Inhalation of hydrogen gas elevates urinary 8-hydroxy-2′-deoxyguanine in Parkinson's disease

Masaaki Hirayama[1], Mikako Ito[2], Tomomi Minato[1], Asako Yoritaka[3], Tyler W. LeBaron[4, 5], Kinji Ohno[2, *]
1 Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan
2 Division of Neurogenetics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan
3 Department of Neurology, Juntendo University Koshigaya Hospital, Saitama, Japan
4 Molecular Hydrogen Institute, Utah, USA
5 Center of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Ropublic

*Correspondence to: Kinji Ohno, MD, PhD, ohnok@med.nagoya-u.ac.jp.
orcid: 0000-0002-1529-2750 (Kinji Ohno)

Abstract

Hyposmia is one of the earliest and the most common symptoms in Parkinson's disease (PD). The benefits of hydrogen water on motor deficits have been reported in animal PD models and PD patients, but the effects of hydrogen gas on PD patients have not been studied. We evaluated the effect of inhalation of hydrogen gas on olfactory function, non-motor symptoms, activities of daily living, and urinary 8-hydroxy-2′-deoxyguanine (8-OHdG) levels by a randomized, double-blinded, placebo-controlled, crossover trial with an 8-week washout period in 20 patients with PD. Patients inhaled either ~1.2–1.4% hydrogen-air mixture or placebo for 10 minutes twice a day for 4 weeks. Inhalation of low dose hydrogen did not significantly influence the PD clinical parameters, but it did increase urinary 8-OHdG levels by 16%. This increase in 8-OHdG is markedly less than the over 300% increase in diabetes, and is more comparable to the increase after a bout of strenuous exercise. Although increased reactive oxygen species is often associated with toxicity and disease, they also play essential roles in mediating cytoprotective cellular adaptations in a process known as hormesis. Increases of oxidative stress by hydrogen have been previously reported, along with its ability to activate the Nrf2, NF-κB pathways, and heat shock responses. Although we did not observe any beneficial effect of hydrogen in our short trial, we propose that the increased 8-OHdG and other reported stress responses from hydrogen may indicate that its beneficial effects are partly or largely mediated by hormetic mechanisms. The study was approved by the ethics review committee of Nagoya University Graduate School of Medicine (approval number 2015-0295). The clinical trial was registered at the University Hospital Medical Information Network (identifier UMIN000019082).

Key words: Parkinson's disease; randomized, double-blinded, placebo-controlled, crossover trial; smell test; hydrogen gas; 8-hydroxy-2′-deoxyguanine; 8-OHdG; oxidative stress; hormesis

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Introduction

Hyposmia in Parkinson's disease (PD) was first reported in 1975 by Ansari and Johnson.[1] Subsequent studies revealed that (i) more than 70 to 80% of PD patients have hyposmia; (ii) hyposmia is one of the earliest symptoms of PD; (iii) hyposmia is correlated with severity of cognitive dysfunction.[2-5] Abnormal aggregation of α-synuclein in dopaminergic neurons in the substantia nigra causes neuronal death and is a hallmark of PD. The abnormal aggregate of α-synuclein starts from the olfactory epithelium and/or intestinal neural plexus, and enters the brain.[6] The expression level of α-synuclein is normally high in the olfactory epithelium.[7] The abnormal aggregate of α-synuclein in the olfactory epithelium is likely due to some environmental toxins and/or genetic factors,[8] but the underlying mechanisms remain to be elucidated. Hyposmia is quantitatively evaluated by the University of Pennsylvania Smell Identification Test[9] or the Sniffin' Sticks odor identification test.[10] Some substances in these tests, however, are culture-specific and are unfamiliar to Japanese people. To circumvent the cultural barrier, the odor stick identification test for Japanese (OSIT-J) was developed and is comprised of 12 odors embedded in microcapsules.[11,12]

Neurodegeneration of the substantia nigra in PD is caused by Lewy bodies that are comprised of abnormally aggregated α-synuclein. Braak et al.[13] reported in 2002 that Lewy bodies start from the dorsal vagal nucleus, and ascend to the pons, the midbrain, the limbic system, and the cerebral cortex. The anterior olfactory nucleus in the olfactory bulb is also the earliest site where Lewy bodies are observed. The formation of Lewy bodies is causally associated with oxidative stress.[14] Urinary excretion of 8-hydroxy-2′-deoxyguanosine (8-OHdG) serves as a marker for oxidative stress, and is increased on average 2.3-fold in PD.[15] We later reported that urinary 8-OHdG is elevated only in PD with hallucinations but not with dementia or other clinical features.[16]

As of 2015, the effects of molecular hydrogen on various diseases have been reported in more than 300 original articles.[17] Reactive oxygen species (ROS)-mediated diseases, inflammatory diseases, and metabolic diseases constituted ~70%, 20%, and 6% of original articles, respectively.[17] In these studies,

hydrogen was administered by ingestion of hydrogen water in ~25% of original articles, inhalation of hydrogen gas in ~20% of original articles, and by other methods in ~55% of original articles (intraperitoneal injection of hydrogen-rich saline, bathing in hydrogen-rich water, *etc*.).[17] We previously reported that drinking hydrogen-rich water almost normalized a rat model of 6-hydroxydopamine-induced hemiparkinsonism.[18] Fujita et al.[19] similarly reported a marked effect of drinking hydrogen-rich water in a mouse model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced PD. We later reported that intermittent inhalation of 2% hydrogen gas for 15 minutes at a 1-hour interval from 6 p.m. to 6 a.m. for 5 weeks ameliorated a rat model of 6-hydroxydopamine-induced hemiparkinsonism, whereas continuous inhalation of 2% hydrogen gas for 24 hours a day for 5 weeks had no effect.[20] A randomized, double-blinded, placebo-controlled, parallel-group clinical pilot study revealed that drinking 1000 mL/d of hydrogen water for 48 weeks significantly ameliorated total Unified Parkinson's Disease Rating Scale (UPDRS) scores in PD patients.[21] The effect of inhalation of hydrogen gas has not been examined in PD patients, but is reported in patients having percutaneous coronary intervention[22] and in patients with acute cerebral infarction.[23]

Here we examined the effect of inhalation of hydrogen gas on hyposmia, non-motor symptoms, activities of daily living (ADLs), and urinary excretion of 8-OHdG. We found that although short-term inhalation of hydrogen gas did not affect any clinical parameters, it significantly increased urinary 8-OHdG.

Subjects and Methods

Subjects

The study was approved by the ethics review committee of Nagoya University Graduate School of Medicine (approval number 2015-0295). The clinical trial was registered at the University Hospital Medical Information Network (identifier UMIN000019082). This study follows the Consolidated Standards of Reporting Trials (CONSORT) guidelines. Written informed consent was obtained from 20 PD patients (**Additional Table 1**). Parkinsonism other than PD was excluded. All PD patients were medicated with levodopa, and no drug was changed during the study.

Procedures

A randomized, double-blinded, placebo-controlled, crossover trial was performed (**Figure 1**). Twenty PD patients were evenly divided into two groups by one of the authors, AY. At baseline, we examined olfactory function, non-motor symptoms, and ADLs, as stated below. We also collected urine for measuring 8-OHdG. Either a true or placebo hydrogen-producing machine was sent to the patient's home and then returned after 4 weeks. The treated group received a true machine, whereas the control group received a placebo machine. After 4 weeks of inhalation (10 minutes twice per day), the same clinical and laboratory markers were examined. After an 8-week washout period, the true and placebo machines were switched, and the markers were examined before and after the 4 weeks of inhalation (10 minutes twice per day). The key was open to evaluators after the crossover trial was finished.

Hydrogen-producing machine and inhalation of hydrogen gas

The true hydrogen-producing machine generated 3.0–3.5% hydrogen gas in 2 L/min of mixed air by electrolysis. Assuming that the PD patients inhaled 5 L/min of air, 3.0–3.5% hydrogen in 2 L/min of air would equate to 1.2–1.4% hydrogen-air mixture. The placebo machine was made by disconnecting an electrode for electrolysis, but still produced 2 L/min of air using the air pump. The true and placebo machines could not be differentiated without a hydrogen gas-detecting device. We previously reported that intermittent inhalation of 2% hydrogen gas for 15 minutes, 12 times a day, but not continuous inhalation of 2% hydrogen gas, improved motor deficits in a rat model of 6-hydroxydopamine-induced hemiparkinsonism.[20] However, the improvement by inhalation of hydrogen gas for 15 minutes, 12 times a day, was not as effective as that by *ad libitum* administration of hydrogen water.[18,20] To simulate a temporal profile of hydrogen concentrations akin to drinking hydrogen water, we instructed participants to inhale the gas produced by the true or placebo machine for 10 minutes in the morning and 10 minutes in the evening using a nasal cannula for 4 weeks. We confirmed after the 4 weeks that all participants complied with the instruction.

Odor examination

In the OSIT-J (Daiichi Yakuhin Sangyo Ltd., Tokyo, Japan), test odorants were microencapsulated and contained within an odorless solid cream dispensed in a lipstick container.[12] The odorant was applied to a strip of paraffin paper within a 2-cm diameter circle. The paper strip was folded with the odorant inside, and rubbed together to release the odorant. The total number of correct answers for the 12 odorants made the OSIT-J score. Correct answers were not disclosed to the subjects after the examination.

Measurement of urinary 8-hydroxy-2′-deoxyguanosine

Urinary samples were kept at –20˚C before analysis. Concentrations of urinary 8-OHdG were measured by the New 8-OHdG Check ELISA (Nikken SEIL, Tokyo, Japan), and

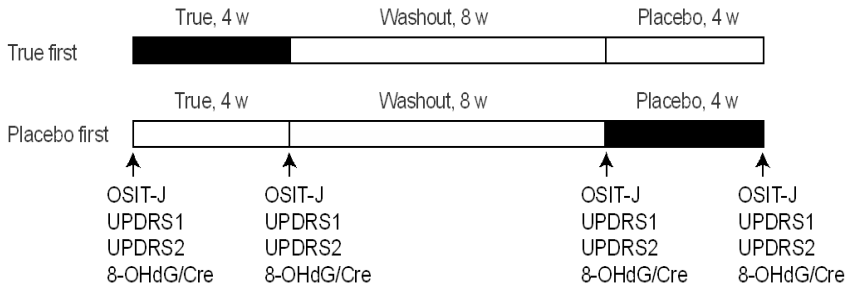

Figure 1: Protocol for randomized, double-blinded, placebo-controlled, crossover trial of inhalation of true and placebo hydrogen gas for 4 weeks.
Note: The odor stick identification test for Japanese (OSIT-J), the Unified Parkinson's Disease Rating Scale scores 1 and 2 (UPDRS1 and UPDRS2), and quantification of urinary 8-hydroxy-2′-deoxyguanine (8-OHdG) normalized for urinary creatinine (Cre) were performed at the indicated time points. w: Weeks.

were normalized by concentrations of urinary creatinine that was measured by the Creatinine (urinary) Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI, USA).

Unified Parkinson's Disease Rating Scale scores

We used UDPRS scores 1 and 2 for quantitative evaluation of clinical severity of PD. UPDRS1 was a rank score for psychomotor functions and mood. UPDRS2 was a rank score for evaluating ADLs.

Statistical analysis

The data were analyzed using JMP Pro 13 (SAS Institute, Cary, NC, USA). *P*-values less than 0.05 by Student's paired *t*-test were designated as having statistical significance. The statistical significance of differences was analyzed using one-way analysis of variance (ANOVA).

Results

A randomized, double-blinded, placebo-controlled, cross-over trial of inhalation of ~1.2–1.4% hydrogen-air mixture or placebo for 10 minutes twice a day for 4 weeks in 20 PD patients revealed that OSIT-J ($P = 0.77$), UPDSR1 ($P = 0.84$), and UPDRS2 ($P = 0.15$) were not changed by hydrogen gas (**Table 1**). In contrast, inhalation of hydrogen gas for 4 weeks increased urinary excretion of 8-OHdG by 16% with statistical significance ($P = 0.02$) (**Table 1** and **Figure 2**).

Discussion

Molecular hydrogen is the smallest molecule in the universe and has the highest diffusibility.[24] Inhaled hydrogen gas readily enters the blood stream through the alveoli.[20,25] The 1.2–1.4% hydrogen-air mixture inhaled by PD patients would increase the hydrogen concentrations at the olfactory bulbs and the alveoli by 9.4–10.9 μM according to Henry's law. Inhalation of hydrogen gas generally elevates hydrogen concentrations more than ingestion of hydrogen water. Drinking 200-mL saturated hydrogen water (0.8 mM)[20] and 530-mL hypersaturated hydrogen water (2.5 mM)[26] increased the hydrogen concentrations in the organs by ~0.016 μM and ~0.21 μM, respectively. The hydrogen concentration from either inhaling or drinking returns to baseline in ~30 to 60 minutes.[20,25,26]

Inhalation of hydrogen gas had no effect on olfactory function, non-motor symptoms, and ADLs. Marginal improvement of OSIT-J scores of 0.15 (3.2%) by hydrogen was likely to represent a high variability of OSIT-J scores. The lack of the effect of hydrogen inhalation on olfactory function, non-motor symptoms, and ADLs may be due to a short trial period, short inhalation time (10 minutes twice a day), and/or low hydrogen concentration (1.2–1.4% hydrogen). Alternatively, these clinical features may not be sensitive enough to detect the marginal effects, if any, of hydrogen inhalation. In contrast to the lack of an improvement of clinical features, inhalation of hydrogen gas significantly increased urinary 8-OHdG levels, suggesting

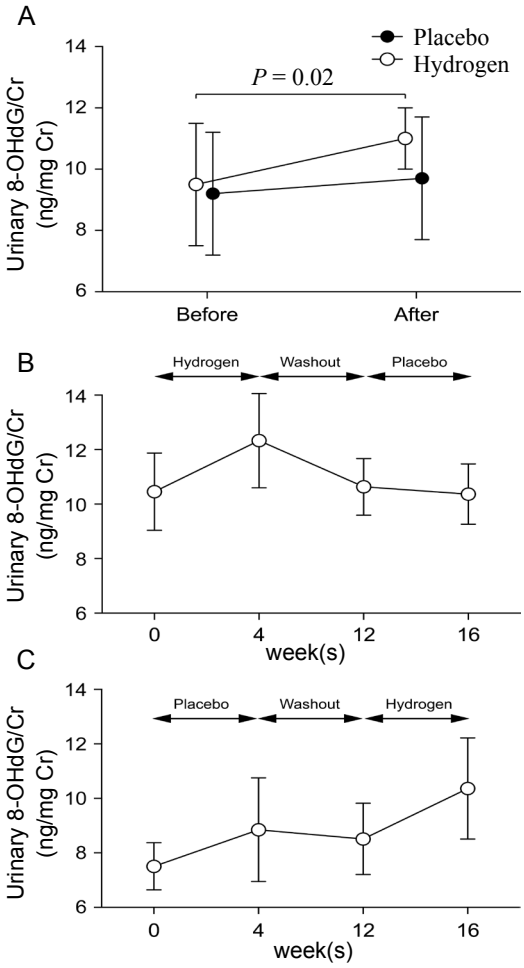

Figure 2: Urinary 8-OHdG/Cr before and after inhalation of true and placebo hydrogen gas for 4 weeks.
Note: (A) Twenty Parkinson's disease (PD) patients. *P* value by Student's paired *t*-test is indicated. (B) Ten PD patients who inhaled hydrogen first. (C) Ten PD patients who inhaled placebo first. (B, C) No statistical significance by one-way analysis of variance. Date are expressed as the mean ± SE. 8-OHdG: 8-Hydroxy-2′-deoxyguanine; Cr: creatine.

Table 1: Metrics before and after inhalation of true and placebo hydrogen gas for 4 weeks

	Hydrogen			Placebo		
	Before	After	*P*	Before	After	*P*
OSIT-J	4.7±2.0	4.85±1.9	0.77	4.55±2.4	4.6±2.2	0.92
UPDRS1	2.2±1.6	2.3±1.9	0.84	2.7±2.4	2.4±1.9	0.41
UPDRS2	13.7±9.0	15.9±7.0	0.15	16.6±9.1	16.8±6.3	0.93
8-OHdG/Cr (ng/mg Cr)	9.5±9.7	11.0±5.9	0.02	9.2±6.9	9.7±6.8	0.59

Note: Date are expressed as the mean ± SD. *P* values are calculated by paired *t*-test. 8-OHdG: 8-Hydroxy-2′-deoxyguanine; Cr: creatine; UPDRS: Unified Parkinson's Disease Rating Scale.

that hydrogen increased production of ROS. The 16% increase of 8-OHdG by hydrogen, however, is much less compared to the 53% increase by smoking 15–20 cigarettes per day,[27] 63% increase in prostate cancer,[28] 95% increase in bladder cancer,[28] 335% increase in non-insulin-dependent diabetes mellitus,[29] and the 355% increase in insulin-dependent diabetes mellitus.[29] Similarly, 30 km/d running for 8 days increases urinary 8-OHdG by 26%,[30] and 60-minute exercise at 70% of maximal O_2 uptake increases urinary 8-OHdG by 276%.[31] In contrast to disease-associated increase in urinary 8-OHdG, the exercise-mediated increase of 8-OHdG is returned to the normal level in 2 days.[31] Exercise-induced ROS[32] and inflammation[33] play important roles in mediating the benefits and cellular adaptations of exercise. Although we did not analyze the temporal profile of the 8-OHdG surge in our patients, rapid dissipation of hydrogen from our body[20] implies that patients were temporarily exposed to ROS. Thus, inhalation of hydrogen gas for 10 minutes in our patients might have mimicked a short and mildly strenuous exercise in regard to ROS exposure.

Ohsawa et al.[34] reported in 2007 that molecular hydrogen reduces hydroxyl radicals and, to a lesser extent, peroxynitrite. However, significant beneficial effects from a radical-scavenging activity of hydrogen are unlikely to occur in our body, because (i) the concentration of hydrogen is too low compared to those of nucleophilic biomolecules, (ii) hydrogen rapidly dissipates from our body, mostly in the breath, and returns to baseline in ~30 minutes,[20,25] (iii) the reaction rate constant between hydrogen and hydroxyl radical is 4.2×10^7 M/s, which is three orders of magnitude slower than most reactions with hydroxyl radicals,[35] and (iv) intestinal bacteria produces 12 L of hydrogen in a day by metabolizing only 40 g carbohydrate,[36,37] which constantly yields on average 10 ppm (2–12 ppm) of hydrogen in our breath.[20,26,38,39]

Hydrogen has been reported to decrease urinary 8-OHdG,[26] and tissue malondialdehyde, a marker of lipid peroxidation.[40] However, in our study, inhalation of hydrogen gas mildly increased ROS production as measured by 8-OHdG. Mild increases in ROS production may provide beneficial effects by evoking hormesis. Hormesis is a physiological process, in which mitochondrial stress activates cytosolic signaling pathways to make the cells less susceptible to oxidative damage.[41] Indeed, exercise-induced ROS production plays critical roles in mediating the benefits and cellular adaptations of exercise training. Consequently, ingestion of antioxidants has been reported to blunt/impair exercise training benefits.[42,43] Perhaps a mild and transient increase in ROS production from H_2 administration may similarly be beneficial. This hypothesis is corroborated by several other studies demonstrating that hydrogen administration increases ROS production, and are accompanied by a beneficial effect. For example, in young athletes, strenuous exercise increased the blood level of derivatives of reactive oxidative metabolites, and administration of hydrogen water mildly potentiated its increase while also suppressing the elevation of blood lactate and improving exercise-induced decline of muscle function.[44] Similarly, in a mouse model of a surgically injured brain, hydrogen increased malondialdehyde in the brain while also improving cerebral edema and the neurobehavioral score.[45] Likewise, inhalation of 2.9% hydrogen gas for 2 hours increased malondialdehyde in normal mouse brain ~4-fold.[46] In Arabidopsis, hydrogen-rich water increased ROS production, which in turn induced drought tolerance.[47] Lastly, Murakami et al. recently reported that hydrogen increases the mitochondrial membrane potential, and the production of superoxide radicals in SH-SY5Y cells.[48] They also demonstrated that hydrogen-induced oxidative stress activates the Nrf2 anti-oxidative pathway, and proposed that hydrogen is a mitohormetic effector. Hydrogen-mediated activation of the Nrf2 signaling pathway has been repeatedly reported in different disease models,[48-56] and no effect of hydrogen has been observed in Nrf2-knockout mice.[50] Hormesis may also be involved in contributing to the protective effect of hydrogen on a mouse model of ventilator-induced lung injury, where hydrogen enhanced DNA binding of NF-κB in the first hour followed by its suppression in the second hour.[57] Similarly, the neuroprotective effect of hydrogen in a model of subarachnoid hemorrhage in rabbits was associated with hydrogen-mediated activation of the NF-κB/Bcl-xL pathway.[58] NF-κB activation in the early phase may have been induced by hydrogen-induced increase in ROS production as the hormetic mediator. We also reported by meta-analysis of gene-expression profiles of normal rodent livers that hydrogen induces heat-shock response, and subsequently leads to transient arrest of cell cycles and upregulation of collagen biosynthesis,[59] which may also be a form of hormesis. Considering that the primary and long term benefits of hydrogen are unlikely due to it acting as a radical scavenger in our bodies, the mitohormetic response is an attractive and a feasible mechanism to explain the effect of hydrogen.

Conclusion

We observed that inhalation of ~1.2–1.4% hydrogen-air mixture for 10 minutes twice a day for 4 weeks increased urinary 8-OHdG levels by 16%, which was markedly less than the over 300% increase in diabetes, and was more comparable to the increase after a bout of strenuous exercise. Increases of oxidative stress by hydrogen have been previously reported, along with its ability to activate the Nrf2, NF-κB pathways, and heat shock responses. Although we did not observe any beneficial effect of hydrogen in our short trial, we propose that the increased 8-OHdG and other reported stress responses from hydrogen indicate that its beneficial effects are partly or largely mediated by hormetic mechanisms.

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Author contributions

Performing clinical studies: MH and TM; analyzing urinary 8-OHdG levels: MI and TM; patient assignment: AY; writing the manuscript: MH, TWL, and KO.

Conflicts of interest

There is no conflict of interest.

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Institutional review board statement

The study was approved by the ethics review committee of Nagoya University Graduate School of Medicine (approval number 2015-0295), and was registered at the University Hospital Medical Information Network (identifier UMIN000019082).

Declaration of patient consent

The authors certify that they have obtained patient consent forms. In the form, patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Reporting statement

This study follows the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Biostatistics statement

The statistical methods of this study were reviewed by the biostatistician of the Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan.

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Data sharing statement

Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices). Study protocol and informed consent form will be available immediately following publication, without end date. Results will be disseminated through presentations at scientific meetings and/or by publication in a peer-reviewed journal. Anonymized trial data will be available indefinitely at www.figshare.com.

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Additional file

Additional Table 1: Demographic features of participants.

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Additional Table 1: Demographic features of participants

Item	Data
Number of participants (Male/female)	20 (8/12)
Age (years)	69.0±6.8
Hoehn and Yahr score	2.3±0.8
OSIT-J score at baseline	4.7±2.2
UPDRS1 score at baseline	2.2±1.7
UPDRS2 score at baseline	13.7±9.2

Note: Date as expressed as the mean ± SD except number of participants. 8-OHdG: 8-Hydroxy- 2'-deoxyguanine; Cr: creatine. UPDRS: Unified Parkinson's Disease Rating Scales.