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Reduced apoptosis by combining normobaric oxygenation with ethanol in transient ischemic stroke

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ABSTRACT

Background and purpose: The effect of normobaric oxygen (NBO) on apoptosis remains controversial. The present study evaluated the effect of NBO on ischemia-induced apoptosis and assessed the potential for improved outcomes by combining NBO administration with another neuroprotective agent, ethanol, in a rat stroke model.

Methods: Rats were subjected to right middle cerebral artery occlusion (MCAO) for 2 h. At the onset of reperfusion, ischemic animals received either NBO (2 h duration), an intraperitoneal injection of ethanol (1.0 g/kg), or both NBO and ethanol. Extent of brain injury was determined by infarct volume, neurological deficit, and apoptotic cell death. Expression of pro- and anti-apoptotic proteins was evaluated through Western immunoblotting.

Results: Given alone, NBO and ethanol each slightly ($p<0.05$) reduced infarct volume to 38% and 37%, respectively, as compared to the impressive reduction of 51% ($p<0.01$) seen with combined NBO–ethanol administration. Neurologic deficits were also significantly reduced by 48% with combined NBO–ethanol therapy, as compared to lesser reductions of 24% and 23% with NBO or ethanol, respectively. Combined NBO–ethanol therapy decreased apoptotic cell death by 49%, as compared to 31% with NBO and 30% with ethanol. Similarly, combination therapy significantly increased expression of anti-apoptotic factors (Bcl-2 and Bcl-xL) and significantly reduced expression of pro-apoptotic proteins (BAX, Caspase-3, and AIF), as compared to the minimal or nil protein expression changes elicited by NBO or ethanol alone.

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Conclusions: In rats subjected to ischemic stroke, NBO administration salvages ischemic brain tissue through evidenced decrease in apoptotic cell death. Combined NBO therapy with ethanol administration greatly improves both degree of neuroprotection and associated apoptosis.

1. Introduction

Apoptosis plays a crucial role in propagating the spread of neuronal cell loss after ischemic stroke. Despite advances that have unveiled the cascade of molecular mechanisms underlying ischemia-induced apoptosis, no effective anti-apoptotic stroke therapies have emerged. Oxygen therapy, that aims to increase oxygen delivery to hypoxic tissue, has long been considered a logical candidate for neuroprotective treatment of ischemic stroke. Accordingly, oxygen treatment, including normobaric (NBO) and hyperbaric oxygenation (HBO), has been evaluated as a therapeutic agent in a variety of experimental studies of ischemic stroke (Michalski et al., 2011; Singhal et al., 2002). However, the clinical relevance of oxygen therapy in mitigating apoptosis remains controversial due to its range of variable effects—with some studies indicating that normobaric hyperoxia induces apoptosis (Jiang et al., 2012; Stuhr et al., 2007), while other studies demonstrate that NBO suppresses apoptosis (Foadoddini et al., 2011; Lu et al., 2012).

In previous studies, neuroprotection has been consistently evidenced by ethanol through reduced sequelae of brain infarction with improved functional outcomes in a rat ischemic stroke model (Wang et al., 2012). Further, in a brain slice model with hypoxia followed by re-oxygenation, we demonstrated that ethanol confers neuroprotection by regulating apoptotic proteins (Yuan et al., 2012). When we previously assessed the dose-response relationship of ethanol administered after middle cerebral artery occlusion (MCAO), it was determined that ethanol at 1.5 g/kg provided the strongest neuroprotection (Wang et al., 2012). In order to explore the beneficial effects of ethanol therapy, a goal of the present study was to employ a combination therapy approach by assessing the effects of administering ethanol with oxygen. Combination therapy may demonstrate profound translational therapeutic potential by allowing the use of lower dosages of agents than when used alone. As such, multiple neuroprotective pathways are triggered and/or related pathways may be synergistically affected, resulting in complementary neuroprotection with improved tolerability (Lo, 2008). As a result, it was determined that NBO administration for 2 h would be appropriate to assess the impact of combination therapy, because it has not been shown to induce significant neuroprotection on its own (Fujiwara et al., 2011). The same rationale was used to select the dosage of ethanol at 1.0 g/kg rather than the higher, optimal neuroprotective dose of 1.5 g/kg determined from our dose–response study (Wang et al., 2012). Thus, this study investigated the potential for enhanced neuroprotection by way of apoptotic modulation through administering combination therapy of short-duration NBO (2 h) and low-dose ethanol (1.0 g/kg). Specifically, we asked how combined NBO–ethanol therapy affects brain damage after ischemia–reperfusion injury and to what extent the apoptotic pathway is mechanistically involved in their combined versus individually-derived effects.

The molecular underpinnings of apoptotic cell death following cerebral ischemia are well established. During periods of reduced oxygen delivery, pro-apoptotic proteins, such as Caspase-3, BAX, and apoptosis-inducing factor (AIF) become up-regulated and are one of the major causes of neuronal breakup during ischemia-reperfusion injury (Wu et al., 2003). Conversely, Bcl-2 and Bcl-xL are examples of anti-apoptotic proteins that play a critical role in cellular survival. Bcl-2 and its relative, Bcl-xL, are oncogene-derived proteins that function as repressors of cell death (Korsmeyer, 1995). Thus, the effects of combined NBO–ethanol therapy on apoptotic cell death and associated apoptotic proteins were determined in the present study.

2. Results

2.1. Physiological parameters

There were no significant differences in blood pH, $PaCO_2$, and MAP among all groups. NBO treatment elevated PaO_2 levels to approximately 400 mmHg at the conclusion of treatment for 2 h ($p<0.01$ as compared to sham-operation and ethanol groups). As part of the stress response, blood glucose significantly increased during MCAO in all stroke groups ($p<0.01$ as compared to before MCAO), but there was no significant difference among stroke groups. Body temperature remained at approximately 37 °C.

2.2. Infarct volume and neurological deficit

After 48 h of reperfusion, both the NBO-only and ethanol-only groups demonstrated a small decrease ($F_{[3,\ 28]}=12$, $p<0.05$) in infarct volume from 51.4±4.0% in the stroke without treatment group to 38.2±3.6% or 36.9±4.1%, respectively (Fig. 1). However, this decrease was significantly ($p<0.01$) enhanced by combination therapy of NBO and ethanol (19.7±3.1%). A similar pattern of neuroprotection was observed in functional outcome (Fig. 1). A significantly high neurological deficit score (8.4±0.7) was observed in the non-treatment group after 48 h of reperfusion ($F_{[3,\ 28]}=8.1$, $p<0.01$). Mildly reduced scores were observed in both the NBO (6.4±0.6) and ethanol (6.5±0.7) monotherapy groups ($p<0.05$).Yet, the NBO–ethanol combination therapy group exhibited the greatest reduction (4.4±0.3) in neurological deficits ($p<0.01$).