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Research Report

The effects of hyperbaric air and hyperbaric oxygen on blood–brain barrier integrity in rats

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ABSTRACT

Hyperbaric oxygen (HBO) treatment yields conflicting results on blood–brain barrier (BBB) integrity under various pathological conditions and the effects of HBO on healthy brain is poorly understood. In this experimental study, the effects of HBO on BBB integrity were investigated in comparison with hyperbaric air (HBA) in intact rats. Four sessions of HBA or HBO were applied to intact rats in 24 h. BBB integrity was functionally and structurally evaluated by determining extravasation of Evans blue (EB) dye and horseradish peroxidase (HRP) tracers. In immunohistochemical evaluation, relative staining intensity for occludin, a tight junction (TJ) protein, and aquaporin 4 (AQP4), a water-channel protein, was detected in the barrier type of microvessels of brain by image analysis. BBB permeability to EB dye significantly increased in animals in HBO treatment group compared to those in HBA and control groups ($p<0.05$). The immunoreactivity of occludin, a tight junction protein, remained essentially unaltered in capillaries of hippocampus in all groups. In animals exposed to HBO, AQP4 immunoreactivity significantly increased in parietal cortex compared to those in HBA and control groups ($p<0.01$). Ultrastructurally, frequent vesicles containing HRP reaction products were observed in capillary endothelial cells in cerebral cortex and hippocampus of rats subjected to both HBA and HBO. Our results indicate that the HBO administration to intact rats increased BBB permeability to both EB and HRP while HBA increased only HRP extravasation in these animals. The results of this study suggest that HBA also impairs the BBB integrity in intact rats as well as HBO.

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1. Introduction

Hyperbaric oxygen (HBO) has been used as a therapeutic option for various diseases such as air or gas embolism, carbon monoxide poisoning, decompression sickness, gas gangrene, acute traumatic ischemias, necrotizing soft tissue infections, acute thermal burn injuries, and delayed radiation injury (Al-Waili et al., 2005; Gesell, 2008; Lin et al., 2008). Although this treatment modality is used for these indications, it is very rare to use it in any human brain disease except intracranial abscesses (Kaide and Khandelwal, 2008). An experimental study suggested that HBO at 2.5 atmosphere absolute (ATA) and normobaric oxygen are able to attenuate hypoxia-induced changes in brain slices of rats, when compared to normobaric and hyperbaric room air (Günther et al., 2004). Moreover, a recent study reported that HBO and hyperbaric air (HBA) increased neuronal survival in gerbil brain after transient forebrain ischemia (Malek et al., 2013). HBO is still under discussion as an adjunctive treatment for several clinical indications such as global and focal brain ischemia, traumatic brain injury as well as carbon monoxide poisoning (Bennett et al., 2012; Buckley et al., 2011; Golden et al., 2002; Nighoghossian et al., 1995).

On the pre-clinical level HBO has been investigated as a treatment modality in various experimental models and has been shown to improve blood–brain barrier (BBB) integrity and neurological recovery by decreasing infarct size and behavioral deficit after cerebral ischemia (Chang et al., 2000; Mink and Dutka, 1995a; Ostrowski et al., 2010; Veltkamp et al., 2005,2010), brain injury and brain edema (Jadhav et al., 2010; Palzur et al., 2008). In one of the early studies, HBO appeared to decrease the mortality and morbidity rate in dogs, providing a protective effect against experimentally produced cerebral edema (Sukoff et al., 1968). It is also reported that HBO preconditioning prevented the increase in the permeability of BBB and brain edema caused by hypoxia exposure in mice (Peng et al., 2008).

The BBB which is mainly formed by endothelial cells maintains the neuronal homeostasis under physiological conditions and alterations of the BBB integrity contribute to the loss of neuronal homeostasis leading to secondary pathophysiological process within the brain (Abbott et al., 2010; Zlokovic, 2008). In addition, pericytes, astrocytes, microglia, neurons and the extracellular matrix constitute a "neurovascular unit" together with barrier type of endothelium supporting the BBB integrity that is essential for the function of the brain (Bechmann et al., 2007; Zhang et al., 2012; Zlokovic, 2008). In many clinical situations the patients undergo HBO for certain indications, whereas their brain function is normal. In these cases, it is not known whether and to what extent BBB integrity is altered. Limited number of studies examining the influence of HBO on BBB permeability in intact animals have reported contradictory results; some claimed no alteration in BBB integrity (Grunenau et al., 1981) whereas others described increased BBB permeability (Avtan et al., 2011; Lanse et al., 1978). On the other hand, normobaric hyperoxia, when administered during 2 h of focal cerebral ischemia and 1 h of reperfusion is found to be neuroprotective and did not change the BBB permeability to Evan's blue (EB) dye (Singhal et al., 2002). The loss of endothelial integrity could be an important mechanism for the observed decrease in BBB integrity during HBO, and one cannot say if the BBB impairment during HBO is due to hyperoxia or hyperbaric condition itself.

However, it remains to be elucidated whether BBB alterations—as addressed by the permeability behavior of dyes—during hyperoxia are caused by an increased percentage of oxygen under hyperbaric conditions or by the increased ambient pressure itself. In the present study, we compared the effects of HBO (100% O_2 at 2.5 ATA) and HBA (~21% O_2 at 2.5 ATA) on the functional and structural properties of BBB in an intact rat model.

2. Results

The EB dye content in left and right cerebral cortex and diencephalon regions was markedly increased in HBO-treated rats compared with those in HBA and control groups ($p<0.01$; Fig. 1). The EB dye content in brain regions of rats in HBA group did not show a statistical significant difference compared with control animals.

To address structural constituents of the BBB, immunostaining for occludin, a tight junction (TJ) protein, has been performed, while immunostaining for aquaporin 4 (AQP4), a water-channel protein, has been performed to provide a further mechanistic explanation to alterations in BBB integrity. The immunostaining for occludin and the relative intensity of occludin immunoreactivity as assessed by image analysis remained essentially unchanged in the brain capillaries in hippocampus of rats in HBA group compared to controls, while an increase, though statistically not significant, was noted in HBO-treated rats (Fig. 2A–D). The observation of the pattern of immunostaining for AQP4 in experimental groups is shown in Fig. 3A–C. AQP4 immunostaining was stronger in the capillary wall in hippocampus of rats exposed to HBO (Fig. 3C) and the relative intensity of AQP4 immunoreactivity increased significantly in animals

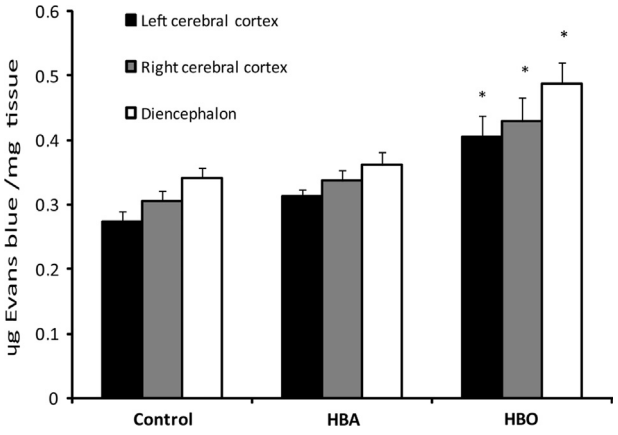

Fig. 1 – EB dye content in the brain regions of animals in experimental groups. Note the significantly increased BBB permeability to EB dye in HBO treated rats. Data are shown as means ± S.E.M. *$p<0.01$ versus other groups.