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Effect of Vascular Function on Alzheimer's Disease

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Abstract

Effect of Vascular Function on Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by a state of reduced cerebral blood flow, which may partly be secondary to cerebral artery vascular dysfunction. Recent studies have shown that systemic vascular dysfunction may precede Alzheimer's disease. We hypothesize that biochemical changes associated with AD progression can impact vascular function. We also hypothesize that AD transgenic rats progressively develop vascular dysfunction and cognitive decline, and can be mediated by candesartan. Homogenates were prepared from temporal lobe samples from patients with or without tauopathies. The chronic effects of brain homogenates on vascular function was characterized using isolated wild-type rat aorta segments incubated with homogenates for 24 hours. Rat aortas were isometrically mounted and the vascular function was assessed by examining contractile responses to KCl and phenylephrine, and relaxation responses to methacholine and sodium nitroprusside. The acute effect of homogenates on vascular function was measured using wild-type rat aorta segments after maximal relaxation by methacholine. AD transgenic and wild-type rats were administered with candesartan daily since 12-months-old. Blood pressures of AD transgenic and wild-type rats were measured using femoral artery catheterization at 12 months and 18 months and compared to controls. Vascular function of AD transgenic rats was studied at 12 months and 18 months with the same methodology.

Preliminary data from chronic studies suggests that, amyloid group patient tissues are less sensitive to methacholine-dependent relaxation compared to tissue from Tau and control groups. Preliminary data from acute studies suggests that tissue from the tau group results in more severe contractile effects on maximally relaxed aortas compared to amyloid and control groups. Results from 12-month blood pressure of AD transgenic rats show no difference before the progression of AD, comparing to the controls. Results from 12-month contractility study of AD transgenic rats show no significant difference in vessel functions.

These results are significant because they suggest that biochemical changes associated with AD can impact vascular function. Therefore, these studies suggest that there is an association between AD and vascular dysfunction and propose new therapeutic targets.

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Introduction

Alzheimer's Disease

Alzheimer's Disease (AD) is a major type of dementia. During the meeting of the Society of Southwest German Psychiatrists on November 3, 1906, Dr. Alois Alzheimer first reported the clinical and pathological characteristics of the disease (Goedert, 2006). AD is a neurodegenerative disease characterized by a progressive deterioration in a patient's cognitive function and memory loss. It is currently the sixth leading cause of death in the US and accounts for 50 to 60 percent of all dementia cases among people over 65 years old (Francis et al, 1999). It is also the primary disease that causes decline in functional capacity in developed countries (Christiane, 2011). It is estimated that over 5.5 million Americans suffered from AD in 2017. (alz.org, 2017).

AD is a neurodegenerative disease that mostly affects the brain regions that are involved in higher mental functions (neocortex and hippocampus). The progression of AD varies among patients, ranging from several years to two decades (Mann, 1992). Many factors affect the rate of progression, including brain atrophy, vascular function, genetic factors, and immune system factors (Thalhauser, 2011). The direct cause of Alzheimer's disease remains unknown, but several factors have been implicated to play a role in disease progression.

Normally, the progression of AD has three stages: early, middle, and late stage. In the early stage, patients experience memory lapses, for example, forgetting common words and names. They also have difficulty remembering new information. This is the stage when patients are mostly being diagnosed. Patients in the middle stage have difficulties expressing their thoughts and ideas. They are more easily angered, and have more trouble communicating with words and sentences. In the late stage, patients have even worse memory and begin to have issues with movement control. They also become incapable of interacting with the environment (what does this mean??). The disease can affect their speech, mood, and behavior. Patients need extensive assistance with their daily tasks (Orta-Salazar, 2016). Although Alzheimer's disease does not directly cause death, patients in the late stage have trouble walking, swallowing, and coughing. They stop eating and become susceptible to infections. They have higher chances of choking, which can cause immediate death.

The cause of non-genetic Alzheimer's disease is still unclear. Many theories have been proposed over decades of research but only a few have persisted. One of the theories is the amyloid hypothesis. It is now known that extracellular deposit of plaques and intracellular deposit of tangles are involved in the progression of the disease (Masters, 1985). A second theory claims that decreased cerebral blood flow (cerebral hypoperfusion) is the major cause of Alzheimer's disease.

Alzheimer's as a neurodegenerative disease

The amyloid hypothesis states that the deposit of insoluble amyloid-β protein (Aβ) is thought to be the cause of Alzheimer's disease. Two histopathological changes associated with the progression of AD are the senile plaques and the neurofibrillary tangles (NFTs) combined with massive loss of neuronal cells and synapses (Selkoe, 2002). Senile plaques mainly consist of amyloid-β protein (Aβ). NFTs consist of abnormally phosphorylated Tau protein. Aβ is a peptide of 36-43 amino acids that derives from proteolysis of amyloid precursor protein (APP) and accumulates into plaques (Selkoe, 1991). APP is an integral membrane protein that is secreted mostly by neurons. It serves normal functions in the synapse of neurons, regulating synapse function, neuron plasticity, and hormones. Plaques can cause the accumulation of Tau protein, known as tangles. The number of tangles in neocortex is positively related to the severity of AD (Arriagada, 1992). Amyloid Tau and total Tau in cerebrospinal fluid (CSF) are two of the biomarkers for AD, and previous studies have shown that the high concentration of CSF Tau protein can predict AD (Kester, Maartje I., 2010).

However, there are still questions about the hypothesis. The insoluble Aβ does not correlate to the severity of the disease, and it is a product of a sick neuron and not the cause of the disease. In addition, patients that do not have any symptoms of AD can have similar levels of Aβ to patients with AD (Arriagada, 1992). Furthermore, many studies on AD animal models have proven that treatments against Aβ do not improve cognition (Austin, 2003).

Alzheimer's as a vascular disorder

AD shares many risk factors with vascular dysfunction, and the onset of these factors can happen decades before sign of cognitive decline. It is proposed that AD may be caused by cerebral hypoperfusion, where the brain does not receive sufficient blood for its metabolic needs. Cerebral hypoperfusion can be caused by cardiovascular diseases.

Many risk factors have been identified acting on the progression of AD. Among the factors, hypertension, stroke, and cardiac disease relates most to the non-genetic AD. Hypertension is increased blood pressure in long term caused by increased cardiac output and/or peripheral vascular resistance. Epidemiologically, it has been shown that hypertension precedes dementia onset by approximately 30 years (Dickstein, 2010). Hypertension can lead to other vascular diseases like stroke, atherosclerosis, and cardiac diseases, which share risk factors with AD as well. Many longitudinal studies have shown an increase of blood pressure years before the onset of AD (Skoog, 1996; Kivipelto, 2001). Some other studies showed opposite results, but also discovered the link between hypertension and dementia (Posner, 2000).

In blood vessels, the endothelium is the inner layer responsible for relaxing blood vessels and controlling nutrient transportation. It controls relaxation by releasing nitric oxide and mediating local angiotensin-II activity. Endothelial dysfunction is characterized by impaired relaxation of blood vessels. Endothelial dysfunction precedes many clinical cardiovascular diseases, predicts cardiovascular disease progression, and shares many risk factors with AD (Bellew KM, 2009). Recent studies suggest that systemic endothelial dysfunction is directly related to AD (Refael H, 2002). Endothelial cells in the brain produce amyloid precursor protein-cleaving secretases,

which increase the production of cerebral amyloid protein and the deposit of plaques and tangles. Amyloid proteins increase oxidative stress and reduce the availability of nitric oxide in smooth muscle. Less nitric oxide results in vascular smooth muscle relaxation and thereby reduces cerebral blood flow.

Cardiovascular disease and Alzheimer's disease share many risk factors, and vascular dysfunction is the central feature of cardiovascular disease. However, its role in AD is still unclear. Additionally, vascular function may be further deteriorated by the progression of AD.

Nitric Oxide (NO)

NO is a free radical that serves multiple roles in biological systems. Because it has an unpaired electron, it is highly reactive and has a transient lifetime, which makes it suitable for paracrine and autocrine functions.

Nitric oxide serves as signaling molecule, neurotransmitter, and immune effector in mammals (Toda, 2005; Tripathi, 2007). It can dilate blood vessels and decrease blood pressure. Nitric oxide is endogenously produced by nitric oxide synthase. In blood vessels, nitric oxide produced in endothelial cells diffuses to smooth muscle cells and activates guanylate cyclase, which converts GTP to cGMP. cGMP dilates smooth muscle in multiple ways. First, increasing cGMP in the cells inhibits Ca^{2+} influx into the cell which reduces myosin contraction. Second, cGMP opens K^{+} channels. Increasing intracellular K^{+} causes hyperpolarization and decreases

contraction. Third, cGMP activates myosin light chain phosphatase, which dephosphorylates myosin light chains and causes relaxation (Klabunde, Cardiovascular Physiology Concepts).

Renin-Angiotensin-Aldosterone System (RAAS)

RAAS regulates blood pressure and fluid balance by angiotensin as a hormone signal. When renal blood flow is low, juxtaglomerular cells in the kidneys convert the precursor – prorenin, already present in the blood into renin and secrete it directly into the circulation. Renin converts angiotensinogen to angiotensin I and then angiotensin converting enzyme, made in tehlungand endothelial cells converts angiotensin I to the biologically active angiotensin II. Angiotensin II constricts blood vessels and increases the release of aldosterone, which increase fluid uptake and raises the blood volume.

RAAS has been proved to be involved in AD progression in both animal models and patients (Hajjar, 2013). In experimental model, increase in systemic and interventricular angiotensin II concentrations results in increase of amyloidosis in the brain (Zhu, 2011). APP and Aβ production increases and the number of angiotensin converting enzyme (ACE) decreases. Consequently, deposition of amyloid aggregates is facilitated and oxidative stress is induced (Hemming, 2005). In addition, angiotensin II can impair endothelial function and vascular regenerative activity by activating angiotensin receptor type 1 (Kou, 2007).

Purpose

In 1984, the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) established diagnostic criteria for Alzheimer's disease. It states that, diagnostics should be based on cognitive impairment, and neuropsychological tests, such as the mini-mental state examination (MMSE). Decisive diagnosis requires histopathologic exam of brain tissue. Currently, the earliest diagnosis of AD is the increasing cerebral amyloidosis (Marilyn, 2011). However, the changes in the brain can take place much earlier than any symptom appears. AD has a long progression time before any sign of cognitive decline (DeCarli, 2003). These diagnostics normally take place after patients show signs of dementia, when the disease has progressed extensively. Treatments that target the possible risk factors that may cause AD are potentially useful, if such factors can be detected earlier. Diagnostics at early stages are necessary for treatments that can slow down or stop the progression of AD.

In this study, we were looking at prodromal AD and what vascular factors might affect pathological progression in early stages. Comparing the current treatments against cognitive decline, interventions that target vascular dysfunction are likely to have better curative effects. The link between systemic vascular function and AD is also studied. Vascular functions of AD transgenic rats were examined at 12 and will also be examined at 24 months to see if vascular dysfunction happens before or after cognitive decline. Candesartan was administered to AD

rats as an angiotensin receptor antagonist and its effect in mediating vascular dysfunction was tested.

Material and Method

Transgenic Rat

Animal models greatly contribute to our understanding of AD. Many transgenic rats and knock out rats have been designed to reveal the mystery of this disease. In this study, a novel rat model (TgF 334) was used to simulate AD progression in human. The rat overexpresses the gene that encodes presinilin-1 and the amyloid precursor proteins (APP), which are the precursor to the beta amyloid. Rats that possess this modification have more beta amyloid production and will develop AD. TgF 334 overexpresses human APP and develop both amyloid plaques and neurofibrillary tangle as in human AD brain (Orta-Salazar, 2016). Behavioral study was conducted and showed that the rats start to have behavioral changes at 18 weeks.

Homogenate

Samples of temporal lobes were collected from patients. Homogenates were made from grinded samples and were divided into 3 groups: amyloid group gained from AD patients that have amyloid proteins, Tau group from patients with high concentrations of Tau protein, and a control group. The concentrations of proteins in the homogenates were normalized using BCA protein essay.

Krebs Buffer

Physiological saline solution (Krebs-Henseleit Buffer) was made in lab (118 mM NaCl, 4.73 mM KCl, 1.2 mM $MgSO_4$, 0.025 mM EDTA, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 5 mM glucose, and 25 mM NaH_2CO_3, pH=7.4). All materials were purchased from Sigma-Aldrich (St. Louis, MO).

Potassium Chloride (KCl)

Potassium Chloride (KCl) is a vasoconstrictor and is frequently used in medical and biological studies. Vessels exposed to a high concentration of KCl constrict rapidly followed by a plateau that slowly decreases (Xiao and Rand, 1989). KCl acts as a vasoconstrictor in two ways. First, the rapid vessel contraction is due to the high concentration of K^+. K^+ induces membrane depolarization that opens Ca^{2+} channels in smooth muscles. The influx of Ca^{2+} activates calmodulin, which activates the Myosin Light Chain (MLC) kinase. The MLC kinase phosphorylates the head of the myosin light chain, causing contraction in smooth muscle (Tansey, 1994). Second, K^+ can release neurotransmitters, including noradrenaline released secreted from sympathetic nerve terminals (Boullin, 1967). The noradrenaline is mainly responsible for mediating the plateau after the contraction, since the second plateau of the vessels treated with contraction-inducing concentrations of KCl and α_2-adrenoceptor antagonist were significantly reduced (Xiao and Rand, 1991).

Phenylephrine (PE)

PE is a selective α_1-adrenoceptor agonist commonly used for dilating pupils and increasing blood pressure.

PE selectively binds to α-adrenergic receptors and activates Gq G protein pathway. G-protein activates phospholipase C (PLC), which selectively catalyzes the hydrolysis of PIP_2 into DAG and IP_3. IP_3 diffuses to the endoplasmic reticulum and opens ligand gated ion channels that allow Ca^{2+} to flow outside the endoplasmic reticulum and increase intracellular Ca^{2+} level. Myosin head is phosphorylated in a calcium-dependent manner, resulting in contraction of blood vessels.

Methacholine (MCh)

MCh is a synthetic choline ester. It activates the parasympathetic nervous system by acting as a non-selective muscarinic receptor.

Comparing to the acetylcholine, which is a neurotransmitter released by nerve cells, methacholine has a β-methyl group that provides affinity to muscarinic receptors due to their stereoselectivity (Wilson, 1998). It also is resistant to esterases.

Methacholine dilates vessels by inducing the release of nitric oxide from endothelial cells. It binds to the muscarinic receptors on the endothelial cell membrane and activates Gq G protein pathway which activates phospholipase-C, which cleaves PIP2 into IP3 and DAG. IP3 diffuses to the endoplasmic reticulum and opens ligand gated ion channels that allow Ca^{2+} to flow outside the endoplasmic reticulum and increase intracellular Ca^{2+} level. Increased Ca^{2+} concentration activates calmodulin, which acts as a molecular switch and activates the nitric oxide synthase (Su, 1995). Nitric oxide diffuses to the smooth muscle cells and induces vessel relaxation.

Sodium Nitroprusside (SNP)

Sodium nitroprusside is a medication used for relaxing blood vessels. It has an immediate onset, no effect on central nervous system, and has little effect on other smooth muscle. These characteristics make sodium nitroprusside the ideal medication to lower blood pressure in emergencies (Palmer 1975). It is usually injected into a vein continuously during surgery.

SNP dilates blood vessels in an endothelium-independent way. SNP is an organometallic complex with an iron center and a NO ligand. The NO ligand is substituted by sulfhydryl-containing molecule and acts on vascular smooth muscle cells (Grossi, 2005). SNP was used to induce endothelium-independent relaxation.

Candesartan

Candesartan is an antihypertensive drug. It acts as an angiotensin II receptor antagonist. It also has effect on heart failure (Pfeffer, 2003).

Because the RAAS is overactivated in AD progression, we are testing whether candesartan can reduce subsequent effect of angiotensin II by blocking its receptors. This hypothesis was tested by daily administration of candesartan to AD transgenic rats. Their vascular functions were tested at 12 and 24 months and compared to control group.

Assessing Vascular Function

Homogenate Chronic Study

Aortas from Sprague Dawley rats were excised, cleaned of loose fat and connective tissue in physiological saline. The aortas were dissected into 5mm segments and were incubated in Opti-MEM (Gibco, Waltham, MA) supplemented with Penicillin- Streptomycin solution in a 1:100 ratio containing homogenates (0.1 % and 1%; amyloid Tau, high Tau and control) and incubated for 24 hours (37˚C, 5% CO_2). Homogenate percentages were normalized to the protein concentrations measured by BCA protein assay. The incubated aorta segments were isometrically mounted on a force transducer apparatus (Harvard Apparatus, Holliston, MA) in an organ bath containing physiological saline solution (Krebs-Henseleit Buffer: 118 mM NaCl, 4.73 mM KCl, 1.2 mM $MgSO_4$, 0.025 mM EDTA, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 11 mM glucose, and 25 mM NaH_2CO_3, pH 7.4, in 95%-5% CO_2 at 37˚C). Resting tension was set to 50 mN to approximate an in vivo pressure of 100 mmHg. Vascular contraction was studied by generating concentration-isometric force responses to KCl (0-80 mM) and phenylephrine (1 nM-30 μM). To

study vasorelaxation vessels were preconstricted with phenylephrine and concentration-relaxation responses generated to the endothelium-dependent vasorelaxant methacholine (0.1 nM-10 μM) and the endothelium-independent vasorelaxant sodium nitroprusside (SNP, 0.1 nM-300 nM). Data were acquired using a Powerlab system (AD Instruments, Sydney, Australia) and analyzed using Graphpad Prism.

Homogenate Acute Study

Aortas from Sprague Dawley rats were excised, cleaned of loose fat and connective tissue, dissected into 5mm segments and isometrically mounted on apparatus in physiological saline solution (Krebs-Henseleit Buffer: 118 mM NaCl, 4.73 mM KCl, 1.2 mM $MgSO_4$, 0.025 mM EDTA, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 5 mM glucose, and 25 mM NaH_2CO_3, pH=7.4, in 95%-5% CO_2 at 37˚C). Resting tension was set at 50mN to approximate an in vivo pressure of 100 mmHg. The acute effect of homogenates (0.001%-1%) was measured after being preconstircted with phenylephrine (1 μM) and maximally relaxed with methacholine (30 μM). Data were acquired using a Powerlab system (AD Instruments, Sydney, Australia) and analyzed using Graphpad Prism. A value of $p<0.05$ is considered significant for all statistical analysis.

AD Transgenic Study

Rats were assigned into groups based on their gender (male, female) and genotype (wild type, AD). Half of each group were treated orally with candesartan cilexitil (DOSE) suspended in

peanut oil consumed in moistened rodent chow daily. The other half were treated with only peanut oil as control. The blood pressure was measured using femoral artery catheterization at 12, 18 months and 24 months. Aortas were excised at 12 or 18 months, cleaned of loose fat and connective tissue, dissected into 5mm segments and isometrically mounted on apparatus in physiological saline solution (Krebs-Henseleit Buffer: 118 mM NaCl, 4.73 mM KCl, 1.2 mM $MgSO_4$, 0.025 mM EDTA, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 5 mM glucose, and 25 mM NaH_2CO_3, pH=7.4, in 95%-5% CO_2 at 37˚C). Resting tension was set at 50mN to approximate an in vivo pressure of 100 mmHg. Resting tension was set to 50mN to approximate an in vivo pressure of 100 mmHg. Vascular contraction was studied by generating concentration-isometric force responses to KCl (0-80 mM) and phenylephrine (1 nM-30 μM). To study vasorelaxation vessels were preconstricted with phenylephrine and concentration-relaxation responses generated to the endothelium-dependent vasorelaxant methacholine (0.1 nM-10 μM) and the endothelium-independent vasorelaxant sodium nitroprusside (SNP, 0.1 nM-300 nM). Data were acquired using a Powerlab system (AD Instruments, Sydney, Australia) and analyzed using Graphpad Prism. A value of $p<0.05$ is considered significant for all statistical analysis.

Results

Effect of chronic brain homogenate exposure on vascular function.

To study the long-term effect of amyloid Tau protein on vascular function, wild type rat aortas were incubated in brain homogenates for 24 hours and the vascular function was assessed.

Aortas incubated in 1% homogenates showed significant decrease in response to methacholine ($p<0.05$; Figure 1A). To calculate the dosage that induces half maximal relaxation, an EC_{50} analysis was made. Results shows that the 1% amyloid group requires significantly more methacholine to reach 50% relaxation ($p<0.05$; Figure 1B). The endothelial-dependent relaxation pathway of aortas in 1% amyloid group are compromised. However, the aortas show no significant difference in response to SNP (Figure 2A and 2B).

Effect of acute brain homogenate exposure on vascular function.

To assess the short-term effect of homogenate on vascular function, aortas were first constricted with PE and maximally relaxed with MCh. Response to various homogenate concentration (0.001% to 1%) was measured. Result shows that when treated with homogenate from the high Tau group, aorta exhibits stronger contraction comparing to the amyloid Tau group and the control group (Figure 3)

Vascular function in an AD transgenic model.

To study the vascular function of AD transgenic rats, aortas from the AD transgenic rats were assessed at age 12 months. This is the time when rats have not developed cognitive decline. Result shows that there is no significant difference in vessel function at 12 months (Figure 4).

To understand the effect of candesartan on vascular function and AD progression, AD transgenic rats and wild type rats were randomly divided in to a treatment group and a control

group, where the treatment group received daily administration of candesartan cilexitil suspended in peanut oil and the control group received only peanut oil. Blood pressure was measured using femoral artery catheterization at 12 months. Result shows that rats have not developed vascular dysfunction nor hypertension at 12 months of age.

Discussion

Amyloid Tau and total Tau in CSF are two of the biomarkers for AD, and previous studies have shown that the high concentration of CSF Tau protein can predict AD (Kester, Maartje I., 2010). However, the quantitative measurement of Tau in the case of hypertension has not been done. In the acute homogenate study, aortas were preconstricted with PE and maximally relaxed by MCh. Certain concentrations of homogenates counteract with the vasorelaxant effect of MCh, and the aortas constrict again due to loss of the vasorelaxant, most likely NO. Result shows that aortas treated with the high Tau group have significant higher increases in force. As we see an acute effect of high Tau in vascular function, increasing Tau might be an early trigger of hypertension before the onset of Alzheimer's. Some studies suggest that Tau can induce vessel abnormality (Bennett, 2018). Once the relationship between the Tau concentration and the hypertension is well understood, the measurement of Tau can potentially become a predictor of vascular dysfunction, and even an indicator of Alzheimer's disease. However, in this study, the small sample size limits us from making a definitive conclusion, and in the future more brain samples will be studied to obtain greater statistical power.

The chronic study, however, shows a different result. Aortas incubated in 1% amyloid homogenate are significantly less sensitive to methacholine, compared to those in high Tau group and control group. This indicates that 24 hours of incubation of the aortas with homogenates from the amyloid group impairs the endothelium-dependent relaxation pathway. Decreased sensitivity means that the blood vessels will dilate less, given a similar amount of vasodilating agent available. As a result, hypertension is more likely to be present in AD patients. The chronic study supports the hypothesis that AD progression deteriorates vascular function.

Because the endothelium-dependent relaxation involves nitric oxide production and signaling, it is possible that the biochemical changes in the AD brain affect the methacholine signaling or the nitric oxide synthase in the endothelial cells. This is supported by the result from SNP relaxation where no significant effect of the homogenate on the actions of the NO donor is present. Data do not show significant difference in SNP sensitivity, which means that the nitric oxide pathway in smooth muscle cells remains intact after incubation. The incubation of the aortas with homogenates are considered to have a long-term effect that represents the interaction between cerebral proteins and blood vessels as AD progresses. Because the nitric oxide synthase in the endothelial cells is a major source of nitric oxide, blocking the synthase will result in a decrease in nitric oxide bioavailability. A study has shown that AD patients have a progressive loss of NO availability that is not confined in the brain. The study also points out that the loss of NO is directly related to AD and not aging (Venturelli, 2018)

A possible explanation is that high concentration of Tau in the brain triggers early events during prodromal AD, making blood vessels less able to relax. Narrowed blood vessels in brain causes hypoperfusion and induce dementia. Amyloid plaques and neurofibrillary tangles build up and these have chronic effects on blood vessels, further deteriorating vessel function. From the results, vascular dysfunction and cognitive decline have a long-term interaction, one promotes another.

From Figure 5, blood pressures measured at 12 months do not show a difference among groups. The vascular function is intact at 12 months. AD transgenic rats develop cognitive declines at later stages and exhibit behavioral changes at 18 months. Data of blood pressure at 18 months is therefore crucial for understanding whether vascular dysfunction contributes to the change in behavior. Hypertension indicates narrowed blood vessels. The brain is expected to receive less blood and therefore less nutrition and oxygen. As a result, brain cell death increases, which is also the result of building up amyloid plaques and NFTs. If vascular dysfunction is in fact a factor that promotes the progression of AD, treatments on hypertension will be potentially effective in blocking the behavioral change. In this study, we proposed candesartan as an antihypertensive drug to control AD progression.

The study on blood pressure shows that the candesartan treatment aims to test the therapeutic potential of antihypertension drug. The blood pressure at 12 months serves as baseline. Blood pressures of all the groups are not significantly from each other. Rats were randomly assigned

in treatment group or control group, where the treatment group was administered with candesartan cilexitil. AD transgenic rats exhibit behavioral changes at 18 months, and blood pressures will be measured to see the effect of candesartan on preventing the change in behavior. This brings the question about whether controlling blood pressure or vascular function can prevent AD to some extent. These effects of blood pressure will be correlated with studies evaluating cognitive decline in rats, so we can correlate the effects of candesartan on BP with behavioral changes. This study helps to determine some detectable factors in prodromal AD. Because the progression time of AD varies significantly, it is hard to conduct a control experiment in patients. Every patient has a different condition from others, and the factors that induce AD also vary. Gathering a statistically significant number of AD patients with similar symptoms is impractical. However, examining cerebral blood flow in AD patients will help to support this study.

Many factors in the study can affect the outcome. First, there are possible sources of contamination in the chronic study. Although the equipment was sterilized before dissection and Penicillin- Streptomycin solution was added into the Opti-MEM media, it is impossible to be completely sterile during dissection. Bacteria can block PE response, resulting in decreased contraction and abnormal relaxation. Bacteria also produce nitric oxide and interfere with relaxation. About 30% of the chronic study did not yield any result because aortas did not respond to PE anymore. Second, the homogenates made are mixtures of all the proteins in the temporal lobe. It is impossible to determine which specific protein is responsible for the result in homogenate study. Third, some parts of the study do not have statistically significant sample

sizes. The numbers of samples of the acute study, 12-month AD transgenic study, and the 24-month blood pressure study were too small to draw conclusions. Third, the complexity of the disease impedes us from studying a single pathway. Many rats suffered from diseases other than AD. 24 months in rat's age is similar to 60 years old in human. As the rats age, they can develop other diseases that can potentially affect vascular function or kill them.

Combining all the results from the study together, there are two possible pathways of AD progression that relates with vascular dysfunction. First, patients start with amyloid plaques, which builds up intercellularly in the brain and induces the accumulation of NFT. NFT has an acute effect on vascular function, blocking NO signaling and causing the narrowing of blood vessels. Reduced blood flow increases blood pressure and reduced the amount of nutrition and oxygen transported to the brain, further deteriorating the brain. On the other hand, patients can start with cardiovascular disease or hypertension that damage the brain cells, which promotes the buildup of Aβ and NFT. As discussed above, the cause of AD is very complicated and diverse, and its mechanism is still unclear. This study suggests a possible direction for further research.

The result provides a new way of preventing AD. New drugs that can target endothelium-dependent relaxation have the potential to mediate the negative effect of Tau protein on vascular function. Pathways that involve in the relaxation are good targets. The repairing mechanism of blood vessels is also a possible aim for controlling vascular dysfunction. If the blood pressure study shows a link between hypertension and AD, and candesartan is effective

in reducing blood pressure, the model will be validated and additional possible drugs can be explored.

In future studies, the sample sizes of acute homogenate study and the 24-month blood pressure study need to be increased. Studies that can test the effect of candesartan on late-stage AD is necessary to determine the therapeutic potential of candesartan in reversing AD. It is also helpful to quantitatively measure the amyloid proteins in the rat brains at various ages. Patient studies on blood pressure and cerebral blood flow can support the findings in this study.

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Figures:

A **B**

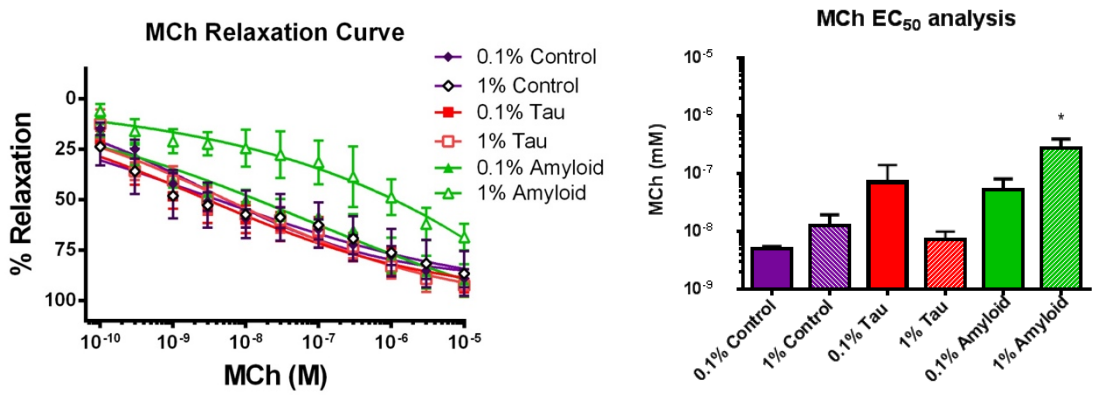

Figure 1: Concentration-relaxation curves (A) and half maximal effective concentration (EC_{50}; B) of methacholine (MCh) in aortas incubated with homogenate for 24 hours. The Endothelial function was studied by preconstircting with PE and measuring relaxation to methacholine. Aortas treated with 1% amyloid homogenates showed a decreased sensitivity to methacholine (One-way ANOVA; $p<0.05$). Results are presented as the mean±SEM. Asterisk indicates significant difference.

A **B**

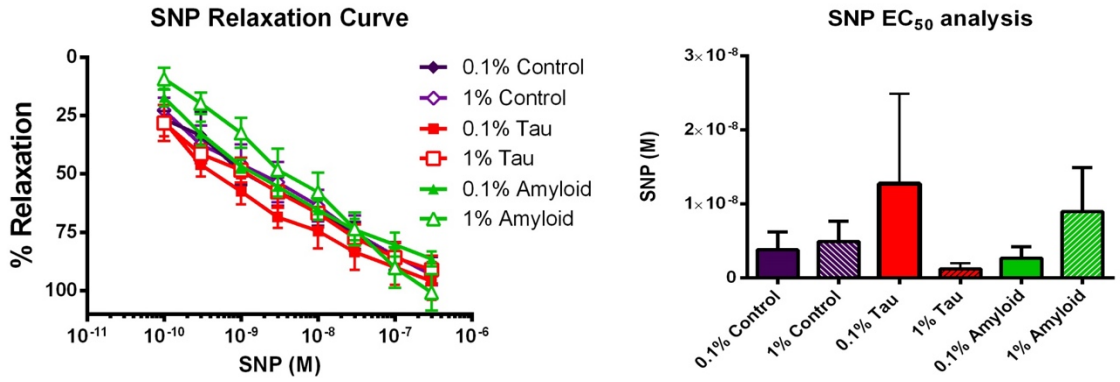

Figure 2: Concentration-relaxation curves (A) and half maximal effective concentration (EC_{50}; B) of sodium nitroprusside (SNP) in aortas incubated with homogenate for 24 hours. The Endothelial function was studied by preconstricting with PE and measuring relaxation to SNP. Aortas do not show significantly different sensitivity to SNP relaxation. Results are presented as the mean±SEM.

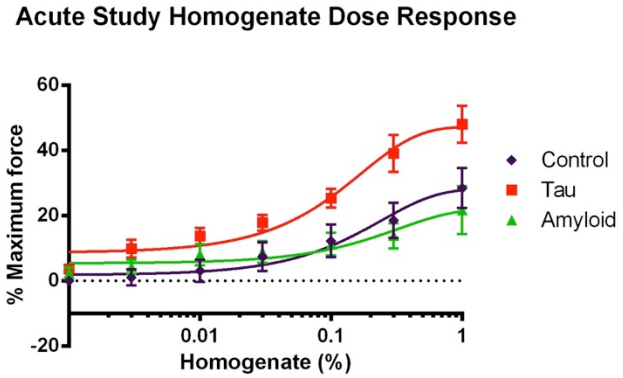

Figure 3: Homogenate Dose-response curves of aortas. Aortas were isometrically mounted and contracted with PE (1 µM) and maximally relaxed with MCh. Their response to increasing concentrations of homogenate was assessed. Aortas incubated in tau homogenates appear to have an increased maximal force compared to aortas incubated in amyloid or control homogenates. Results are presented as the mean±SEM.

A B

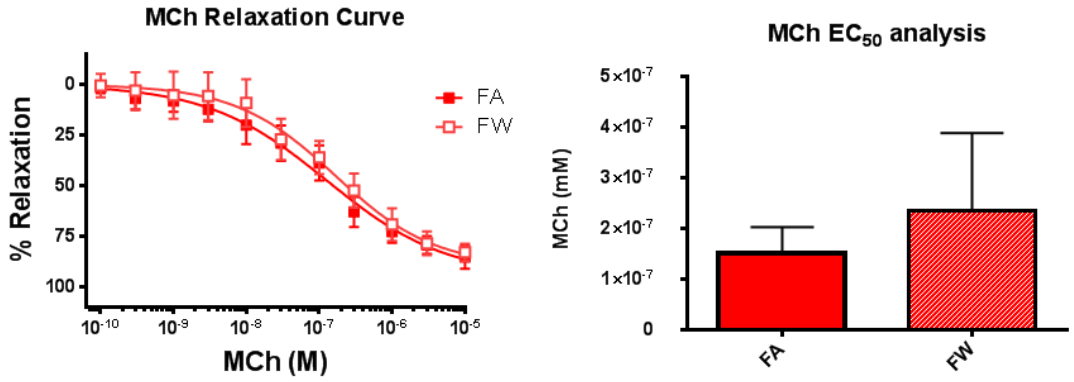

Figure 4: Concentration-relaxation curves (A) and half maximal effective concentration (EC_{50}; B) of methacholine (MCh) in AD female transgenic rats (FA) and wild-type female rats (FW). The endothelial function was studied by preconstircting with PE and measuring relaxation to methacholine. Results show that there is no significant difference in endothelial-dependent relaxation. Results are presented as the mean±SEM.

A B

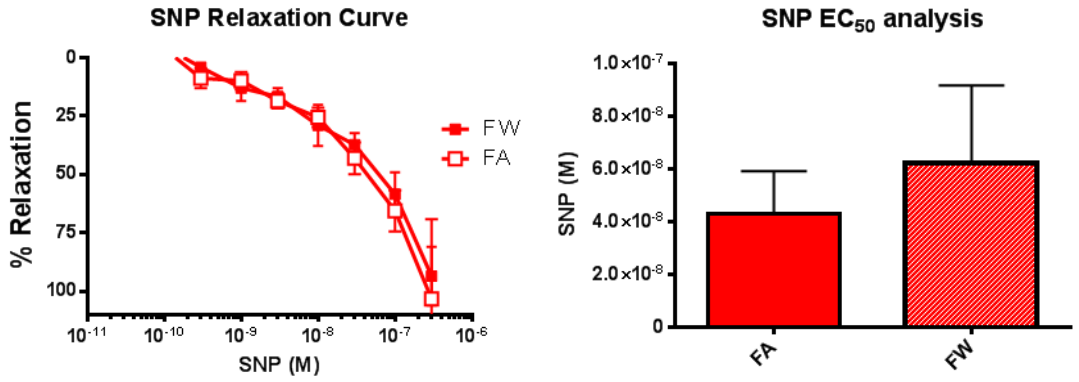

Figure 5: Concentration-relaxation curves (A) and half maximal effective concentration (EC_{50}; B) of sodium nitroprusside (SNP) in AD female transgenic rats (FA) and wild-type female rats (FW). The relaxation function was studied by preconstircting with PE and measuring relaxation to methacholine. Results show that there is no significant difference in endothelial-dependent relaxation. Results are presented as the mean±SEM.

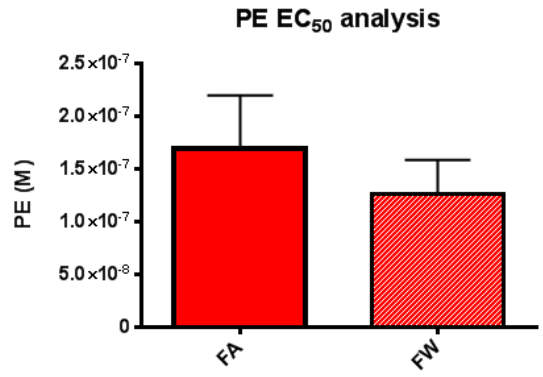

Figure 6: Half maximal effective concentration (EC_{50}) of phenylephrine (PE) in AD female transgenic rats (FA) and wild-type female rats (FW). The contractile function was studied by measuring contraction to PE. Results show that there is no significant difference in endothelial-dependent relaxation. Results are presented as the mean±SEM.

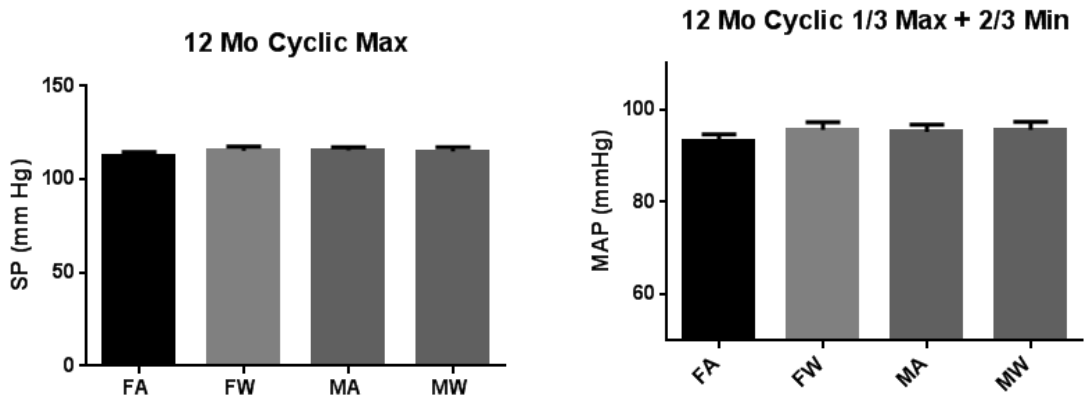

Figure 7: Systolic pressure (A) and mean arterial pressure (B) of AD transgenic rats and wild-type rats at 12 months. Blood pressures were measured using femoral artery catheterization. Results show no difference at 12 months. Results are presented as the mean±SEM.