

New Insights into Possible Role of NOS-NO-ADMA Pathway Dysfunction in the Development of Cognitive Decline and Dementia: Exploring the Vascular Features of Alzheimer's Disease

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ABSTRACT

In recent years significant overlap between cardio-metabolic risk factors and cognitive decline has been reported. Cardio-metabolic and vascular factors shown to be associated with Alzheimer's disease (AD) and other forms of dementia include midlife diabetes and hypertension, cerebrovascular lesions, diminished vascular function, dyslipidemia, obesity, and cigarette smoking. Accordingly, it has been recently proposed that amyloid is not the cause of AD but merely a marker and a later consequence of upstream changes that lead to neuronal and synaptic losses. However, although the idea that features of vascular dysfunction and injury are present in cognitive decline and AD patients was suggested over 25 years ago, the role of cardio-metabolic and cerebrovascular mechanisms in the pathogenesis of AD is far from being fully elucidated. Based on newly proposed vascular hypothesis, there is an impaired structure and function of cerebral blood vessels and cells in patients with cognitive decline and AD which is mediated by vascular oxidative stress. Consistent with these observations, the importance of endothelial dysfunction in the development of AD has been highlighted. One of the most prominent features of endothelial dysfunction is decreased production and bioavailability of Nitric

Oxide (NO). Based on versatile properties in physiological as well as in pathophysiological conditions NO is regarded as a key molecule for longevity and cardiovascular health. NO is produced by three different isoforms of the enzyme NO synthase (NOS), namely: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Reduced NO bioavailability and altered vascular expression and activity of NOS enzymes are implicated as major molecular players in the process of vascular aging. In the process of aging, the mechanisms by which NOS enzymes promote vascular dysfunction are specific for each NOS isoform. Normal activity of eNOS is required for the balanced production/bioavailability of NO, which is the main prerequisite for optimal vascular function. Conversely, excessive amount of NO produced by iNOS contributes to vascular dysfunction. Furthermore, although the possible role of nNOS-derived NO in aging-associated vascular dysfunction is far from being fully elucidated, experimental findings indicate that impaired perivascular NO release from nNOS increases vasoconstriction in aged arterioles. Oxidative stress and inhibitors of NO synthase are regarded as most potent initiators of disturbed production and bioavailability of NO. Important endogenous inhibitor of all three isoforms of NOS is asymmetrical dimethylarginine (ADMA). It has been recently proposed that ADMA may be a possible link between vascular disease and dementia. Based on this assumption ADMA might represent a unifying pathophysiological pathway linking the presence of vascular risk factors with the onset and progression of cognitive decline and dementia. These observations were made based on good evidence from literature that higher plasma ADMA concentrations favor atherosclerosis and independently predict adverse cardiovascular and cerebrovascular outcomes in several patient groups. However, possible role of ADMA in pathophysiology of cognitive decline and AD is still not fully understood. Prospective studies are warranted aiming at further clarification of ADMA involvement in development and progression of dementia. Moreover, use of ADMA in CSF and /or plasma as prospective biomarker of

AD should be explored. According to newly introduced hypothesis disturbed NOS-NO-ADMA pathway is one of the most important attributes of cerebrovascular dysfunction that plays a pathogenic role in the development of cognitive decline and dementia. Experimental data from cell cultures and animal models have demonstrated that dysfunction of the NO-NOS-ADMA pathway results in cell death, blood-brain barrier (BBB) disruption, and brain edema via different pathological mechanisms. However, exact underlining mechanisms through which disturbed NOS-NO-ADMA pathway contributes to cognitive decline and dementia remain to be elucidated. Clarification of these mechanisms may be helpful in the identification of new therapeutic targets for aging and neurodegenerative diseases. Future research should also explain whether endothelial dysfunction precede or follow neurological changes which characterize cognitive decline and dementia. Moreover, clinical and epidemiological studies have shown that healthy nutrition and physical exercise are important non-pharmacological, lifestyle-related interventions that could help in maintaining appropriate vascular tone, adequate cerebral blood flow and normal cognition during aging. It is reasonable to suggest that prevention of cardio-metabolic diseases via endorsement of healthy aging determinants such as control of blood pressure, lipids and glucose levels as well as maintenance of optimal body weight achieved by regular physical activity and nutrition with high anti-oxidant capacity might be effective strategy in the preservation of cognitive function.

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Key words: Nitric Oxide (NO); Nitric Oxide Synthase (NOS); asymmetrical dimethylarginine (ADMA); NOS-NO-ADMA pathway dysfunction, cognitive decline; Dementia; Alzheimer's disease

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REVIEW

In recent years significant overlap between cardio-metabolic risk factors and cognitive decline has been reported. Accordingly, numerous studies have demonstrated that midlife diabetes and hypertension predispose individuals to Alzheimer's disease (AD) and vascular dementia^[1-3]. Other cardio-metabolic and vascular factors shown to be associated with AD include cerebrovascular lesions, diminished vascular function, dyslipidemia, obesity, and cigarette smoking^[4]. Although the idea that features of vascular dysfunction and injury are present in AD patients was suggested over 25 years ago, the role of cardio-metabolic and cerebrovascular mechanisms in the pathogenesis of AD is far from being fully elucidated. Nevertheless, the linkage of vascular contributions to cognitive impairment and dementia has been now well recognized and there is necessity for better understanding how vascular factors contribute to development of AD and related dementias.

In this line of evidence, the significance of vascular contributions to cognitive impairment and dementia has been underlined in recently published Statement for Healthcare Professionals by the American Heart Association/American Stroke Association^[5]. The American Academy of Neurology as well as The Alzheimer's Association

supported the value of guidelines reported in this Statement as an important attempt in the understanding of causes of dementia as well as the factors that contribute to its progression. The authors of mentioned Statement have introduced term vascular cognitive impairment (VCI) in order to capture the entire spectrum of cognitive disorders associated with all forms of cerebral vascular brain injury ranging from mild cognitive impairment through fully developed dementia. Based on the reported findings within the Statement, cerebral amyloid angiopathy is emerging as an important marker of risk for AD and VCI. Authors propose that the neuropathology of cognitive impairment in later life is often a mixture of microvascular brain damage and classical AD features. Furthermore, mechanisms regulating cerebral blood flow as well as dysfunction of the neurovascular unit are suggested as important factors in VCI development.

Brain endothelial cells, pericytes or vascular smooth muscle cells, glia and neurons constitute the neurovascular unit (NVU) that controls blood-brain barrier (BBB) permeability and cerebral blood flow. Dysfunction of BBB results in reduction of cerebral blood flow and consequent hypoxia alongside with accumulation of diverse vasculotoxic and neurotoxic molecules within brain parenchyma^[1]. All of these factors are thought to be associated with neurodegeneration seen in AD and related disorders.

Pathogenesis of Alzheimer's disease is multifactorial and still not known entirely. This most common form of dementia is neuropathologically characterized by presence of neurofibrillary tangles and senile neuritic plaques, amyloid- β peptide ($A\beta$) deposits and widespread neuronal loss. In everyday clinical practice reliable peripheral biochemical markers for diagnosis of AD are still not available and the disease itself can be manifested in wide range of neurological and psychiatric symptoms^[4]. Hence, definitive diagnosis of AD can only be confirmed by postmortem examination of the brain that must contain sufficient number of plaques and tangles. Plaques are extracellular deposits of fibrils and amorphous aggregates of $A\beta$ -peptide, whereas neurofibrillary tangles are intracellular fibrillar aggregates of the microtubule-associated protein tau which exhibits hyperphosphorylation and oxidative modifications^[6]. Pivotal characteristic of AD brain is altered proteolytic processing of amyloid precursor protein (APP) that leads to the production and aggregation of neurotoxic forms of $A\beta$ ^[4]. Thus, based on the "amyloid hypothesis", the toxic cause of neural/synaptic damage and dementia is thought to be amyloid- β . However, it has been recently proposed that amyloid is not the cause of AD but merely a marker and a later consequence of upstream changes that lead to neuronal and synaptic losses^[7]. According to this assumption impaired microvasculature, decreased cerebral blood flow and lack of vascular endothelial-derived trophic factors are causing atrophy of neural structures in already vulnerable brains of elderly with multiple age-related changes. Authors suggest that improvement of cerebral microvascular endothelial function may ameliorate the precipitating cause of AD and that future therapeutic strategies should focus on supporting normal angiogenesis.

Consistent with these observations, the importance of endothelial dysfunction in the development of AD has been highlighted in a recent review by Kelleher *et al*^[8]. According to newly proposed vascular hypothesis, there is an impaired structure and function of cerebral blood vessels and cells in AD which is mediated by vascular oxidative stress. These changes can have detrimental effects on the cognitive function in patients with coexisting neurodegeneration.

Main features of vascular hypothesis for AD have been nicely summarized in a paper by Zlokovic^[1]. In short, vascular risk

factors lead to blood–brain barrier (BBB) dysfunction and a reduction in cerebral blood flow. These detrimental changes result in diminished amyloid- β clearance as well as in its increased production from the amyloid- β precursor protein with consequent amyloid- β accumulation. The increase in amyloid- β leads to neuronal dysfunction and injury causing cognitive dysfunction and neurodegeneration with consequent dementia.

Pivotal role in vascular hypothesis for AD development has been given to vascular/endothelial dysfunction that according to numerous evidences from literature is closely related to cognitive decline and dementia^[9]. One of the most prominent features of endothelial dysfunction is decreased production and bioavailability of Nitric Oxide (NO).

Nitric oxide is a small, highly diffusible and reactive signaling molecule with a short life-time. NO mediates numerous physiologic processes including, among others, vasodilatation, regulation of immune response, neuronal development, neurotransmission, as well as memory and learning^[10]. NO also regulates gene transcription and mRNA translation, and produces post-translational modifications of proteins^[11].

NO is produced by three different isoforms of the enzyme NO synthase (NOS), namely: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). These NOS isoforms can be distinguished by their origin from different genes, and they also have distinct tissue localization, as well as diverse properties including specific regulation and various sensitivity to inhibitors^[12]. All three isoforms of NOS utilize L-arginine as the substrate whereas molecular oxygen and reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) serve as co-substrates in the process of NO formation. Several cofactors are necessary for NO synthesis such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and 5,6,7,8-tetrahydrobiopterin (BH4)^[11].

Endothelial NOS (eNOS) apart from endothelial cells can also be found in cardiomyocytes, vascular smooth muscle cells, thrombocytes, lung epithelial cells, hepatocytes and others^[13-15]. NO synthesized by eNOS has numerous physiological functions including vasodilatation, inhibition of thrombocyte adhesion and aggregation, as well as potent antiatherogenic effects^[16].

Neuronal NOS (nNOS) is expressed in specific neurons of the central nervous system (CNS), but it can also be expressed in hypophysis, skeletal muscles, kidneys, adrenal glands, skin, pancreatic islet cells, lung epithelial cells, vascular smooth muscle cells and other cells and tissues^[17]. Significance of nNOS in diverse synaptic signaling events including neurotransmission and neuromodulation has been verified in numerous studies^[18-21]. Learning, memory, neurogenesis, long-term regulation of synaptic transmission, as well as important role in stress and adaptive responses of the organism are some of the most prominent functions of nNOS-derived NO.

Both eNOS and nNOS isoforms are considered to be expressed constitutively and at least in some tissues their activity is Ca²⁺-dependent. Inducible NOS (iNOS) has been long thought to be Ca²⁺-independent and expressed only after induction under inflammatory conditions. After stimulation which requires a complex cytokine combination, a variety of cells are able to express iNOS including monocytes, mast cells, cardiac myocytes, vascular smooth muscle cells, hepatocytes or glial cells^[22,23]. Contrary to earlier opinion, it is now known that some specific cells such as human airway epithelium also show constitutive expression of iNOS. Expression of iNOS can be further promoted by presence of certain factors such as oxidative stress and, on the contrary, be suppressed by glucocorticoid

treatment^[12]. Upon induction, high amount of iNOS-derived NO is produced with cytotoxic effects in antiviral, antimicrobial, antiparasitic and tumoricidal processes. NO produced by iNOS is thought to mediate inflammation and non-specific immune defense and also has important role in septic shock^[24-26].

Although NO has numerous functions within human body, best studied and confirmed so far are those in vasculature and central nervous system. Studies have shown that constitutively synthesized NO plays an obligatory role in maintaining vascular homeostasis in the cerebral circulation^[27]. One of the most prominent features of cerebrovascular dysfunction seen in AD is cerebral hypoperfusion/hypoxia with impaired NO release^[28].

Reduced NO bioavailability and altered vascular expression and activity of NOS enzymes are implicated as major molecular players in the process of vascular aging. The term vascular aging reflects structural and functional disturbances of the vasculature with advancing age^[29]. It is of note that in the process of aging, the mechanisms by which NOS enzymes promote vascular dysfunction are specific for each NOS isoform. Normal activity of eNOS is required for the balanced production/bioavailability of NO, which is the main prerequisite for optimal vascular function. Conversely, excessive amount of NO produced by iNOS contributes to vascular dysfunction. Results from experimental studies suggest that decreased NO bioavailability as well as increased reactive nitrogen species (RNS) production contribute to aging-associated vascular dysfunction^[30]. Furthermore, although the possible role of nNOS-derived NO in aging-associated vascular dysfunction is far from being fully elucidated, experimental findings indicate that impaired perivascular NO release from nNOS increases vasoconstriction in aged arterioles^[31,32]. Based on versatile properties in physiological as well as in pathophysiological conditions NO is regarded as a key molecule for longevity and cardiovascular health.

Possible role of disturbed production and bioavailability of NO in the development and progression of dementia has been suggested by Colton *et al.*^[33] based on the findings from the study in a mouse model of Alzheimer's disease. Reduced synthesis and availability of endothelial NO may contribute to the development of dementia by at least two mechanisms: favoring the onset and progression of atherosclerosis, vasoconstriction, and impaired cerebral blood flow regulation; and by reduced neuroprotection^[34].

Oxidative stress and inhibitors of NO synthase are regarded as most potent initiators of disturbed production and bioavailability of NO^[35]. Important endogenous inhibitor of all three isoforms of NOS is asymmetrical dimethylarginine (ADMA)^[36]. Most prominent feature of ADMA actions besides inhibition of NO synthesis is promotion of oxidative stress and vascular damage^[34]. Moreover, ADMA is regarded as reliable non-invasive marker of endothelial dysfunction. Accordingly, studies conducted so far have shown increased plasma levels of ADMA in numerous diseases related to endothelial dysfunction such as coronary heart disease, hypercholesterolemia, hypertension and diabetes mellitus^[37]. However, only few studies so far have been evaluating alterations of ADMA concentrations related to cognitive decline and AD.

Results of a large case-control study including 80 AD patients and 80 healthy controls, matched for age, body mass index (BMI), and gender conducted by Arlt *et al.*^[35] have shown increased plasma ADMA concentration but decreased ADMA concentration in cerebrospinal fluid (CSF) of AD patients. Furthermore, significant association between decreasing ADMA levels in CSF and the severity of cognitive impairment was observed. One of the most important findings of this study is that CSF and plasma ADMA levels

are independently regulated and have to be regarded separately. In short, based on obtained data authors suggest that decreased ADMA levels in CSF might be related to oxidative stress and protein damage through increased peroxynitrite production in the brain. On the other hand, increased plasma ADMA concentration levels might independently contribute to AD pathophysiology by decreasing brain microperfusion.

Importance of decreased cerebrovascular perfusion as contributing factor to AD pathology has been suggested by other authors as well^[38-40]. In line with these remarks, Asif *et al*^[34] recently proposed ADMA as a possible link between vascular disease and dementia. Authors suggested that ADMA might represent a unifying pathophysiological pathway linking the presence of vascular risk factors with the onset and progression of cognitive decline and dementia. Their observations were made based on good evidence from literature that higher plasma ADMA concentrations favor atherosclerosis and independently predict adverse cardiovascular and cerebrovascular outcomes in several patient groups.

However, possible role of ADMA in pathophysiology of cognitive decline and AD is far from being fully elucidated. Prospective studies are warranted aiming at further clarification of ADMA involvement in development and progression of dementia. Moreover, use of ADMA in CSF and /or plasma as prospective biomarker of AD should be explored. In difference to NO measurement, potential usefulness of ADMA as possible biomarker of cognitive decline is reflected by its relative stable concentration in plasma which can be accurately measured.

Previous studies have shown that disturbed NOS-NO-ADMA pathway is closely related to endothelial dysfunction which has pivotal role in the development of cardio-metabolic diseases^[41]. Furthermore, according to recently introduced hypothesis disturbed NOS-NO-ADMA pathway is also one of the most important attributes of cerebrovascular dysfunction that plays a pathogenic role in the development of cognitive decline and dementia. Experimental data from cell culture and animal models have demonstrated that dysfunction of the NO-NOS-ADMA pathway results in cell death, blood-brain barrier (BBB) disruption, and brain edema via different pathological mechanisms^[42]. However, exact underlining mechanisms through which disturbed NOS-NO-ADMA pathway contributes to cognitive decline and dementia remain to be elucidated. Clarification of these mechanisms may be helpful in the identification of new therapeutic targets for aging and neurodegenerative diseases.

In summary, numerous findings indicate that cardio-metabolic and vascular risk factors, as well as advanced aging contribute to the development of endothelial dysfunction. The main features of endothelial dysfunction are reduced eNOS activity, decreased NO production, increased NO degradation as well as increased plasma ADMA levels. Within brain vasculature endothelial dysfunction results in hypoperfusion leading to reduced oxygen supply, metabolic dysfunction, increased amyloid- β accumulation and enhanced production of reactive oxygen species. These detrimental processes are thought to be associated with neurodegeneration, apoptosis, cognitive failure and memory loss^[27]. According to present notion, important role in this setting has disturbed NOS-NO-ADMA pathway. However, more basic and clinical research on the vascular features of cognitive dysfunction and AD is required. Future research should also clarify whether endothelial dysfunction precede or follow neurological changes which characterize cognitive decline and dementia.

Based on the findings summarized in this paper, increasing evidence point to significant role of endothelial dysfunction

in the pathogenesis of cognitive decline and dementia. Both cognitive decline and dementia are related to cardio-metabolic risk factors which is well documented in the literature. Clinical and epidemiological studies have shown that healthy nutrition and physical exercise are important non-pharmacological, lifestyle-related interventions that could help in maintaining appropriate vascular tone, adequate cerebral blood flow and normal cognition during aging^[43]. It is reasonable to suggest that prevention of cardio-metabolic diseases via endorsement of healthy aging determinants such as control of blood pressure, lipids and glucose levels as well as maintenance of optimal body weight achieved by regular physical activity and nutrition with high anti-oxidant capacity might be effective strategy in the preservation of cognitive function.

CONFLICT OF INTERESTS

The author has no conflicts of interest to declare.

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