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Research Report

Perfluorocarbon-facilitated CNS oxygen toxicity in rats: Reversal by edaravone

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ABSTRACT

Perfluorocarbon (PFC) has been hypothesized to potentially increase the risk of central nervous system oxygen toxicity (CNS-OT) under hyperbaric oxygen (HBO) conditions. However, little is known about the effects, mechanism and prevention of PFC-facilitated CNS-OT. A rat model of CNS-OT was used to evaluate the effects of intravenously-administered PFC emulsion. The electroencephalogram (EEG) was recorded during treatment with HBO_2 at 6.0 ATA in the presence and absence of PFC. Concentrations of malondialdehyde (MDA), nitric oxide (NO) and hydrogen peroxide (H_2O_2) in the brain cortex and hippocampus were quantified. Changes in the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and NO synthase (NOS) in the brain cortex and hippocampus were also determined. Edaravone, a potent antioxidant, was used to prevent PFC-facilitated CNS-OT. The results showed that after PFC administration, the latency to first electrical discharge in EEG was significantly shortened; MDA, H_2O_2, NO levels and NOS activity increased; and SOD, GPx and CAT activities decreased. Edaravone effectively protected against CNS-OT and the adverse effects of PFC. The results clearly demonstrate that PFC administered before HBO_2 would promote the occurrence of CNS-OT, and edaravone could serve as a promising chemoprophylactic agent to prevent CNS-OT.

1. Introduction

Since the 1960s, it has been known that perfluorocarbons (PFCs) could dissolve and transport large amounts of non-polar gases, including O_2 and N_2. It was demonstrated that a mouse could survive in O_2-saturated liquid PFC (Clark and Gollan, 1966). The potential medical applications of PFCs has drawn much attention. PFC emulsions were extensively studied as a possible, near ideal blood substitute and potential treatment for a variety of disorders, including decompression sickness (DCS) (Castro and Briceno, 2010). DCS usually occurs when gas molecules (mainly N_2), which are dissolved in bodily tissues, leave solution

Abbreviations: PFC, Perfluorocarbon; CNS-OT, Central nervous system oxygen toxicity; HBO_2, Hyperbaric oxygen; MDA, Malondialdehyde; NO, Nitric oxide; H_2O_2, Hydrogen peroxide; SOD, Superoxide dismutase; GPx, Glutathione peroxidase; CAT, Catalase; NOS, NO synthase

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in sufficient numbers to form bubbles during decompression. This condition frequently occurs during transition from a hyperbaric environment to normal atmospheric pressure (such as during emergence from deep diving) or from normal atmospheric pressure to hypobaric surroundings (such as during extravehicular activity) (Francis, 2002). One obvious treatment for DCS is hyperbaric oxygen (HBO_2) therapy, which can reduce bubble size, improve tissue oxygenation and enhance the elimination of dissolved inert gases (Brubakk, 2004). However, under some conditions, such as submarine escape, HBO_2 treatment may be immediately unavailable at the sites where DCS is encountered. Until the victims are transported to hyperbaric treatment facilities, the critical time for therapy may be delayed, leading to irreversible organ damage and even death. Hence, there is a desperate need for effective and convenient non-compression treatments for DCS such as PFC. Because of its ability to increase the solubility of respiratory gases, PFC emulsions can reduce the risk and severity of DCS (Randsoe and Hyldegaard, 2009). As PFC can transport excess N_2 from tissues to the lungs, the formation of harmful N_2 gas emboli could be effectively prevented. Additionally, the improved O_2 delivery can attenuate tissue hypoxia in DCS patients, thus protecting them from hypoxia-induced injury. With improvements in the pharmaceutical properties of PFC emulsions, it is appreciable that they will be applied in the first aid of DCS in near future.

However, HBO_2 remains the most effective treatment for DCS-related injuries. Therefore, once available to DCS victims, HBO_2 should be immediately applied, although it is possible that, in the interim, PFC emulsions might be administered to dissolve and transport O_2. In this situation, the toxic effects of HBO_2 are likely to be exacerbated. It was found that after being given 6 ml/kg PFC, swine exhibited a higher incidence of seizures when subjected to HBO_2 treatment at 2.8 ATA (Mahon et al., 2006). However, little research has been conducted regarding the exact effects and prevention of PFC-facilitated CNS-OT.

CNS oxygen toxicity (CNS-OT) is one of the main concerns in HBO_2 therapy (Hampson and Atik, 2003). It is characterized by epileptic-like seizures (Beckman and Crittenden, 1981) and loss of consciousness. Although the mechanism of CNS-OT is poorly understood, it is generally accepted that increased levels of reactive oxygen species (ROS) and weakened antioxidant defenses may be the underlying etiology (Bitterman, 2004). Therefore, antioxidants, such as vitamin C and E, have been reported to improve cellular tolerance for CNS-OT in vitro (Kann et al., 1964; Zirkle et al., 1965) but are not as effective when given systemically in vivo (Bitterman, 2004).

Edaravone, a potent free-radical scavenger, has been shown to be neuroprotective in cerebral ischemia and approved for treatment of cerebral infarction (Watanabe et al., 1994). Moreover, recent researches have demonstrated that edaravone produced antiepileptogenic and neuroprotective effects in the rat hippocampus following pilocarpine-induced status epilepticus (Kamida et al., 2009a, 2009b). However, its potential in the prevention or treatment of hyperoxic seizure has hitherto not been explored.

The main purpose of this study was to explore the impact of PFC emulsion on CNS-OT and the preventive effects of edaravone on CNS-OT in the presence and absence of PFC emulsion.

2. Results

2.1. HBO_2-induced changes in the EEG latency of CNS-OT

An EEG characteristic of HBO_2-induced seizures in rats is shown in Fig. 1A. The typical pattern consists of three phases. The first phase (indicated by (a)) is the latency time to appearance of the FED, which is a transitory increase in the amplitude (b), followed by a series of electrical discharges (c). The latency to FED is a reliable indicator of O_2 convulsions (Arieli et al., 2005). In the present study, the latency to FED in HBO_2-treated rats was significantly shortened both immediately or 1 h—but not 3 h—after PFC administration ($P<0.01$) compared to that of the rats treated with saline. Conversely, the latency to FED in edaravone-treated rats was significantly prolonged by more than 60% ($P<0.01$). In rats treated with both PFC and edaravone, the latency was significantly greater than that of the PFC/0 h and Control rats ($P<0.05$) and comparable to that of the edaravone only group (Fig. 1B).

2.2. HBO_2-induced changes in oxidative biomarkers

A 12-min HBO_2 treatment at 6.0 ATA significantly increased the levels of MDA, NO and H_2O_2 in both cortex and hippocampus ($P<0.01$). These HBO_2-induced increases were potentiated after PFC administration ($P<0.05$ for MDA; $P<0.01$ for NO and H_2O_2). Edaravone reversed the effects of HBO_2 and PFC+HBO_2 on the production of MDA, NO and H_2O_2 ($P<0.01$) (Figs. 2 and 3).

2.3. HBO_2-induced changes in antioxidase activities

The CAT, GPx and SOD activities in the brain of saline-treated rats were all significantly impaired by the HBO_2 treatment ($P<0.05$ for GPx in the cortex; $P<0.01$ for CAT and SOD), and PFC further decreased these enzyme activities ($P<0.05$ for GPx; $P<0.01$ for CAT and SOD). Edaravone counteracted these effects of HBO_2 on CAT, GPx and SOD. In rats receiving PFC+HBO_2, edaravone restored SOD activity to normal levels (Fig. 4).

2.4. HBO_2-induced changes in NOS activities in the brain

The enzyme activities of total NOS (TNOS) and constitutive form of NOS (cNOS) in brain cortex and hippocampus increased after HBO exposure ($P<0.01$), and further increased in rats treated with HBO plus PFC ($P<0.01$). Edaravone significantly lowered these increased NOS activities by HBO_2 ($P<0.05$ in the hippocampus; $P<0.01$ in the cortex) and PFC ($P<0.01$) but not to within normal values. The activity of inducible form of NOS (iNOS) was also increased by HBO_2 exposure ($P<0.01$), but this was unaffected by treatment with either PFC or edaravone (Fig. 5).

3. Discussion

PFC emulsion is considered a promising treatment for DCS (Randsoe and Hyldegaard, 2009). However, its high efficiency of O_2 transmission increases the risk of CNS-OT when DCS victims also undergo HBO_2 treatment after PFC administration.