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Research Article

Exercise training reduces chronic dizziness by inhibiting NADPH oxidase

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Abstract

Dizziness is a one of the common clinical symptoms noted in internal medicine clinics. Recent studies suggested that patients with dizziness receive a high level of oxidative stress. However, the probable mechanism of this finding is still largely unknown. In fact, exercise has been proven to bean efficient non-pharmacological approach for improving good health in humans. Previous studies revealed that exercise cures dizziness and reduces the circulatory oxidative stress in patients with chronic dizziness. In this study, we confirmed that the exercise intervention inhibits the NADPH oxidase activation through gp91 and p22 mRNA repression. We also reported that the exercise intervention promotes Hemo oxygenase-1 (HMOX-1), an antioxidant gene, expression level by using the real-time PCR assay. Our results demonstrated that exercise cures chronic dizziness through NADPH oxidase inhibition and HMOX-1 activation.

Keywords: exercise, chronic dizziness, NADPH oxidase, HMOX-1

INTRODUCTION

Dizziness is a common clinical symptom studied by general neurologists and practitioners (Bisdorff et al., 2009). Moreover, dizziness disorders can present without a prior organic vestibular dysfunction or, as a result, of an organic vestibular dysfunction. Indeed, both vestibular migraine and Menière's disease contribute dizziness in patients with relevantdisorders(Eckhardt-Henn et al., 2008). For the 75 years and over patients, those affected with these problems are likely to visit doctors more. The previous reports revealed that about 30–50% of complex dizziness disorders are not diagnosed yetwith a distinguishable medical disease.In fact, dizziness disorders may highly correlated with phobias, panic attacks, anxieties, depressions, dissociative or somatoform disorders(Dieterich and Eckhardt-Henn, 2004). Most importantly, the medical histories from those patients whom have dizziness disorderswere diagnosed with inflammatory diseases, including the peripheral labyrinthine abnormalities or inflammation located in the inner ears (Paparella et al., 1990).

In addition, the up-regulated oxidative stress had been found in patients with dizziness disorders (Calabrese et al., 2010; Tian et al., 2013). The NOX family of NADPH oxidases is the main donor of oxidative stress in humans. The NADPH oxidase is composed of the two membrane components, Nox2 (also called gp91) and p22phox as well asthe three cytoplasmic proteins, p47phox, p67phox, and the small GTPase Rac-1. The process, which the NADPH oxidase enzyme complex is activated, begins with the phosphorylation of p47phox, which induces the translocation of the p47phox/p67phox complex to the plasma membrane (Tsai et al., 2013)

In general, exercise is known as the clinical management of dizziness disorders (Telian and Shepard, 1996). It is useful and functional as a clinical intervention for patients with persistent vertigo due to vestibular disorders. The main principle of vestibular rehabilitation is aexercise programs, thereby promotingthe compensation of central nervous

system. This intervention is reported to correct the dizziness symptoms and gazes, to retrain the balance of gait, and to enhance the physical fitness (Luxon, 2004). Boyer et al suggested that exercise training led a positive feedback for patients suffering from vertigo and dizziness disorders (Boyer et al., 2008). Moreover, exercise training is considered for repressing pro-inflammatory responses in many studies. For example, itrepresses the pro-inflammatory responses Aβ-induced inflammation of neuron and mitigates the pro-inflammatory parameter in LPS-injected rats (Kim et al., 2013; Kang et al., 2013). Besides, exercise training is known to increase the function of antioxidant genes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH) in human study (Franzke et al., 2015).

Assuredly, exercise training has been an efficient non-pharmacological treatment for health improvement. Besides, many studies have validated that exercise training improved physiological function in humans(De Angelis et al., 2002; Ghosh et al., 2005). However, the effects of exercise intervention on NADPH oxidase inhibition in patients with dizziness disorders have not yet been revealed. Therefore, we pursued to test whetherthe exercise intervention inhibits the NADPH oxidase level in patients with dizziness disorders.

MATERIALS AND METHODS

Subjects

Patients with the chief complaint of dizziness for more than one year were recruited from the Department of Physical Medicine& Rehabilitation, Taipei Veterans General Hospital. The diagnosis was based on the patients' self-reported histories and the results of head thrust tests, horizontal and vertical head shaking nystagmus tests and caloric examinations (AIRSTAR, Micromedical Technologies, Illinois, USA). Age-matched subjects without any history of dizziness or vertigo were recruited as controls. However, subjects with benign paroxysmal positional vertigo and cerebrovascular diseases were excluded from the study. All the participants were asked to sign informed consent forms that were approved by Taipei Veterans General Hospital Institutional Review Board.

Exercise protocol

Each subject received exercise training three days each week for six weeks. The program lasted a total of 30 to 40 minutesfor each session. Each subject was asked to read a target two meters away from them. The training session started with a horizontal head movement at a comfortable speed for each subject. The head movements then increased to at least 120 degrees/second to achieve a therapeutic goal. The participants changed positions from sitting to standing and from standing on a stable floor to standing on an unstable cushion surface. For strengthening and balance exercises, the subjects were asked to sit on a chair, raise both legs with full knee extension and hold for 30 seconds, rest for 10 seconds and repeat for a total of 10 minutes. Then, the subjects were asked to stand on one leg for 30 seconds, hold onto a chair to keep balance if necessary, rest for 20 seconds, then change legs. Overall, the exercise was repeated for 10 minutes.

Clinical assessments for evaluation of dizziness severity

We used the visual analogue scale (VAS), the Dizziness Handicap Inventory (DHI) (Jacobson and Newman, 1990) and the Tinetti Fall Risk Performance Scale (POMA)[17]for evaluating the self-perceived dizziness level and balance functions before and after six weeks of exercise training. The DHI is a validated 25-item questionnaire for evaluating the individuals' self-perceived levels of dizziness-related handicap. Specifically, a high DHI score indicates a greater level of handicap caused by dizziness. The POMA is a 2-point scale for evaluating static and dynamic functions. In this case, a high POMA score indicates good balance function.

Isolation of mRNA and quantitative real-time PCR

The 6 ml amount of human blood was collected (BD Vacutainer system, K2-EDTA tubes; BD Diagnostics, Franklin Lakes, NJ, USA). Total RNA was isolated using a RN easy Plus minikit (Qiagen, Hilden, Germany).After isolation, RNA quality was assessed by an Experion Automated Electrophoresis Station (Bio-Rad).The oligonucleotides were designed using the computer software package Primer Express 2.0 (Applied Biosystems, Foster City, CA). All of the oligonucleotides were synthesized by Invitrogen (Breda, The Netherlands). The oligonucleotide specificity was determined by a homology search within the human genome (BLAST, National Center for Biotechnology Information, Bethesda, MD) and confirmed by dissociation curve analysis. The oligonucleotide sequences were shown in Table1.

Table 1. provide table title

Gene	**Sense**	**Anti-sense**
gp-91	5′-GGATGAATCTCAGGCCAA-3′	5′-TTAGCCAAGGCTTCGG-3′
p22	5’-GTTTGTGTGCCTGCTGGAGT -3’	5’-TGGGCGGCTGCTTGATGGT-3’
HMOX-1	5’-CTCAAACCTCCAAAAGCC-3’	5’-TCAAAAACCACCCCAACCC-3’
b-actin	5’-CGGGAAATCGTGCGTGAC-3’	5’-TGCCCAGGAAGGAAGGCT-3’

PCR was performed with SYBR Green in an ABI 7000 sequence detection system (Applied Biosystems) according to the manufacturer’s guidelines.

Statistical analyses

Results are expressed as mean ± SE. Differences between the groups were analyzed using one-way ANOVA followed by Student’s *t* test. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Subject characteristics

Fifteen patients (mean age 70.73 ± 5.48 years) who suffered from chronic dizziness (mean disease duration 3.2 ± 1.62 years; 12 patients with and 3 patients without a history of vestibular hypofunction) and 15 subjects (mean age 69.12 ± 3.62 years) without a history of dizziness were enrolled in this study. No age difference was found between groups (ANOVA, p > 0.05). After exercise training, clinical assessments were used to evaluate the effects of exercise training in patients with dizziness. The VAS scores before and after six weeks of exercise training were 4.46 ± 3.24 and 3.46 ± 2.43, respectively (p=0.018).The total DHI score changed from 35.07 ± 24.73 to 24.53 ± 24.70 (p = 0.014), and the POMA score changed from 17.80 ± 3.86 to 22.80 ± 2.80 (p < 0.0001). These results suggested that exercise significantly decreased the symptoms of dizziness and improved the balance functions.

The expression level of gp91 and p22 are repressed is attenuated in patients with dizziness disorders

Dizziness disorders are highly related to oxidative stress. We presumed that the NADPH oxidase expression level is up-regulated in patients with dizziness disorders. Human blood mRNA was isolated and real time PCR was used to confirm our hypothesis. In Figure 1, we revealedthat the mRNA expression levels of gp91 (Figure 1A) and p22 (Figure 1B) are largely lower in patients with dizziness disorders in comparison with health control. However, those genes expression levels were reversed after the exercise intervention.

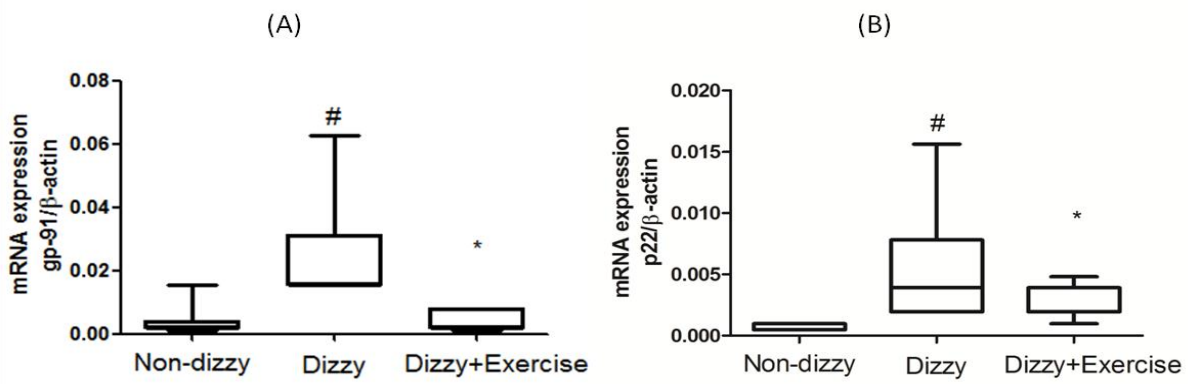

Figure 1. Expression levels of gp91 and p22 in the blood samples of patients with dizziness before or after exercise training. Total blood mRNAs were isolated, and the (A) gp91, (B) p22 expression of the three different groups (non-dizzy, dizzy, and dizzy with exercise) were investigated using real-time PCR. Box-and-whisker plot illustrating the mean, standard deviation (S.D) and upper/lower extreme values for each group. #p< 0.05 compared with the control group. *p< 0.05 compared with the dizzy group.

Total blood mRNAs were isolated, and the HMOX-1 expression of the three different groups (non-dizzy, dizzy, and dizzy with exercise) were investigated using real-time PCR. Box-and-whisker plot illustrating the mean, standard deviation (S.D) and upper/lower extreme values for each group. #p< 0.05 compared with the control group. *p< 0.05 compared with the dizzy group

Exercise training enhances antioxidant gene in patients with dizziness disorders

Next, we focused our attention in investigating the relative antioxidant gene mRNA expression level in each group. Hemo oxygenase-1 (HMOX-1) acts a major role in the repression against oxidative injuries through mitigating pro-apoptotic events. As shown in Figure 2, we found that the expression level of HMOX-1 is repressed in patients with dizziness disorders compared with health cases. However, those genes expression levels were reversed after the exercise treatment.

DISCUSSION

The physical exercise intervention for managing symptoms, associated with the vestibular dysfunction that manifests itself as dizziness and imbalance relating to the position or movement of the body, is recognized as an effective strategy in patients with dizziness disorders (Porciuncula et al., 2012). In this present study, we demonstrate for the first time the blood NADPH oxidase expression level is up-regulated in patients with dizziness disorders. Moreover, we also prove that exercise training provide a non- pharmacological effect to mitigatethe blood NADPH oxidase expression level in patients.

In fact, previous studies suggested that around 30–50% of complex dizziness disorderswere not well-explained with having a distinguishable medical disease, because clinical dizziness disorders may highly linked with phobias, panic attacks, anxieties, depressions, dissociative and somatoform disorders, or other chronic inflammatory diseases(Dieterich and Eckhardt-Henn, 2004; Paparellaet al., 1990). Taken together, oxidative stress and chronic inflammation play critical roles in dizziness disorders.The NADPH oxidase consists of the membrane-bound gp91phox and p22hox as well as the cytosolic subunits such as p47phox, p67phox, and the small GTPaseRac. NADPH oxidase–generated ROS production appears to be a driving force in the development of chronic diseases, which suggests that the NADPH oxidase–facilitated ROS plays as a secondary mediator to activate the downstream signal transduction pathways leading to human diseases (Tsai et al., 2012). In Figure 1, we reported that the gp91 and p22 expression wererepressed via exercise intervention in patients with chronic dizziness.

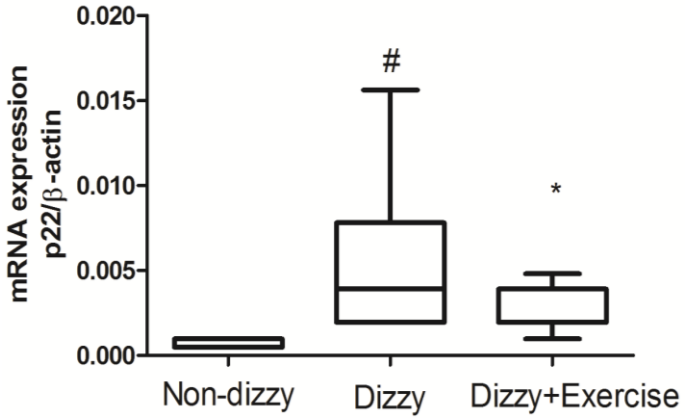

Figure 2. Expression levels of HMOX-1 in the blood samples of patients with dizziness before or after exercise training

In general, exercise training is an important non-pharmacological management for improving human health (Flynn et al., 2007). Furthermore, exercise training has been shown to reverse the functional and molecular abnormalities associating with human pathology(McMullen et al., 2007). HMOX-1 acts as a main role in regulating anti-inflammation, cyto-protection, and anti-oxidative stress function(Lien et al., 2014). Moreover, previous study suggested that exercise training increases the SOD_level also known as the antioxidant gene, thereby mitigating the blood MDA concentration (Zhao et al., 2013). HMOX-1 is regulated byNuclear factor 2 (Nrf2) during oxidative signaling. Nrf2 mediates ROS formation by activation of NADPH oxidase is reported (Kovac et al., 2007). In this study, we are the first group identified that the HMOX-1 expression is inhibited in patients with chronic dizziness. However, this finding was

reversed by utilizing the exercise intervention (Figure 2). We presumed this finding might due to NADPH oxidase inhibition by exercise treatment. This conclusion is supported by previous study. Kao et al revealed exercise mitigates oxidative stress through reducing ROS level and MDA concentration in human circulatory system (Kao et al., 2014). The limitation of our study is that this is a cross-sectional design. A longitudinal study would be valuable to determine the long term effects of exercise training. Future research may need to include kinase activity assays or Western blotting for NADPH oxidase proteins to further validate the roles of other NADPH oxidase activities.

CONCLUSION

In summary, we found that the blood NADPH oxiadse expression level is up-regulated in patients with chronic dizziness in this present study. However, the exercise intervention not only corrects the dizziness symptoms but also mitigates the NADPH oxidase blood level. Additionally, our data confirmed that exercise also increases the HMOX-1 expression level. Therefore, this therapeutic strategy suggests that exercise training might be beneficial to reduce the NADPH oxidase expression as novel approach to manage chronic dizziness.

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Conflict-of interest: The authors declare no competing financial interests.

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