JOURNAL OF SCIENTIFIC RESEARCH IN ALLIED SCIENCES
ISSN NO. 2455-5800

Contents available at: www.jusres.com

BIOLOGICAL MECHANISMS OF APOA-I MIMETIC PEPTIDE IN IMPROVING COGNITIVE FUNCTION IN ALZHEIMER'S DISEASE

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ARTICLE INFO **ABSTRACT** **ORIGINAL RESEARCH ARTICLE**

Article History
Received: July 2017
Accepted: June 2020
Keywords:
hyperlipidemia, HDL cholesterol, mimetic peptide, cognitive impairment, immune system, Alzheimer's disease, Western diet.

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Alzheimer's disease (AD) is the most common cause of cognitive dementia, demonstrated by a progressive decline in cognitive function and memory. AD has no current curative treatments in overcoming the underlying pathology of the disease, although palliative treatments are available. This paper reviews the potential mechanism of the ApoA-I mimetic peptide for the prevention and treatment of cognitive impairment induced by hyperlipidemia and AD. Projected mechanisms suggest the peptide may: 1) reduce inflammation and wall thickness in small arteries and 2) prevent synaptic and neuronal loss induced by immune system response. Considering 'Western diet' (42% fat, 0.15% cholesterol)-induced cognitive dysfunction and the underlying pathogenesis of AD, targeting vascular pathology and immune response may prove to be clinically effective in treating AD and hyperlipidemia-induced cognitive dysfunction.

ABBREVIATIONS: AD, Alzheimer's disease; BBB, blood-brain barrier; $LDLR^{-/-}$, low-density lipoprotein receptor-deficient mice; MIP, macrophage inflammatory protein; Amyloid-β (Aβ); APP, Aβ precursor protein.

INTRODUCTION:

Dementia is a major public health concern. Alzheimer's disease (AD) is the leading cause of cognitive dementia, demonstrated by memory loss and progressive decline in cognitive and functional impairment [1]. The global prevalence of AD is estimated to be as high as 24 million and is predicted to double every 20 years until 2040, imposing a large economic burden on the healthcare system worldwide [1]. The U.S. Food and Drug

Administration has approved several medications for the management of AD symptoms, however, there are no effective medications for reversal or treatment of underlying pathogenesis of the disease [2]. Conversely, lifestyle interventions such as exercise have been shown to benefit cognitive function [3]. Investigators in the past have mainly focused on decreasing the level of amyloid-βeta (Aβ) as a treatment target for AD, but unfortunately, no compounds have produced meaningful results [4]. Targeting inflammation in treating AD has recently attracted more interest [5-7].

Based on current knowledge of underlying AD pathogenesis, targeting immune system response and vascular pathology may be clinically effective [8-10]. According to recent data published by Cui et al, Buga et al, and Handattu et al, the HDL mimetic peptide D-4Fhas been effective in reducing inflammation in neurovascular pathways, as well as preventing synaptic and neuronal loss induced by innate immune response[11-13]. Furthermore, investigations by Handattu et al suggest ApoA-I mimetic peptides have therapeutic potential in a variety of diseases affecting very small arterial vessels, including AD [12, 13]. The usage of D-4F, therefore, appears to be a novel and realistic route for AD treatment. This article aims to review potential mechanisms wherein ApoA-I mimetic peptides may ameliorate cognitive impairment induced by hyperlipidemia, with a focus on vascular pathology and immune-driven neuro-inflammation.

The inflammation hypothesis in AD and Western diet-induced cognitive dysfunction

AD is characterized by the aggregation of distinctive lesions throughout the brain parenchyma, which are caused by protein misfolding. These lesions include senile plaques (an extracellular amyloid-β (Aβ) deposit), neurofibrillary tangles (predominantly composed of hyperphosphorylated tau protein), and cerebroamyloid angiopathy (deposition of Aβ within the arterial walls) [14]. For the last two decades, the “amyloid hypothesis” has been looked to as the main factor in AD pathogenesis. New data published has demonstrated that inflammation via vascular pathways and immune system responses may contribute to AD pathogenesis [15, 16]. Though neuroinflammation is postulated to contribute, the nature of this inflammatory pathogenic influence is not well understood, specifically in the spectrum of macro phagocytic microglial cell action.

Studies in mouse models indicate that inflammation affects blood vessels, which is similarly modeled in atherosclerosis and coronary heart disease [17, 18]. Buga et al showed that administering a high-fat Western diet (42% fat, 0.15% cholesterol)to low-density lipoprotein (LDL) deficient mice ($LDLR^{-/-}$) causes endothelial dysfunction and LDL, monocyte, and macrophage accumulation in very small arteries such as brain arterioles, eventually leading to arterial thickening and dysfunction[12]. This result is similarly observed in large arteries such as coronary arteries and the aorta, demonstrated in studies by Breslow and Schiller at al[19, 20]. Ou et al furthermore demonstrated that administering apolipoprotein A-I (ApoA-I) mimetic peptide D-4F markedly reduced lipid accumulation in arteries and significantly reduced facial arterial wall thickening [21]. D-4F’s action in reducing pro-inflammatory lipids is likely the mechanism through which D-4F significantly reduces lesions in mouse models with atherosclerosis [2]. This may also explain why D-4F reduces inflammation after viral infections.[22, 23].

High cholesterol levels are reported to be a prognostic risk factor for neurodegenerative diseases, particularly AD [24], as cholesterol levels may modulate Aβ formation and aggregation in the brain [25-29]. D-4F’s ability to reduce hyperlipidemia-induced inflammation both in large arteries, such as the aorta and in small arteries, such as facial arteries, has been shown in animal models [21, 30, 31]. Buga et al[12] reported the

ability of D-4F to reduce hyperlipidemia-induced inflammation of brain arterioles; this was the first example of D-4F action in very small vessels.

The innate immune system, the primary line of defense that acts as the initial immunological response in the brain, is strongly implicated in the pathogenesis of AD [32]. Microglial cells, the resident macrophages of the brain, survey for pathogens and support the central nervous system (CNS) homeostasis and plasticity in a healthy brain [33]. The microglial cells play an essential role in removing Aβ in various ways [34]but it is now evident that their function in neurological diseases is more complex [34]. Namely, the enhanced activity of microglial cells is reported to contribute to neuronal cell death and drive AD pathogenesis [8, 35].

The protein aggregates in AD are associated with neuroinflammation, including activation of complement pathways, increased cytokine production, and microgliosis [36-39]. Because the inflammatory response is a series of destructive and rebuilding processes in the brain, the balance between these processes determines if the immune process is detrimental or beneficial [38, 40, 41]. Such insights suggest novel potential therapeutic targets for the treatment of AD and 'Western diet'-induced cognitive dysfunction through modulation of the immune response.

ApoA-I mimetic peptides

For over a quarter of a century, apolipoprotein mimetic peptides, which are short synthetic peptides that share structural and biological features of native apolipoproteins, have been studied as potential therapeutic agents[42]. ApoA-I, the main protein component of high-density lipoproteins (HDL), has proven to be an effective compound in the treatment of vascular conditions such as atherosclerosis and endothelial dysfunction[2, 43, 44]. ApoA-I mimetic peptide is shown to enhance HDL protective capacity and decrease LDL-induced monocyte chemotactic activity. Additionally, it has been established to exhibit anti-inflammatory and antioxidant properties that reduce inflammation in animal models [2, 45-47]. In recent years, in addition to atherosclerosis, apolipoprotein mimetic peptides have shown to be beneficial in various disease models such as AD, asthma, cancer, colitis, insulin resistance, sickle cell disease, and endotoxemia [48-50]. It is currently unclear whether a common mechanism of action is responsible for their effectiveness in these different diseases; however, the anti-inflammatory properties of these peptides could certainly play a role [48, 51].

Navab et al. have formulated and tested mimetic peptides containing two to eight phenylalanines (2F to 8F) in the investigation of their anti-inflammatory properties regarding lipid vascular pathology [2, 43-45]. Peptide nomenclature indicates the number of phenylalanines on the hydrophobic face, which contributes to differing anti-inflammatory properties as phenylalanine residues render hydrophobicity and increase hydrophobic action [2]. The peptide may be used as a lipid-carrier with its hydrophobic face, effectively reducing inflammation by picking up oxidized lipids to decrease inflammatory LDL and improve the anti-inflammatory properties of HDL[2, 43-47, 52]. Figure 1 displays the sequences of 4F and 6F peptides. The helical wheel structure of ApoA-I mimetic peptide is shown in Figure 2.

D-4Fpeptide and ApoA-I protein (postulated to possess eight tandem repeating 22mer sequences) may be considered examples of class A amphipathic helical structures (93). The D-4F peptide is the most studied apolipoprotein mimetic peptide [52]. It is commonly referred to as L-4F if made with the L-amino acids and D-4F if made with D-amino acids. D-4F, an18-amino acid peptide, mimics the tertiary structure of the main protein component of HDL, apolipoprotein A-I, and is easily absorbed without liver toxicity [11]. A potential advantage of D-4F peptide is that is resistant to proteolysis because it contains all

D-amino acids. Thus, gut peptidases cannot recognize it in the same way as naturally occurring proteins that are composed of L-amino acids. D-4F is therefore not degraded in the gut and is additionally orally bioavailable [43, 53]. The ApoA-I mimetic peptide D-4F showed promise in early human trials [53], however, due to the potential of long-term tissue accumulation, its clinical development has been halted [54]. L-4F would not have this potential problem. Niclosamide, a tineacide in the anthelmintic family used to treat tapeworms, in combination with L-4F appears to be a promising method for oral delivery of this peptide[55]. The impacts of D-4F and L-4F on biomarkers and lesion areas were comparable in cholesterol-fed rabbits when administered by subcutaneous (SC) injection[56]. Hence, clinical trial studies of L-4F administered to humans by either SC injection (ClinicalTrials.gov number, NCT00907998) or intravascular (IV) infusion (ClinicalTrials.gov number, NCT00568594) were initiated to assess the HDL anti-inflammatory effects of ApoA-I mimetic peptides [54].

Neuroinflammation and neurodegenerative disease

Heneka et al. [35]discussed the pivotal role of the innate immune system in neurodegenerative diseases. Misfolded proteins throughout the brain divert resting microglia to become activated microglial cells. These cells secrete a wide range of inflammatory factors, including Th1 cytokines such as IL-6, Interferon γ (INF-γ), tumor necrosis factor TNF-α, IL1β, chemokines such as macrophage inflammatory protein 1α (MIP-1α), monocyte chemotactic protein 1 (MCP-1), along with secreting reactive oxygen species and complement species such as C1q, C3, C4 and C9 [38]. Sustained exposure of neurons to pro-inflammatory mediators such as these eventually lead to neuronal damage in structure and function, ultimately ending in cell death [8].

4F peptide and modulation in the innate immune system

D-4F and its stereoisomer L-4F most likely influence multiple pathways in the cells. It has been suggested that serum levels of HDL are not increased by D-4F oral administration but that HDL function is enhanced.D-4F does increase pre-beta HDL formation, efflux of cholesterol, and conversion of dysfunctional pro-inflammatory HDL to functional anti-inflammatory HDL[57-59]. D-4F also alters the particle size distribution and metabolism of HDL [60]. Buga et al. previously proposed a role of inflammation and the innate immune system in Western diet-induced neuroinflammation. Feeding a Western diet provided to ($LDLR^{-/-}$) mice resulted in a marked increase in the number of microglia within arterioles which was statistically significant and was prevented in mice on a supplemental D-4F diet ($p=0.01$) [12]. Active D-4F was effective in this respect while scrambled D-4F peptide (a control inactive peptide with the same D-amino acids in scrambled order which contributes no specific hydrophobic face and no lipid-binding) did not affect. One potential mechanism of action forD-4F might be triggering the innate immune system. We suggest that D-4F reduces neuronal damage by affecting the number and the function of CNS-resident and/or blood-derived innate immune cells. The schematic view of this concept is shown in Figure 3.

D-4Fpeptide reduces MCP-1 and MIP-1α expression in vascular and non-vascular brain cells

In hyperlipidemia, LDL phospholipids and fatty acids in the artery walls undergo oxidation in inflammatory pressure, which induces chemotactic factors including MCP-1, a regulator of monocyte migration from the bloodstream across the vascular endothelium [61]. Monocytes, in turn, can oxidize LDL lipids, thus generating a cycle. The increase in MCP-1 levels and macrophages in the brain is similar to the inflammatory changes observed in the kidney and the aorta after feeding a

Western diet to LDLR$^{-/-}$mice, which is shown to be decreased by D-4F therapy [62, 63]. It has been reported that even during human fetal life, large arteries including the aorta contain macrophages in their walls [33].

Inflammation affects large and small arteries, as well as arterioles that range from 10-100 micrometers in diameter [64]. MCP-1 and MIP-1α are both found in atherosclerotic lesions of mouse models [65]. In cultured human arterial cell walls, oxidized lipids, such as the products of arachidonic acid oxidation, induce LDL to stimulate the release of the chemokines MIP-1α and MCP-1. These factors are inactivated by the HDL-associated antioxidant enzyme paraoxonase and are removed or inactivated by ApoA-I or normal HDL [44, 66]. These oxidized phospholipids also inhibit several HDL-associated antioxidant enzymes [2]. The ApoA-I peptides, D-4F and L-4F, have four to six times higher affinity for oxidized and oxidizable lipids in comparison with native ApoA-I [67], but not for native lipids, and removes oxidized lipids from oxidized lipoproteins [2]. Following the removal of the oxidized lipids, HDL associated antioxidant enzymes are reactivated [2]. After oral administration in mice, the ApoA-I peptides have been shown to improve the ability of HDL to inhibit LDL-induced production of MCP-1 by human aortic endothelial cells [30, 43, 45, 55, 56, 67, 68].

In mice placed on a Western diet, the inflammatory markers such as MCP-1 and MIP-1α are found to be elevated. MCP-1 was shown to be markedly reduced upon treatment with active D-4F, but not scrambled D-4F ($p=0.001$) [62]. The chemokine receptors expressed by mature neurons have been suggested to regulate neuronal survival and synaptic transmission [69, 70]. Therefore, it is possible that chemokine signaling in the brain has functions beyond a role in neuroinflammation. Interestingly, F4/80 immunostaining was only reduced by D-4F in arterioles. It is therefore suggested that arterioles can be regarded as centers of hyperlipidemia-induced inflammation, from which soluble chemokines can spread to adjacent nonvascular cells and induce dysfunction; this concept is novel, and still undergoing further research. The spread of chemokines from brain arterioles to adjacent nonvascular brain cells can also be considered to play a partial role in observed cognitive changes, as seen by Buga and colleagues [12].

The vascular hypothesis of AD

The amyloid-beta(Aβ)peptides of 36–43 amino acids are crucially important contributing factors in AD pathogenesis[71, 72]. However, AD pathology is not limited to the distribution of amyloid plaques and neurofibrillary tangles. The vascular hypothesis of AD, first proposed by de la Torre et al. [73] in 1993suggests that pathogenesis commences with hypoperfusion or reduced cerebral blood flow. This, in turn, results in a crisis among non-vascular brain cells (neurons and glia), eventually culminating in neurodegeneration and cognitive impairment[74]. This concept has become a rich source of interdisciplinary research involving primarily the brain and cardiovascular system since 1993 [75-79]. There is ample evidence indicating that vascular risk factors cause blood-brain barrier (BBB) dysfunction and a reduction in blood flow to the brain, initiating a cascade of events that precede cognitive decline and dementia [16, 80, 81]. The earliest stages of AD involve oxidation-induced inflammatory damage to small blood vessels involved in the BBB [4, 82, 83]. Vascular aging, arterial stiffness, and endothelial dysfunction leading to BBB dysfunction impairing several protective mechanisms in the brain and subsequently ending in neuronal damages and dementia [15, 83]. Recent studies have shown that more than 30% of AD cases exhibit vascular pathology [84]. Figure 4 demonstrates the overlap that exists between AD and vascular diseases, in tandem with Aβ deposition and brain atrophy.

There is a growing body of evidence that vascular risk factors including hyperlipidemia, obesity, diabetes, and

hypertension lead to vascular disease and endothelial dysfunction [22, 85]. These changes result in BBB dysfunction and reduction in blood flow, leading to reduced Aβ clearance. Vascular injuries also induce Aβ production from the Aβ precursor protein (APP). These factors cause the accumulation of Aβ in the neurovasculature and brain tissue [16]. Conversely, hypoperfusion contributes to the accumulation of toxic substances that increase the risk of neurodegeneration (non-Aβ pathway). Accumulation of Aβ and/or hypoperfusion can lead to hyperphosphorylation of tau (p-tau), inducing neurofibrillary tangle formation that ultimately contributes to neuronal dysfunction, accelerated cell death, and dementia [4, 16, 85]. Figure 5 shows hypothesized pathways of decreased cognitive dysfunction in AD originating from cerebrovascular disorder.

D-4F ameliorates Western diet-induced increases in brain arterial wall thickness

In a recent study, we reported that brain arteriole wall thickness in $LDLR^{-/-}$ mice was greater when compared to wild-type mice, and further thickening was observed in $LDLR^{-/-}$ mice on a Western diet. Western diet-induced increase in arterial wall thickness was additionally reduced by treatment with active D-4F[12]. Additionally, smooth muscle α actin was markedly increased in arteriolar walls, which was subsequently reduced in the group receiving active D-4F, but not in the group treated with scrambled D-4F. Our finding of a positive linear correlation between wall thickness and the number of microglia per arteriole indicates microglia directly contributes to wall thickness[12]. However, although highly significant ($P< 0.0001$), the correlation ($r = 0.65$ and $r^2 = 0.42$) suggests that only 42% of the variation in wall thickness is explained by the number of microglia per arteriole, and other factors must have been involved in determining arterial wall thickness. Wall thickness increases with increasing vessel size; however, vessel diameter was not a factor in this study since all arterioles studied had the same internal luminal diameter [12]. The increased arterial wall thickness may be partly due to increased collagen synthesis, which may be induced by oxidized lipids and prevented by D-4F[21, 86]. Western diet has been found to increase the level of MCP-1 in brain arterioles, which can also be reduced by D-4F [12]. MCP-1 can potentially play a role by increasing smooth muscle-α actin and is reported to contribute to vascular hypertrophy [70]. A Western diet therefore clearly induces inflammatory response throughout the arterial tree in $LDLR^{-/-}$ mice. It is notable that although cerebral blood flow was not directly measured in our study, we did not find an alteration in blood pressure, suggesting these inflammatory effects are independent of mechanisms related to blood perfusion regulation[12].

D-4F improves Western diet-induced cognitive impairment in mouse models

It has been shown that feeding a Western diet to $LDLR^{-/-}$ mice induces brain arteriole inflammation and causes cognitive impairment [12]. All mice were initially tested for cognitive performance in the standard T-maze continuous alternation task system [87] and the Morris Water Maze [17, 18, 88]. The mice showed significantly reduced mental capacity in both systems following 6-8 weeks on the Western diet. $LDLR^{-/-}$mice treated with oral D-4Fexhibited significant reduction in inflammation and cognitive impairment without significant changes in plasma lipids and lipoproteins, blood pressure, or arteriole lumen size [12, 63].D-4F also ameliorates cognitive function and reduces inflammatory properties (TNFα and IL-1β) and amyloid burden in the brain of APPSwe-PS1 Delta E9 mice [13]. Although D-4F treatment was shown to improve cognitive behavior in both the T-maze continuous alteration test and the Morris water maze test, the mechanism by which it reversed cognitive dysfunction is unknown.

As noted above, administering a Western diet to $LDLR^{-/-}$ mice causes LDL,

monocyte, and macrophage accumulation, and endothelial dysfunction in very small arteries such as brain arterioles. These processes eventually lead to arterial thickening and dysfunction. This result is similarly observed in large arteries such as coronary arteries and the aorta [19, 20]. Administering D-4F is shown to markedly reduce lipid accumulation in arteries and significantly reduce facial arterial wall thickening [21]. The role of peptide D-4F in reducing pro-inflammatory lipids is likely the mechanism through which D-4F significantly reduces lesions in mouse models with atherosclerosis[2]. This may also explain why D-4F reduces inflammation after viral infections[22, 23]. It has been shown that hyperlipidemia induces inflammation of brain microvessel, and this inflammation in such small vessels is similar to that previously seen in large blood vessels. However, the location of macrophages associated with small arterioles differs from that observed in larger vessels. In coronary arteries or the aorta, macrophages were located between endothelial cells and medial smooth muscle cells, whereas the microglia associated with the brain arterioles were associated with the adventitial layer [12].

However, the mechanism of action of D-4F in AD mice is still unclear. Although it has been suggested that D-4F modulates the inflammatory properties of circulating lipoproteins, in particular HDL, and to reduce amyloid deposition, it is not clear whether the peptide crosses the BBB and enters the brain [13]. It is also unclear whether the peptide alone would reduce the Aβ burden. Handattu et al. [13]demonstrated that anti-inflammatory peptide D-4F in combination with pravastatin (to enhance the effectiveness of the peptide) improves cognitive function and inhibits amyloid β deposition in the hippocampus of AD mouse models. It is still possible that D-4F alone would inhibit Aβ deposition in this mouse model, and experiments are currently underway[2].

D-4F has been demonstrated to reduce the number of activated microglia and astrocytes that are involved in generating cytokines, oxidized lipids, and other pro-inflammatory molecules [13]. It can be concluded that hyperlipidemia-induced inflammation of brain arterioles leads to the production of chemokines by the arterioles, which spreads from brain arterioles to adjacent nonvascular brain cells and deteriorates their function, causing deterioration in cognitive performance [12]. Handattu et al. [13]observed that apo A-I mimetic peptide D-4F was effective in reducing vascular wall inflammation and improving cognitive performance, additionally showing effectiveness in treating animal AD models. Improved cognitive function can be interpreted to be partly due to decreased MCP-1 and MIP-1α levels, resulting in a reduction in the inflammatory response in the group receiving D-4F treatment.

Figure 6 demonstrates the hypothesized pathways through which Apo-AI mimetic peptides such as D-4F reduce the risk of AD. As it is shown, Western diet-induced microgliosis, MCP-1 and MIP-1α increases, and arterial wall thickening could be effectively prevented by D-4F. In defining the possible mechanism of D-4F action, it should be recognized that D-4F improves cognitive impairment in AD by targeting and reversing vascular pathology [13]. Furthermore, D-D-4F can affect the role of HDL in modulating inflammatory response or nonspecific innate immunity [89, 90], which plays a crucial role in neurodegenerative disease as shown by Heppner et al. [8].

Similar to beliefs regarding hyperlipidemia, data suggest that vascular disease risk factors, potentially including inflammation in individuals with chronic kidney disease (CKD), may contribute to cognitive dysfunction [62, 63, 91]. Regarding CKD inflammation, it has also been exhibited that D-4F reduces renal oxidized phospholipids, decreasing renal inflammation [63]. Additionally, investigators have found a relationship between kidney disease and risk

for AD development and cognitive impairment [62, 92].

CONCLUSIONS

ApoA-I mimetic peptides may be promising new agents for the treatment of a variety of diseases of small arterial vessels. As noted above, an oral ApoA-I mimetic peptide called D-4F has been demonstrated to be effective in ameliorating hyperlipidemia-induced inflammation in large, small, and very small (e.g., the brain arterioles) vessels. It has been shown that feeding a high-fat diet (Western diet) to $LDLR^{-/-}$ mouse models caused brain arteriole inflammation and cognitive impairment. This was significantly improved with orally administered D-4F without altering plasma lipids, blood pressure, or arteriole lumen size. Although the best-understood mechanism of action of ApoA-I mimetic peptides is through their ability to promote cholesterol efflux from cells and to act as anti-oxidants, their effectiveness in animal models of several various diseases suggests that they may work through various mechanisms. Western diet resulted in a two-fold increase in the percent of microglia associated with brain arterioles, along with a significant increase in chemokine MIP -1α and MCP-1 levels. D-4F peptide has been shown to reduce the levels of MIP-1α and MCP-1 and microglia-induced pro-inflammatory mediators. It is reasonable to presume that such properties of D-4F contribute to beneficial effects demonstrated in terms of reducing wall thickness, altering chemokine secretion, and improving cognitive performance to counteract detriments induced by Western diet and/or AD. Because only MIP-1α and MCP-1 levels were measured, relations with other circulatory cytokines could not be assessed; therefore, there is a possibility that D-4F peptide may also play a role in reducing other cytokine levels and microglia-induced pro-inflammatory mediators. Hopefully, this pathway will be a fruitful line of investigation for future studies. However, due to the complex pathogenesis of AD, accurate interpretation of the peptide mechanism of action is potentially problematic. Finally, Phase I trials on these peptides have only just started, in the recent years, and considerable investigation in terms of behavioral and biochemical studies in animal models, and eventually clinical trials, needs to be performed in developing apolipoprotein mimetic peptides into therapy for neurodegenerative disorders.

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Peptide Name	Amino acid Sequences
4F peptide	Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH2
6F peptide	Ac-D-W-L-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH2

Figure 1: ApoA-I mimetic peptide. Naming - such as 4F or 6F - is dependent on the number of phenylalanines (amino acid code F).

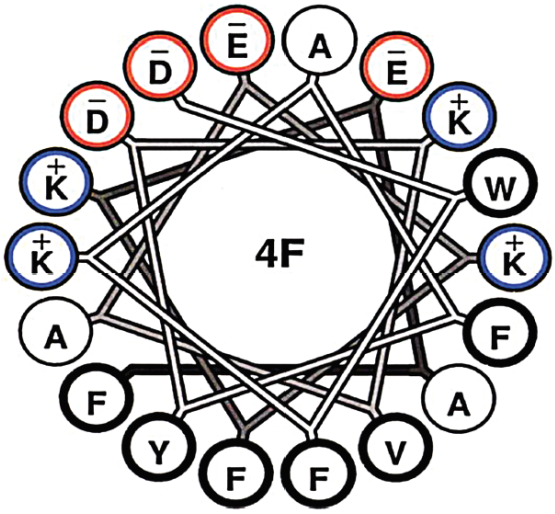

Figure 2: Helical wheel model of D-4F peptide of ApoA-I mimetic peptide**.**

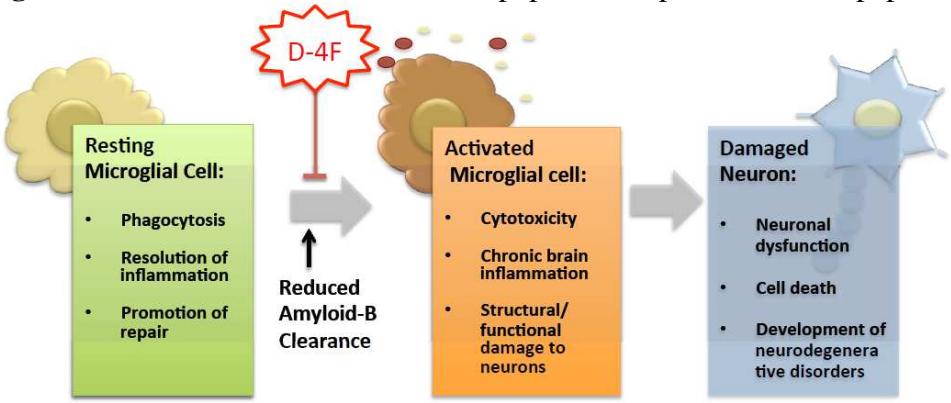

Figure 3: Pathway of chronic neuroinflammation contribution to neurodegenerative disease. Resting microglial cells convert to activate microglial cells. Activated microglial cells produce inflammatory mediators such as reactive oxygen species, nitric oxide, cytokines (TNF-α, IL-Iβ), and chemokines (MIP-1α, MCP-1). Inflammatory mediators take action which leads to structural and functional neuronal damage, eventually initiating cell death and contributing to neurodegenerative disease. D-4F is shown to reduce both the percent of microglia located on the adventitial side of the vessels and the level of chemokines MIP-1α and MCP-1.

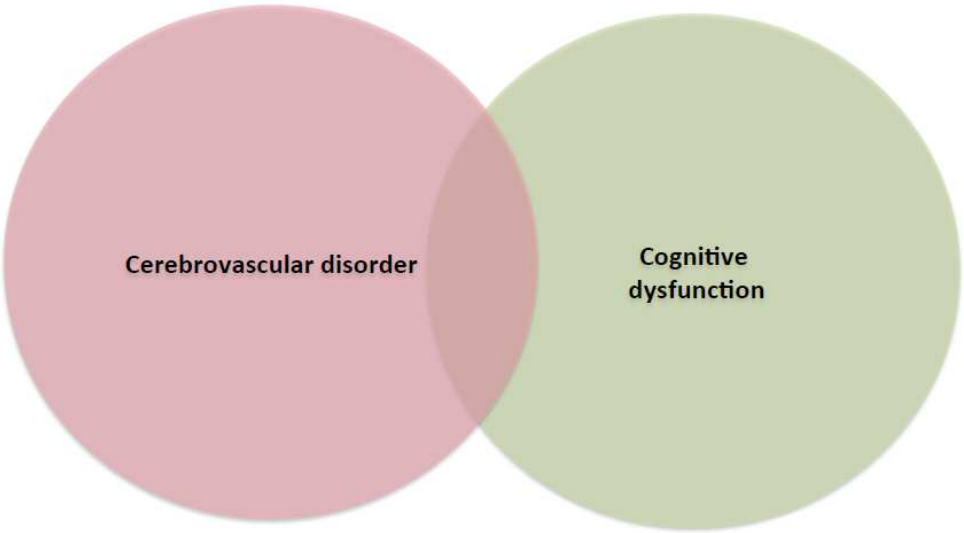

Figure 4: Essential overlaps exist between Alzheimer's disease and vascular disease. It is recognized that vascular risk factors predispose individuals to Alzheimer's disease (details in text below).

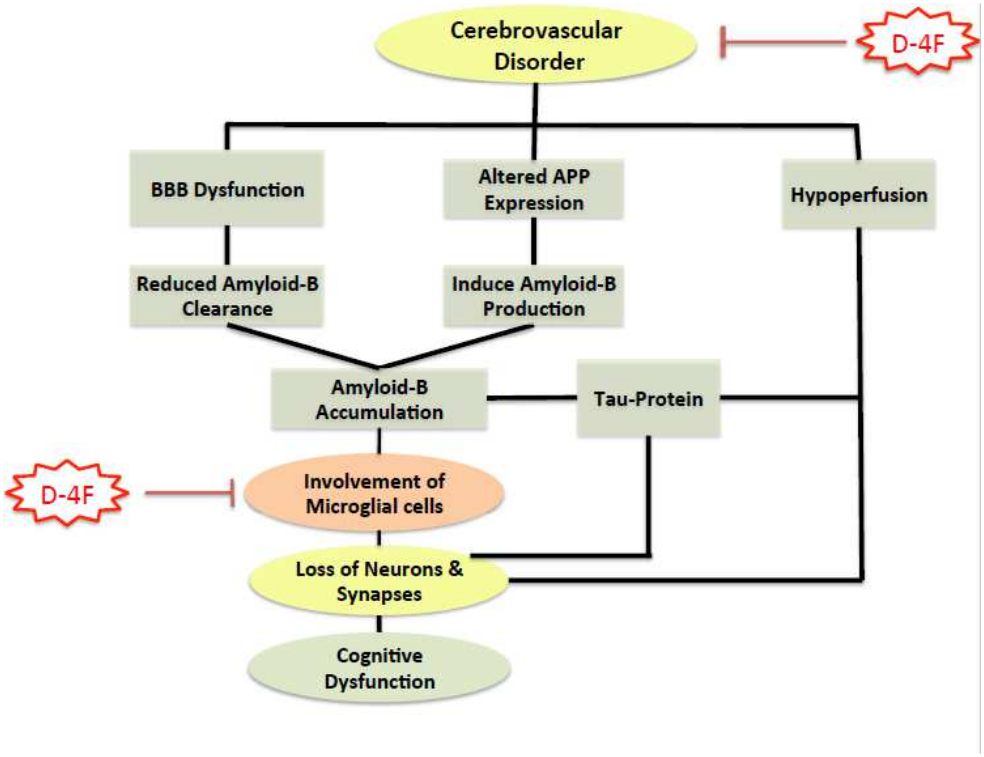

Figure 5: Role of hypothesized pathways targeting vascular pathology in cerebral arterioles for the risk of Alzheimer's disease (for details see text).

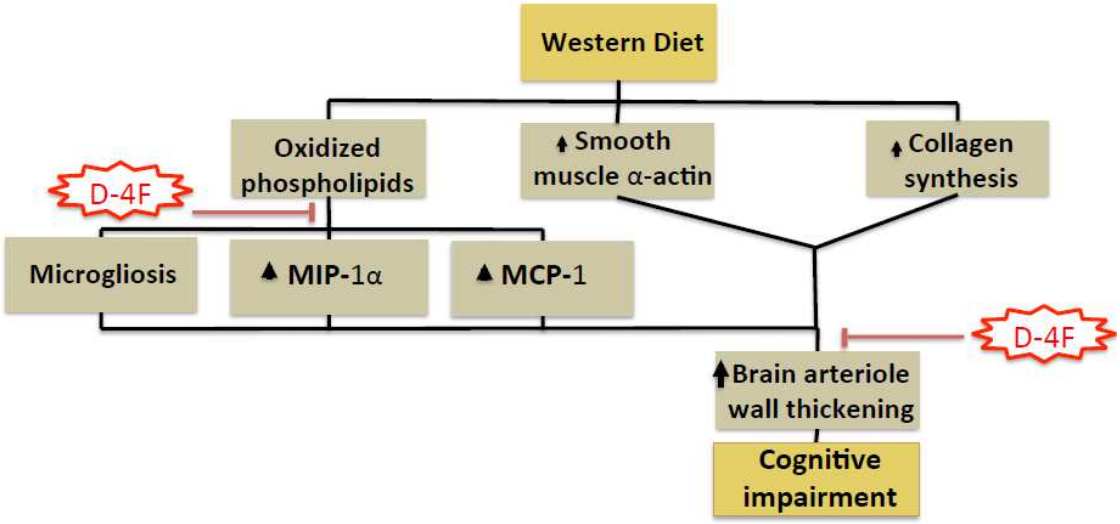

Figure 6: Adding Western diet to LDL receptor deficient mice leads to inflammation in brain arterioles. Western diet increases the number of arterioles with microglia on the adventitial side of the vessels, which was ameliorated by D-4F. Additionally, MIP-1 α and MCP-1 are induced by Western diet and are reduced by D-4F. Western diet resulted in arterial wall thickening by inducing inflammation in brain arterial walls, increasing the amount of smooth muscle-α actin and collagen synthesis, both of which are reduced by D-4F. As a result, D-4F improves hyperlipidemia-induced cognitive impairment in the LDL receptor deficient mouse (for details see text).