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Neuroprotective effects of methane-rich saline on experimental acute carbon monoxide toxicity

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ABSTRACT

Background: Methane has been reported to play a protective role in ischemia-reperfusion injury via *anti*-oxidation, anti-inflammatory and anti-apoptotic activities. This study was designed to determine the protective effects of methane-rich saline (MRS) on acute carbon monoxide (CO) poisoning.
Methods: A total of 36 male Sprague-Dawley rats were randomly divided into 3 groups: sham group, CO group and MRS group. Acute CO poisoning was induced by exposing rats to 1000 ppm CO in air for 40 min and then to 3000 ppm CO for an additional 20 min until they lost consciousness. MRS at 10 ml/kg was intraperitoneally administered at 0 h, 8 h and 16 h after CO exposure. Rats were sacrificed 24 h after CO exposure. Brains were collected for Nissl staining. The cortex and hippocampus were separated for the detections of malondialdehyde (MDA), 3-nitrotyrosine (3-NT), 8-hydroxydeoxyguanosine (8-OHdG), tumor necrosis factor-α (TNF-α), interleukin1-β (IL-1β), interleukin-6 (IL-6) and superoxide dismutase (SOD) activities.
Results: The results showed that MRS treatment improved neuronal injury, reduced MDA, 3-NT and 8-OHdG, and increased SOD activity of the hippocampus and cortex compared with normal saline-treated rats. In addition, MRS reduced the expression of TNF-α and IL-1β in the brain but had no effect on IL-6 expression.
Conclusion: These findings suggest that MRS may protect the brain against acute CO poisoning-induced injury via its *anti*-oxidative and anti-inflammatory activities.

1. Introduction

Acute carbon monoxide (CO) toxicity is a leading cause of gas poisoning-related deaths worldwide due to the increased use of carbon-based fuels [1]. It has been reported that CO poisoning is responsible for approximately 15,000 visits to emergency departments and nearly 500 deaths annually in the United States [1–3]. Acute CO poisoning may produce severe brain damage, which can lead to high mortality and delayed neurological syndrome (DNS) [4]. Numerous studies have indicated that an increase in the production of reactive oxygen species (ROS) following CO poisoning is of crucial relevance to the pathophysiology of CO poisoning [5–7]. Although ROS play important roles in the clearance of invaded pathogens, they seem to produce substantial damage if they are produced in excess, which can lead to DNA strand breaks or to lipid and protein oxidation [8,9]. The brain is highly vulnerable to oxidative stress compared with other organs due to the high metabolic rate required to meet the energy consumption of the brain; and this high metabolic rate can lead to increased ROS production. When the defense is insufficient to scavenge these ROS, they may inevitably cause the oxidation of unsaturated fatty acids, which results in lipid peroxidation [10,11]. Enhanced ROS generation following brain insults, including cerebral ischemia/hypoxia, brain trauma, and CO poisoning, may disrupt the balance between ROS generation and scavenging, which in turn accelerates neural injury, expands the injured area, and leads to poorer outcomes [5,12].

Abbreviations: ANOVA, analysis of variance; CO, carbon monoxide; COHb, carboxyhemoglobin; DNS, delayed neurological syndrome; HBO, hyperbaric oxygen; •OH, hydroxyl radical; IL-1β, interleukin 1 –β; IL-6, interleukin-6; MRS, methane-rich saline; MDA, Malondialdehyde; NS, normal saline; 3-NT, 3-nitrotyrosine; 8-OHdG, 8-hydroxydeoxyguanosine; ROS, reactive oxygen species; SOD, superoxide dismutase; $ONOO^-$, peroxynitrite; O_2^-, superoxide anion; TBA, thiobarbituric acid; TNF-α, tumor necrosis factor-α.

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Methane is the simplest aliphatic hydrocarbon and a major component of the natural gas used to heat homes and cook food. Certain microbes, called methanogens, may use carbon dioxide, acetate, or other small organic molecules as terminal electron acceptors under strictly anaerobic conditions and produce methane as a metabolic product [13]. Interestingly, it was reported that methane can be generated by rat liver mitochondria and formed from choline in the presence of hydrogen peroxide, catalyticiron, and ascorbic acid [14,15]. In addition, it has been shown that methane has *anti*-oxidative and anti-nitrosative activities in mesenteric ischemia-reperfusion injury [16]. In animal models, our team found that methane could protect the liver against ischemia-reperfusion injury [17] and the myocardium against myocardial infarction [18] via its *anti*-apoptotic, anti-oxidative and anti-inflammatory activities. In addition, the protective effects of methane on diabetes mellitus were also found to be related to *anti*-inflammatory pathways [19]. Recently, it was reported that methane protected liver against Con A-induced injury through anti-inflammatory and anti-oxidative pathways [20]. Our previous study showed methane-rich saline (MRS) was able to exert long term protection on the brain injury of rats after CO poisoning [21], but whether it protects the acute injury to the brain after CO poisoning is still unclear.

This study was done to evaluate the protective effects of methane-rich saline (MRS) on the brain injury secondary to acute CO poisoning and the potential protection mechanisms in a rat model.

2. Materials and methods

All surgical procedures were approved by the Ethics Committee for Animal Experimentation and conducted according to the Guidelines for Animal Experimentation of our institute. All efforts were made to minimize the number of animals used in this study, and every effort was taken to reduce animal suffering.

2.1. Animals and groups

A total of 36 male Sprague-Dawley rats weighing 250–280 g were used in the present study. The animals were kept in a humidity- and temperature-controlled room with a 12-hour light/dark cycle and given ad libitum access to food and water. Animals were randomly distributed into three groups as follows: control group, CO poisoning plus normal saline (NS) (CO) group and CO poisoning plus MRS (CO + CH4) group (Fig. 1).

2.2. CO exposure and carboxyhemoglobin detection

The establishment of acute CO poisoning in rats has been described previously [6]. Briefly, the rats were placed in a 7-L Plexiglass chamber and exposed to 1000 ppm CO (Shanghai Gas CO, China) at a rate of 4 L/min for 40 min, followed by 3000 ppm CO for another 20 min until they lost consciousness. Then, these rats were allowed to breathe fresh air and regain consciousness. The rats in the control group inhaled fresh air for 1 h. Hypothermia was avoided when poisoning was discontinued. Immediately after CO exposure, approximately 0.3 ml of whole blood was drawn for carboxyhemoglobin (COHb) assay after intraperitoneal anesthesia with 3% pentobarbital sodium (50 mg/kg). A Blood Gas Analyzer (Cobasb 221 system, Roche Diagnostics GmbH, Germany) was used for COHb detection.

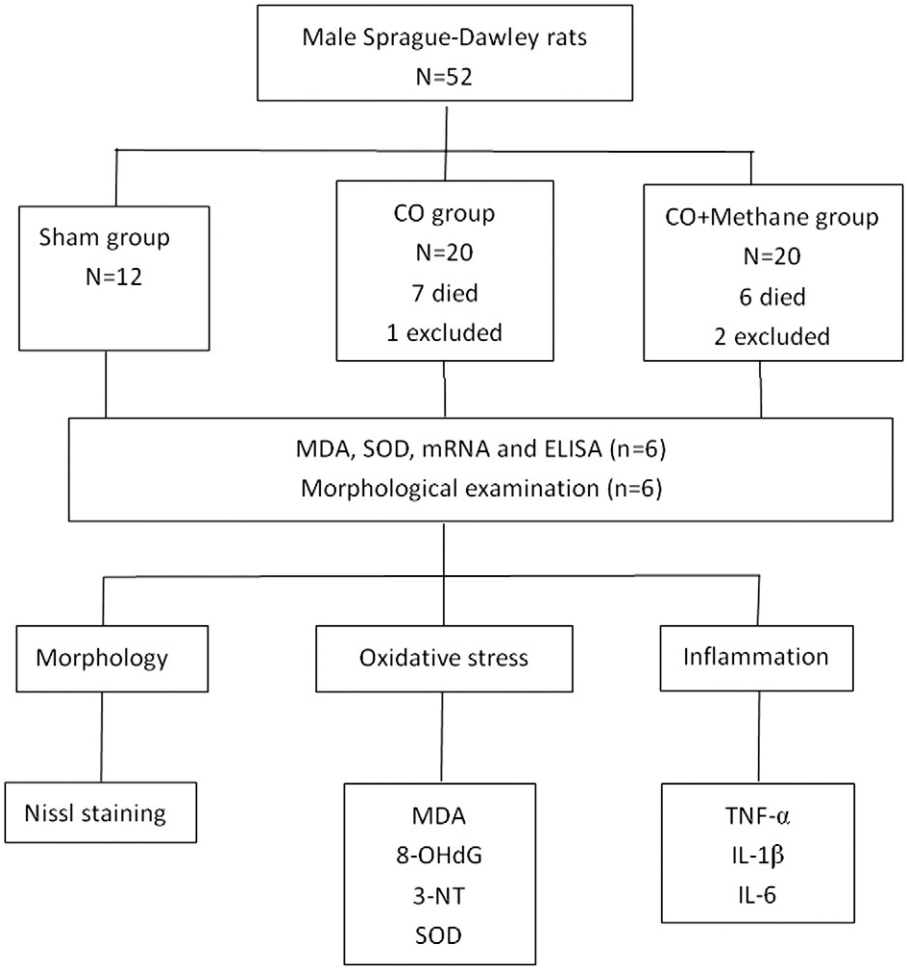

Fig. 1. Flow chart of the study.