Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Neuroprotective effects of exogenous methane in a rat model of acute carbon monoxide poisoning

Dan-feng Fan[a,b,1], *Hui-jun Hu*[a,b,1], *Qiang Sun*[b], *Yan Lv*[b], *Zhou-heng Ye*[a], *Xue-jun Sun*[a,*], *Shu-yi Pan*[b,**]

[a]*Department of Naval Aeromedicine, Faculty of Naval Medicine, Second Military Medical University, No. 800, Xiangyin Road, Shanghai 200433, PR China*

[b]*Department of Hyperbaric Oxygen, Navy General Hospital, No. 6, Fucheng Road, Beijing 100048, PR China*

ARTICLE INFO

Article history:
Accepted 11 December 2015
Available online 19 December 2015

Keywords:
Methane
Carbon monoxide poisoning
Neuroprotection
Oxidative stress
Apoptosis

ABSTRACT

Objective: Delayed neuropsychological sequelae (DNS) are the most common and serious effects of severe carbon monoxide (CO) poisoning, occurring in approximately half of all CO poisoning cases. Growing evidence suggests that oxidative stress and secondary reactions in delayed brain injury are crucial to CO toxicity, similar to ischaemia–reperfusion injury. Exogenous methane plays a protective role in ischaemia–reperfusion injury by affecting key events through anti-oxidant, anti-inflammatory, and anti-apoptosis actions. Our study aimed to explore the potential of exogenous methane to relieve the severity of DNS.

Methods: Thirty-six male Sprague–Dawley (SD) rats were divided into three groups of normal-, CO- and CO plus methane-treated rats. The rats in the latter two groups were exposed to 1000 ppm CO for 40 min and then to 3000 ppm CO for another 20 min. Following CO exposure, saline or methane saline (10 ml/kg) was intraperitoneally administered to rats in the CO group or the CO plus methane group, respectively. On the ninth day after CO exposure, Morris water maze testing, histological analysis, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) and immunohistochemical labelling were performed on 6 rats in each group. The remaining 6 rats in each group were used to detect oxidative damage markers, inflammatory cytokines and apoptosis proteins.

Results: Methane significantly improved CO-impaired pathological characteristics as well as learning and memory performance. In addition, methane significantly increased the superoxide dismutase (SOD) activity, lowered the CO-increased level of malondialdehyde (MDA) 3-nitrotyrosine (3-NT) and 8-hydroxy-2-deoxyguanosine (8-OHdG), inhibited levels of tumour necrosis factor-α (TNF-α), interleukin 1-β (IL1-β) and caspase-3 in the rat cerebral cortex and hippocampus but had no effect on IL-6 levels.

Conclusion: The hippocampus was the main target of CO-induced alterations in the rat brain compared to the cerebral cortex. Methane treatment protected the rat brain from the

*Corresponding author.
**Corresponding author.
E-mail addresses:
sunxjk@hotmail.com (X.-j. Sun), hbofandf@163.com (S.-y. Pan).

[1]Dan-feng Fan and Hui-jun Hu contributed equally to this work.

http://dx.doi.org/10.1016/j.brainres.2015.12.019

harmful effects induced by CO exposure and improved the outcome of DNS through anti-oxidant, anti-inflammatory and anti-apoptosis activities.

1. Introduction

Carbon monoxide (CO) poisoning is one of the major causes of poisoning morbidity and mortality in the modern world. CO is a tasteless, odourless and non-irritating but highly toxic gas that is produced by the incomplete burning of carbon-containing fossil fuels. CO poisoning might occur as a result of faulty furnaces, inadequate ventilation of heating sources, and exposure to engine exhaust. The normal concentration of CO in the atmosphere is less than 0.001%, and a concentration of 0.1% can be lethal.

The symptoms of CO poisoning are nonspecific. Mild exposure can result in headache, myalgia, dizziness, nausea, or neuropsychological impairment. Severe exposure to CO can result in confusion, coma, and even death (Weaver, 2009). Over half of those with serious poisoning develop an encephalopathy from 14 to 45 days after exposure (Hsiao et al., 2004), with features of delayed impairment ranging from subtle abnormalities, such as personality changes or mild cognitive deficit, to severe dementia, psychosis, Parkinsonism, incontinence or other abnormalities. These impairments are referred to as delayed neuropsychological sequelae (DNS). Among patients with DNS, 50–75% recover completely or improve considerably within 1 year (Choi, 1983).

Because CO has a 200× greater affinity for haemoglobin (Hb) compared to oxygen (O_2) (Weaver, 2009), CO quickly diffuses into the blood via the lungs and binds to Hb to form carboxyhaemoglobin (COHb). The easy displacement of O_2 from Hb reduces the amount of Hb available to carry O_2, causing hypoxaemia. Meanwhile, COHb shifts the oxyhaemoglobin dissociation curve to the left, further decreasing the amount of the O_2 released (Ernst and Zibrak, 1998) and worsening the histanoxia. The toxic effects of CO on the cytochromes plays a minor role in the pathophysiological mechanisms of CO poisoning, as the amount of CO required to significantly impair the cytochromes is 1000 times higher than the lethal dose (Prockop and Chichkova, 2007). Hypoxia caused by CO is key for injury to the brain.

Some of the pathophysiological effects of CO poisoning are consistent with ischaemic–reperfusion injury, in that for both conditions, there is a hypoxic phase usually followed by reoxygenation (Mannaioni et al., 2006). In the CO-impaired brain, xanthine dehydrogenases are converted to xanthine oxidases, suggesting that xanthine oxidase-derived reactive oxygen species (ROS) are responsible for the lipid peroxidation of neuronal membranes (Thom, 1992). Hydroxyl radicals are also present in a brain that is subjected to CO-induced hypoxia and then reoxygenated in both the hypoxic phase and the reoxygenation phase (Zhang and Piantadosi, 1992). Increasingly, studies suggest that in the late stages of brain damage due to CO poisoning, leucocytes play a key role in non-bacterial inflammation (Thom, 1993). A 10-fold increase in nitrotyrosine production in the brains of CO-poisoned rats further demonstrates that peroxynitrite may be generated during CO poisoning due to the enhanced rates of production of both nitric oxide (NO) and superoxide (Thom, 1993). Together, the findings above suggest that one of the important mechanisms of brain injury in CO poisoning is free radical formation, which results in neuronal death, thereby causing DNS. CO-mediated brain damage is the result of a free radical cascade, according to the classical mechanism of a redox reaction, and is strongly dependent on disrupting the balance between the anti-oxidant system and oxidative stress. Acute anti-oxidant reinforcement may be a novel therapeutic strategy for DNS after acute CO poisoning.

In recent years, a novel and exciting trend in the evolving putative therapeutic treatment of oxidative stress is the use of medical gas. Nitric oxide (NO) (Culotta and Koshland, 1992; Padmaja and Huie, 1993), CO (Fujita et al., 2001), and hydrogen sulphide (H_2S) (Oh et al., 2006) are the classic therapeutic medical gases with anti-oxidant properties used in disease therapy. However, all of the gases have different toxic effects in humans and cells. Hydrogen (H_2) is the lightest and most abundant of the chemical elements. Ohsawa et al. showed that H_2 (~4%) has anti-oxidant and anti-apoptotic properties, protecting the brain from ischaemia-induced injury and stroke by selectively neutralizing cytotoxic ROS (Ohsawa et al., 2007). Further studies have demonstrated that H_2 has numerous protective effects in various animal disease models and in clinical treatment (Ohta, 2008, 2011). Importantly, H_2 has no cytotoxicity, even at high concentrations (Lillo and Parker, 2000), which has been thoroughly researched in diving medical science. With respect to our interest, H_2, via anti-oxidant and anti-apoptotic mechanisms, also has a protective effect on brain injury induced by CO poisoning (Shen et al., 2013; Sun et al., 2011).

Methane is the simplest aliphatic hydrocarbon and the main gas energy. The anaerobic bacteria in our bodies produce significant amounts of methane, using carbon dioxide (CO_2), acetate, or other small organic molecules as terminal electron acceptors under strictly anaerobic conditions, via the methanogenesis pathway (Liou et al., 2013). It was previously thought that humans do not use methane. However, Ghyczy et al. (2003) reported for the first time the generation of methane by rat liver mitochondria and the formation of methane from choline in the presence of hydrogen peroxide, catalytic iron, and ascorbic acid. Besides these, in recent year, there are more and more evidences supporting the notion that methane liberation may be linked to redox regulation and may be connected with hypoxic events leading to, or associated with a mitochondrial dysfunction (Boros et al., 2015). Boros et al. (2012) demonstrated that exogenous methane significantly ameliorates histological damage to the intestinal mucosa of dogs subjected to