The role of argon in stroke

Xiang Li[#], Zhu-Wei Zhang[#], Zhong Wang[*], Jin-Quan Li[*], Gang Chen
Department of Neurosurgery & Brain and Nerve Research Laboratory, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, China.

#These authors contributed equally to this work.
*Correspondence to: Jin-Quan Li, M.D., or Zhong Wang, M.D., jingquanlisdfyy@163.com.
orcid: 0000-0002-6317-2980 (Jin-Quan Li)

Abstract

Stroke, also known as "cerebrovascular accident", is an acute cerebrovascular disease that is caused by a sudden rupture of blood vessels in the brain or obstruction of the blood supply by blockage of blood vessels, thus including hemorrhagic and ischemic strokes. The incidence of ischemic stroke is higher than that of hemorrhagic stroke, and accounts for 80% of the total number of strokes. However, the mortality rate of hemorrhagic stroke is relatively high. Internal carotid artery and vertebral artery occlusion and stenosis can cause ischemic stroke, and especially males over 40 years of age are at a high risk of morbidity. According to the survey, stroke in urban and rural areas has become the first cause of death in China. It is also the leading cause of disability in Chinese adults. In a word, stroke is characterized by high morbidity, high mortality and high disability rates. Studies have shown that many noble gases have the neuroprotective effects. For example, xenon has been extensively studied in various animal models of neurological injury including stroke, hypoxic-ischemic encephalopathy. Compared to xenon, Argon, as a noble gas, is abundant, cheap and widely applicable, and has been also demonstrated to be neuroprotective in many research studies. In a variety of models, ranging from oxygen-glucose deprivation in cell culture to complex models of mid-cerebral artery occlusion, subarachnoid hemorrhage or retinal ischemia-reperfusion injury in animals. Argon administration after individual injury demonstrated favorable effects, particularly increased cell survival and even improved neuronal function. Therefore the neuroprotective effects of argon may be of possible clinical use for opening a potential therapeutic window in stroke. It is important to illuminate the mechanisms of argon in nerve function and to explore the best use of this gas in stroke treatment.

Key words: argon; stroke; experimental research; underlying mechanism; clinical research; stroke treatment; neuroprotective effects; therapeutic implications

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Introduction

Stroke is one of the most common causes of death and disability in the world and creates a significant social and economic burden for our society.[1] As we know, while stroke could be divided into two types, ischemic and hemorrhagic, the ischemic stroke accounts for approximately 80% of them.[2,3] The majority of ischemic strokes are caused by a large artery occlusion due to thromboembolism.[4] Currently, more and more attention has been paid to the role of inert gases such as xenon, helium and argon in the protection of nerve function. All of them possess biological effects that have shown to be neuroprotective in pre-clinical models of ischemic stroke.[5] Therefore we can focus on their beneficial effects to solve clinical problems caused by ischemia-induced neuronal death.[6] Similar to helium and other noble gases, argon was investigated for its therapeutic properties, as early as in 1930s.[5] Argon has a lot of advantages compared to other medical gases. It is more abundant than other noble gases and generates inexpensive costs; what's more, the lack of hypnotic effects in normobaric pressures allows it to become a useful therapeutic agent for many conditions including cerebral ischemia. The effects of argon were tested in different *in vitro* and *in vivo* models. Regarding cell-culture based research, injury of neuronal cells by deprivation of glucose and oxygen is a frequently used model, investigating neuroprotective properties of gaseous molecules.[7] So, in order to explore the practicability of treatment. we will discuss the impact of argon on stroke injury and the potential mechanisms of neuroprotection on the basis of experimental and clinical studies in this article.

Experimental Studies of Argon in Stroke

As for animal experiments, we have successfully established animal models of hemorrhagic and ischemic stroke, especially ischemic, and applied the gas of argon to treat the subjects after stroke insult. The researchers detect the effects of argon on stroke and explore the potential mechanisms by which this gas can protect the nervous system.[8] As everyone knows, animal experiments also belong to basic medical and preclinical research and most of them were aimed to investigate the mechanism of objective gas on neurological status following stroke. Now we find that conclusions of these studies are different to a great extent, and we think this is owing to different experimental conditions and methods. We will analyze several recent experimental studies related to this gas for stroke treatment in this paper (**Table 1**), and then summarize the outcomes. In the in vitro model of oxygen-glucose deprivation (OGD), it was found that 75% vol/vol argon protected against the cell death of neurons, while however neon and krypton had no effect, and helium exacerbated cell death.[9] In a hippocampal organotypic slice subjected to OGD, according to studies, argon (at doses of 25, 50 or 75% vol) was neuroprotective, and this protection could be maintained with a delayed treatment.[10] An *in vivo* transient rat middle cerebral artery occlusion (MCAO) study showed that 50% vol argon administration for 1 hour in ischemia could reduce infarct volume and neurological deficit.[11] In brain slices, treatment of argon in different vol (37%, 50% and 75% vol) in post-OGD could produce a reduction in cell death.[12] Recently,

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Table 1: Experimental studies of argon in stroke

Type of disease	Model	Main results	Animals/cells
Stroke	OGD	75% vol argon protect against neuronal cell death	Neurons
Stroke	OGD	Argon at doses of 25, 50 or 74% vol is neuroprotective, and its protection might be maintained with delayed treatment	Neurons
Stroke	MCAO	50% vol agron administration for 2 hours during ischemia can reduce the infarct volume and neurological deficit	Rats
Stroke	MCAO	Agron treatment at 50% vol in post-ischemia can reduce cortical injury	Rats
Asphyxia	Perinatal asphyxia	Agron treatment at 70% vol in post-HI reduce infarct volume linked to an increase in the anti-apoptotic protein Bcl-2	Pigs

Note: OGD: Oxygen-glucose deprivation; MCAO: middle cerebral artery occlusion; HI: hypoxic-ischemic.

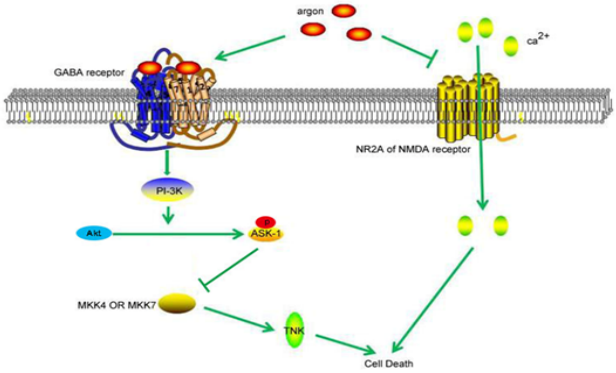

Figure 1: The potential mechanisms of the inert gas on protecting neuron system after ischemia.
Note: Argon activates the γ-aminobutyric acid (GABA) receptor by acting at the benzodiazepine binding site and GABA receptor agonists can come into being neuroprotection after ischemia *via* the phosphoinositide 3-kinase (PI-3K)/Akt(protein kinase B) pathway or by abating the phosphorylation of the NR2A subunit of the N-methyl D-aspartic acid (NMDA) receptor. ASK-1: Apoptosis signal-regulating kinase 1; MKK: mitogen-activated protein kinase kinase; TNK: tenecteplase.

some studies have shown that in a model of perinatal asphyxia, argon treatment (70% vol) post-hypoxic-ischemic (HI) could reduce infarct volume associated with an increase in the anti-apoptotic protein Bcl-2.[13]

According to the above experiments, the promising preclinical results of argon applied following ischemia, suggest that argon could be further examined as a potential neuroprotective gas for ischemic stroke in the clinical setting, which we believe it would succeed.

Mechanism of Argon in Stroke

The underlying mechanism of the neuroprotective function of argon has not been fully elucidated. However, there is ample evidence to show that argon activates the γ-aminobutyric acid (GABA) receptor by acting at the benzodiazepine binding site,[14] and GABA receptor agonists can confer neuroprotection after ischemia via the phosphoinositide 3-kinase pathway[15] or by abating the phosphorylation of the NR2A subunit of the N-methyl D-aspartic acid (NMDA) receptor.[16] Here we will discuss both pathways of how argon protects neurons. Firstly, as we all know, the survival or death of neurons is determined by the balance between pro-survival and pro-apoptosis signals. The Akt (protein kinase B) serine/threonine kinases, as the downstream of phosphatidylinositol 3-kinase (PI-3K), are important mediators of cell survival in brain ischemia. The c-Jun N-terminal kinase (JNK) pathways are stress-activated mitogen-activated protein kinase modules which can be stimulated by brain ischemia. Therefore, one of the mechanisms of cell survival may be the inhibition of the activity of stress-activated kinase cascades. A lot of pro-apoptotic proteins have already been identified as direct Akt substrates, including glycogen synthase kinase 3 (GSK-3), BAD, caspase-9, and apoptosis signal-regulating kinase 1 (ASK1), which were suppressed upon phosphorylation by Akt.[17-20] Specifically, by increasing Akt activity, the suppression of the ASK1-mitogen-activated extracellular signal-regulated kinase (MEK)-JNK pathways dependent on phosphorylation of serine 83 of ASK1 would be activated,[20,21] otherwise ASK1 would phosphorylate and activate mitogen-activated protein kinase kinase 4 (MKK4) or MKK7 which in turn induce JNK kinase activities.[15] So we believe that argon can exerted neuroprotective effect *via* PI-3K/Akt pathway, which could inhibit the ASK1-JNK cascade, by activating the GABA receptor.

Secondly, glutamate, as a major excitatory neurotransmitter in the central nervous system (CNS), is frequently researched because it is related to the development of cerebral ischemia induced cell death. Glutamate receptors are mainly classified into two groups: metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs). The ionotropic glutamate receptors have been divided into three classes pharmacologically: NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (KA) receptors. The NMDA receptor acts as a ligand-gated calcium channel, which plays an important role in neuronal development, addiction, learning and memory.[22] Over-excessive release of glutamate will cause over-activation of glutamate receptors, mainly NMDA-type, and subsequent calcium overload, all known as excitotoxicity, and then will activate calcium-dependent signaling cascade reactions of postsynaptic neurons that eventually leads to neuronal death.[23] According to the study by other researchers, the NMDA receptors are formed by two classes of subunits, a principal NR1 subunit and modulatory NR2 subunits (NR2A–NR2D).[24] NMDA receptors are phosphorylated on tyrosine residues of NR2 subunits, especially NR2A and NR2B.[25] The protective effect of nerve function is mediated by argon through intervening in the above two pathways (**Figure 1**).

Clinical Studies and Possible Therapeutic Implications of Argon in Stroke

A decade of investigation on the effects of xenon has led to

a clinical trial that may yet change clinical care of perinatal asphyxia. The findings of Loetscher and his colleagues should encourage the pursuit of argon as a neuroprotective alternative/supplement to xenon.[26] The potential value for the clinical application of argon needs to be further explored through tests of clinical safety and translational research. According to the data of the current research, there is an increasing amount of evidence that at the vol of 50−70% argon may have potentially neuroprotective and therapeutic properties and be beneficial for preventing brain injury, by protecting neurons in the brain through above two pathways. Although there is insufficient clinical evidence that argon can effectively treat stroke. Future studies should focus on clinical experiments to further explore the use method, dosage and adverse reactions of argon gas. We believe that the argon will open up a new method to protect nervous system.

Author contributions
XL and ZWZ were responsible for writing the manuscript. JQL were responsible for its revision. ZW and GC were responsible for its drafting and revision. All authors read and approved the final version of the manuscript for publication.
Conflicts of interest
The authors declare that they have no competing interests.
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