Comments and hypotheses on the mechanism of methane against ischemia/reperfusion injury

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Abstract

As we all know, methane is a kind of fuel. Previous studies have shown that methanogens in the colon can react with carbon dioxide and hydrogen to produce methane. In a recent study, the anti-inflammatory effects of methane were shown in a dog model of small intestinal ischemia/reperfusion. The mechanism of this anti-inflammatory effect needs further investigation. Recently, studies have shown anti-inflammatory, anti-apoptotic and anti-oxidative effects of methane on different organic injuries. According to the results of these studies, we hypothesize that the initial effects of methane are to react with free radicals and enhance expression of antioxidase through forkhead box transcription factor class O pathway. The anti-inflammatory effect is following the anti-oxidative effect, and the anti-apoptotic effect relies on anti-inflammatory and anti-oxidative effects.

Key words: methane; ischemia/reperfusion injury; anti-apoptosis; anti-inflammation; anti-oxidative; hypotheses; mechanism; forkhead box transcription factor class O pathway

INTRODUCTION

Ischemia/reperfusion (I/R) injury is a cellular damage induced by hypoxia following the restoration of blood flow and oxygen delivery after transplantation surgery, hemorrhagic shock or tissue resections.[1,2] I/R injury is one of the cause of impairment of organs. The mechanism of the impairment is related to oxidative stress, inflammation and apoptosis.[3] Oxidative stress is induced by the production of reactive oxygen species (ROS) especially hydroxyl radicals. Inflammation is caused by the accumulation of pro-inflammatory cytokines and the activation of leucocyte.[4] Apoptosis can also be found in tissues after I/R injury.[5]

Methane is the simplest alkane and the most abundant organic compound. Studies have proved that methanogens in the colon can produce methane in the presence of carbon dioxide and hydrogen.[6] Methane is generally considered as a kind of biologically inactive gas. Excitingly, much attention has been paid to the application of methane as a therapeutic gas recently. Boros and colleagues[7] found that exhaled methane exerted protective effects against intestinal I/R-induced oxidative stress and inflammation. Since then, lots of studies about methane against I/R injury have been made, including its protective effect on liver, abdominal skin flap and myocardium I/R injury.[8-10] These studies indicate that the mechanism of methane is related to anti-inflammation, anti-apoptosis and anti-oxidative effect.

ANTI-INFLAMMATORY EFFECT OF METHANE

Boros and colleagues[7] found that exogenous methane modulates leucocyte activation and ameliorates oxidative and nitrosative stress induced by I/R, which shows an anti-inflammation effect. Thus, anti-inflammation is an impor-

tant mechanism for methane against I/R injury.

Recently, Ye and colleagues[8] demonstrated the anti-inflammatory effect of methane in a rat liver I/R model. PCR was used to detect the *in situ* mRNA levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin (IL-1β) and enzyme-linked immunosorbent assay (ELISA) was used to detect the serum levels of these factors. The result showed that the mRNA expression of these factors significantly increased after I/R and methane-rich saline reduced the expression. The levels of TNF-α and IL-6 in serum were increased significantly after I/R injury and such a significant increase was reduced by methane. Interestingly, the level of IL-1β in serum had no significant change after I/R or methane-rich saline treatment.

In another study by Chen et al.,[10] the anti-inflammation effect of methane was also demonstrated in a rat myocardial infarction (MI) model. Although MI is different from I/R, there are many points in common in their mechanism. For instance, both of them are related to inflammation, oxidative stress and apoptosis. So, the effects of methane on MI model also have reference value for I/R treatment. In this study, immunohistochemistry of myeloperoxidase (MPO) and CD68 and the levels of TNF-α, IL-1β, and MPO in the heart of rats were detected to evaluate the inflammation level and the anti-inflammation effect of methane-rich saline. The immunohistochemistry results showed that the percentages of MPO and CD68 positive cells were increased significantly in I/R group and methane attenuated the increase of the percentage. The amount of TNF-α, IL-1β, and MPO also increased significantly after I/R and such significant increasewas reduced by methane treatment. The author wanted to find the best dose of methane-rich saline against MI induced inflammation, but the results were not clear.

Accordingly, anti-inflammation is an important mechanism of methane against I/R injury in different organs.

Anti-Oxidative Effect of Methane

The study by Boros et al.[7] also mentioned that the anti-inflammation effect of methane may be related to its anti-oxidative effect.

In the study by Ye and colleagues,[8] the anti-oxidative effect of methane in liver tissue was measured. Malondialdehyde (MDA) is an expression of lipid oxidation. Superoxide dismutase (SOD) levels are used to reflex antioxidant level, and 8-hydroxyguanosine (8-OHG) is a marker of DNA oxidation. The study showed that the level of MDA increased in I/R group and decreased in I/R + methane-rich saline (MS). The level of SOD was decreased in I/R and increased after MS treatment. These results showed that MS treatment ameliorated lipid oxidation and enhanced anti-oxidative capability of liver cells. This result was confirmed by the 8-OHG staining result which showed a decreased number of positive cells in the I/R + MS group compared with that in the I/R group.

Another study by Liu et al.[9] demonstrated the anti-oxidative effect of methane-rich saline in a rat model of retinal I/R. In this study, methane treatment significantly reduced the levels of oxidative stress biomarkers such as 8-hydroxy-2-deoxyguanosine, 4-hydroxy-2-nonenal and MDA according to ELISA and immunofluorescence staining results. The levels of antioxidant enzymes such as SOD, catalase and glutathione peroxidase (GPx) were also measured by ELISA. Expressions of these antioxidant markers were increased after methane-rich saline treatment. Interestingly, this study found that methane had no significant effect on the redox system of normal retina.

A study about the effect of MS on MI by Chen et al.[10] also showed the increase of antioxidant markers could also be observed.

In conclusion, the anti-oxidative effect is an important link in the mechanism of methane treatment. This effect may be related to methane-induced antioxidant expression.

Anti-Apoptotic Effect of Methane

Recently, several studies have demonstrated the anti-apoptosis effect of methane.

In a study by Ye et al.,[8] the apoptosis condition of liver tissue was valued by Western blots of caspase-3. The apoptosis level was significantly reduced by MS treatment compared with I/R group.

Another study by Liu et al.[9] detected the expressions of apoptosis-related genes in retinal. As results, the levels of B cell leukemia/lymphoma-2 (Bcl-2) and Bcl-2 associated X protein (Bax) mRNA were significantly increased in I/R group. Bax was reduced by methane treatment remarkably, and Bcl-2 was additionally enhanced by methane treatment. The activities of caspase-9 and caspase-3, measured by fluorescent quantitative detection, were significantly increased in I/R group and methane treatment significantly reduced the activities of them.

In a study by Song et al.,[11] they confirmed the anti-apoptosis effect of methane on abdominal skin flap I/R injury in rats. TdT-mediated dUTP-X nick end labeling (TUNEL) staining was used to detect the number of apoptotic cells in tissue. MS treatment significantly reduced the TUNEL-positive cells compared with I/R group. Immunohistochemical stain of apoptotic markers such as Bax, phosphorylated apoptosis signal-regulating kinase 1 and phosphorylated c-Jun N-terminal kinase showed a significant reduce of apoptotic markers after methane treatment compared with I/R group, and Bcl-2 was significantly increased after methane treatment compared with I/R group. The result of caspase-3 activity was just consistent with the result of TUNEL.

In another study by Chen and colleagues,[10] western blot assay showed that MS administration increased Bcl-2 and decreased Bax, caspase-3, and caspase-9 expression significantly. These results were additionally confirmed by quantitative RT-PCR of Bax, Bcl-2 and caspase-3. Result of TUNEL staining showed methane treatment reduced the number of positive cells after I/R injury.

These results demonstrated the anti-apoptotic effect of methane against I/R injury.

DISCUSSION AND HYPOTHESIS

As we all know, methane is a kind of fuel and plays a role in global warming. Previous studies have demonstrated that methane can be synthesized biologically.[12] Methanogens in the colon can produce methane with CO_2 and H_2.[6] It has been demonstrated that endogenous methane has effect on constipation-predominant irritable bowel syndrome, diverticulosis, and colon cancer.[13-15] In 2012, Boros et al.[7] illustrated the treatment effect of exogenous methane on intestinal I/R injury. This research pointed out the anti-oxidative and anti-inflammatory effects of methane on I/R injury.

I/R injury happens when the blood flow and oxygen supply of organs are interrupted for some time and restored subsequently. Recently, many studies have demonstrated the anti-oxidative, anti-inflammatory and anti-apoptotic effects of methane against I/R injury. However, these studies have always isolated these important effects of methane when illustrating the mechanism of methane.[1] Thus, the mechanism of methane treatment is still unclear and the order is still unknown by which these three effects take place.

The accurate mechanism of methane needs to be investigated. I/R injury induced accumulation of leucocytes generated free radicals, and free radicals can induce inflammation and accumulation of leucocytes.[16,17] We hypothesize that anti-oxidative effect is the initial effect of methane for the following reasons: First, oxidation is the initial mechanism of I/R injury.[18] Additionally, methane is a kind of gas with reducibility, which is similar to hydrogen. According to previous studies, hydrogen can react with free radicals and show anti-oxidative effect.[19] Thus, methane may also react with free radicals (**Figure 1**).

If the hypothesis is true, the reaction with free radicals may be the initial mechanism of the treatment effect of methane toward I/R injury. To confirm anti-oxidative effect is the initial effect, *in vitro* experiment is needed. The cells of organs need to be separated and cultured in medium. Inflammation is avoided *in vitro*. If the anti-oxidative effect still exists, it may be the independent initial effect against I/R injury.

The mechanism of the expression methane inducement of antioxidase is still unclear. This may include several signal transduction pathways. A forkhead box transcription factor
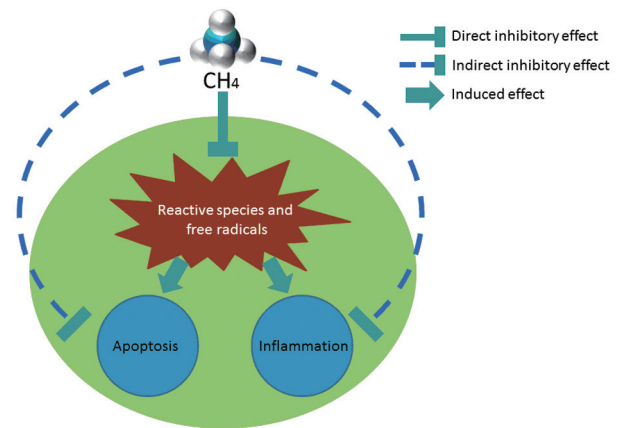

Figure 1: Anti-oxidative effect is the initial effect of methane (CH_4), and anti-apoptotic and anti-inflammatory effects are induced by anti-oxidative effect.

class O (FoxO) is a modifier of the antioxidant genes. Previous studies illustrated that FoxO is involved in the production of antioxidants including SOD-2, GPx-1 and catalase.[20,21] Accordingly, we hypothesize that the effect of methane is related to FoxO pathway (**Figure 2**). To confirm our hypothesis, I/R models of animals is required. The activity of FoxO and its downstream factors needs to be measured. If FoxO and its downstream factors are induced by methane and the expression enhancement of antioxidase is blocked by a FoxO inhibitor, the hypothesis will be confirmed.

Previous studies indicated that I/R induced apoptosis is related to inflammation and oxidation. Methane has anti-oxidative and anti-inflammatory effects. Thus, its anti-apoptotic effect may be based on these two effects.

Therefore, the anti-oxidative, anti-inflammatory and anti-apoptotic effects of methane have been demonstrated. To illustrate the consequence and causality of these effects, additional investigation of methane is required. Thus, we hypothesize that the initial effect of methane is the reaction with free radicals and expression enhancement of antioxidase through FoxO pathway. Anti-inflammatory effect comes after anti-oxidative effect, and anti-apoptotic effect relies on anti-inflammatory and anti-oxidative effects. To confirm the hypotheses above, further investigation is needed.
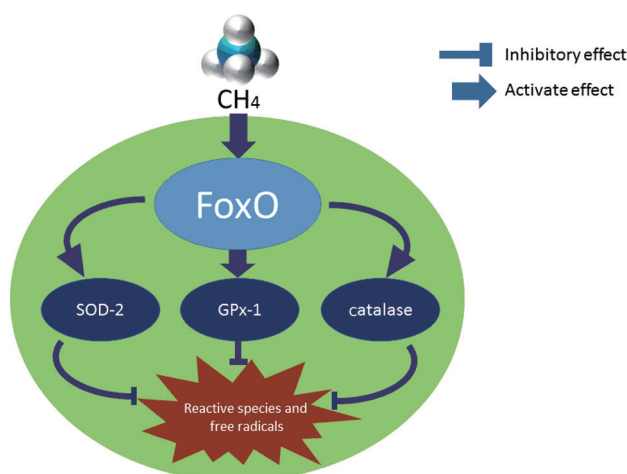
Figure 2: FoxO pathway may be involved in the anti-oxidative effect of methane (CH_4), and this effect may induce by antioxidants including SOD-2, GPx-1 and catalase.

Note: FoxO: Forkhead box transcription factor class O; SOD-2: superoxide dismutase-2; GPx-1: glutathione peroxidase-1.

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