The effects of nitric oxide in Alzheimer's disease

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Abstract

Alzheimer's disease (AD), the most prevalent cause of dementia, is a progressive neurodegenerative condition that commences subtly and inexorably worsens over time. Despite considerable research, a specific drug that can fully cure or effectively halt the progression of AD remains elusive. Nitric oxide (NO), a crucial signaling molecule in the nervous system, is intimately associated with hallmark pathological changes in AD, such as amyloid-beta deposition and tau phosphorylation. Several therapeutic strategies for AD operate through the nitric oxide synthase/NO system. However, the potential neurotoxicity of NO introduces an element of controversy regarding its therapeutic utility in AD. This review focuses on research findings concerning NO's role in experimental AD and its underlying mechanisms. Furthermore, we have proposed directions for future research based on our current comprehension of this critical area.

Key words: Alzheimer's disease; apoptosis; cell death; mitochondrial fission; nitric oxide synthase; nitric oxide; nitroxidative stress; S-nitrosylation

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Introduction

Alzheimer's disease (AD), an insidious and progressive neurodegenerative disease, commonly afflicts the elderly population.[1,2] It is characterized by memory impairment, aphasia, apraxia, agnosia, impaired visual spatial ability, executive dysfunction, as well as personality and behavioral changes, and other comprehensive dementia.[3,4] The underlying mechanism of AD remains unclear, despite reports on the following hypotheses: cholinergic theory, amyloid-β (Aβ) toxicity theory, oxidative stress theory, and tau protein hyperphosphorylation theory.[5] Although the pathogenic factors of AD are yet to be definitively established, it is widely accepted that the pathogenic factors of AD include excessive deposition of Aβ, abnormal phosphorylation of tau protein, inflammatory response, neuronal apoptosis and oxidative stress.[6] Aβ is the major component of amyloid plaques in AD patients.[7] To date, the prevention and treatment of AD remains a huge challenge. The patient's condition typically progresses over time, often leading to death due to the complications.[8] Recently, extensive research has been conducted on AD, with increasing focus on the nitric oxide synthase (NOS)/nitric oxide (NO) system to treat neurodegenerative diseases.[9]

At the onset of the 20th century, small-molecule NO was initially considered to be only an atmospheric pollutant and was also used in warfare as a chemical poison. In the 1970s, Murad et al. discovered that after nitroglycerin, upon entering the blood vessels, is converted into a transient gas called NO, which expands the blood vessels, accelerates blood flow, and increases blood supply to the heart.[10] In the 1980s, Furchgott et al.[11] identified molecules in vascular endothelial cells that induce blood vessel relaxation. These molecules, named endothelial-derived relaxation factors, exist for an extremely brief duration, usually less than 1 second.[11] Furchgott et al.[11] named them the endothelial-derived relaxation factor. Later in 1986, Ignarro et al.[12] found that these relaxation factors were, in fact, NO. They also found that vascular endothelial tissue can produce NO to regulate blood pressure.[12] NO can dissolve in water and fat, and can be freely dispersed inside and outside cells.[13] It is highly active and has a short half-life, and is easily inactivated in the presence of oxygen and superoxide ions, but is relatively stable under superoxide dismutase and acidic conditions. NO has a high affinity for hemoglobin, the heme molecule of soluble guanylate cyclase (sGC). Despite its simple chemical structure, NO can control a variety of cellular or physiological functions in a relatively specific manner, including: (1) maintaining vasodilation and regulation of blood flow and pressure; (2) inhibition of platelet aggregation and adhesion, controlling leukocyte adhesion, and regulation of smooth muscle cell proliferation; (3) serving as a neurotransmitter synthesized in the central nervous system, playing roles in memory formation, neuronal activity, and pain control; (4) serving as a ubiquitous mediator in the peripheral nervous system, mediating neurovascular vasodilation and certain functions of the gastrointestinal, respiratory, and genitourinary tracts, considered to be non-adrenergic, non-acetylcholinergic effects.[14-17] This review focused on the role of NO in AD and summarized the possible molecular mechanisms based on a literature retrieved from PubMed using (Alzheimer's disease [MeSH Major Topic]) AND nitic oxide [All Fields]) AND neurogenerative diseases [MeSH Terms].

Biosynthesis of Nitric Oxide

The L-arginine (L-Arg)-NO pathway is the main route for *in vivo* biosynthesis of NO. NOS employs L-Arg and molecular

oxygen as substrates, reacting with nicotinamide adenine dinucleotide phosphate to create NO and L-guanidine.[18] NOS-1 or neuronal NOS, ubiquitously expressed in human and vertebrate central and peripheral nervous systems, is a constitutively expressed enzyme primarily regulated by intracellular calcium.[19] Primarily found in vascular endothelial cells, NOS-3, also known as endothelial NOS, is similarly regulated by intracellular calcium. NO produced by NOS-3 critically facilitates systemic circulation and cerebral blood flow by relaxing blood vessels and restraining platelet aggregation and leukocyte adhesion.[20] Unlike NOS-1/NOS-3, NOS-2 (inducible NOS) is not constitutively expressed. Its expression relies on various signaling pathways and is mainly found in phagocytic cells such as macrophages. However, it can also be induced in other cell types, including microglia and astrocytes. Upon induction, NOS-2 generates NO persistently, regardless of calcium concentrations, yielding 100- to 1000-fold more NO than constitutive isoforms.[21,22] The activity of NOS-2 eventually ceases due to enzymatic degradation. Its ability to produce substantial NO amounts aligns with its role as a cytotoxic inflammation mediator. Notably, NOS-2 contributes significantly to AD development and progression.[23,24]

The Role and Mechanism of Nitric Oxide in Alzheimer's Disease

The Aβ cascade hypothesis postulates that Aβ deposition in the brain is a critical step leading to AD.[25,26] Multiple studies have suggested that soluble Aβ oligomers, more toxic than Aβ aggregates, could be linked with cognitive impairment.[27-29] Such oligomers can stimulate inducible NOS (iNOS) overexpression in astrocytes, triggering NO production.[30] Concurrently, Aβ inhibits glutamate reuptake, precipitating pathological activation of N-methyl-D-aspartate-type glutamate receptors. This drives Ca^{2+} influx, leading to neuronal NOS activation and a NO overload.[31,32] NO exhibits dual functions in this context: First, it regulates the release of pro-inflammatory molecules and interacts with reactive oxygen species (ROS), resulting in reactive nitrogen species formation associated with neuronal inflammation and oxidative stress-related neurodegeneration.[33] Second, NO can post-translationally modify proteins by S-nitrosylating thiol amino acids, thereby regulating AD-related protein functions. It is crucial to note that NO's functions in AD progression are interconnected, influencing and transforming each other.[34]

NO and nitroxidative stress

The oxidative stress theory, one of the primary etiological theories for AD, suggests free radical accumulation as an AD clinical marker.[17] However, whether oxidative stress is a cause or a consequence in the brains of patients with AD remains uncertain. Oxidative stress-derived free radicals can promote the phosphorylation of Aβ and tau proteins and alter neuronal DNA and RNA. Nitrosative stress, closely associated with oxidative stress, involves the interplay of ROS and reactive nitrogen species formation and scavenging pathways.[35] ROS, including superoxide anion (O_2^-), hydrogen peroxide, and hydroxyl radical, can interact with high ROS levels to create a series of potent oxidative free radicals and nitro groups, including pernitrite anion (ONOO–) and peroxynitrite (HOONO).[36] These compounds contribute to the production of 3-nitrotyrosine from various biomolecules (lipids, DNA, RNA, proteins), causing cellular damage or apoptosis—commonly known as nitrosative stress. Its mechanisms leading to cell death include target protein tyrosine nitration, mitochondrial dysfunction, and cell membrane disruption.[37]

Recent studies have underscored the role of nitrosative stress in the progression of neurodegenerative diseases, with significantly increased levels of 3-nitrotyrosine reported in AD brains and cerebrospinal fluid (6-fold higher than same-age counterparts).[38-42] Oxidative and nitrosative stress, protein aggregation, and inflammation are common molecular markers in most neurodegenerative diseases.[39] For instance, in mice and human PD brains, S-nitrosylation of peroxiredoxin-2 is significantly increased.[40] This disrupts the normal Prx2 redox cycle, causing cellular peroxide accumulation and thus inducing oxidative stress.[40] Research also indicates that abnormal production of oxygen and nitrogen free radicals can cause significant damage to crucial cellular components and contribute to neuronal death in various neurodegenerative diseases.[41] Proanthocyanidin B2 (PB2), a natural polyphenolic compound, has shown a notable dose-dependent protective effect against sodium nitroprusside-induced nitrosation stress, suggesting PB2's potential as a neuroprotective agent for treating neurodegenerative diseases.[42]

NO and neurotransmitter release

In conventional NO signaling, NO binds to the sGC receptor. The activated sGC creates cyclic guanosine monophosphate (cGMP), directly interacting with downstream effectors such as cGMP-dependent protein kinases (cGKI or PKG), cyclic nucleotide-gated channels, and cGMP-dependent phosphodiesterases.[43,44] Tissue cGMP level, used in experiments, sometimes indirectly indicates tissue NO concentration.[45] NO-mediated signal transduction occurs via direct binding to iron in the Fe-S cluster of the enzyme or heme group in heme proteins, enabling reversible enzyme activation or inhibition.[46]

NO indirectly regulates acetylcholine release from the nucleus accumbens by activating nearby glutaminergic neurons. It exhibits dual, concentration-dependent effects: at basal levels, it reduces γ-aminobutyrate release in a Ca^{2+} and Na^+ dependent manner, while high NO concentrations increase γ-aminobutyrate release. NO donors in the hippocampus activate norepinephrine and glutamate release.[47] In rat premedial area, NO stimulation of the sGC-cGMP pathway increases dopamine and serotonin release.[48] Several studies have suggested that sGC/cGMP signaling is essential for acquiring new learning and memory.[49,50]

Cholinergic system damage, the earliest proposed theory of AD pathogenesis, plays an important role in the pathogenesis of AD. In patients with AD, the loss of cholinergic neurons and subsequent defects in cholinergic neurotransmission in the hippocampus and cerebral cortex are strongly associated with cognitive impairment and clinical symptoms of dementia.[51,52] Acetylcholine is an important central excitatory neurotransmitter that is related to several advanced behaviors such as learning and memory. The central cholinergic nervous system affects central acetylcholine levels by regulating its synthesis

and release. Acetylcholine is involved in neuromodulation by activating NO/sGC/cGMP signaling.[53] As previously mentioned, several reports have demonstrated a direct link between abnormal signaling of NO and brain damage in patients with AD.[54,55] NO-induced sGC activity is severely reduced in the cortex of patients with AD. There is increasing evidence that NOS inhibition or N-methyl-D-aspartate receptor blockade leads to the impairment of spatial working memory. However, the administration of L-Arg, an NO precursor, via intracerebroventricular injection was found to ameliorate this impairment by activating cholinergic receptors to normalize the function of descending signaling pathways.[53]

NO and protein S-nitrosylation

Recent studies have found that another major mechanism by which NO exerts its signal transduction effect is S-nitrosylation of reactive cysteine residues. In 1992, Stamler et al.[56] found that NO reacts with the sulfhydryl (–SH) group of protein cysteine residues to form S-nitrosothiol (SNO), which exerts more stable biological activity than does NO. In the same year, Stamler et al.[57] found that NO acts on the cysteine sulfhydryl group of glyceraldehyde-3-phosphate dehydrogenase and affects its enzymatic activity. In 1994, Stamler[58] first proposed the concept of protein sulfhydryl nitrosylation, in which NO or partial nitroso (–NO) acts on the cysteine sulfhydryl (–SH) side chains of proteins and is associated with specific thiol (–SH) side chains. Sulfhydryl residues (-S) of proteins are covalently linked to form S-nitroso groups (-SNO).[58] Protein S-nitrosylation plays a pivotal role in the regulation of protein post-translational modifications, which can regulate more than 3000 proteins and participate in a variety of biological processes, including protein stability/transformation, steroid synthesis, transcriptional regulation, DNA damage repair, cell growth/differentiation, apoptosis and redox regulation.[59,60] The whole-brain SNO proteome of the APP/PS1 (APPswe, PSEN1dE9) mouse model was characterized and 135 SNO proteins were identified, 11 of which were differentially modified between wild type and AD mouse.[61]

In the AD context, the roles of Aβ and microtubule-associated protein tau are pivotal. Both, alongside neuronal hyperexcitability and aging-related neuroinflammation, trigger NO production and S-nitrosylation.[62] For example, S-nitrosylation of cyclin-dependent kinase 5 at residues 83 and 157 was found to be associated with Aβ peptide-induced dendritic spine loss.[63] Excess NO also encourages nitrosylation of glyceraldehyde-3-phosphate dehydrogenase, leading to tau hyperacetylation and the formation of neurofibrillary tangles, hallmarks of AD.[64-66] S-nitrosylation of protein disulfide isomerase is notably increased in the brain tissue of patients with AD. A high-glucose medium-induced SH-SY5Y cell model showed that glucose load triggered S-nitrosylation at the protein disulfide isomerase C343 site, inducing neurodegeneration through IRE1α phosphorylation.[67,68] NO can drive mitochondrial fragmentation via S-nitrosylation of dynamin-related protein 1, leading to bioenergetic compromise, insufficient neurotransmission energy, and synapse loss.[69] This is linked with cognitive decline in AD patients.[70] Importantly, Tomohiro et al.[71] discovered that S-nitrosylation of deubiquitinase ubiquitin carboxy-terminal hydrolase isoenzyme-L1 led to synaptic damage in AD. Taken together, NO-mediated S-nitrosylation of proteins is instrumental in AD progression.

Application of Nitric Oxide Synthase/Nitric Oxide System in Alzheimer's Disease Treatment

Anti-nitroxidative stress

Nitroxidative stress is crucial in AD development. Increased iNOS expression in AD elevates NO levels, which, upon reaction with excessive oxygen free radicals from oxidative stress, amplifies cytotoxicity.[72] Accordingly, drug research to counter oxidative stress targets interrupting NO production and/or iNOS activity and expression to decrease oxygen free radical generation.

iNOS is key in immune responses, especially through macrophage defenses. However, its expression in non-immune cells can cause tissue damage. Overexpression of iNOS enhances NO production. The three NOS types share 50–60% structural similarity, posing a significant challenge in creating selective iNOS inhibitors for clinical use. New iNOS inhibitors have demonstrated promising therapeutic effects in animal models, but clinical trial effectiveness is yet unproven. Some inhibitors have shown high toxicity in humans due to their non-selectivity.[73]

Antioxidants like vitamin E, C, beta-carotene, B12, alpha-lipoic acid, coenzyme Q10, berberine, and melatonin are extensively used to combat oxidative stress and may delay AD onset and progression.[74] Additionally, natural dietary antioxidants – rich in vitamins, carotenoids, flavonoids, and polyphenols – could play a significant role in slowing AD progression.[75,76]

NO mimetics

NO is crucial for learning and memory, regulating synaptic plasticity and long-term potentiation.[77] Despite its benefits, the application of NO is limited due to its short half-life in water (less than 3 seconds).[18] and little effect on systemic circulation. Direct inhalation of NO is typically used to treat patients with AD, however, with limited therapeutic effect. The NO precursor L-Arg has been shown to be useful under experimental conditions, but large-scale clinical trials are lacking; further, it may lead to iNOS production and synthesis of more harmful NO, aggravating the process of nitroxidative stress after AD.[78] In recent years, research has focused on NO mimetics or chimeras, which combine the biological activity of nitrate and auxiliary pharmacophores.[79,80] These and other drugs that enhance NO/cGMP signaling, bypassing cholinergic receptor activation, show potential for AD treatment. Unlike NO produced by iNOS, NO derived from endothelial and neuronal NOS seems to protect against AD neuropathology.[81-84] L-Arginine, another NO precursor, enhances synaptic plasticity and long-term potentiation in the hippocampus, improving cognitive deficits in AD patients.[85] In the intra-cerebroventricular streptozotocin AD model, S-nitrosoglutathione, an endogenous NO donor, reduces cognitive deficits and Aβ load in the hippocampus.[86] The novel nitrate, GT 1061, initially targeted at treatment of mild to moderate AD, reverses some cognitive deficits caused by chemical damage.[53,87] Furoxans

(oxadiazole-4 N-oxides), a thiol-dependent NO mimetic, offers a slow-onset NO effect, crossing the blood-brain barrier and improving passive avoidance memory.[88] Despite their promise in peripheral and central nervous systems, NO chimeras have limited application, with only one U.S. Food and Drug Administration-approved treatment for glaucoma. The allosteric regulatory potential of sGC in brain diseases remains underexplored.[79]

Protein S-nitrosylation

S-nitrosylation of proteins can modify cellular signaling and neuronal function by affecting protein activity, conformation, folding, interaction, and subcellular localization.[89,90] Studies indicate that S-nitrosylation increases mitochondrial fragmentation rates and inhibits mitophagy, linked to aging-related diseases.[91,92]

Experiments show that the ROS scavenger neohesperidin prevents $A\beta_{25-35}$ induced apoptosis by blocking S-nitrosylation of protein disulfide isomerase and associated neurotoxicity.[68] S-nitrosoglutathione, an NO donor, increases Aβ uptake and clearance in bEND3 cells through the formation of SNO-dynamin 2.[93] Furthermore, mutation of the SNO site of Dexras1 reduces damage in $A\beta_{1-42}$ mice.[94] Hence, S-nitrosylation, as a novel post-translational modification, selectively modulates mitophagy and could be a potential target in age-related pathologies. Details of experimental studies are presented in **Table 1**.

Conclusion

NO, a gaseous signaling molecule, plays a pivotal role in the progression of AD. As highlighted in this review, NO is implicated in nitrosative stress, neural signal transduction, and S-nitrosylation of proteins relevant to AD. Interventions that reduce nitroxidative stress, such as the use of NO mimetics to enhance NO bioavailability and regulation of protein S-nitrosylation, can mitigate neuronal apoptosis, synaptic damage, and the overall progression of AD. Consequently, the importance of NO's role in AD continues to gain recognition. This review provides a comprehensive overview of the interplay between AD and NO, outlining the key protective and aggravating factors in AD and their correlation with NO. However, the intricate pathogenesis of AD and NO's role within it results in diverse mechanisms of potential drug treatments, limiting the progress of clinical research. Future studies should aim to deepen our understanding in this area, ultimately leading to the development of novel and effective therapeutic strategies for AD.

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No additional data are available.

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Table 1: The main experimental study of nitric oxide in AD

Study	Animals/cells	Results
Szeliga[41]	Rats	PB2 has shown a notable dose-dependent protective effect against sodium nitroprusside-induced nitrosation stress
Sen et al.[65]	Mice	Excess NO leads to increased nitrosylation of GAPDH, enhancing pathological tau acetylation after exposure to amyloid-β
Wang et al.[68]	Primary hippocampal neurons	Excessive NO induces RNS and induces the formation of SNO-PDI, inhibits PDI activity, forms unfolded protein responses, and leads to neuronal cell death
Cho et al.[69]	Primary cortical neurons	NO acts on the Cys644 site of Drp1 to form SNO-Drp1, which overactivates GTPase, resulting in mitochondrial fragmentation and synapse loss
Qu et al.[71]	Primary cortical neurons	NO acts on the Cys152 site of Uch-L1 to form SNO-Uch-L1, which then trans-nitrosylates Cdk5. Next, SNO-Cdk5 trans-nitrosylates Drp1, leading to synaptic damage in AD
Paul et al.[85]	Rats	Reduced NO synthesis in the hippocampus is responsible for impaired memory formation in aged animals
Dubey et al.[86]	Rats	NO donor ameliorates cognitive deficits and associated brain biochemical changes in this experimental model of sporadic AD, and NO-BDNF interaction may play a key role in these effects

Note: AD: Alzheimer's disease; BDNF: brain derived neurotropic factor; Cdk5: cyclin-dependent kinase 5; Drp1: dynamin-related protein 1; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GTPase: guanosine triphosphatase; MG: methylglyoxal; NO: nitric oxide; PDI: protein disulfide isomerase; RNS: reactive nitrogen species; SNO: S-nitrosothiol; TPI: triose phosphate isomerase; Uch-L1: ubiquitin carboxy-terminal hydrolase isoenzyme-L1.

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