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Hyperbaric oxygen therapy ameliorates pathophysiology of 3xTg-AD mouse model by attenuating neuroinflammation

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ABSTRACT

There is a real need for new interventions for Alzheimer's disease (AD). Hyperbaric oxygen therapy (HBOT), the medical administration of 100% oxygen at conditions greater than 1 atmosphere absolute, has been used successfully to treat several neurological conditions, but its effects on AD pathology have never been thoroughly examined. Therefore, we exposed old triple-transgenic (3xTg) and non-transgenic mice to HBOT followed by behavioral, histological, and biochemical analyses. HBOT attenuated neuroinflammatory processes by reducing astrogliosis, microgliosis, and the secretion of proinflammatory cytokines (IL-1β and TNFα) and increasing expression of scavenger receptor A, arginase1, and antiinflammatory cytokines (IL-4 and IL-10). Moreover, HBOT reduced hypoxia, amyloid burden, and tau phosphorylation in 3xTg mice and ameliorated their behavioral deficits. Therefore, we suggest that HBOT has multifaceted effects that reduce AD pathologies, even in old mice. Given that HBOT is used in the clinic to treat various indications, including neurological conditions, these results suggest HBOT as a novel therapeutic intervention for AD.

1. Introduction

Dementia is a common and growing problem, affecting 47.5 million people worldwide. Alzheimer's disease (AD), the most common form of dementia in the elderly, accounts for 60%–70% of cases. Globally, the number of people living with dementia is expected to escalate to 65.7 million by 2030 and 115.4 million by 2050, based on current rates of mortality and treatment availability (Prince et al., 2013). Despite many advances in our understanding of the pathophysiology of AD in the last decades, management is still mainly symptomatic, and there is a real need for new interventions.

The main pathological hallmarks characterizing AD are extracellular amyloid plaques, intracellular accumulation of neurofibrillary tangles, and progressive loss of synapses and neurons (Braak and Braak, 1997; Heinonen et al., 1995; Jellinger and Bancher, 1996). Another major component of AD is neuroinflammation. Microglia and astrocytes are key players in the inflammatory response and have been shown to be altered in postmortem brains of patients with AD and in animal models of the disorder (Hoozemans et al., 2006; Nazem et al., 2015). In early stages of the disease, the acute inflammatory response is thought to mitigate the clearance of amyloid plaques and restore tissue homeostasis. However, in advanced stages of AD, sustained activation of microglia and astrocytes (Rama Rao and Kielian, 2015; Villegas-Llerena et al., 2015) ultimately leads to chronic neuroinflammation. Excessive activation of glia due to persistent exposure to proinflammatory cytokines induces synapse loss and neuronal degeneration (Hickman et al., 2008; Iram et al., 2016; Kitazawa et al., 2011; Shi et al., 2011).

Hypoxia is intimately entwined in the pathogenesis of AD (Zhang and Le, 2010). Following hypoxic conditions, such as cerebral ischemia and stroke, the incidence of AD and vascular dementia is greatly increased (Altieri et al., 2004; Honig et al., 2005; Schneider et al., 2003). Reduced cerebral perfusion has been observed in preclinical and early stages of AD and correlates with both structural and functional deterioration as the disease progresses (Alsop et al., 2010; Binnewijzend et al., 2013; Chao et al., 2010; Chen et al., 2011; Roher et al., 2012; Thomas et al., 2015; Tosun et al., 2010). Cerebral hypoxia may arise due to hypoperfusion and has been shown to contribute to the accumulation of β-amyloid (Aβ), hyperphosphorylation of tau, dysfunction of the blood-brain barrier, and

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degeneration of neurons (Zhang and Le, 2010). Interestingly, hypoxia has been shown to activate microglia and astroglia and induce proinflammatory cytokine secretion, thus leading to neuronal death (Kaur et al., 2013; Lai and Todd, 2006; Teo et al., 2015; Tikka et al., 2001; Yrjanheikki et al., 1998, 1999). Therefore, reducing hypoxia and neuroinflammation might be the key to ameliorate AD pathophysiology.

Hyperbaric oxygen therapy (HBOT)—the administration of 100% oxygen at environmental pressure greater than 1 ATA—is utilized for a wide range of medical conditions in which oxygen is the rate-limiting factor for tissue recovery. This includes carbon monoxide poisoning, crush injuries, and decompression sickness (Gill and Bell, 2004; Thom, 2011). Recently, HBOT has emerged as a valuable treatment in neurological conditions (Boussi-Gross et al., 2015; Figueroa and Wright, 2015; Hadanny et al., 2015; Huang and Obenaus, 2011; Tal et al., 2015). The principal effect of HBOT is an increase in the solubility of plasma oxygen to a level sufficient to support tissues with minimal oxygen supply carried out by hemoglobin (Gill and Bell, 2004; Thom, 2011). Breathing oxygen under hyperbaric conditions has been shown to be a potent means of increasing arterial oxygen tension, as well as brain oxygen tension, thereby allowing a 6-fold increase in the amount of oxygen reaching the brain tissue (Calvert et al., 2007; Meirovithz et al., 2007; Tibbles and Edelsberg, 1996; van Hulst et al., 2003). Studies suggest that HBOT reduces intracranial pressure, improves survival, and promotes neurobehavioral recovery in traumatic brain injury patients, even years after the injury (Efrati et al., 2013; Huang and Obenaus, 2011; Lin et al., 2008; Rockswold et al., 2013; Sahni et al., 2012; Tal et al., 2015). In stroke patients, HBOT significantly improved neurological functions and life quality, even at chronic late stages after the occurrence of the stroke (Boussi-Gross et al., 2015; Efrati et al., 2013; Hadanny et al., 2015; Heneka et al., 2013). In traumatic brain injury and stroke animal models, HBOT exerted part of its beneficial effect by attenuating astrogliosis and microgliosis, suppressing proinflammatory cytokine secretion and increasing antiinflammatory cytokine secretion (Chen et al., 2014; Gunther et al., 2005; Harch, 2015; Lavrnja et al., 2015; Lim et al., 2013; Liu et al., 2013). As neuroinflammation is one of the hallmarks of AD, we examined whether HBOT can reduce it in a mouse model of AD.

Although HBOT has been shown to mitigate reduced blood flow, hypoxia, and neuroinflammation in a variety of brain disorders, its effects on AD pathology have never been thoroughly studied. We investigated the effects of HBOT on AD by exposing triple transgenic—AD (3xTg-AD) mice—a mouse model of AD—to HBOT and testing for behavioral, pathological, and biochemical changes due to the treatment. Our data suggest that HBOT ameliorates cognitive deficits; reduces the presence of hypoxia, amyloid load, and phosphorylated tau; and suppresses neuroinflammation.

2. Materials and methods

2.1. Mice

Triple-transgenic (3xTg) mice, harboring the $PS1_{M146V}$, $A\beta PP_{swe}$, and tau_{P301L} transgenes (kindly received from Prof. Frank M. LaFerla), and non-transgenic (non-Tg) C57BL/6 mice were used for this study. All animal experiments were performed in accordance with animal protocols approved by the Tel Aviv University Animal Care Committee. Every effort was made to relieve animal stress and minimize animal use. Tel Aviv University follows the international ARRIVE guiding principle for biomedical research involving animals, developed by the International Organizations of Medical Sciences. All animals received humane care as outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institute of Health.

2.2. Hyperbaric oxygen therapy

Male homozygous 3xTg mice (17-month old) and non-Tg C57BL/6 mice (14-month old) were assigned to 2 groups: HBO-treated and control non-treated mice. For the HBOT, animals were administered 100% oxygen at a pressure of 2 ATA in a custom-made monochamber intended for small animals for 60 minutes daily for 14 consecutive days ($n = 14$). Before compression was initiated, the monochamber was washed with 100% oxygen for 5 minutes to enrich oxygen content. Compression and decompression were performed gradually within 5 minutes. Oxygen level inside the chamber following compression reached saturation of $\geq$96%, as measured by an oxygen analyzer (320BRC model; Teledyne Analytical Instruments). The animals in the control, non-treated group, were placed inside the monochamber for 60 minutes without additional treatment (at 1 ATA; $n = 14$). Temperature, measured with a temperature controller (N322; Novus) during all sessions for HBO and control groups in the monochamber, was similar between groups (HBO: $21.64 + 0.1699$ °C, $n = 14$, control: $21.79 + 0.1710$ °C, $n = 14$, $p = 0.5201$).

2.3. Behavioral testing

The effects of HBOT on memory and behavior in mice were evaluated using a series of behavioral tests. Tests were performed during the 7 days preceding sacrifice with a 24-hour delay after the last HBOT/control treatment and a 48-hour delay after the last task to reduce stress.

2.3.1. Y-maze test

The test was performed in a symmetrical black Plexiglas Y maze with 3 arms (30 cm long × 10 cm wide × 15 cm high) set at 120° angles, designated entrance, familiar, and novel. The mice were placed in the distal end of the entrance arm and allowed to explore the maze for 10 minutes with only the familiar arm available to explore. After a 2-minutes delay, mice were reintroduced into the maze with 2 arms (familiar and novel) available to explore and documented for 2 minutes. The ratio of time spent or frequency of visits to the novel arm was calculated as time or frequency in the novel arm divided by the sum of time or frequency in both novel and familiar arms. The maze was cleaned with 40% ethanol between sessions. Arms were changed randomly between animals but kept similar for each animal.

2.3.2. Open-field test

Animals were placed in the center of an open field (40 cm × 40 cm × 30 cm), and exploration was assessed for 15 minutes. Cages were cleaned with ethanol following each session.

2.3.3. Novel object–recognition test

Mice were allowed to explore an empty arena (40 cm × 40 cm × 30 cm walls), and 24 hours later, 2 identical objects were added for a familiarization session of 10 minutes. Either 5 minutes (short-term memory test) or 24 hours (long-term memory test) later, the mice were reintroduced into the arena with one of the objects having been replaced by a novel one. The behavior of the mice was then monitored using the EthoVision XT 9 program for 5 minutes, and the time and number of visits to each of the objects were measured. The results are presented as the ratio of percentage of time spent