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Research Report

Preconditioning with prolonged normobaric hyperoxia induces ischemic tolerance partly by upregulation of antioxidant enzymes in rat brain tissue

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ABSTRACT

Recent studies suggest that normobaric hyperoxia (HO) results in brain ischemic tolerance (BIT), reducing ischemic brain injury. We have attempted to determine the time course of HO-induced upregulation of antioxidant enzymes. Rodents comprised five groups, breathing room air (RA; O_2=21%), or 95% oxygen (hyperoxia, HO) for 4, 8, 16, and 24 h (RA, 4HO, 8HO, 16HO, 24HO respectively) in the same chamber. Each main group was subdivided to MCAO-operated (middle cerebral artery occlusion), and intact (without any surgery) subgroups. After 24 h, MCAO-operated subgroups were subjected to 60 min of right MCAO. After 24 h reperfusion, neurologic deficit score (NDS), mortality rate, and infarct volume were measured in MCAO-operated subgroups. 48 h after pretreatment, antioxidant enzymes activities were assessed in MCAO-operated, sham-operated, and intact subgroups. Preconditioning with 16HO and 24HO decreased NDS, mortality rate, infarct volume, and increased antioxidant enzymes activities (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) significantly. Although further studies are needed to clarify the mechanisms of ischemic tolerance, the prolonged HO seems to partly exert their effects via increase in antioxidant enzymes activities.

1. Introduction

Noxious stimuli applied at doses close to but below the threshold of cell injury induce adaptive responses that protect the brain against additional stress from the same (tolerance) or other (cross-tolerance) stimuli. Ischemic tolerance (IT) is an endogenous phenomenon in which brief periods of ischemia render a tissue more resistant to subsequent severe ischemic injury (Romera et al., 2004). This phenomenon (ischemic preconditioning, IPC) has been demonstrated in a variety of organs including the brain (Kitagawa et al., 1990). IPC is clearly an attractive target for therapeutic development, and can be induced by means other than simple ischemia, such as exposure to diverse pharmacological agents, changes in inspired oxygen tension, and lipopolysaccharide-induced low-grade inflammation (Valen, 2003). Specifically, hypoxia (Gidday et al., 1994), ischemia (Kitagawa et al., 1990), anoxia (Perez-Pinzon et al., 1996), oxidative stress (Ohtsuki et al., 1992), inhibitors of oxidative phosphorylation (Riepe et al., 1997), and normobaric hyperoxia (Bigdeli et al., 2007) induce brain ischemic tolerance (BIT).

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Recent studies show that BIT is mediated by the synthesis of proteins which promote neuronal survival, including heat shock protein 70 (Warner et al., 2004), Bcl-2 (Shimazaki et al., 1994), glutamate transporters (Bigdeli et al., in press-a; Pradillo et al., 2006), superoxide dismutase (SOD) (Bigdeli et al., in press-b; Toyoda et al., 1997), antiapoptotic factors (Shimazaki et al., 1994), reactive oxygen species (Ravati et al., 2001), NF-κB and proinflammatory cytokines (Bigdeli et al., 2008).

Antioxidant enzymes activities can be stimulated by various modalities of cellular stress such as mild ischemia-reperfusion (Das et al., 1994), and hyperbaric oxygenation (Oh et al., 1997) were reported to increase antioxidant enzyme activity in experimental animals. Among these, mild ischemic stress was shown to enhance mRNA expression of several genes, including the catalase gene, which was significantly increased as early as 30 min after the ischemic stress (Das et al., 1994). On the other hand, in some of these experiments, it was also observed that the activation of antioxidant enzymes is accompanied by the suppression of ischemia/reperfusion injuries, showing that the increased antioxidant enzyme capacity actually induces the tolerance to oxidative insults (Kim et al., 2002).

One of the manifestations of central nervous system (CNS) damage after cerebral ischemia is the formation of brain edema caused by the breakdown of the blood brain barrier (BBB) that is improved by normobaric hyperoxia preconditioning (Bigdeli et al., 2007). SOD prevents vasogenic brain edema after several kinds of injuries (Kinouchi et al., 1991), suggesting that O_2^- is an important factor for disruption. Another manifestation of CNS damage is the direct injury of neural cells including excitatory events that are induced by glutamate release after cerebral ischemia that is improved by normobaric hyperoxia preconditioning via upregulation of glutamate transporters (Bigdeli et al., 2008). Glutamate elevates free calcium (Ca^{2+}), which activates Ca^{2+}-dependent enzymes and leads to free radical production (Orrenius et al., 1992). Resent studies suggest that excitotoxic injury causes apoptotic neuronal cell death in some neuronal subpopulations (Ankarcrona et al., 1995). Recently, it has been also shown that antioxidant enzymes inhibit apoptotic neuronal cell death, suggesting the possibility that oxygen-free radical may modulate neuronal apoptosis. Therefore, apoptotic neuronal cell death may play an important role in focal cerebral ischemia injury (Linnik et al., 1993). Therefore, there have been some attempts to enhance endogenous antioxidant system to prevent oxidative damage.

In our laboratory, we have recently shown that pretreatment with intermittent and prolonged HO induces IT and upregulates glutamate transporters, serum TNF-α levels, and TNF-α converting enzyme (TACE) in the rat brain (Bigdeli and Khoshbaten, 2008; Bigdeli et al., 2008) and confer different degrees of neuroprotection in the rat brain. Intermittent HO also reduces brain edema and Evans Blue (EB) extravasation significantly (Bigdeli et al., 2007).

This study was designed to (i) explore the necessary duration of HO to induce upregulation antioxidants enzymes and (ii) to explore the association of such BIT with changes in antioxidants enzymes activities. Such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPOX).

2. Results

2.1. *Experimental conditions parameters*

The oxygen concentration inside the container was continuously monitored. Arterial blood gas analysis confirmed clinical hyperoxia in the pretreated groups (Table 1). Oxygen concentration was maintained at 95% and 21% for HO and RA conditions, respectively. Cerebral blood flow was reduced to less than 24% of base line in each group (Fig. 1).

2.2. *Effects of various durations of HO on mortality rate and neurologic deficit scores*

16HO and 24HO decreased mortality from 26.3% in MCAO-RA to 16.9 and 13.4% in MCAO-16HO and MCAO-24HO groups, respectively (Fig. 2). Animals which were subjected to sham surgery showed no neurological deficits. Median neurologic deficit scores (NDS) were reduced by hyperoxic exposure, and significantly so when MCAO-16HO, MCAO-24HO were compared to their controls (Table 2). Those with no deficit all showed EB extravasation, confirming the fact that focal cerebral ischemia had been induced. The putative beneficial effects of HO were confirmed by a reduction in infarct volume not seen in RA (Fig. 3). The neuroprotection exerted by HO was mainly seen in the penumbra (cortex).

2.3. *Effects of various durations of HO on infarct volume*

Infarct volume was not reduced in MCAO-4HO, MCAO-8HO groups, whereas 16 h, 24 h HO 24 h before MCAO resulted in a reduction of infarct volume, when compared to the respective RA groups (Fig. 3). The infarct volume was reduced by 80.5, 85.5% in penumbra and 21.4, 31.9% in the core of MCAO-16HO and MCAO-24HO subgroups respectively, when compared to the respective MCAO-RA subgroup. Neuroprotection exerted by HO was mainly seen in the penumbra (cortex) (Fig. 3).

2.4. *Effects of various durations of HO on superoxide dismutase (SOD) activity*

Enzyme activity analysis showed that SOD is expressed in rat brains. The activation of SOD was increased in Int-16HO and Int-24HO, when compared to the respective Int-RA groups (Fig. 4). At 24 h after ischemia-reperfusion, activity of SOD in the core or penumbra in the MCAO-RA group was lower than that of the Sham-RA group (Fig. 4). At 48 h after HO, activity of SOD in the penumbra and core in the Int-16HO and Int-24HO

Table 1 – ABG tests at the end of pretreatment ($P<0.001=^{***}$)

Experimental groups	pH	PCO_2 (mmHg)	PO_2 (mmHg)	Respiratory rate (Hz)
RA	7.4±0.04	43.5±2.1	94.3±4.5	1.54±0.10
4HO	7.3±0.03	38.9±2.8	343.1±25.1***	1.29±0.15
8HO	7.3±0.02	39.0±2.2	351.3±27.2***	1.18±0.13
16HO	7.3±0.03	38.1±2.6	355.2±24.2***	1.19±0.12
24HO	7.3±0.03	38.9±2.8	363.1±23.1***	1.20±0.11