Neuroscience Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Short Communication

Mild hyperbaric oxygen inhibits the decrease of dopaminergic neurons in the substantia nigra of mice with MPTP-induced Parkinson's disease

Yuina Kusuda [a], Ai Takemura [a], Masaki Nakano [b], Akihiko Ishihara [a,*]

[a] Laboratory of Cell Biology and Life Science, Graduate School of Human and Environmental Studies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan
[b] Laboratory of Functional Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form
15 November 2017
Accepted 24 November 2017
Available online xxx

Keywords:
Balance beam test
Dissolved oxygen
Enhanced atmosphere absolute
Immunohistochemistry
Increased oxygen concentration
Oxidative metabolism
Rotarod test

ABSTRACT

We examined whether exposure to mild hyperbaric oxygen inhibits the decrease of dopaminergic neurons in the substantia nigra of a neurotoxic animal model with Parkinson's disease. Mice injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride and probenecid twice a week were divided into two groups: mice with mild hyperbaric oxygen and those without. The mice with mild hyperbaric oxygen were exposed to 1317 hPa with 45% oxygen for 3 h, three times a week. The decrease in dopaminergic neurons of mice with Parkinson's disease was inhibited by 11 weeks of exposure to mild hyperbaric oxygen. We conclude that exposure to mild hyperbaric oxygen is effective in preventing the progression of Parkinson's disease.

Parkinson's disease is a progressive neurodegenerative disorder in the elderly that is characterized by typical motor symptoms such as resting tremors, rigidity, bradykinesia, and gait disturbances (Adler, 2005). The conventional therapy using levodopa and dopamine agonists for Parkinson's disease focuses primarily on relieving motor symptoms. Another therapy for Parkinson's disease includes chronic exercise (Bergen et al., 2002; Hirsch et al., 2003; Pothakos et al., 2009). However, it is difficult to completely prevent the degeneration of dopaminergic neurons with these therapies.

Exposure to mild hyperbaric oxygen at 1266–1317 hPa with 35–45% oxygen increases the level of oxygen in blood, especially the oxygen dissolved in blood plasma, and facilitates oxidative metabolism in the mitochondrial tricarboxylic acid cycle in cells and tissues (Ishihara et al., 2005; Matsumoto et al., 2007). Metabolic syndrome (Takemura and Ishihara, 2017), lifestyle-related diseases (Gu et al., 2010; Nagatomo et al., 2010a, 2011), and arthritis (Nagatomo et al., 2010b) are inhibited and/or improved when rodents are exposed to mild hyperbaric oxygen.

Parkinson's disease results from the progressive decrease of dopaminergic neurons in the substantia nigra (Dauer and Przedborski, 2003) although the mechanisms for this decrease have not fully been clarified. It is suggested that exposure to mild hyperbaric oxygen inhibits the decrease in dopaminergic neurons, which would result in an improvement of Parkinson's disease because of the enhancement of oxidative metabolism in dopaminergic neurons.

All experimental and animal care procedures were approved by the Guidelines for the Care and Use of Laboratory Animals issued by the Institutional Animal Experiment Committee of Kyoto University (Kyoto, Japan) and conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize both the number of animals used and their suffering.

Four-week-old male C57BL/6JJmsSlc mice were divided into the control (CON), Parkinson's disease (PD), and Parkinson's disease with mild hyperbaric oxygen (PDO) groups ($n = 12$ in each group). All mice were housed under normobaric conditions, that is, 1013 hPa with 20.9% oxygen. The PD and PDO groups were injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP, 20 mg/kg body weight) and probenecid (250 mg/kg body weight) twice a week (Tuesday and Thursday)

* Corresponding author.
E-mail address: ishihara.akihiko.8s@kyoto-u.ac.jp (A. Ishihara).

https://doi.org/10.1016/j.neures.2017.11.008

from 7 to 18 weeks of age (Meredith et al., 2008). In addition, the PDO group was exposed to mild hyperbaric oxygen at 1317 hPa with 45% oxygen using a chamber for exposure to mild hyperbaric oxygen (Japan Patent No. 5076067 dated September 7, 2012; Inventor: Akihiko Ishihara) for 3 h (11:00–14:00 h), three times a week (Monday, Wednesday, and Friday).

All mice were evaluated for motor functions using rotarod and balance beam tests at 16, 17, and 18 weeks of age. The rotarod test consisted of a 3 cm-diameter cylinder rotating at 22 rpm. The amount of time before the mouse dropped off the cylinder was recorded. For the balance beam test, the time taken by the mouse to move a distance of 50 cm on a 5 mm-diameter stick and the number of times that the mouse's feet slid off the stick were recorded.

Food and water were provided *ad libitum* to all mice. The room was maintained in a controlled 12-h light/dark cycle (dark period from 20:00–08:00 h) at 22 ± 2 °C with 45–55% relative humidity.

After 11 weeks of the experimental period, the mice were anesthetized using sodium pentobarbital (30 mg/kg body weight, via intraperitoneal injection). The mice were perfused using phosphate buffered saline (pH 7.4), followed by 4% paraformaldehyde and 2% glutaraldehyde. The brains were isolated, fixed, and embedded in paraffin. Serial 4 μm-thick sections were cut using a microtome and mounted onto slides. The sections were incubated in primary antibody (anti-tyrosine hydroxylase (TH) antibody; EMD Millipore Corporation, Germany) overnight, washed with tris-buffered saline with Tween 20 (TBST), and incubated in secondary antibody (biotinylated rabbit immunoglobulin G; VECTOR Laboratories, USA) for 60 min. After washing with TBST, the sections were incubated using VECTASTAIN Elite ABC kit (VECTOR Laboratories, USA) for 30 min. They were then rinsed with TBST, incubated using a DAB substrate kit (VECTOR Laboratories, USA) for 6 min, cleared, and mounted. For each mouse, TH-positive neurons in the substantia nigra were counted in five consecutive sections. Repeated sampling of the same neurons on the serial sections was avoided by confirming the position of individual neurons.

The data are expressed as mean and standard deviation. One-way analysis of variance (ANOVA) was used to evaluate the differences among the CON, PD, and PDO groups. When the differences were found to be significant by ANOVA, individual group comparisons were made using *Scheffé's* post hoc test. Statistical significance was set at $P < 0.05$.

There were no differences in the body weight among the three groups at 7 (CON, 22.0 ± 0.6 g; PD, 22.0 ± 0.9 g; PDO, 22.7 ± 1.0 g; $F = 2.368$, $P = 0.109$) and 18 (CON, 26.9 ± 1.4 g; PD, 26.5 ± 1.1 g; PDO, 27.0 ± 1.1 g; $F = 0.467$, $P = 0.632$) weeks of age (the first and last stages of the experiment, respectively).

There were no differences in the amount of time before the mouse dropped off the cylinder during the rotarod test among the CON, PD, and PDO groups at 16 ($F = 0.369$, $P = 0.696$), 17 ($F = 1.028$, $P = 0.378$), and 18 ($F = 0.672$, $P = 0.521$) weeks of age (Fig. 1A).

During the balance beam test, there were no differences in the time taken by the mouse to move a distance of 50 cm on the stick among the CON, PD, and PDO groups at 16 ($F = 0.218$, $P = 0.805$) and 18 ($F = 0.217$, $P = 0.807$) weeks of age (Fig. 1B); however, there were differences at 17 weeks of age ($F = 3.694$, $P < 0.05$). The time taken by the mouse to move a distance of 50 cm on the stick was shorter in the PDO group than in the CON group at 17 weeks of age ($P < 0.05$).

There were differences in the number of times that the mouse's feet slid off the stick during the balance beam test among the CON, PD, and PDO groups at 16 ($F = 14.590$, $P < 0.05$), 17 ($F = 11.313$, $P < 0.05$), and 18 ($F = 8.135$, $P < 0.05$) weeks of age (Fig. 1C). The number of times that the mouse's feet slid off the stick was higher in the PD group than in the CON and PDO groups at 16, 17, and 18 weeks of age ($P < 0.05$ for all weeks).

There were differences in the number of dopaminergic neurons in the substantia nigra among the CON, PD, and PDO groups

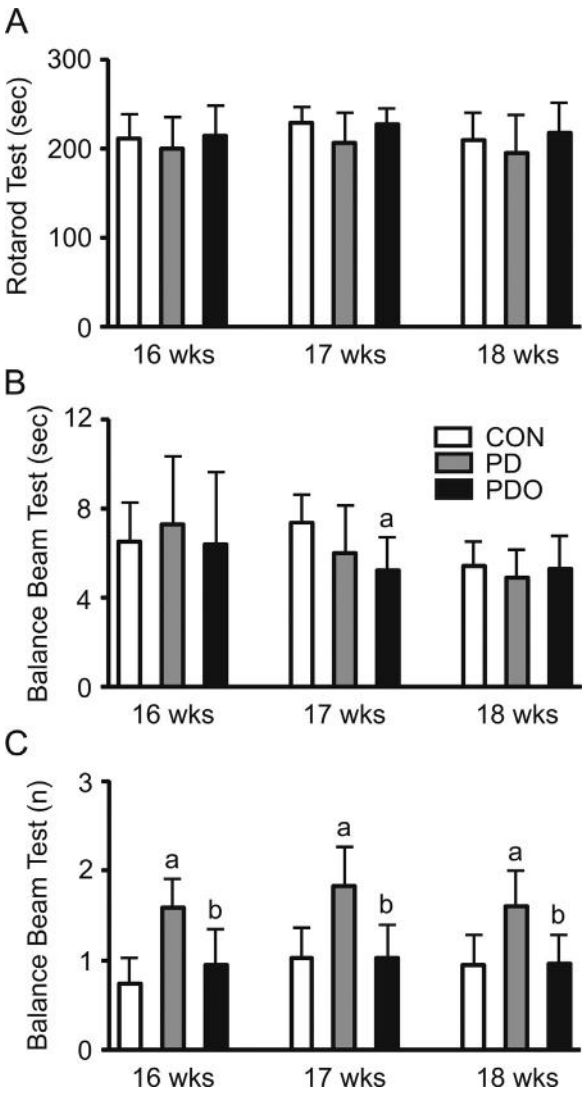

Fig. 1. Rotarod and balance beam tests for the CON, PD, and PDO groups. The amount of time before the mouse dropped off a cylinder rotating at a speed of 22 rpm was recorded for the rotarod test (A). The time taken by the mouse to move a distance of 50 cm on a 5 mm-diameter stick (B) and the number of times that the mouse's feet slid off the stick (C) were recorded for the balance beam test. CON, control; PD, Parkinson's disease; PDO, Parkinson's disease with mild hyperbaric oxygen. Data are expressed as mean and standard deviation of 12 mice. [a] $P < 0.05$ compared with CON; [b] $P < 0.05$ compared with PD.

(Figs. 2 and 3, $F = 39.230$, $P < 0.05$). The number of dopaminergic neurons in the substantia nigra was lower in the PD group than in the CON ($P < 0.05$) and PDO ($P < 0.05$) groups. The number of dopaminergic neurons in the substantia nigra was lower in the PDO group than in the CON group ($P < 0.05$).

This study examined the effects of exposure to mild hyperbaric oxygen on motor functions and dopaminergic neurons in the substantia nigra of mice with MPTP-induced Parkinson's disease. Exposure to mild hyperbaric oxygen improved motor functions in mice with Parkinson's disease; the number of times that the mouse's feet slid off the stick decreased following exposure to mild hyperbaric oxygen in the PDO group (Fig. 1C). We observed that exposure to mild hyperbaric oxygen facilitates oxidative metabolism, particularly in pathways in the mitochondrial tricarboxylic acid cycle, thus enhancing the oxidative capacity of skeletal muscle fibers and the spinal motoneurons innervating them (Ishihara et al., 2005; Matsumoto et al., 2007). It is suggested that exposure to mild hyperbaric oxygen has an effect on the neu-

Please cite this article in press as: Kusuda, Y., et al., Mild hyperbaric oxygen inhibits the decrease of dopaminergic neurons in the substantia nigra of mice with MPTP-induced Parkinson's disease. Neurosci. Res. (2017), https://doi.org/10.1016/j.neures.2017.11.008

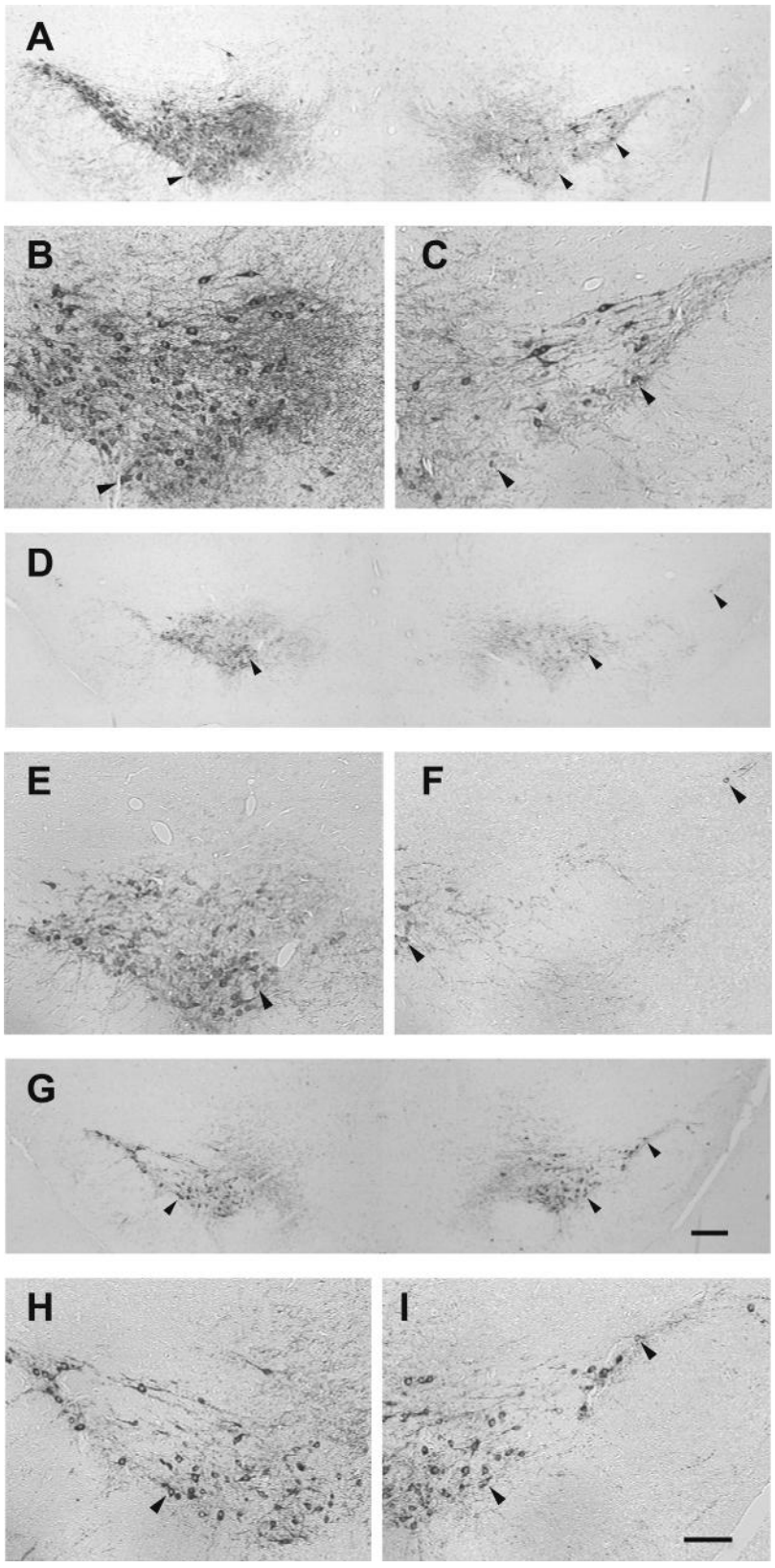

Fig. 2. Representative sections of the substantia nigra in control (A, B, C), Parkinson's disease (D, E, F), and Parkinson's disease with mild hyperbaric oxygen (G, H, I) mice, stained for tyrosine hydroxylase activity. B and C, E and F, and H and I extended A, D, and G, respectively. Scale bars on G and I are 200 and 100 μm, respectively.

Please cite this article in press as: Kusuda, Y., et al., Mild hyperbaric oxygen inhibits the decrease of dopaminergic neurons in the substantia nigra of mice with MPTP-induced Parkinson's disease. Neurosci. Res. (2017), https://doi.org/10.1016/j.neures.2017.11.008

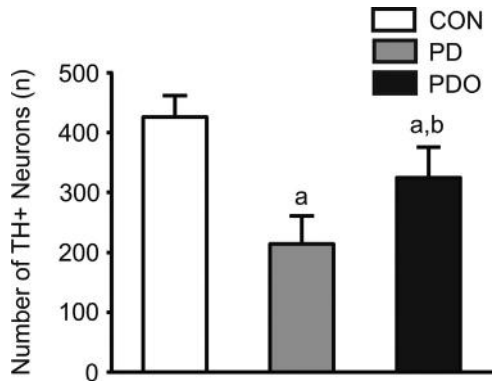

Fig. 3. Numbers of anti-tyrosine hydroxylase antibody positive neurons in the substantia nigra for the CON, PD, and PDO groups. TH+, tyrosine hydroxylase positive. Data are expressed as mean and standard deviation of 12 mice. [a]$P < 0.05$ compared with CON; [b]$P < 0.05$ compared with PD.

romuscular system, which is largely related to motor functions in mice with Parkinson's disease. Therefore, the number of times that the mouse's feet slid off the stick was decreased by exposure to mild hyperbaric oxygen. In contrast, there were no effects of exposure to mild hyperbaric oxygen on the amount of time before the mouse dropped off the cylinder (Fig. 1A) or the time taken by the mouse to move a distance of 50 cm on the stick (Fig. 1B). The reasons for differences in the motor function of these tests were not elucidated in this study. Multiple performance tests (e.g., rotarod, balance beam, pole, and open-field tests) are required for the evaluation of motor functions in mice with Parkinson's disease, because of behavioral anomalies in individual mice.

The decrease in the number of dopaminergic neurons in the substantia nigra of mice with Parkinson's disease was inhibited by exposure to mild hyperbaric oxygen (Fig. 3). Increased atmospheric pressure, accompanied by a high oxygen concentration, enhances the partial pressure of oxygen and increases dissolved oxygen in blood plasma (Ishihara et al., 2014). When water vapor pressure, concentration of carbon dioxide in the alveolus, and the respiratory exchange ratio are set at 47 mmHg, 40 mmHg, and 0.85, respectively, the estimated levels of oxygen dissolved in blood plasma under mild hyperbaric oxygen conditions at 1317 hPa with 45% oxygen (1.18 ml/dL blood plasma) are 3.7 times those under normobaric conditions at 1013 hPa with 20.9% oxygen (0.32 ml/dL blood plasma). Exposure to mild hyperbaric oxygen enhances the oxidative capacity of pathways in the mitochondrial tricarboxylic acid cycle, thus improving oxidative metabolism in cells and tissues. Therefore, we hypothesized that exposure to mild hyperbaric oxygen inhibits the decrease in dopaminergic neurons induced by Parkinson's disease, because it activates oxidative metabolism in the dopaminergic neurons. It is suggested that peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) is one of the factors that contributes to the improvement in oxidative metabolism of dopaminergic neurons in Parkinson's disease, because cell oxidative metabolism, mitochondrial biogenesis, oxidative stress, and gene expression are regulated by PGC-1α, which is a transcriptional coactivator (Wu et al., 1999; Liang and Ward, 2006). A previous study (Mudò et al., 2012) observed that transgenic mice overexpressing PGC-1α are resistant to both dopaminergic neuron degeneration in the substantia nigra and dopamine disappearance in the striatum.

Hyperbaric oxygen therapy, which is an established medical treatment and is usually conducted under conditions of 2026–3039 hPa with 100% oxygen, induces many diseases and complications, including atherosclerosis, cataract, retinopathy, myocardial infarction, hypertension, diabetes, renal failure, and uremia (Padgaonkar et al., 2000; Griendling and FitzGerald, 2003; Oter et al., 2005, 2007; Gesell and Trott, 2007). Excessive atmospheric pressure and oxygen concentration, especially in treatments with 100% oxygen, have been shown to increase the number of invasive inflammatory cells (Folz et al., 1999) and cause excessive production of reactive oxygen species in several tissues and organs (Narkowicz et al., 1993). In this study, the condition of mild hyperbaric oxygen was produced using 1317 hPa and 45% oxygen. Such side effects, including oxidative stress and barotrauma, are not induced by these conditions (Nagatomo et al., 2012). Exposure to mild hyperbaric oxygen also poses negligible risk of fire and explosion because of relatively low atmospheric pressure and oxygen concentration.

We conclude that exposure to mild hyperbaric oxygen at 1317 hPa with 45% oxygen inhibits the decrease of dopaminergic neurons, which results in the improvement of Parkinson's disease because it activates oxidative metabolism in dopaminergic neurons.

Conflict of interest

The authors have no conflict of interest to report.

References

Adler, C.H., 2005. Nonmotor complications in Parkinson's disease. Mov. Disord. 20 (Suppl. 11), S23–S29.
Bergen, J.L., Toole, T., Elliott 3rd, R.G., Wallace, B., Robinson, K., Maitland, C.G., 2002. Aerobic exercise intervention improves aerobic capacity and movement initiation in Parkinson's disease patients. Neurorehabiitation 17, 161–168.
Dauer, W., Przedborski, S., 2003. Parkinson's disease: mechanisms and models. Neuron 39, 889–909.
Folz, R.J., Abushamaa, A.M., Suliman, H.B., 1999. Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. J. Clin. Invest. 103, 1055–1066.
Gesell, L.B., Trott, A., 2007. De novo cataract development following a standard course of hyperbaric oxygen therapy. Undersea Hyperb. Med. 34, 389–392.
Griendling, K.K., FitzGerald, G.A., 2003. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation 108, 1912–1916.
Gu, N., Nagatomo, F., Fujino, H., Takeda, I., Tsuda, K., Ishihara, A., 2010. Hyperbaric oxygen exposure improves blood glucose level and muscle oxidative capacity in rats with type 2 diabetes. Diabetes Technol. Ther. 12, 125–133.
Hirsch, M.A., Toole, T., Maitland, C.G., Rider, R.A., 2003. The effects of balance training and high-intensity resistance training on persons with idiopathic Parkinson's disease. Arch. Phys. Med. Rehabil. 84, 1109–1117.
Ishihara, A., Kawano, F., Okiura, T., Morimatsu, F., Ohira, Y., 2005. Hyperbaric exposure with high oxygen concentration enhances oxidative capacity of neuromuscular units. Neurosci. Res. 52, 146–152.
Ishihara, A., Nagatomo, F., Fujino, H., Kondo, H., 2014. Exposure to mild hyperbaric oxygen increases blood flow and resting energy expenditure but not oxidative stress. J. Sci. Res. Rep. 3, 1886–1896.
Liang, H., Ward, W.F., 2006. PGC-1α: a key regulator of energy metabolism. Adv. Physiol. Educ. 30, 145–151.
Matsumoto, A., Okiura, T., Morimatsu, F., Ohira, Y., Ishihara, A., 2007. Effects of hyperbaric exposure with high oxygen concentration on the physical activity of developing rats. Dev. Neurosci. 29, 452–459.
Meredith, G.E., Totterdell, S., Potashkin, J.A., Surmeier, D.J., 2008. Modeling PD pathogenesis in mice: advantage of a chronic MPTP protocol. Parkinsonism Relat. Disord. 14 (Suppl. 2), S112–S115.
Mudò, G., Mäkelä, J., Liberto, V.D., Tselykh, T.V., Olivieri, M., Piepponen, P., Eriksson, O., Mälkiä, A., Bonomo, A., Kairisalo, M., Aguirre, J.A., Korhonen, L., Belluardo, N., Lindholm, D., 2012. Transgenic expression and activation of PGC-1α protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. Cell. Mol. Life Sci. 69, 1153–1165.
Nagatomo, F., Fujino, H., Takeda, I., Ishihara, A., 2010a. Effects of hyperbaric oxygenation on blood pressure levels of spontaneously hypertensive rats. Clin. Exp. Hypertens. 32, 193–197.
Nagatomo, F., Gu, N., Fujino, H., Okiura, T., Morimatsu, F., Takeda, I., Ishihara, A., 2010b. Effects of exposure to hyperbaric oxygen on oxidative stress in rats with type II collagen-induced arthritis. Clin. Exp. Med. 10, 7–13.
Nagatomo, F., Roy, R.R., Takahashi, H., Edgerton, V.R., Ishihara, A., 2011. Effect of exposure to hyperbaric oxygen on diabetes-induced cataracts in mice. J. Diabetes 3, 301–308.
Nagatomo, F., Fujino, H., Kondo, H., Ishihara, A., 2012. Oxygen concentration-dependent oxidative stress levels in rats. Oxid. Med. Cell. Longev., 10:1155/2012/381763.
Narkowicz, C.K., Vial, J.H., McCartney, P.W., 1993. Hyperbaric oxygen therapy increases free radical levels in the blood of humans. Free Radic. Res. Commun. 19, 71–80.

Please cite this article in press as: Kusuda, Y., et al., Mild hyperbaric oxygen inhibits the decrease of dopaminergic neurons in the substantia nigra of mice with MPTP-induced Parkinson's disease. Neurosci. Res. (2017), https://doi.org/10.1016/j.neures.2017.11.008

Oter, S., Korkmaz, A., Topal, T., Ozcan, O., Sadir, S., Ozler, M., Ogur, R., Bilgic, H., 2005. Correlation between hyperbaric oxygen exposure pressures and oxidative parameters in rat lung, brain, and erythrocytes. Clin. Biochem. 38, 706–711.

Oter, S., Topal, T., Sadir, S., Ozler, M., Uysal, B., Ay, H., Yaren, H., Korkmaz, A., Akin, A., 2007. Oxidative stress levels in rats following exposure to oxygen at 3 atm for 0–120 min. Aviat. Space Environ. Med. 78, 1108–1113.

Padgaonkar, V.A., Leverenz, V.R., Fowler, K.E., Reddy, V.N., Giblin, F.J., 2000. The effects of hyperbaric oxygen on the crystallins of cultured rabbit lenses: a possible catalytic role of copper. Exp. Eye Res. 71, 371–383.

Pothakos, K., Kurz, M.J., Lau, Y.S., 2009. Restorative effect of endurance exercise on behavioral deficits in the chronic mouse model of Parkinson's disease with severe neurodegeneration. BMC Neurosci., 10, http://dx.doi.org/10.1186/1471-2202-10-6.

Takemura, A., Ishihara, A., 2017. Mild hyperbaric oxygen inhibits growth-related decrease in muscle oxidative capacity of rats with metabolic syndrome. J. Atheroscler. Thromb. 24, 26–38.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C., Spiegelma, B.M., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.

Please cite this article in press as: Kusuda, Y., et al., Mild hyperbaric oxygen inhibits the decrease of dopaminergic neurons in the substantia nigra of mice with MPTP-induced Parkinson's disease. Neurosci. Res. (2017), https://doi.org/10.1016/j.neures.2017.11.008