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Review

# Mitochondrial mechanisms in amyloid beta peptide-induced cerebrovascular degeneration

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ABSTRACT

Prevailing evidence suggests that amyloid beta peptide (Aβ), a key mediator in age-dependent neuronal and cerebrovascular degeneration, activates death signaling processes leading to neuronal as well as non-neuronal cell death in the central nervous system. A major cellular event in Aβ-induced death of non-neuronal cells, including cerebral endothelial cells, astrocytes and oligodendrocytes, is mitochondrial dysfunction. The death signaling cascade upstream of mitochondria entails Aβ activation of neutral sphingomyelinase, resulting in the release of ceramide from membrane sphingomyelin. Ceramide then activates protein phosphatase 2A (PP2A), a member in the ceramide-activated protein phosphatase (CAPP) family. PP2A dephosphorylation of Akt and FKHRL1 plays a pivotal role in Aβ-induced Bad translocation to mitochondria and transactivation of Bim. Bad and Bim are pro-apoptotic proteins that cause mitochondrial dysfunction characterized by excessive ROS formation, mitochnondrial DNA (mtDNA) damage, and release of mitochondrial apoptotic proteins including cytochrome *c*, apoptosis inducing factor (AIF), endonuclease G and Smac. The cellular events activated by Aβ to induce death of non-neuronal cells are complex. Understanding these death signaling processes will aid in the development of more effective strategies to slow down age-dependent cerebrovascular degeneration caused by progressive cerebrovascular Aβ deposition.



## 1. Introduction

Stroke is the second leading cause of death and the leading cause of adult disability worldwide according to World Health Organization statistics. Age is the most important stroke risk factor [1,2]. The burden of stroke on the health care system and the society as a whole is likely to aggravate with accelerated aging of the population. Vascular dementia is also a serious burden to families and the society. This review on Aβ in cerebrovascular degeneration focuses on mitochondrial dysfunction affecting cerebral endothelial cells (CECs) with selected ancillary findings on other cell types. Extensive works by a number of distinguished investigators on Aβ effects in vascular smooth muscles, particularly Dr. W.E. Van Nostrand's group, are not extensively covered because of space constraints.

### 1.1. Aging and cerebrovascular diseases

Prevailing evidence suggests that cerebrovascular function declines with aging [1,3–8]. Atherosclerosis is an important cause of age-dependent degeneration of large arteries. However, another aging process that affects primarily the microvessels has emerged in the study of the aging brains, particularly those with Alzheimer's disease (AD). The cerebral vasculature appears to share a common fate with the brain parenchyma in age-dependent amyloid deposition. With aging, cerebrovascular changes include decreasing number of endothelial cells, thinning of the capillary wall, and reduced endothelial mitochondrial density [9,10].

### 1.2. Amyloid in the cerebral vasculature

Amyloid deposition in the brain is an aging process noted widely in primates and other species [11,12]. Research on the pathology and molecular mechanisms of AD has focused primarily on amyloid deposition in the brain parenchyma. The cerebral vasculature is also a primary target of amyloid deposition resulting in cerebral amyloid angiopathy (CAA) in the aging brains with or without AD [7,13–19]. Studies in hereditary types of CAA (Dutch and Icelandic, and others) show that amyloid deposition may occur predominantly in the cerebral vasculature [20–26]. Recent data from brain imaging studies in humans and animal models suggest that cerebrovascular dysfunction may precede cognitive decline and onset of neurodegenerative changes in AD and AD models [27]. Cerebral hypoperfusion and impaired amyloid β-peptide (Aβ) clearance across the blood brain

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barrier (BBB) may contribute to the onset and progression of AD [18,28–30]. CAA is a major stroke risk factor in the elderly causing hemorrhagic or ischemic strokes [1,19,25,31–33]. Patients with CAA may present clinically with recurrent strokes with or without dementia [34–38]. Vascular amyloid deposition is accompanied by dysfunction and loss of mitochondria in endothelial cells. Endothelial cell death may contribute to vascular degeneration [3,9,10,15,39]. Other features of amyloid angiopathy are thickening of the basement membrane, irregularities of vasculature, alteration of collagen content [3,7,14,40], and vascular smooth muscle degeneration [40–43]. Understanding the molecular mechanism of amyloid-induced cerebrovascular degeneration may contribute to the development of stroke preventive measures in the elderly.

### 1.3. Amyloid β peptide (Aβ)

The major component of amyloid deposits in the brain parenchyma and cerebral vasculature is a small and unique peptide, amyloid β (Aβ) [33,44]. The cellular origin of Aβ that causes CAA remains to be fully defined [45,46]. Aβ is a 39–43 amino acid peptide derived from proteolytic cleavage of the amyloid precursor protein (APP) [47]. Aβ has been consistently demonstrated in senile plaques and leptomeningeal and intracortical vessels in the AD brains but to a lesser extent in the aging brain without AD. Aβ peptides that accumulate in the AD brains are heterogeneous. $Aβ_{1-40}$ and $Aβ_{1-42}$ are the most commonly encountered species. In some AD brains $Aβ_{1-42}$ may be the dominant peptide in the senile plaques, while the dominant species in cerebral vasculature is $Aβ_{1-40}$ [7,48–53]. The demonstration of APP mRNA in the vascular wall supports the contention that locally derived Aβ contributes to cerebrovascular degeneration in aging brains including those with AD and hereditary CAA [54]. Recent studies suggest that Aβ accumulation in the AD brain is likely due to its impaired clearance from the brain [55,56]. LDL receptor-related protein 1 (LRP1) is a major Aβ transporter at the BBB [30,57]. Binding of Aβ to LRP1 initiates Aβ clearance from brain to blood via transcytosis across the BBB [18,28]. Aβ may also be eliminated by proteolytic degradation [58]. In addition, soluble Aβ probably originates from the peripheral circulation as well as the cells within the central nervous system [59,60]. Human platelets contain high levels of APP, which may contribute to more than 90% of the circulating APP [61]. Platelet APP may also be the major source of Aβ detected in whole blood [62]. Aβ is released upon platelet activation [63]. The main species of Aβ released from activated human platelets is $Aβ_{1-40}$, consistent with the contention that circulating Aβ contributes to vascular amyloid deposits dominated by $Aβ_{1-40}$ [64]. However, exceptions have been noted [23]. The relative importance of different Aβ fragments in CAA remains to be fully defined.

### 1.4. Aβ and cerebrovasculature degeneration

Aβ has been implicated as the primary neurotoxic factor in the pathogenesis of AD [65]. Many lines of studies have shown that Aβ is also cytotoxic to non-neuronal cells including CECs [66–76], cerebrovascular smooth muscle cells [77], oligodendrocytes [78,79], and astrocytes [80]. In addition to neuronal degeneration, cerebrovascular alterations indicative of damage to vascular endothelial cells and disruption of the BBB occur in AD [5,81]. Aβ also impairs BBB function in vitro [82] and in vivo [72]. Since CECs and astroglia are the two major constituents of BBB to shield the brain from damage by harmful circulating toxins or deleterious cellular elements, Aβ induced death of these two cell types may lead to the disruption of BBB. [16,18,30,56]. Other detrimental or angiopathic effects of Aβ include arterial hypercontractility, cerebral blood flow dysregulation, [17], enhancement of endothelial permeability and defective glucose transport [72,82]. Aβ deposition may also increase brain vulnerability to ischemic injury [83,84], probably related to its additional vascular effects, including platelet aggregation [85], leukocyte activation, promotion of inflammatory reaction [86,87], inhibition of endothelial proliferation [16,88], alteration of cerebrovascular reactivity [89], disruption of the basement membrane [90] and increase in BBB permeability [91]. Overall, Aβ appears to cause multiple detrimental effects in the pathogenesis of age-dependent angiopathy. Findings characterizing the cell death pathways that underlie Aβ cytotoxicity in non-neuronal cells including CECs and astrocytes may aid in preserving BBB integrity and slow down age-dependent cerebrovascular degeneration.

## 2. Mitochondrial mechanisms in Aβ-induced non-neuronal cell death

### 2.1. Aβ induction of mitochondrial dysfunction

Mitochondrial dysfunction may be a major mechanism of aging and neurodegenerative disorders including stroke. Accumulating evidence suggests mitochondrial dysfunction may trigger apoptosis [92–95] and is a key mechanism of cell death in disease states [96]. This has led to a renaissance on the study of this organelle. Because mitochondria are the major consumers of molecular oxygen within cells, they stand as one of the most important generators of reactive oxygen species (ROS). Mitochondria are a primary target of therapeutic interventions in pathologic states involving oxidative stress and apoptosis [96–100].

Aβ induced CEC death is characterized by a number of biochemical and morphologic features indicative of apoptosis [73,76]. $Aβ_{1-40}$ was noted to be more potent than $Aβ_{1-42}$ in causing endothelial cell death [101,102]. $Aβ_{25-35}$, a synthetic fragment of Aβ, which shares selected Aβ effects is also cytotoxic to CECs [71,73,76]. Aβ cytotoxicity was thought to be related to excessive formation of ROS such as superoxide and can be suppressed by antioxidants [73,103]. Aβ activation of caspases is accompanied by mitochondrial DNA (mtDNA) damage, and mitochondrial dysfunction [73,104]. Events associated with Aβ-induced mitochondrial dysfunction leading to apoptosis include excessive ROS formation, and release of mitochondrial apoptotic proteins such as cytochrome *c*, apoptosis inducing factor (AIF), endonuclease G (endoG) [105] and Smac [73,74,105]. A consequence of excessive mitochondrial ROS formation is mtDNA damage. Cumulative mtDNA damage is a cellular marker of aging [106]. mtDNA damage caused by Aβ can further compromise mitochondrial function, feeding another positive loop of apoptosis.

### 2.2. The neutral sphingomyelinase-ceramide cascade

The molecular mechanism of Aβ induced mitochondrial dysfunction and subsequent cell death in non-neuronal cells remains to be fully defined. It appears oxidative stress induced by Aβ contributes to its cytotoxicity [73,101]. Ceramide is a lipid mediator that also causes excessive ROS formation and subsequent apoptosis in a number of cell types [107]. Since both Aβ [73,104] and ceramide [108] share common features in death signaling processes (mitochondrial dysfunction and excessive ROS generation), efforts have been devoted to explore whether ceramide is a mediator of Aβ-induced apoptosis in non-neuronal cells including CECs, astrocytes, or oligodendrocytes [79,80,109]. To define the causal role of a ceramide synthetic pathway in Aβ-induced cell death, there are at least 3 putative enzymes for cellular ceramide formation to explore. Two of these involve the degradation of sphingomyelin by sphingomyelinase (SMases) to release ceramide. Neutral SMase (nSMase) and acidic (aSMase) are respectively implicated in a number of cell death paradigms [108,110,111]. The 3rd cascade entails de novo ceramide synthesis catalyzed by ceramide synthase [109]. Increase in nSMase activity is linked to cellular senescence [112]. nSMase has also been identified in cerebral microvessels [113]. We demonstrated that Aβ-induced non-