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Research report

1400 W ameliorates acute hypobaric hypoxia/reoxygenation-induced cognitive deficits by suppressing the induction of inducible nitric oxide synthase in rat cerebral cortex microglia

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HIGHLIGHTS

- 1400 W ameliorated spatial memory deficits caused by acute HH/R in rats.
- 1400 W inhibited iNOS overexpression in cerebral cortex microglia after acute HH/R.
- 1400 W reduced NO and MDA generation, 3-NT, and apoptosis after acute HH/R.
- 1400 W inhibited overexpression of iNOS and NO production by microglia after H/R.
- 1400 W reduced 3-NT, MDA production, and decreased apoptosis in microglia after H/R.

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ABSTRACT

Nitric oxide (NO) is involved in neuronal modifications, and overproduction of NO contributes to memory deficits after acute hypobaric hypoxia-reoxygenation. This study investigated the ability of the iNOS inhibitor 1400 W to counteract spatial memory deficits following acute hypobaric hypoxia-reoxygenation, and to affect expression of NOS, NO, 3-NT and MDA production, and apoptosis in rat cerebral cortex. We also used primary rat microglia to investigate the effect of 1400 W on expression of NOS, NO, 3-NT and MDA production, and apoptosis. Acute hypobaric hypoxia-reoxygenation impaired spatial memory, and was accompanied by activated microglia, increased iNOS expression, NO, 3-NT and MDA production, and neuronal cell apoptosis in rat cerebral cortex one day post-reoxygenation. 1400 W treatment inhibited iNOS expression without affecting nNOS or eNOS. 1400 W also reduced NO, 3-NT and MDA production, and prevented neuronal cell apoptosis in cerebral cortex, in addition to reversing spatial memory impairment after acute hypobaric hypoxia-reoxygenation. Hypoxia-reoxygenation activated primary microglia, and increased iNOS and nNOS expression, NO, 3-NT, and MDA production, and apoptosis. Treatment with 1400 W inhibited iNOS expression without affecting nNOS, reduced NO, 3-NT and MDA production, and prevented apoptosis in primary microglia. Based on the above findings, we concluded that the highly selective iNOS inhibitor 1400 W inhibited iNOS induction in microglial cells, and reduced generation of NO, thereby mitigating oxidative stress and neuronal cell apoptosis in the rat cerebral cortex, and improving the spatial memory dysfunction caused by acute hypobaric hypoxia-reoxygenation.

1. Introduction

A main feature of the natural environment of the plateau is low air pressure, with accompanying low air oxygen content. Most people entering the plateau elevation of 4000 m above sea level will show different degrees of acute high altitude reactions [1,2], and have differing degrees of cognitive and neuropsychological dysfunctions, including slow reflection, memory deficits, and attention deficit disorder [3–5]. Cognitive dysfunction caused by the high altitude plateau is also related to hypoxia-reoxygenation, which happens during mountaineering, plateau rescue and emergency, and oxygen inhalation therapy. In addition, hypoxia-reoxygenation occurs after return to normal elevations after a period on the plateau; however, the mechanism of cogni-

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tive dysfunction due to plateau-related hypoxia-reoxygenation is not well understood. Previous studies have confirmed that hypobaric hypoxia-reoxygenation can reduce the learning and memory capacity of the brain, which is related to the increase of free radical production and apoptosis or necrosis [6–9]. At the same time, there is evidence indicating that endogenous nitric oxide (NO) is directly correlated with cognitive dysfunction caused by hypobaric hypoxia-reoxygenation [10–14].

NO is a bioactive molecule and is an important messenger molecule in the brain [15]. NO plays a role in cognition, which may be related to synaptic plasticity of the hippocampus [16,17]. NO is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS) [18]. NOS has three subtypes; neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) are expressed under normal conditions, and inducible nitric oxide synthase (iNOS) is expressed after injury. Under anoxic conditions, because of the decrease of oxygen supply, the formation of NO is reduced because NOS requires oxygen [19]. Hypoxia-reoxygenation triggers a number of events, including release of glutamate and activation of free radical, Ca^{2+} influx, upregulated NOS expression, and increased NO generation. During hypoxia-reoxygenation, the activated microglial cell (especially the M1-type) is the main source of NO [20–23]. Activated microglia upregulate expression of iNOS through the p38/MAPK pathway [24] and the PI3-kinase/AKT/mTOR pathway [25]. With the increase in oxygen supply during reoxygenation, the level of NO increases via enhanced iNOS activity. This excessive NO is toxic to neurons through the combination of NO and oxygen free radical to produce peroxynitrite anion ($ONOO^-$). Because $ONOO^-$ is involved in cellular damage, energy exhaustion, and cell death [26–29], reoxygenation results in brain dysfunction including cognitive deficits.

Because excessive NO generated by hypoxia-reoxygenation is caused by iNOS activity, previous studies have investigated pharmacological strategies to prevent acute hypobaric hypoxia-reoxygenation damage by inhibiting iNOS activity and reducing NO production. Melatonin can suppress hippocampal iNOS, nNOS, and eNOS overexpression, with reduced NO production after acute hypobaric hypoxia-reoxygenation [12]; previous studies indicated that melatonin has an antioxidant and free radical scavenging effect [30]. However, the mechanism of how NOS inhibitors or melatonin prevent NOS expression has not been studied thoroughly. Other studies showed that aminoguanidine could prevent the expression of iNOS of cerebral cortex of rats caused by acute hypobaric hypoxia-reoxygenation, improving retrograde memory disorders [11]. However, aminoguanidine also has some selectivity over nNOS and eNOS, and whether nNOS and eNOS play a role in the hypoxia-reoxygenation process remains to be determined.

Based on previous research strategies, we aimed to determine the source of NO and the impact of a selective inhibitor on NOS expression and NO release through in vitro and in vivo experiments. We chose the highly selective iNOS inhibitor N-[3-(Aminomethyl)benzyl]-acetamidine Dihydrochloride (1400 W), which is more effective in preventing iNOS expression and activity than are eNOS and nNOS (5000 and 200 times higher respectively), and at a conventional dose, 1400 W has no effect on the biological activity of eNOS and nNOS [31–33]. In the present study, rats were pretreated with 1400 W before exposure to hypobaric hypoxia-reoxygenation to determine whether 1400 W could improve the spatial memory deficits typically seen after this hypoxic treatment, and whether 1400 W could inhibit iNOS overexpression in microglia cells, and the subsequent excessive NO levels causing oxidative stress and neuronal cell apoptosis in the rat cerebral cortex. We then utilized primary microglia in in vitro experiments to observe the effect of 1400 W pretreatment on the transcription and expression of NOS, the release of NO and the impact on oxidative stress and cell apoptosis after hypoxia-reoxygenation.

2. Materials and methods

2.1. Animal source and ethics statement

Adult Sprague–Dawley (SD) rats (200–250 g) were used for the in vivo experiments and for obtaining primary microglia. Rats were maintained in polypropylene cages with a 12 h light/12 h dark schedule and were provided with food and water ad libitum. Animals were housed at the Laboratory of Animals in the Medical Research Center, Southwest Hospital, Third Military Medical University. All experiments were carried out in accordance with the Provisions and General Recommendation of the Chinese Experimental Animals Administration Legislation, which were approved by the Animal Ethics Committee of Chongqing.

2.2. In vivo hypobaric hypoxia/reoxygenation (HH/R)

2.2.1. HH/R and drug treatment

Animals were randomly assigned to one of four experimental groups: vehicle-treated normoxia group, 1400 W-treated normoxia group, vehicle-treated hypoxia group, and 1400 W-treated hypoxia group. The 1400 W-treated groups were pretreated with ip injections of 1400 W (20 mg/kg, optimum dose; Code No. W4262, SIGMA-ALDRICH LLC) at 12 h intervals as previously described [34]. 1400 W was dissolved in sterile distilled water at a concentration of 20 mg/ml. Vehicle-treated groups were pretreated with ip injections of an equal volume of sterile distilled water. Two hours after administration of vehicle or 1400 W, normoxia groups were maintained in a normoxic environment while hypoxia groups were exposed to simulated hypobaric hypoxia (HH) and reoxygenation as previously described [11,12]. In brief, rats were exposed to simulated HH for 12 h at 8000 m (267 Torr) in an animal decompression chamber (Aviation Industry Corporation of China, China) with the temperature and humidity maintained at $22 \pm 2\,^{\circ}C$ and $30 \pm 5\%$, and animals were provided with food and water ad libitum. After 12 h of HH, the hypoxia groups were brought down to sea level. Subjects from each experimental group were assessed at 0, 1 or 3 days post-HH with behavioral experiments or by resection of the cerebral cortex for embedding in paraffin and preparing tissue homogenate. Treatment of all 1400 W treated animals was stopped prior to spatial memory retention trial or resection of the cerebral cortex.

2.2.2. Behavioral experiments

2.2.2.1. Morris Water Maze (MWM) test. Spatial memory was tested using a MWM [35]. The water maze apparatus consisted of a circular pool 150 cm in diameter, 60 cm deep, filled to the height of 30 cm with water (temperature 22–24 °C) to cover a platform (diameter 10 cm). The platform was submerged approximately 2 cm below the surface of the water, and was camouflaged by adding non-toxic white paint to the water. The rat's head was painted yellow using picric acid, and an overhead camera and computerized video imaging analysis system (Chengdu TME Technology Co., Ltd, China) were used to record the swimming paths of the marked rat in the maze.

Before the HH insult, rats were trained in the MWM for 7 days. The platform was always placed into the water in the center of the north quadrant; the pool wall from the nearest platform posted a visual cue [4]. Each rat was trained to find the platform with three trials a day from three different fixed locations at east, south, and west quadrants. On each trial, the rat was lowered gently into the water facing the pool wall at one of the three fixed locations, and allowed to find the hidden platform. In cases where the rat did not succeed in finding the platform within 60 s, it was placed onto the platform. Once on the platform, the rats were allowed to remain