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Original Article

Normobaric hyperoxia preconditioning attenuates streptozotocin - induced impairments in spatial learning

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Abstract

Introduction: A large body of evidence points to oxidative stress as prime candidate mediating the behavioral impairments and memory deficits in Alzheimer's disease (AD). It has been demonstrated that hyperoxia preconditioning activates complex endogenous neuroprotective mechanisms including an increase in capacity of antioxidant defence mechanisms. The aim of this study was to investigate the beneficial effects of normobaric hyperoxia preconditioning in streptozotocin (STZ)- induced memory impairment in rats.

Materials and Methods: Male Wistar rats were first exposed to air with high oxygen concentration (>90%) or atmospheric air for 24 hours and then STZ (3 mg/kg) was bilaterally infused in lateral ventricles of the brain. Two weeks later Morris Water Maze (MWM) test was performed to assess spatial learning and memory consolidation.

Results: STZ increased escape latency ($P<0.05$), distance and number of crossed quadrants ($P<0.05$) especially on 1st and 2nd days. However, hyperoxia preconditioning significantly attenuated STZ-induced learning and memory deficits during training sessions in the MWM ($P<0.05$). Preconditioning also increased time spent and swimming distance in the target quadrant in probe test ($P<0.05$). However, hyperoxia preconditioning had no effect on the swimming speed.

Conclusion: Hyperoxia preconditioning significantly attenuated STZ-induced impairments in spatial learning and memory. These results suggest that hyperoxia may have a potential therapeutic effect at the early stage of AD and possibly the prevention of memory deficits.

Keywords:
Alzheimer's Disease;
Streptozotocin;
Hyperoxia;
Preconditioning;
Morris Water Maze;
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Introduction

Alzheimer's disease (AD) is a chronic and irreversible neurodegenerative disease characterized by progressive decline in cerebral functions, including memory. As the global prevalence of AD will reach 106.2 million by 2050, there is an urgent need to examine/explore various therapeutic methods for treatment of AD. The pathogenesis of AD is multi-factorial, including oxidative injury, inflammation, extracellular β-amyloid (Aβ) plaques formation, intracellular neurofibrillary tangles development, and abnormal cholesterol metabolism (Yamasaki et al., 2012).

A large body of evidence point to oxidative stress as

prime candidate causing the behavioral impairments and memory deficits observed in AD (Launer et al., 1998, Cantuti-Castelvetri et al., 2000). Free radicals such as hydroxyl radicals and hydrogen peroxide can be formed in cells during aerobic metabolism of endogenous and exogenous substances. Cells have enzymatic and non-enzymatic scavenger systems to remove these free radicals. However, if free radical production and scavenger systems remain unbalanced, cells are exposed to oxidative stress damage, resulting in cell injury (Parinandi et al., 1990, Griesmacher et al., 1995). Thus, antioxidants have been considered as having putative positive benefits in altering, reversing or a potential treatment of Alzheimer's dementia.

It is well known that if the noxious stimulus below the threshold of damage to the tissue is applied, of damage.(sentence may need to be rephrased) This phenomenon which is called preconditioning has been demonstrated in a variety of organs such as heart, kidney, and brain. preconditioning involves complex endogenous protective mechanisms including an increase in capacity of antioxidant defense mechanisms (Cadet et al., 2009), inhibition of mitochondrial permeability, transition pore opening (Hausenloy et al., 2009), and inhibition of excitotoxicity/(cytotoxicity??) through down regulation of NMDA and AMPA receptors (Tanaka et al., 2002). Preconditioning has attracted the interest of clinical and basic neuroscientists and the studies over the past decade have resulted in various promising strategies for the treatment of patients with acute brain injury. Several strategies have been tested in randomized clinical trials (Dirnagl et al., 2009). although the causes of neurodegeneration are different both in acute (such as stroke) and chronic n forms (such as Alzheimer's disease (AD) and Parkinson's disease), but the mechanisms leading to neuronal death including mitochondrial dysfunction, apoptosis, and caspase activation are the same (Soane et al., 2007). Therefore, preconditioning strategies may prevent or attenuate the neuronal death in chronic neurodegenerative disorders too.

In this study, we examined the effect of normobaric hyperoxia preconditioning on learning and memory deficits in streptozotocin (STZ)-induced AD in rats. Intracerebroventricular (i.c.v.) injection of STZ causes behavioral impairments including prolonged impairment in learning and memory which resemble AD (Blokland et al., 1993, Lannert et al., 1998). Moreover, i.c.v. injection of STZ, in a subdiabetogenic dose produces oxidative stress and several authors have attributed the cognitive impairment in STZ-induced AD to STZ-induced oxidative stress (Sharma et al., 2001, Raza et al., 2012).

Materials and methods

Animals

Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 200–300 g at the beginning of study were housed in large cages (38 × 59 × 20 cm) at a temperature-controlled colony room (23 ±1^{0}C) under light/dark cycle (light on 07:00) with free access to tap water and standard food. Animals were divided into 3 experimental groups as follows: STZ, STZ + normal O2, STZ + hyper O2. In addition, the data of another group of rats marked as healthy rats, was also used in this study. This extra group was consisted of intact rats subjected to no intervention and did not received STZ. n for each group was 8. Each animal was used only once and killed immediately after the experiment. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Qazvin University of Medical Sciences. STZ were purchased from SIGMA-ALDRICH Company and anesthetic drugs (ketamine and xylazine) are products of to Alfasan Company, Holland. Oxygen containers were prepared domestically.

Preconditioning procedure

In this study we used prolonged preconditioning in which the animals either continuously breathed atmospheric air (STZ + normal O2 group) or air with high oxygen concentration (>90%, STZ + hyper O2 group) for 24 hours. The whole cage of animals with food and water were placed in an air-tight Plexiglas box (45 × 70 × 25 cm) with a gas inlet and outlet port. Pure

humidified oxygen or atmospheric air was delivered at a rate of ≤ 5 L/ min through the inlet port. The oxygen concentration inside the container was continuously monitored (Lutron-DO5510 oxygen sensor, Taiwan) and the carbon dioxide cleared using soda lime (Romina, Marlic Company, Iran) on the bottom of the box.

Induction of experimental dementia of AD by i.c.v. administration of STZ in rats

Two to 24 hours after the termination of preconditioning, animals were first anesthesized with intraperitoneal (i.p.) injection of ketamine 10% (100 mg/kg) and xylazine 2% (10 mg/kg) and then STZ (3 mg/kg, 10 µl/injection site) was injected bilaterally into ventricles by stereotaxic surgery and through a Hamilton syringe. According to Paxinos and Watson's atlas (Paxinos and Watson, 1997), the following coordinates were used for icv injection: 0.8 mm posterior to the bregma, 1.5 mm lateral to the sagittal suture, and 3.6 mm ventral from the surface of the brain. STZ was dissolved in normal saline shortly before application. Surgeries were performed alternatively i.e. first surgery on a rat from STZ group, the next one on a rat from STZ + normal O2 group and third one on a rat from STZ + hyper O2 group. Then, the surgeries were continued.

Assessment of learning and memory using the Morris water maze

To assess spatial learning and memory of animals, Morris water maze (MWM) tests were performed according to Morris (Morris et al., 1982). Briefly, in MWM animal learns to escape to a hidden platform by swimming in circular water tank. This tank consisted of a large circular black colored pool of 150 cm diameter, 60 cm height, filled to a depth of 40 cm with water at 25±1 °C. A black colored round platform of 10 cm diameter was placed 1 cm below the surface of water in a fixed position in the middle of the target quadrant (Q2) in the pool; the starting point was in the Q1 quadrant in all the trials. The rats could climb on the platform to escape from the necessity of swimming. Only distal visual cues were available. The task was divided into two sessions: Initial learning test (place learning) and without platform test (probe test). Trials were given for 6 consecutive days in order to train rats in the Morris water maze. The rats were given a maximum time of 60 s (cut-off time) to find the hidden platform and were allowed to stay on it for 20 s. Once the animal found it, it was allowed to remain there for 20 s; if it did not find the platform after 60s, it was guided gently onto the platform and allowed to remain there for 20s. The animals were given a daily session of 4 trials. The numbers of quadrants traversed by the animals and course length (swimming paths) and latency time to reach the platform were recorded in each trial by water maze software. A significant decrease in latency time compared with the 1st session was considered as successful learning (Saxena et al., 2007). The animals were subjected to the probe test on the 8th day; 48h after the last trial of (the sixth session) The platform was removed and each rat was allowed to explore the pool for 60s. The mean time spent in all four quadrants was noted. The mean time spent by the animal in the target quadrant (Q2) searching for the hidden platform was measured and noted as an index of retrieval or memory.

Statistics

Data are expressed as mean ± SEM (standard error of mean). In order to compare the latency time, the number of quadrants that the animals crossed and path length to reach the platform (distance) obtained in all control and experimental groups. Then, one-way repeated measure ANOVA followed by the Tukey post hoc test multiple group comparison, was used to analyze data. *P* values less than 0.05 were considered to be statistically significant.

Results

Oxygen concentration in the preconditioning box was between 91 to 96% for hyperoxia group and 21% for normal O2 group. No rat died during preconditioning and it caused no seizure or other unusual behaviors in animals indicating that hyperoxia preconditioning caused no marked toxic effect.

fig.1

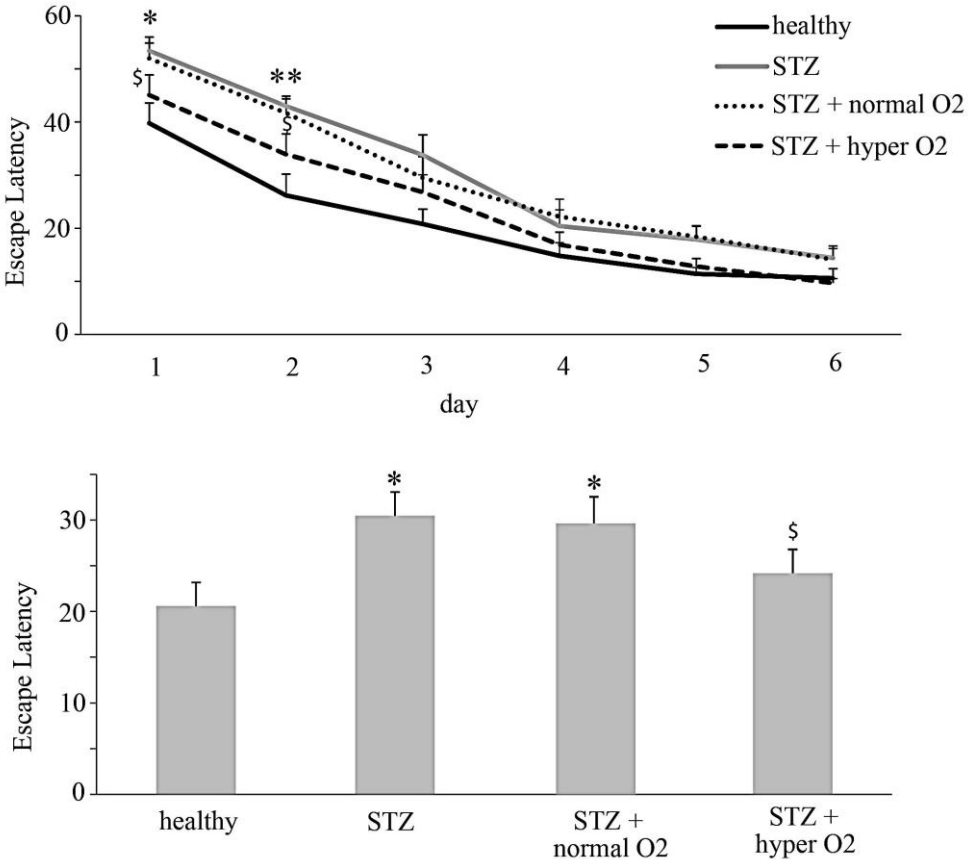

Fig.1. Place learning. Upper panel shows the escape latency (The latency time to find the hidden platform) of the experimental groups during successive training days (four sessions per day). Lower panel shows the escape latency in all of the training days. *p<0.05; **p<0.001; relative to healthy group, $ p<0.05; relative to STZ group, one-way repeated measure ANOVA followed by the Tukey post hoc test.

Place learning

Figures 1 to 3 display place learning of different experimental groups in the MWM. As these figures show, all parameters of place learning (escape latency, distance and number of crossed quadrants) in the all groups improved in the successive training days. However, there were significant differences between the groups. First of all, all of these parameters in STZ group, especially in days 1 and 2 of the training days, were significantly higher than those in healthy group indicating STZ treatment reduced the ability of rats for place learning. On the other hand, preconditioning of rats with hyperoxia air before of the STZ administration attenuated significantly STZ-induced impairment in learning processes. In contrast, preconditioning of STZ -received/administered rats with normal air failed to improved learning compared to STZ group.

Also, our results show that swimming speed of all groups of rats increased in the consecutive training days. But there was no significant difference among the experimental groups indicating both STZ and preconditioning had no effect on the motor activity of rats.

In the probe test, healthy rats spent the most time and swum the most in the target quadrant indicating memory consolidation were taking place well in this group (fig. 4). However, in the STZ and STZ+ normal O2 groups, time spent and swimming distance in the target quadrant were significantly less than those in

fig.2

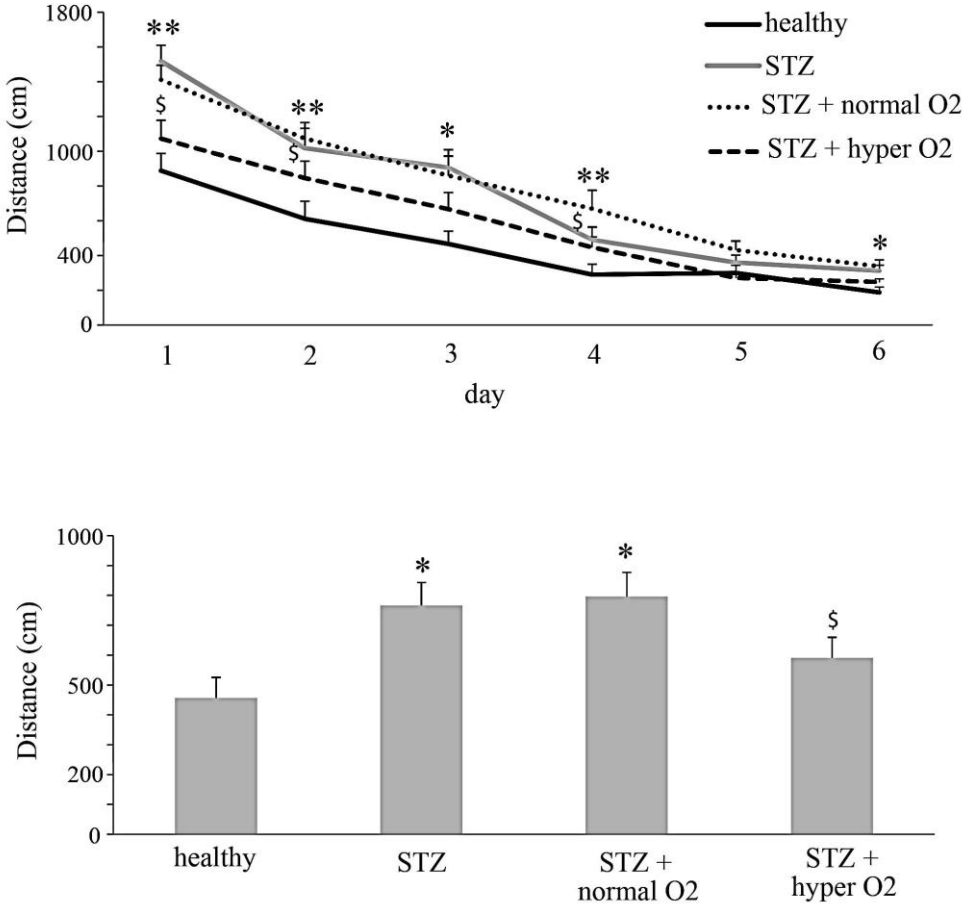

Fig. 2. Place learning .Upper panel shows distance (the path length to find the platform) of the experimental groups during successive training days. Lower panel shows the distance in all of the training days. *p<0.05; **p<0.001; relative to healthy group, $ p<0.05; relative to STZ group, one-way repeated measure ANOVA followed by the Tukey post hoc test.

healthy group. On the other hand, in the STZ + hyper O2 group, time spent and swimming distance in the target quadrant were close to those in healthy rats indicating hyperoxia preconditioning attenuated/ reduced STZ- induced impairment in memory consolidation.

Discussion

The main finding of this study is that normobaric hyperoxia preconditioning is capable ofattenuate STZ-induced impairment in spatial learning. Since there was no significant difference in the swimming speed between/among experimental groups including STZ and STZ + hyper O2 groups, the effect of hyperoxia preconditioning was not due to improve in motor activity of rats. Therefore, hyperoxia preconditioning probably attenuated STZ-induced neuronal damage in brain. Previously, we demonstrated that hyperoxia preconditioning attenuates behavioral symptoms of 6-OHDA-induced Parkinsonism (Hamidi et al., 2012). Here in this study, we extend our findings and found that normobaric hyperoxia preconditioning is capable of attenuating STZ-induced neurotoxicity and impairment in spatial learning and memory. Confirming our results, Prass et al., (2000) showed that hyperoxia preconditioning provides a neuronal protection mechanism against CNS ischemic damages (Prass et al., 2000). Also, Ohtsuki et al., (1992) showed that production HBO or NBO, is capable ofinducing ischemic tolerance in gerbil hippocampal neurons of brain (Ohtsuki et al., 1992).

normobaric hyperoxia has been considered as a noninvasive model as a defense mechanism against

fig.3

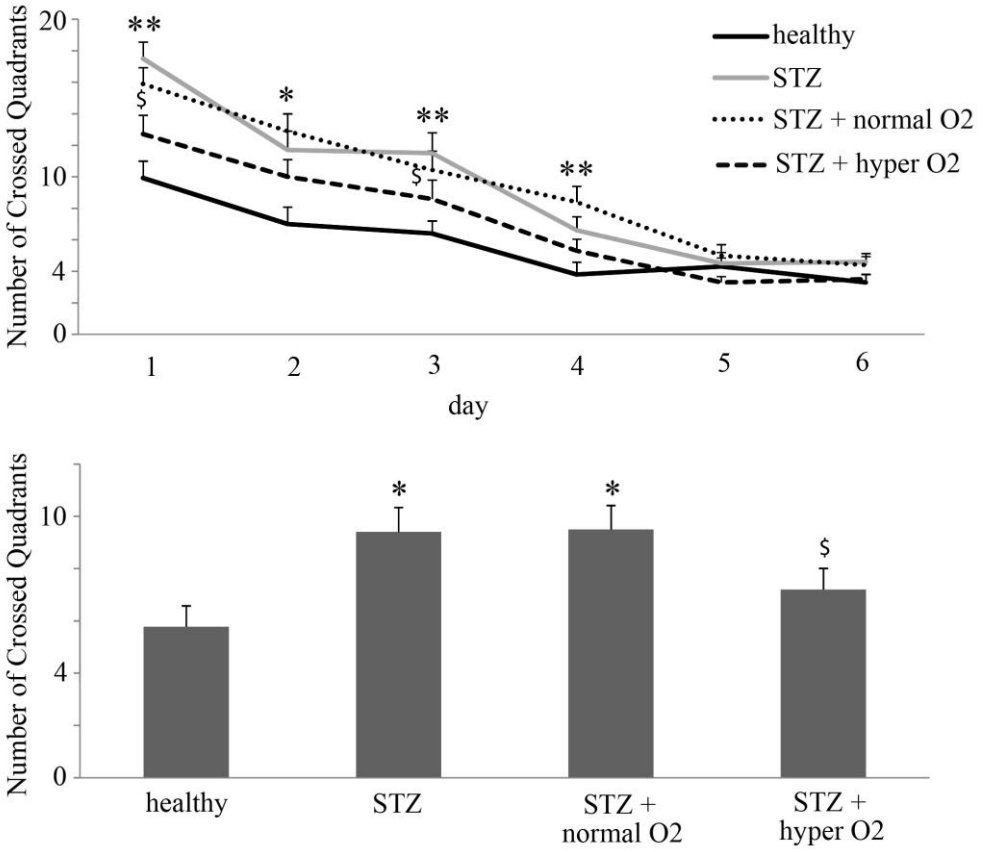

Fig 3. Place learning. Upper panel shows the number of crossed quadrants in the experimental groups during successive training days. Lower panel shows the number of crossed quadrants in the all of the training days. *p<0.05; **p<0.001; relative to healthy group, $ p<0.05; relative to STZ group, one-way repeated measure ANOVA followed by the Tukey post hoc test.

oxidative stress and widely used to study the effects of oxidant injury to the brain. Reactive oxygen species (ROS) alter the intrinsic membrane properties and synaptic transmission which can produce seizure in animals (D'Agostino et al., 2009). However, in our experiments no seizure or unusual behaviors was observed during or after breathing of the hyperoxic air in rats indicating that the level of ROS was below the toxic level. toto confirm this, considerable evidence shows that both HBO and NBO treatment can significantly increase the brain tissue level of oxygenation, as well without cerebral oxygen toxicity (Rockswold et al., 2010).

it is well known that hyperoxia preconditioning via generation of a nonlethal level of ROS produces specific adaptation responses which may lead to neuroprotection during the actual insult of oxidative stress. These adaptation responses involve number of cellular and biochemical mechanisms including upregulation of activities of antioxidant enzymes, induction the nitric oxide synthase (NOS) and heat shock proteins (Wang et al., 2009) . Several studies have shown that STZ in addition to impairingt of glucose utilization, produces free radicals, thereby inducing oxidative stress which causes neuronal damage in the brain (Sharma et al., 2001a; Sharma et al., 2001b; Sharma et al., 2002; Shoham et al., 2003). Hyperoxia also can improve mitochondrial function (Rockswold et al., 2001, Daugherty et al., 2004). Hyperoxia may also trigger activation of multiple factors that decrease levels of Aβ deposit. It may inhibit the process of the apoptotic pathway and increase clearance of Aβ (Zhu et al., 2007). Aβ pis generatedby the proteolytic cleavage of AβPP y the action of β and γ-secretases. Gao et al., (2011) have shown that NBO treatment had no significant effect on AβPP level and

fig.4

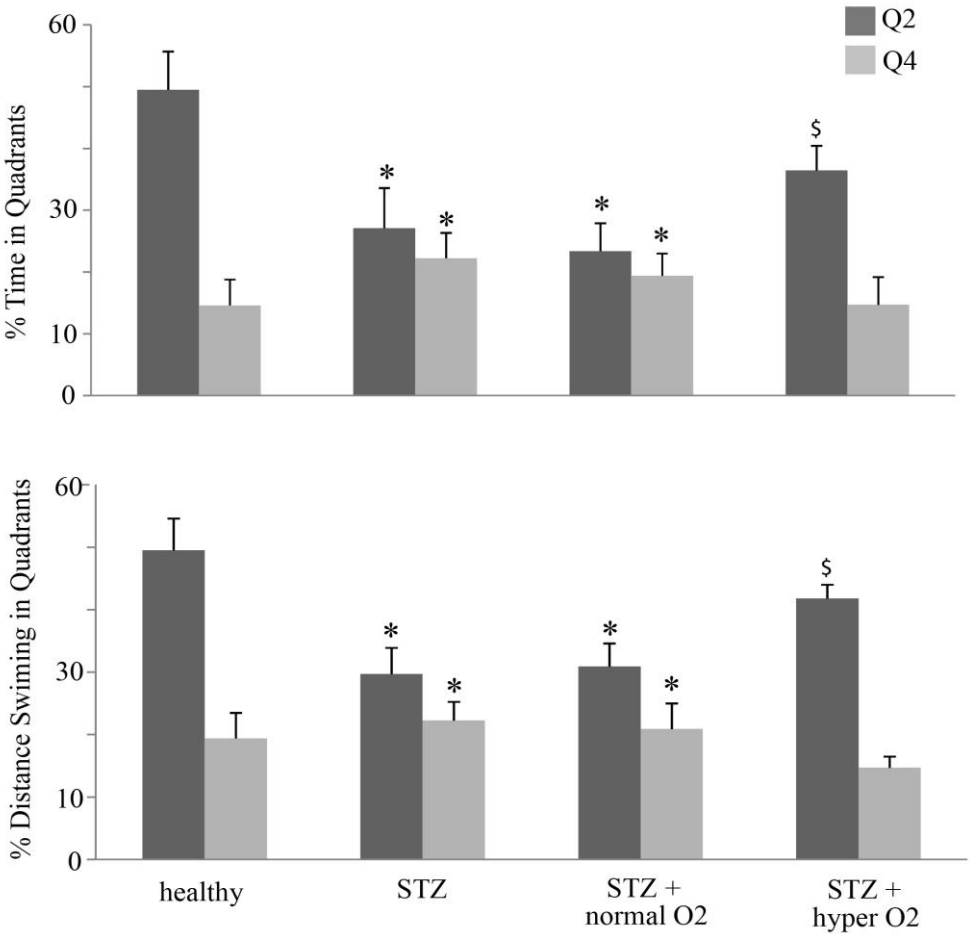

Fig. 4. Probe test. Upper panel shows the percentage of time spent in the target quadrant (Q2) and opposite target quadrant (Q4) during only one day in experimental groups. Lower panel shows the percentage of distance swimming in the target quadrant and opposite target quadrant during only one day in different experimental groups. *p<0.05; relative to healthy group, $ p<0.05; relative to STZ group, one-way repeated measure ANOVA followed by the Tukey post hoc test.

β-secretase activity while the production of Aβ was notably reduced by NBO, through inhibition of γ-secretase cleavage of AβPP proteins (Gao et al., 2011). As the abnormal accumulation of Aβ plaques is a sign of AD neuropathology, it is predicted that one effective strategy and method of emerging experimental therapies for AD in future will be the reduction of Aβ production probably by hyperoxia that inhibits γ-secretase cleavage of AβPP proteins.

Also, there are several evidence indicating that both hyperbaric oxygen preconditioning and hypoxia preconditioning against focal cerebral ischemia elevate autophagic activity which elicits a neuroprotective effect against ischemic injury in the brain. Autophagy is a catabolic process for maintaining the balance between production and degradation of cellular components, is critical for cell homeostasis (Klionsky et al., 2007) and beneficial to neuronal survival. A reduction in the level of autophagy leads to a huge loss of neurons that may eventually lead to or worsen neurodegenerative diseases (Hara et al., 2006; Komatsu et al., 2006). In neonatal rats, autophagy is responsible for preventing neuronal death and reducing the brain injury after brain hypoxia–ischemia (Carloni et al., 2008).

Recent studies indicate that short ischemic episodes in the brain induce neural protection against a subsequent lethal ischemic injury. Unfortunately, induction of a transient f sub lethal ischemia before an anticipated cerebral ischemic event is hardly practical clinically. Therefore HBO and NBO preconditioning which have

minimal side effects were used instead of short ischemic preconditioning. In the current study we observed a significantly more refined behavioral outcome in rats that received normobaric hyperoxia preconditioning (for 24h) than in the normal oxygen preconditioning and STZ groups. The results indicate that pretreatment with normobaric hyperoxia can induce neuroprotection against cerebral ischemia.

In conclusion, the current study clearly demonstrates that normobaric hyperoxia preconditioning attenuates learning and memory deficits in streptozotocin -induced Alzheimer's disease in rats. Hyperoxia preconditioning might be clinically useful for the treatment of cognitive impairments in Alzheimer's disease.

Acknowledgments

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Conflict of interest

All authors declare that there are no actual or potential conflicts of interest for this study.

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