The role of nitric oxide in stroke

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Abstract

Stroke is considered to be an acute cerebrovascular disease, including ischemic stroke and hemorrhagic stroke. The high incidence and poor prognosis of stroke suggest that it is a highly disabling and highly lethal disease which can pose a serious threat to human health. Nitric oxide (NO), a common gas in nature, which is often thought as a toxic gas, because of its intimate relationship with the pathological processes of many diseases, especially in the regulation of blood flow and cell inflammation. However, recent years have witnessed an increased interest that NO plays a significant and positive role in stroke as an essential gas signal molecule. In view of the fact that the neuroprotective effect of NO is closely related to its concentration, cell type and time, only in the appropriate circumstances can NO play a protective effect. The purpose of this review is to summarize the roles of NO in ischemic stroke and hemorrhagic stroke.

Key words: nitric oxide; neuronal nitric oxide synthase; inducible nitric oxide synthase; endothelial nitric oxide synthase; ischemia stroke; hemorrhagic stroke; neuroprotection; neurotoxicity

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Introduction

Stroke has a high morbidity and mortality, which is defined as a kind of acute cerebrovascular disease.[1,2] According to the existing data, ischemic stroke and hemorrhagic stroke have a record of more than one million new cases each year. These new cases bring a heavy burden on the family and society because of a substantial expenditure for complication treatment in healthcare systems.[3] With the aging of population and the change of diet structure, the incidence of stroke increases year by year, which further aggravates the burden. A large number of animal experiments and clinical studies have shown primary brain injury and secondary brain injury caused by stroke are complex pathophysiological processes, involving inflammatory reaction, neuronal apotosis/death, ischemia-reperfusion injury, blood-brain barrier damage, neurotoxic substance release, the generation of free radical, oxidative stress and brain edema.[4-11] At present, ischemic stroke therapies mainly concentrate on translator mechanical thrombectomy, stenting and angioplasty, surgical treatment (decompressive craniectomy and carotid endarterectomy), thrombolytic agents, neuroprotective drugs[12] and rehabilitation training.[13] The comprehensive therapy may also improve the prognosis and quality of life to a certain extent, and early thrombolysis or mechanical thrombectomy may also improve the prognosis of stroke. However, the therapeutic effect of stroke is still unsatisfactory. Therefore, some new treatment strategies and the pathogenesis of stroke need to be further studied.

Nitric oxide (NO) is commonly considered as a toxic gas, but it was found to transmit biological information as a signal

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molecule 40 years ago. At first, it was recognized that the endothelium released a factor which relaxed vascular smooth muscle cells and subsequently caused vasodilatation in the late 1970s.[14,15] During that time, as the molecular structure of this factor was unknown, it was named endothelium-derived relaxing factor (EDRF). Furchgott and his colleagues[16] conformed that EDFR was NO, a colorless, odorless gas until 10 years later. Since then, NO has been gradually recognized as a gas signaling molecule and its mechanisms of action in the laboratory animals and humans have been extensively researched. The main physiological functions of NO include the maintenance of vascular tone, the reduction of inflammation response, the balance of thrombotic-thrombolytic homeostasis and the regulation cell growth.

NO has a close relationship with stroke. There are three kinds of NO synthases (NOS) produced by NO during the stroke. Inducible NOS (iNOS)-derived NO and neuronal NOS (nNOS)-derived NO play neurotoxicity, but endothelial NOS (eNOS)-derived NO plays a neuroprotective role in acute ischemic stroke. The toxic effects of NO produced by iNOS and nNOS are mainly due to the production of nitrates and the release of free radicals, which directly damage mitochondrial enzymes and genetic materials.[17-20] On the contrary, neuroprotective effects of NO produced by eNOS are achieved primarily by regulating vascular bed and peripheral nerve tissue.[21,22] In hemorrhagic stroke, NO is extensively studied in subarachnoid hemorrhage (SAH). The poor prognosis of SAH is due to cerebral vasospasm and delayed ischemic neurologic deficits (DIND).[23-25] Cerebral vasospasm and DIND are related to complex pathophysiological processes. For exemple, DIND is involved in ruptured aneurysm, cerebral ischemia, blood-brain barrier dysfunction, increased intracranial pressure, and macro- and microcirculatory embolism and spasm.[26-29] Some studies have suggested that NOS dysfunction in the vicinity of cerebral vascular beds leads to cerebral vasospasm, DIND and clearance of deoxyhemoglobin.[30-32] Some studies have shown that the concentration of NO is associated with cerebral vasospasm.[33,34] Current research shows that the activation of the NO may improve vascular diameter, but it remains unclear in regard to the survival of patients.

Mechanisms of NO in Stroke

Mechanisms of NO in ischemic stroke

NO has a dual identity including neuroprotectiion and neurotoxicity during ischemia reperfusion. The distribution and concentration of NO in brain tissue was significantly changed after cerebral ischemia. NO is mainly synthesized by three subtypes of NOS in brain tissue: nNOS, eNOS and iNOS. Among them, nNOS and eNOS are calcium-dependent NOS, iNOS is a calcium-independent.[35,36] In the acute phase of ischemic stroke, the increase of NO was mainly caused by nNOS, followed by eNOS, but the formation of NO mediated by iNOS did not increase significantly, especially in 30 minutes after ischemia stroke.[37]

In general, nNOS and iNOS play a neuronal injury role in the early and late stage of ischemic stroke, while the activation of eNOS mainly exerts neuroprotection effects. NO is produced in several different types of cells, such as endothelial cells, neurons, glial cells and neutrophils. It plays a dual role in different time and space in ischemic stroke.[38] The beneficial or harmful role NO played in brain tissue of ischemic stroke depending on the cell type, the concentration of NO and microenvironment of ischemia.[39-41]

nNOS derived NO

In ischemic stroke, the concentration of NO decreases rapidly due to blocked blood flow.[37] Once the blood flow is restored, the production of NO will increase, which is mainly mediated by nNOS. Scientists used nNOS gene-deficient mice and nNOS-specific inhibitors to verify above view.[39] The synthesis of NO through nNOS is mainly related to calcium overload induced by glutamate in ischemic neurons.[42] Within one hour after reperfusion, the concentration of NO returned to physiological level. However, the defect of nNOS gene or the inhibition of nNOS can reduce the area of ischemic penumbra and the number of neuronal necrosis.[39,43,44] Inhibition of nNOS is also able to produce oxygen free radicals[45] and nitrosative stress,[46] reduce excitotoxicity and down regulate the expression caspase-3 in ischemic stroke.[44]

iNOS derived NO

The activation of iNOS increases from 12 hours after the onset of ischemic stroke and lasts for 1 week.[47] At this stage, iNOS is mainly produced by microglia, astrocytes, endothelial cells and infiltrating lymphocytes. The amount of NO released by iNOS is 1,000 times than that by nNOS.[48] Additionally, the production of NO induced by iNOS leads to brain damage during ischemia reperfusion.[49] The overexpression of iNOS can promote the secretion of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and subsequently induce secondary inflammatory reaction and the generation of oxygen free radicals.[50,51] After ischemic stroke, iNOS produces a large amount of NO, and NO elevates nitrous oxide levels and causes nitrosation damage within 12 hours to 8 days.[52,53]

eNOS derived NO

Unlike the other two subtypes of NOS, NO derived from eNOS often plays a neuroprotective role in ischemic stroke. In brain tissue, eNOS is mainly produced by the vascular endothelial cells and the choroid.[54] Although eNOS generates a small amount of NO, it plays a critical role in the regulation of cerebral microvascular tone, the protection of the blood-brain barrier, the reduction of oxidative stress and

the alleviation of procoagulant stimulation. It has been proved that NO released by eNOS can scavenge oxygen free radicals, inhibit the expression of adhesion molecules, and promote the aggregation of platelet and the adhesion of lymphocyte.[55-58] The inhibition of eNOS activity achieved by employing knockout mice ($eNOS^{-/-}$) and eNOS-specific inhibitors leads to hypertensive-prone organism, and more severe ischemia-reperfusion injury, significantly reduced cerebral blood flow, and thus subsequently result in greater infarct size.[21,39,59] On the contrary, flavonoids induced overexpression of eNOS and therefore exerted neuroprotection effects.[60]

Non-selective inhibition of NOS did not significantly alter the infarct volume in the permanent model, but the total infarct volume in the transient ischemic model was reduced. Although inhibition of NOS may have a negative effect on cerebral blood flow,[61] further investigations are required. Selective nNOS and iNOS inhibitors can be candidates for acute ischemic stroke treatment.[61]

NO plays a dual role in ischemic stroke, and the production of NO in the early stage of transient cerebral ischemia has a positive effect on the neuroprotection of stroke, but nNOS and iNOS play a negative role in the later stage.[62,63] eNOS plays a key role in the protection of neurovascular system. The production of NO derived from eNOS around the nerve vessels is capable to regulate the tension between the cerebral vessels and plays a positive role in improving the blood supply of the brain tissue (**Figure 1**).[64]

Mechanisms of NO in hemorrhagic stroke

At present, the study of NO in hemorrhagic stroke is mainly focused on SAH. We further discuss the case of SAH. About 1/4 of SAH patients in the first week will produce vascular spasm, which makes blood flow reduce to half of the normal blood flow.[65] Although there are many studies on DIND around the world last century, its pathological mechanism remains to be further explored.[66] In any case, it is thought that hemoglobin may be the cause of cerebral vasospasm because the affinity of eNOS-derived NO and hemoglobin is 1,000 times than that of oxygen and hemoglobin.[14,67] The amount of NO produced by eNOS is decreased after SAH and thereby reduced the affinity between NO and hematoma in the cerebrospinal fluid, reduced the concentration of NO around the blood vessel, and increased cerebral vasospasm.[66] Recent research has suggested that there is a close relationship between NO and cerebral vasospasm after SAH, and it is of great significance to study the basic role of NO after SAH.[68]

nNOS derived NO

As to whether SAH would change the expression of nNOS and therefore affect the production of NO, there were some related researches dedicated to explore this pathophysiological effect. The expression of nNOS was decreased from the onset of vasospasm, it resulted the decrease of the concentration of NO in the arterial adventitia and ultimately leaded to vasoconstriction. The latest data suggest that elevated intracranial pressure may cause transient cerebral ischemia after SAH, which may subsequently promoted the phosphorylation of Ser^{847} of nNOS *via* Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) pathway in the hippocampus. The phosphorylation of nNOS reduces ischemic injury and plays a neuroprotective effect in early brain injury.[69]

iNOS derived NO

In the early study of human SAH, it was found that the genenation of iNOS is a consequence of SAH and plays a major role in the pathogenesis of vasospasm.[70] Hyperglycemia increases the chance of cerebral vasospasm after SAH, mainly through the NO pathway as a potential underlying mechanism *via* the dysregulation of eNOS and iNOS.[71] A study found that aminoguanidine inhibits iNOS activity and reduces cerebral vasospasm after SAH in rabbits after abnormal endothelial cell repair.[72]

eNOS derived NO

Cerebral vasospasm is a common complication of SAH, and eNOS has the effect of regulating vascular tone. At present, the study of NOS after SAH is mainly focused on eNOS subtype. Early stenosis of the spastic artery was able to stimulate eNOS due to increased shear stress.[73] Therefore, the production of NO in the early stage counteracts the decrease of NO and leads to vasodilation. However, the persistence of delayed cerebral vasospasm in the arterial wall lowered the levels of cyclic guanosine monophosphate (GMP) and nitrites in the cerebrospinal fluid (CSF), which companied with the dysfunction of vascular endothelial cell and the reduction of eNOS and the decreased levels of NO around the arterial wall.[66,74-77] The functional defect of eNOS may be due to the increased activation of phosphodiesterase and the quick elimination of 3′,5′-cGMP, which may activate endogenous inhibitors of eNOS through asymmetric dimethylarginine, an endogenous inhibitor of NOS that produced by the fault of the oxidative cleavage fragment of bilirubin in haemorrhagic cerebrospinal fluid.[74,78,79] The interaction between asymmetric dimethyl-L-arginine (ADMA) and bilirubin-oxidation products (BOXes) in the CSF is related to the degree and time course of vasospasm in patients with SAH.[78,80] The levels of ADMA in late CSF are reduced by the clearance of BOXes, and increased NO levels resulting from eNOS ultimately lead to the relaxation of vascular endothelial.[66,74,81]

The levels of NO are closely related to cerebral vasospasm

after SAH, and more and more studies have assumed that low levels of NO could contribute to cerebral vasospasm.[66,82] Increased NO levels and increased NO donors (NODs) can reverse cerebral vasospasm.[83] In conclusion, the present studies suggest that increased concentration of NO after SAH is expected to improve the prognosis of patients with cerebral vasospasm after SAH (**Figure 1**).

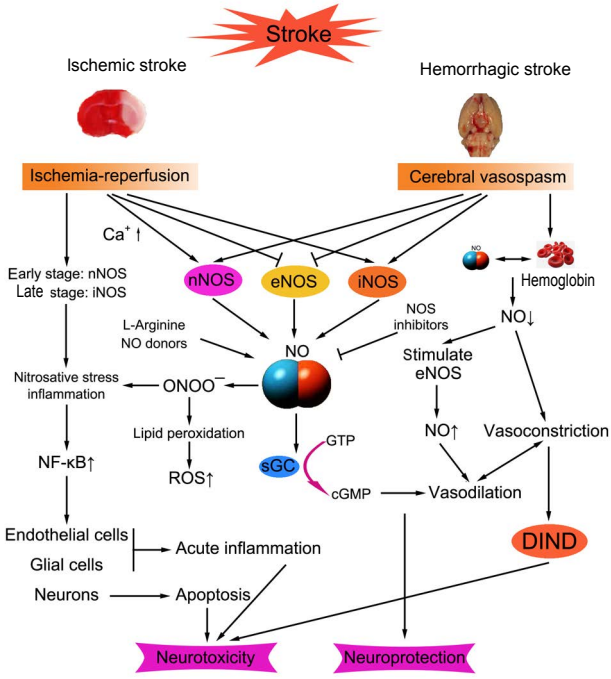

Figure 1: Schematic diagram of NO synthesis and action on ischemic stroke and hemorrhagic stroke.

Note: NO: Nitric oxide; NOS: nitric oxide synthase; nNOS: neuronal NOS; eNOS: endothelial NOS; iNOS: inducible NOS; sGC: soluble guanylate cyclase; ROS: reactive oxygen species; DIND: delayed ischemic neurological deficits; onoo⁻: peroxynitrite anion; NF-κB: nuclear factor-κB; GTP: guanosine triphosphate; cGMP: cyclic guanosine monophosphate.

Therapeutic approaches of NO donors and inhibitors in stroke

NOD is a class of drugs which is generally characterized by the production of NO or NO-related substances independently *in vivo* or *in vitro*, such as nitro anions (NO^-) or nitroonium ions (NO^+).[84] NODs are the most commonly used donors in basic and clinical studies: organic nitrate, S-nitrosothiols, sydnonimines, NONOates and sodium nitroprusside.[85] NOD has many neurotoxic effects that are not associated with NO and the neurotoxicity of the media molecules carried by the NOD themselves.[86] Therefore, it is important to avoid the adverse influence of NOD medium on the treatment of stroke.

At present, some common inhibitors include: NOS inhibitors (*e.g.*, Nω-nitro-L-arginine methyl ester hydrochloride, pan-NOS inhibitors, 7-nitroindazole); statins (HMG-CoA-reductase inhibitors); Rho kinase (ROCK)-inhibitors; and phosphodiesterase inhibitors. However, most of the NOS inhibitors are nonselective and may cause toxic side effects to eNOS, so they have not been applied clinically. It is necessary to develop highly selective inhibitors of NOS that will be better applied clinically.[87] Statins do improve cerebral perfusion during the acute phase of ischemia stroke, but it may increase the risk of infection.[88] The limiting factor for ROCK inhibitors in stroke treatment is that it has the potential to cause hypotension. Thus, the development of ROCK inhibitors with selectively targeted cerebral blood flow may improve ROCK therapeutic value in stroke.[89]

NO and neonatal hypoxia-ischemia (HI) brain injury

In neonatal HI brain injury, NO plays a different role in different studies. Some studies suggest that NO has neuroprotective effects in neonatal rat brain hypoxia. In 2012, Zhu et al.[90] reported that inhalation of NO in neonatal mice with HI brain damage had protective effects on male mice, but had no protective effect on female mice. The neuroprotective effect of NO on neonatal HI rat model after helium pretreatment (He-PC) suggests that the treatment of He-PC may induce the production of NO and activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which plays a neuroprotective role on neonatal HI.[91] Study on the relationship between low dose lipopolysaccharide (LPS) pretreatment and neonatal HI showed that low-dose LPS-mediated neuronal activation and enhanced endothelial cell eNOS activity can improve hypoxia tolerance through AKT pathway and thus play a neuroprotective effect.[92]

In addition, some studies about the negative effects of NO on neonatal HI are reported. NO is responsible for the death of neuronal cells in neonatal HI brain injury by disrupting the homeostasis of iron metabolism and generating more free radicals.[93] In a recent study, it has been shown that the activation of nNOS leads to microcirculation injury and the reduction of blood flow after recanalization and exacerbates brain damage.[94] Neonatal HI brain damage leads to overexpression of iNOS and cause white matter damage. The expression of iNOS may be involved in the ischemic cellular events including apoptosis, and play a role in the pathophysiological process of white matter damage.[95]

Neuroprotection and Neurotoxic Studies

It is well known that a large number of animals and cell studies must be tested before NO enters the clinical application. These studies further elaborate on the molecular mechanisms of NO from several aspects such as neuroprotection, neurotoxicity and biological effects. The study summarizes that most of the neuroprotective effects of NO are associated with eNOS, and the neurotoxicity is primarily related to nNOS and iNOS. In our review we will systematically summarize the existing animal and human studies on the role of NO in stroke (**Table 1**).

Table 1: The neuroprotective and neurotoxic effects of hydrogen nitric oxide (NO) in stroke

Author	Year	Animals/ cells	Model	Results
Neuroprotection				
Watanabe et al.[96]	2016	Mouse	Middle cerebral artery occlusion (MCAO)	Serine racemase inhibition induces NO-mediated neurovascular protection during cerebral ischemia
Yan et al.[97]	2015	Rats	MCAO	CXC195 induced phosphorylation of endothelial NO synthase (eNOS) by activation of PI3K/Akt signaling under pathological cerebral ischemia-reperfusion conditions, which provided a novel explanation for the neuroprotective effect of CXC195
Fu et al.[98]	2014	Rats	MCAO	Calycosin-7-O-β-D-glucoside could protect blood-brain barrier integrity in experimental cerebral ischemia-reperfusion injury *via* regulating NO/caveolin-1/ matrix metalloproteinases pathway
Yu et al.[99]	2013	Rats	MCAO	Prior exposure to enriched environment reduced inducible NO synthase (iNOS) and neuronal NO synthase (nNOS) mRNA and protein and improved neurological status after MCAO without affecting infarct volume, suggesting that enriched environment may provide neuroprotection *via* ischemic preconditioning
Koh[100]	2012	Rats	MCAO	iNOS and nNOS expression levels increased during MCAO, and ferulic acid prevented injury-induced increase of these isoforms. Thus, these findings suggest that the up- and down-modulation of three isoforms by ferulic acid is associated with a neuroprotective mechanism
Li et al.[101]	2012	Rats	MCAO	Autologous transplantation of adipose-derived mesenchymal stem cells attenuates cerebral ischemia and reperfusion injury through suppressing apoptosis and inducible NO synthase
Zhao et al.[102]	2012	Rats	MCAO	(S)-ZJM-289, a NO-releasing derivative of 3-n-butylphthalide, protects against ischemic neuronal injury by attenuating mitochondrial dysfunction and associated cell death
Cheng et al.[103]	2010	Rats	MCAO	Ferulic acid significantly enhances gamma-aminobutyric acid type B receptor subunit 1 receptor expression at early reperfusion and thereby provides neuroprotection against p38 mitogen-activated protein kinase-mediated NO-induced apoptosis at 24 hours of reperfusion
Munakata et al.[104]	2016	Rabbit	Subarachnoid hemorrhage (SAH)	Cyclooxygenase-2 might be involved in the pathogenesis of cerebral vasospasm due to up-regulation of endothelin-1 and endothelin A receptor and down-regulation of eNOS, and celecoxib may potentially serve as an agent in the prevention of cerebral vasospasm after SAH
Terpolilli et al.[105]	2016	Mice	SAH	NO inhalation reduces brain damage, prevents mortality, and improves neurological outcome after subarachnoid hemorrhage by resolving early pial microvasospasms
Chang et al.[106]	2015	Rats	SAH	Arctigenin, a potent ingredient of *Arctium lappa* L., induces nNOS and attenuates SAH-induced vasospasm through PI3K/Akt pathway in a rat model
Huang et al.[107]	2015	Rats	SAH	Memantine attenuates delayed vasospasm after experimental SAH via modulating eNOS
Rameshn et al.[108]	2015	Humans	SAH	In SAH patients reduced levels of NO are associated with increased incidence of cerebral vasospasm and poor outcome
Nevzati et al.[109]	2015	Endothelial cells	SAH	Estrogen induces NO level increases in cerebral and peripheral endothelial cells *in vitro via* eNOS activation and through E2 receptor-mediated mechanisms. Further *in vivo* studies are warranted to evaluate the therapeutic value of estrogen for the treatment of SAH-induced vasospasm
Chang et al.[110]	2014	Rats	SAH	Progesterone attenuates experimental SAH-induced vasospasm by upregulation of eNOS *via* Akt signaling pathway
Osuka et al.[111]	2012	Rats	SAH	Adiponectin is significantly increased in the cerebrospinal fluid after SAH, resulting in the activation of adenosine monophosphate-activated protein kinase (AMPK)α and eNOS. Adiponectin plays an important role against cerebral vasospasm via the AMPK/eNOS signaling pathway
Sabri et al.[112]	2011	Mice	SAH	Simvastatin re-couples eNOS after SAH, leading to decreased secondary complications such as vasospasm, microthromboemboli and neuronal injury
Vellimana et al.[41]	2011	Humans	SAH	Endothelial NO synthase mediates endogenous protection against SAH-induced cerebral vasospasm

Table 1: Continued

Author	Year	Animals/ cells	Model	Results
Neurotoxicity				
Mohammadi et al.[113]	2016	Rats	MCAO	Overproduction of NO intensifies brain infarction and cerebrovascular damage through reduction of claudin-5 and zonula occludens-1 (ZO-1) expression in striatum of ischemic brain
Zheng et al.[114]	2016	Rats	MCAO	Effects and mechanism of action of iNOS on apoptosis in a rat model of cerebral ischemia-reperfusion injury
Fabian et al.[115]	2012	Rats	MCAO	Hyperglycemia accentuates persistent "functional uncoupling" of cerebral microvascular NO and superoxide following focal ischemia-reperfusion in rats
Mohammadi et al.[116]	2012	Rats	MCAO	Contribution of NO synthase (NOS) in blood-brain barrier disruption during acute focal cerebral ischemia in normal rat
Mohammadi et al.[117]	2011	Rats	MCAO	The harmful actions of NOS activity on cerebral microvascular integrity are intensified by ischemia-reperfusion injuries during acute hypertension
Iqbal et al.[118]	2016	Humans	SAH	Inducible NOS-2 in subarachnoid hemorrhage: regulatory mechanisms and therapeutic implications
Zhao et al.[119]	2015	Rats	SAH	NO is involved in the pathophysiological events of early brain injury after SAH by increasing caspase-12, which results in neuronal apoptosis
Sabri et al.[120]	2011	Mice	SAH	Artery constriction by SAH upregulates eNOS but that it is uncoupled and produces peroxynitrite that may generate microemboli that travel distally and contribute to brain injury

NO and neuroprotection

The neuroprotection of NO in the model of middle cerebral artery occlusion (MCAO) is exhibited as follows: (1) Watanabe and his colleagues[96] reported that the inhibition of serine racemases induce NO-mediated neurovascular protection in cerebral ischemia. (2) Yan et al.[97] reported that CXC195 induced phosphorylation of eNOS by the activation of PI3K/Akt signaling pathway under pathological cerebral ischemia-reperfusion conditions, which provided a novel explanation for the neuroprotective effect of CXC195. (3) Calycosin-7-O-β-D-glucoside could protect BBB integrity in experimental cerebral ischemia-reperfusion injury by regulating NO/caveolin-1/matrix metalloproteinases pathway.[98] (4) Neuroprotection was achieved by reducing the mRNA and protein levels of iNOS and nNOS.[99] (5) Ferulic acid can inhibit the expression of nNOS and iNOS, prevent the increase of isomer and therefore achieve neuroprotection.[100] (6) Mutologous adipose derived from mesenchymal stem cells (MSCs) transplantation can inhibit brain injury by inhibiting apoptosis and iNOS in ischemia-reperfusion injury.[101] (7) (S)-ZJM-289, a NOD, reduces neuronal mitochondrial dysfunction and reduces cell death in ischemic stroke.[102] (8) Ferulic acid significantly enhances the expression of gamma-aminobutyric acid type B receptor subunit 1 receptor during early reperfusion and thereby provides neuroprotection against p38 mitogen-activated protein kinase-mediated and NO-induced apoptosis at 24 hours of reperfusion.[103]

The neuroprotection of NO in the SAH is exhibited as follows: (1) The pathophysiological mechanism of Cyclo-oxygenase-2 may be involved in cerebral vasospasm. The upregulation of endothelin-1, down regulation of eNOS and ETAR and the regulation of celecoxib might contribute to the prevention of cerebral vasospasm after SAH.[104] (2) Inhaled NO can alleviate the early cerebral microvasospasms, reduce brain injury and improve the prognosis of neurological impairment after SAH.[105] (3) Arctigenin induced eNOS and alleviated vasospasm after SAH through PI3K/Akt signaling pathway.[106] (4) Memantine alleviates cerebral vasospasm by regulating the eNOS in experimental SAH.[107] (5) Reduced levels of NO are associated with increased incidence of cerebral vasospasm and poor outcome in SAH patients.[108] (6) Estrogen increases the level of NO in brain and peripheral vascular endothelial cells, and alleviates the vascular spasm after SAH.[109] (7) Progesterone reduces cerebral vasospasm induced by SAH via upregulating eNOS through Akt signaling pathway.[110] (8) Adiponectin was significantly increased in the cerebrospinal fluid after SAH. It results in the activation of AMPKα and eNOS, which played an important role in antagonizing cerebral vasospasm.[111] (9) Simvastatin improves the expression of eNOS after SAH and leads to reduced complications such as cerebral vasospasm, thrombosis, and neuronal injury.[112] (10) eNOS mediates endogenous protection against SAH-induced cerebral vasospasm.[41]

NO and neurotoxicity

However, the role of NO in different concentrations, different environments is quite different, even in different time periods and different cells will get different or even the opposite consequences. The neurotoxicity of NO in the model of MCAO is exhibited as follows: (1) Excess NO increases infarct size and cerebral vascular injury.[113] (2) The activation of iNOS induced cell apoptosis in a rat model of cerebral ischemia-reperfusion injury.[114,121] (3) Persistent hyperglycemia after ischemic stroke is associated with an excess of

NO and peroxides which leads to microvascular dysfunction and poor prognosis.[115] (4) NOS leads to the damage of blood brain barrier in acute ischemic stroke.[116] (5) The activation of NOS leads to the damage of blood-brain barrier and brain edema in acute ischemic stroke.[117] The neurotoxicity of NO in the SAH is exhibited as follows: (1) Imbalance of NOS and its product NO leads to adverse factors in SAH.[118] (2) NO increases the production of caspase-12 which induced neuronal apoptosis in early brain injury after SAH.[119] (3) SAH leads to the upregulation of eNOS and results in the generation of microemboli. It reaches to the end of blood vessels and leads to brain injury.[120]

Conclusion

There is growing evidence that NO has an important role in neuroprotection in stroke, even if NO is usually considered as a toxic gas. Therefore, we need to dialectically treat NO, and further research including animal and clinical research can provide us with a new insight into the treatment of stroke and other central nervous system diseases such as multiple sclerosis, Parkinson's disease and traumatic brain injury.

Author contributions

ZQC and RTM were responsible for writing the manuscript. DXF was responsible for its revision. ZW and GC were responsible for its drafting and revision. All authors read and approved the final version of the paper for publication.

Conflicts of interest

The authors declare that they have no competing interests.

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