Protective role of methane in traumatic nervous system diseases

Methane, an odorless, colorless, tasteless gas under standard state, is the simplest hydrocarbon.[1] It is a very potential fuel that constitutes the main component of natural gas. Since the discovery of methane in 1778, it is generally believed that methane is a physiologically inert gas with stable chemical properties and does not participate in biological metabolism.[2] So for years, little research has focused on the role of methane in the clinical field.[3] Until 2012, Boros et al.[4] carried out a preliminary research on the physiological effects of methane and found that methane has anti-inflammatory and anti-oxidation stress effects in intestinal ischemia/reperfusion injury. Subsequently, Ye et al.[5] found that methane could inhibit inflammation and apoptosis after hepatic ischemia/reperfusion injury. Since then, an increasing number of papers have shown that methane has biological activity and can protect cells and organs from inflammation, oxidation stress and apoptosis damage, indicating that methane has a certain therapeutic effect on various clinical diseases during the basic research stage.

Methane is a lipid-soluble gas molecule that has the ability to pass through the cell membrane and spread to the organelles,[6] which means that methane can reach the DNA and mitochondria, let alone the BBB and the blood-spinal cord barrier. Wang and his colleagues[7] used methane-rich saline (MS) to treat spinal cord injury (SCI) and found that MS could stay in the spinal cord for 10 hours after only 72 minutes of irrigation, showing good biocompatibility. Therefore, MS is suitable for the treatment of nervous system diseases. In addition, methane in the body is essentially non-toxic. In this way of injecting intraperitoneally at a micromolar dose, methane does not change the arterial oxygen tension, arterial carbon dioxide tension, pH, plasma glucose and hematocrit, which confirms the security of methane.[8] In summary, methane has hypertonicity, good biocompatibility and safety, which makes it a very promising method for treating nervous system diseases.

It is worth noting that although human clinical experiments on the function of methane in neurological diseases have not yet begun, the basic research on methane in traumatic neurological diseases, including traumatic SCI (TSCI),[7] traumatic brain injury (TBI)[9] and optic nerve crush (ONC),[10] has become more profound in recent years (**Table 1**). Therefore, giving further clarification on the neuroprotective effects is of great significance for methane as a new drug to treat nervous system diseases. In this context, we summarize and discuss the protective effects of methane on traumatic nervous system diseases and its possible mechanism in treating neurological diseases by retrieving previously published documents and databases. At the same time, in this perspective, to promote safe and effective use in clinical area, we propose the existing problems in the application of methane and suggest future directions for methane research.

Methane plays a crucial role following central nervous system trauma

Neuroprotective role of methane in TSCI: TSCI is a destructive nervous system disease.[11] The neurological mechanism of TSCI is very complicated. Initial trauma leads to acute cell dysfunction or death, and a large number of inflammatory cells and microglial cells, once activated, can release inflammatory factors such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α, leading to the inflammatory cascade, which thereby results in secondary SCI.[12] At the same time, reactive oxygen species (ROS) can cause cell damage and spinal cord dysfunction. Therefore, the focus of SCI is to minimize the oxidation stress and inflammatory response of secondary damage.[7] At the same time, a related study has shown that the reduction of apoptosis, especially neuronal cell apoptosis, could significantly improve the prognosis of TSCI.[7]

Wang et al.[7] constructed SCI rat models and confirmed that the levels of superoxide dismutase and malondialdehyde, as well as the levels of TNF-α, IL-1β, and IL-6, were significantly increased after 72 hours of damage. MS could downregulate the content of oxidation stress factors and inflammatory factors 72 hours after SCI, and also found that intraperitoneal injection with MS could significantly inhibit the apoptosis, and its concentration was significantly effective at 20 mL/kg. To further explore the protection mechanism of MS for SCI, researchers tested microglial cells after SCI, and found them activated in SCI areas 72 hours and 2 weeks, and MS could weaken microglial activation. In addition, Wang et al.[7] confirmed that the use of MS to treat SCI could help improve locomotor function, especially in the early stages of SCI. The sooner the MS was administered, the better the protection effects. However, these results *in vivo* have not yet received the support of methane's neurological protection *in vitro*. The results of the above research suggested that after SCI, MS could reduce the oxidation stress, inflammatory level and apoptosis by inhibiting the microglial activation, and protecting against SCI. Nevertheless, since the exact molecular and signaling pathway mechanisms of the neuroprotective effect of methane in the SCI animal model is unclear, further research should be conducted to clarify how methane plays neuroprotection against SCI. According to reports, MS could alleviate traumatic brain damage by blocking the activation of the Janus kinase 1/signal transducer and activator of transcription 1/nuclear factor-κB-P65 pathway.[13] Methane was used in cerebral ischemia/reperfusion injury damage resting upon the phosphoinositide 3-kinase/Akt/heme oxygenase-1 pathway.[14] Another study reported that bone marrow mesenchymal stem cell transplantation could effectively restore motor function after SCI by activating the phosphoinositide 3-kinase/AKT/mammalian target of rapamycin pathway.[15] The verification of conduction pathways as described above opened the idea for research in the field of SCI. Clearing this problem may help find the new target of methane effects, so as to find a new method for treating neuronal damage after SCI.

Neuroprotective effects of methane in TBI: TBI is one of the main causes of death and disability. Ameliorate inflammation reactions, oxidation stress and apoptosis are very important for the treatment of TBI.[16] However, no effective drugs have been approved. Methane may be a potential drug for this purpose.

The research results showed that the neurological severity score in the MS group increased, the residual neurons and the superoxide dismutase increased, while the expression of TNF-α, IL-1, IL-6 and apoptosis signaling molecules decreased, which proved that MS could inhibit the release of inflammatory factors, exert antioxidant effects, maintain the normal structure of neurons, and restrain cell apoptosis after TBI, thereby promoting the improvement of the nerve function.[9] The group found that a concentration of 20 mL/kg had the strongest protective effect, and increasing the dosage to 30 mL/kg did not improve its effects. In a subsequent study, Li et al.[9] also found that these functions of MS may be mediated by the Wnt pathways. Because the Wnt pathway suppression could weaken the moderating effects of MS on oxidative stress, neuroinflammation, and apoptosis, it could not be eliminated, which highlights the possibility of other signal routings.[9] This study was conducted the role of MS in the early and middle stages of TBI. Therefore, further experiments should focus on the post-stage of TBI. In addition, the significant results between the 2 mL/kg MS

Table 1: Mechanisms of action of methane in disease treatment

	Anti-inflammation	Anti-oxidation	Anti-cell death	Reference
Spinal cord injury	Interleukin-1β, tumor necrosis factor-α, interleukin-6, ionized calcium binding adaptor molecule-1	Malondialdehyde, superoxide dismutase	Caspase-3	Wang et al.[7]
Traumatic brain injury	Interleukin-1β, tumor necrosis factor-α, interleukin-6, Wnt pathway	Superoxide dismutase, Wnt pathway	Caspase-3, Wnt pathway	Li et al.[9]
	Tumor necrosis factor-α, interleukin-1β, interleukin-6, Janus kinase 1/signal transducer and activator of transcription 1/ nuclear factor-κB-p65 pathway	Malondialdehyde, 3-nitrotyrosine, 8-hydroxy-2-deoxyguanosine, superoxide dismutase	Caspase-3, Bax, Bcl-2	Wang et al.[13]
Optic nerve crush			Phosphorylated Bad, phosphorylated glycogen synthase kinase-3β, Bax, Bcl-xL	Wang et al.[10]

treatment group and the 20 mL/kg MS treatment group reflected the dose-dependent effects of methane protection, but Fan and others[17] believed that 10 mg/kg was the best dose. This different result might be due to using different animal models and dosing frequency. The specific dose still needs further studies to confirm it, so that methane can be used in clinical trials.

Secondly, in another study by Wang et al.[13] the intraperitoneal injection of methane was proved to improve the cognitive function of TBI in the Morris water maze test, reduce the blurring of brain tissues boundary, the number of necrotic plaques and neurons in TBI rats. Treatment with methane can downregulate malondialdehyde, 3-nitrotyrosine and 8-hydroxy-2-deoxyguanosine, also caspase-3 and Bax protein levels, change over TNF-α, IL-1β and IL-6, while upregulating superoxide dismutase and Bcl-2 levels, by blocking the activation of Janus kinase 1/signal transducer and activator of transcription 1/nuclear factor-κB-P65. Subsequently, MS improved cognitive function, reduced inflammatory response and oxidation stress, and inhibited apoptosis.[13] In addition to cell death, oxidation stress and inflammation, recent research results show that ferroptosis is a new form of secondary brain injury after TBI, which provides new therapeutic targets for protecting damaged brain.[18] Whether methane can act on ferrostatin-1, thereby inhibiting ferroptosis and exerting neuroprotective effects will be the next step. Also, MS had a protective effect on brain injury by inhibiting the apoptosis guided by the endoplasmic reticulum stress. Further exploration can be made to see if the protective effect of methane against TBI is related to endoplasmic reticulum stress-mediated apoptosis.[19]

Neuroprotective effects of methane in ONC: ONC is extremely common among patients with trauma at the head and face region. Due to the limitations of nerve regeneration capacity, the therapeutic options focus on protecting early neuron cells and inhibiting later apoptosis.[20] Constructing a mouse ONC model, Wang et al.[10] subjected the mice to a battery of tests that were used routinely to measure the nerve protection effect of methane, and proved that methane therapy could promote the survival of the retinal ganglion cells after ONC, improve the visual dysfunction caused by ONC. Also, slowing down the apoptosis process of retinal ganglion cells, promoting mitochondrial steady state caused by ONC, these above could testify that the nerve protection of methane might be related to the improvement of mitochondrial dysfunction.[10] Methane might be a potential neuroprotective agent of traumatic optic neuropathy and glaucomatous optic atrophy.

Points of interaction between traumatic neurological disorders

Microglia run through the traumatic neurological disorders: Microglia, are the resident macrophages of the central nervous system, which are also congenital immune cells in the human brain.[21] They play a vital role in secondary damage after the traumatic injury of the central nervous system. Microglia are essential for the restoration of tissue stability and the best recovery after SCI.[22] Research shows that microglia could also promote the neuron stabilization of TBI.[23] When microglia are activated, they will polarize to proinflammatory M1 and anti-inflammatory M2 phenotypes. Under the influence of certain pathological factors, if microglia are in activation for a long time, the proportional disorders of M1 and M2 cells will lead to an imbalance in the central immune system.[24] The final ending is to cause neuronal lesions or death. In addition to being activated by neuroinflammation, microglia are also major contributors to the oxidative stress of central nervous system, and the sources of ROS production are diverse,[25] such as intracellular peroxidase and membrane surface nicotinamide adenine dinucleotide phosphate oxidase.[26] Besides that, studies have shown that oxygen-glucose deprivation-induced microglia produce large amounts of ROS and malondialdehyde, damaging the microglia themselves, and causing glial cell dysfunction, which may affect the fixed process of nerves.[27]

It can be said that the double-sided roles of microglia play a pivotal role in traumatic neurological diseases, and given the anti-inflammatory and oxidative stress effects of methane, researchers can further study the role of methane in the balance of M1 and M2 types.[28] By harnessing the protective or repairing effects of these reactions while inhibiting their harmful effects, we may be able to control the progression and exacerbation of inflammatory and oxidative stress, and protect living brain tissue.

Methane is effective in traumatic neurological disorders: Methane is effective in terms of being against inflammation, oxidative stress and apoptosis in traumatic neurological disorders. In neurological diseases, the occurrence of neuroinflammation is often accompanied by the participation of oxidative stress, and the two are interdependent. ROS is necessary for neuronal differentiation and neuronal signaling.[29] It has been reported that nicotinamide adenine dinucleotide phosphate oxidase and myeloperoxidase can be expressed in many types of inflammatory cells.[30,31] Both can be activated to induce chlorination reaction to produce hypochlorous acid[32] and ROS,[29] which has high oxidative activity, causing damage to substances such as lipids, proteins and DNA.[33] In subarachnoid hemorrhage, neutrophil oxidases from inflammatory sources such as nicotinamide adenine dinucleotide phosphate oxidase and myeloperoxidase can act as interaction points between neuroinflammation and oxidative stress, which deserve further study. At the same time, oxidative stress could also aggravate inflammatory damage, heme can activate neutrophils and microglia, promote the release of inflammatory factors and the expression of ROS.[34]

Neuroinflammation can lead to the occurrence and development of oxidative stress, and the products of oxidative stress can also aggravate the infiltration of inflammation, which lead to a poor prognosis of traumatic neurological diseases. For the treatment of patients, perhaps more attention should be paid to such "interaction points", so as to more effectively inhibit the development of brain injury. With the advancement of basic medicine and clinical medicine, new therapies to improve the prognosis of patients with traumatic neurological disorders have a wider applied prospect.

Delivery of methane

Inhalation of methane: Methane itself is a non-toxic gas, which can be transported into the human body through a ventilator or a mask. Strifler and others[35] found that 2.2% methane inhalation could significantly reduce the severity of hepatic ischemia/reperfusion injury. A study by Boros and others[4] had shown that the gas mixture of oxygen and methane (21% oxygen + 2.5% methane) was safe for rodents. The hybrid gas of methane and air would explode when igniting. The safe limit for methane inhalation is 25% to 30%,[36] which has become the limitation of methane in clinical applications. Therefore, choosing reliable tools for using and storing methane is very important.

Injection of MS: Due to the volatile characteristics of methane, MS under high-pressure conditions has similar properties.[9] This form is easy to store and transport. In addition, delivering methane into the body by intraperitoneal injection can increase its safety and accuracy. The MS injection method in the research literature can be summarized as follows, dissolving methane into the normal saline, and then pressing it at 0.4 or 0.6 MPa for 4 or 8 hours to reach the level of saturation and putting it at 4°C to store.[7,9,10] It is worth mentioning that transporting nitric oxide to the body in the form of isosorbide dinitrate, can relax the smooth muscle of the blood vessels and relieve the patient's symptoms.[37] Therefore, another methane transmission method can be explored by oral methane or developing "methane pills." The intrathecal injection is also a way of administration by the nervous system, and whether methane can be administered by intrathecal injection is worth further studying.[38,39] Further clarification of the dose dependence of methane will provide the basis for its clinical application.

Conclusion: This article reviews the related mechanisms by which methane exerts a neuroprotective role in traumatic neurological diseases and briefly elaborates some commonalities between the three diseases. Secondary injury associated with traumatic nervous system disease is the main cause of death and disability after admission. But the current clinical treatment is still mainly using surgery to relieve post-traumatic compression,[40] or using hypothermia therapy to reduce brain metabolism,[41] but there are no effective drugs to treat them. Although some promising neuroprotective therapies have been developed, they have not been translated into the clinic unfortunately, and the prognosis of patients urgently needs to be further improved.

In the process of searching the literature, we found that methane can play a neuroprotective role in the progression of traumatic nervous system diseases by inhibiting oxidative stress, neuroinflammation and apoptosis (**Figure 1**), but the connection between them is very complex and lacks a clear and systematic mechanism. Iron deposition, lipid accumulation, and oxidative stress have been reported to be secondary injuries following traumatic injuries to the central nervous system, in which ferroptosis is the key point connecting all such secondary injuries.[42] In basic experiments, inhibition of ferroptosis has shown significant efficacy in protecting against neurotraumatic injury,[42] it has been reported that mitophagy plays a protective role in traumatic neurological diseases, and upregulating mitophagy to reduce downstream cascade reactions, such as oxidative damage, inflammatory response, and cell death, may improve neurological dysfunction.[43,44] Mitophagy and ferroptosis may be the key mechanisms of the connection. Therefore, it is bold to imagine that the two may be new targets for methane. In addition, except for the Wnt pathway and the Janus kinase 1/signal transducer and activator of transcription 1/nuclear factor-κB-P65 pathway,[13] the phosphoinositide 3-kinase/AKT pathway,[45] the nuclear factor erythroid 2-related factor 2 pathway,[8] the nuclear factor-κB and the mitogen-activated protein kinase pathway[46] have been confirmed to be involved in the neuroprotective effect of methane, indicating that the beneficial effects of methane are synergistic through a variety of signal-guided pathways, and the next step may be to verify whether these pathways mediate the role of methane in traumatic neurological diseases. Clarifying the connection between the guiding pathways of methane action and its interaction points has great significance for making up for the deficiencies in neuroprotective therapy for traumatic neurological diseases in clinical practice.

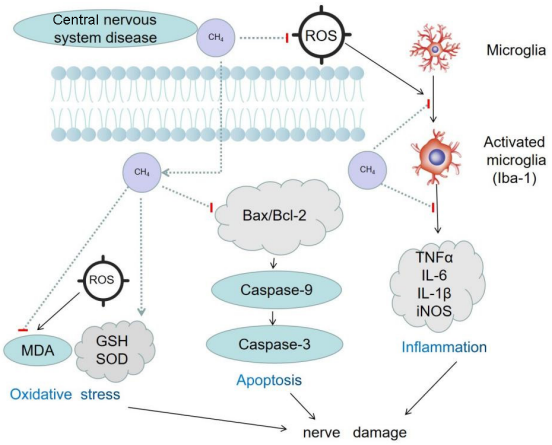

Figure 1: Possible neuroprotective mechanisms of methane.
Note: Created with BioRender.com. CH_4: Methane; GSH: glutathione; Iba-1: ionized calcium binding adaptor molecule-1; IL: interleukin; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; ROS: reactive oxygen species; SOD: superoxide dismutase; TNF: tumor necrosis factor.

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***doi:** 10.4103/mgr.mgr_23_23*
***How to cite this article:** Lu X, Hu W, Wang X, Wang Z, Yang P, Wang W. Protective role of methane in traumatic nervous system diseases. Med Gas Res 2024;14(3):159-162.*

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Date of submission: April 19, 2023
Date of decision: May 15, 2023
Date of acceptance: November 24, 2023
Date of web publication: March 20, 2024