Targeting gaseous molecules to protect against cerebral ischaemic injury: 
Mechanisms and prospects

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SUMMARY

1. Ischaemic brain injury is a leading cause of death and disability in many countries. However, the pathological mechanisms underlying ischaemic brain injury, including oxidative stress, calcium overload, excitotoxicity and neuronal apoptosis, are perplexing and this makes it difficult to find effective novel drugs for the treatment of the condition.

2. Recently, gaseous molecules such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulphide (H₂S) and hydrogen (H₂) have attracted considerable interest because of their physiological and pathophysiological roles in various body systems. Emerging evidence indicates that gaseous molecules are involved in the pathological processes of ischaemic brain damage.

3. In the present review, we summarize evidence regarding the involvement of gaseous molecules in ischaemic brain injury and discuss the therapeutic potential of targeting gaseous molecules.

4. Collectively, the available data suggest that the application of these biological gas molecules and their pharmacological regulators may be a potential therapeutic approach for the treatment of ischaemic brain injury.

Key words: carbon monoxide, cerebral ischaemia, gaseous molecule, hydrogen, hydrogen sulphide, nitric oxide.

INTRODUCTION

Because of its high metabolic demand, the brain is very susceptible to damage following reductions in blood supply. After the onset of ischaemia, brain concentrations of glucose, glycogen, ATP and phosphocreatine fall immediately and are nearly completely depleted within 10–12 min.1 Transient or permanent reductions in cerebral blood flow (CBF) following ischaemia can lead to severe and irreversible brain damage. Brain damage has been observed within several minutes or over a longer period of time after the onset of global ischaemia.2 More severe brain injury can be caused by reperfusion,3 which occurs when blood flow to the ischaemic brain is restored, resulting in multiple pathophysiological processes that include further oxidative stress injury and neuronal apoptosis.4 These ischaemic brain injuries can result in vascular dementia, stroke and even death.

Considering the complexity of the mechanisms underlying ischaemic brain injury5 and the severity of the subsequent conditions, adequate therapeutic approaches for a certain type of ischaemic brain damage need to be developed carefully and optimized. Generally speaking, there are two pivotal points in the therapeutic intervention for brain ischaemia: (i) to increase CBF and improve the supply of oxygen and nutrients, which improves the cerebral microcirculation and terminates the pathological process caused by ischaemia;6 and (ii) to attenuate the loss of ischaemic neurons by using calcium antagonists and neuroprotective agents that exhibit anti-oxidant, anti-excitotoxic or anti-inflammatory effects when administered during reperfusion.7 Commonly used neuroprotective drugs include excitatory amino acid receptor
antagonists, GABA receptor agonists, free radical scavengers, calcium channel blockers and inflammatory cytokine receptor antagonists. In addition, in certain cases, patients with brain ischaemia may be candidates for surgical treatment, such as interventional therapy for ischaemic cerebral diseases, endovascular treatment of neurosurgical diseases or bone marrow-derived matrix cell transplantation therapy. It has also been found that, at the onset of cerebral ischaemia, central neuronal stem cells are activated and undergo compensatory neurogenesis and functional repair. Therefore, the application of neurotrophic factors to promote the proliferation of neuronal stem cells and to improve the survival of neurons may be an efficient way in which to treat cerebral ischaemic diseases.

Despite the abundance of studies on the subject, many therapeutic interventions for ischaemic brain injury have proved disappointing in clinical trials. The very nature of brain ischaemia (i.e. a lack of blood) restricts the ability to deliver pharmacological agents to the site of need. Furthermore, the drugs used traditionally have several disadvantages for use in the treatment of cerebral ischaemic diseases. First, the drugs may have trouble crossing the blood–brain barrier (BBB). The BBB is essentially impenetrable for many chemical drugs and neurotrophins because of their large molecular mass and poor liposolubility. Crossing the BBB is the most problematic aspect in translating neurotrophin neuroscience into clinically effective neurotherapeutics. Second, orally administered drugs have to move down the alimentary canal and pass through the gut wall and liver, which decreases drug availability. Third, some candidate molecules with a short half-life in vivo are not stable enough to reach the brain, a typical example being brain-derived neurotrophic factor. Fourth, irreversible neuronal damage occurs during the early stages of cerebral ischaemia (within minutes), but the onset of action of most orally administered drugs is relatively slow (within tens of minutes).

In recent decades, endogenous gaseous molecules have been found in mammals (including humans). These molecules include nitric oxide (NO), carbon monoxide (CO), hydrogen sulphide (H₂S) and molecular hydrogen (H₂) and they are collectively referred to as gasotransmitters. Endogenous gas molecules can be produced and degraded by special pathways within the body. The gaseous signalling molecules play important roles in various physiological and pathological processes. Using small molecules, such as gaseous molecules, to treat central nervous system disorders is a feasible option. These gaseous molecules are lipid soluble, endogenously produced and can freely cross cell membranes and the BBB. The gaseous molecules diffuse easily into target cells, where they bind directly to intracellular targets and induce biochemical and physiological responses. For example, NO binds directly to cytoplasmic soluble guanylate cyclase (sGC), activating a variety of physiological responses, such as cardiovascular effects and regulation of neuronal synaptic plasticity, through signalling pathways. In addition, gaseous molecules have a relatively longer duration of action because they persistently interact with and structurally modify many functional biological macromolecules. Gaseous molecules have a rapid onset of action, which is appropriate for the treatment of acute ischaemic diseases. For example, nitroglycerine (an NO donor) tablets are used in the treatment of myocardial ischaemia and angina attacks. Gaseous molecules can also be administered via inhalation and sublingually. They are absorbed rapidly into the systemic circulation, reaching the brain and escaping degradation in the gastrointestinal tract and first-pass metabolism in the liver.

Over the past two decades, more and more reports have confirmed that biologically active gaseous molecules are involved in the pathological processes of cerebral ischaemic disorders. The present article reviews the roles of four biological gaseous molecules in cerebral ischaemic diseases, along with potential therapeutic applications.

NITRIC OXIDE

Synthesis of NO

Nitric oxide is a multimodal endogenous mediator that is synthesized from its precursor L-arginine by the action of NO synthase (NOS). Three NOS isoforms have been characterized in the brain. Neurons produce NO mostly via neuronal (n) NOS, which is constitutively expressed in these cells. Glial cells (astrocytes, microglia and macrophages) synthesize NO mainly through the action of inducible (i) NOS (or NOS2), which is expressed in response to various stimuli. Finally, endothelial cells produce NO via endothelial (e) NOS (or NOS3). In addition to this commonly accepted distribution of NOS isoforms in different cell types, most brain cell types are able to express several NOS isoforms. For example, neurons and endothelial cells in the brain can also express iNOS, whereas astrocytes are able to express nNOS. Several splice variants of nNOS (nNOS-1 and nNOS-2) have been identified in the central nervous system.

Cytoprotective effects and underlying mechanisms

The role of the neural messenger NO in cerebral ischaemia has been investigated extensively. Nitric oxide may have either a protective or destructive role in ischaemia and many
contradictory findings have been reported in the literature. Recent evidence challenges the idea that NO from neurons builds up to levels (micromolar) that are sufficient to directly elicit cell death during the postischaemic period. Concomitantly, the case has been strengthened for a role of NO in delayed postischaemic death mediated by iNOS. Mechanistically, it seems unlikely that NO is released in high enough quantities to inhibit respiration in vivo, with the formation of reactive nitrogen species (RNS), such as peroxynitrite, representing a more likely pathway to cell death. The results of another study suggest that NO mediates N-methyl-D-aspartate (NMDA)-induced persistent inhibition of protein synthesis via the dephosphorylation of eukaryotic initiation factor 4E-binding protein 1 and proteolysis of eukaryotic initiation factor 4G and that these effects contribute to neuronal damage after a transient ischaemic attack.

The protective and restorative properties of NO have become the focus of increasing interest. By stimulating cGMP production, NO from endothelial cells may protect the ischaemic brain by augmenting blood flow in the short term and by helping form new blood vessels over the longer term (angiogenesis). Elevated cGMP production may also inhibit apoptosis and help repair damage by stimulating neurogenesis. Nitric oxide may also act as a direct anti-oxidant and trigger protective gene expression programmes that underlie cerebral ischaemic preconditioning. The beneficial role of NO after cerebral ischaemia may be attributed largely to its vascular effects. As a potent vasodilator and inhibitor of platelet aggregation, NO is beneficial in the early stages of focal cerebral ischaemia because it facilitates collateral blood flow to the ischaemic territory. It has been reported that the effects of the inhibition of NO synthesis on focal cerebral ischaemic damage are time dependent; that is, inhibition of NO synthesis worsens ischaemic damage when instituted shortly after the induction of ischaemia but does not affect (or even reduces) damage when started at later times. The vascular actions of NO are beneficial only during the early stages of permanent focal cerebral ischaemia. Ischaemia–reperfusion (IR) activates constitutive NOS, suggesting that NO production during reperfusion is related to neuronal degeneration and that NOS inhibitors may be a new therapeutic strategy during reperfusion. Nitric oxide is involved in the autoregulation of CBF, as well as in the modulation of respiratory chain activity in the normal brain. It has also been demonstrated to play an important role in the autoregulation of CBF and mitochondrial activity in the partially ischaemic brain. Other studies have provided evidence for a regulatory role of NO in initial leucocyte–endothelial interactions in the cerebral microcirculation under basal and ischaemic conditions. After transient focal ischaemia, early NO production exerts a neuroprotective effect by modulating neutrophil infiltration in male Sprague-Dawley rats subjected to 2 h occlusion of the left middle cerebral artery and the left common carotid artery. Thus, endogenous NO production during and after transient focal cerebral ischaemia is neuroprotective by limiting the process of neutrophil infiltration and its deleterious consequences. In addition, it has been reported that prior sublethal ischaemia (preconditioning) results in neuronal tolerance to subsequent lethal ischaemia. Nitric oxide production via nNOS during preconditioning is crucial for the downregulation of astrocytic glutamate transporter-1 (GLT-1) and coincides with the increased survival of neurons in neuron–astrocyte cocultures.

It has been demonstrated that the administration of NO in experimental models of both permanent and transient stroke reduces stroke lesion volume. This may be mediated, in part, by increased CBF in models of permanent stroke and the data support clinical trials in stroke patients, although the presence of a narrow therapeutic window may be a limiting factor. Moreover, inhaled NO has been shown to exhibit neuroprotection in neonatal excitotoxicity-induced brain damage in a chronic stroke model in the rat and this effect appears to be mediated via the vascular endothelial growth factor–phosphorylated (p-) Akt–p-cAMP response element-binding protein pathway and subsequent modulation of glutamate receptor subunit expression.

Existing evidence suggests that NO can exert both protective and deleterious effects depending on factors such as the NOS isoform and the cell type in which NO is produced and/or the temporal stage after the onset of ischaemic brain injury (Fig. 1). Immediately after brain ischaemia, NO produced via eNOS exhibits protective effects mainly by promoting vasodilatation; however, as ischaemia develops, NO produced by overactivation of nNOS and, later, NO production by the de novo expression of iNOS contribute to brain damage. In stroke, NO produced by nNOS and iNOS can be neurotoxic, in part as a consequence of the formation of peroxynitrite, a free radical, which causes direct damage to mitochondrial enzymes and DNA. In contrast, NO produced by eNOS is beneficial in acute stroke. Endothelial-derived NO may limit neuronal damage through effects in vascular beds or within the brain itself. In the intravascular space, NO acts as a powerful vasodilator that modulates blood flow. In addition, NO inhibits leucocyte adhesion and/or migration to the endothelium and has antiplatelet effects. In the brain, NO may exert its neuroprotective effects through several mechanisms, including the scavenging of reactive oxygen species (ROS), anti-inflammatory effects and possibly via attenuation of NMDA receptor activity. In experimental stroke models, such as focal cerebral ischaemia in the rat, the administration of exogenous NO

**"eNOS is protective against ischaemic injury"**
limits metabolic derangement, reduces apoptosis and stimulates neurogenesis.

**Potential clinical application of NO in the treatment of ischaemic cerebral injury**

Theoretically, manipulation of the NO system could be useful in the treatment of stroke. Nitric oxide donors may be useful within the first few hours after the onset of ischaemia; NO release from NO donors could help by inducing vasodilatation and improving blood flow in the penumbra. In addition, NO donors could induce neurogenesis after chronic treatment. However, clinical experience with the use of NO donors for the treatment of human stroke is limited. In one study, following the administration of sodium nitroprusside to 22 patients with acute ischaemic stroke (and 12 matched control subjects) at a dose that caused a 10 mmHg fall in mean arterial blood pressure, improvements in CBF were noted in four patients only. In other studies, the intravenous administration of S-nitrosoglutathione, an NO donor that is relatively platelet specific, was shown to reduce the rate of embolization in humans. A better understanding of the molecular mechanisms by which NO is beneficial or detrimental in brain ischaemia may ultimately result in the identification of potential new therapeutic targets.

**CARBON MONOXIDE**

**Synthesis of carbon monoxide**

Heme oxygenase (HO) enzymes catalyse the breakdown of heme to iron, carbon monoxide (CO) and biliverdin. Heme oxygenases have been implicated in the protection against oxidative stress and ischaemic brain injury. Heme oxygenase-1 (or heat shock protein (HSP) 32), the first form of the enzyme discovered, is an inducible protein found predominantly in tissues involved in the degradation of red blood cells. It is stimulated by haemolysis and numerous other toxic perturbations to eliminate potentially toxic heme. In contrast, HO-2 is constitutively expressed and is most abundant in neural tissues. Endogenous CO, formed mainly from HOx, is a putative neurotransmitter in the brain and peripheral autonomic nervous system. At high concentrations CO is toxic, but at physiological concentrations CO acts as a vasodilator and modulates GC activity, subsequently generating the second messenger cGMP.

**Cytoprotective effect and underlying mechanisms**

Results from a variety of experimental models, including a cerebral ischaemic model, have suggested that HO-2 is a cytoprotective enzyme. Levels of HO-2 are enriched in neurons and, under normal conditions, HO-2 accounts for nearly all the brain HO activity. Deletion of HO-2 in mice (HO-2 knockout) results in increased neurotoxicity of cultured brain cells and damage following transient cerebral ischaemia. Moreover, pharmacological inhibition of HO activity significantly augments focal ischaemic damage in wild-type mice, but does not further exacerbate it in HO-2 knockout mice. There are some similarities between the HO and NOS systems, most notably the synthesis of CO and NO, respectively, which are both diffusible gases with numerous biological actions, including neurotransmission and vasodilatation. The outcome of focal cerebral I/R in double-knockout (HO-2 knockout × nNOS knockout) mice suggests that the neuroprotective role of HO-2 could counteract the deleterious action of nNOS.

Many consider HO-1 to be a potential target in ischaemic damage. Heme oxygenase-1 is a highly regulated cytoprotective enzyme that responds to numerous chemical and/or physical stressors, many of which decrease oxygen availability and generate oxidative stress. Disruption of HO-1 activity is associated with a bad outcome in many disorders, such as...
enteritis and cancer, and a protective role via the heme catabolism products of HO-1 has been seen following transplantation, cardiac ischaemia and limb I/R, as well as in other conditions that involve I/R events. Increased HO-1 immunoreactivity is detected in hippocampal and cortical neurons after 1 h ischaemia, lasting for >1 day and the induction of HO-1 protein protects cerebral tissues from ischaemic damage. In an HO-1 transgenic mouse model, it was shown that overexpression of HO-1 was neuroprotective in permanent middle cerebral artery occlusion (MCAO). Because CO is regarded as a gaseous molecular messenger, like NO, the neuroprotective effects of HO-1 and HO-2 may be mediated, at least in part, by the production and signalling of CO (Fig. 2).

Potential clinical application for ischaemic cerebral injury

It has been demonstrated that low doses of CO protect against experimental focal brain ischaemia injury following transient MCAO in mice. It has also been reported that exposure of ischaemic neurons to CO increases intracellular levels of cGMP, resulting in the suppression of caspase 3 activity and increased neuronal survival. These results describe a potentially important paracellular pathway through which astrocytes may rescue nearby neurons from ischaemic death, suggesting the potential for CO inhalation in the treatment of brain ischaemic stroke and other cerebral ischaemic diseases.

HYDROGEN SULPHIDE

Synthesis of hydrogen sulphide

Hydrogen sulphide (H2S) has been regarded as a toxic gas and environment pollutant for a long time. It is a colourless gas that can cross plasma membranes because its solubility in lipophilic solvents is fivefold greater than in water. Therefore, the gas can easily diffuse through cells, reaching intracellular compartments. Following the discovery of the presence and enzymatic production of H2S in mammalian tissues, considerable attention has focused on H2S as a physiological signalling molecule. Accumulating evidence suggests that H2S is a gaseous messenger that serves as an important neuromodulator in the central nervous system. In mammalian tissues, H2S is generated via the degradation of l-cysteine primarily by two enzymes, namely cystathionine β-synthase and cystathionine γ-lyase. The former is expressed mainly in the brain, peripheral nervous system, liver and kidney, whereas the latter is found mostly in the liver, vascular smooth muscle and endothelial cells. In recent years, it has been reported that endogenous H2S can also be produced by 3-mercaptopropionate sulphydryltransferase and cysteine aminotransferase in the brain.

Cytoprotective effect and underlying mechanisms

In some pathological conditions, H2S-synthesizing enzymes are activated, thus changing H2S dynamics, which may regulate pathological processes. Controversial effects of H2S have been observed in different pathophysiological states. For example, exogenous H2S has been shown to be beneficial in animal models of hypertensive diseases, whereas inhibition of the endogenous synthesis of H2S is beneficial in shock of various aetiologies. Under ischaemic conditions, inhibitors of H2S synthesis reduce the infarct volume caused by MCAO. Therefore, controlling H2S production may be a useful approach in stroke therapy (Fig. 3a). However, H2S production can also be induced by myocardial ischaemia in sufficient amounts to limit myocardial injury and exogenous H2S has been reported to protect neurons against hypoxic injury by stimulating the ATP-sensitive potassium channel–protein kinase C-extracellular signal-regulated kinase–Hsp90 pathway.

The physiological and pathological roles of H2S in the central nervous system have been well documented and many studies have uncovered the cytoprotective effects of H2S for both in vitro and in vivo ischaemic injury. Oxidative stress associated with I/R injury results in major damage to the neurons. An in vitro study has demonstrated that sodium hydrosulphide (NaHS; 0.1 mmol/L) protects neurons against glutamate toxicity and oxidative stress, with the anti-oxidant effect of H2S mediated, in part, by enhanced glutamate uptake. In addition, H2S has been shown to have an anti-inflammatory effect by inhibiting the activity of microglia. Exogenous H2S has been shown to inhibit the oedema around pyramidal neurons and the nuclear shrinking induced by ischaemia, as well as to enhance the expression of growth-associated protein–43 and synaptic plasticity in the hippocampus to improve spatial learning and memory deficits induced by brain ischaemia. Administration of sodium sulphide...
(Na₂S) 1 min prior to the start of cardiopulmonary resuscitation (CPR) markedly improved myocardial and neurological function and survival 24 h after normothermic cardiac arrest – CPR in mice.⁹³ This effect may have been mediated via an eNOS-dependent mechanism.

It has been reported recently that H₂S content is elevated in the first 12 h and then decreased in the followed 24 h during reperfusion following transient global cerebral ischaemia, suggesting that application of low doses of H₂S may have beneficial effects on cerebral I/R injury.⁹⁴ Preconditioning with H₂S inhalation has been demonstrated to attenuate apoptosis after retinal I/R injury in rat.⁹⁵ Thus, H₂S may have both beneficial and detrimental effects depending on the different stages of a given pathological condition. In this way, administration of physiological concentrations of exogenous H₂S during the early phase of brain ischaemia, or preconditioning with low concentrations of H₂S, may rescue ischaemic injury (Fig. 3b).

Many studies have also reported that garlic extract has antioxidant properties and exhibits beneficial effects in the prevention of I/R injury, including cerebral I/R injury, in the rat.⁹⁶,⁹⁷ Because garlic contains high levels of sulphur compounds, including H₂S, the beneficial effects of garlic on I/R injury may be mediated mainly or in part via an H₂S signalling pathway.

Potential clinical application for ischaemic cerebral injury

Both in vitro and in vivo studies have demonstrated that low concentrations of H₂S can attenuate oxidative stress by restoring levels of reduced glutathione in the brain,⁹⁸ whereas higher concentrations of H₂S exert effects ranging from the exacerbation of cerebral damage in the ischaemic stroke model to the induction of a state of neuroprotection.⁹⁹ Studies investigating the role of H₂S-producing enzyme systems in cerebral I/R injury could potentially elucidate the role of H₂S in modulating cerebral ischaemia and may be of considerable use in developing H₂S-derived drugs for the treatment of brain ischaemic disorders.

**MOLECULAR HYDROGEN**

**Synthesis of molecular hydrogen**

In mammals, endogenous molecular hydrogen (H₂) is produced mainly in the large intestine (~150 mL daily) as a result of the fermentation of non-digestible carbohydrates by intestinal bacteria; the H₂ produced is then absorbed into the systemic circulation.¹⁷,¹⁸ It has been shown that H₂ gas neutralizes free radicals, a potential cytoprotective action against many oxidative injuries,⁹⁹ and reduces oxidative stress in heart and liver I/R damage.¹⁰⁰,¹⁰¹ It seems that H₂ selectively reduces hydroxyl radical and peroxynitrite in vitro and exerts its anti-oxidant effect in the rat MCAO model by decreasing 4-hydroxynonenal, a specific marker of lipid peroxidation and 8-hydroxy guanosine (8-OHG), a marker of nucleic acid oxidation.¹⁰²

**Cytoprotective effect and underlying mechanisms**

In recent years, it has been shown that H₂ provides significant protection against oxidative stress and inflammation-associated brain ischaemic disorders, such as cerebral I/R injury¹⁰³,¹⁰⁴ and stress-induced nervous impairment.¹⁰⁵ It has also been reported that administration of H₂ after brain ischaemia protects the brain by inhibiting microglia activation and caspase activity, consequently preventing neuronal apoptosis.¹⁰⁶ Moreover, it has been demonstrated that intraperitoneal injection of hydrogen-rich saline has a strong protective effect against transient global cerebral I/R in rats.¹⁰⁷ However, because most damage is caused between 6 and 24 h after I/R, the period when hydrogen-rich saline was effective was much shorter than that of 6 h after I/R and the protective effect of hydrogen-rich saline is quite limited.¹⁰⁷

Acute oxidative stress induced by brain I/R causes serious damage to brain tissues. Molecular hydrogen has been shown to have preventive and therapeutic effects as an anti-oxidant in a rat model of acute focal ischaemia and reperfusion.¹⁰³ Inhalation of H₂ gas markedly suppressed focal ischaemic brain injury in the rat by buffering the effects of oxidative stress because of its ability to rapidly diffuse across membranes and react with cytotoxic ROS.¹⁰⁴ Moreover, H₂ selectively reduces levels of the...
hydroxyl radical, the most cytotoxic of ROS and does not react with other ROS, which may have a role in normal physiological processes. The results of various studies suggest that the effect of H2 is superior to that of edaravone, a widely used free radical scavenger, in the treatment of cerebral I/R injury. Thus, H2 protects against brain ischaemic diseases by selectively scavenging destructive free radicals.

**Potential clinical application for ischaemic cerebral injury**

There are various pathological mechanisms involved in the process of cerebral I/R, including oxidative stress. Ischaemia disrupts the balance of endogenous oxidants and antioxidants, with a resultant overproduction of toxic free radicals. Reperfusion is also associated with a significant production of ROS and RNS that potentiate initial brain damage. Hydroxyl radicals easily react with cellular macromolecules, including DNA, proteins and lipids, to exert a strong cytotoxic effect. Reactive oxygen species, other free radicals and/or oxidants and pro-apoptotic cytokines (released by inflammatory cells) can severely threaten tissue viability in the ischaemic brain. Because H2 exhibits potent free radical-scavenging, anti-inflammatory and anti-apoptotic activity in the central nervous system, it may become a potential remedy for cerebral ischaemia-related disorders.

**INTERACTIONS BETWEEN GASEOUS MOLECULE SYSTEMS**

The enzymes involved in the synthesis of NO, CO and H2S are heme-containing proteins. In addition, sGC, a primary target and/or receptor for NO and CO, is a heme protein. Although these functional proteins have adapted ways to recognize specific substrates, cofactors or ligands, there are interactions between these gaseous molecules that contribute to the complex and refined regulation of various systems in the body, especially that of vascular tone.

The cross-talk between the NO and CO transduction systems has been reviewed in detail elsewhere. Although CO inhibits NOS at relatively high concentrations, NO can bind to and inhibit HO at much lower concentrations. There are three different ways by which CO controls sGC activity and vascular tone. With a low local amount of NO, CO modestly stimulates sGC, thereby reducing the tonic contractile tension of vascular walls. In contrast, when a sufficient amount of NO exists, CO partially inhibits sGC. In addition, CO interferes with NOS activities and subsequently reduces NO, resulting in sGC inhibition.

**Heme and gas molecular cross-talk**

Increasing evidence demonstrates the critical role of biological gaseous molecules in physiological and pathological conditions, including ischaemic brain injury. The molecular weights of gases are much smaller than those of general chemical drugs and the gaseous molecules can easily cross the BBB. For example, NO is an electrically neutral molecule that rapidly crosses biological membranes and diffuses freely among all kinds of cells and tissues. Moreover, NO is highly reactive and has a very short half-life. Therefore, inhalation of NO may avoid systemic side-effects. In addition, inhalation of NO will result in the rapid accumulation of NO metabolites in the blood and tissues to

**CONCLUSION AND PROSPECTS**

Increasing evidence demonstrates the critical role of biological gaseous molecules in physiological and pathological conditions, including ischaemic brain injury. The molecular weights of gases are much smaller than those of general chemical drugs and the gaseous molecules can easily cross the BBB. For example, NO is an electrically neutral molecule that rapidly crosses biological membranes and diffuses freely among all kinds of cells and tissues. Moreover, NO is highly reactive and has a very short half-life. Therefore, inhalation of NO may avoid systemic side-effects. In addition, inhalation of NO will result in the rapid accumulation of NO metabolites in the blood and tissues to
protect against cardiac I/R injury. The concentration of NO metabolites achieved within a target tissue may be more important than the absolute amount of NO absorbed, providing a good example for the application of gaseous molecules in the prevention and treatment of ischaemic brain injury.

However, many gas donors are not suitable for clinical application for the treatment of ischaemic disorders because the gas-releasing agents exhibit significantly different dynamics compared with endogenously produced gases. For example, many studies suggest that exogenous regulation of H2S may represent a novel approach for the administration of gaseous molecules in the regulation of endogenous H2S system. Thus, innovative approaches for the administration of gaseous molecules are urgently required.

In recent years, many natural components of traditional herbal medicines have been shown to be effective in protecting against cerebral ischaemic damage. Although the underlying mechanisms remain unknown, it would be of considerable interest to investigate whether gasotransmitter signalling is involved in these protective mechanisms.

In conclusion, sufficient evidence has been accumulated to support gaseous signalling molecules acting as functional molecules in the mammalian central nervous system, especially in ischaemic cerebral injury. However, further studies are needed to identify more potential therapeutic properties and the underlying mechanisms of action of these gaseous molecules. The safety, dose(s), routes of administration and stability of gaseous molecules, as well as their pharmacological regulators, need to be determined.

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