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Research Article
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Blood Pressure-Lowering Effect of Daily Hydrogen Gas Inhalation in Spontaneously Hypertensive Rats

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Abstract

Objective: Hydrogen (H_2) is a unique inert gas that has versatile effects including anti-oxidative and anti-inflammatory properties, in many diseases and conditions. A recent study demonstrated that H_2 inhalation attenuates hypertension by ameliorating the dysregulation of the autonomic nervous system in a rat model of chronic kidney disease. The aim of this study was to confirm the blood pressure-lowering effect of H_2 inhalation in other models of hypertension which the pathophysiology is the overactivity of the sympathetic nerve in the brain.

Methods: We examined whether H_2 has a salutary effect on hypertension in spontaneously hypertensive rats (SHR). We subjected SHR to inhalation of either an H_2 (1.3% H_2+21% O_2+77.7% N_2) or control (21% O_2+79% N_2) gas mixture for 1 h every day for 2 weeks (N=3, each group). Blood pressure and heart rate were measured weekly for a total of 3 weeks: during 2 weeks of H_2 inhalation and then for 1 week without H_2 inhalation, using the telemetry system in freely moving rats.

Results: In SHR, daytime blood pressure and heart rate increased over the time, but daily 1-h H_2 inhalation suppressed these changes (P=0.001, P=0.03, respectively). The effect of lowering blood pressure and heart rate was associated with the suppression of sympathetic overactivity and restoration of parasympathetic underactivity (P=0.02). The therapeutic effect of H_2 on the autonomic imbalance was also observed at night, and this effect persisted even 1 week after the discontinuation of H_2 inhalation (P=0.006).

Conclusion: Daily 1-h H_2 inhalation has therapeutic effects on autonomic imbalance and hypertension in SHR.

Keywords: H_2 inhalation; Pathophysiology; Hypertension; Therapeutic effect

Key Questions

What is already known about this subject?

Haemodialysis using H_2-containing dialysate reduces post-dialysis high blood pressure in patients with end-stage renal disease. In a rat hypertension model of chronic kidney disease, daily 1-h H_2 inhalation lowers blood pressure by restoring autonomic imbalances.

What does this study add?

Daily H_2 inhalation for 2 weeks lowers blood pressure in spontaneously hypertensive rats by attenuating autonomic imbalances. The blood pressure-lowering effect of inhaled H_2 was conserved among different models of hypertension. The effect of inhaled H_2 on autonomic balance remained 1 week after discontinuation of H_2 inhalation.

How might this impact on clinical practice?

We confirmed the blood pressure-lowering effect of H_2 in another animal model of hypertension, which serves as a counterpart for clinical essential hypertension. This unique antihypertensive effect of H_2 that possibly occurs through attenuation of autonomic imbalances in the central nervous system warrants further investigation in humans.

Introduction

Hypertension is a leading risk factor of cardiovascular and cerebrovascular diseases and is expected to affect approximately 29% of the worldwide population by 2025 [1]. Hypertension is the top modifiable risk factor for disability-adjusted life years lost worldwide [2,3]. The current treatment strategy for hypertension includes primary prevention through lifestyle modifications complemented by antihypertensive pharmaceutical treatments. However, more than 60% of patients with hypertension fail to achieve the target blood pressure with antihypertensive treatments [4].

Molecular hydrogen (H_2) is an inert gas with versatile effects, including antioxidative and anti-inflammatory properties [5]. H_2 has several unique features, including high diffusivity owing to the low molecular weight, no obvious side effects, and a variety of administration routes such as inhalation, drinking water, and intravenous administration [5,6]. H_2 inhalation is a promising therapeutic option in animal models of ischaemia-reperfusion injury such as acute myocardial infarction (AMI), cardiac arrest, and haemorrhagic shock [6-9]. We further conducted clinical translational studies and successfully validated the efficacy and safety of H_2 gas inhalation therapy in patients with ST-elevation myocardial infarction and post-cardiac arrest syndrome [10,11]. A large-scale randomised control study evaluating the efficacy of H_2 in post-arrest brain

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injury is underway [12]. Recently, haemodialysis with H_2-enriched dialysate improved the post-dialysis blood pressure compared to standard dialysate in patients with end-stage renal disease [13,14]. In response to these studies, we performed a reverse-translational study investigating the mechanism by which H_2 exerts an anti-hypertensive effect in a rat model of chronic kidney disease (CKD) [15]. We confirmed that inhalation of H_2 does not improve renal function after 5/6 nephrectomy, but that H_2 inhalation has a therapeutic effect on hypertension associated with renal dysfunction. However, whether this blood pressure-lowering effect of H_2 is conserved in other models of hypertension is not clear. Herein, we evaluated the anti-hypertensive effect of H_2 in spontaneously hypertensive rats (SHR). SHR is a well-established genetic hypertension model in which the overactivity of sympathetic nerve activity in the central nervous system plays a pivotal role in the pathogenesis of hypertension [16,17].

Materials and Methods

Animals

Male 10-week-old male SHR/Izumo rats were used for the experiments (CLEA Japan, Tokyo, Japan). Animals were fed standard chow ad libitum with free access to water and were not fasted before the experiments. Animals were housed under standardised temperature (22 ± 1°C) and humidity (55 ± 5%) conditions with a 14-h:10-h light:dark period. The rats were acclimatised to the above-mentioned conditions for at least 1 week before the experiments. Allocation to the H_2 and control groups was performed randomly. The study was approved by the Institutional Animal Care and Use Committee (Nippon Veterinary and Life Science University [Tokyo, Japan], No. 30K-61; and Keio University [Tokyo, Japan], No. 13002-4). All animal experiments were performed per ARRIVE guidelines [18].

The sample size calculation for this study was based on t-test for testing the difference in the daytime mean blood pressure. The effect size was determined as 2.0 by our preliminary experiment showing mean blood pressure (mean ± SD) of 160 ± 10 and 140 ± 10 mm Hg in the control and H_2 groups, respectively. A study with an effect size of 2.0 and a power of 80% will require a total sample of 6 to test the difference at 5% levels using one tailed test. The power calculation was performed using G*Power 3.1.9.

Gas inhalation

H_2 or control gas was inhaled using a previously established system [15]. Briefly, a rat was placed in an anaesthetic box and gas was injected at a rate of 10 L/min into the box from gas cylinders. Gas cylinders were filled with premixed H_2 (1.3% H_2+21.0% O_2+ 77.7% N_2; H_2 group) or control gas (21.0% O_2+79.0% N_2; control group) (Taiyo Nippon Sanso Corporation, Tokyo, Japan). The day of initiation of gas inhalation was defined as day 0. The treatment was repeated approximately at the same time of the day during the light period for an hour, every day for 2 weeks followed by a one-week observational period without the gas inhalation.

Implantation of telemetry transmitter

Rats underwent a telemetric transmitter (HD-S10, Physiotel HD Telemetry, Data Science International, St. Paul, MN, USA) implantation one week before day 0 using the previously described method [15,19].

Non-invasive continuous haemodynamic monitoring

Blood pressure and heart rate were continuously monitored using a telemetry system (Ponemah Ver. 6.3, Data Science International, St. Paul, MN, USA) as previously described [15]. Briefly, during the light period, 1-h pressure waveform data were obtained at 6 h after the gas inhalation, weekly from week 0 to week 2. On week 3, 1-h pressure waveform data were obtained without the gas inhalation. During the dark period, pressure waveform data were recorded throughout the dark period on weeks 0 (before the first gas inhalation), 2, and 3. The 1-h pressure waveform data during middle of the dark period were used for analyses. Of the recorded 1 h data, data from first 15 min were discarded, and the remaining 45-min data were divided into 5-min blocks. The first 1 min of measurement without body movement and any parts missing data due to loss of signal were used.

Spectral analysis of arterial blood pressure variability

Arterial blood pressure variability was analysed using telemetry system software (Ponemah Ver. 6.3, Data Science International, St. Paul, MN, USA) as previously described [15]. Frequency domain analysis (500 Hz sampling rate; very low frequency [VLF], 0.05 Hz-0.25 Hz; LF, 0.25 Hz-1.0 Hz; HF, 1.0 Hz-3.0 Hz) was performed using arterial pressure waveform. The same pressure waveform data used for haemodynamic parameters analyses were used.

Statistical analysis

Descriptive statistics are presented as mean ± standard error. Comparisons of two groups were conducted using paired t-test. The mixed-effect model was used to analyse repeated measures. All tests were two-tailed, and a P-value <0.05 was considered as statistically significant. All statistical analyses were conducted using GraphPad Prism 9.0.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Anti-hypertensive effect of H_2 in SHR during light period

One week after the telemetry transmitter insertion, 10-week-old SHR were randomly divided into H_2 and control groups (N=3 in each group). Each rat was subjected to daily 1-h gas treatment for 2 weeks (Figure 1). The mean blood pressure 6 h after the gas inhalation in the control group was 142.4 ± 3.3 mm Hg on day 0, which was the first day of the gas inhalation, and 169.9 ± 0.9 mm Hg after 2 weeks (Figure 2A). In contrast, in the H_2 group, the mean blood pressure was 153.3 ± 4.4 mm Hg on day 0 and 151.8 ± 1.9 mm Hg after 2 weeks. H_2 suppressed the rise in mean blood pressure over time in SHR of the H_2 group in comparison to that in the control group (P=0.001). After the termination of daily gas inhalation at 2 weeks, SHR were housed without gas inhalation for observation of haemodynamic changes. One week after the termination of gas inhalation, their blood pressure was compared. The mean blood pressure was lower in the H_2 group (136.9 ± 2.6 mm Hg) than in the control group (151.4 ± 4.8 mm Hg); however, the difference was marginally significant (P=0.06).

Similar therapeutic effects of H_2 on the blood pressure were also observed for the heart rate. Namely, in the control group, the heart rate was 298.8 ± 3.7 beats per minute (bpm) on day 0 and 338.3 ± 4.9 bpm after 2 weeks. In contrast, their respective values were 301.1.3 ± 7.2 bpm and 296.5 ± 7.6 bpm in the H_2 group. Daily 1-h H_2 inhalation suppressed the increase in heart rate over time in SHR of the H_2 group in comparison to that of the control group (P=0.03) (Figure 2B). One week after the discontinuation of gas inhalation, the heart rate was 302.5 ± 9.8 bpm in the control group and 269.7.9 ± 8.5 bpm in the hydrogen group, with the hydrogen group showing a trend towards a lower heart rate than the control group (P=0.06).

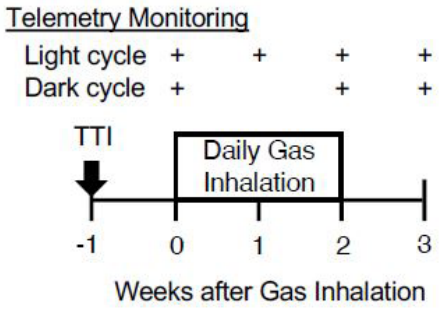

Figure 1: Schematic illustration of the experimental protocol. A telemetric transmitter was implanted in spontaneously hypertensive rats (SHR) 1 week before the first day of gas inhalation (week 0). SHR were randomly divided to the H_2 (1.3% H_2+21% O_2+77.7% N_2, N=3) and control (21% O_2+79% N_2, N=3) groups. Intermittent 1-h daily gas inhalation was repeated for 2 weeks, and SHR were housed without gas inhalation for another 1 week. The ambulatory haemodynamic parameters were recorded weekly for an hour using the telemetry system. During the light period, haemodynamic recording was performed 6 h after the gas inhalation, weekly from 0 to 2 weeks, and at the same time of the day without a gas inhalation on week 3. For the dark period, data were recorded between 1:30 AM and 2:30 AM on weeks 0,2, and 3. The week 0 data for dark period were recorded before the first gas inhalation. TTI, telemetric transmitter implantation.

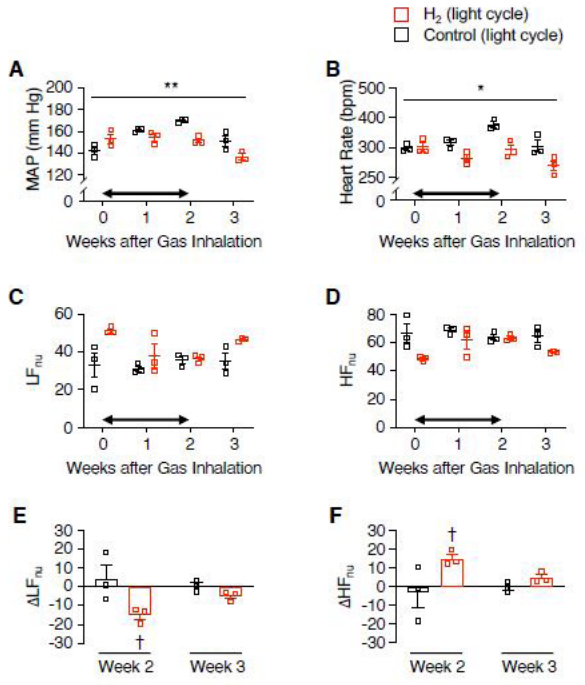

Figure 2: H_2 attenuates the elevation of mean blood pressure and heart rate during light period in spontaneously hypertensive rats. A) Mean blood pressure, B) Heart rate, C) Normalized LF power (LFnu), D) Normalized HF power (HFnu), E) Delta change of LFnu from week 0 to week 2 and week 3, F) Delta change of HFnu from week 0 to week 2 and week 3. LF power and HF power indicate the sympathetic nerve activity and parasympathetic nerve activity, respectively. Double arrow indicates the period of the daily gas inhalation. Data are shown as mean ± SE (N=3, each group). *p<0.05; A and B, mixed effect model, **p<0.01; E and F, mixed effect model; †p<0.05; paired-t test between week 0 and week 2 of respective group.

Next, we quantitatively examined the effects of H_2 on autonomic nervous system function by using blood pressure variability. In agreement with the high blood pressure and heart rate in the H_2 group compared to those in the control group on day 0, sympathetic nerve activity was higher and parasympathetic activity was lower in the H_2 group than in the control group (Figures 2C and 2D). After 2 weeks of H_2 inhalation, sympathetic nerve activity was suppressed, and parasympathetic nerve activity was augmented in the H_2 group compared to day 0 (P=0.02). These changes were not evident in the control group. The salutary effect of H2 on the autonomic nervous system was no longer apparent 1 week after the discontinuation of H_2inhalation.

Anti-hypertensive effect of H_2 in SHR during dark period

Next, we examined the effects of H_2 on the blood pressure and heart rate in SHR during the dark period. Temporal variations in blood pressure and heart rate during the dark period were analysed at three points: before starting inhalation treatment, after 2 weeks of treatment, and 1 week after its discontinuation (Figure 3A). There were no significant changes in the temporal variability of the mean blood pressure between the two groups (H_2 group: 146.2 ± 3.8 mm Hg and 167.8 ± 2.8 mm Hg; control group: 148.8. ± 1.5 mm Hg and 171.7 ± 5.9 mm Hg at day 0 and 2 weeks, respectively) (Figure 3B). Moreover, the mean blood pressure 1 week after the discontinuation of gas inhalation was not significantly different between the two groups (H_2 group: 158.9 ± 2.1 mm Hg vs. control group: 163.9. ± 2.3 mm Hg).

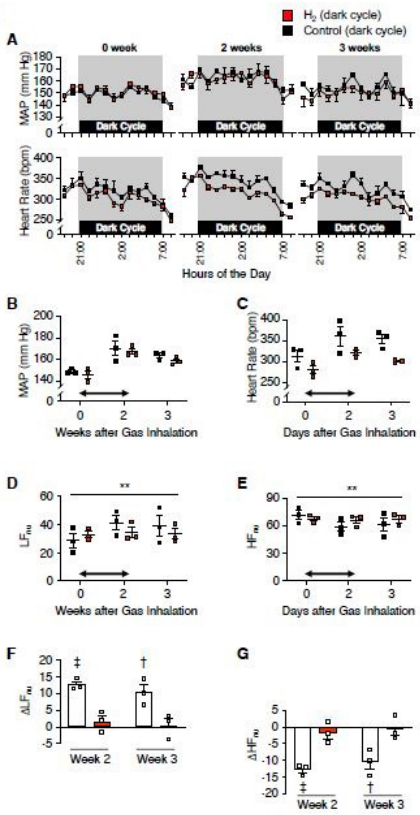

Figure 3: H2 balances the autonomic nerve activity during the dark period. A) Hourly plot of mean blood pressure and heart rate during the dark period. B)-E) weekly haemodynamic data during 1:30 AM-2:30 AM. B) Mean blood pressure, C) heart rate, D) normalized LF power (LFnu), E) normalized HF power (HFnu). Data are shown as mean ± SE (N=3, each group). D and E, mixed effect model; **P<0.01.

However, there was a tendency for H_2 to suppress the increase in heart rate compared to the control group (H_2 group: 281.2 ± 9.5 bpm and 321.8 ± 5.7 bpm; control group: 314.6 ± 14.5 and 348.9 ± 34.8 bpm at day 0 and 2 weeks, respectively; P=0.49) (Figure 3C).

Spectral analysis results of blood pressure variability at these three points were compared between the two groups. During the 2-week gas inhalation period, sympathetic nerve activity increased, and parasympathetic nerve activity decreased in the control group, but these changes were significantly suppressed in the H_2 group (Figures 3D-3G).

In the dark period, the inhibitory effect of H_2 inhalation on sympathetic overactivity and parasympathetic underactivity remained even 1 week after the inhalation was stopped. Accordingly, there was significant suppression of the sympathetic nerve hyperactivity and augmentation of parasympathetic nerve activity (P=0.006) for 3 weeks.

Discussion

This study revealed that the daily inhalation of 1.3% H_2 for 2 weeks in SHR significantly improved the autonomic imbalances and suppressed the increase in blood pressure and heart rate. These results are in line with the 5/6 nephrectomy model higjlighting the universal antihypertensive effect of H_2 inhalation therapy in different models of hypertension.

Dysregulation of the autonomic nervous system, namely, sympathetic overactivity and parasympathetic underactivity, plays a central role in the pathogenesis of essential hypertension [20,21]. In animal research, SHR are widely used as an animal model of primary or essential hypertension [16,17,22]. H_2 inhalation for 1 h per day improved sympathetic hyperactivity and parasympathetic underactivity in SHR. Notably, the therapeutic effect of H_2 on autonomic imbalance was observed both in the light and dark periods. Moreover, even when H_2 inhalation was terminated after 2 weeks, the therapeutic effect of H_2 on autonomic imbalance was sustained for at least another 1 week, especially in the dark period.

Previous studies have shown that oxidative stress and inflammation in the brain can increase sympathetic output from the brain, resulting in excessive sympathetic activation throughout the body [23,24]. H_2 is known to have antioxidative and anti-inflammatory properties [5,25]. We recently reported that inhaled H_2 is transported to the whole body by blood flow rather than by simple diffusion, and that H_2 concentration in the carotid artery increases immediately after a single inhalation of H_2 in a porcine experimental model [26]. This pharmacokinetic data clearly support the notion that inhalation is an efficient method of delivering H_2 to the brain. In the 5/6 nephrectomy model, a rodent model of hypertension which mimics human CKD, it is possible that H_2 acts directly on the injured kidneys to suppress the excitation of renal afferent nerves, thereby suppressing subsequent sympathetic nerve output from the brain. In contrast, in SHR, H_2 likely acts directly on the brain to suppress sympathetic output from the vasomotor centre. H_2 may lower blood pressure by suppressing the sympathetic nerve output from the brain.

Hypertension is one of the greatest risk factors of cardiovascular disease, yet merely lowering blood pressure is not sufficient to prevent cardiovascular death [27]. The underlying dysregulation of the autonomic nervous system increases the risk of developing cardiovascular diseases through metabolic, hemodynamic, trophic, and rheologic (thrombotic) abnormalities [28]. The increase in heart rate in patients with hypertension is precisely the result of autonomic imbalance [28]. H_2 can restore the autonomic imbalance in SHR, lower the heart rate, and lower the blood pressure. In this respect, H_2 inhalation is an ideal therapeutic method for hypertension. Our study has several limitations. We used the same H_2 concentration (1.3%) as our recent study [15]. While this was a sufficiently high concentration to test consistency in the anti-hypertensive effect of inhaled H_2 in another model of hypertension, future dose-response studies should be conducted to reveal the optimal H_2 concentration for treating hypertension. Likewise, an optimal duration of daily H_2 inhalation needs to be further explored. Since this is an animal model study, results cannot be generalised to humans. Hence, a clinical translational research is needed.

Conclusion

In conclusion, daily H_2 inhalation therapy lowered the blood pressure in a rat model of genetic hypertension and prevented the overactivation of sympathetic nervous activity. Clinical studies are warranted to verify whether H_2, alone or in combination with antihypertensive drugs, can reduce the number of patients with poorly controlled hypertension and improve clinical outcomes.

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Author Contributions

M. Sano, E.K., and Y.H. conceived the experiments; K.S., T.T., S.U., M.F., E.K., and Y.H. conducted the experiments; K.S. and T.T. analysed the results; M. Sano and Y.H. supervised the experiments and analyses. T.T. and M. Sano wrote the manuscript; and J.Y., Y.K., J.E., K.H., M. Suzuki, M. Sano, J.S., and Y.H. critically revised the manuscript. All authors reviewed the manuscript and consented to its publication.

Competing Interests

M. Sano receives advisory fees and commissioned research fees from Taiyo Nippon Sanso Co., Ltd. And Doctors Man Co., Ltd.; M. Suzuki received advisory fees and commissioned research fees from the Taiyo Nippon Sanso Co., Ltd. E.K receives advisory fees and commissioned research fees from Doctors Man Co., Ltd. The other authors declare no competing interests.

Data Availability

All data are available upon reasonable request to the corresponding author.

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