Medical gases for stroke therapy: summary of progress 2015–2016

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Abstract

Stroke is a cerebrovascular disease with high mortality and morbidity. Despite extensive research, there are only a very limited number of therapeutic approaches suitable for treatment of stroke patients as yet. Mounting evidence has demonstrated that such gases as oxygen, hydrogen and hydrogen sulfide are able to provide neuroprotection after stroke. In this paper, we will focus on the recent two years’ progress in the development of gas therapies of stroke and in understanding the molecular mechanisms underlying protection induced by medical gases. We will also discuss the advantages and challenges of these approaches and provide information for future study.

Key words: medical gases; stroke; neuroprotection

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INTRODUCTION

Stroke is a leading cause of death and acquired adult disability worldwide. Approximately 80% of all strokes are ischemic, which result from obstruction of the cerebral arteries. Currently, tissue plasminogen activator (tPA) is the only approved drug by the US Food and Drug Administration (FDA) for treatment of acute ischemic stroke.1 However, encouraging data from recent clinical studies have demonstrated that endovascular management strategies such as thrombectomy can restore the blood flow and, hence, be beneficial for the acute stroke treatment.2,3 Although blood supply recovery can rescue distant penumbra area, it is inevitable that penumbra cells in the rim of infarct core will undergo death triggered by complex ischemic cascades.4 These underscore the needs of development of additive treatments that are able to increase the therapeutic window of advanced reperfusion strategies and, on this way, to preserve brain functions after stroke.2

In past decades, neuroprotective abilities of certain gases have been observed. Several publications reported about neuroprotective effects of oxygen, hydrogen, carbon monoxide (CO) and nitric oxide (NO) as well as some volatile anesthetics (isoflurane, sevoflurane, xenon and nitrous oxide).5-8 Protective properties of inert gases (helium and argon) and even gases classically considered as toxic (hydrogen sulfide, H2S; CO) have been also investigated (Table 1).9

Among protective medical gases, oxygen, isoflurane, H2S and hydrogen are the most studied ones. In this paper, we briefly summarize the newest reports regarding application of these medical gases in the stroke treatment and discuss their challenges and advantages needed for future study.

OXYGEN

Oxygen can be administrated normobaric (normobaric
oxygen therapy, NBOT) or under pressure (hyperbaric oxygen therapy, HBOT). Neuroprotective effects of oxygen therapy have been observed in various experimental models of brain injury and neurological diseases. Neuroprotective effects of oxygen therapy have been observed in various experimental models of brain injury and neurological diseases. A systematic review and meta-analysis of the literature published prior to September 2015 showed hyperbaric oxygen (HBO) had a neuroprotective effect and improved survival in animal models of middle cerebral artery occlusion (MCAO), especially in animals given more than 6 hours of HBO and given HBO at 2.0 ATA (101.3 kPa) immediately after MCAO. In tPA-induced thrombolysis in vitro, HBO increased tPA-induced thrombolysis; and in rats subjected to thromboembolic MCAO, 5-minute HBO reduced infarct volume and brain edema. However, HBOT in human stroke is still not sufficiently evidence-based, due to the insufficient randomized double-blind

### Table 1: Original articles of medical gases in stroke from 2015 to 2016

| Medical gases | Paradigm | Models | Effects | References |
|---------------|----------|--------|---------|------------|
| **O₂**       |          |        |         |            |
| HBOT         | 2.5 ATA for 1 h daily for 5 days | MCAO in rats | Improved neurological function | Bian et al.¹⁰ |
|              | 2.5 ATA for 1.5 h | Recombinant tPA-induced thrombolysis in vitro | Provided neuroprotection by promoting thrombolysis | Chazalvio et al.¹¹ |
|              | 2.5 ATA for 1 h daily for 2 days | Transient cerebral ischemia in gerbils | Reduced inflammation and inhibited apoptosis | Gamdzik et al.¹² |
|              | 2.8 ATA for 1 h | BBB damage model in vitro | Protected the integrity of BBB | Hao et al.¹³ |
|              | 2.5 ATA for 1 h daily for 5 days | MCAO in rats | Reduced infarct volume ratio and neurobehavioral deficit | Xue et al.¹⁴ |
|              | 2.4 ATA for 1.5 h | OGD in cells | Decreased infarction area, lessened neuronal injury, and reduced apoptosis | Zhai et al.¹⁵ |
|              | 2.5 ATA for 1 h by four times at an interval of 12 h | MCAO in rats | Provided neuroprotection by promoting thrombolysis |            |
| NBOT         | 33%, 45% or 61% O₂ for either 3, 6, 12, 24, 48 or 72 h during reperfusion | MCAO in rats | Reduced apoptosis and promoting neurological functional recovery | Chen et al.¹⁶ |
|              | > 90% O₂ for 1 h daily for 5 days | BBB damage ICH in rats | Reduced intracerebral edema | Fang et al.¹⁷ |
|              | 95% O₂ for 2 h | MCAO in rats | Prevented BBB damage and improved the outcome of brain injury | Liu et al.¹⁸ |
|              | 60% O₂ for 3 h | 60% O₂ for 3 h | Decreased infarct volume and neurological deficit | Cai et al.¹⁹ |
| Volatile anesthetics | 1.5–4.5% ISPOC | OGD | Provided better neuroprotection | Wang et al.²⁰ |
|              | 1.5–4.5% ISPOC for 1 h | MCAO in rats | Decreased the neurobehavioral deficit scores and infarct volume | Wang et al.²¹ |
|              | 2.5% ISPOC for 1 h | Embolic stroke model in rabbits | Improved neurological outcome | Chen et al.²² |
| Hydrogen sulfide | Blocking endogenous H₂S | MCAO in rats | Reduced brain edema and improved BBB disruption, and the neurological outcome | Jiang et al.²³ |
|              | 40 ppm or 80 ppm H₂S inhalation for 3 h | MCAO in rats | Reduced neurological deficits, infarct size, and brain edema | Wei et al.²⁴ |
|              | H₂S donors | OGD in cells | Reduced tPA-induced cerebral hemorrhage | Liu et al.²⁵ |
| Hydrogen     | Ingestion of HRW | SHRSP | Improved neurological function outcome and attenuated BBB disruption | Takeuchi et al.²⁶ |
| Injection of HRW | MCAO in rats | Reduced brain infarct volume and improve neurological function | Han et al.²⁷ |
| Other gas    | 25–75% helium | MCAO in rats | Reduced ischemic brain damage and brain hemorrhages | Haelewyn et al.²⁸ |

Note: HBOT: Hyperbaric oxygen therapy; NBOT: normobaric oxygen therapy; O₂: oxygen; H₂S: hydrogen sulfide; OGD: oxygen-glucose deprivation; MCAO: middle cerebral artery occlusion; BBB: blood-brain barrier; ICH: intracerebral hemorrhage; ISPOC: isoflurane postconditioning; tPA: tissue plasminogen activator; SHRSP: spontaneously hypertensive stroke-prone rats; HRW: hydrogen-rich water; SAH: subarachnoid Hemorrhage; h: hour(s). 1 Atmosphere absolute (ATA) = 101.3 kPa.
controlled clinical studies.\textsuperscript{33} HBO has also been investigated as a pre-conditioning agent.\textsuperscript{34} HBO pre-conditioning is neuroprotective and able to attenuate hemorrhagic transformation after MCAO by upregulation of peroxisome proliferator activated receptor gamma (PPAR-\textgamma), downregulation of aquaporin-4 (AQP-4) and oxidative stress reduction.\textsuperscript{10,12-15,17} Yan et al.\textsuperscript{35} reviewed the status of clinical and experimental HBOT research in China and concluded that HBOT could increase the oxygen supply to ischemic tissue, improve blood oxygen partial pressure and reduce irreversible tissue damage.

Similar to HBOT, NBOT provides therapeutic benefits and is likewise effective in the treatment of stroke.\textsuperscript{36} NBOT can inhibit the apoptotic pathway by reducing the expression of caspase-3 and -9, thereby promoting neurological functional recovery in MCAO rats.\textsuperscript{16} In transient ischemic attack rat model, normobaric oxygen therapy (NBO) administered during ischemia nearly completely prevented the neuronal death, microglial inflammation and sensorimotor impairment.\textsuperscript{18} Cai et al.\textsuperscript{19} reported that combining NBO (60\% for 3 hours) with ethanol (1.0 g/kg) or hypothermia (33°C for 3 hours) reduced post-stroke hyperglycolysis in thromboembolic stroke rats. NBOT is a promising therapy for short-lasting ischemia. Since it can be initiated at home in at-risk patients or in the ambulance in subjects suspected of transient ischemic attack/early stroke, it is clinically very attractive. It may also be a straightforward support or combination to reperfusion therapies, and help prevent brain damage, attenuating the long-term cognitive and sensorimotor impairment in at-risk populations.\textsuperscript{37}

The major concern with oxygen therapy in acute ischemic stroke is the potential increase of reactive oxygen species (ROS), however, the review paper on NBOT in animal models of stroke by Weaver and Liu\textsuperscript{38} showed that NBO does not increase ROS or oxidative stress if applied for a short duration.

It has been reported recently that chronic hypoxia activated the hypoxia inducible factor-1\textalpha (HIF-1\textalpha) response in zebrafish embryos and alleviated death caused by mitochondrial dysfunction.\textsuperscript{19} Hypoxia may represent the promising therapeutic strategy with vital clinic significance by triggering innate adaptive programs.\textsuperscript{5} Repetitive intermittent hypoxic exposures have been shown to provide protective effects against ischemic stroke in pre-clinical models.\textsuperscript{40,41} The major hurdle in translating of hypoxia into the clinical settings is the negative attitude of clinicians and patients towards the breathing of low levels of oxygen.\textsuperscript{42}

**Volatile Anesthetics**

Volatile anesthetics routinely used in clinic. Hence, the volatile anesthetics such as isoflurane and sevoflurane, are considered as low risk-bearing gaseous agents. Application of commonly used volatile anesthetics after brain ischemia onset provides neuroprotection in experimental stroke research.\textsuperscript{43} Wang et al.\textsuperscript{44} reported that post-conditioning with 3.0\% isoflurane provided better neuroprotection than 1.5\% and 4.5\% isoflurane. Effects of isoflurane post-conditioning were mediated by activation of activin A/Smad 2/3 and activin A/extracellular signal-regulated kinase (ERK) 1/2 signaling pathway. However, in another study, 1.5\% isoflurane post-conditioning was showed to be more effective than 3.0\%, and 4\% isoflurane in reducing infarct volume and improving neurological deficits, which were associated with up-regulated expression of transforming growth factor beta 1 (TGF-\textbeta1) and down-regulated phosphorylated c-Jun N-terminal kinase (p-JNK) expression.\textsuperscript{45} To determine the translational potential, Chen et al.\textsuperscript{29} used 2.5\% isoflurane post-conditioning in rabbit model of embolic stroke and confirmed its neuroprotection. Rabbit post-conditioned with isoflurane tolerated more clots than control animals in the dose-response study, and isoflurane post-conditioning reduced infarct volume and improved neurological deficit scores in animals received intra-carotid injection of 5 mg clots. Although preclinical studies provide strong evidences that isoflurane induces neuroprotection, clinical results, especially long-term neurological outcome, are disappointing. Translation of positive animal findings to patients has been hampered by the associated clinical comorbidity and concurrent medication of patients.\textsuperscript{46}

**H\textsubscript{2}S**

H\textsubscript{2}S is a signaling molecule and exert antioxidant, anti-inflammatory and vasodilatory actions.\textsuperscript{47-49} These results lend credence to the notion that H\textsubscript{2}S provide neuroprotection in ischemic brain tissue. Wei et al.\textsuperscript{21} revealed that 40 ppm and 80 ppm H\textsubscript{2}S inhalation reduced brain edema and infarct volume through inhibiting the expression of AQP-4 via activating protein kinase C (PKC) in MCAO rats. Co-administration of two H\textsubscript{2}S donors, 5-(4-hydroxyphenyl)-3H-1,2-dithiocyclopentene-3-thione (ADT-OH) and sodium hydrosulphide (NaH\textsubscript{2}S) with tPA attenuated hemorrhagic transformation following MCAO in mice.\textsuperscript{24} Controversially, Hadadha et al.\textsuperscript{22} indicated that the administration of oxicacetic acid, an inhibitor of H\textsubscript{2}S synthesis, at a low dose significantly reduced the infarct volume brain edema and improved the neurological outcome in transient MCAO rats. Similarly, Jiang et al.\textsuperscript{21} found that endogenous production of H\textsubscript{2}S results in post-ischemic cerebral vasodilation and early blood-brain barrier (BBB) disruption in MCAO mice. These results
suggest that the role of H₂S in cerebral ischemic might be double-sided depends on the concentration. Hadadha et al.²² believed that while low-concentration of H₂S is beneficial, the high-concentration is harmful and aggravates brain injury after ischemic stroke. At present, H₂S inhalation has not found its use in clinic practice. Further investigations leading to better understanding of whether modulating of H₂S activity can be an option for the treatment of cerebral ischemia, are needed.

**HYDROGEN**

After the discovery of antioxidant properties of hydrogen by Ohsawa et al.⁵⁹ in 2007, hydrogen therapy has been a new hotspot of medical gas application in stroke.⁵¹ Most studies in regards of the hydrogen application after experimental stroke have been conducted in Japan, China, and the USA.⁵² The mechanisms underlying the hydrogen-induced neuroprotection include inhibition of various inflammatory molecules, such as ERK, inducible nitric oxide synthase and nuclear factor-κB.⁵²,⁵³ Takeuchi et al.⁵⁵ demonstrated that hydrogen-rich water improved neurological function and survival rate in spontaneously hypertensive stroke-prone rats, which was associated with reduction of ROS production and suppressing the activity of matrix metalloproteinase-9, leading to the stabilization of BBB. Furthermore, Han et al.⁵⁶ demonstrated that hydrogen-rich water reduced infarct volume and improved neurological function of rats after MCAO. They also demonstrated that hydrogen-rich water maintained the levels of parvalbumin and hippocalin (two calcium buffering proteins) and attenuated the glutamate toxicity-induced elevation of intracellular Ca²⁺ levels.⁵⁶ The usage of therapeutic hydrogen for treatment of ischemic stroke is promising, but its positive effects need to be replicated in the clinic studies and underlying mechanisms need to be further investigated.

**OTHER GASES**

Besides oxygen, isoflurane, H₂S and hydrogen, neuroprotective effects of other gases also have been studied recently. Abraini’s group reported that helium is an efficient neuroprotective agent able to attenuate tPA-induced thrombolysis consequently decreasing brain injury in thromboembolic model of stroke in rats.²⁷ In the clinic practice, helium would be a safe treatment for patients receiving thrombolytic agent.

**ADVANTAGES AND CHALLENGES**

Medical gases are one of the promising therapy strategies for stroke. Among the medical gases, oxygen and volatile anesthetics (isoflurane and sevoflurane) are of the greatest interest, for all because they are routinely used in clinical settings. H₂S, hydrogen and helium are more popular than other medical gases due to numerous pre-clinical studies demonstrating benefits of these gases. Even though NBO and HBO has been studied in clinical trials, the usage of therapeutic gases in clinic has received little attention.²⁹,⁵⁴-⁵⁷ Given the complex pathophysiology of stroke, it is unlikely that a single intervention strategy will result in clinical relevant protection. Medical gases have distinct advantages over pharmaceutical drugs such as the ease of diffusibility across the BBB and the mechanism of action may be via multiple pathways. Medical gases might be a valuable support therapy to the tPA therapy or thrombectomy after stroke. However, timing and dosing of gas administration investigated in animal studies not necessarily extrapolate with clinical settings.⁵⁸ These facts represent the major challenge for development of stroke related medical gas treatments and point the direction for the future studies.

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