cognitive scores, entry self care depression score</td>
<td>Non-significant effect on cognitive function</td>
</tr>
<tr>
<td>Forrette et al, Syst-Eur I, 1998[113]</td>
<td>2 years</td>
<td>Dementia, AD, VaD, or mixed; DSM-III-R</td>
<td>Nitrendipine +/or enalapril, chlorothiazide</td>
<td>BMI, BP, MMSE score, atrial fibrillation</td>
<td>Reduced dementia risk by 50%, (3.8 vs 7.4 cases per 1000 patient years; p=0.05)</td>
</tr>
<tr>
<td>Forrette et al, Syst-Eur II, 2002[114]</td>
<td>3.9 years</td>
<td>Dementia, AD, VaD, or mixed; DSM-III-R</td>
<td>Nitrendipine +/or enalapril, chlorothiazide</td>
<td>Entry DBP</td>
<td>Reduced dementia risk by 55% (p&lt;0.001), and reduced incidence of AD (1.9 vs 5.0 cases per 1000 patient years)</td>
</tr>
</tbody>
</table>
Table 2: Randomised controlled studies investigating the effect of AH medication on cognitive impairment or dementia (Cont’d)

<table>
<thead>
<tr>
<th>Study population</th>
<th>F/U</th>
<th>Outcomes</th>
<th>Medications</th>
<th>Covariates*</th>
<th>Main Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tzourio et al, PROGRESS study, 2003[126]</td>
<td>6105 patients with previous stroke or TIA in 5 years (mean age 64 years)</td>
<td>3.9 years</td>
<td>Dementia or dementia with recurrent stroke, DSM-IV</td>
<td>Perindopril +/- or indapamide</td>
<td>BMI, APOE, alcohol, smoking, MMSE</td>
</tr>
<tr>
<td>Lithell et al, SCOPE study, 2003[127]</td>
<td>4964 non-demented elderly (mean age: 76.4 yrs)</td>
<td>3.7 years</td>
<td>Cognitive impairment, (decline in MMSE ≥ 4 points)</td>
<td>Candesartan +/- or chlorothiazide</td>
<td>..</td>
</tr>
<tr>
<td>Peters et al, HYVET-COG, 2008[117]</td>
<td>3336 non-demented patients (aged ≥80 years) with SBP 160-200mmHg and DBP&lt;110 mmHg</td>
<td>2.2 years</td>
<td>Dementia; AD; VaD, DSM-IV, neuro-radiological findings, mHIS</td>
<td>Indapamide +/- perindopril</td>
<td>Stroke, BP, smoker MMSE</td>
</tr>
</tbody>
</table>

Demographic variables (i.e. age, sex, and education were included as covariates in all studies. AH= antihypertensive; AD= Alzheimer’s dementia; VaD= vascular dementia; APOE= apolipoprotein E genotype; BP=blood pressure; TIA= transient ischaemic attack; DSM-IV= Diagnostic and Statistical Manual of Mental Disorders, 4th edition; RR=risk ratio; DSM-III(R)= Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (revised); BMI=Body Mass Index; AHR= adjusted hazard ratio; CI= confidence interval; mHIS=modified Hachinski ischaemic scores; BMI=body mass index; SBP= systolic blood pressure; DBP=diastolic blood pressure; MMSE= mini mental state examination; PALT= paired associate learning test; TMT= trail making test, MHIS= modified Hachinski ischaemic scores
The authors combined the results of the HYVET in a meta-analysis with three other placebo-controlled trials of AH treatment that assessed incident dementia: Syst-Eur, PROGRESS, and the SHEP trial (see above). The pooled ratio was borderline significant (with the random effects model); relative risk 0.87 (95% CI 0.76-1.00; p=0.045), although this meta-analysis includes data from primary and secondary prevention studies. A previous meta-analysis which included data from the SCOPE trial was inconclusive [129]. An updated Cochrane review by the same group included the HYVET study, and focussed in particular on trials where antihypertensives were used for primary prevention [355].

The benefit of this rational is that it excludes the potential confounding effect on cognition attributable to vascular disease burden, and ensures a more homogenous study population. The authors concluded there was no convincing evidence from the trials that blood pressure lowering in late-life prevented the development of dementia or cognitive impairment in hypertensive patients with no apparent prior cerebrovascular disease. There were significant problems identified with analysing the data, however, due to the number of patients lost to follow-up and the number of placebo patients who received active treatment. This introduced bias and more robust results may be obtained by conducting a meta-analysis using individual patient data.

**Methodological considerations**

Several methodological factors may explain the lack of benefit of AH treatment on the incidence of dementia in some of these trials. The majority only measured effects of short term treatment (2-5 years), whereas the results from observational studies suggests that treatment of hypertension many years earlier in midlife has the greatest effect. Thus, the length of follow-up may have been too short to detect any effect on the
incidence of dementia. Most of these AH trials have included participants who are mentally healthy at baseline, but selective dropout in relation to dementia, and the difficulties associated with the diagnosis of dementia in large trials limits their power to detect a relationship. Comparisons between placebo and treatment groups in some of the trials have been hampered because of crossover from placebo to active treatment, resulting in some trials comparing different AH treatments, rather than treatment versus no treatment as per the original design. The HYVET trial has added further to the field in that it focussed on individuals older than 80 years, an age group with a high incidence of dementia. Reassuringly HYVET demonstrated that treatment did not increase the risk of dementia or cognitive decline in this group. There may however be a selection bias in that hypertensive subjects who survive to 80 years with no prior vascular event may not be representative of most elderly patients with vascular disease. Owing to the benefit of treatment on cardiovascular outcomes, future long-term, placebo controlled studies will probably not be possible for ethical reasons.

Summary of methodological limitations

- No study had cognition as primary endpoint
- Very heterogeneous groups
- Variable follow up periods
- Selective drop-out and crossovers- no class effect
- All active groups had ↓ in BP- no clear correlation between reduction and improvement in cognition
- Methods of monitoring BP and measuring cognition varied greatly
Potential mechanisms of action for certain antihypertensive classes

Are these potential benefits of AH treatment solely related to their blood pressure lowering properties, or are other mechanisms involved? Given that none of the randomised placebo control trials were mechanistic, it is not possible to draw any conclusions on how antihypertensives contribute to dementia prevention. The ACE inhibitor perindopril and the diuretic indapamide were the treatment arm in the PROGRESS and HYVET studies, while the DHP-CCB nitrendipine was used as the primary treatment in the Syst-Eur trial with the possible addition of an ACE inhibitor or diuretic. The SHEP and MRC trials failed to detect any improvement in cognitive outcomes following treatment with diuretics and β blockers or reserpine, despite significant decreases in blood pressure on treatment. The SCOPE trial also failed to demonstrate any benefit on cognitive scores following treatment of elevated BP with the ARB candesartan, although a follow on sub-study did describe a possible benefit. As a result of the general limitations of these studies (outlined above); one couldn’t attribute a treatment effect on the ability of these medications to reduce blood pressure alone. Given that some of these trials had positive results after a relatively short duration of treatment it begs the question do certain AH medications confer benefits above and beyond their ability to reduce BP?

- **Dihydropyridine calcium channel blockers (DHP-CCB)**

Several studies have proposed that calcium channel blockers (CCBs) may possess neuroprotective properties, especially DHP-CCB’s [62, 120]. A recent study investigating the association between different classes of antihypertensives and cognitive performance in 378 elderly hypertensive patients (Mean age 70.4 ± 6.3 years) with subjective memory
complaints, reported that CCB use was associated with better memory performance independently of blood pressure and white matter changes on MRI [130]. The majority of these patients were on DHP-CCB. In a Cochrane review of the clinical efficacy of nimodipine in treating dementia performed in 2001, and updated in 2010 [131], the authors reported on outcome data from nine trials (2492 patients) covering the domains of cognitive function, activities of daily living, global clinical state, safety and tolerability. This review found benefit associated with nimodipine (90 mg/day at 12 weeks) compared with placebo on the SCAG scale (WMD -7.59, 95% CI -9.87 to -5.31, P<0.00001), on clinical global impression (WMD -0.87, 95% CI -1.07 to -0.67, P<0.00001) and cognitive function (SMD 0.61, 95% CI 0.42 to 0.81, P<0.00001), but not on scales assessing activities of daily living. When the AD trials and the VD trials were pooled separately, similar significant results were found for the 90 mg/day dose of nimodipine at 12 weeks. The authors concluded that trials assessing longer-term benefits were necessary. Lipophillic CCBs cross the blood brain barrier with ease enabling more local effects within the brain. In a comparison between two DHP-CCBs the highly lipophillic nilvadipine but not amlodipine improved cognitive outcomes and increased regional cerebral blood flow in a group of patients with mild cognitive impairment (MCI), despite similar reductions in blood pressure in both groups [132]. Over a further follow up period of 20 months patients treated with nilvadipine were less likely to progress to AD [133]. It is hypothesized that DHP-CCBs exert these effects by correcting the cerebral hypoperfusion that can precede clinical symptoms of both AD and VaD. DHP-CCBs also appear to antagonise the β-amyloid induced vasoconstriction associated with AD [134]. The aging brain loses its ability to efficiently regulate intracellular calcium levels, leading to cell death [135], and
contributing to the development of AD [136]. It is hypothesized that DHP-CCBs may alter this disruption [137].

- **Potassium (K⁺) sparing diuretics**

  Several observational studies noted an improvement in cognitive outcomes following treatment with diuretics [118], and potassium (K⁺) sparing diuretics in particular [62]. It is unclear why potassium sparing diuretics in particular should be associated with a reduced risk of AD, but it is well known that both loop and thiazide diuretics reduce potassium concentration, whereas K⁺ sparing diuretics typically lead to increased concentrations. Low potassium concentrations are associated with increased oxidative stress [138], increased inflammation [138], platelet aggregation [139], and vasoconstriction [140]; all of which are potential contributors to AD pathogenesis.

- **Inhibition of the renin-angiotensin system**

  There is some evidence that components of the renin-angiotensin system have a role in learning and memory processes [141]. ACE inhibitors are effective and well tolerated antihypertensive medication, which inhibit the formation of angiotensin II, a potent vasoconstrictor [142]. Angiotensin-receptor blockers (ARB), which are newer agents, act on the same biological pathway as ACE inhibitors, and are widely used antihypertensives. Conflicting results have been reported for the effect of ACE inhibitors on cognition. Findings from the Syst-Eur, PROGRESS and HYVET trials suggest that ACE inhibitors with and without diuretics seem to reduce cognitive decline, especially in stroke-related dementia. However, ACE inhibitors were the only AH drug class potentially associated with a slight increased risk for AD (Adjusted hazard Ratio = 1.13), in the Cache
County cohort [62]. One explanation for these conflicting results may be that lipophilic ACE inhibitors, capable of crossing the blood brain barrier (e.g. captopril, perindopril) are associated with decreased rates of cognitive impairment and dementia, whereas non brain penetrating ACE inhibitors fail to exert the same effects [143]. Studies in hypertensive rats have shown that treatment with the ACE inhibitor captopril, but not the AH drug hydralazine, significantly attenuated age related impairment in learning and memory, despite similar effects by both drugs on lowering blood pressure [144]. Recently Sink et al, reported on participants in the Cardiovascular Health Study Cognition Sub-study, with treated HTN, and no diagnosis of congestive heart failure (n = 1054; mean age, 75 years), who were followed up for a median of 6 years to determine whether cumulative exposure to ACE inhibitors (as a class and by central activity), compared with other anti-HTN agents, was associated with a lower risk of incident dementia, cognitive decline (by Modified Mini-Mental State Examination [3MSE]), or incident disability in instrumental activities of daily living (IADLs) [356]. Among 414 participants who were exposed to ACE inhibitors and 640 who were not, there were 158 cases of incident dementia. Compared with other anti-HTN drugs, there was no association between exposure to all ACE inhibitors and risk of dementia (hazard ratio [HR], 1.01; 95% confidence interval [CI], 0.88-1.15), difference in 3MSE scores (-0.32 points per year; P = 0.15), or odds of disability in IADLs (odds ratio [OR], 1.06; 95% CI, 0.99-1.14). Adjusted results were similar. However, centrally active ACE inhibitors (Captopril, Perindopril) were associated with 65% less decline in 3MSE scores per year of exposure (P = .01), and non-centrally active ACE inhibitors were associated with a greater risk of incident dementia (adjusted HR, 1.20; 95% CI, 1.00-1.43 per year of exposure) and greater odds of
disability in IADLs (adjusted OR, 1.16; 95% CI, 1.03-1.30 per year of exposure) compared with other anti-HTN drugs. It’s likely that an additional mechanism to blood pressure lowering accounts for these variable neuroprotective observations. Possible candidates are modulation of cerebral blood flow [145], pleiotropic effects on the musculoskeletal system and nervous system, or effects on inflammation and oxygen free radicals [146, 147]. The progression of white matter intensities on MRI, implicated in the pathogenesis of both AD and VaD, is also modified by ACE inhibitors [148].

Drugs that selectively inhibit the AT-1 receptor are termed angiotensin receptor blockers (ARB’s). Transgenic mouse models of AD given the ARB valsartan demonstrated improved spatial learning and attenuated oligomerisation of Aβ [357], while low doses of the ARB olmesartan improved hippocampal synaptic plasticity and Aβ-mediated cerebrovascular dysfunction, which included impairment of the autoregulatory mechanisms involved with cerebral blood flow [358]. Losartan, an ARB that can cross the BBB, was found to improve the cognitive function and quality of life in people with hypertension aged up to 73 years [359, 360], but also has been found to demonstrate positive benefits in both animal models and human paradigms of cognition and anxiety that appear to be independent of blood pressure changes. A recent US veterans affairs study reported on 819 491 predominantly male participants (98%) aged 65 or more with cardiovascular disease, treated with angiotensin receptor blockers, lisinopril (ACE-I), and other cardiovascular drugs- the “cardiovascular comparator” [361]. Patients were followed longitudinally for 4 years. Time to incident Alzheimer’s disease or dementia was recorded. Disease progression was the time to admission to a nursing home or death among participants with pre-existing Alzheimer’s disease or dementia. Results were
adjusted using Cox proportional hazard models for age, diabetes, stroke, and cardiovascular disease. Hazard rates for incident dementia in the angiotensin receptor blocker group were 0.76 (95% CI: 0.69-0.84, p< 0.001) compared with the cardiovascular comparator and 0.81 (0.73-0.90, p< 0.001) compared with the lisinopril group.

Compared with the cardiovascular comparator, angiotensin receptor blockers in patients with pre-existing Alzheimer’s disease were associated with a significantly lower risk of admission to a nursing home (HR 0.51, 0.36-0.72, p< 0.0001) and death (HR 0.83, 0.71-0.97, p< 0.022). Angiotensin receptor blockers exhibited a dose-response as well as additive effects in combination with angiotensin converting enzyme inhibitors. This combination compared with angiotensin converting enzyme inhibitors alone was associated with a reduced risk of incident dementia (HR 0.54, 0.51-0.57, p< 0.001) and admission to a nursing home (HR 0.33, 0.22-0.49, p< 0.001). Minor differences were shown in mean systolic and diastolic blood pressures between the groups. Similar results were observed for Alzheimer’s disease. Therefore within this study, treatment of a predominantly male population with angiotensin receptor blockers was associated with a significant reduction in the incidence and progression of Alzheimer’s disease and dementia.

As discussed previously decreased cerebral blood flow is a common and early observation among people with Alzheimer’s disease. Several studies suggest that angiotensin receptor blockers offer an important advantage over angiotensin converting enzyme inhibitors and other antihypertensive agents in improving outcomes following stroke [354, 362-364]. Recent results from the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET), which examined the
effects of telmisartan plus ramipril, observed a decrease in secondary stroke associated with use of telmisartan, but the lowering of stroke rates did not reach significance [365]. Animal studies report that angiotensin receptor blockers elicit neuroprotective responses that are independent of decreases in blood pressure and are apparent even in cell culture [363, 366]. Vascular dysfunction induced by amyloid β, the protein that accumulates in Alzheimer's disease, plays an important part in the disease. As has been discussed in chapter 1, loss of cerebral blood flow is a prominent and early feature of the disease, perhaps as a result of the accumulation of amyloid β. Angiotensin receptor blockers seem to be particularly effective at preventing vascular damage induced by amyloid β [357, 358, 367]. Diabetes is a strong risk factor for Alzheimer's disease and dementia [368]. Clinical studies also indicate that angiotensin receptor blockers offer superior protection against diabetes compared with other antihypertensive drugs [369, 370]. Angiotensin receptor blocker mediated protection against deleterious effects of diabetes might also contribute to the positive results observed.

- β-Blockers

There have been several studies regarding the impact of β-blockers on cognitive function in patients with [149] and without [150, 151] cognitive impairment. While the studies examining the use of β-blockers in cognitively normal subjects failed to demonstrate any negative influence, a more recent study of patients with cognitive impairment and dementia at baseline, found that lipophillic central nervous system (CNS) β-blocker use was associated with poorer cognitive scores [149]. Growing evidence suggests that adrenergic signalling plays a role in the retrieval of intermediate-term contextual memories, because the hippocampus receives dense input from adrenergic
terminals [152]. In particular norepinephrine may be important in retrieval of memories
that are at the early stages of consolidation [152]. Theoretically this process could be
affected by the use of β-blockers with negative consequences. There is insufficient
evidence for this assertion currently, but further studies should investigate the effects of
β-blockers in patients with cognitive impairment or dementia.

Conclusion

Midlife hypertension is a significant risk factor for the later development of both AD
and VaD. Accumulating evidence suggests that treatment with AH medications may
lower the incidence of dementia, but the exact mechanisms of action of these
compounds remains unclear. Recent recommendations from British Association of
Psychopharmacology reported there was type II evidence (evidence from small, non­
replicated RCT’s) for a preventative effect of antihypertensive therapy on the incidence of
dementia [371]. In particular these recommendations commented on the outcomes from
the nimodipine and nitrendipine studies discussed above, and conclude that further trials
were required to confirm these potential benefits. Ethical approval is unlikely for future
placebo controlled trials of AH therapy in hypertensive patients, given the clear benefits
for these treatments on cardiovascular disease and mortality. Whether certain AH
medications may benefit normotensive patients with cognitive impairment or dementia,
given that these compounds may have neuroprotective properties independent of blood
pressure lowering effect remains unclear. This doctoral investigation will examine the
safety and tolerability of the antihypertensive agent nilvadipine in the treatment of AD.
Patients will be included in the trial on the basis of their diagnosis of AD, rather than in
previous trials of AH agents, where inclusion was based on baseline BP values. Within this
open label study I'll examine the cognitive effects of nilvadipine treatment over six weeks versus no treatment, and explore some potential mechanisms of action.
Chapter 3: Demonstration of safety in Alzheimer’s patients for intervention with an antihypertensive drug Nilvadipine: results from an eight-week open label study

Abstract

Background: Nilvadipine may lower rates of conversion from mild-cognitive impairment to Alzheimer’s disease (AD), in hypertensive patients. However, it remains to be determined whether treatment with nilvadipine is safe in AD patients, given the higher incidence of orthostatic hypotension (OH) in this population, who may be more likely to suffer from symptoms associated with the further exaggeration of a drop in BP.

Objective: The aim of this study was to investigate the safety and tolerability of the nilvadipine in AD patients.

Methods: AD patients in the intervention group (n= 56) received nilvadipine 8mg daily over 6-weeks, compared to the control group (n = 30) who received no intervention. Differences in systolic (SBP) and diastolic (DBP) blood pressure, before and after intervention, was assessed using automated sphygmomanometer readings and ambulatory BP monitors (ABP), and change in OH using a finometer. Reporting of adverse events was monitored throughout the study.

Results: There was a significant reduction in the SBP of treated patients compared to non-treated patients but no significant change in DBP. Individuals with higher initial blood pressure (BP) had greater reduction in BP but individuals with normal BP did not experience much change in their BP. While OH was present in 84% of the patients, there was no further drop in BP recorded on active stand studies. There were no significant differences in adverse event reporting between groups.
Conclusion: Nilvadipine was well tolerated by patients with AD. This study supports further investigation of its efficacy as a potential treatment for AD

Introduction

Approximately 36 million individuals worldwide have Alzheimer’s disease (AD), and in the absence of disease modifying therapies, this figure is estimated to increase to in excess of 80 million by 2040 [153] [372]. This increase in AD prevalence represents a considerable economic and public health burden, where the current estimated annual global cost already exceeds $200 billion [154]. The emerging AD epidemic coupled with recent failures of potentially disease modifying therapies to show clinical efficacy [155] clearly highlight an urgent need to explore other therapeutic options to delay or halt the disease progression. Evidence from several longitudinal studies suggests that the use of particular dihydropyridine calcium channel blockers (DHP-CCBs) as antihypertensive treatment is associated with a lower incidence of AD [120, 156]. For instance, the Syst-Eur study, a prospective randomized clinical trial, showed a decline in the incidence of dementia (mostly AD) with nitrendipine intervention in hypertensive individuals [113, 114]. However, the Canadian Study of Health and Aging, also using a prospective design, showed cognitive decline with the use of another DHP-CCB, nifedipine, in elderly hypertensive individuals [157]. A meta-analysis from the Cochrane database of systematic review suggested that over a short time-period, the DHP-CCB nimodipine was effective in preserving cognitive function in patients with primary progressive dementia [131], but its derivate MEM1003 failed to demonstrate any clinical efficacy in a Phase Ila
trial (http://www.memorypharma.com). These findings clearly suggest that there is no class effect for DHP-CCBs on protection against AD and that the beneficial effect of certain DHP-CCBs in AD is unlikely to be related to their anti-hypertensive activity alone. The role of anti-hypertensive treatment *per se* has been explored in one head-to-head comparison of two anti-hypertensive drugs in patients with hypertension and amnestic mild cognitive impairment (aMCI). Nilvadipine, but not amlodipine, was associated with conversion of fewer patients from aMCI to AD in hypertensive patients, despite similar blood pressure (BP) reduction in both groups [133]. Nilvadipine may thus be beneficial as a potential therapy for slowing AD progression, unlike amlodipine, it is able to cross the blood-brain-barrier [158, 159] and as such, it may have restorative effects on calcium homeostasis, dysregulation of which is widely implicated in AD pathogenesis [160].

Nilvadipine is currently licensed as an antihypertensive agent and has a better tolerability profile than nitrendipine, amlodipine or nifedipine, despite similar efficacy for reducing BP in hypertensive individuals [161-163]. However, the treatment of normotensive individuals with nilvadipine might cause an undesired reduction in BP and/or orthostatic hypotension (OH), particularly in AD patients many of whom have impaired cerebrovascular responses and are already prone to hypotension [109, 164]. Therefore, it was important to establish the safety of nilvadipine in AD patients, prior to commencing a large-scale investigation into its efficacy as a treatment for AD. I therefore investigated the safety and tolerability of nilvadipine in mild to moderate AD patients in a single site, non-randomized open label trial.
Participants

Excluded if...
- History of syncope; unexplained falls
- SBP ≤ 100 mmHg, DBP ≤ 65 mmHg
- Already taking β blocker or CCB or > 1 AH medication
- Significant behavioural symptoms
- Heart block, coronary artery disease

Included if...
- Alzheimer’s Disease: Consensus meeting: History, neuropsychological testing; neuroimaging
- MMSE ≥ 14
- Male/Female;
  - Age 50-90 years
- Sufficient hearing/vision/language
- Reliable caregiver
- Stable on current medications

Mean age 70.3 years; 30 (35%) patients aged ≤ 65 years (6 had + Family Hx of AD); Females 49 (57%); Mean MMSE 22.0

Figure 2: Inclusion/Exclusion criteria and overall population demographics
Participants and methodology

The Irish Medicines Board (IMB) and the Federated Hospitals Ethics Committee approved this study. Community-based mild-to-moderate AD patients from a national tertiary referral centre in St. James Hospital were recruited for participation in an eight-week open label study. Inclusion criteria included a diagnosis of probable AD according to NINCDS-ADRDA [165], age between 50-90 years, sufficient hearing, vision, language fluency and having a reliable caregiver to ensure compliance with the study guidelines (Figure 2). Subjects with a history of syncope or unexplained falls, concurrent use of more than one other anti-hypertensive agent, significant behavioural symptoms or atrial fibrillation were excluded. The main outcomes of this study was to determine if nilvadipine caused an inappropriate reduction in blood pressure in AD patients and to assess any adverse events associated with nilvadipine treatment in patients with a diagnosis of AD.

Prior to enrolment, I met with each potential subject and his/her next of kin to discuss the trial and they were provided with a consent form and a detailed patient information sheet, which outlined all aspects of the trial, including the study procedures and potential expected adverse events associated with the medication. Once consented, each participant was assigned sequentially to either treatment with nilvadipine 8mg CR (Nivadil®) once daily or no treatment in a 2:1 ratio. Two subjects screened to participate in the treatment group withdrew their consent prior to commencement in the trial. Subjects were examined on a bi-weekly basis over the eight-week period. During the trial period, patients and their caregivers were given the medication in a tapered fashion (14 tablets at a time), and the study doctor or nurse performed a pill count at least every 2
weeks to monitor compliance. Subjects were asked to return all unused pills and the study drug compliance was calculated by dividing the number of doses removed from the bottle by the number of days of the treatment period. If the compliance fell below 80% or extra doses were taken in the interim, the reason(s) were noted, and the participant and caregiver were counselled accordingly. Only one subject (in the control group) failed to complete all the visits. However, as the majority of baseline and follow up tasks were completed, these data were included in the analyses. The study medication compliance was 98.2% (+/- 4.3%) overall. One person had a compliance of only 73% and was also included in the analyses.

Physical examination and routine laboratory tests were administered at the baseline and at the 6-week time-point. A trained nurse or physician performed isolated BP measurements at screening (Visit 1) and 4 weeks (Visit 4) (Figure 1) into the study using an automated sphygmomanometer, an A&D* digital blood pressure monitor (Model UA-767 Plus 30), which has been validated by the Association for the Advancement of Medical Instrumentation (AAMI) and British Hypertension Society (BHS). Participants in the treatment group were instructed to take the tablet at 11:00 AM each day, and where possible the BP measurements were performed at approximately the same time for the baseline and follow up visits. Elevated BP status for sphygmomanometer results was defined as a SBP of 140 mmHg or above and/or a DBP of 90 mmHg at visit 1 [166].
Ambulatory blood pressure (ABP) measurements were performed at baseline (Visit 2) and six weeks later (Visit 5, final treatment visit). Ambulatory blood pressure monitoring is a non-invasive, fully automated technique, which has been in use for over 20 years for the assessment and management of hypertension [167-169]. The A&D TM-2430* ABP device was used, which has been validated by the British Hypertension Society (BHS) and the Association for the Advancement of Medical Instrumentation (AAMI) [170]. The ABP measurements were taken at every 30 minutes during the daytime (08:00-22:00 Hrs) and every hour during the night (22:01-07:59 Hrs). At least 14 daytime and 7 nighttime readings were required for inclusion of the results in the analysis [169]. Elevated BP status for ABP results was defined as a mean SBP of 140 mmHg or above and/or a DBP of
90 mmHg or above on daytime ABP values at visit 2 [166].

All subjects underwent lying-to-standing orthostatic testing (active stand) with non-invasive beat-to-beat blood pressure monitoring using the Finometer™ Pro device (Finapress Medical Systems Amsterdam). Orthostatic Hypotension was defined as a drop in SBP ≥ 20mmHg and/or a drop in DBP ≥10mmHg [171]. For the active stand measurements, subjects were resting in a supine position for at least 5 minutes, followed by standing, upon which their blood pressure was monitored for 3 minutes, as they stood motionless. After the test, each subject was asked to report whether he/she felt dizziness, faintness or light-headedness. Active stand data was exported with the Beatscope™ 1.1a software according to the 5-second averages method, as one study demonstrated that this had the best association with a history of falls [172]. The following variables were extracted for SBP and DBP analysis:

1. Baseline BP: Averaged BP between 60 and 30 seconds before standing.
2. Nadir BP: The lowest BP reached following the active stand.
3. Delta (Δ): The difference between the nadir BP and the baseline BP (mmHg).

All participants had 4 visits over the 6-week period covering the treatment phase, and at each visit, participants were specifically asked about the occurrence of falls and orthostatic symptoms. Subjects were also followed for an additional 2 weeks after discontinuation of the study medication. The study physician determined the severity (mild/ moderate/ severe), and causality (definitely not related/ probably not related/ probably related) for each reported adverse event. A serious adverse event was classified as any adverse event (AE) that was fatal, life threatening, or permanently disabling, or that resulted in new or prolonged hospitalization.
**Statistical analysis**

*A priori* power calculations were performed using the G-power software based on the primary outcome of nilvadipine's effect on isolated BP measurements in hypertensive patients [173]. A sample size of 100 subjects (60 for treatment; 40 for no treatment) was suggested based on Cohen’s d of 0.90, an alpha of 0.05 and a β score of 0.90. A post-hoc power calculation suggested that a β score of 0.74 was achieved at an alpha of 0.05 for the sample size used in the current study. The non-parametric Mann Whitney test was used for the analysis of asymmetric Mini Mental State Examination test scores. Symmetric quantitative values, such as BP measurements, age, and education were compared using the students T-test. Adjusted analyses were performed using ANOVA to account for gender differences, concomitant use of other anti-hypertensive agents and elevated BP at screening/baseline. For qualitative data the Pearson’s Chi square test was used if the cell size requirement for the contingency tables was met, otherwise the Fisher’s Exact test was used. All statistical analyses were performed using the SPSS 16.0 statistical package. A p value of less than 0.05 was considered statistically significant.

**Results**

Eighty-six patients with mild/moderate AD qualified for the study. Fifty-six participants received treatment with nilvadipine 8mg CR daily for six weeks and thirty participants received no treatment. There was no difference in the age, baseline MMSE score, presence of elevated SBP or DBP (referred to hereon as elevated BP) or use of antihypertensives and cholinesterase inhibitors between the groups (Table 1). There were a greater number of females in the control group.
<table>
<thead>
<tr>
<th></th>
<th>No treatment n=30</th>
<th>Nilvadipine n=56</th>
<th>Difference (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at baseline, years (SD)</strong></td>
<td>71.2 (8.7)</td>
<td>69.3 (8.4)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Female, n (%)</strong></td>
<td>22 (73.3)</td>
<td>27 (48.2)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>MMSE (SD)</strong></td>
<td>22.6 (3.4)</td>
<td>21.5 (4.1)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Systolic BP mmHg (SD)</strong></td>
<td>139.1 (17.0)</td>
<td>145.8 (20.6)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Diastolic BP mmHg (SD)</strong></td>
<td>78.5 (6.9)</td>
<td>80.5 (7.1)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Elevated blood pressure, n (%)</strong></td>
<td>12 (40.0)</td>
<td>31 (55.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>**Use of antihypertensives at baseline, n (%) **</td>
<td>3 (10)</td>
<td>4 (7.1)</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Drop in SBP on active stand mmHg (SD)</strong></td>
<td>33.4 (14.7)</td>
<td>32.2 (21.1)</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Drop in DBP on active stand mmHg (SD)</strong></td>
<td>17.5 (10.6)</td>
<td>17.3 (12.7)</td>
<td>0.95</td>
</tr>
<tr>
<td><strong>Cholinesterase use, n (%)</strong></td>
<td>29 (96.6)</td>
<td>55 (98.2)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

§ Based on baseline isolated automated sphygmomanometer readings; *SBP ≥140 mmHg and/or DBP ≥90 mmHg on screening automated sphygmomanometer readings, SD Standard Deviation; **Patients on more than one concomitant antihypertensive were excluded from participation.

- **Isolated blood pressure monitoring**

Compared to the control group, a significant reduction in SBP was observed within the treatment group on isolated BP measurements (Nilvadipine: -11.6 ± 18.4 mmHg versus Control: +6.1 ± 18.6 mmHg, p < 0.01), but no such difference was observed for the DBP (Nilvadipine: -5.0 ± 9.5 mmHg versus Control: -2.7 ± 7.6 mmHg, p = 0.27). Once adjusted
for gender differences, concomitant use of other anti-hypertensive agents and elevated BP at screening, the main treatment effect for SBP remained significant (F = 15.25, p < 0.001, table 2), but no main treatment effect was evident for DBP (F = 0.81, p = 0.37, table 2). A confounding effect of elevated BP status at screening was observed for SBP (F = 9.75, p < 0.01) and DBP (F = 3.91, p = 0.05).

- Ambulatory blood pressure monitoring

Results of ABP monitoring were available for 77 participants (89.6%) due to inability of 9 participants (n=8 treated; n=1 untreated) to tolerate the monitor for the required number of readings (n=8) and drop out prior to their six-week review (n=1). A further 12 subjects (n=7 treated; n=5 untreated) had sufficient readings for inclusion in the daytime analysis, but didn’t tolerate wearing the monitor during the night. One patient had sufficient night-time readings but not enough daytime readings.

There were no significant differences between the two groups on daytime SBP (Nilvadipine: +0.4 ± 17.6 mmHg versus Control: +2.6 ± 12.7 mmHg, p = 0.57) and DBP (Nilvadipine: -0.7 ± 10.0 mmHg versus Control: +3.1 ± 9.9 mmHg, p = 0.11) for unadjusted analyses. Once adjusted for gender differences, concomitant use of other anti-hypertensive agents and elevated BP at baseline, no main treatment effect was observed for daytime SBP measurements (F = 0.86, p = 0.36, table 2), however a trend for a main treatment effect was observed for daytime DBP readings (F = 3.65, p = 0.06, table 2). A significant confounding effect of elevated baseline BP was observed for both daytime SBP (F = 8.08, p < 0.01) and daytime DBP (F = 3.97, p = 0.05).

For unadjusted analyses of night-time ABP measurements, there was no difference
between the groups for SBP (Nilvadipine: +1.9 ± 16.0 mmHg versus Control: +2.9 ± 11.6
mmHg, p = 0.80) and DBP readings (Nilvadipine: +1.0 ± 10.3 mmHg versus Control: +1.5 ±
8.2 mmHg, p = 0.85). For adjusted analyses, no main treatment effect was observed for
night-time SBP (F = 0.14, p = 0.71, table 2) or night-time DBP (F = 1.16, p = 0.29, table 2)
measurements. A significant interaction between treatment and elevated baseline BP
was evident for both night-time SBP (F = 4.99, p = 0.03), and night-time DBP (F = 12.15, p
< 0.01).
<table>
<thead>
<tr>
<th>Automated Isolated BP</th>
<th>Nilvadipine (n = 56)</th>
<th>No treatment (n = 30)</th>
<th>Difference</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Screening Visit 4</td>
<td>Screening Visit 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>145.84 (2.7)</td>
<td>134.27 (2.1)</td>
<td>139.07 (3.1)</td>
<td>145.20 (3.7)</td>
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<td></td>
<td>(n = 47)</td>
<td>(n = 29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>80.54 (1.0)</td>
<td>75.57 (1.2)</td>
<td>78.50 (1.3)</td>
<td>75.7 (1.0)</td>
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<tr>
<td></td>
<td>(n = 47)</td>
<td>(n = 29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambulatory 24 hour BP</td>
<td>Baseline Visit 5</td>
<td>Baseline Visit 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>132.87 (2.4)</td>
<td>133.30 (2.5)</td>
<td>134.17 (2.7)</td>
<td>136.76 (2.7)</td>
</tr>
<tr>
<td></td>
<td>(n = 47)</td>
<td>(n = 29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>78.49 (1.3)</td>
<td>77.74 (1.6)</td>
<td>76.34 (1.9)</td>
<td>79.45 (1.7)</td>
</tr>
<tr>
<td></td>
<td>(n = 47)</td>
<td>(n = 29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night-time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>117.95 (2.5)</td>
<td>119.88 (3.1)</td>
<td>121.62 (3.6)</td>
<td>124.50 (3.4)</td>
</tr>
<tr>
<td></td>
<td>(n = 47)</td>
<td>(n = 29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>69.05 (1.5)</td>
<td>70.07 (1.7)</td>
<td>70.00 (2.3)</td>
<td>71.50 (2.1)</td>
</tr>
<tr>
<td></td>
<td>(n = 47)</td>
<td>(n = 29)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: * P values are from adjusted analyses accounting for gender differences, use of other anti-hypertensive agents and screening/baseline elevated BP.
Table 3: Relative changes in BP values in treated patients with elevated baseline BP versus those with BP within normal limits.

<table>
<thead>
<tr>
<th></th>
<th>Elevated BP&lt;sup&gt;1&lt;/sup&gt; (n = 31)</th>
<th>Normal BP (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± SE)</td>
<td>Mean (± SE)</td>
</tr>
<tr>
<td>Automated Isolated BP</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>158.7 (3.3)</td>
<td>139.3 (3.1)</td>
</tr>
<tr>
<td></td>
<td>-19.4* (3.5)</td>
<td>130.0 (1.7)</td>
</tr>
<tr>
<td></td>
<td>128.0 (2.3)</td>
<td>-2.0 (2.3)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>82.8 (1.4)</td>
<td>75.9 (1.7)</td>
</tr>
<tr>
<td></td>
<td>-6.9* (1.9)</td>
<td>77.7 (0.9)</td>
</tr>
<tr>
<td></td>
<td>75.1 (1.6)</td>
<td>-2.6 (1.5)</td>
</tr>
<tr>
<td>Ambulatory&lt;sup&gt;2&lt;/sup&gt;BP (n = 15)</td>
<td></td>
<td>(n = 32)</td>
</tr>
<tr>
<td>24 hour BP</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>151.1 (2.9)</td>
<td>145.2 (4.2)</td>
</tr>
<tr>
<td></td>
<td>-5.9* (4.5)</td>
<td>124.3 (9.7)</td>
</tr>
<tr>
<td></td>
<td>127.7 (15.0)</td>
<td>3.4 (3.0)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>86.3 (2.1)</td>
<td>83.1 (2.3)</td>
</tr>
<tr>
<td></td>
<td>-3.2* (2.3)</td>
<td>74.8 (7.1)</td>
</tr>
<tr>
<td></td>
<td>75.2 (10.9)</td>
<td>0.4 (1.8)</td>
</tr>
<tr>
<td>Night-time&lt;sup&gt;3&lt;/sup&gt;(n = 12)</td>
<td></td>
<td>(n = 29)</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>131.7 (4.8)</td>
<td>138.3 (4.2)</td>
</tr>
<tr>
<td></td>
<td>6.6** (6.1)</td>
<td>112.3 (11.7)</td>
</tr>
<tr>
<td></td>
<td>112.2 (16.1)</td>
<td>0.03 (2.5)</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>73.9 (1.8)</td>
<td>80.3 (2.3)</td>
</tr>
<tr>
<td></td>
<td>6.4** (2.7)</td>
<td>67.0 (9.9)</td>
</tr>
<tr>
<td></td>
<td>65.9 (8.7)</td>
<td>1.2 (1.9)</td>
</tr>
</tbody>
</table>

Note: <sup>1</sup>Mean difference is based on visit 4 values subtracted from screening (visit 1) values for automated BP measurements, and for ambulatory BP measurements visit 5 values were subtracted from baseline (visit 2) values. *Significant confounding effect based on adjusted analyses (p < 0.05). **Significant interaction, based on adjusted analyses (p < 0.05).
• **Stratification by screening/baseline elevated blood pressure status**

Due to the presence of statistically significant confounding and/or interactive effects of elevated screening/baseline BP on treatment outcome, we subsequently stratified the treatment group by this variable (see table 3) and observed a greater reduction in BP within the group with elevated screening/baseline BP.

Furthermore, a significant correlation between initial BP values and the subsequent change in BP in response to nilvadipine was also observed (isolated BP: SBP: \( r = -0.68, p < 0.01 \); DBP: \( r = -0.45, p < 0.01 \) and daytime ABP: SBP: \( r = -0.48, p < 0.01 \); DBP: \( r = -0.36, p = 0.014 \)), whereby patients with higher initial BP had a greater reduction and patients with lower BP had little or no response. No such pattern was observed in the untreated group.

• **Active Stand monitoring for orthostatic hypotension**

Results were analysed for 75 (87.2%) of the 86 participants who had active stands performed at baseline and at 6 weeks. Data was unavailable for 11 subjects due to participant refusal at either visit (n=5), or instrument recording errors (n=6). Sixty-three subjects (84%) had OH diagnosed at baseline (see table 1). There was no significant difference in the incidence of OH in either group at baseline (Fisher’s Exact test \( p=0.52 \)). There were also no significant differences in the drop in blood pressure in either group over the study period for both unadjusted (SBP: Nilvadipine -2.8 ± 20.3 mmHg versus Control -4.0 ± 20.4 mmHg, \( p = 0.80 \), where minus indicates less of a drop in blood pressure from baseline to follow up), (DBP: Nilvadipine +1.5 ± 14.7 mmHg versus Control -0.7 ± 13.7 mmHg, \( p = 0.51 \), and adjusted analyses (SBP: \( F = 0.02, p = 0.89 \) and DBP: \( F = \)
No subjects reported symptoms during orthostasis at baseline, and only 1 subject reported mild symptoms associated with orthostasis following treatment.

- **Adverse event monitoring**

Twenty-two (39.0%) participants treated with nilvadipine reported at least one adverse event versus seven (23.3%) in the no treatment group (Pearson Chi-Square value 1.04, \( p = 0.31 \)). Similarly, there were no differences in adverse events by screening/baseline BP status (Pearson Chi-Square value = 0.71, \( p = 0.4 \) for the treatment group; Pearson Chi-Square value = 2.52, \( p = 0.11 \) for the control group). Among the adverse events reported by treated participants, fifteen (26.8%) were adjudged "probably related" to nilvadipine. The majority of adverse events were expected (Table 4) as defined by those previously recorded on the Nivadil® product insert. There were 4 reported cases of pre-syncopal symptoms (e.g. dizziness, light-headedness), one fall (mechanical trip), and one case of syncope (in the control group) throughout the trial. Only 3 of these episodes were deemed probably related to the nilvadipine treatment, with the others occurring in the control group. The expected mild adverse effects of flushing and headache were the most commonly reported events. One patient treated with nilvadipine had an episode of symptomatic bradycardia while off the medication. Eight unexpected adverse events were recorded, the majority of which were mild urinary or respiratory tract infections, and were distributed evenly between the two groups (Fishers Exact Test \( p = 0.74 \)). There were no reports of behaviour disturbances in either group.
Table 4: Summary of adverse events in participants either treated with Nilvadipine 8mg OD vs no treatment

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Expected /Unexpected</th>
<th>Events (No.)</th>
<th>Control</th>
<th>Nilvadipine</th>
<th>Serious</th>
<th>Severity</th>
<th>Relation to study Rx*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial Flushing</td>
<td>Expected</td>
<td>5b</td>
<td>0</td>
<td>5</td>
<td>No</td>
<td>Mild</td>
<td>5=PR</td>
</tr>
<tr>
<td>Headache</td>
<td>Expected</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>No</td>
<td>Mild</td>
<td>1=DNR; 1=PNR; 4=PR</td>
</tr>
<tr>
<td>Low BP* (asymptomatic)</td>
<td>Expected</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>No</td>
<td>Mild</td>
<td>3=PR</td>
</tr>
<tr>
<td>Bradycardia/Collapse^</td>
<td>Expected</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Yes</td>
<td>Severe</td>
<td>1=PNR</td>
</tr>
<tr>
<td>Syncope</td>
<td>Expected</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>No</td>
<td>Mod</td>
<td>1=DNR</td>
</tr>
<tr>
<td>Fall</td>
<td>Expected</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>No</td>
<td>Mild</td>
<td>1=DNR</td>
</tr>
<tr>
<td>Pre-syncope</td>
<td>Expected</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>No</td>
<td>Mild</td>
<td>1=DNR; 1=PNR; 2=PR</td>
</tr>
<tr>
<td>Orthostatic Hypotension</td>
<td>Expected</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>No</td>
<td>Mild</td>
<td>1=PR</td>
</tr>
<tr>
<td>Urinary tract / Respiratory tract infection</td>
<td>Unexpected</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>No</td>
<td>Mild</td>
<td>3=DNR; 2=PNR</td>
</tr>
<tr>
<td>Elevated y-glutamyl transferase</td>
<td>Unexpected</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>No</td>
<td>Mild</td>
<td>1=PNR</td>
</tr>
<tr>
<td>Back/ Joint pain</td>
<td>Unexpected</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>No</td>
<td>Mild</td>
<td>2=PNR</td>
</tr>
</tbody>
</table>

* Expected events were defined as events expected based on Nilvadipine medication package insert. b Two events occurred in the same participant. ^ Patient was not on medication at the time and had been asymptomatic on medication. *DNR = definitely not related, PR = probably related, PNR = probably not related. *BP recorded on automated sphygmomanometer at visits 1, 3, 4 and 6.
Discussion

Currently approved AD medications are limited in their effects, often only providing symptomatic relief at best, and fail to alter the course of the disease [174]. Nilvadipine treatment in hypertensive aMCI patients resulted in fewer patients converting to AD, suggesting a possible neuroprotective influence. However, nilvadipine is an antihypertensive agent, and it is unclear whether inappropriate lowering of BP could have adverse consequences in the AD patient population who are already prone to hypotension and associated symptoms [175]. These findings suggest that administration of nilvadipine to subjects with AD does not cause an inappropriate drop in BP with associated adverse effects. Treatment with nilvadipine led to selective reductions in blood pressure in participants with higher baseline BP values but virtually no change in subjects with normal BP. Within the treatment group some individuals experienced an increase in their night-time DBP, values which may be due to a rebound effect on BP as the plasma levels of nilvadipine diminished later in the day. This has previously been described with controlled release calcium channel antagonists [176, 177].

Anti-hypertensive medications are known to cause OH [178, 179] and the symptoms associated with OH include dizziness, light-headedness, falls, visual disturbances, and loss or near-loss of consciousness [180, 181]. In this study population, the presence of OH in 84% of the subjects at baseline is in accord with previous reports that this condition is extremely common in patients with AD, which is higher than in non-demented individuals [109, 182][373]. A recent study by Mehrabian et al studied the association between cognitive function and OH in 495 consecutive elderly outpatients attending a memory centre [374]. Blood pressure (BP) was measured in a sitting and
standing position. A significant relationship was observed between OH and cognitive status (normal cognitive function, MCI, AD, or VaD). OH was present in 22% in VaD subjects, 15% in AD subjects, 12% in MCI subjects and 4% in normal control subjects (p<0.01 for overall test). As such, patients with AD may be at high risk of suffering adverse events and symptoms if their orthostatic drop in BP is further exaggerated by intervention with anti-hypertensive agents. In this study there was no additional drop in BP on active stand in treated participants. Similarly, there were no significant differences between the treated and untreated individuals in the frequency of reporting of adverse events related to BP lowering (i.e. falls, syncope and pre-syncopal episodes). Consistent with previous reports on nilvadipine, there were no reports of peripheral oedema [162].

It is important that before embarking on large-scale expensive and time consuming clinical trials, small safety trials are conducted in a community-based population which reflects the full spectrum of AD patients. A small open-label trial, such as this one, represents a first-step in understanding the physiological response to potentially new interventions in AD patient populations. As the outcome measure was a drop in blood pressure upon treatment with nilvadipine, which is a very distinguishable property of this drug, I opted not to use a double-blind placebo-controlled design, as it would not have been possible to keep study staff blinded to the outcome. Given the presence of OH in this population and a lack of other reports on nilvadipine intervention in generally normotensive AD patients, concerns over safety issues further precluded use of such a study design in this vulnerable patient population. Despite these limitations, my confidence in these findings is increased since similar observations were made using two different methodologies for BP measurement and the frequency of reported adverse
events did not differ significantly between the treated and non-treated participants and suggest no systematic reporting bias.

This preliminary study suggests that nilvadipine intervention does not drop BP in AD patients with normal BP and is safe and well tolerated in this patient population. Given the possible cognitive enhancing and potential disease modifying effects of nilvadipine described in previous studies, this study supports further investigation of its safety and treatment effects in double-blind randomised controlled trials.

Summary: Main safety results

1. Study medication compliance = 98.2% (4.3)
2. BP reduced in patients with higher baseline BP, but no significant effect in non-hypertensive patients
3. High incidence OH (84%) overall, but no increase in the drop in BP on standing
4. Well tolerated- no patient required or requested discontinuation of trial medication
Chapter 4: APOE ε4 specific short-term cognitive benefits of treatment with the antihypertensive nilvadipine in Alzheimer’s patients- an open-label trial

Abstract

Background: Evidence suggests that dihydropyridine (DHP) calcium channel blockers may be useful in preventing and treating Alzheimer’s disease (AD).

Method: In an open-label safety and tolerability trial, we also investigated change in cognition using the MMSE, and executive function using the EXIT25 in 55 AD patients who received nilvadipine 8mg daily for 6-weeks compared with 30 non-treated AD subjects. APOE genotyping was performed and the study team and caregivers were kept blinded to APOE ε4 status during the trial.

Results: Aside from differences in gender and education, both the treatment and the control groups were similar in general demographics and on cognition status at baseline. After correction for potential confounders, APOE ε4 status, and use of other antihypertensive medications, a significant impact of study intervention was observed on MMSE (F = 8.67, p < 0.01) and EXIT (F = 8.77, p < 0.03) scores. An interaction between APOE ε4 carrier status and treatment (p ≤ 0.05) was observed for both outcome measures where APOE ε4 non-carrier treated participants demonstrated overall stabilization in cognition and improvement in executive functions.

Conclusion: Consistent with other randomized AD trials, in this open-label trial we observe modulation of cognitive outcome by APOE ε4 carrier status. As the genetic data were double blinded the genotype dependent effects are unlikely to be attributable to placebo or participant or investigator bias. These findings provide additional evidence for
potential therapeutic efficacy of nilvadipine in treating AD and warrant further investigation.

**Introduction**

Alzheimer's disease (AD) is clinically characterized by progressive cognitive decline and pathologically by presence of beta-amyloid (Aβ) plaques and neurofibrillary tangles comprised of hyperphosphorylated and aggregated tau, both Aβ and tau are now considered key proteins involved in AD pathogenesis. Prevalence rates of AD are estimated to be 27 million worldwide and, in the absence of therapies that prevent or slow disease progress, rates are projected to quadruple by the year 2050 [183]. Currently approved treatments only provide modest symptomatic relief without modifying the natural disease course. Therefore, recent efforts have focused on identifying disease modifying therapies that target the underlying pathogenic factors for AD, with the aim of halting disease progression or delaying its onset indefinitely.

Over the last decade, numerous clinical and basic science studies have substantiated that vascular risk factors contribute significantly to the pathogenesis of AD [10]. Several longitudinal studies have demonstrated that elevated blood pressure is associated with an increased risk of AD [32, 63] and this risk is noticeably reduced with the use of certain anti-hypertensive regimens [113, 119]. In particular, the Syst-Eur study demonstrated that the treatment of hypertension with the dihydropyridine (DHP) calcium channel blocker, nitrendipine reduced the incidence of dementia by 55% [114]. A systematic review of the use of the DHP nimodipine in patients with primary neurodegenerative dementia, suggested that over a short duration of 12 to 24 weeks,
nimodipine treatment resulted in improved cognition [131]. However, not all DHPs have demonstrated favourable outcomes for AD treatment, despite similar effects on blood pressure. For instance treatment with nifedipine is associated with reduced cognitive function in the elderly, while amlodipine treatment offers no improvement in cognition [133, 157]. This suggests that certain DHP calcium channel blockers may possess specific neuroprotective properties separate from their antihypertensive effects, which may potentially be beneficial as a treatment for AD.

Treatment with nilvadipine, but not amlodipine, in hypertensive patients with mild cognitive impairment (MCI) was shown to prevent further cognitive decline for up to 20 months [132] and was associated with an improvement in CBF measured using SPECT [132]. However treatment groups were rather small (less than 10 subjects) and subjects who progressed to AD were omitted from the longitudinal analyses. Furthermore, several other case-reports suggest improved cognition and CBF in AD patients receiving nilvadipine [184, 185]. However, larger scale clinical trials in AD subjects have hitherto not been undertaken. Therefore, I conducted an open-label trial of nilvadipine for safety and tolerability in AD (Chapter 3). Nilvadipine treatment was safe and well tolerated in AD patients [186]. In this current report I detail the analyses of cognitive measures performed over 6 to 7 weeks within the same trial. Furthermore, given the recent reports on modification of treatment response by APOE ε4 allele (a known risk factor of AD) [187], I analyzed the cognitive outcome data by APOE ε4 carrier status as well.
Methods and participants

The Irish Medicines Board (IMB) and the Federated Hospitals Ethics Committee approved this study. Subjects with a diagnosis of mild/moderate AD attending the memory clinic (a national tertiary referral centre) in St. James Hospital, Dublin, were recruited between November 2006 and December 2008 for participation in an eight-week open label study. All subjects were diagnosed with probable AD according to NINCDS-ADRDA criteria following “consensus opinion” [165]. Consensus diagnosis involved recommendations from a panel consisting of a geriatrician, psychiatrist, neuropsychologist, and where necessary, a neurologist. All subjects had prior neuroimaging consistent with a diagnosis of AD. Individuals between the ages of 50-90 years, with sufficient hearing, vision, and language fluency and with a reliable caregiver who was willing to ensure compliance with the study guidelines were invited to participate in the study. Subjects with a history of syncope, unexplained falls, or receiving treatment with more than one antihypertensive agent were excluded. Subjects already on DHP calcium channel blockers or beta-blockers were also excluded.

Before consenting, all potential participants and their caregivers were provided an IMB approved detailed patient information sheet, which outlined all aspects of the trial, including the potential expected adverse events associated with the medication. Following consent (assent), enrolled subjects were assigned by the study physician, in a 2:1 ratio, to either nilvadipine 8mg CR (Nivadil®) once daily or no treatment. Two patients randomised to the treatment group withdrew consent for participation prior to commencement and were excluded from analysis. During the trial period, patients and their caregivers were given the medication in a tapered fashion (14 tablets at a time), and
the study doctor or nurse performed a pill count at least every 2 weeks to monitor compliance. Subjects were asked to return all unused pills and the study drug compliance was calculated by dividing the number of doses removed from the bottle by the number of days of the treatment period. If the compliance fell below 80% or extra doses were taken at any visit, the reason(s) were noted and the patient and caregiver were counselled accordingly. After removing one subject with a poor compliance rate (73%) the overall medication compliance rate was 98.67% (± 2.65). Analyses were performed with and without this subject; no differences in overall findings were found and therefore we present the data with the exclusion of this patient. Only one subject (in the control group) failed to complete all the visits. However, as the majority of baseline and follow up tasks were completed, this data was included in the analyses.
Subjects were examined on a bi-weekly basis over the eight-week period; both in the hospital and in their own home (Figure 1). Physical examination and routine laboratory tests were administered at the baseline (visit 2) and at the 6-week time-point (visit 5). Modified versions of the Mini-Mental Status Examination (MMSE) [188] were administered at the screening visit prior to treatment (visit 1) and at visit 5 (7.4 weeks ± 8 days) (Figure 1). At both visits, subjects were asked to spell "World" backwards and perform serial sevens and the higher score was included in the overall performance score. Subjects were also given full credit for the 3-word registration regardless of the number of trials needed to accurately reproduce all 3 words. At visit 5 follow-up, alternative
words (i.e. orange, chair, and euro) were used. Although this approach represents a variation in standardized instructions, the scoring method above is commonly utilized and was applied consistently to all participants at both visits. This approach was used due to the large variation in literacy levels and education within the study group [189]. Word registration was ensured so that the 3-word delayed recall part of the test could be assessed confidently. The MMSE was performed in a quiet location in the subject’s home, while all other tests were administered in the hospital. The Executive Interview (EXIT25) was also administered at visit 2 (also prior to treatment) and visit 5 (6 weeks later ± 2 days) (Figure 1). The EXIT25 is a 25-item questionnaire assessing dysexecutive signs and behaviours (e.g., frontal release signs, utilization behaviour, perseverative errors, echopraxia) often seen in AD [190]. Cognitive measures were administered by trained nurses and the study physician, who were trained by a senior neuropsychologist. When possible the same examiner administered all tests at baseline and follow-up for each individual to eliminate any inter-rater variability over the course of the study period. A US board-certified neuropsychologist and clinical research monitor, independent of administration of these tests, periodically audited the cognitive data including scoring and administration variations.

Ambulatory blood pressure (ABP) measurements were performed at baseline (Visit 2) and six weeks later (Visit 5) (Figure 1). Ambulatory blood pressure monitoring is a non-invasive, fully automated technique in which BP is recorded over an extended period of time and has been in use for over 20 years for the assessment and management of hypertension [169]. The BP readings obtained using ABP are considered superior over isolated measurements as they are recorded over a prolonged time period, away from
the office, and at night [191]. We used the A&D TM-2430* ABP device, which has been independently validated to British Hypertension Society (BHS) and Association for the Advancement of Medical Instrumentation (AAMI) recommendations [170]. The readings from ABP measurements were divided into 2 categories, daytime (08:00-22:00 Hrs), and night-time (22:01-07:59Hrs). The recorders were set to take readings every 30 minutes during the daytime interval, and every hour during the night-time interval. At least 14 daytime and 7 night-time readings were required to include the results in the analysis.

Mean daytime and night time systolic (SBP) and diastolic BP (DBP) was analyzed. Hypertension was diagnosed according to international guidelines as a SBP of ≥ 140 mmHg and/or a DBP of ≥ 90 mmHg [166]. Categorization of participants by baseline hypertension status as defined by above criteria was performed at the completion of the study for final analyses.

Blood samples were collected for APOE genotyping. Pure Gene Kits (Gentra Systems, Minneapolis, MN, USA) were used for extracting DNA from whole blood and PCR analyses for APOE were performed using previously established methods [192]. The study staff and the family members were kept blinded to the APOE ε4 status and only revealed to the key study investigators at the completion of the study for analyses.

The Mini-Mental State Examination (MMSE)

The MMSE [188] is presently the most widely used screening instrument for the detection of an individual’s cognitive impairment, and quantification of its severity [193]. The MMSE has nineteen individual tests of eleven domains covering orientation, registration, attention or calculation (serial sevens or spelling), writing and construction. The test can be administered in 10 minutes and is commonly used by specialists [194] and
non-specialists [195]. The most common application of the MMSE is as a brief method to help detect suspected dementia but recently it has also been used in the diagnosis of mild cognitive impairment (MCI) [196]. A score of 23 out of 30 or less has generally been accepted as indicating the presence of cognitive impairment, however a cut-off of less than 23 had greater accuracy in an Irish community sample [189]. Most studies that have used this criterion score to identify cases with dementia, report moderate to high levels of sensitivity and specificity of the MMSE in clinical series [188, 197]. Despite its popularity the MMSE has several well-known shortcomings, in particular a bias against older people and those with lower levels of education [198, 199]. The MMSE has been successfully used to assess the cognitive efficacy of many treatment interventions over varying study periods, in patients with AD [200-203].

The delayed recall section of the MMSE is of particular interest in patients with AD. Patients are asked to remember three items, which are repeated until the patient has registered them and can repeat them back. Several moments later the patient is asked to recall these items. A decline in delayed recall is often the first sign of cognitive impairment in patients who go on to develop AD [204-206]. Delayed recall performance on the MMSE has been shown to correlate with the development and severity of AD [207-210]. In this study, as well as looking at the overall changes in MMSE scores, we also analysed results from the delayed recall subsection to examine for any treatment effects specific to this area.

The Executive Interview (EXIT-25)

Patients with AD are impaired on a variety of tasks that have commonly been considered a measure of executive function [211-214]. Executive function encompasses a
number of cognitive abilities, which are generally associated with controlling or guiding behaviour in a top-down fashion such as decision making, planning of tasks, self-monitoring, behaviour initiation, organisation and inhibition. Neuro-anatomically, executive functions are associated with the pre-frontal cortex and its basal ganglia-thalamic connections [215]. Patients with AD may display executive function deficits early in the course of the illness [213, 216]. Impairment of executive function in patients with mild AD, may precede the disturbance of sustained attention, language, and constructional abilities [213].

The EXIT25 is a screening instrument developed for the assessment of executive dysfunction [190]. Items assess verbal fluency, design fluency, frontal release signs, motor/impulse control, imitation behaviour, and other clinical signs associated with frontal system dysfunction. It requires 15 minutes, and can be administered by non-medical personnel. Inter-rater reliability is high (correlation coefficient r=0.90). It also correlates well with other executive function measures including the Wisconsin Card Sorting Task [217] (r = -0.54), Trail Making Part B (r = 0.64), Lezak’s Tinker Toy test (r = -0.57) and the Test of Sustained Attention (time: r =0.82; errors: r =0.83). EXIT25 scores range from 0 to 50. A score of 10/50 reflects the fifth percentile for young adults. Scores of 15/50 or greater suggest clinically significant executive control impairment [218]. In non-institutionalised older people, a decline in EXIT25 performance has been associated with a decline in activities of daily living [219]. The EXIT25 has been used to assess the cognitive efficacy of several medications in trials of patients with cognitive impairment and dementia [220, 221]. A pressing need has been identified for the inclusion of
executive function outcome measures in clinical dementia trials, given its correlation to an individual’s ability to function independently [215].

**Statistical Analysis**

*A priori* power calculations focused on the primary aim of the study which was to investigate the safety and tolerability of nilvadipine treatment in AD patients. The *a priori* calculations were performed using the G-power software based on the primary outcome of nilvadipine’s effect on isolated BP measurements in hypertensive patients [173]. A sample size of 100 subjects (60 for treatment; 40 for no treatment) was suggested based on Cohen’s d of 0.90, an alpha of 0.05 and a β score of 0.90. *Post-hoc* power calculations examining the cognitive effects in this study (G-power software) suggest a power of 96% for the sample size used in these analyses. Differences in baseline characteristics between control and treated participants were explored using either the student’s t-test or the χ²-statistics. The means and standard deviations were used to summarize symmetric continuous variables. Unadjusted analyses were performed using the student’s t-test on mean differences in cognitive test scores between the baseline visit and the final visit. Mixed linear regression modelling was employed to examine the treatment effects on MMSE and EXIT25 scores, while controlling for potential confounders. All analyses were performed using SPSS 16.0 and the significance level was set at 0.05.
Table 1: Baseline characteristics of the study group

<table>
<thead>
<tr>
<th></th>
<th>No Treatment (n = 30)</th>
<th>Nilvadipine (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>22 (73)</td>
<td>27 (49)*</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>30 (100)</td>
<td>55 (100)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>11 (37)</td>
<td>20 (36)</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>3 (10)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0 (0)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Anti-hypertensive use, n (%)</td>
<td>3 (10)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Cholinesterase inhibitors, n (%)</td>
<td>29 (97)</td>
<td>54 (98)</td>
</tr>
<tr>
<td>APOE ε4 (+) genotypes, n (%)</td>
<td>24 (80)</td>
<td>39 (72)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.20 (8.7)</td>
<td>69.04 (8.3)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.30 (2.7)</td>
<td>16.93 (3.5)*</td>
</tr>
<tr>
<td>Total Cholesterol mmol (SD)</td>
<td>5.2 (1.0)</td>
<td>5.2 (1.0)</td>
</tr>
<tr>
<td>MMSE†</td>
<td>22.60 (3.4)</td>
<td>21.49 (4.1)</td>
</tr>
<tr>
<td>MMSE Recall †</td>
<td>0.50 (0.9)</td>
<td>0.44 (0.8)</td>
</tr>
<tr>
<td>EXIT25†</td>
<td>14.79 (1.59)</td>
<td>16.55 (8.5)</td>
</tr>
<tr>
<td>Daytime SBP (mmHg)</td>
<td>134.17 (2.7)</td>
<td>132.87 (2.4)</td>
</tr>
<tr>
<td>Daytime DBP (mmHg)</td>
<td>76.34 (1.9)</td>
<td>78.49 (1.3)</td>
</tr>
<tr>
<td>Night-time SBP (mmHg)</td>
<td>121.62 (3.6)</td>
<td>117.95 (2.5)</td>
</tr>
<tr>
<td>Night-time DBP (mmHg)</td>
<td>70.00 (2.3)</td>
<td>69.05 (1.5)</td>
</tr>
</tbody>
</table>

Note: *One participant with the compliance rate of 73% was excluded from the analyses. †Performed at screening, all other tests performed at baseline before medication administration. Recall is a component of Mini-Mental Status Exam (MMSE). ‡For EXIT25, 2 participants were unable to complete this test. *p < 0.05 for education. **APOE genotype was unavailable for one subject.
Results

Baseline characteristics of the study population are presented in table 1. The nilvadipine and control groups were similar with respect to general demographics, cholinesterase inhibitor use, and antihypertensive use and baseline cognitive scores.

However, a significant difference was observed in gender and education between the two groups (Table 1). Educational level was determined by the reported age an individual was when they completed their formal education (range = 13 – 25 years). Because formal education in Ireland was highly variable when this cohort was young, education level was re-coded as a categorical variable (13-14 yrs = 1; 15-16 yrs = 2, 17 or more = 3) to roughly correspond to primary and early post-primary level, post primary secondary level, and third level education. The categorical variable was utilized for subsequent analyses. Both MMSE and EXIT25 scores were correlated with each other at baseline (r = -0.71, p < 0.01) and at visit 5 (r = -0.72, p < 0.01).

In a comparison between baseline and visit 5, in an unadjusted model, no significant effect of treatment was seen on the MMSE (Table 2, Nilvadipine: +0.22 ± 2.8 versus Control: -0.9 ± 2.7, p = 0.08), and the EXIT25 tests (Table 2, Nilvadipine: -1.87 ± 5.6 versus Control: -0.62 ± 3.1, p = 0.21). Differences in the 3-word recall component of the MMSE were significant (Table 2, Nilvadipine: +0.20 ± 1.0 versus Control: -0.23 ± 0.8, p = 0.04). Mean post-treatment scores for the treatment group were: MMSE = 21.7 (± 4.9 SD); EXIT25 = 14.7 (± 7.5 SD). For control post-treatment scores were: MMSE = 21.7 (± 4.1 SD); EXIT25 = 14.2 (± 6.0 SD).
Table 2: Change in Nilvadipine group compared to the no treatment group.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No Treatment</th>
<th>Nilvadipine 8mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 30)</td>
<td>(n = 55)*</td>
</tr>
<tr>
<td>Mean difference ± SD</td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td></td>
<td>t-test</td>
<td>p value</td>
</tr>
<tr>
<td>MMSE①</td>
<td>-0.90 (2.7)</td>
<td>0.22 (2.8)</td>
</tr>
<tr>
<td>MMSE Recall</td>
<td>-0.23 (0.8)</td>
<td>0.20 (1.0)</td>
</tr>
<tr>
<td>EXIT25①</td>
<td>-0.62 (3.1)</td>
<td>-1.87 (5.6)</td>
</tr>
</tbody>
</table>

Note: Mean difference is calculated by subtracting visit 5 of the treatment phase from the screening visit where the negative score indicates decline and positive score indicates improvement. ①Mean difference for EXIT25 was calculated by subtracting visit 5 on the treatment phase from the baseline visit where a negative score indicates improvement. Analyses adjusted for age, gender, education, presence of APOE ε4 allele and use of anti-hypertensives.

We subsequently analyzed the MMSE and EXIT25 data using a mixed linear model to include age, gender, education, APOE, and use of other anti-hypertensive medications [e.g. ACE inhibitors (n = 2), Angiotensin II receptor blockers (n = 3) or thiazide diuretics (n = 3)], as covariates in order to adjust for their potentially confounding effects on cognitive outcome. After correction for these factors, a significant impact of study intervention was observed on MMSE scores (Table 2, F = 8.67, p < 0.01) and EXIT25 scores (Table 2, F = 8.77, p < 0.01). Furthermore a significant interaction between APOE ε4 status and the study intervention was also observed for MMSE (Table 3, F = 9.29, p < 0.01). A statistically marginal interaction between APOE ε4 allele presence and treatment was also observed on the EXIT25 (Table 3, F = 3.82, p = 0.05). Subsequent exploratory testing in
APOE stratified data revealed cognitive stabilization and improvement in executive function in APOE non-ε4 carriers but APOE ε4 carriers did not improve (Table 3).

Given the antihypertensive properties of nilvadipine, we also investigated whether the cognitive benefit was related to hypertension status in this cohort. Therefore, in a second model, we determined whether baseline hypertension status had any impact on the relationship between treatment and cognitive outcomes.

| Table 3: Change in cognitive measures by presence of APOE ε4 allele among the nilvadipine and untreated controls |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Characteristics                | APOE ε4-                        | APOE ε4+                        | APOE ε4-                        | APOE ε4+                        |
|                                | Nilvadipine (n = 15)            | No treatment (n = 39)           | Nilvadipine (n = 6)             | No treatment (n = 24)           |
| Mean difference ± SD           |                                |                                |                                |                                |
| MMSE                           | 0.67 ± 3.58*                   | 0.08 ± 2.56                    | 0.50 ± 1.97                    | -1.30 ± 2.87                   |
| MMSE Recall                    | 0.53 ± 1.25                    | 0.08 ± 0.84                    | -0.50 ± 0.84                   | -0.17 ± 0.76                   |
| EXIT25                         | -3.38 ± 6.78*                  | -1.35 ± 5.46                   | 0.33 ± 1.97                    | -1.04 ± 3.26                   |

Note: *Interactive terms significant at p < 0.05 in mixed linear model for EXIT25 and for MMSE.

Participants were categorized by the presence or absence of hypertension at baseline (as measured by mean “daytime” ABP measurements outlined above, n = 77). There was no significant difference between the treatment and the control group due to the presence of hypertension at baseline (Table 1). While adjusting for potential confounders as described above, hypertension and the interactive term for hypertension...
and treatment were also included in this second model. No impact of hypertension or the
interactive term for treatment and hypertension was found for MMSE ($F = 1.64, p = 0.20$
and $F = 1.29, p = 0.26$, respectively) or EXIT25 scores ($F = 2.01, p = 0.16$ and $F = 0.10, p =$
0.75, respectively) over the duration of the trial. In this model, the main treatment effect
for MMSE ($F = 4.29, p = 0.04$) and for EXIT25 ($F = 5.58, p = 0.02$) remained significant. In
addition, the interactive term for treatment and APOE ε4 status remained significant for
MMSE ($F = 5.57, p = 0.02$); however for EXIT25, this term became marginally insignificant
($F = 3.14, p = 0.08$).

Discussion

Our findings, similar to Hanyu and colleagues [132, 133], indicate that nilvadipine
has modifying effects on cognition. In this open label study, patients treated with
nilvadipine scored significantly better than the untreated control group over the trial
period. Subjects who were not treated, on average declined on the MMSE total score and
3-word delay recall score. Furthermore, on the EXIT25 treated subjects tended to exhibit
less dysexecutive signs over the course of treatment, while untreated controls did not
change. As this was an open-label and unblinded trial, we further adjusted our analyses
for potential confounding factors known to be associated with AD pathogenesis and
cognitive outcomes, and the overall findings remained significant. Also, consistent with
other reports we found that APOE ε4 non-carriers demonstrated larger treatment gains
over APOE ε4 carriers [187] [375, 376]. Furthermore, within this study interval, the
untreated individuals who were APOE ε4 carriers experienced a higher rate of cognitive
decline than the APOE ε4 non-carriers.
In the Hanyu study a comparative anti-hypertensive (amlodipine) was administered to the control group demonstrating that treatment of hypertension per se was not responsible for the favourable cognitive outcome [133]. Similarly in this study I found no significant interaction between treatment and the presence of baseline hypertension. I have previously reported that non-hypertensive patients in this study had little or no change in their overall BP, while hypertensive patients had a modest reduction. I also reported a high incidence of baseline orthostatic hypotension in this group however; there was no significant increase in the drop in BP or orthostatic symptoms in response to nilvadipine treatment. Overall the medication was well tolerated, with no patient requiring or requesting discontinuation [186].

Functional imaging techniques, such as Positron Emission Tomography (PET) or Single Photon Emission Tomography (SPECT), have demonstrated specific regional abnormalities in brain perfusion in patients with established AD. Nilvadipine increases cerebral blood flow (CBF) and oxygen metabolism in the ischemic brain regions of patients affected with both hypertension and cerebral artery occlusion [222, 223]. Experimental evidence for the potential use of nilvadipine in AD treatment is suggested by a study showing improvement in CBF in a transgenic mouse model of AD. This effect may be attributed to nilvadipine’s ability to oppose β-amyloid (Aβ) induced vasoconstriction in isolated vessels [134]. Similarly, in rats subjected to cerebral occlusion and Aβ treatment, nilvadipine prevented cognitive impairment and neuronal apoptosis [224]. As discussed earlier, several small clinical studies and case reports have observed that treatment of hypertensive patients with nilvadipine resulted in improved cognitive outcomes and cerebral perfusion [132, 133, 184, 185]. CBF reduction and
hypometabolism correlate positively with cognitive decline in AD patients [225-227]. Although the exact mechanism behind this remains to be determined, it is possible that impaired CBF may impede the delivery of nutrients and oxygen to the brain resulting in neuronal damage and subsequently neurodegeneration [228]. In the current study, MMSE scores remained essentially unchanged in the treatment group while the control group declined and the scores on EXIT25 modestly improved in the treatment group and the control essentially remained unchanged. As other reports have shown a relationship between global cognitive and executive function changes and CBF [229, 230], the cognitive stabilization in this study may be due to a general improvement in CBF. Collectively, these findings suggest that restoration of blood flow to the brain by nilvadipine may partly explain the observed changes in cognition, although further clinical studies are required to explore this hypothesis.

The observation of an interaction between APOE and treatment on cognitive outcome is consistent with the findings from other clinical trials, including the recent bapineuzumab phase II study, showing a lack of therapeutic response in APOE ε4 carriers compared to APOE non-carriers [187, 231, 232, 375, 376]. The fact that I observe an APOE dependent treatment effect on cognitive and functional outcomes is in agreement with these larger randomized double-blinded studies and provides external validation of the results, despite the unblinded nature of the overall study design. This is particularly so because the genotype data results were not known to either the participants, their families nor the medical staff carrying out the study which therefore argues against a placebo effect in the treatment group or systematic bias by the investigators. It is unlikely these differences are due to coexistent vascular disease, given the overall low burden of
vascular risk factors in this population, and all participants had neuro-imaging consistent with AD (Table 1). I did not find a significant dose response relationship between APOE ε4 genotype and cognitive scores, but our findings in this regard were limited by the small numbers of homozygous APOE ε4 carriers in the control group. Given that APOE-genotype dependent treatment effects have been reported with other compounds (i.e., Tacrine, Glitazones, and a monoclonal antibodies), and now nilvadipine, it is unlikely that this response is entirely treatment related, as all these treatments have different mechanisms of action. It is far more likely that APOE genotype heterogeneity in individuals dictates this variable response to treatment and that there is a difference between APOE ε4 positive dementia, and APOE ε4 non-carrier dementia. Interestingly, previous SPECT studies have identified that APOE ε4 carriers with AD have lower regional cerebral perfusion than APOE ε4 non-carriers [233]. Recent studies have reported that APOE carrier status was associated with early and late cognitive response to cholinesterase inhibitor treatment in mild AD (Mini-Mental State Examination (MMSE) ≥/21) (P<0.01) [377]. Interestingly, this group also reported that in moderate-to-severe AD (MMSE </15), presence of the (Butyrylcholinesterase) BCHE-K genotype variant was associated with late response to cholinesterase inhibitor treatment (P=0.02). Future studies with nilvadipine and other treatments for AD patients need to focus on the apparent APOE and other genotype dependent responses to treatment. It may be that certain genotype groups such as APOE ε4 carriers require higher doses or, longer treatment periods to examine therapeutic potential.

While this is a preliminary study, these findings suggest that nilvadipine may provide cognitive improvement in AD patients and a double-blinded randomized placebo
controlled trial is required to fully explore the potential therapeutic benefits of nilvadipine as a new therapy for AD.

**Summary: Main cognitive outcomes**

1. A signal suggesting cognitive **stabilisation** in treated versus untreated over 6 weeks
2. APOE ε4 non-carriers appear to have greater benefit
3. **No association** between baseline BP values and cognition
4. **No association** between changes in BP and changes in cognition following treatment
Chapter 5: The relationship between cerebral haemodynamics and APOE ε4 allele expression in patients with Alzheimer’s disease- a transcranial doppler study

Abstract

Background: A complex relationship exists between cerebral perfusion and Alzheimer’s disease. Cerebral hypoperfusion is associated with AD and in many occasions it pre-dates the onset of clinical symptoms. However there is evidence that some patients with AD have a relative hyperperfusion early on in the disease. APOE is a significant risk factor for AD, and APOE genotype may influence cerebral perfusion.

Objectives: The aim of this study was to investigate the effects of APOE ε4 genotyping on cerebral haemodynamics in patients with AD.

Methods: Forty-two of sixty-two patients (67.7%) with AD (MMSE 23.1 ± 3.4; age 71.5 ± 8.3 years) were recruited from a tertiary referral memory clinic. Transcranial colour-coded doppler (TCD) was performed to assess blood flow velocities (Peak systolic Velocity, Vs; Mean systolic velocity, Vmean; and End diastolic velocity, Vdia) and the resistance (RI) and pulsatility (PI) indices in the middle cerebral artery (MCA). Blood samples were collected for APOE genotyping. Patients were sub-grouped according to their number of APOE ε4 alleles. The TCD operator was blinded to patients APOE ε4 status.

Results: Thirty- three patients (78.5%) had at least one APOE ε4 allele. There was a dose response effect between APOE ε4 carrier status and cerebral haemodynamics. Patients with two ε4 alleles had significantly greater perfusion velocities than individuals with one or no ε4 alleles. Patients with two ε4 alleles also had lower resistance indices (RI: ε4 ++ 0.58 (0.05) versus ε4 +/− 0.65 (0.08) and ε4 −/− 0.70 (0.07); F= 5.15, p=0.01).
**Conclusion:** APOE ε4 homozygous patients appear to have greater middle cerebral artery velocities than APOE ε4 non-carriers. This may represent an attempted compensation for hypo-perfusion elsewhere in the brain. This relationship between APOE ε4 expression and cerebral perfusion merits further investigation as it may have treatment implications in the future.

**Introduction**

Clinical, pathological and genetic studies suggest that Alzheimer’s disease (AD) is a heterogeneous entity [234-236]. The ε4 allele of apolipoprotein E (APOE) is a significant risk factor for AD, and accelerates the onset of dementia [237, 238]. The APOE gene, located on chromosome 19, has three major alleles: ε2, ε3, and ε4. APOE is present in senile plaques, neurofibrillary tangles, and cerebrovascular amyloid, the major neuropathological changes seen in AD. APOE gene is a “susceptibility gene”. While homozygosity for APOE ε4 alleles increases ones risk of developing AD from 20 to 90% [237], carrying an APOE ε2 allele seems to reduce the risk [239]. The exact mechanism of how APOE genotype influences AD pathogenesis is still a subject of intense research. APOE is a β-amyloid chaperone protein, and APOE ε4 expression is associated with impaired transport of β-amyloid across the blood brain barrier (BBB) [240, 241]. This slower clearance results in cerebral β-amyloid accumulation, which is a pathological hallmark of AD. In addition, the extent of the deficit of acetylcholine containing neurons in brains of patients with AD is related to the number of ε4 alleles present [242, 243]. Interestingly, the distribution of cerebrovascular amyloid in AD varies with APOE
genotype and specifically, an increasing dose of APOE ε4 alleles has been associated with increased cerebral amyloid angiopathy [244].

The association between cerebral hypoperfusion and AD is well established [245, 246]. Functional imaging techniques, such as Positron Emission Tomography (PET) or Single Photon Emission Tomography (SPECT), have demonstrated specific regional abnormalities in brain perfusion in patients with established AD. Most consistently decreased perfusion in the temporoparietal cortex is observed [247-251]. These perfusion deficits often exist before overt clinical symptoms of dementia are evident, emphasising the possible role of impaired cerebral perfusion in the overall pathogenesis of AD [252, 253]. These perfusion abnormalities also correlate accurately with the extent of AD pathology identified at autopsy [254].

A previous SPECT study on patients with AD performed in 1996, suggested that APOE ε4 allele carriers appear to have more diminished regional perfusion than non-carriers [233, 255]. APOE ε4 status also appears to influence perfusion and cerebral velocities in non-demented "at risk" individuals [256], and patients with mild cognitive impairment [257]. Previous studies have highlighted the usefulness of TCD in identifying altered cerebral haemodynamics in patients with AD versus controls [258], and even for distinguishing AD from vascular dementia [259], however no study to date has investigated the cerebrovascular dynamics of APOE ε4 carriers and non-carriers with AD. The purpose of this study is to examine for differences in the cerebrovascular velocities and resistance and pulsatility indices in AD patients with or without APOE ε4 alleles.
Participants and methodology

The Irish Medicines Board (IMB) and the Federated Hospitals Ethics Committee approved this study. Details on the inclusion and exclusion criteria have been discussed elsewhere. The primary study commenced in October 2006 and the transcranial Doppler study commenced in May 2007. APOE genotyping has been discussed elsewhere (Chapter 3 and 4). Study investigators performing blood pressure and TCD measurements were blinded to patients APOE ε4 status.

Transcranial Doppler Ultrasonography

Imaging TCD ultrasonography studies were performed at baseline using an HDI 5000 sonographic scanner (Philips Medical Systems, Bothell, Washington) equipped with a 2.0 MHz 90° phased-array probe for both B-mode and Doppler imaging (Figure 1).
The same sonographer (SK) performed all measurements in a quiet, controlled environment, with the patients resting supine with their eyes closed, for at least 10 minutes prior to the examination. This eliminated any inter-observer variation that can occur with this methodology [260]. Recordings were performed at the same time of the day at baseline and follow up, and patients were instructed to avoid heavy exercise and caffeinated or alcoholic beverages for the twenty-four hours prior to examination. The right middle cerebral artery (MCA) was identified through the temporal acoustic window in an oblique axial plane with colour Doppler ultrasonography [261] (Figure 2).

The sampling volume of the pulsed Doppler system was set at 3mm-wide, and was...
placed in the MCA at a depth of 45-60 mm. The angle of insonation was determined by placing a linear marker provided by the scanner software, under visual guidance, on the colour image of the artery being studied, and fitting its direction along the long axis of the vessel. This allowed for angle-corrected measurement of CBFV, which is more reliable than uncorrected measurements [262, 263]. In the absence of an acoustic temporal window on the right side, the left temporal window was used to perform the same protocol on the left MCA. Blood flow velocity measured at the MCA has a high correlation coefficient (0.995) with invasive blood flow measurements and a high reliability in older patients [264]. Recordings were taken for at least one minute and recorded onto DVD for analysis later. The peak systolic velocity (Vsys), end diastolic velocity (Vdia), and mean systolic velocity (Vmean) were determined as an average over the number of total recorded cardiac cycles. Traditionally these measures are used to describe pressure, flow, and flow velocity in the arterial system. Of these values Vmean carries the greatest physiological significance because it depends less on central cardiovascular factors such as heart rate, contractility, total peripheral resistance and aortic incompetence, than do Vsys or Vdia. The Vmean correlates better with overall perfusion than the peak and trough values [265]. Middle cerebral artery Vmean in healthy adults ranges from 35-90 cm/sec under normal physiological conditions (Figure 3) [266]. The values of cerebral blood flow velocities decrease with age, with an average decrease of 20% described from healthy adults aged 30, to similar adults aged 80 years [267].
The pulsatility index (PI), and resistance index (RI) were automatically calculated and also were averaged over the total recorded cardiac cycles. The pulsatility index is a measure of the variability of blood velocity in a vessel and is defined as $\text{PI} = \frac{\text{Vsys} - \text{Vdia}}{\text{Vmean}}$ [268]. The resistance index was determined as described by Pourcelot as $\text{RI} = \frac{\text{Vsys} - \text{Vdia}}{\text{Vsys}}$ [269]. Originally PI and RI were regarded as measures of distal vascular resistance or impedance [270], however they are also a function of cerebral perfusion pressure, heart rate and pulsatility of arterial pressure as well [271].

The main limitation of TCD is that it measures blood flow velocity instead of actual blood flow volume. Therefore under conditions of increased flow velocity it is unable to distinguish between decreased or increased CBF, e.g. vascular constriction or hyperperfusion [272, 273] (Figure 4).
Figure 4: Vasoconstriction/ Stenosis of MCA; Doppler measures flow velocities along a vasoconstricted artery. Even if overall cerebral blood flow rates are lower, given the narrowing in blood vessel diameter it is like the velocities will be higher. Velocities may not reflect overall cerebral perfusion.

Similarly, if a vessel is vasodilated and has an increase in vessel diameter, then despite an increase in cerebral blood flow volume, the velocities may be lower than expected (Figure 5).

Figure 5: MCA vasodilated: Doppler measures flow velocity along a correspondingly dilated artery with increased vessel diameter. Even if overall cerebral blood flow is increased velocities may only remain similar to steady state velocities or may be lower if the rate of increase in cerebral blood flow hasn’t matched the increase in vessel diameter. Velocities may not reflect overall cerebral perfusion.
To date there is no widely accepted, reliable method of assessing vessel diameter using TCD. A technical limitation of TCD is the absence of adequate acoustic windows. Inadequate windows are more common in women and older subjects [274].

**Statistical Analysis**

Baseline characteristics were tested for normality of distribution. The non-parametric Mann Whitney test was used for the analysis of differences in asymmetric MMSE-recall scores, and education. Symmetric quantitative values were compared using the students t-test. We used the Students t-test and analysis of variance (ANOVA) to detect differences in means of TCD values in different APOE ε4 groups. For qualitative data the Pearson’s Chi square test was used if the cell size requirement for the contingency tables was met, otherwise the Fisher’s Exact test was used. All statistical analyses were performed using the SPSS 16.0 statistical package. A p value of less than 0.05 was considered statistically significant.

**Results**

Transcranial Doppler studies were performed successfully at baseline and follow up in 42/62 subjects (67.7 %). Transcranial studies were not possible in 20 participants due to the absence of a trans-temporal window (Figure 4).
All patients without an acoustic window were female, slightly younger, (see table 1).

Within the group who had TCD performed successfully 33/42 (78.6%) had at least one APOE ε4 allele.
Table 1: Characteristics of participants without an acoustic window

<table>
<thead>
<tr>
<th></th>
<th>Subjects with acoustic window (n=42)</th>
<th>Subjects without acoustic window (n=20)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline, years (SD)</td>
<td>71.5 (8.3)</td>
<td>67.3 (6.8)</td>
<td>0.05</td>
</tr>
<tr>
<td>Female (%)</td>
<td>16 (38)</td>
<td>20 (100)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MMSE (SD)</td>
<td>23.1 (3.4)</td>
<td>22.4 (3.6)</td>
<td>0.42</td>
</tr>
<tr>
<td>Baseline SBP mmHg (SD)*</td>
<td>144.6 (18.5)</td>
<td>145.7 (19.5)</td>
<td>0.82</td>
</tr>
<tr>
<td>Baseline DBP mmHg (SD)*</td>
<td>80.0 (7.1)</td>
<td>81.4 (7.7)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* automated sphygmomanometer values

There were no significant differences in the baseline demographics of APOE ε4 carriers and non-carriers (Table 2).
# Table 2: Baseline demographics of APOE ε4 carriers and non-carriers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>APOE ε4 + (n=33)</th>
<th>APOE ε4 - (n=9)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>70.9 (8.7)</td>
<td>73.8 (6.6)</td>
<td>0.37</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>11 (33.3)</td>
<td>5 (55.5)</td>
<td>0.27</td>
</tr>
<tr>
<td>MMSE (SD)</td>
<td>22.9 (3.4)</td>
<td>24.0 (3.2)</td>
<td>0.38</td>
</tr>
<tr>
<td>MMSE-recall (SD)</td>
<td>0.27 (0.6)</td>
<td>0.78 (1.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>EXIT-25 (SD)</td>
<td>14.2 (8.3)</td>
<td>12.4 (2.6)</td>
<td>0.20</td>
</tr>
<tr>
<td>Education, years (SD)</td>
<td>16.2 (3.6)</td>
<td>17.6 (4.0)</td>
<td>0.35</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>2 (6.0)</td>
<td>1 (11.1)</td>
<td>0.53</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol, mmol (SD) (3.0-5.2)</td>
<td>5.1 (1.1)</td>
<td>5.1 (1.0)</td>
<td>0.99</td>
</tr>
<tr>
<td>LDL, mmol (SD) (2.0-3.36)</td>
<td>2.8 (1.1)</td>
<td>2.5 (1.0)</td>
<td>0.50</td>
</tr>
<tr>
<td>Elevated BP*, n (%)</td>
<td>6 (18.2)</td>
<td>2 (22.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Systolic BP*, mmHg (SD)</td>
<td>127.7 (15.5)</td>
<td>125.1 (15.5)</td>
<td>0.68</td>
</tr>
<tr>
<td>Diastolic BP*, mmHg (SD)</td>
<td>73.8 (10.4)</td>
<td>71.1 (11.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>Antihypertensive use**, n (%)</td>
<td>1 (3.0)</td>
<td>2 (22.2)</td>
<td>0.11</td>
</tr>
<tr>
<td>Cholinesterase inhibitor use, n (%)</td>
<td>32 (97.0)</td>
<td>9 (100)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* 24 Hour ABP monitor; SD Standard Deviation; **Patients on more than one concomitant antihypertensive were excluded from participation

APOE ε4 carriers with AD had statistically significant higher mean systolic (ε4+: 45.7 ± 12.8 cm/sec; versus ε4-: 36.6 ± 9.1 cm/sec, p = 0.05), and end diastolic (ε4+: 27.1 ± 8.8 cm/sec; versus ε4-: 21.9 ± 5.2 cm/sec, p = 0.02).
cm/sec; versus ε4-: 19.5 ± 7.3 cm/sec, p = 0.02) velocities. Patients who were APOE ε4 carriers also had lower resistance (ε4+: 0.63 ± 0.07; versus ε4-: 0.70 ± 0.08, p = 0.02), and pulsatility (ε4+: 1.03 ± 0.2; versus ε4-: 1.22 ± 0.3, p = 0.04) index scores (Table 3).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>APOE ε4 + (n = 33)</th>
<th>APOE ε4 - (n = 9)</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Systolic Velocity cm/sec</td>
<td>45.7 (12.8)</td>
<td>36.6 (9.1)</td>
<td>-1.99</td>
<td>0.05</td>
</tr>
<tr>
<td>Peak Systolic Velocity cm/sec</td>
<td>73.2 (19.4)</td>
<td>63.9 (12.0)</td>
<td>-1.37</td>
<td>0.18</td>
</tr>
<tr>
<td>End Diastolic Velocity cm/sec</td>
<td>27.1 (8.8)</td>
<td>19.5 (7.3)</td>
<td>-2.36</td>
<td>0.02</td>
</tr>
<tr>
<td>Resistance Index</td>
<td>0.63 (0.07)</td>
<td>0.70 (0.08)</td>
<td>2.54</td>
<td>0.02</td>
</tr>
<tr>
<td>Pulsatility Index</td>
<td>1.03 (0.21)</td>
<td>1.22 (0.33)</td>
<td>2.14</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Given this difference we examined the differences in velocities relative to APOE ε4 genotype groups (Table 4). Patients with two APOE ε4 alleles had higher mean and end diastolic velocities, and lower pulsatility and resistance indices than patients with only one or no APOE ε4 allele, indicating a significant dose response relationship between cerebral haemodynamics and APOE genotype.
Table 4: TCD values according to the number of ApoE ε4 alleles

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ε4 ++</th>
<th>ε4 +/-</th>
<th>ε4 --</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 21)</td>
<td>(n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Systolic Velocity cm/sec</td>
<td>50.3</td>
<td>44.8</td>
<td>31.9</td>
<td>3.36</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(14.4)</td>
<td>(12.9)</td>
<td>(6.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Systolic Velocity cm/sec</td>
<td>76.6</td>
<td>72.9</td>
<td>57.0</td>
<td>1.70</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>(21.7)</td>
<td>(20.2)</td>
<td>(10.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End Diastolic Velocity cm/sec</td>
<td>32.1</td>
<td>25.6</td>
<td>17.4</td>
<td>4.71</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(9.4)</td>
<td>(8.6)</td>
<td>(5.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance Index</td>
<td>0.58</td>
<td>0.65</td>
<td>0.70</td>
<td>5.15</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.08)</td>
<td>(0.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatility Index</td>
<td>0.90</td>
<td>1.08</td>
<td>1.28</td>
<td>4.40</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(0.19)</td>
<td>(0.23)</td>
<td>(0.27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Transcranial Doppler studies were successfully performed at baseline and follow up in 42/62 (67.7 %) subjects, which is in keeping with results from previous transcranial Doppler studies in similarly aged populations [103, 275]. As expected, all patients in whom an acoustic window couldn't be identified were female. Overall middle cerebral artery velocities were lower in this patient population (Vmean = 43.8 ± 12.6; Vsys = 71.2 ±
18.3; $V_{dia} = 25.4 \pm 9.0$ cm/sec), compared to reported results from similarly aged non-demented patients ($V_{mean} = 46.9 \pm 5.7$; $V_{sys} = 75.0 \pm 12.0$; $V_{dia} = 30.4 \pm 8.7$ cm/sec) [276]. This is consistent with other clinical studies that have reported decreased MCA velocities in patients with AD [258, 277, 278].

APOE ε4 carriers have significantly higher MCA velocities, and lower RI and PI, when compared to APOE ε4 non-carriers (Table 4). This relationship appears all the more significant given that the number of APOE ε4 alleles appears to influence these haemodynamic parameters in a dose-dependent fashion. Only one previous study group has examined perfusion abnormalities in APOE ε4 carriers and non-carriers with AD (MMSE scores 22.0 ± 4.0) [233, 255]. This SPECT study found that perfusion ratios for patients with AD versus controls were significantly reduced in all regions other than the occipital cortices. On subgroup analysis the perfusion pattern for APOE ε4 homozygote AD patients did not differ from the control group in the frontal and right temporal cortices, whereas patients with one or no ε4 alleles had significantly reduced perfusion in these regions compared to the control group [255]. The APOE ε4 homozygous AD patients had the greatest reduction in perfusion overall on longitudinal follow up (3 years) [233]. Middle cerebral artery velocities as measured in this TCD study are considered representative of cerebral blood flow, although TCD as an imaging modality only gives information on blood flow velocities rather than blood flow volume [264]. The combination of increased flow velocities and reduced resistance and pulsatility indices does suggest increased blood flow. It’s possible the cerebrovascular haemodynamic changes detected in this current study and the perfusion variations observed by Lehtovirta et al, indicate an early compensatory regional hyperperfusion in APOE ε4
positive AD patients, which as the disease progresses diminish rapidly. Previous PET [279] and arterial spin-labelling MRI [280] studies have also proposed an increase in hippocampal rCBF (regional cerebral blood flow) in very mild AD which subsides with disease progression, although these studies did not specifically examine for genotype dependent effects. This hyperperfusion appears even more significant following adjustment for hippocampal atrophy [280]. These changes may be a response to localised regions of under-perfusion elsewhere in the brain and may be driven by the vasoactive effect of β-amyloid deposition in the brain. APOE ε4 allele homozygosity is associated with increased β-amyloid deposition in the brain [281], and prior studies have shown that the application of exogenous β-amyloid to normal blood vessels ex vivo causes endothelium-dependent vasoconstriction [105, 282]. Therefore the differences in cerebral haemodynamics seen in this study may be due to local effects of previously documented variations in cerebral β-amyloid deposition in APOE ε4 carriers and non-carriers [283].

Some of these effects also appear to extend to non-demented “at-risk” groups. A functional MRI study demonstrated that non-demented individuals with at least one APOE ε4 allele and a family history of AD had higher resting temporal lobe perfusion than APOE ε4 non-carriers, but despite this increased resting perfusion, APOE ε4 carriers had comparably lesser increases in perfusion in response to cognitive tasks [284]. A recent PET study examining changes in regional CBF in non-demented APOE ε4 carriers and non-carriers longitudinally over 8 years, also reported higher baseline values in the APOE ε4 carrier group, however, this group also had the greatest decline in rCBF over time [256].
It is unlikely the differences between APOE ε4 carriers and non carriers in this study are as result of coexistent vascular disease in either group, as all recruited patients had neuro-imaging consistent with a diagnosis of AD, and in general they had a very low burden of vascular risk factors (Table 2). APOE ε4 homozygosity is an independent vascular risk factor in its own right, and is associated with an increased risk of stroke, atherosclerosis and ischaemic heart disease [285], primarily through association with elevated total cholesterol and low density lipid (LDL) protein levels [286], but this was not the case in this group. The validity of these findings is strengthened in that the operator performing the TCD studies was completely blinded to the APOE status of the patients until after the study.

APOE is not the only gene which confers a risk for AD. Genome-wide association studies have identified the CLU gene, which encodes for clusterin, as a genetic locus involved in Alzheimer disease (AD) [378]. The protein clusterin, also known as apolipoprotein J, appears to be involved in the pathogenesis of AD [379]. Clusterin has been found in the frontal cortex and hippocampus of postmortem AD brains, and is increased in the cerebrospinal fluid of patients with AD [380, 381]. Plasma clusterin was reported to be associated with brain atrophy, baseline disease severity, and rapid clinical progression in AD, suggesting its possible use as a biomarker of AD [382]. However, a recent population-based cohort study reported that plasma levels of clusterin were associated with the prevalence and severity of AD, but not with the development of incident AD during follow-up thereby limiting its usefulness as a biomarker [383]. There was a similar association between clusterin levels and both vascular and all-cause dementia suggesting the increased expression of clusterin in AD and dementia may
reflect a neuroprotective response [379]. Several protective effects of clusterin on the
brain that may play a role in AD have been described in in vitro or in vivo studies. These
include inhibition of amyloid formation through binding amyloid-beta [384], or enhancing
its clearance over the blood-brain barrier [385], clearance by endocytosis of amyloid-beta
aggregates and cell debris to brain phagocytes, and inhibition of complement activation
[379]. The neurodegenerative changes that occur in AD may trigger an increased
expression of clusterin [379]. Furthermore, APOE and CLU have been shown to cooperate
in suppressing Aβ deposition [386] and APOE and CLU may critically modify Aβ clearance
at the blood brain barrier acting as chaperone proteins, suggesting a role for clusterin in
the amyloidogenic pathway [385]. Levels of APOE protein appear to be inversely
proportional to APOE-ε4 allele dose levels, i.e. expression levels are reduced in ε4
homozygotes compared with heterozygotes. Conversely, CLU levels are increased in
proportion to APOE-ε4 allele dose suggesting an induction of clusterin in individuals with
low APOE levels [387]. This further underlines the interplay of these genetic risks for AD,
and it would be useful to measure plasma clusterin levels in future trials.

This study once again highlights how apolipoprotein E polymorphism is a major
contributor to the heterogeneity of AD. Much recent attention has focused on the
interplay between vascular disease and AD, and several studies have identified regional
hyperperfusion in early AD. No previous study has identified the association between
APOE ε4 expression and this hyperperfusion. Future therapeutic strategies may focus on
augmenting or improving cerebral perfusion in the pre-clinical and early stages of AD.
This APOE ε4 dependent effect on cerebral haemodynamics needs further exploration to
understand the biological pathways linking APOE allele expression and changes in
cerebral perfusion. We only examined cerebral haemodynamics in the middle cerebral artery; future studies should examine how these APOE-dependent perfusion differences evolve over time, and use TCD or other neuro-imaging techniques to investigate global cerebral blood flow patterns in patients with AD.
Chapter 6: The effects of treatment with the antihypertensive nilvadipine on cerebral haemodynamics in patients with AD - a transcranial doppler study

Abstract

Background: Nilvadipine is a dihydropyridine calcium channel blocker (DHP-CCB) antihypertensive that has been shown in previous studies to reduce the rates of progression of cognitive impairment and increase regional cerebral blood flow in patients with mild cognitive impairment and hypertension.

Objective: In this study I use transcranial doppler (TCD) to assess the cerebrovascular impact of treatment with nilvadipine in patients with Alzheimer's disease (AD).

Methods: AD patients in the intervention group (n= 25) received 8mg Nilvadipine daily, compared to the control group (n=17) who received no treatment. Cerebral haemodynamics were measured at baseline and after 6 weeks treatment with nilvadipine using a Philips HDI 5000 colour duplex transcranial doppler. Peak (Vsys) and mean (Vmean) systolic, and end diastolic (Vdia) velocities were measured as well as the resistance and pulsatility indices, over at least one minute in the right or left middle cerebral artery (MCA) via a transtemporal window.

Results: Forty-two of sixty-two (67.7%) screened patients had transtemporal windows suitable for TCD analysis. More patients without a window were female and were younger. There were proportionately more women in the treatment group, but otherwise both groups were matched. There were no significant differences in baseline TCD values. Over six weeks there were no significant differences in the change of Vmean (Nilvadipine -0.13 ± 7.9 versus Control +0.35 ± 6.3; p = 0.84), Vsys (Nilvadipine +1.76 ±
10.9 versus Control +0.52 ± 8.9; p = 0.70), or Vdia (Nilvadipine -0.42 ± 5.7 versus Control +0.88 ± 4.7; p = 0.44). Similarly there was no differences in pulsatility (Nilvadipine +0.07 ± 0.25 versus Control -0.01 ± 0.13; p = 0.23), or resistance (Nilvadipine +0.02 ± 0.06 versus Control -0.01 ± 0.03; p = 0.09) indices. This remained the case following adjustment for potential confounders.

**Conclusion:** I found no clear impact of nilvadipine treatment on cerebral haemodynamic measures on TCD. Future studies need to examine the impact of intervention over a longer treatment period and with alternative neuro-imaging techniques.

**Introduction**

There is a consistent relationship between cerebral hypoperfusion and AD [245, 246], which may even exist before overt clinical symptoms of dementia is evident, emphasising its potential role in the overall pathogenesis of AD [252, 253]. As discussed in previous chapters nilvadipine is a lipophillic DHP-CCB that crosses the blood-brain-barrier (BBB) with ease, allowing it to exert local effects on cerebral blood flow [173, 287]. Several pre-clinical studies have demonstrated that it restores cerebral perfusion following ischaemic insults [288-296]. This has also been supported in clinical trials [222, 223], and these effects are not evident with other DHP-CCB's [297, 298]. Further studies in AD transgenic mice models demonstrate that nilvadipine antagonizes β-amyloid (Aβ) mediated cerebral vasoconstriction, and improves the associated hypoperfusion [134]. Hanyu and colleagues reported that 12-16 weeks treatment with nilvadipine, but not amlodipine, improved cognitive function and cerebral perfusion, especially in the left
frontal lobe of hypertensive patients with amnestic mild cognitive impairment (aMCI) [132]. In two recent case reports Nilvadipine treatment for 3 and 6 months respectively, improved cognitive performance, and increased rCBF in two patients with hypertension and AD [184, 185]. All the above studies demonstrate the ability of nilvadipine to increase cerebral blood flow to hypoperfused areas of the brain.

Given the association between the development of AD and reduced blood flow to the brain, one would be hopeful that medications that increase cerebral perfusion might be of benefit in treating AD patients. In this study I investigate the effects of 6 weeks treatment with nilvadipine on cerebral haemodynamics in patients with AD, using colour coded transcranial doppler (TCD).

**Participants and methods**

As per all aspects of the study, approval was granted by the Irish Medicines Board (IMB) and the Federated Hospitals Ethics Committee. Details on the inclusion and exclusion criteria have been discussed elsewhere. The primary study commenced in October 2006 and the TCD study commenced in May 2007.

Trial participants were assigned to nilvadipine (Nilvadil®) 8mg CR once daily or no treatment in a 2:1 ratio. The methodology for monitoring blood pressure changes, measuring cognitive outcomes, and APOE genotyping has been discussed elsewhere (Chapter 3, 4 and 5). The study investigators performing blood pressure and TCD measurements were blinded to patients APOE ε4 status.
Transcranial Doppler Ultrasonography:

Imaging TCD ultrasonography studies were performed at baseline (Visit 2) and six weeks later (Visit 5) using an HDI 5000 sonographic scanner (Philips Medical Systems, Bothell, Washington) equipped with a 2.0 MHz 90° phased-array probe for both B-mode and Doppler imaging. The same sonographer (SK) performed all measurements in a quiet, controlled environment, with the patients resting supine with their eyes closed, for at least 10 minutes prior to the examination. This eliminated any inter-observer variation that can occur with this methodology [260]. Recordings were performed at the same time of the day at baseline and follow up, and patients were instructed to avoid heavy exercise and caffeinated or alcoholic beverages for the twenty-four hours prior to examination. The right middle cerebral artery (MCA) was identified through the temporal acoustic window in an oblique axial plane with colour Doppler ultrasonography [261]. The sampling volume of the pulsed Doppler system was set at 3mm-wide, and was placed in the MCA at a depth of 45-60 mm. The angle of insonation was determined by placing a linear marker provided by the scanner software, under visual guidance, on the colour image of the artery being studied, and fitting its direction along the long axis of the vessel. This allowed for angle-corrected measurement of CBFV, which is more reliable than uncorrected measurements [262, 263]. The exact depth and angle of insonation were recorded, to ensure the same segment of the MCA was studied under identical conditions at baseline and follow-up. In the absence of an acoustic temporal window on the right side, the left temporal window was used to perform the same protocol on the left MCA. Recordings were taken for at least one minute and recorded onto DVD for analysis later. The peak systolic velocity (Vsys), end diastolic velocity (Vdia), and mean systolic velocity
(Vmean) were determined as an average over the number of total recorded cardiac cycles. The pulsatility index (PI), and resistance index (RI) were automatically calculated and also were averaged over the total recorded cardiac cycles. The pulsatility index is a measure of the variability of blood velocity in a vessel and is defined as PI= (Vsys-Vdia)/Vmean [268]. The resistance index was determined as described by Pourcelot as RI= (Vsys-Vdia)/Vsys [269]. The reasons for selecting these measurements and the limitations of TCD have been discussed previously in chapter 5.

**Statistical Analysis**

Baseline characteristics were tested for normality of distribution. The non-parametric Mann Whitney test was used for the analysis of differences in asymmetric MMSE-recall scores, and education. Symmetric quantitative values, such as BP measurements, age, MMSE and EXIT-25 scores, and baseline TCD values were compared using the students T-test. Unadjusted analyses were performed using the students T-test. Adjusted analysis was performed using ANOVA. For qualitative data the Pearson’s Chi square test was used if the cell size requirement for the contingency tables was met, otherwise the Fisher’s Exact test was used. All statistical analyses were performed using the SPSS 16.0 statistical package. A p value of less than 0.05 was considered statistically significant.
**Results**

Transcranial Doppler studies were performed successfully at baseline and follow up in 42/62 (67.7 %) subjects (Figure 1).

Transcranial studies were not possible in 20 participants due to the absence of a trans-temporal window. All patients without an acoustic window were female, slightly younger, (Table 1), and were spread across both treatment and control groups (n=13 no treatment group, n=7 nilvadipine group).
### Table 1: Characteristics of participants with versus without an acoustic window

<table>
<thead>
<tr>
<th></th>
<th>Subjects with acoustic window (n=42)</th>
<th>Subjects without acoustic window (n=20)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline, years (SD)</td>
<td>71.5 (8.3)</td>
<td>67.3 (6.8)</td>
<td>0.05</td>
</tr>
<tr>
<td>Female (%)</td>
<td>16 (38)</td>
<td>20 (100)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MMSE (SD)</td>
<td>23.1 (3.4)</td>
<td>22.4 (3.6)</td>
<td>0.42</td>
</tr>
<tr>
<td>Years of education (SD)</td>
<td>16.5 (3.7)</td>
<td>15.5 (2.2)</td>
<td>0.82</td>
</tr>
<tr>
<td>Baseline SBP mmHg (SD)*</td>
<td>144.6 (18.5)</td>
<td>145.7 (19.5)</td>
<td>0.82</td>
</tr>
<tr>
<td>Baseline DBP mmHg (SD)*</td>
<td>80.0 (7.1)</td>
<td>81.4 (7.7)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Screening (Visit 1) automated sphygmomanometer values

Of the participants who successfully had transcranial Doppler measurements, proportionately more in the treatment group were male, but otherwise the two groups were similar (Table 2).
<table>
<thead>
<tr>
<th></th>
<th>No treatment n=17</th>
<th>Nilvadipine n=25</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>71.9 (8.5)</td>
<td>71.3 (8.4)</td>
<td>0.82</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>11 (64.7)</td>
<td>5 (20)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MMSE (SD)</td>
<td>23.0 (3.2)</td>
<td>23.2 (3.5)</td>
<td>0.85</td>
</tr>
<tr>
<td>MMSE recall (SD)</td>
<td>0.41 (0.8)</td>
<td>0.36 (0.8)</td>
<td>0.83</td>
</tr>
<tr>
<td>EXIT (SD)</td>
<td>15.8 (6.3)</td>
<td>15.0 (8.4)</td>
<td>0.75</td>
</tr>
<tr>
<td>Education, years (SD)</td>
<td>14.8 (2.6)</td>
<td>15.6 (4.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>APOE ε4 allele + (%)</td>
<td>13 (76.5)</td>
<td>20 (80.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Use of antihypertensives at baseline (%)**</td>
<td>1 (6)</td>
<td>2 (8)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cholinesterase Inhibitor use (%)</td>
<td>16 (93.8)</td>
<td>25 (100)</td>
<td>0.41</td>
</tr>
<tr>
<td>Systolic BP mmHg (SD)*</td>
<td>128.9 (17.5)</td>
<td>125.6 (12.5)</td>
<td>0.56</td>
</tr>
<tr>
<td>Diastolic BP mmHg (SD)*</td>
<td>73.4 (13.0)</td>
<td>72.9 (8.7)</td>
<td>0.90</td>
</tr>
<tr>
<td>Elevated Baseline BP (%) *</td>
<td>5 (29.4)</td>
<td>3 (12.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>0.26</td>
</tr>
<tr>
<td>Total Cholesterol mmol (SD)</td>
<td>5.2 (1.2)</td>
<td>4.9 (1.0)</td>
<td>0.22</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
</tbody>
</table>

SD Standard Deviation; ** Patients on more than one concomitant antihypertensive were excluded from participation, * Based on 24 Hour ABP values
There were no statistically significant differences between both groups in their baseline transcranial doppler values, or in the angle of insonation and the number of cardiac cycles used to average the data (Table 3).

<table>
<thead>
<tr>
<th>Table 3: Comparison of baseline transcranial doppler values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>----------------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Mean Systolic Velocity cm/sec (SD)</td>
</tr>
<tr>
<td>Peak Systolic Velocity cm/sec (SD)</td>
</tr>
<tr>
<td>End Diastolic Velocity cm/sec (SD)</td>
</tr>
<tr>
<td>Pulsatility Index (SD)</td>
</tr>
<tr>
<td>Resistance Index (SD)</td>
</tr>
<tr>
<td>Angle of Insonation ° (SD)</td>
</tr>
<tr>
<td>Number of Cycles (SD)</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups on mean systolic (Nilvadipine -0.13 ± 7.9 versus Control +0.35 ± 6.3 cm/sec; p = 0.84), peak systolic (Nilvadipine +1.76 ± 10.9 versus Control +0.52 ± 8.9 cm/sec; p = 0.70), or end diastolic (Nilvadipine -0.42 ± 5.7 versus Control +0.88 ± 4.7 cm/sec; p = 0.44) velocities, over the six weeks. Similarly there was no differences between the two groups in changes in pulsatility (Nilvadipine +0.07 ± 0.25 versus Control -0.01 ± 0.13; p = 0.23), or resistance (Nilvadipine +0.02 ± 0.06 versus Control -0.01 ± 0.03; p = 0.09) indices over the
six weeks. Following adjustment for the potential confounders of age, gender, use of other antihypertensives, blood pressure, and APOE ε4 carrier status there remained no significant differences between the groups (Table 4). There was no significant effect of APOE ε4 status within the model for any of the TCD variables examined.

There was no statistically significant correlation evident between baseline cognitive scores (MMSE, MMSE-recall, EXIT 25) and baseline TCD values. Similarly, there was no statistically significant correlation between the change in scores on cognitive tests, and the change in TCD values in response to treatment with nilvadipine over the trial period.

<table>
<thead>
<tr>
<th>Table 4: Between group changes in transcranial doppler values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean difference ± SD</td>
</tr>
<tr>
<td>Mean Systolic Velocity cm/sec</td>
</tr>
<tr>
<td>Mean difference ± SD</td>
</tr>
<tr>
<td>Peak Systolic Velocity cm/sec</td>
</tr>
<tr>
<td>End Diastolic Velocity cm/sec</td>
</tr>
<tr>
<td>Pulsatility Index</td>
</tr>
<tr>
<td>Resistance Index</td>
</tr>
<tr>
<td>Mean difference ± SD</td>
</tr>
<tr>
<td>Resistance Index</td>
</tr>
<tr>
<td>* adjusted for age, gender, use of other antihypertensives, blood pressure and APOE ε4 status</td>
</tr>
</tbody>
</table>
Discussion

Transcranial Doppler studies were successfully performed at baseline and follow up in 42/62 (67.7 %) subjects, which as discussed previously is in keeping with results from previous transcranial Doppler studies in similarly aged populations [103, 275]. There were no statistical differences between the baseline velocity and resistance values in the nilvadipine and no treatment group. Consistent with other clinical studies, overall baseline middle cerebral artery velocities were lower in this group of AD patients (Vmean = 43.8 ± 12.6; Vsys = 71.2 ± 18.3; Vdia = 25.4 ± 9.0 cm/sec), compared to reported results from similarly aged non-demented patients (Vmean = 46.9 ± 5.7; Vsys = 75.0 ± 12.0; Vdia = 30.4 ± 8.7 cm/sec) [258, 276-278].

Unadjusted and adjusted analysis demonstrated no statistically significant differences in the change in blood flow velocities and pulsatility and resistance indices, in the nilvadipine and untreated groups over the six weeks. Previous studies that investigated the effect of nilvadipine treatment on cerebral perfusion, demonstrated improved perfusion following treatment periods of 12-16 weeks [132], 3 months [184], and six months [185]. The treatment duration in this trial may have been too short to demonstrate significant changes in blood flow velocities in either group. These changes in blood flow were identified using SPECT imaging which allows a more global assessment of cerebral perfusion as opposed to being limited to observations of blood flow changes in the temporal region alone.

In the previous chapter I discussed the apparent APOE ε4 dependent effect on cerebral haemodynamics. The vast majority of patients in this group (78.6%) had at least one APOE ε4 allele. All previous clinical trials examining the effects of nilvadipine in
patients with cognitive impairment and dementia were performed in Asian population groups. The APOE ε4 allele is far more common in people of Northern European ancestry than in those of Asian ancestry [299, 300]; therefore it is possible that the subjects investigated in the previous studies with nilvadipine may have had a smaller proportion of APOE ε4 carriers than in our study population. Unfortunately none of the studies specified the APOE status of the participants, but given the apparent effects of APOE ε4 carrier status on cerebral perfusion this is also important to consider when assessing the effect of nilvadipine on cerebral perfusion in this population when compared to other studies. Patients with AD and no APOE ε4 alleles appear to have lower baseline cerebral artery velocities and higher resistance and pulsatility indices versus APOE ε4 carriers. It may be that APOE ε4 non-carriers have the greatest scope for initial improvement in their cerebral blood flow in response to treatment; however we were not powered to identify that. It is worth noting that the APOE ε4 non-carriers demonstrated the greatest cognitive gains over the short trial period (Chapter 4).

Further studies are required to examine the effects of nilvadipine on cerebral perfusion with particular attention to the differences in APOE ε4 carriers and non-carriers. Alternative neuro-imaging techniques such as arterial spin labelling MRI or perfusion CT scans will give more detail on the overall impact of nilvadipine treatment on cerebral perfusion than TCD.
Conclusions from TCD study

- APOE ε4 carriers had significantly higher MSV, EDV, and lower PI and RI = ↑ MCA perfusion !!
- ? How would this progress longitudinally
- Nilvadipine had no apparent effect-? Correct modality or APOE effect, duration of treatment
- No correlation between baseline TCD values and scores on baseline cognitive tests or change in test scores longitudinally
Chapter 7: The effect of treatment with the antihypertensive nilvadipine on cerebrospinal fluid levels of Aβ-40 and Aβ-42 in patients with mild/moderate Alzheimer’s disease

Abstract

Introduction: The use of certain antihypertensive medications appears to reduce the incidence of AD. This effect appears to be independent of their effects on blood pressure. The cerebral accumulation of neuro toxic forms of β-amyloid is a significant step in the pathogenesis of AD.

Objective: The purpose of this study was to examine the effects of treatment with the antihypertensive nilvadipine for six weeks, on cerebrospinal fluid (CSF) levels of Aβ-40 and Aβ-42.

Methods: Twenty-five patients (mean age 68.5 ± 7.6 years) with mild/moderate AD had CSF samples taken at baseline and six-weeks later for measurement of CSF Aβ-40 and Aβ-42 (n=18: nilvadipine 8 mg OD and n=7: no treatment). These participants also had blood pressure monitoring, cognitive testing, and transcranial doppler ultrasonography performed.

Results: There were no significant differences in the baseline characteristics between the two groups including baseline Aβ-40 (Control: 3205.0 ± 2424 pg/ml, versus Nilvadipine: 4567.7 ± 2046 pg/ml, p =0.17), and Aβ-42 (Control: 198.7 ± 124.1 pg/ml, versus Nilvadipine: 168.6 ± 77.9 pg/ml, p=0.57). There were no significant effects of treatment in the overall changes in Aβ-40 (Control: -38.3 ± 444.1 pg/ml, versus Nilvadipine: +12.5 ±
473.9 pg/ml, p=0.81) or Aβ-42 (Control: -19.8 ± 39.6 pg/ml, versus Nilvadipine: +15.0 ± 67.7 pg/ml, p=0.22) over the trial period, and this remained the case after controlling for potential confounders. There was an association between treatment with nilvadipine and a proportional increase in CSF Aβ-42 levels, although this did not reach statistical significance (Nilvadipine: +10.6 ± 39.5%, versus Control: -9.2 ± 12.5%, p = 0.07).

**Conclusion:** There was an association between nilvadipine treatment and a proportional increase in CSF Aβ-42 levels. When considered along with data from other pre-clinical trials, these results suggest that certain antihypertensives may interfere with Aβ processing and aggregation within the brain, although this exact mechanism remains to be determined.

**Introduction**

As discussed in previous chapters Alzheimer’s disease (AD) is a progressive neurodegenerative disorder. The diagnosis of AD is largely based upon clinical assessment, with definitive diagnosis still requiring pathological evaluation at autopsy. Intensive research efforts over the last three decades have given detailed knowledge on the molecular pathogenesis of AD. This pathogenesis involves sequentially interacting pathological cascades, including the aggregation of β-amyloid (Aβ) with plaque development, hyperphosphorylation and aggregation of tau protein with formation of tangles, together with downstream processes such as inflammation and oxidative stress, all of which contribute to loss of synaptic integrity, effective neural network connectivity and progressive regional neurodegeneration [301]. Pathological, neurochemical, and
genetic studies give support to the “amyloid cascade hypothesis” [302], which states that an imbalance between the production, degradation, or clearance of Aβ is a significant contributory event to the onset and progression of AD (Figure 1).

![Figure 1: Amyloid cascade hypothesis](image)

Cerebrospinal fluid (CSF) is a translucent bodily fluid that occupies the subarachnoid space and the ventricular system around the brain. CSF has more contact with the brain than any other fluids, as it is not separated from the brain by the tightly regulated blood-brain barrier (BBB). As a result, proteins or peptides that may reflect AD disease pathology are most likely to diffuse into the CSF, than other bodily fluids. Some of these proteins can serve as excellent biomarkers of AD.
The 42-residue-long Aβ isoform (Aβ-42) is highly hydrophobic and forms oligomers and fibrils that accumulate as extracellular plaques [303]. The pathogenic mechanisms that allow Aβ monomers to self-associate to form oligomeric and ultimately polymeric structures are not yet understood, but it is clear that Aβ can exist in several forms [304]. Because Aβ-42 is the dominant component of the plaques seen in AD [303], many groups have investigated its use as a diagnostic tool. The amount of total Aβ in CSF is not well correlated with the diagnosis of AD [305]. The majority of studies have demonstrated a decrease (approximately 50%) of CSF Aβ-42 in AD patients versus normal controls [306-308]. However, a few reports have suggested increased [309], or unchanged [310], CSF Aβ-42 levels in AD, although the difference in observations in these studies may have been due to the variations in simple assaying protocols, and selection of patient groups at varying stages of the disease. In healthy subjects the concentration of Aβ-42 in the CSF exceeds 500 pg/ml in all age groups. The reduced level of CSF Aβ-42 is believed to be caused by deposition of Aβ-42 in senile plaques “amyloid sinks”, with lower levels diffusing into the CSF. Accordingly, studies have found a strong correlation between low Aβ-42 in the CSF, and high numbers of plaques in the neocortex and hippocampus [311], or high retention of Pittsburgh Compound-B in positron emission tomography (PET) scans that directly reflect plaque pathology in the living brain [312, 313]. Some studies have also found a reduction in Aβ-42 levels in disorders without Aβ plaques such as Creutzfeldt-Jakob disease (CJD) [314], amyotrophic lateral sclerosis [315], and multiple systems atrophy [316]. These findings suggest that there may be other reasons for low CSF Aβ-42, in addition to deposition of Aβ in plaques. Factors that may contribute to this include, (a) formation of Aβ-42 oligomers that escape ELISA (enzyme linked
immunosorbant assay) detection [317], (b) association with other molecules that block access to epitopes recognised by detection antibodies, e.g. binding of Aβ-42 to apolipoprotein e4 (APOE) or other chaperone-like amyloid-binding proteins, such as β-trace protein or cystatin-C [318, 319], or (c) sequestering of Aβ-42 in the plasma membrane or intracellularly with lower levels diffusing to the CSF [320]. CSF levels of Aβ-42, especially together with total tau (t-tau) can distinguish subjects with mild cognitive impairment (MCI) who are likely to progress to AD with high sensitivity, specificity, and predictive values, and may even be useful as markers for pre-clinical AD [321, 322].

CSF Aβ-40 is unchanged or slightly increased in AD [323-325]. Consequently, a decrease in the ratio of CSF Aβ-42/Aβ-40 has been found in AD patients in several papers [323-325]. This decrease in the ratio of Aβ-42/Aβ-40 seems more pronounced and predictive than the reduction in Aβ-42 alone [324, 326].

While much of the research has focused on the utility of CSF Aβ-42 and Aβ-40 as diagnostic aids, several trials have studied the possible effects of potential disease modifying drugs, using these markers as an indication of efficacy [327-329]. The benefit of using these CSF biomarkers as markers of efficacy is that they offer a rapid, objective and biologically plausible indication of a therapeutic effect, where changes in cognitive outcomes may be more subtle and lag behind the intervention. They also offer an opportunity to inform us on potential mechanisms of action of the intervention. Despite evidence that usage of certain antihypertensives may decrease the incidence of AD (Chapter 2) no previous clinical trial has investigated the effects of any antihypertensive therapy on CSF Aβ-42 or Aβ-40 levels. There is support from pre-clinical data using
animal models of AD that certain antihypertensive medications, including dihydropyridine calcium channel blockers, interfere with Aβ oligomerization and aggregation [330-332]. In this study we investigate the effects of six weeks treatment with the antihypertensive nilvadipine, on CSF levels of Aβ-42 and Aβ-40.

**Participants and methods:**

As per all aspects of the study, approval was granted by the Irish Medicines Board (IMB) and the Federated Hospitals Ethics Committee. Details on the inclusion and exclusion criteria have been discussed in previous chapters. The primary study investigating the safety and tolerability of nilvadipine was commenced in October 2006 and approval from the ethics committee and IMB to perform CSF studies was granted in November 2007. The study was completed in December 2008.

Trial participants were assigned to nilvadipine (Nilvadil®) 8mg CR once daily or no treatment in a 2:1 ratio. All patients and their carers were given a separate patient information and consent sheet which outlined the specifics of performing a lumbar puncture and the risks attached. Patients were advised that they could withdraw from this aspect of the study at any point. Thirty-one (63%) patients consented to baseline lumbar punctures; one patient declined follow up lumbar puncture (for reasons unrelated to the procedure), and was excluded from the analysis; CSF samples were not obtained from five patients who consented to lumbar puncture due to technical limitations (Figure 2). The methodology for monitoring blood pressure changes, measuring cognitive outcomes, performing transcranial doppler studies and APOE genotyping has been
discussed elsewhere (Chapter 3, 4, 5, and 6). The study investigators performing blood pressure and TCD measurements were blinded to patients APOE ε4 status and their CSF β-amyloid results.

**Figure 2: Schema of CSF study**

- **AD patients screened and recruited**
  - (N = 49)
- **Successful Pre / Post CSF sample**
  - (N = 25)
- **No Pre / Post CSF sample**
  - (N = 24)
  - No consent n = 19
  - Technical limitation n = 5
- **Nilvadipine 8mg OD**
  - (N = 18)
- **No treatment**
  - (N = 7)

* One patient had baseline CSF but declined follow up

**Methodology for CSF collection and analysis:**

Following consent, all CSF samples were obtained by lumbar puncture (LP) between the L3/L4 and L4/L5 intervertebral space on visit 2 and visit 5 six weeks later. All LP’s were performed in the same room by the same physician (sk), using a 24 gauge atraumatic needle which is associated with the lowest incidence of post-LP headache and is well tolerated in patients with AD [333]. All patients taking nilvadipine had been instructed to take the medication at eleven o’clock each morning. LP’s were performed at the same time of day at baseline and follow up to limit any potential confounder of diurnal variations of CSF levels [334]. Exactly ten millilitres of CSF was collected from each
individual at every sample collection. Previous studies have demonstrated that extracting this volume was not associated with an Aβ spinal cord gradient [335]. The CSF was gathered in polypropylene tubes and then divided into smaller aliquots for storage at -80 °C. It was then transported frozen to the Roskamp Institute laboratories for Aβ measurements. This protocol was adhered to for all samples.

Following thawing at room temperature and centrifugation, the Aβ content of CSF was determined, as per manufacturer’s instructions, using enzyme linked immunoassay (ELISA) kits for human Aβ-40 and Aβ-42. The inter-assay and intra-assay coefficients of variance were reported to be ≤ 10% (Invitrogen, Carlsbad, CA, USA). All the samples for each individual were processed in lots to reduce the confounder of inter-assay variability [336].

Statistical Analysis

Baseline characteristics were tested for normality of distribution. The non-parametric Mann Whitney test was used for the analysis of differences in asymmetric MMSE-recall scores. Symmetric quantitative values, such as CSF Aβ levels, BP measurements, age, MMSE and EXIT-25 scores, were compared using the students T-test. Unadjusted analysis on changes in ratios, levels, and percentages of CSF Aβ was performed using the students T-test. Adjusted analysis was performed using ANOVA. For qualitative data the Pearson’s Chi square test was used if the cell size requirement for the contingency tables was met, otherwise the Fisher’s Exact test was used. Correlations were examined using Pearson correlation analysis with a two-tail p value. All statistical
analyses were performed using the SPSS 16.0 statistical package. A p value of less than 0.05 was considered statistically significant.

Results

Twenty-five (51%) patients had CSF samples retrieved at baseline and follow-up. Patients who didn’t have an LP, were older, more dysexecutive, and more likely in the control group (Table 1). All patients were on acetyl cholinesterase inhibitors (AChI). Overall medication compliance was 96.7% (± 6.4) in the nilvadipine group. Consistent with previous reports of lower levels of Aβ-42 in AD populations, mean baseline Aβ-42 was 177.0 ± 91.3 pg/ml while, Aβ-40 was 4186.2 ± 2196.4 pg/ml.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre/Post Cerebrospinal Fluid (n =25)</th>
<th>No Cerebrospinal Fluid (n=24)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>15 (60)</td>
<td>17 (71)</td>
<td>0.42</td>
</tr>
<tr>
<td>Nilvadipine Group, n (%)</td>
<td>18 (72)</td>
<td>11 (46)</td>
<td>0.06</td>
</tr>
<tr>
<td>Age, years (SD)</td>
<td>68.5 (7.6)</td>
<td>72.3 (7.9)</td>
<td>0.09</td>
</tr>
<tr>
<td>APOE ε4 +*, n (%)</td>
<td>22 (92)</td>
<td>19 (79)</td>
<td>0.42</td>
</tr>
<tr>
<td>MMSE (SD)</td>
<td>23.8 (3.0)</td>
<td>22.3 (3.4)</td>
<td>0.13</td>
</tr>
<tr>
<td>EXIT-25 (SD)</td>
<td>12.5 (6.5)</td>
<td>16.5 (7.7)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

SD, Standard Deviation; APOE, apolipoprotein E; * at least one ε4 allele

There were no significant differences in the baseline characteristics and demographics in the treatment and control group (Table 2). There were no statistically
significant differences in the baseline values of CSF Aβ-40 and Aβ-42, or the Aβ-42/Aβ-40 ratio in both groups (Table 2).

### Table 2: Baseline demographics of nilvadipine and no treatment groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No treatment (n=7)</th>
<th>Nilvadipine 8 mg (n=18)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>6 (86)</td>
<td>9 (50)</td>
<td>0.18</td>
</tr>
<tr>
<td>APOE ε4 + *, n (%)</td>
<td>6 (86)</td>
<td>17 (94)</td>
<td>0.50</td>
</tr>
<tr>
<td>Antihypertensive use, n (%)</td>
<td>2 (29)</td>
<td>2 (11)</td>
<td>0.55</td>
</tr>
<tr>
<td>Smoker</td>
<td>2 (29)</td>
<td>2 (11)</td>
<td>0.55</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>-</td>
</tr>
</tbody>
</table>

**Mean ± SD**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No treatment</th>
<th>Nilvadipine 8 mg</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>68.1 (6.0)</td>
<td>68.7 (8.3)</td>
<td>0.88</td>
</tr>
<tr>
<td>CSF Aβ42/40 ratio</td>
<td>0.067 (0.02)</td>
<td>0.063 (0.09)</td>
<td>0.90</td>
</tr>
<tr>
<td>CSF Aβ-40 pg/ml</td>
<td>3205.0 (2424)</td>
<td>4567.7 (2046)</td>
<td>0.17</td>
</tr>
<tr>
<td>CSF Aβ-42 pg/ml</td>
<td>198.7 (124.1)</td>
<td>168.6 (77.9)</td>
<td>0.57</td>
</tr>
<tr>
<td>Education, years</td>
<td>14.7 (1.1)</td>
<td>16.0 (3.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>MMSE</td>
<td>23.1 (3.2)</td>
<td>24.0 (3.0)</td>
<td>0.54</td>
</tr>
<tr>
<td>MMSE-recall</td>
<td>0.14 (0.4)</td>
<td>0.28 (0.6)</td>
<td>0.63</td>
</tr>
<tr>
<td>EXIT-25</td>
<td>11.6 (4.5)</td>
<td>12.8 (7.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>Systolic BP, mmHg °</td>
<td>124.7 (14.2)</td>
<td>122.9 (9.9)</td>
<td>0.74</td>
</tr>
<tr>
<td>Diastolic BP, mmHg °</td>
<td>72.3 (5.7)</td>
<td>71.7 (8.3)</td>
<td>0.87</td>
</tr>
<tr>
<td>Total Cholesterol, mmol</td>
<td>4.93 (1.0)</td>
<td>5.1 (1.2)</td>
<td>0.68</td>
</tr>
<tr>
<td>Mean Systolic Velocity, cm/sec</td>
<td>49.1 (6.6)</td>
<td>49.2 (11.4)</td>
<td>0.99</td>
</tr>
<tr>
<td>Pulsatility Index §</td>
<td>0.89 (0.3)</td>
<td>1.1 (0.2)</td>
<td>0.19</td>
</tr>
<tr>
<td>Resistance Index §</td>
<td>0.61 (0.08)</td>
<td>0.65 (0.07)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

APOE, Apolipoprotein E; * at least one ε4 allele; SD, standard deviation; ° based on 24 hour ambulatory blood pressure values (Overall values not available for 4 patients in nilvadipine group), § Middle cerebral artery measured by transcranial doppler (n =15, 4 patients in Control group)
There was no significant difference in the change in MMSE (Nilvadipine: -0.3 ± 1.8 versus Control: +0.3 ± 2.1, p = 0.49), MMSE-recall (Nilvadipine: -0.18 ± 0.7 versus Control: 0.0 ± 0.1, p = 0.51), or EXIT-25 scores (Nilvadipine: -1.4 ± 3.2 versus Control: -0.7 ± 2.7, p = 0.62), in both groups over the trial period. There was also no significant difference in the change in either systolic (Nilvadipine: 0.0 ± 14.3 mmHg versus Control: +5.7 ± 10.6 mmHg, p = 0.36), or diastolic BP (Nilvadipine: -1.0 ± 7.9 mmHg versus Control: +5.3 ± 7.0 mmHg, p = 0.10), over the trial period. There was also no significant difference between the two groups in changes in middle cerebral artery haemodynamics over the six-weeks (MSV; Nilvadipine: -2.7 ± 8.1 cm/sec versus Control: -2.6 ± 2.3 cm/sec, p = 0.99), (RI; Nilvadipine: 0.2 ± 0.03 versus Control: 0.3 ± 0.04, p = 0.40), and (PI; Nilvadipine: 0.09 ± 0.15 versus Control: 0.11 ± 0.16, p = 0.87). There was no correlation evident between changes in CSF levels of Aβ-40 and Aβ-42, and changes on cognitive scores or transcranial doppler values (n = 15) in the treatment and control groups.

<table>
<thead>
<tr>
<th>Table 3: Between group changes in CSF amyloid levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CSF Aβ-40 pg/ml</td>
</tr>
<tr>
<td>CSF Aβ-42 pg/ml</td>
</tr>
</tbody>
</table>

* adjusted for age, gender, use of other antihypertensives, blood pressure and APOE ε4 status

There was no statistically significant treatment effect on the overall mean difference changes, for either Aβ-40 (Figure 3), or Aβ-42 (Figure 4) on unadjusted analysis, or following adjustments for the potential confounders of age, APOE ε4.
genotype, gender, and the use of other antihypertensives (Table 3). There was also no significant effect of treatment on the Aβ-42/ Aβ-40 ratio (Nilvadipine: -0.019 ± 0.09 versus Control: -0.004 ± 0.01, p = 0.65). There was an association between treatment and a proportional increase in CSF Aβ-42 levels, although this did not reach statistical significance (Figure 6: Nilvadipine: +10.6 ± 39.5% versus Control: -9.2 ± 12.5%, p = 0.07). Patients with nilvadipine appear to have a proportional increase in CSF Aβ-42 following treatment. There was no such significant effect noted on Aβ-40 levels (Figure 5: Nilvadipine: -0.5 ± 15.3% versus Control: -4.9 ± 15.6%, p = 0.46). There was no significant interactive or confounding effect of APOE ε4 status, although this analysis was limited as only one patient in the control group, and one patient in the treatment group were ε4 non-carriers.

![Figure 3: Aβ-40 CSF levels; T-test = - 0.25, p = 0.81](image-url)
**Figure 4:** Aβ-42 CSF levels; T-test = -1.30, p = 0.22

**Figure 5:** Aβ-40 levels (% change); T-test = -0.75, p = 0.46
Baseline TCD and Aβ-42 values

Fifteen of the patients who had CSF samples received also had TCD measurements performed. Thirteen (86.6%) patients had at least one APOE ε4 allele. There was no significant APOE ε4 effect on baseline levels of Aβ-40 (F= 0.94, p= 0.41), or Aβ-42 (F= 0.37, p= 0.70). There was a significant correlation between baseline Aβ-42 levels and baseline mean systolic velocity (Vmean) (Pearson correlation= 0.61, p= 0.02), peak systolic velocity (Vsyst) (Pearson correlation= 0.65, p= 0.009), and end diastolic velocity (Pearson correlation= 0.57, p= 0.03). Patients with higher levels of CSF Aβ-42 appeared to have higher Vmean (Figure 7), Vsyst, and Vdia values. There was no such correlation evident with the PI and RI. There was also no correlation evident between any TCD measurements and Aβ-40 levels.
**Discussion:**

Twenty-five of a possible forty-nine (51%) patients underwent baseline and follow-up lumbar punctures to retrieve CSF. There were no incidents of post-LP headache reported, and consistent with previous literature the LP procedure was very well tolerated in this group overall [333, 337]. Only one patient declined a follow up LP (for reasons unrelated to the procedure) after having a baseline sample. Both the treatment and control groups were well matched for baseline characteristics and demographics (Table 2). As reported in previous studies there was marked inter-individual variability in 153
baseline levels of Aβ-40 and Aβ-42 [338]. However, previous studies have also established that there is good intra-individual stability in repeat measures of CSF Aβ-40 and Aβ-42 for up to two years, with coefficients of variation of 7.2 to 8.7% [339]. Within this subgroup there was no noticeable effect of treatment on cognitive scores, blood pressure or middle cerebral artery haemodynamics. These outcomes have been discussed elsewhere within the context of the overall trial.

Within this study, while there was no significant change in the overall values of Aβ-40 and Aβ-42 (Table 2), although there was an association between treatment and a proportional increase in CSF Aβ-42 levels, although this did not reach statistical significance (Figure 2). As discussed previously, several studies have suggested that the use of certain antihypertensives appear to reduce the incidence of AD by mechanisms independent of changes in blood pressure (Chapter 2) [62, 114]. Recent papers have looked at the ability of certain antihypertensives to attenuate the oligomerization of Aβ peptides into high-molecular-weight (HMW) peptides that are implicated in the progression of AD [330, 340]. While deposition of Aβ peptides into extracellular amyloid plaques in the brain is a key neuropathological feature of AD, recent evidence suggests that the accumulation of soluble extracellular HMW oligomeric Aβ species in the brain, rather than deposition of amyloid plaques per se, may be specifically related to spatial memory reference deficits in mouse models of AD [341, 342]. Neutralisation of soluble extracellular HMW Aβ peptides via immunotherapy may causally improve spatial memory functions in a mouse model of AD [343]. This suggests that preventing oligomerisation of Aβ in the brain, rather than dissociating or preventing plaque formation deposition, may be a more productive approach in preventing or treating AD memory dysfunction [344].
Passinetti and colleagues have identified four antihypertensives, valsartan, frusemide, candesartan cilextil and the DHP-CCB nitrendipine, which are capable of inhibiting Aβ-40 and Aβ-42 aggregation \textit{in vitro}. While the potential of nilvadipine to attenuate this aggregation of Aβ peptides has not yet been formally investigated, given its molecular similarity to nitrendipine, it's possible it may exert neuroprotective properties in a similar fashion.

The vast majority of clinical trials have demonstrated a reduction in the CSF Aβ-42 levels of patients with AD when compared to normal controls [345]. The proposed reason for this is that Aβ-42 is deposited in "sinks" of amyloid plaques. Our findings are consistent with data from a recent trial of a humanised monoclonal antibody (Solanezumab) which reported increased CSF Aβ levels following treatment [329]. The trend towards a proportional increase in CSF Aβ-42 levels in patients who received nilvadipine may reflect a reduced uptake of Aβ-42 into amyloid plaques and therefore a higher circulation in the CSF. There is a significant inverse relationship between levels of circulating Aβ-42 in the CSF and cerebral amyloid plaque burden [311-313].

Only two patients in this study were APOE ε4 non-carriers, and this limited our ability to investigate for an APOE dependent effect. A clear APOE ε4 allele dose effect has been reported previously, with ε4 carriers having significantly lower CSF Aβ-42 [346]. There was no significant APOE confounder or interactive effect noted in the adjusted analysis. Repeating the analysis minus the two APOE ε4 non-carriers did not alter the results, and the trend towards a proportional increase in Aβ-42 levels from baseline was still evident. Given the apparent APOE dependent effects of nilvadipine treatment on
cognition, it would be useful to repeat this study with a larger proportion of APOE ε4 non-carriers. APOE and CLU (discussed in chapter 5) are chaperone proteins that facilitate transport of Aβ across the BBB, and it would be useful to measure plasma clusterin levels in future patients treated with nilvadipine.

No differences in middle cerebral artery velocities were noted between the treatment and control group. This is consistent with my finding from the overall TCD study reported in Chapter 6. Previous SPECT and PET studies have demonstrated that nilvadipine increases cerebral perfusion in patients with hypertension and MCI or AD, however this effect on perfusion was not captured using TCD. Nilvadipine may increase perfusion by antagonising Aβ-mediated cerebral artery vasoconstriction [134]. There was a significant correlation between levels of CSF Aβ-42 at baseline and Vmean, Vsys, and Vdia velocities, with patients with higher levels of CSF Aβ-42 appearing to have higher velocities. No previous TCD study has reported this association. This finding may be reflective of the APOE dependant effect on TCD measures reported in chapter 5; however I found no APOE ε4 effect on levels of CSF Aβ-42 in this sample. In chapter 5, I reported findings of increased velocities in APOE carriers, and most previous studies have reported that APOE ε4 carriers generally have lower levels of Aβ-42 than non-carriers, so therefore one would expect them to have lower velocities. This relationship between Aβ-42 levels and cerebral artery velocities may reflect differences between patients at different stages of the disease, with patients with more advanced AD and lower CSF levels of Aβ-42 also having lower perfusion velocities. Previous studies have described the vasoactive effect of Aβ, and according to the “amyloid-sink” hypothesis, patients with lower CSF levels of Aβ are more likely to have greater cerebral accumulation [134]. This correlation between
CSF A\(\beta\)-42 and TCD readings may be a response to greater levels of Intracerebral A\(\beta\). A small cross sectional sample such as this cannot attribute causality, but these findings merit further investigation. A recent study found no correlation between brain perfusion (using SPECT) and CSF A\(\beta\)-42 levels, but did report a strong correlation between CSF total (t-tau) and phosphorylated tau (p-tau) concentrations, and diminished perfusion in the left parietal cortex \[347\]. Future nilvadipine studies should include CSF levels of t-tau and p-tau, and investigate for correlations with regional cerebral perfusion changes.

This preliminary study suggests that there may be a treatment related effect of nilvadipine on A\(\beta\)-42 levels. Given the reduction in A\(\beta\)-42 levels associated with AD, an increase in those levels may propose a neuromodulatory effect, but this requires further investigation. No previous study has examined the effect of treatment with an antihypertensive on CSF A\(\beta\) peptides in humans. The medication used in this study was a controlled release formula of nilvadipine 8mg daily, which is the usual starting antihypertensive dose. All subjects took the tablet at the same time each day but there may have been a dose response relationship relative to the timing of the LP which I have not examined for. To limit this as a confounder, patients had their LP performed at the same time of day pre and post treatment. This also limited any potential confounding attributable to a possible diurnal variation in CSF A\(\beta\) levels \[334\]. Further studies are required to investigate the relationship between medication ingestion and levels in the CSF over time.

The evidence from this study is not conclusive, but when examined within the context of existing pre-clinical data suggesting that certain antihypertensive medication may be neuroprotective or disease modifying for AD, it merits further investigation. The
LP procedure was well tolerated in this group of patients and given the objective clinical information that can be yielded it should be incorporated into all study protocols investigating potential treatments for AD.

**Overall study summary**

1. Blood pressure values (high and low) impact on the incidence of AD - probably via diminished cerebral perfusion
2. Certain antihypertensives may reduce the incidence of AD but not by their effect on BP
3. Overall nilvadipine treatment is safe and well tolerated by patients with AD
4. Possible treatment effect on cognition - may be APOE ε4 carrier status dependent
5. Possible APOE ε4 effects on MCA velocities
6. Possible treatment effect of nilvadipine on CSF Aβ-42 levels
7. Possible association between CSF Aβ-42 levels and MCA velocities
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173


180


