PATHOGENESIS OF NEURODEGENERATIVE DISEASES AND THE EFFECT OF NATURAL PRODUCTS ON NITRIC OXIDE PRODUCTION IMPLICATING IN THESE DISEASES

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Nitric oxide (NO) is a free radical synthesized from L-arginine by three isoforms of NO synthase (NOS). NO is involved in a wide range of physiological functions. It functions as neurotransmitter by modulating the release of glutamate and the neurotransmission of *N*-methyl-D-aspartate (NMDA) receptor, as neuro-endothelial-derived relaxing factor through the action on guanylyl cyclase (sGC), and as inflammatory molecule in response to proinflammatory cytokines or bacterial endotoxin. NO is also implicated in multiple pathological conditions such as acute and chronic neurodegeneration. In spite of the different mechanisms of pathogenesis for these neurodegenerative diseases, NO may play a pivotal role in the pathological conditions of these diseases. Under pathological conditions, NO is produced either from activated neuronal NOS (nNOS) in neuron or inducible NOS (iNOS) in glial cells by increased intracellular calcium concentration through glutamate-NMDA receptor interaction or by inflammatory cytokine-mediated signaling pathway, respectively. NO and its toxic metabolite peroxynitrite ($ONOO^-$) directly injure membrane integrity or impair mitochondrial function leading to DNA break, lipid peroxidation, and protein nitrosylation. These events consequently activate caspase-cascade or poly-(ADP-ribose) polymerase 1 (PARP) resulting in apoptotic or necrotic cell death. Much effort is devoted to search the specific inhibitors of NOS from natural resource as neuroprotectants. We summarized here the reported natural products that target NO/NOS pathway by blocking iNOS expression, attenuating the activity of nNOS, disturbing upstream signaling of nNOS, scavenging $ONOO^-$, or inhibiting tyrosine nitration. These natural products may offer possible therapeutics to prevent and/or to treat these neurodegenerative diseases.

Key words: Nitric oxide, Acute neurodegeneration, Neurotoxicity, Oxidative stress, Inflammation, Mitochondrial dysfunction, Natural products.

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PHYSIOLOGICAL ROLE Of NO IN CENTRAL NERVOUS SYSTEM (CNS)

NO is synthesized from L-arginine by NOS. At least three distinct NOS isoforms, neuronal NOS (nNO S, Type I), inducible NOS (iNOS, Type II), and endothelial NOS (eNOS, Type III) have been identified in mammalian cells. The expression of iNOS in glial cells of CNS is elicited during inflammatory reactions. The eNOS and nNOS, two constitutively active calcium-dependent NOS isoforms, are originally identified in vascular endothelium[1] and neural tissue, respectively (Fig. 1). The first target established for NO signaling is soluble guanylyl cyclase (sGC) because NO binds to the heme-containing transition metal center at the active site of the enzyme, altering the conformation of the protein to stimulate the formation of cyclic guanosine monophosphate (cGMP)[2]. Moreover, NO functions by modifying transition metal centers of a wide variety of proteins. NO can also selectively and reversibly *S*-nitrosylate

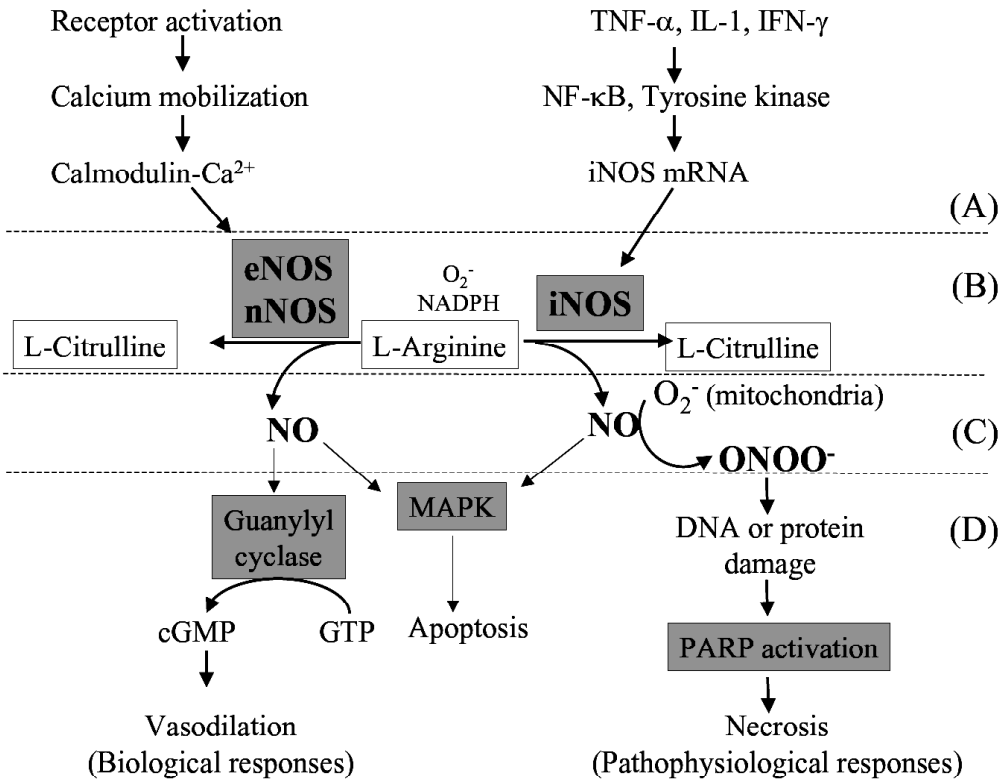

Fig. 1. The generation and action mechanisms of NOS. The activity of eNOS and nNOS are regulated predominantly at the post-transcriptional level by calmodulin in a calcaium-dependent manner, whereas iNOS is an inducible calmodulin containing but calcium-independent enzyme (A). NO is synthesized from L-arginine by NOS, which oxidatively removes the terminal guanidine-nitrogen from L-arginine to form L-citrulline and NO (B). NO combines with O_2^- generated from mitochondria to form more toxic metabolite $ONOO^-$ (C). NO generated from eNOS modulates vasodilation through action on guanylyl cyclase (D). Excessive NO and $ONOO^-$ directly injure membrane integrity or impair mitochondrial function leading to DNA break, lipid peroxidation, and protein nitrosylation. These events consequently activate caspase-cascade or PARP resulting in apoptotic or necrotic cell death.

the cysteine residues in a wide variety of proteins with precise spatial and temporal resolution[3].

NO FUNCTIONS AS NEUROTRANSMITTER

Neuronal NOS requires calmodulin as a cofactor[4] and is regulated by multiple kinases including cAMP-dependent protein kinase, calcium/calmodulin-dependent protein kinase, and protein kinase C[5]. Convincing data have shown that localization of nNOS activity emerges from the association of adaptor proteins with the PDZ domain of nNOS. *N*-methyl-D-aspartate (NMDA) receptor binds to postsynaptic density protein (PSD95), which in turn binds to nNOS[6]. This event enables NMDA receptor activation to promote calcium entry in proximity to nNOS, which facilitates robust, rapid activation of the enzyme.

Glutamate released from glutamatergic neurons stimulates NMDA receptors located on nitrergic neurons thus enhancing the release of NO. The released NO stimulates cGMP within glutamatergic neurons. Depending on the concentration of NO, glutamate release is either enhanced or decreased. The released glutamate stimulates NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors located on cholinergic neurons, thereby enhancing the release of acetylcholine, while inhibition of glutamate release exerts the opposite effect on cholinergic transmission. NO also either promotes or diminishes the release of γ-aminobutyric acid (GABA) from GABAergic neurons. GABA stimulates $GABA_A$ receptors located on cholinergic neurons and inhibits acetylcholine release. Inhibition of GABA release enhances acetylcholine output. The release of biogenic amines seems to be modulated in a similar manner[7].

NO ACTS AS ENDOTHELIAL-DERIVED RELAXING FACTOR IN CNS

Endothelial NOS in endothelial cells is thought to be cerebroprotective due to a possible vasodilating effect that improves cerebral perfusion under ischemic conditions. Increasing the enzymatic activity of eNOS has proven to be neuroprotective in experimental models of cerebral ischemia. Furthermore, NO produced by eNOS exerts beneficial effects through limiting aggregation of platelets and adhesivity of white blood cells, thus impeding microvascular flow during stroke[8].

NO FUNCTIONS AS INFLAMMATORY MOLECULE

In CNS, iNOS is expressed mainly in activated astrocytes and microglia. iNOS is induced in response to a series of proinflammatory cytokines including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and bacterial lipopolysaccharide (LPS).

The expression of iNOS is predominantly regulated at the transcriptional level. Evidences have shown that nuclear factor-κB (NF-κB), signal transducer and activator of transcription 1 (Stat1), and activating protein-1 (AP-1) are important for the expression of iNOS[9-12]. In most cell types, NF-κB is present as a heterodimer composed of p50 (NF-κB1) and p65 (RelA) subunits. NF-κB is sequestered in the cytoplasm by association with a member of the inhibitor family of NF-κB (IκB)[13,14]. The dissociation of NF-κB from IκB results in NF-κB translocation into the nucleus and interacts with the NF-κB binding motif in the promoters of iNOS and so regulates its transcription[10,11].

Binding of IFN-γ to its receptor induces oligomerization and activation of the receptor-associated Janus kinas 1 (JAK1) and JAK2 by trans-phosphorylation. JAK-mediating tyrosine phosphorylation of Stat1 triggers the dimerization. Dimerized Stat1 translocates into the nucleus and regulates iNOS expression by binding to IFN-stimulated-response element (ISRE) or γ-activated site (GAS)[15-19].

The main AP-1 proteins in mammalian cells are c-fos and c-jun. AP-1 activity has been described being implicated in the expression of iNOS[9,20]. Cytokines stress activates various transcription factors that induce the expression of fos and jun. Post-translational phosphorylation of jun by jun N-terminal kinase (JNK) also activates the AP-1 activity[21]. The activation of protein kinases are also involved in the induction of iNOS expression. These include protein kinase C-ε (PKCε), PKCη, PKCδ, mitogen-activated protein kinase (MAPK), and p38[22-25].

NO is involved in a variety of physiological functions. It also participates in multiple pathological conditions specially the neurodegenerative diseases, such as acute neurodegeneration (stroke), chronic neurodegeneration (Alzheimer's disease and Parkinson's disease). The role of NO in the neurodegenerative diseases is described as below.

ACUTE NEURODEGENERATION

Acute neurodegeneration describes clinical conditions in which neurons are rapidly damaged and ultimately die in response to a sudden insult, such as lack of oxygen or essential nutrients, elevated temperature, contact with toxic compounds and excessive mechanical strain[26]. Among these initiators, acute energy depletion is one of the most potent necrosis-triggering conditions for neuron in the case of ischemia after stroke. Ischemic stroke is characterized by complex spatial and temporal events evolving within hours to several days. Cells in the core of ischemic territory die progressively due to severely compromised blood flow, ATP depletion, ionic disruption, and metabolic failure. However, the peripheral zones of the ischemic territory, the so-called ischemic penumbra, suffer milder insults due to residual perfusion from collateral blood vessels. Major fundamental mechanisms leading to cell death during ischemic brain injury include excitotoxicity, ionic imbalance, oxidative/nitrosative stress, inflammation, and apoptotic cell death[27,28]. Possible signaling pathways involved in acute stress-induced neurodegeneration are also summarized in Fig. 2.

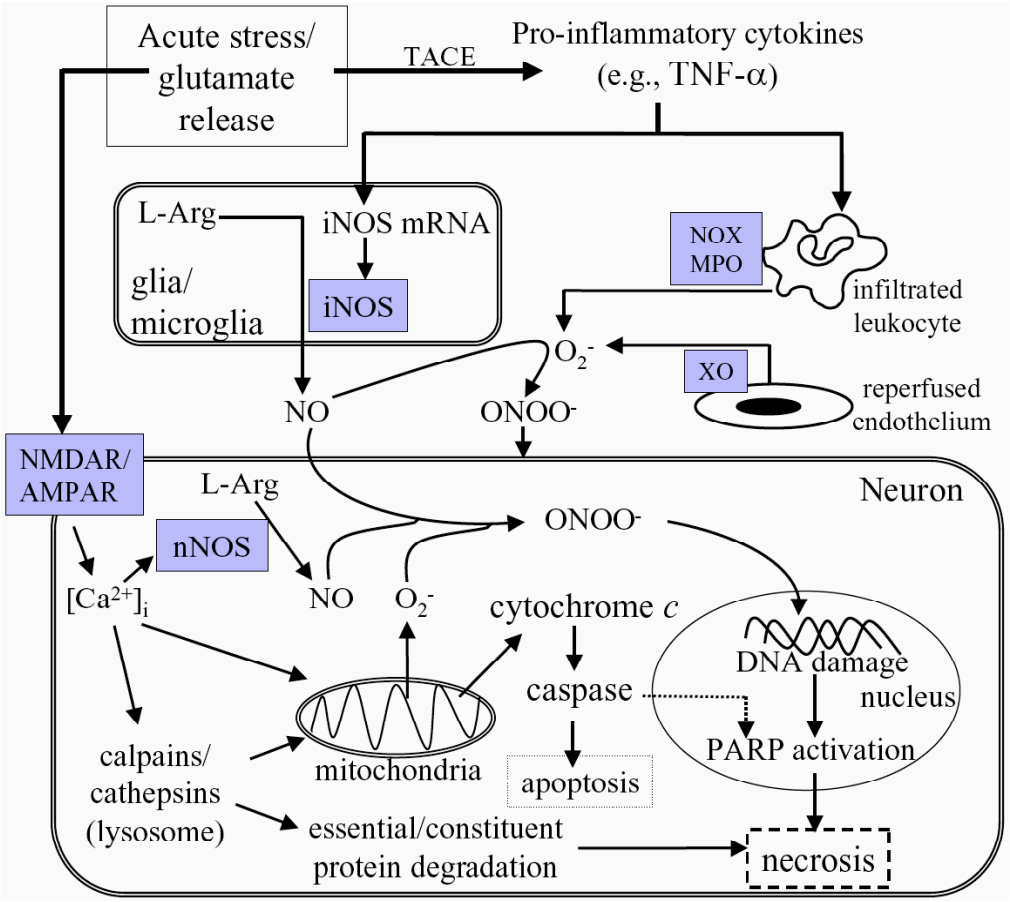

Fig. 2. The possible signaling pathways involved in acute stress-induced neurodegeneration. Acute cerebral stress triggers excessive glutamate release when binding to NMDA or AMPA receptor (NMDAR/AMPAR), induces overloading of intracellular calcium ($[Ca^{2+}]_i$) that further damages mitochondria, lysosome and other organelles through calpains/cathepsins pathway. O_2^- released from damaged mitochondria combines with NO produced from activated nNOS (in neuron) or iNOS (in glial cells) to form more toxic metabolite $ONOO^-$. $ONOO^-$ directly injures cell membrane or induces DNA break, lipid peroxidation, and protein nitrosylation. Consequently, limited DNA damage activates caspase for induction of apoptotic cell death, while massive DNA damage over-activates PARP that results in necrotic cell death. TNF-α, a pro-inflammatory cytokine produced by activated TNF-α converting enzyme (TACE), recruits leukocytes especially neutrophils for excess production of O_2^- by their NADPH oxidase (NOX) or myeloperoxidase (MPO). Reperfused-endothelial cells also release enormous amount of free radicals by xanthine oxidase (XO) that also ruins cells by the formation of $ONOO^-$.

EXCITOTOXICITY

Energy depletion results in ionic imbalance, neurotransmitter activation and impairment of reuptake by transporters. The excessive amount of glutamate binds to ionotropic NMDA receptors promoting excessive calcium influx. Overloading of the intracellular calcium concentration ($[Ca^{2+}]i$) activates certain calcium-dependent enzymes (e.g., calpains/capthesins), phospholipases, and proteases that subsequently disturb the membrane integrity causing the

generation of oxygen radicals, protein misfolding and cytoskeletal damage, the process called excitotoxicity[29,30]. In addition, glutamate excessively binding to AMPA or kainate receptor promotes an excessive influx of sodium with concomitant cell swelling and edema. The sharp increase of $[Ca^{2+}]i$ is a principal death-signaling event involved in both necrosis and apoptosis.

OXIDATIVE AND NITROSATIVE STRESS

Ischemia and reperfusion induce production of reactive oxygen species (ROS), including superoxide and hydroxyl radicals. Mitochondrial damage has been strongly implicated in the generating oxygen radical at least in part due to the failure of mitochondrial electron transport *via* the increase of permeability of mitochondrial transition pore (MTP)[26]. Oxygen radicals are also produced during enzymatic conversions, such as the inducible cyclooxygenase-2 (COX-2)-dependent conversion of arachidonic acid to prostanoids and the degradation of hypoxanthine (accumulated during ischemia) by xanthine oxidase[31], especially upon reperfusion. Furthermore, free radicals are also generated by infiltrated leukocytes *via* activating NADAPH oxidase (NOX). Nitrosative stress comes from the activation of the NOS family during ischemia. NO combining with superoxide to generate the strong oxidant peroxynitrite[32]. Therefore, a series of events, oxidative/nitrosative stress, excitotoxicity, energy failure and ionic imbalances are crossly linked, and consequently contribute to ischemic cell death.

INFLAMMATION

Several pro-inflammatory cascades are initiated within minutes after the onset of stroke. These events are promoted, in part, by the binding of cell adhesion molecules (e.g., selectin and intercellular adhesion molecule (ICAM)) expressed in endothelial cells with integrins expressed on the neutrophil surface[33]. The inflammatory mediators are generated by reactive microglia, neurons, astrocytes, or macrophages and leukocytes recruited into the ischemic brain. There is transient upregulation of immediate early genes encoding transcription factors (e.g., fos and jun) within minutes of occlusion. This is followed by a wave of expression of heat shock genes (e.g., Hsp70, Hsp72) within 1-2 hours. A third wave follows hours to days after the onset of stroke, at these time intervals several chemokines and cytokines are expressed [e.g., IL1, IL6, IL8, TNF-α, monocyte chemo attractant protein-1 (MCP1), etc][34].

APOPTOTIC CELL DEATH PATHWAYS

Necrotic cell death usually predominates after severe ischemia perhaps due to rapidly decreasing cellular ATP levels, cell swelling, and ionic imbalances[35]. Ischemia also triggers apoptotic cell death under certain conditions[36].

Acute ischemia and trauma activate executioner caspases (caspases 3 and 7). Caspase 3 is the most abundant cysteine protease in brain. It is cleaved acutely in neurons and is present in the ischemic core as well as the penumbra at the acute and sub-acute stages of reperfusion[37]. Caspase cleavage usually occurs from hours to days after ischemia, and probably mediates delayed ischemic cell death. Cytosolic Bid, a proapoptotic Bcl2 family member lying as an upstream cascade of the mitochondrial activation, results in cytochrome c release and promotes formation of apoptosome[38]. Additionally, the TNF super family of death receptors regulates upstream caspase processes in brain and spinal cord ischemia, as well as in CNS trauma. In spinal cord ischemia and brain trauma, caspase 8 is activated through the action of death induce signaling complex composed of Fas and Fas-associated protein with death domain (FADD)[39]. Recently, a caspase-independent apoptosis triggered by apoptosis-inducing factor (AIF), a mitochondrial component, has been suggested as another important component of cell death pathway[40]. Mitochondria also possesses membrane recognition elements for pro-apoptotic signaling molecules, such as Bid, Bad and Bax, that reside upstream of the cascade.

ROLE OF NO IN ACUTE NEURODEGENERATION

Recent findings indicated a pivotal role of NO and the excess of pro-oxidants in neuronal functional impairment and structural damage. This is mainly caused by the oxidative/nitrosative effects produced by extensive release of NO and the generation of other oxidant species such as peroxynitrite ($ONOO^-$)[32]. $ONOO^-$ is a strong tissue-damaging agent that acts through initiation of lipid peroxidation, oxidation of sulfhydryl groups and nitrosation of tyrosine-containing molecules. The activation of nNOS and iNOS pathways largely depend on the activation of the NMDA subtype of glutamate receptor and on the activation of the transcription factor, nuclear factor-κB (NF-κB), respectively[34,41]. The activation of NF-κB also signals the expression of several responsive genes, some of which are involved in oxidative/nitrosative products, e.g., cyclooxygenase-2 (COX-2)[42]. Conversely, eNOS, a constitutive enzyme in endothelial cells is thought to be neuroprotective mainly due to a possible vasodilating effect. Increasing eNOS expression or eNOS enzymatic activity has been shown to be neuroprotective in experimental models. Furthermore, NO produced by eNOS exerts beneficial effects through limiting the aggregation of platelets and the adhesivity of white blood cells[10].

ALZHEIMER's DISEASE

Pathogenesis of Alzheimer's Disease

The hallmark of Alzheimer's disease (AD) is the neuronal degeneration associated to senile plaques. The major protein component of senile plaques is amyloid β protein (Aβ), which plays an important role in the pathogenesis of AD. Aβ, a 39- to 42-amino acid peptide, is derived proteolytically from amyloid precursor protein (APP) by the action

of β- and γ-secretases. The Aβ-derived fragments ($A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{25-35}$) are toxic to neurons[43,44]. The neurotoxic effects may attributable to the disturbance of calcium homeostasis and the accumulation of ROS and reactive nitrogen species (RNS)[45-47]. ROS and RNS not only provoke membrane damage that compromises membrane integrity but also increase the permeability of ions, including calcium. The increase of calcium influx further leads to more generation of ROS and RNS, thereby initiating a positive feedback loops. Cultured neurons treated with Aβ renders neurons vulnerable to apoptosis, indicating that caspase activation plays an essential role in Aβ-induced neurotoxicity[48].

The Role of NO in Aβ-mediated Brain Inflammation

In the CNS, iNOS is abundantly present in the cerebellum, hippocampus, striatum, hypothalamus, and medulla oblongata. NO reacts with superoxide anion to form peroxynitrite. Peroxynitrite production has been implicated as one of the important factors worsening neuronal degeneration in the inflammatory hypothesis of AD[49]. The senile plaque is surrounded by abundant reactive microglia and astrocytes. Aβ not only activates microglia or astrocytes directly, but also activates $CD4^+$ T cells to produce cytokines that subsequently activate microglia. Several lines of evidence have shown that NF-κB in microglia is stimulated by Aβ[50]. Activation of NF-κB can stimulate transcription of genes expressing TNF-α, interleukin-1 (IL-1), IL-6, monocytes chemo-attractant protein-1, and iNOS. The activation of microglia causes iNOS-mediated NO release[51]. CD40L and Aβ synergistically promotes the secretion of NO from activated microglia[52]. Aβ also acts synergistically with IFN-γ to trigger the production of NO by microglia and to induce neurotoxicity *in vitro*[53-55]. Other neurotoxic substances, such as TNF-α or glutamate, secreted from microglia may also act to induce neuronal toxicity.

Pharmacological studies have shown that induction of iNOS by $A\beta_{1-42}$ is associated with selective loss of cholinergic neurons[56]. Direct injection of Aβ into the rat brain also results in increase of iNOS. These results strongly support that iNOS may play an important role in the neurodegenerative process of AD.

The Role of NO in Aβ-mediated Neurotoxicity

Ca^{2+} homeostasis dysfunction is another mechanism that link NO and AD, since Ca^{2+} is a primary regulator of nNOS activity. Slow progressive increase of intracellular Ca^{2+} concentration in cultured neurons upon Aβ exposure has been observed. Furthermore, Aβ pretreatment enhanced the effect of glutamate on intracellular Ca^{2+} concentration[57]. In addition to the ability of Aβ itself in generating oxidative stress[58], Aβ has also been shown to have synergistic action with glutamate to induce neuronal damage *via* NO-mediated pathway[59,60].

The ability of NO to damage mitochondrial function as a free radical and by producing peroxynitrite is well-documented[61]. In addition to the direct cellular damage caused by oxidative stress, mitochondrial dysfunction may also present in a ROS-mediated pathology. Studies have shown that NO modulates acetylcholine release[62]. It is not known whether this phenomenon has any pathological impact in AD to date, even though cholinergic pathology in

the basal forebrain is coincident with AD. Nevertheless, the presence of NOS-positive neurons in close proximity to cholinergic neurons may be closely related to the selected vulnerability of the basal forebrain cholinergic system secondary to NO neurotoxicity.

NO stimulates the mitochondrial production of ROS, primarily superoxide anion ($O_2^{\cdot-}$) and H_2O_2, and generation of $ONOO^{\cdot}$. $ONOO^{-}$ is a powerful oxidant that can directly nitrate tyrosine residues of proteins, forming the stable compound 3-nitrotyrosine (3-NT) and tyrosine dimer [3,3′-dityrosine (diTyr)][63]. An elevation in both diTyr and 3-NT levels in the hippocampus and cortical regions of AD have been reported[64].

In summary, NO play an important role in many of the AD pathogenic pathways. With being activated by Aβ and elevated intracellular Ca^{2+}, NO is excessively produced by either nNOS of neurons or iNOS of glial cells. NO may react with $O_2^{\cdot-}$ to form $ONOO^{\cdot}$. Both NO and $ONOO^{-}$ are neurotoxic and perhaps playing a key role in AD, which compromise cellular integrity and viability by various mechanisms. Furthermore, Aβ has synergistic action with cytokines and glutamate to induce neuronal damage *via* NO-mediated pathway. NO may also be involved in AD pathogenesis by modulating acetylcholine release, which results in cholinergic deficit and subsequent dementia. The pathway of NO-mediated neurotoxicity in relation to AD is summarized in Fig. 3.

PARKINSON's DISEASE

Parkinson's disease (PD) is a progressive, neurodegenerative disorder that is characterized by severe motor symptoms, including uncontrollable tremor, postural imbalance, slowness of movement and rigidity. The main pathological hallmark of this disorder is a selective loss of dopamine-producing neurons in the substantia nigra, which results in a drastic depletion of dopamine in the striatum. Another pathological characteristic of PD is the presence of eosinophilic, cytoplasmic inclusions of fibrillar, misfolded proteins, termed Lewy bodies (LB), in the affected brain areas.

Molecular Pathogenesis of PD

There are various familial forms of PD, including those linked to mutations in α-synuclein (*PARK1*), parkin (*PARK2*), DJ-1 (*PARK7*), and PTEN (phosphatase and tensin homolog deleted on chromosome 10)-induced kinase 1 (*PINK1*, also known as *PARK6*)[65,66].

Both wild-type and mutant α-synucleins form amyloid fibrils as well as nonfibrillary oligomers termed as protofibrils[67]. Protofibrils may cause toxicity by permeabilizing synaptic vesicles[68], allowing dopamine to leak into the cytoplasm and participate in reactions that generate oxidative stress. Furthermore, the selective vulnerability of nigrostriatal neurons in PD may derive from the ability of dopamine or dopamine-quinone to stabilize α-synuclein protofibrils[69].

Parkin functions as an E3 ligase, catalyzing the ubiquitination of specific substrates that targets them for

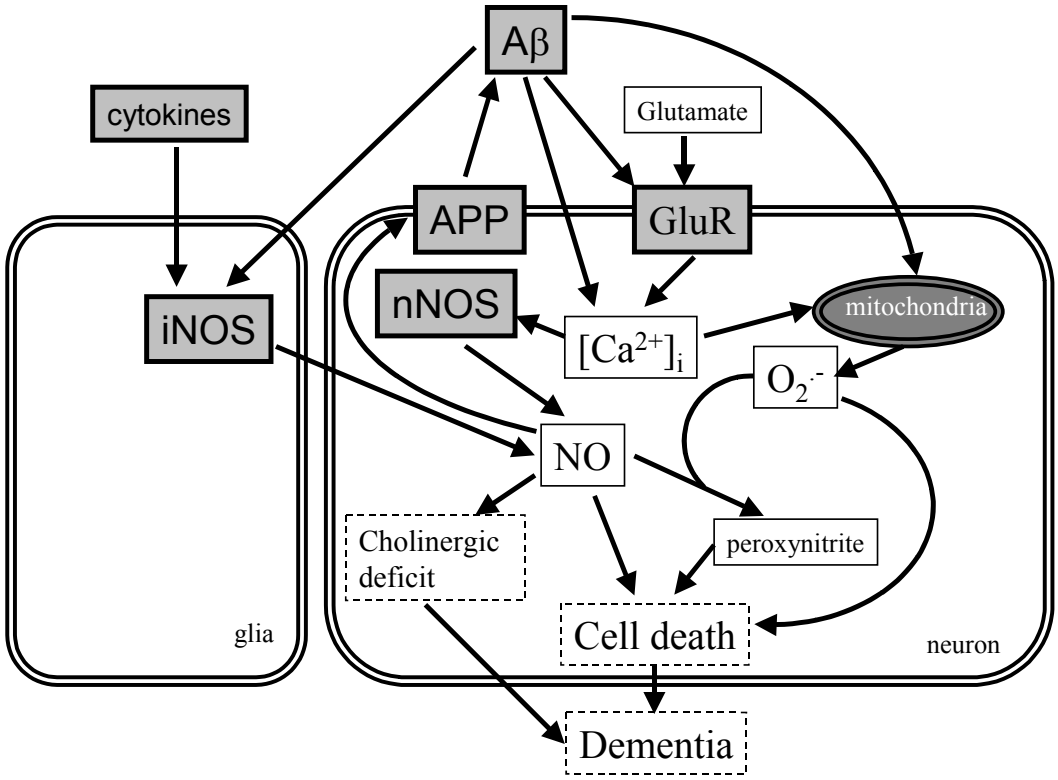

Fig. 3. NO mediated neurotoxicity in relation to AD. Mutations of amyloid precursor protein (APP) are associated with increased production of Aβ. Cholinergic deficit, which may be induced by NO-mediated toxicity, is implicated in dementia observed in AD. Increased production of Aβ induces NO production either by disrupting Ca^{2+} homeostasis and subsequent increase in intracellular Ca^{2+} (nNOS-mediated NO release) or by interacting with glial cells (iNOS-mediated NO release). NO is a free radical and can produce peroxinitrite in the present of $O_2^{\cdot-}$. All three radicals induce a variety of neurotoxic mechanisms that are likely to be involved in cell death and dementia observed in AD. Furthermore, NO may induce more Aβ formation by activating a variety of signaling molecules.

degradation by the ubiquitin-proteasome system (UPS). Many mutations affecting parkin can abolish its E3 ligase activity[70]. Cysteine residues in the RING domains of the C-terminal half of parkin are sensitive to nitrosative and oxidative modifications. Recent findings indicate that parkin's E3 ligase activity is modified by NO, thus linking environmental stress to a molecular abnormality and a clinical phenotype similar to that seen in hereditary forms of PD[71,72].

DJ-1 is a homodimeric, multifunctional protein ubiquitously expressed in human tissues including brain. Eleven different mutations affecting DJ-1 have been linked to an autosomal recessive form of PD[73]. DJ-1 may also function both as a sensor for oxidative stress and as an antioxidant[74].

Recent data revealed that mutations in the gene encoding PTEN-induced kinase 1 (PINK1) are linked to autosomal recessive form of PD[66]. Normal and mutant PINK1 are localized to mitochondria. PINK1 contains a highly conserved kinase domain similar to serine/threonine kinases in the Ca^{2+}-calmodulin family. This finding may be relevant, as a defect in mitochondrial function has been implicated in the pathogenesis of PD[70].

A variety of factors including environmental toxins, oxidative stress, and mitochondrial dysfunction have been implicated in the pathogenesis of sporadic PD[70,75]. The first neurodegenerative change that occurs in this disorder is a loss of terminals in the striatum, accompanied by the accumulation of aggregated proteins in nigral processes known as Lewy neurites. The appearance of Lewy neurites might be followed by retrograde degeneration, further accumulation of aggregated proteins in nigral cell bodies (LBs) and, finally, reactive gliosis and cell death.

OXIDATIVE STRESS

Nigral dopaminergic neurons are particularly vulnerable to oxidative stress because the metabolites of dopamine can act as endogenous toxins. Dopamine auto-oxidizes at normal pH into toxic dopamine-quinone species, O_2^- radicals and H_2O_2[76]. Superoxide can be converted to H_2O_2 by superoxide dismutase, or into labile $ONOO^-$ radicals in the presence of NO. H_2O_2 can be broken down into cytotoxic hydroxyl radicals in a chain of iron-mediated reactions. In PD, nigral cells seem to be under a heightened state of oxidative stress, as evidenced by the presence of high level of by-products of lipid, protein and DNA oxidation[77,78].

MITOCHONDIA AND PD

Sporadic PD can be initiated by environmental toxins such as the exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Astrocytes metabolize MPTP to 1-methyl-4-phenyl pyridium (MPP^+) *via* monoamine oxidase B. MPP^+ is selectively imported into dopaminergic neurons *via* dopamine transporter, where it targets mitochondria, inhibits respiratory complex I and promotes ROS production. Additional support for mitochondrial dysfunction in PD pathogenesis comes from the evidence that the insecticide rotenone induces a parkinsonian-like syndrome in animal models and probably humans[79]. Rotenone also inhibits mitochondrial complex I, and dopaminergic neurons seem to be most severely affected. Current evidences suggest that mitochondrial complex I inhibition may be the central cause of sporadic PD and the derangements in complex I cause α-synuclein aggregation, which contributes to the demise of dopamine neurons.

NO AND PD

Piles of evidences suggest that NO overproduction also plays a key role in the pathogenesis of PD. NO and its toxic metabolite $ONOO^-$ impair the mitochondrial respiratory chain by affecting the function of complex I, complex II, and complex IV, leading to energy failure and ultimately cell death[80].

Exposure of astrocytes to cytokines leads to the induction of iNOS, generation of NO, and mitochondrial

respiratory chain damage. $O_2^{\cdot-}$ generated by the respiratory chain further react with NO, forming $ONOO^-$ and causing further mitochondrial damage[81]. Despite this mitochondrial damage, astrocytic ATP levels are relatively maintained by switching to glycolysis. Diffusion of NO into neighboring neurons causes neuronal glutamate release, which subsequently activates the NMDA-type glutamate receptors of the same or neighboring neurons. Activation of the neuronal NMDA receptor triggers Ca^{2+} influx and further activation of nNOS. The subsequent generation of $NO/ONOO^-$ initiates neuronal mitochondrial damage, lipid peroxidation, and ATP depletion. Since damaged neurons are unable to proceed glycolysis to maintain their energy demands, cell death may ultimately occur[80]. Therefore, NO is implicated in the pathogenesis of PD by inhibiting the mitochondrial respiratory chain (Fig. 4).

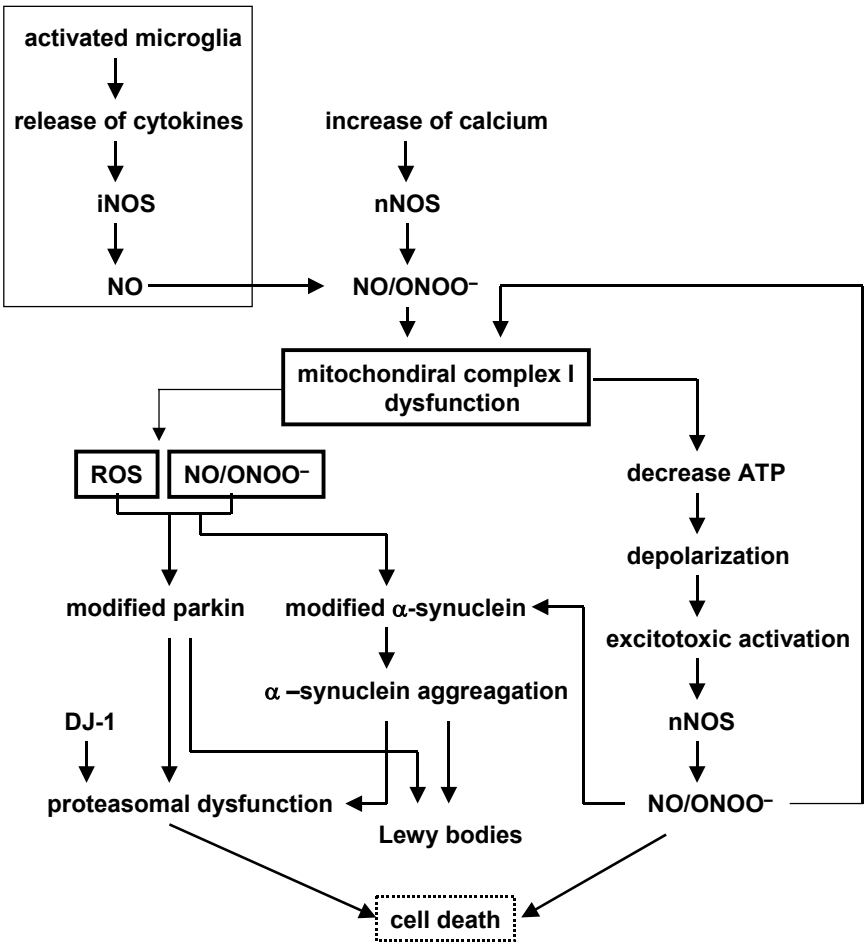

Fig. 4. The relationship of NO and dysfunction of mitochondrial complex I in the pathogenesis of PD. The elevation of intracellular calcium of dopaminergic neurons or activated microglia contribute to the activation of nNOS and iNOS, respectively. Excessive production of NO coincides the generation of cytotoxic metabolite $ONOO^-$. $ON/ONOO^-$ causes the dysfunction of mitochondrial complex I leading to increased oxidative stress, free radical formation, and reducing intracellular level of ATP. Decrements in ATP lead to membrane depolarization and contribute to excitotoxic neuronal injury and further free radical – mediated injury involving $ON/ONOO^-$ and a feed forward cycle of increasing oxidative stress and injury. The deficiency of complex I causes the modification of parkin and aggregation of α-synuclein, which lead to dysfunction of the proteasome and contributes to cell death.

Natural Products Function as NOS Inhibitors and Modulator of NO Action

NO is produced by the oxidation of L-arginine that is catalyzed by NOS using molecular oxygen and a number of cofactors (NADPH, FAD, tetrahydrobiopterin, and heme) that aid in the generation or transfer of electrons. Three isozymes of NOS (iNOS, eNOS, and nNOS) are expressed in specific tissues to generate NO for specific physiological processes. In addition, each isozyme has been implicated in specific diseases in terms of either excess or inappropriate levels of NO. Therefore, much effort has been dedicated to the design of selective inhibitors of NOS isozymes.

Most inhibitors of NOS used to date are synthesized chemicals. These NOS inhibitors are classified into at least three groups: 1) L-arginine (Arg) derivatives that compete with the active site[82-84]; 2) metabolic inhibitors such as aminoguanidine, citrulline analogs[85,86], N-iminoethyl-L-lysine[87], and a cyclic amidine derivative[88] that react either with the heme residue at the NOS active site and/or a nucleophilic amino acid residue that projects into the active site[89,90]; and 3) inhibitors of homodimerization, since the iNOS and nNOS monomers are inactive[91-94].

The selective inhibitors of NOS are also searched from the natural sources (Table 1). Since the expression of iNOS is predominantly regulated at the transcriptional level, numerous inhibitors of iNOS targeting the upstream signaling of iNOS expression have been reported. These iNOS inhibitors include diterpenes (andrographolide[95], staminols C, D, orthosiphonone C, D, 14-deoxo-14-*O*-acetylorthosiphol Y[96], andalusol[97], spiramine T[98]), flavonoids (quercetin, (-)-epigallocatechin gallate, (+)-catechin[99], baicalein[100]), polyphenols (morin, sesamol, chlorogenic acid, fisetin, (+)-taxifolin, ellagic acid, and caffeic acid[99]), sesquiterpene lactones (artemisinin[101], nepalolide A[102], hydroxyachillin[103]), polyacetylenes (panaxynol, falcarindiol, panaxydol[104,105]), glycoside (ginsenoside-Rd[106]); stilbenes (rhapontigenin, piceatannol, resveratrol[107]), and the orthers (sporogen, S14-95, S-curvularin[108], ginkgolide A, B, huperzine A[108-111], triptolide[112]), etc. The mechanisms of inhibition of NO production are mediated by blockade of NF-κB activation[97,101,103,105], suppression of NF-IL6 activation[100], inhibition of IκB kinase activity[102], or interference of the JAK-STAT pathway[105,108].

The inhibitor directly targeting to the activity of iNOS from natural products has not been found to date. However, two novel aplysinopsin-type indole alkaloids, 5,6-dibromo-2′-demethylaplysinopsin (Z) and 5,6-dibromo-2′-demethylaplysinopsin (E) with selective inhibitory activity against the nNOS were isolated from the marine sponge *Hyrtios erecta*[113]. The activity of nNOS is also inhibited by 9,10-phenanthraquinone[114]. Since the activity of nNOS is regulated by calcium/calmodulin, numerous inhibitors of nNOS have been reported targeting the upstream signaling of nNOS. These nNOS inhibitors include flavones (baicalin, norwogonoside, oroxylin A-glucuronide (oroxyloside), and wogonoside[115]), glycosides (ginsenosides Rb1 and Rg3[116]), and the others (l-*S.R*-daurisoline[117], nicotine[118], ginkgolide A, B, huperzine A[119]).

Many natural products possess scavenging activity of $ONOO^-$ or inhibition of tyrosine nitration. The natural products exert $ONOO^-$ scavenging activity are sinapic acid ((3,5-dimethoxy-4-hydroxycinnamic acid) from *Brassica*

Table 1. The natural products function as NOS inhibitors

NO targeting model	NOS involved	Mechanism of NO	Natural Products	Mode of drug's effect (A-D)*	References
Acute					
Cerebral ischemia	iNOS	Inflammation	spiramine T	A	98
Cerebral ischemia	iNOS	neuroprotective effect	wogonin	A	127
Cerebral ischemia	iNOS	Inflammation	catechins	A	128
Cerebral ischemia	nNOS	Neurotoxicity	quisqualic acid	C	129
Ischaemia-reperfusion/ $ONOO^-$, LPS	$ONOO^-$	oxidative stress	berberine	C	121
Microglia	iNOS		caffeic acid phenethyl ester, helenalin	A	130
Microglia (BV-2)	iNOS	inflammation	sesamin, sesamolin	A	131
Hippocampal neurons	nNOS	glutamate toxicity	l-S.R-daurisoline	B	117
Hippocampal Neurons	nNOS	glutamat toxicity	baicalin, norwogonoside, oroxylin A-glucuronide (oroxyloside), and wogonoside	B	115
Cortical neurons	nNOS	glutamat toxicity	ginsenosides Rb1 and Rg3	B	116
Cortical neurons	nNOS	glutamat toxicity	nicotine	B	118
Glutamate-neurons	iNOS nNOS	neuroinflammation	8-O-E-p-methoxycinnamoyl-harpagide (iridoid)	C	132
NMDA-striatal neurons			genistein	D	133
In vitro biological activities	nNOS	inflammation	proanthocyanidins, procyanidin B2, procyanidin B3, catechin, epicatechin	B	134
	iNOS		curcumin, EGCG, resveratrol	A	135
	nNOS	glutamate toxicity	baicalin, baicalein, wogonin	A	136
Chronic					
Substantia nigra	nNOS		EGCG (tea)	A	137
Microglia	iNOS	inflammation	ginkgolides	A	109
Microglia	iNOS	inflammation	ginkgolide A and B	A	110
Microglia	iNOS	inflammation	triptolide	A	112
Microglia	iNOS	inflammation	andrographolide	A	95
Microglia	iNOS	inflammation	baicalein	A	100
Neurons	NOS	neurotoxicity	resveratrol, quercetin, and (+)-catechin,	C	123
Abeta-hippocampal neurons	iNOS		Ginkgo biloba extract EGb 761 and red wine-derived constituents	C	138
Neuroblastoma	iNOS	inflammation	ginkgolide A, B (GA, B), and huperzine A	A	119

Astrocytoma	iNOS	inflammation	ginkgolide A, B (GA, B), and huperzine A	A	111
Astrocytoma	iNOS	inflammation	artemisinin	A	101
Astrocytoma	iNOS	inflammation	ginsenoside Rd	A	106
Astrocytoma	iNOS	inflammation	nepalolide A, panaxynol, falcarindiol, panaxydol, and panaxytriol	A	102,104,105
Astrocytoma	iNOS	inflammation	quercetin, (-)-epigallocatechin gallate, morin, curcumin, apigenin, sesamol, chlorogenic acid, fisetin, (+)-taxifolin, (+)-catechin, ellagic acid, and caffeic acid	A	99
PC12	NOS	neurotoxicity	bilobalide	C	127
Epithelial A549/8 cells	iNOS	inflammation	sporogen, S14-95, S-curvularin	A	108
nNOS	nNOS	neurotoxicity	9,10-phenanthraquinone	B	114
nNOS	nNOS	neurotoxicity	5,6-dibromo-2′-demethylaplysinopsin (Z), (E)	B	113
$ONOO^-$, $ONOO^-$ donor (SIN-1)	$ONOO^-$	oxidative stress	sinapic acid	C	120
$ONOO^-$	$ONOO^-$	oxidative stress	chlorogenic acid, genkwanin, scopoletin	C	122
$ONOO^-$	$ONOO^-$	oxidative stress	lithospermate B	C	125
Albumin, LDL	$ONOO^-$	nitration	lithospermate B	C	125
albumin, LDL	$ONOO^-$	nitration	sinapic acid	C	120
Tyrosine	$ONOO^-$	nitration	caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid, ocoumaric acid, *m*-coumaric acid	C	126

* The mode of drug effect indicated by:
A, modulate upstream signaling of NOS expression
B, modulate NOS activity
C, modulate NO effectors function
D, modulate target of NO

juncea)[120], berberine (the main alkaloid in *Coptidis Rhizoma*)[121], chlorogenic acid/genkwanin/scopoletin, (from *Artemisia iwayomogi*)[122], and quercetin[123], (+)-catechin[123], resveratrol[123], bilobalide[124], and lithospermate B (from *Salvia miltiorrhiza*)[125]. The natural products modulate the nitration activity of $ONOO^-$ may be potential candidates to prevent the oxidative damage. For example, lithospermate B protects bovine serum albumin and low-density lipoprotein (LDL) against $ONOO^-$-mediated nitration *via* an electron donation mechanism[125]. Hydroxycinnamate antioxidants inhibit the nitration of tyrosine in the order of caffeic acid > or = chlorogenic acid > or = ferulic acid > *p*-coumaric acid > *o*-coumaric acid > *m*-coumaric acid. Hydroxycinnamates can act through one of two possible

mechanisms: preferential nitration for monophenolates and electron donation by catecholates[126]. Sinapic acid from *Brassica juncea* suppresses the formation of $ONOO^-$-mediated tyrosine nitration through an electron donation mechanism[120].

NO participates in numerous neurodegenerative diseases mediated by oxidative/nitrosative stress, neuroinflammation, and mitochondrial dysfunction. NO/$ONOO^-$ plays a pivotal role in the progressive and exacerbated process of these neurodegenerative diseases. Numerous natural products that target NO/NOS pathway by blocking iNOS expression, attenuating the activity of nNOS, disturbing upstream signaling of nNOS, scavenging of $ONOO^-$, or inhibiting tyrosine nitration are summarized. These natural products may be useful as alternative therapies to treat these neurodegenerative diseases.

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一氧化氮與神經退化性疾病之關聯性與天然物對一氧化氮產生之影響

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一氧化氮(nitric oxide, NO)是一種氣態的自由基，有三種 NO 生成酵素(NO synthase, NOS)可以將 L-arginine 轉變成 NO。NO 具有多種生理功能，它是一種神經傳遞物質，可以調節 glutamate 釋放和調整 N-methyl-D-aspartate（NMDA）受體之神經傳遞功能；由內皮細胞衍生出之 NO 藉由與 guanylyl cyclase 作用而擔任一種血管鬆弛因子；NO 在發炎性細胞激素或細菌內毒素所引發的發炎反應中扮演重要的角色；NO 更參與多種急性或慢性中樞神經退化性疾病之致病機制。僅管各種中樞神經退化性疾病的致病機制不同，NO 在其中都扮演著樞紐的角色。在病理情況下，glutamate 與 NMDA 受體結合，增加神經元內鈣離子濃度而活化 neuronal NOS（nNOS），或是發炎性細胞激素與細菌內毒素所間介的訊息途徑引發膠細胞之 inducible NOS（iNOS）表現，皆會使細胞產生 NO。NO 與其更具有毒性的代謝產物，peroxynitrite（$ONOO^-$）會直接破壞細胞膜完整性或間接地經由損害粒線體功能而造成 DNA 斷裂、脂質過氧化、和蛋白質硝基化。這一些作用接著活化 caspase-cascade 或 poly-(ADP-ribose) polymerrase（PARP），最後導致細胞凋亡或壞死。大量的研究致力於由天然物篩選 NOS 抑制劑以做爲神經元保護藥物。在此，我們將已發表之有關天然物作用在 NO/NOS 訊息途徑包括：阻斷 iNOS 表現、降低 nNOS 活性、干擾 nNOS 上游訊息、清除 $ONOO^-$、或抑制 tyrosine 硝基化等論文做一摘要討論。這一些天然物可能提供我們將來用於中樞神經退化性疾病之預防或治療。

關鍵詞：一氧化氮，急性神經退化，神經毒性，氧化壓力，發炎，粒線體功能異常，天然物。

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