Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Therapeutic effect of methane and its mechanism in disease treatment

Zhou-heng YE[1], Ke NING[2], Bradley P. ANDER[3], Xue-jun SUN[†‡2]

[1]*Department of Aviation and Diving Medicine, the Sixth Medical Center, General Hospital of People's Liberation Army, Beijing 100048, China*

[2]*Department of Navy Aviation Medicine, Faculty of Naval Medicine, the Naval Military Medical University, Shanghai 200433, China*

[3]*Department of Neurology, University of California at Davis, Sacramento, California, USA*

[†]E-mail: sunxjk@126.com

Received Nov. 4, 2019; Revision accepted Feb. 19, 2020; Crosschecked July 20, 2020

Abstract: Methane is the simplest hydrocarbon, consisting of one carbon atom and four hydrogen atoms. It is abundant in marsh gas, livestock rumination, and combustible ice. Little is known about the use of methane in human disease treatment. Current research indicates that methane is useful for treating several diseases including ischemia and reperfusion injury, and inflammatory diseases. The mechanisms underlying the protective effects of methane appear primarily to involve anti-oxidation, anti-inflammation, and anti-apoptosis. In this review, we describe the beneficial effects of methane on different diseases, summarize possible mechanisms by which methane may act in these conditions, and discuss the purpose of methane production in hypoxic conditions. Then we propose several promising directions for the future research.

Key words: Methane treatment; Ischemia and reperfusion injury; Inflammation; Methanogenesis
https://doi.org/10.1631/jzus.B1900629 **CLC number:** R459.9

1 Introduction

Methane (CH_4) is the simplest hydrocarbon, comprising one carbon and four hydrogen atoms. These atoms form a tetrahedral structure with H–C–H angles of 109.5°. Methane is combustible and its combustion products include carbon dioxide (CO_2), water, carbon monoxide (CO), and hydrogen. It is rich in marsh gas, livestock rumination, and combustible ice.

Methane is found in many parts of the human body. Methanogens belong to archaea, producing methane during anaerobic respiration. In nature, there are seven orders of methanogens found in the environment, plants, mammals, and humans (Sogodogo et al., 2019). They can use the carbon present in small molecules as the final acceptor of electrons in energy metabolism (Lyu et al., 2018). In the human body, five known orders have been discovered, being distributed in the oral cavity, gastrointestinal tract, lungs, and skin (Koskinen et al., 2017). This diversity of localization means that methane is present almost throughout the human body.

Methane is associated with human disease and is used in the field of diagnosis. Methane detected in breath has been applied diagnostically in common gastroenterology. A breath test for methane is useful in the diagnosis of carbohydrate maldigestion, methane-associated constipation, and oral-cecal transit evaluation (Rezaie et al., 2017). Small intestinal bacterial overgrowth (SIBO) was diagnosed using a hydrogen/methane breath test to explore the relationship between SIBO and deep vein thrombosis (DVT) (Cheng et al., 2017). Furthermore, methane production is correlated with cancer of the large bowel. Eighty percent of patients with large bowel cancer had

[‡] Corresponding author
ORCID: Xue-jun SUN, https://orcid.org/0000-0003-2387-5531

considerable methane production in their breath, while only about 40% of patients without the disease had detectable levels of methane (van de Pol et al., 2017). However, Basson et al. (2017) found no correlation between methane and the risk of colon cancer among different populations. These associations need further research, but demonstrate that methane measurements are already being clinically applied in diagnosis and research of human disease.

For years, little research focused on the protective properties of methane in mammals. Only since 2012 has research started to examine the protective effects of methane on clinical diseases. An increasing number of papers demonstrate the beneficial effects of methane in disease treatment with thorough exploration of mechanisms. In this review, we surveyed the protective effects of methane on different diseases, summarized and compared possible mechanisms of methane protective action, discussed the relationship of methane production to hypoxic conditions, and suggested future directions for methane research.

2 Methane protections in different kinds of diseases

2.1 Methane in treating organ ischemia and reperfusion injury

Boros et al. (2012) first characterized the anti-inflammatory effects of methane on intestinal ischemia and reperfusion injury (IRI). The partial pressure of the CO_2 (pCO_2) gap between the intestinal tissue and arteries was related to the severity of intestinal IRI. Methane inhalation significantly reduced the pCO_2 gap after IRI compared with the IRI group. Another research method, methane-rich saline, proposed by Ye et al. (2015), was widely applied in subsequent research. Their work demonstrated that methane could be used to treat liver IRI. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are representative signals of liver injury (Ye et al., 2015). Methane decreased the levels of ALT and AST significantly in hepatic IRI. Chen et al. (2016) showed that methane brought down the level of cardiac troponin (c-TnI), improved cardiac function, and inhibited myocardial remodeling after heart ischemia injury. Methane also showed protective effects against retinal IRI by significantly reducing the loss of retinal ganglion cells (RGCs) and the thinning of the total retinal layer, and improving visual function (Liu et al., 2016). Moreover, methane showed protective effects in renal IRI (Meng et al., 2018). Methane significantly decreased blood creatinine (CRE) and blood urea nitrogen (BUN) levels and recovered renal histology in renal IRI.

2.2 Methane in treating inflammatory disease

Methane has been protective in different inflammatory disease models, including endotoxic shock, dextran sulfate sodium (DSS)-induced colitis, and bacteria-induced sepsis. Zhang et al. (2016) showed that methane significantly attenuated tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), used as markers of the severity of inflammatory disease. They also proposed that methane could probably be protective in systemic inflammatory diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Other scientists have focused primarily on complications of sepsis. Results showed that methane improved survival rate and organ function, and alleviated pathological damage induced by sepsis (Li et al., 2019). Also, kidney dysfunction induced by sepsis was significantly improved by methane along with BUN and CRE levels (Jia et al., 2018). He et al. (2016) modeled murine concanavalin A (Con A)-induced autoimmune hepatitis (AIH) and tested the effect of methane on injury. Their results showed that methane significantly reduced the serum ALT level and decreased liver tissue damage. Methane treatment reduced the levels of proinflammatory cytokines including TNF-α, interferon-γ (IFN-γ), IL-6, and IL-1β, and increased the anti-inflammatory cytokine, IL-10.

2.3 Methane in treating neuronal disease

Fan et al. (2016) proposed that methane-rich saline could be used to relieve delayed injury after carbon monoxide (CO) poisoning. Methane-rich saline significantly reduced delayed neuropsychological sequelae following severe CO poisoning in rats. Oxidation, inflammation, and cell death markers were significantly improved along with learning and memory performance (Fan et al., 2016). Other research work focused on cognitive dysfunction after surgery. Methane has ameliorated postoperative cognitive dysfunction (POCD) as indicated by improvement of

Morris water maze (MWM) performance (Zhang D et al., 2019). Methane also showed neuroprotective effects on myenteric neurons against transient superior mesenteric artery occlusion (Poles et al., 2018). The oxidoreductive reaction of xanthine was well analyzed including the activity of xanthine oxidoreductase (XOR). Methane reduced XOR activity and nitrotyrosine formation, resulting in nitrergic neuron protection.

2.4 Methane in treating other diseases

In diabetic retinopathy, which commonly occurs after years of diabetes mellitus, methane showed protective effects by improving retinal thickness and reducing cell loss and blood-retinal barrier break down (Wu et al., 2015). Methane also showed protective effects on lung injury (Sun et al., 2017), acute pancreatitis (Xie et al., 2017), carbon tetrachloride (CCl_4)-induced liver injury (Yao et al., 2017), allergic asthma (Zhang N et al., 2019), and ulcerative colitis (Wang et al., 2019). Moreover, methane has a beneficial analgesic effect that was uncovered using a monoarthritis (MA)-induced chronic pain model (Zhou et al., 2018).

3 Mechanisms of methane action in disease protection

3.1 Anti-inflammatory mechanism

Inflammation is the mechanism through which methane most often appears to act. In pioneering research work, the regulation of inflammatory markers was observed. Boros et al. (2012) showed in vitro that methane inhibited leukocyte activation, suggesting a mechanism for protective effects in intestinal IRI. In liver IRI, methane decreased TNF-α, IL-6, and the number of CD68-positive cells (Ye et al., 2015). These results indicated that methane decreased inflammatory markers and inhibited infiltration of inflammatory cells. A study by Chen et al. (2016) of myocardial ischemia similarly showed that methane reduced the levels of inflammatory markers. The inhibition of inflammatory markers, including TNF-α, IL-6, IFN-γ , IL-10, was also demonstrated in retinal injury (Liu et al., 2016), sepsis (Li et al., 2019), autoimmune colitis (Zhang et al., 2016), AIH (He et al., 2016), CO poisoning-induced delay injury (Fan et al., 2016), spinal cord injury (Wang et al., 2017), cognitive dysfunction (Zhang D et al., 2019), diabetic retinopathy (Wu et al., 2015), lung injury (Sun et al., 2017), acute pancreatitis (Xie et al., 2017), ulcerative colitis (Wang et al., 2019), chronic inflammatory pain (Zhou et al., 2018), and allergic asthma (Zhang N et al., 2019).

Upstream pathways of these inflammatory markers were explored in some elaborate research work. These signaling pathways, including Toll-like receptor 4 (TLR4)/nuclear factor-κB (NF-κB)/mitogen-activated protein kinases (MAPKs), phosphoinositide-3-kinase (PI3K)/AKT/glycogen synthase kinase-3β (GSK-3β), and Janus-associated kinase 1 (JAK1)/signal transducer and activator of transcription 3 (STAT3), represent a molecular mechanism which may explain the increase in inflammatory markers of the assumed molecular target of methane. In lipopolysaccharide (LPS)-induced sepsis, methane down-regulated p38 MAPK, NF-κB, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) protein expression, leading to reduced production of TNF-α and IL-6 (Zhang et al., 2016). In Con A-induced AIH, the up-regulated protein expression levels of phosphorylation of inhibitor of NF-κB (p-IκB), p-NF-κB, p65, and p-p38 were effectively suppressed by methane administration (He et al., 2016). In cognitive dysfunction after operation (Zhang D et al., 2019) and CCl_4-induced acute liver injury (Yao et al., 2017), phosphorylation levels of the NF-κB/MAPKs pathway showed the same anti-inflammatory trend that methane demonstrated in previous work. In ulcerative colitis, myeloid differentiation primary response protein 88 (MyD88) and TLR4 levels were also significantly reduced by methane (Wang et al., 2019). The PI3K-AKT-GSK-3β pathway was also explored in several papers. The protein levels of GSK-3β and AKT were first tested in macrophages in vitro. Methane significantly increased levels of GSK-3β phosphorylation at Ser9 and induced levels of AKT at all time-points in the presence of LPS (Zhang et al., 2016). In CCl_4-induced liver injury, phosphorylation levels of AKT and GSK-3β were also increased by methane (Yao et al., 2017).

The molecular targets of methane have also been investigated. IL-10, acting as a protective inflammatory cytokine, was increased after methane administration in several diseases including renal IRI,

LPS-induced sepsis, Con A-induced AIH, acute pancreatitis, MA, and chronic inflammatory pain. Using the anti-IL-10 blockade method, the protective effects of methane on disease were abrogated (Zhang X et al., 2016; Yao et al., 2017; Zhang D et al., 2019). This might indicate that methane acts through elevation of IL-10 to provide protection from inflammatory diseases (Table 1).

3.2 Anti-oxidation mechanism

Oxidation has been another important focus for studies of the mechanism of methane action in disease protection. Almost every study has explored oxidation products, such as superoxide dismutase (SOD), and malondialdehyde (MDA) as well as myeloperoxidase (MPO), 8-hydroxy-2-deoxyguanosine (8-OhG) and glutathione (GSH)/GSH disulfide (GSSG). Chen et al. (2016) demonstrated that 8-OhG-positive cells were increased in the heart tissue of a myocardial ischemia rat group, and that methane significantly reduced the number of these 8-OhG-positive cells. This effect of methane was dose-dependent. The SOD level increased significantly after methane administration compared with SOD level in the myocardial ischemia group, and MDA content was significantly decreased by methane in heart tissue (Chen et al., 2016). Besides oxidative markers, there has also been a research focus on microRNA which can show major activation in oxidative injury. Wu et al. (2015) found that methane up-regulated the levels of microRNA-335 (miR-335), which is related to oxidative stress in diabetic retinopathy. Results of genome analysis suggested that miR-335 is related to oxidative stress, cell proliferation, and leukocyte activation. The miR-335 levels in the methane-treated group were down-regulated significantly compared with those in the diabetic retinopathy group. Further signaling and pathway exploration is needed to better understand this mechanism (Table 1).

3.3 Anti-apoptotic mechanism

A number of papers have proposed inhibition of apoptotic cell death as a protective mechanism of methane in several diseases (Table 1). The major marker was the number of cells lost, illustrated by hematoxylin-eosin (HE) staining or terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining (Ye et al., 2015). Caspase-3, the effector molecule of apoptosis, was commonly used in these studies to demonstrate the involvement of cell death. The protein of pro-caspase-3 was significantly decreased by methane administration according to all papers that considered caspase-3 (Wang et al., 2017). Also, capsase-9, a downstream molecule of apoptosis, and the ratio of B-cell lymphoma 2 (Bcl-2)-associated X protein (Bax)/Bcl2 were other markers studied in relation to an apoptotic mechanism of methane protection. The same anti-apoptotic trends were observed: methane significantly decreased caspase-9 content compared to the injury group. Methane can also attenuate endoplasmic reticulum (ER)-related apoptosis by reducing glucose-regulated protein 78 (GRP78), activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), and caspase-12 levels (Jia et al., 2018).

Since anti-inflammation and anti-apoptotic mechanisms have attracted attention in studies exploring the protective action of methane, we have attempted to connect these two mechanisms and illustrated commonalities and overlap. TNF-α might be associated with both mechanisms, by activating the NF-κB pathway to increase the expression of inflammatory factors, and activating apoptosis through caspase-8 and the caspase-9/caspase-3 pathways (Fig. 1).

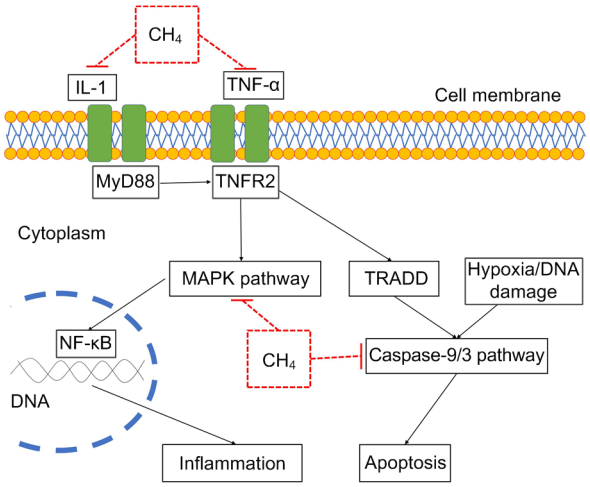

Fig. 1 Possible connecting points between anti-apoptotic and anti-inflammatory effects of methane in disease protection

Dotted line means action of inhibition and arrow line means activation. IL-1: interleukin-1; TNF-α: tumor necrosis factor-α; MyD88: myeloid differentiation primary response protein 88; TNFR2: TNF receptor 2; MAPK: mitogen-activated protein kinase; TRADD: TNF receptor type 1-associated death domain protein; NF-κB: nuclear factor-κB

Table 1 Mechanisms of action of methane in disease treatment

Disease	Direction of mechanism				Reference
	Anti-inflammation	Anti-oxidation	Anti-cell death	Other	
Intestinal IRI	Leukocyte activation	SOX, MPO		Nitrotyrosine formation	Boros et al., 2012
Liver IRI	TNF-α, IL-6, CD68	SOD, MDA, 8-OhG	Caspase-3, apoptotic cells		Ye et al., 2015
Myocardial ischemia	CD68, TNF-α, IL-1β	8-OhG, MDA, MPO, SOD	Bax/Bcl-2, caspase-3, caspase-9, cytochrome-C		Chen et al., 2016
Renal IRI	TNF-α, IL-6, IL-10, macrophages cells	MDA, SOD, CAT, MPO, 8-OhG	Apoptotic cells		Meng et al., 2018
Retinal IRI		8-OhG, 4-HNE, MDA, SOD, CAT, GPx	Bcl-2, Bax, caspase-3, caspase-9		Liu et al., 2016
LPS-induced and bacteria-induced sepsis, autoimmune colitis	TNF-α, IL-6, NF-κB/MAPK pathway, GSK-3β, AKT, IL-10, IL-10 abrogation				Zhang et al., 2016
CLP-induced sepsis	TNF-α, IL-6	MDA, SOD, MPO, GSH	Apoptotic cells, caspase-3, caspase-9, cytochrome-C	Pyroptosis, NLRP3/caspase-1/IL-1β signaling pathway	Li et al., 2019
Sepsis-induced kidney injury			Caspase-3, Bcl-2/Bax, PARP, number of cell loss, apoptosis signaling pathway		Jia et al., 2018
Con A-induced AIH	TNF-α, IFN-γ, IL-6, IL-1β, IL-10, IκB, NF-κB, p38 MAPK	MDA, 8-OhG, SOD, CAT			He et al., 2016
CO poisoning delay injury	TNF-α, IL-1β	SOD, MDA, 3-NT, 8-OhG	Caspase-3		Fan et al., 2016
Mesenteric IRI				XOR activity	Poles et al., 2018
Spinal cord injury	TNF-α, IL-6, IL-1β, Iba-1	SOD, MDA	Caspase-3, apoptotic cells		Wang et al., 2017
Cognitive dysfunction	TNF-α, IL-6, IL-10, NF-κB/MAPK pathway, IL-10 blockade				Zhang D et al., 2019
DR	TNF-α, IL-1β	miR-335	Number of cell loss, miR-192-5p	VEGF	Wu et al., 2015
Lung injury	TNF-α, IL-1β	MDA, SOD	Caspase-3		Sun et al., 2017
Acute pancreatitis	TNF-α, IL-6, IFN-γ, IL-10	MPO, SOD	Caspase-3		Xie et al., 2017
CCl_4-induced acute liver injury	IL-6, TNF-α, IL-1β, IFN-γ, ICAM-1, CXCL1, MPO, NF-κB/MAPK pathway, PI3K-AKT-GSK-3β pathway, IL-10 blockade				Yao et al., 2017
Ulcerative colitis	TNF-α, IL-6, IL-1β, IL-10, TLR4/NF-κB/MAPK signaling pathway, IL-10/JAK1/STAT3 signaling pathway	MDA, MPO, SOD, GSH	Apoptotic cells		Wang et al., 2019
MA chronic inflammatory pain	IL-1β, TNF-α, MMP-2, IFN-γ, IL-10	MDA, 8-OhG, SOD			Zhou et al., 2018
Allergic asthma	IL-4, IL-5, IL-13, TNF-α, CXCL15	MDA, MPO, 8-OhG, SOD, GSH	Number of cell loss		Zhang N et al., 2019

IRI: ischemia and reperfusion injury; LPS: lipopolysaccharide; CLP: cecal ligation and puncture; Con A: concanavalin A; AIH: autoimmune hepatitis; CO: carbon monoxide; DR: diabetic retinopathy; CCl_4: carbon tetrachloride; MA: monoarthritis; TNF-α: tumor necrosis factor-α; IL: interleukin; NF-κB: nuclear factor-κB; MAPK: mitogen-activated protein kinase; GSK-3β: glycogen synthase kinase-3β; IFN-γ: interferon-γ; IκB: inhibitor of NF-κB; Iba-1: ionized calcium-binding adaptor molecule 1; ICAM-1: intercellular adhesion molecule-1; CXCL: C-X-C motif ligand; MPO: myeloperoxidase; PI3K: phosphoinositide-3-kinase; TLR4: Toll-like receptor 4; JAK1: Janus kinase 1; STAT3: signal transducer and activator of transcription 3; MMP-2: matrix metalloproteinase 2; SOX: superoxide; SOD: SOX dismutase; MDA: malondialdehyde; 8-OhG: 8-hydroxy-2-deoxyguanosine; CAT: catalase; GPx: glutathione (GSH) peroxidase; 3-NT: 3-nitrotyrosine; miR: microRNA; Bax: B-cell lymphoma 2 (Bcl-2)-associated X protein; PARP: poly ADP-ribose polymerase; NLRP3: NOD-like receptor protein 3; XOR: xanthine oxidoreductase; VEGF: vascular endothelial growth factor

3.4 Other mechanisms of methane protective action

There are other potential mechanisms of methane treatment and protection such as cell proliferation, pyroptosis, and XOR activity. Proliferation is a concern in diabetic retinopathy. Expression of vascular endothelial growth factor (VEGF) was higher in a diabetic retinopathy group, but ameliorated by methane administration (Wu et al., 2015). Pyroptosis is a process of cell death that differs from both apoptosis and necrosis, and has been proposed in research of cecal ligation and puncture (CLP)-induced sepsis (Li et al., 2019). It has its own pathway and is activated by the inflammasome. Li et al. (2019) found that methane alleviated pyroptosis by down-regulating the NOD-like receptor protein 3 (NLRP3)/caspase-1/IL-1β pathway. The protein levels of pro-p65, NLRP3, pro-caspase-1, and pro-IL-1β were significantly decreased by methane treatment compared to those in the sepsis group.

Methane may also act through mechanisms related to nitrosative stress (Poles et al., 2018). Methane decreased XOR activity and nitrotyrosine formation to protect the nitrergic neuron population in mesenteric IRI. The rising level of XOR activity was significantly reduced in the ileum, duodenum, and colon following methane inhalation.

4 Production of methane in eukaryotes under anoxic conditions

Methane may be produced in organisms outside of methanogens. Mitochondria, the crucial organelle for electron transport chains, were isolated and studied for in vitro research. Treating with choline in the presence of hydrogen peroxide, catalytic iron, and ascorbic acid, methane formation was first reported in rat liver mitochondria (Ghyczy et al., 2003). Furthermore, under hypoxic conditions, bovine endothelial cells in the presence of hydroxyl radical generation formed methane that was detected by gas chromatography (Ghyczy et al., 2008). This phenomenon showed that methane production was possible in anoxic condition. In an in vivo study of methane production, rats were grouped by sham, sodium azide (NaN_3), and NaN_3+antibiotic treatments. NaN_3 administration is a common method used to produce hypoxic conditions in cells and animals, and the antibiotics specifically targeted the potential methane-emitting gastrointestinal bacterial flora. The results showed that methane production was higher after NaN_3 administration than that in the sham group and still well over the level of the sham group after addition of antibiotics (Tuboly et al., 2013).

Results such as these suggest varied ranges of methane production, likely resulting from immune reactions and inflammatory processes, which might indicate non-microbial methane formation under anoxic conditions (Polag and Keppler, 2018). This supports the assumption that in hypoxic conditions methane might have another production source. The chemical mechanism underlying this result was well analyzed by Althoff et al. (2014). With iron (II/III), hydrogen peroxide, and ascorbic acid provided as reagents, S-methyl groups of organosulfur compounds can be converted to produce high amounts of methane. The idea that methane can come from oxidation of chemical compounds is also supported by experimental results suggesting that electrophilic methyl groups (EMGs) such as S-adenosylmethionine, betaine, carnitine, and phosphatidylcholine might be responsible for protection against reductive stress (Ghyczy and Boros, 2001). The chemical reaction described above could serve as the mechanism of methane formation in living organisms (Althoff et al., 2014). Because of the lack of data showing exactly how much methane was yielded from each organ, this point is still unproven as there are methanogens located in organs outside of the gut. Therefore, future research could examine methane formation in specific independent organs or related in vitro models. Nevertheless, there is a need to understand the overall purpose of methane formation in hypoxic situations.

Methane has been on earth from the time that seas changed into mulberry fields and mulberry fields into seas. Geochemical evidence suggests that roughly 3.5 billion years ago methanogenesis occurred, with methane produced by methanogens (Lyu et al., 2018). Increases in methane formation under anaerobic conditions are also observed in plants. Methane is emitted significantly from both intact plants and detached leaves (Boros and Keppler, 2019). This may indicate conserved ways of responding to hypoxic conditions for both animals and plants. It also means that methanogenesis can occur in both archaea and eukaryotes. Here too, we need to learn the overall purpose of this conserved mechanism of producing methane in anoxic conditions (Fig. 2).

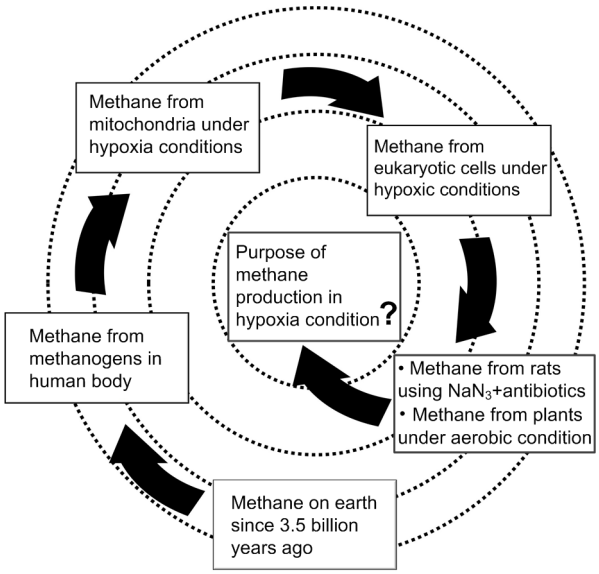

Fig. 2 What is the purpose of producing methane in hypoxic conditions?

Methane is an ancient gas existing on earth since 3.5 billion years ago, far before humans and the presence of oxygen in the atmosphere. Methane has a close relationship with the human body since methanogens are present throughout. This means that methane may have been connected with the human body since the beginning of human being. Methane could be emitted from mitochondria, eukaryotic cells, rats and plants in anoxic conditions, but we do not know the purpose of methane production in hypoxic conditions. Therefore, the purpose of methane production in hypoxic conditions needs further exploration

Three ideas are presented that might help explain the basis and purpose of methane production. First, methane may exist as an active intermediate molecule during signal transduction. This means that methane could become more active and important than is currently known. Evidence supports that methane in the body can turn into organically bound tritium (OBT), organically bound carbon (OBC), CO_2 and water (Carlisle et al., 2005). It is possible that metabolism of methane to other active molecules within the cell could then also be involved in the signal transduction. Transformed methane after biochemical reactions could work as a key molecule in signal transduction.

Second, methane might work directly as a signal for unknown specific receptors. As a similar example, soluble guanylyl cyclase (sGC) works as a receptor of nitric oxide (NO) signaling during vasodilation. Specific cells could have certain receptors that respond to methane and activate downstream mechanisms against hypoxic condition. Boros et al. (2015) thoroughly discussed this topic in their review. They indicated methane liberation may be linked to redox regulation and suggested that methane production might be a surviving evolutionary trait in eukaryote cells. Although the target of methane action in the body has not yet been found, a possible receptor still needs further exploration.

Third, the concentration of methane might work as transduction signals. Methane is a gas from ancient times. Methane production was an increasing response to anoxic environments. A change in the concentration of methane might itself be a signal that an organism should temporarily stop consuming energy. With its property of small size, methane could penetrate nuclear membrane, mitochondria, ER, and other cellular components to exert protection under hypoxic conditions. A study showed that after normal air inhalation of 3% radioactive methane, 0.33% of the methane is taken up by the body and can be converted to OBT, OBC, CO_2, and water (Carlisle et al., 2005). Other work has confirmed this uptake portion as being 0.325%–0.330%. Results indicate retention of methane in several parts of the body, including the liver with high ^{3}H and ^{14}C contents (Didychuk et al., 2014). This supports the overall observation that methane is capable of being taken up by mammals and after absorption can be converted to other active molecules and water, highlighting the involvement of methane in cellular metabolism.

5 Future research and clinical application

In fundamental research, more attention is needed to explore efficacy and the mechanisms underlying the protective effects of methane. Advanced research methods could be employed such as gene regulation using knockouts or clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated protein 9 (Cas9). Breakthroughs could be made in relation to its role in inflammatory or oxidative mechanisms. The Nrf2 knockout mouse has been used to explore the mechanisms underlying the protective effects of hydrogen. Those effects were blocked in the knockout, indicating the role of Nrf2 in response to oxidative injury and its beneficial effect (Kawamura et al., 2013). This may also be a very

promising approach for focusing on the products of methane reactions. Methods such as isotope labelling could be applied to search for isotope-labelled reaction products such as methanol, formaldehyde, or CO_2, and screening for specific monooxygenase reactions.

Another basic research approach might be to apply oral administration of methane or develop pills of methane that are taken orally for continued release. Like the application of NO, isosorbide dinitrate orally taken 2–3 times per day can relax vascular smooth muscle and thus relieve the symptoms of the patient. This method of drug administration is the most common and practical way of delivering drugs. Also, the "methane pill" method could benefit basic research by helping to clarify the dose-dependent manner of methane.

For clinical research of methane, the focus may need to be on improving understanding of the safety of methane in the human body. Demonstration of the safety of methane in humans could lead to trials on human disease treatment and expand methane research in clinical trials. In a manner similar to hyperbaric oxygen, equipment for hyperbaric oxygen application is used in many hospitals. Millions of patients have received and benefitted from hyperbaric oxygen therapy, but the mechanism of its protective effects on inflammatory disease and other chronic diseases is, like methane, still obscure.

6 Conclusions

Methane treatment shows many novel benefits in human diseases, such as organ IRI, inflammatory disease, and neuronal disease. Although different kinds of diseases have shown the protective effects of methane, the mechanisms underlying methane benefits in the human body might share similar processes. There may be three or more possible modes of action of methane including anti-inflammation, anti-oxidation, and anti-apoptosis mechanisms. Research areas such as cell proliferation and pyroptosis need further investigation. Note that it can take years for some gas-based therapies such as hyperbaric oxygen or NO to have healing effects on patients. In future research, more experiments could focus on exploring the mechanisms of action of acute or chronic methane administration. For clinical application, more confidence in methane treatment is needed from clinical trials.

Methane has existed on earth since ancient times. Methanogens, the archaea that emit methane, live alongside humans and are located in many parts of the body. Detecting changes in the production of methane in the breath has already offered practical help in disease diagnosis. Furthermore, injured eukaryotic cells or hypoxic environments can induce production of methane from cells. Therefore, exploration of the way methane may act in human diseases is of great clinical need and significance.

Contributors

Zhou-heng YE and Xue-jun SUN came up with the idea of the paper. Zhou-heng YE, Ke NING, and Bradley P. ANDER wrote the paper. Bradley P. ANDER and Xue-jun SUN edited the paper. All authors have read and approved the final manuscript and therefore, take responsibility for the integrity of the study.

Compliance with ethics guidelines

Zhou-heng YE, Ke NING, Bradley P. ANDER, and Xue-jun SUN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Althoff F, Benzing K, Comba P, et al., 2014. Abiotic methanogenesis from organosulphur compounds under ambient conditions. *Nat Commun*, 5:4205. https://doi.org/10.1038/ncomms5205

Basson AR, Lam M, Cominelli F, 2017. Complementary and alternative medicine strategies for therapeutic gut microbiota modulation in inflammatory bowel disease and their next-generation approaches. *Gastroenterol Clin North Am*, 46(4):689-729. https://doi.org/10.1016/j.gtc.2017.08.002

Boros M, Keppler F, 2019. Methane production and bioactivity-A link to oxido-reductive stress. *Front Physiol*, 10:1244. https://doi.org/10.3389/fphys.2019.01244

Boros M, Ghyczy M, Érces D, et al., 2012. The anti-inflammatory effects of methane. *Crit Care Med*, 40(4):1269-1278. https://doi.org/10.1097/CCM.0b013e31823dae05

Boros M, Tuboly E, Mészáros A, et al., 2015. The role of methane in mammalian physiology—is it a gasotransmitter? *J Breath Res*, 9(1):014001. https://doi.org/10.1088/1752-7155/9/1/014001

Carlisle SM, Burchart PA, McCauley C, et al., 2005. Biokinetics of inhaled radioactive methane in rats: a pilot study. *Appl Radiat Isotopes*, 62(6):847-860. https://doi.org/10.1016/j.apradiso.2005.01.010

Chen OY, Ye ZH, Cao ZY, et al., 2016. Methane attenuates

myocardial ischemia injury in rats through anti-oxidative, anti-apoptotic and anti-inflammatory actions. *Free Radic Biol Med*, 90:1-11. https://doi.org/10.1016/j.freeradbiomed.2015.11.017

Cheng X, Zhang L, Xie NC, et al., 2017. Association between small-intestinal bacterial overgrowth and deep vein thrombosis in patients with spinal cord injuries. *J Thromb Haemost*, 15(2):304-311. https://doi.org/10.1111/jth.13583

Didychuk C, Burchart PA, Carlisle SM, et al., 2014. Retention and excretion of inhaled ^{3}H and ^{14}C radiolabeled methane in rats. *Health Phys*, 107(1):18-35. https://doi.org/10.1097/hp.0000000000000048

Fan DF, Hu HJ, Sun Q, et al., 2016. Neuroprotective effects of exogenous methane in a rat model of acute carbon monoxide poisoning. *Brain Res*, 1633:62-72. https://doi.org/10.1016/j.brainres.2015.12.019

Ghyczy M, Boros M, 2001. Electrophilic methyl groups present in the diet ameliorate pathological states induced by reductive and oxidative stress: a hypothesis. *Br J Nutr*, 85(4):409-414. https://doi.org/10.1079/bjn2000274

Ghyczy M, Torday C, Boros M, 2003. Simultaneous generation of methane, carbon dioxide, and carbon monoxide from choline and ascorbic acid: a defensive mechanism against reductive stress? *FASEB J*, 17(9):1124-1126. https://doi.org/10.1096/fj.02-0918fje

Ghyczy M, Torday C, Kaszaki J, et al., 2008. Hypoxia-induced generation of methane in mitochondria and eukaryotic cells—an alternative approach to methanogenesis. *Cell Physiol Biochem*, 21(1-3):251-258. https://doi.org/10.1159/000113766

He R, Wang LP, Zhu JL, et al., 2016. Methane-rich saline protects against concanavalin A-induced autoimmune hepatitis in mice through anti-inflammatory and anti-oxidative pathways. *Biochem Biophys Res Commun*, 470(1):22-28. https://doi.org/10.1016/j.bbrc.2015.12.080

Jia YF, Li ZY, Feng Y, et al., 2018. Methane-rich saline ameliorates sepsis-induced acute kidney injury through anti-inflammation, antioxidative, and antiapoptosis effects by regulating endoplasmic reticulum stress. *Oxid Med Cell Longev*, 2018:4756846. https://doi.org/10.1155/2018/4756846

Kawamura T, Wakabayashi N, Shigemura N, et al., 2013. Hydrogen gas reduces hyperoxic lung injury via the Nrf2 pathway in vivo. *Am J Physiol Lung Cell Mol Physiol*, 304(10):L646-L656. https://doi.org/10.1152/ajplung.00164.2012

Koskinen K, Pausan MR, Perras AK, et al., 2017. First insights into the diverse human archaeome: specific detection of archaea in the gastrointestinal tract, lung, and nose and on skin. *mBio*, 8(6):e00824-17. https://doi.org/10.1128/mBio.00824-17

Li ZY, Jia YF, Feng Y, et al., 2019. Methane alleviates sepsis-induced injury by inhibiting pyroptosis and apoptosis: in vivo and in vitro experiments. *Aging*, 11(4):1226-1239. https://doi.org/10.18632/aging.101831

Liu L, Sun QL, Wang RB, et al., 2016. Methane attenuates retinal ischemia/reperfusion injury via anti-oxidative and anti-apoptotic pathways. *Brain Res*, 1646:327-333. https://doi.org/10.1016/j.brainres.2016.05.037

Lyu Z, Shao NN, Akinyemi T, et al., 2018. Methanogenesis. *Curr Biol*, 28(13):R727-R732. https://doi.org/10.1016/j.cub.2018.05.021

Meng Y, Jiang ZY, Li N, et al., 2018. Protective effects of methane-rich saline on renal ischemic-reperfusion injury in a mouse model. *Med Sci Monit*, 24:7794-7801. https://doi.org/10.12659/msm.911156

Polag D, Keppler F, 2018. Long-term monitoring of breath methane. *Sci Total Environ*, 624:69-77. https://doi.org/10.1016/j.scitotenv.2017.12.097

Poles MZ, Bódi N, Bagyánszki M, et al., 2018. Reduction of nitrosative stress by methane: neuroprotection through xanthine oxidoreductase inhibition in a rat model of mesenteric ischemia-reperfusion. *Free Radic Biol Med*, 120:160-169. https://doi.org/10.1016/j.freeradbiomed.2018.03.024

Rezaie A, Buresi M, Lembo A, et al., 2017. Hydrogen and methane-based breath testing in gastrointestinal disorders: The North American Consensus. *Am J Gastroenterol*, 112(5):775-784. https://doi.org/10.1038/ajg.2017.46

Sogodogo E, Drancourt M, Grine G, 2019. Methanogens as emerging pathogens in anaerobic abscesses. *Eur J Clin Microbiol Infect Dis*, 38(5):811-818. https://doi.org/10.1007/s10096-019-03510-5

Sun AJ, Wang WH, Ye XJ, et al., 2017. Protective effects of methane-rich saline on rats with lipopolysaccharide-induced acute lung injury. *Oxid Med Cell Longev*, 2017:7430193. https://doi.org/10.1155/2017/7430193

Tuboly E, Szabó A, Garab D, et al., 2013. Methane biogenesis during sodium azide-induced chemical hypoxia in rats. *Am J Physiol Cell Physiol*, 304(2):C207-C214. https://doi.org/10.1152/ajpcell.00300.2012

van de Pol JA, van Best N, Mbakwa CA, et al., 2017. Gut colonization by methanogenic archaea is associated with organic dairy consumption in children. *Front Microbiol*, 8:355. https://doi.org/10.3389/fmicb.2017.00355

Wang GH, Xu B, Shi FY, et al., 2019. Protective effect of methane-rich saline on acetic acid-induced ulcerative colitis via blocking the TLR4/NF-κB/MAPK pathway and promoting IL-10/JAK1/STAT3-mediated anti-inflammatory response. *Oxid Med Cell Longev*, 2019:7850324. https://doi.org/10.1155/2019/7850324

Wang LP, Yao Y, He R, et al., 2017. Methane ameliorates spinal cord ischemia-reperfusion injury in rats: antioxidant, anti-inflammatory and anti-apoptotic activity mediated by Nrf2 activation. *Free Radic Biol Med*, 103:69-86. https://doi.org/10.1016/j.freeradbiomed.2016.12.014

Wu JC, Wang RB, Ye ZH, et al., 2015. Protective effects of methane-rich saline on diabetic retinopathy via anti-inflammation in a streptozotocin-induced diabetic rat

model. *Biochem Biophys Res Commun*, 466:155-161. https://doi.org/10.1016/j.bbrc.2015.08.121

Xie Q, Fei MM, Fa ZZ, et al., 2017. Methane-rich saline alleviates cerulein-induced acute pancreatitis by inhibiting inflammatory response, oxidative stress and pancreatic apoptosis in mice. *Int Immunopharmacol*, 51:17-24. https://doi.org/10.1016/j.intimp.2017.07.022

Yao Y, Wang LP, Jin PP, et al., 2017. Methane alleviates carbon tetrachloride induced liver injury in mice: anti-inflammatory action demonstrated by increased PI3K/Akt/GSK-3β-mediated IL-10 expression. *J Mol Histol*, 48(4):301-310. https://doi.org/10.1007/s10735-017-9728-1

Ye ZH, Chen OY, Zhang RJ, et al., 2015. Methane attenuates hepatic ischemia/reperfusion injury in rats through antiapoptotic, anti-inflammatory, and antioxidative actions. *Shock*, 44(2):181-187. https://doi.org/10.1097/shk.0000000000000385

Zhang D, Li N, Wang YY, et al., 2019. Methane ameliorates post-operative cognitive dysfunction by inhibiting microglia NF-κB/MAPKs pathway and promoting IL-10 expression in aged mice. *Int immunopharmacol*, 71:52-60. https://doi.org/10.1016/j.intimp.2019.03.003

Zhang N, Lu HT, Zhang RJ, et al., 2019. Protective effects of methane-rich saline on mice with allergic asthma by inhibiting inflammatory response, oxidative stress and apoptosis. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 20(10):828-837. https://doi.org/10.1631/jzus.B1900195

Zhang X, Li N, Shao H, et al., 2016. Methane limit LPS-induced NF-κB/MAPKs signal in macrophages and suppress immune response in mice by enhancing PI3K/AKT/GSK-3β-mediated IL-10 expression. *Sci Rep*, 6:29359. https://doi.org/10.1038/srep29359

Zhou SZ, Zhou YL, Ji F, et al., 2018. Analgesic effect of methane rich saline in a rat model of chronic inflammatory pain. *Neurochem Res*, 43(4):869-877. https://doi.org/10.1007/s11064-018-2490-2

中文概要

题　目：甲烷治疗疾病效应及其机制

概　要：甲烷是最简单的有机烃类，由 1 个碳原子和 4 个氢原子组成。甲烷在沼气、家畜反刍和可燃冰中含量丰富。甲烷在疾病治疗中的作用还未被大家熟悉。最近的研究表明甲烷可以治疗多种疾病，包括缺血再灌注损伤和炎症疾病。甲烷治疗疾病的机制可能包括抗氧化、抗炎症和抗凋亡。本文将描述甲烷对不同疾病的治疗效应，总结甲烷治疗效应的可能作用机制，并讨论甲烷在低氧环境下产生的目的。最后，我们也将提出甲烷研究的前景及探索方向。

关键词：甲烷治疗；缺血再灌注损伤；炎症；甲烷生成