Focused Review

**Hyperbaric oxygen preconditioning reduces ischemia–reperfusion injury by stimulating autophagy in neurocyte**

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**ABSTRACT**

Cerebral ischemia–reperfusion injury (IRI) is a complex process resulting in cellular damage and death. Many studies have reported that an ischemic preconditioning could induce protection against ischemic insult. However, the safety concerns and practical feasibility have limited the application of ischemia preconditioning in practice. Subsequently, a number of substances including endotoxin and cytokines etc. have proven effective in inducing ischemic tolerance in the neurocyte. Unfortunately, the application of these substances to the clinical practice of neurosurgery still remains questionable for their toxicity or side effects. Therefore, a novel therapy to protect against cerebral IRI requires further study. Several recent studies confirmed that repeated hyperbaric oxygen preconditioning (HBO-PC) prior to cerebral ischemia or spinal cord ischemia can provide neuroprotection. HBO as a therapeutic measure has been widely accepted for its convenience and safety. However, information about the mechanism of how this neuroprotection works is still very limited. We hypothesize that autophagy induction is involved in HBO-PC induced neuroprotection on IRI in neurocyte. The hypothesis reveals that autophagy may be a new therapeutic target for cerebral IRI.

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**KEYWORDS:** Hyperbaric oxygen preconditioning, Ischemia–reperfusion, Brain injury, Autophagy, Reactive oxygen species

1. **Introduction**

Cerebral ischemia–reperfusion injury (IRI) is a complex process resulting in cellular damage and death. Neuroprotection has been a major theme in neurosurgery for decades. Since Kitagawa et al. (1990) first reported that neurocyte developed resistance to brain injury against a subsequent lethal ischemia after a brief period of sublethal ischemia, cerebral ischemic tolerance has been widely investigated. This protective effect, which named as ischemic preconditioning or ischemic tolerance, has attracted rapidly the interest of clinical and basic neuroscientists for the hope of leading to new therapy of ischemic neuronal damage. However, the method of exposure to brief periods of reversible ischemia prior to ischemic event cannot be used in clinical operation for not knowing the exact periods of ischemia preconditioning. Despite a number of substances such as endotoxin (Tasaki et al., 1997), cytokines (Nawashiro et al., 1997) and potassium...
chloride (Yanamoto et al., 1998) which have proven effective in inducing ischemic tolerance in the brain, the application to the clinical practice of neurosurgery still remains questionable for their toxicity or side effects (Dong et al., 2002). Therefore, a new substance or treatment which is convenient and atoxic should be developed.

Hyperbaric oxygen (HBO) is attractive because it has already been used safely for various disorders such as treating carbon monoxide poisoning and air embolism. One of the earliest studies to examine the effects of HBO on IRI was conducted by Sterling et al. (1993). Furthermore, several recent studies reported that repeated HBO preconditioning prior to cerebral ischemia (Wada et al., 2001) or spinal cord ischemia (Nie et al., 2006) provide neuroprotection. Although upregulation of catalase, HSP72 and production of free oxygen radicals might play some roles in HBO preconditioning inducing ischemic tolerance, information about the mechanism of how this neuroprotection works is still very limited (Li et al., 2008; Dennog et al., 1999).

2. The hypothesis

Autophagy is a highly conserved regulated process of degrading long-lived proteins and organelles, participating in organelle turnover and in the bioenergetic management of starvation or other damaging stimuli (Yoshimori, 2004). Although apoptosis and necrosis are frequently analyzed in focal cerebral ischemia (FCI), cases of autophagic cell death known as type II programmed cell death are relatively few. Despite various functions of autophagy have been studied, the role of enhanced autophagy acting as a pro-survival or pro-death still remains controversial. We hypothesize that HBO preconditioning provides neuroprotection against cerebral IRI partly by stimulating autophagy. The hypothesis reveals that autography may be a new therapeutic target for cerebral IRI. We postulate that it might be an effective treatment for cerebral IRI by the use of stimulation of autophagy.

3. Theoretical fundamental of the hypothesis

Autophagy is another potential form of cell death that can be seen under a variety of adverse environments depending on different physiological and pathological conditions. It is reported that activation of autophagy mediated by AMP-activated protein kinase represents a potential protective mechanism in the early stage of the brain injury (Carloni et al., 2008). Because ischemia induces lack of essential nutrients, it is not surprising that autophagic pathways are rapidly activated after FCI to promote cell survival by degrading the toxic metabolites. However, the autophagy, which should be downregulated for the enough blood resupply, is also activated in the stage of reperfusion. Matsui et al. (2007) reported that activation of autophagy induced by reperfusion is mediated by Beclin-1, a protein required for autophagy, which is different from that in the stage of ischemia. Autophagy may be involved in the mechanisms of cell death through excessive self-digestion and degradation of essential cellular constituents in this stage (Reggiori and Klionsky, 2002).

Although hypoxic preconditioning (HPC) and autophagy are proven to be enhanced by mild hypoxic insults, the association between autophagy and HBO preconditioning remains unclear. Since HPC and HBO pretreatment share several common features (John et al., 2006), we hypothesize that autophagy is also activated in HBO preconditioning. Reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻) and/or hydroxyl radical (OH⁻), were initially thought to play a crucial role in the development of many neurological disorders and brain dysfunctions, including the pathogenesis of cerebral ischemia–reperfusion injury. In recent years, however, this view of ROS being detrimental in the process of IR injury was challenged. Recent studies showed that the endogenous ROS acted as a neuroprotective role in several in vitro models involving ischemic preconditioning. Accumulated evidence showed that HBO preconditioning increased ROS generation (Benedetti et al., 2004; Conconi et al., 2003) and the HBO-induced tolerance can be attenuated by the administration of the oxygen free radical scavenger (Xiong et al., 2001) or the antioxidant enzyme (Nie et al., 2006). These results imply that the generation of a nonlethal level of ROS may be involved in the mechanism of protective effect produced by HBO preconditioning. Despite numerous reports about this, the manner of how ROS causes neuroprotection is poorly characterized. It is reported that ROS generated in HBO preconditioning triggers the cascade in cellular events leading to activation of endogenous antioxidant enzyme, which scavenge the excessive accumulation of ROS and protect neurocyte from ischemia–reperfusion injury. In addition to

Fig. 1 – Hyperbaric oxygen (HBO) preconditioning-induced neuroprotection due to the generation of ROS activated autophagy.
the upregulation of activities of antioxidant enzyme, ROS also induces the nitric oxide synthase (NOS) (Wang et al., 2009) and heat shock protein (HSP) during HBO treatment, which may lead to neuroprotection during the actual insult of ischemia and reperfusion. Several studies have indicated that the ROS generated in mitochondria may be involved in the induction of autophagy, leading to either survival or cell death, depending on different circumstance and the level of ROS. Recent findings show that ROS also regulate starvation-induced autophagy, which is clearly a survival mechanism, partly through class III phosphoinositide 3-kinase pathway (Scherz-Shouval et al., 2007). Based on the results above, we postulate that autophagy can be activated by ROS and may play a neuroprotection role in HBO preconditioning, which also enhances the level of ROS (Fig. 1). These findings imply that autophagy might provide a means of neuroprotection against cerebral ischemia–reperfusion injury.

Next, we design a series of experiments to test our hypotheses. Rabbits will be randomly assigned to one of three groups: sham, middle cerebral artery occlusion (MCAO) for 120 min, and HBO preconditioning before MCAO groups. We can evaluate our hypotheses by determining the protein level and distribution of Beclin-1 and microtubule-associated protein II light chain 3 (LC3-II) which were previously found to partly through class III phosphoinositide 3-kinase pathway in rabbits. The hypotheses include the generation of ROS is involved in the induction of autophagy, leading to either survival or cell death, depending on different circumstance and the level of ROS. We can evaluate and compare the degree of neurocyte injury by regulation of autophagy, we examine the changes of autop-

Next, we design a series of experiments to test our hypotheses. Rabbits will be randomly assigned to one of three groups: sham, middle cerebral artery occlusion (MCAO) for 120 min, and HBO preconditioning before MCAO groups. We can evaluate our hypotheses by determining the protein level and distribution of Beclin-1 and microtubule-associated protein II light chain 3 (LC3-II) which were previously found to promote autophagy in rabbits of three groups, respectively. To investigate whether the generation of ROS is involved in the regulation of autophagy, we examine the changes of autophagy by addition of ROS scavenger. Furthermore, we can evaluate and compare the degree of neurocyte injury by addition of autophagy inhibitors such as 3-methyladenine, rapamycin and wortmannin in three groups. With the experiments above, we can examine whether autophagy is involved in mechanisms of neuroprotection in IRI after pretreatment with HBO.

Acknowledgments

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References


