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BRAIN RESEARCH

Research Report

Therapeutic effects of hyperbaric oxygen in a rat model of endothelin-1-induced focal cerebral ischemia

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ABSTRACT

It has been established that hyperbaric oxygen (HBO) treatment reduces brain edema, decreases infarct volume, contributes to neurological functional recovery and suppresses apoptosis in suture-induced focal cerebral ischemic animal models. In the present study, we evaluated the therapeutic effect of HBO in an endothelin-1-induced focal cerebral ischemia in rats and explored the associated mechanisms of HBO-induced brain protection. One hundred twenty male Sprague–Dawley rats (280 to 320 g) were randomly assigned to sham, focal cerebral ischemia and focal cerebral ischemia treated with HBO groups. Brain water content, neurological function, morphology and molecular biological markers were assessed. HBO (100% O_2, 2.5 atmosphere absolute for 2 h) was initiated at 1 h after focal cerebral ischemia. Rats were killed at 24 h to harvest tissues for Western blot or for histology. In HBO-treated animals, an enhanced ratio of Bcl-2 and Bax and a reduced expression of hypoxia-inducible factor-1α (HIF-1α) in the hippocampus after focal cerebral ischemia were observed. These results indicate that HBO provides brain protection that is probably associated with the inhibition of HIF-1α and the elevation of Bcl-2.

1. Introduction

Since Koizumi et al. (1986) reported the method of middle cerebral artery occlusion by a suture in 1986, most researchers have produced the models of focal cerebral ischemia by filament intraluminal occlusion of the middle cerebral artery (Macrae, 1992; Kuge et al., 1995; Longa et al., 1989; Kawamura et al., 1991). Although this method does not require opening of the skull and has good reproducibility, the suture may directly injure the endothelium. In this study, we used endothelin-1 (ET-1) to induce focal cerebral ischemia, which was first reported by Sharkey et al. (1993). This method is advantageous because the manipulation is simple and tissue damage is limited. This method does not produce surgical complications and therefore decreases surgery-related brain injuries, especially those related to neurological dysfunctions. This model is

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Abbreviations: ANOVA, analysis of variance; ATA, atmosphere absolute; ET, endothelin; ET-1, endothelin-1; HIF-1α, hypoxia-inducible factor-1α; HBO, hyperbaric oxygen; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; PBS, phosphate-buffered saline; PDVF, polyvinylidene; BNIP3, Bcl-2/adenovirus E1B 19-kDa protein-interacting protein 3

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closer to mimicking human stroke (Biernaskie et al., 2001) in that it induces stroke when animals are conscious (Sharkey et al., 1993).

The aim of the study was to evaluate the therapeutic effects of HBO on focal cerebral ischemia ET-1 induced. In our study we found HBO treatment can lessen brain edema, decrease infarct volume, contribute to neurologic function recovery and suppress apoptosis. Using this more clinically relevant model, we also revisited hyperbaric oxygen (HBO)-induced brain protection, especially in the areas of apoptosis and hypoxia-inducible factor-1α (HIF-1α). We studied two apoptotic factors Bax and Bcl-2; the former induces the release of cytochrome c (Gogvadze et al., 2001; Sugawara et al., 2004) and the latter prevents the formation of Bax homodimers (Reed et al., 1996), which inhibits the cytosolic accumulation of cytochrome c and caspase-3 activation (Zhao et al., 2003). We also studied the expression of HIF-1α because HIF-1α increases after focal cerebral ischemia (Bergeron et al., 1999) and because HIF-1α induces apoptosis (Carmeliet et al., 1998; Moritz et al., 2002) by the activation of BNIP3 (Ostrowski et al., 2005; Bruick, 2000; Greijer and van der Wall, 2004; Boyd, 1994) or the stabilization of p53 (Greijer and van der Wall, 2004; Chen et al., 2003).

2. Results

2.1. Neurological score

The neurological score was 1.83±0.58 in the sham group versus the minimum of 1 that is obtainable. A significant increase in neurological score (9±0.93) was found in animals 24 h after focal cerebral ischemia (Fig. 1A). Treatment with HBO alleviated the increase in neurological score, even though the level calculated in this group (5.76±0.89) was significantly higher than the control value ($P<0.01$ versus control, ANOVA).

The body weight variance is shown in Fig. 1B. After 24 h, the mean body weight decreased 10.75±3.58 g in the ET group and 8.00±3.77 g in the ET+HBO group ($P<0.05$).

2.2. Brain water content

There was no significant difference in water content of the right hemisphere, brain stem and cerebellum between the three groups. Mean brain water content values of the left hemispheres were 78.76±0.27% in the sham group, 80.08±0.67% in the ET group and 79.27±0.22% in the ET+HBO group (Fig. 2A). The left hemisphere water content of the ET group was significantly higher than the sham ($P<0.01$, ANOVA) and ET+HBO groups ($P<0.05$). There was no obvious difference in brain water content between the sham and ET+HBO groups ($P>0.05$).

2.3. TTC

Infarct volumes derived from postmortem TTC staining at 24 h (Fig. 2C) were 13.34%±2.89% (ET, $n=7$) vs. 5.39%±2.21% (ET+HBO, $n=8$) ($P<0.01$). No cerebral infarction was observed in the sham group ($n=8$). Six sections were cut for each brain. Section 1 represented the most anterior area and section 6 represented the most posterior area. Decreased infarction occurred primarily in sections 1, 2, 3 and 4 (data not shown).

2.4. Morphology and Nissl staining

Fig. 3 demonstrates Nissl staining of the cortex (A1–C1) and CA1 sector (A2–C2) in rats at 24 h after focal cerebral ischemia. There were extensive neuronal changes in the CA1 sector of the hippocampus and cortex (Figs. 3B1, B2). Considerable dark, pyknotic neurons were shown. HBO (2.5 ATA, 2 h) applied at 1 h after focal cerebral ischemia reduced the deletion of neuron structure and retained the number of cells in the CA1 and the cortex (C2, C1). All the figures were obtained from the left hemisphere.

2.5. TUNEL staining

The fragmentation of nuclear DNA in cells has been identified extensively with TUNEL staining (Banasiak et al., 2000; Johnston et al., 2001). No detectable TUNEL-positive cells were found in the sham-operated animals (Fig. 4A). In samples collected from the ET group, the damaged cells were characterized by a round and shrunken morphology. The processus disappeared and the neuronal body became rounded with strong TUNEL staining in the nucleus. At higher magnification, the nuclei of cells were clearly stained in the cortex (Fig. 4B). After HBO treatment, the number of positive cells observed in the cortex had decreased dramatically by 24 h (Fig. 4C).

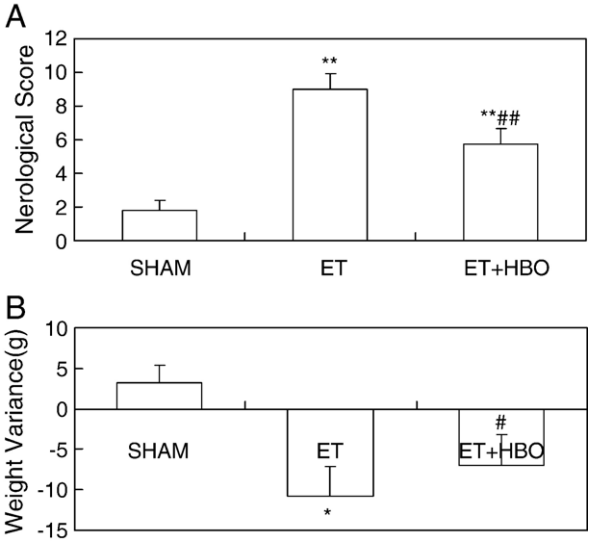

Fig. 1 – (A) The bar graph shows the neurological scores of animals at 23 h after focal cerebral ischemia. The neurological score was profoundly increased in the endothelin (ET) group (9±0.93; $n=26$, $P<0.01$ vs. sham). HBO alleviated the neurological scores (5.76±0.89; $n=26$. $P<0.01$ vs. ET groups) although they remained higher as compared with sham values (1.83±0.58; $n=26$, $P<0.01$ vs. sham). (B) Body weight sharply reduced after focal cerebral ischemia ($P<0.05$ vs. sham), while HBO partially relieved the decrease ($P<0.05$ vs. ET and sham).