Molecular Hydrogen Alleviates Cellular Senescence in Endothelial Cells

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Background: Substantial evidence indicates that molecular hydrogen (H₂) has beneficial vascular effects because of its antioxidant and/or anti-inflammatory effects. Thus, hydrogen-rich water may prove to be an effective anti-aging drink. This study examined the effects of H₂ on endothelial senescence and clarified the mechanisms involved.

Methods and Results: Hydrogen-rich medium was produced by a high-purity hydrogen gas generator. Human umbilical vein endothelial cells (HUVECs) were incubated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for various time periods in normal or hydrogen-rich medium. The baseline H₂ concentration in hydrogen-rich medium was 0.55±0.07 mmol/L. This concentration gradually decreased, and H₂ was almost undetectable in medium after 12 h. At 24 h after TCDD exposure, HUVECs treated with TCDD exhibited increased 8OHDG and acetyl-p53 expression, decreased nicotinamide adenine dinucleotide (NAD+)/NADH ratio, impaired Sirt1 activity, and enhanced senescence-associated β-galactosidase. However, HUVECs incubated in hydrogen-rich medium did not exhibit these TCDD-induced changes accompanying Nrf2 activation, which was observed even after H₂ was undetectable in the medium. Chrysin, an inhibitor of Nrf2, abolished the protective effects of H₂ on HUVECs.

Conclusions: H₂ has long-lasting antioxidant and anti-aging effects on vascular endothelial cells through the Nrf2 pathway, even after transient exposure to H₂. Hydrogen-rich water may thus be a functional drink that increases longevity.

Key Words: Antioxidant; Endothelial senescence; Molecular hydrogen; Nrf2

Molecular hydrogen (H₂) has no polarity and is not highly reactive and therefore was believed to have no effects on organisms. However, in 2007, it was reported that hydrogen gas inhalation relieved brain ischemiareperfusion injury in rats by selectively removing cytotoxic radicals and hydroxyl radicals (OH). Since then, further research has examined hydrogen gas inhalation and hydrogen-rich water consumption, a simplified method of H₂ intake. A study using apolipoprotein E (ApoE) –/– mice found that hydrogen-rich water intake relieved oxidative stress and significantly reduced atherosclerosis formation by improving lipid metabolism and reducing inflammation in the vascular wall. Furthermore, a clinical study of hydrogen-rich water intake showed that it decreased oxidative stress and improved lipid and glucose metabolism in patients with metabolic syndrome, type 2 diabetes, and impaired glucose tolerance. On the basis of these findings, H₂ is now regarded as a signaling gaseous molecule with physiological activity similar to that of nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H₂S). Animal studies have shown that blood concentrations of orally administered H₂ peak at approximately 15 min and return to baseline levels at 20–30 min after administration. In humans drinking hydrogen-enriched water, H₂ concentrations in exhaled air reach a peak at approximately 10 min and return to baseline levels at 1 h after administration. These findings indicate that although H₂ is only transiently present in the body, it may nevertheless have sustained antioxidant effects. However, the exact mechanisms underlying these effects are unclear.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a family of dioxins — persistent organic pollutants that are widely present in the environment — and has the strongest biological toxicity among dioxins. TCDD accumulates in the human body and is associated with carcinogenicity, teratogenicity, liver disorders, reproductive function disorders, immunotoxicity, and endocrine dysfunction, among other conditions. In addition, recent studies showed that the prevalences of coronary and hypertensive diseases were higher in people who had been exposed to relatively high dioxin concentrations in an accident or at work. Diseases attributable to dioxins, including...
TCDD, might be associated with macrophage activation through aryl hydrocarbon receptor (AhR) activation and subsequent induction of inflammation in blood vessel(s) or with premature cell senescence mediated by production of reactive oxygen species (ROS).1,14

AhR is a ligand-activated transcription factor for which dioxins act as ligands. In its steady state, AhR is localized in the cytoplasm but migrates to the nucleus when it binds to a ligand such as TCDD and forms a complex with AhR nuclear translocator (Arnt) localized in the nucleus. The AhR-Arnt complex binds to a gene xenobiotic response element and induces drug-metabolizing enzymes — including cytochrome P450 (CYP) 1A1 — and expression of a large number of genes, such as those for cytokines and signal transducers.15,16

We previously reported that in vascular endothelial cells, the uremic toxin, indoxyl sulfate (IS), triggers nuclear translocation of AhR by acting as a ligand of AhR, which leads to ROS production. Increased ROS decreases intracellular nicotinamide adenine dinucleotide (NAD+), thereby impairing activation of AhR by nuclear translocator (Arnt) localized in the nucleus. The AhR-Arnt complex binds to a gene xenobiotic response element and induces drug-metabolizing enzymes — including cytochrome P450 (CYP) 1A1 — and expression of a large number of genes, such as those for cytokines and signal transducers.15,16

In light of previous findings, we examined the effects of H2 on human umbilical vein endothelial cell (HUVEC) senescence and the AhR-ROS pathway in relation to time-dependent change in H2 concentration in medium and found that H2 exerts sustained effects despite its transient presence in medium.

**Methods**

**Reagents**

Medium 199, endothelial cell growth supplement, TCDD, CH223191 (CH) and chrysin were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Biological Industries (Kibbutz Beit Haemek, Israel).

**Production of Hydrogen-Rich Medium**

A tank was filled with hydrogen gas to a pressure of 0.4 MPa, and hydrogen-rich medium was then produced by spraying the medium with a high-pressure pump from the hydrogen gas tank at a pressure of 0.8 MPa (TED KK, Toyama, Japan). The saturated hydrogen-rich medium was stored under atmospheric pressure at 4°C in an aluminum bag with no dead volume and was used within 1 week.

**Measurement of Hydrogen Content in Cell Culture Medium**

Briefly, 50 μl of medium was injected into a 10-ml glass container with a lid, and H2 levels in the gas phase (1 ml) were determined using gas chromatography with a semiconductor detector (TRI lyzer™ MBA-3000; Taiyo KK, Osaka, Japan).

**Cell Culture**

HUVECs were purchased from Lonza Walkersville Inc. (Walkersville, MD, USA) and cultured in a type I collagen-coated plate (Asahi Glass, Tokyo, Japan) with medium 199 supplemented with 10% FBS, 10mmol/L glutamine, 100 μg/ml heparin, 20 μg/ml endothelial growth factor, 100 μg/ml gentamicin, and 100 μg/ml amphotericin B in 5% CO2 at 37°C. The cells were used for experiments between passages 4 and 7.

HUVECs passaged 4 to 7 times were cultured in type I collagen-coated culture flasks with a surface of 25 cm2 (Asahi Glass, Tokyo, Japan). When HUVECs reached confluence, the cells were pretreated for 1h in normal or hydrogen-rich medium and then incubated with TCDD (1nmol/L) for 1, 6, 24, or 48h. To determine the underlying mechanism responsible for the effects of H2, cells were pretreated for 1h with CH (10nmol/L) or chrysin (10 μmol/L) and then incubated with TCDD (1nmol/L) for 1, 24, or 48h. After incubation, total RNAs, DNA, total proteins, and nuclear fractions were collected.

**Measurement of Cellular 8-Hydroxydeoxyguanosine**

DNA was extracted from HUVECs using a DNA Extractor TIS Kit (Wako, Osaka, Japan). The 8-hydroxydeoxyguanosine (8OhdG) concentration in HUVECs was measured using a Highsensitive 8OhdG Check ELISA Kit (Japan Institute for the Control of Aging, Shizuoka, Japan) after preparation with an 8OhdG Assay Preparation Reagent Set (Wako, Osaka, Japan). The results are expressed as picograms per microgram of DNA, corrected by the amount of DNA in each sample.

**Measurement of mRNA**

ISOGEN (Nippon Gene Co, Ltd, Toyama, Japan) was used to extract total RNA. The CYP1A1 and CYP1B1 mRNA expressions were analyzed by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) using an iQ5 Real-Time PCR Detection System (Bio-Rad, CA, USA) and an iScript One-Step RT-PCR Kit with SYBR Green (Bio-Rad). The sequences of the sense and antisense primers used for amplification were CYP1A1: 5' CTTGGACCTTTTGGAGCTG-3', 5'-CGAAGAAGAGTCGGAAG-3', CYP1B1: 5'-CACCAAGCTGAGACAGTGA-3', 5'-GATGACGACTGGGCCTACAT-3'; and RPL13A: 5' AAGCCTACAAGAAAGTGTTGCTATC-3', 5'-TGTTTCCGTAGCCTCATGAGC-3'.

The fluorescent signal from SYBR Green was detected immediately after the extension step, and the threshold cycle (Ct) was recorded. The Ct value from RPL13A served as the internal control for normalization.

**Protein Collection**

**Total Proteins**

HUVECs were homogenized in ice-cold lysis buffer (1% Triton X-100, 50mmol/L HEPES, pH 7.4, 100mmol/L sodium pyrophosphate, 100mmol/L sodium fluoride, 10mmol/L EDTA, 10mmol/L sodium vanadate, 1mmol/L phenylmethysulfonyl fluoride, 1 μg/ml aprotinin, and 5 μg/ml leupeptin). After centrifugation at 15,000rpm for 30min at 4°C, the supernatant was collected as the total protein.

**Nuclear Fractions**

HUVECs were suspended in ice-cold phosphate-buffered saline and homogenized for 10 strokes using a glass Dounce homogenizer. The homogenates were then centrifuged at 12,000g for 30min at 4°C, the supernatant was collected as the total protein.

**Western Blot Analysis**

The protein sample (2 or 10μg) of each fraction was electrophoretically blotted to poly (vinylidene fluoride)
membranes in 7.5% or 10% SDS-PAGE and then detected by using anti-AhR antibody (R&D Systems, MN, USA), anti-phospho-NF-E2-related factor 2 (Nrf2) antibody (Epitomics, Inc, CA, USA), anti-total Nrf2 antibody (Cell Signaling Technology, Beverly, MA, USA), anti-heme oxygenase-1 (HO-1) antibody (Stressgen Biotechnologies Inc, CA, USA), anti-NAD(P)H:quione oxidoreductase-1 (NQO-1) antibody (R&D Systems), anti-acetyl-p53 antibody (Merck Millipore, Billerica, MA, USA), anti-total p53 antibody (Merck Millipore), anti-lamin A/C antibody (Cell Signaling Technology), or β-actin antibody (Santa Cruz Biotechnology, CA, USA). Images were acquired in a ChemiDoc XRS system (Bio-Rad) and analyzed with PDQuest software (Bio-Rad). Data on the expressions of specific proteins were always normalized to β-actin, and nuclear expression of AhR was particularly normalized to lamin A/C. The results are expressed as fold increase relative to control.

Measurement of Cellular NAD+
Cellular NAD⁺ was measured using a NAD⁺/NADH Quantification Kit (BioVision, Inc, Milpitas, CA, USA) in accordance with the manufacturer’s instructions. This kit uses an enzyme-cycling reaction to specifically detect NAD⁺ and NADH, and is a convenient method for sensitive measurement of NAD⁺, NADH, and their ratio.

Assay of Sirt1 Activity
HUVECs were homogenized in an ice-cold lysis buffer (50mmol/L Tris-HCl, pH 7.4, 150mmol/L NaCl, 1mmol/L phenylmethylsulfonyl fluoride, 5µg/ml leupeptin, and 1µg/ml aprotinin), and total protein in the supernatant was obtained after centrifugation at 15,000rpm for 10 min at 4°C. Sirt1 activity in the total protein was determined using a histone deacetylase colorimetric assay kit (Enzo Life Sciences Inc, NY, USA) in accordance with the manufacturer’s instructions.

Senescence-Associated β-Galactosidase Staining
To assess senescent changes in the phenotype of HUVECs, we used a senescence-associated β-galactosidase (SA β-gal) staining kit (BioVision, Inc) to stain for SA β-gal, a well-established biomarker of cellular senescence. The percentage of SA β-gal-positive cells was determined by using a microscope (×100) to count the number of blue cells and total cells in 2 different fields per sample. Cell numbers were measured by using image analysis software (WinROOF; Mitani Corporation, Japan).

Statistical Analysis
Results are expressed as mean±standard error of the mean. Comparisons between groups were performed by using one-way ANOVA. A P value of <0.05 was considered to indicate statistical significance.

Results
Change in H₂ Concentration
H₂ concentration was 0.55±0.07mmol/L in fresh hydrogen-rich medium. HUVECs were cultured in this hydrogen-rich medium with or without TCDD exposure, and temporal change in H₂ concentration was investigated. A TCDD concentration of 1nmol/L was selected, in accordance with previous reports. As shown in Figure 1, H₂ concentration did not significantly change in hydrogen-rich medium. Levels of H₂ were determined using gas chromatography with a semiconductor detector (TRI lyzer™ mBA-3000; Taiyo KK, Osaka, Japan). Data are expressed as mean±SEM of 10 different experiments. *P<0.05 vs. normal medium.

Figure 1. Time-dependent change in hydrogen (H₂) concentration in hydrogen-rich medium. Levels of H₂ were determined using gas chromatography with a semiconductor detector (TRI lyzer™ mBA-3000; Taiyo KK, Osaka, Japan). Data are expressed as mean±SEM of 10 different experiments. *P<0.05 vs. normal medium.

Figure 2. Assessment of oxidative stress. HUVECs were pretreated with normal medium in the absence or presence of CH (10μmol/L) or hydrogen-rich medium for 1h, after which TCDD (1nmol/L) was added. Concentrations of 8OHdG in HUVECs were measured by using an ELISA kit after incubation with TCDD for 1, 24, and 48h. Data are expressed as mean±SEM of 4 different experiments. *P<0.05 vs. control, †P<0.05 vs. TCDD-treated cells. HUVECs, human umbilical vein endothelial cells; 8-OHdG, 8-hydroxydeoxyguanosine; CH, CH223191; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
indicates that the antioxidative effect of H\textsubscript{2} had ceased. CH significantly suppressed the increase in intracellular 8OHdG until 48 h (Figure 2).

Effect of Hydrogen-Rich Medium on Cellular Oxidative Products

We used an ELISA to evaluate intracellular 8OHdG, a marker of oxidative stress to nucleic acid. HUVECs were pretreated for 1 h in normal medium in the presence or absence of AhR-inhibitor CH or hydrogen-rich medium, and then incubated with TCDD for 1, 24, or 48 h. The 8OHdG content of HUVECs was then assayed. 8OHdG content did not differ between HUVECS cultured for 48 h in the presence or absence of H\textsubscript{2}; however, as compared with control values, 8OHdG was significantly higher from 1 to 48 h after TCDD exposure. H\textsubscript{2} significantly suppressed the TCDD-induced increase in intracellular 8OHdG until 24 h, after H\textsubscript{2} was undetectable in the medium. At 48h, intracellular 8OHdG increased to a level similar to that seen during TCDD exposure without H\textsubscript{2}, which indicates that the antioxidative effect of H\textsubscript{2} had ceased. CH significantly suppressed the increase in intracellular 8OHdG until 48 h (Figure 2).

Effect of Hydrogen-Rich Medium on AhR Activity by TCDD

TCDD toxicity is mostly induced through the AhR-ROS pathway.\textsuperscript{13-15} We examined if the antioxidative effect of H\textsubscript{2} was dependent on inhibition of AhR activity. After placing HUVECs for 1 h in normal medium with or without the CH or hydrogen-rich medium, the cells were exposed to TCDD. Cells were collected at 1 h after TCDD exposure (while the H\textsubscript{2}-rich medium still contained substantial H\textsubscript{2}) and 24 h after TCDD exposure (when H\textsubscript{2} was undetectable in the medium). Nuclear translocation in AhR, a marker of AhR activation, was evaluated using Western blot analysis, and mRNA activation of CYP1A1 and CYP1B1 was evaluated using real-time RT-PCR. The presence of CH in medium suppressed the increase in AhR nuclear translocation at 1 and 24 h after TCDD exposure; that is, translocation remained at control levels. In

![Figure 3](image-url)
Molecular Hydrogen and Endothelial Senescence

**Effect of Hydrogen-Rich Medium on p53 Acetylation**

Activation of p53 by acetylation was reported to be associated with cellular senescence caused by oxidative stress. We used Western blot analysis to evaluate acetyl-p53 expression at 24 and 48 h after TCDD exposure, to assess the possible role of H2 in suppressing cellular senescence. Total p53 expression in HUVECs did not significantly differ between normal and hydrogen-rich media or between media with and without TCDD. However, at 48 h after TCDD exposure, both Nrf2 phosphorylation and HO-1/NQO-1 expression returned to baseline levels. These results suggest that the sustained antioxidative effects of H2 are related to activation of Nrf2 and its associated antioxidant enzyme group.

**Effect of Hydrogen-Rich Medium on Nrf2 Activation**

The transcription factor, Nrf2, controls expression of detoxifying and antioxidant enzymes, thereby contributing to the body’s ability to maintain homeostasis during stress. We therefore investigated whether the antioxidant effects of H2 might depend on activation of Nrf2, using Western blot analysis to evaluate expression of phospho-Nrf2 and its antioxidant enzymes, HO-1 and NQO-1, after exposure of HUVECs to TCDD. In the H2-enriched medium, phospho-Nrf2 expression was increased at 1 h after TCDD exposure (ie, when H2 was abundant) (data not shown). Expression remained elevated at 24 h after TCDD exposure, when H2 had returned to baseline levels. There were also significant increases in HO-1 and NQO-1 expressions. However, at 48 h after TCDD exposure, phospho-Nrf2 expression and HO-1/NQO-1 expression returned to baseline levels. These results suggest that the sustained antioxidative effects of H2 are related to activation of Nrf2 and its associated antioxidant enzyme group.

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Effects of Hydrogen-Rich Medium on NAD+/NADH Ratio and Sirt1 Activity

We previously reported that, in vascular endothelial cells, oxidative stress induced by AhR activation decreases intracellular NAD+, which reduces NAD-dependent deacetylase Sirt1 activity and promotes cellular senescence.\(^{18,19}\) In the present study, we evaluated the NAD+/NADH ratio and Sirt1 activity by colorimetric assay. As shown in Figure 6A, at 48h, the NAD+/NADH ratio did not significantly differ between HUVECs cultured in the presence and absence of H\(_2\). However, the NAD+/NADH ratio was significantly lower at 6–48h after TCDD exposure, as compared with the control. By contrast, in HUVECs cultured in H\(_2\) and exposed to TCDD, the NAD+/NADH ratio was equivalent to that of the control until
24 h; that is, after H₂ had disappeared from the medium. No significant difference was noted in Sirt1 activity between cells cultured in the presence and absence of H₂ for 48 h, as was the case for the NAD⁺/NADH ratio. However, TCDD exposure significantly reduced Sirt1 activity from 6 to 24 h, as compared with the control. By contrast, Sirt1 activity in the H₂+TCDD group remained similar to that of the control from 6 to 24 h, after H₂ was undetectable in the medium, as was the case for the NAD⁺/NADH ratio (Figure 6B). This effect had ceased at 48 h after TCDD exposure.

Effects of Hydrogen-Rich Medium on Cellular Senescence
We used SA β-gal staining to determine the number of senescent cells at 24 and 48 h after exposing HUVECs to TCDD. The number of SA β-gal-positive cells was significantly higher at 24 h after TCDD exposure (18.6±3.8% vs 5.5±1.4% SA β-gal-positive cells in the control). By contrast, in media with H₂ and TCDD, the percentage of SA β-gal-positive cells was similar to that for the control, 5.5±1.9% (Figure 7A). At 48 h after TCDD exposure, the percentage of SA β-gal-positive cells was significantly higher than in the control (19.7±3.6% vs 5.2±1.3%), and the percentage of SA β-gal-positive cells was 16.4±3.2% in TCDD-exposed cells cultured in hydrogen-rich medium, which indicates that, at 48 h, H₂ no longer suppressed the increase in SA β-gal-positive cells (Figure 7B).

Effects of Chrysin on H₂-Induced Changes in HUVECs
We performed experiments using a pharmacological inhibitor of Nrf2 (chrysin) to determine if the antioxidative and anti-senescence effects of hydrogen were dependent on Nrf2 activation. A chrysin concentration of 10 μmol/L was selected, in accordance with a previous report. As shown in Figure 8, chrysin treatment abolished the antioxidative and anti-senescence effects of hydrogen on HUVECs at 24 h after TCDD exposure.

Discussion
Our results indicate that even after H₂ concentration in the medium had decreased to that of the control medium, oxidative stress and cellular senescence caused by activation of the TCDD-AhR pathway continued to be inhibited in HUVECs. These findings suggest that the prolonged antioxidative and anti-senescence effects caused by transient hydrogen exposure are dependent on sustained activation of Nrf2 and HO-1/NQO-1 in HUVECs.

As is the case for other endogenous bioactive gases, such as NO, CO, and H₂S, H₂ has beneficial effects in a variety of diseases. Several animal studies have found that the administration of hydrogen-rich water reduces oxidative stress, protects cognitive function, and suppresses atherosclerosis. In studies of humans, oral intake of hydrogen-rich water 3–5 times daily decreased oxidative stress in patients with chronic diseases such as type 2 diabetes, metabolic syndrome, and Parkinson disease and improved their quality of life. These findings suggest that H₂-enriched water has benefits for such patients. In animals, blood concentrations of orally administered H₂ reach a peak at 5–15 min and return to baseline 30 min after administration. In humans given H₂-rich water orally, the H₂ concentration in exhaled air reached a peak at approximately 10 min and returned to baseline 1 h after intake. In sum, past and present findings indicate that although the increase in H₂ in the body is transient, its...
antioxidant effects are maintained even when supplemental \( \text{H}_2 \) is no longer detectable. However, the mechanisms responsible for this sustained effect have been unclear. We thus assessed the effects of dissolved \( \text{H}_2 \) in cell cultures with hydrogen-rich medium. \( \text{H}_2 \) concentration decreased to 2.5% of its peak at 12h and was undetectable at 16h, which indicates that \( \text{H}_2 \) degasses quickly in hydrogen-rich medium.

Existing evidence indicates that TCDD exposure is associated with development of coronary and hypertensive diseases, through AhR activation.\(^{11,12}\) Diseases attributable to dioxins, including TCDD, may result from macrophage activation through AhR activation and subsequent induction of inflammation in blood vessels or premature cell senescence mediated by ROS production.\(^{13,14}\) Interestingly, TCDD exposure is strongly associated with cellular senescence. We previously demonstrated that ROS production through AhR activation by

\( \text{TCDD} \) reduced cellular NAD\(^+ \) and suppressed Sirt1 activity, thereby accelerating cellular senescence.\(^{18,19}\) When we first examined whether \( \text{H}_2 \) suppresses oxidative stress by means of the TCDD-AhR pathway, we found that in hydrogen-rich medium, the TCDD-induced increase in 8OHdG in HUVECs was significantly suppressed and that these effects were similar to those of the AhR-inhibitor, CH. We then sought to determine whether the antioxidative effects of \( \text{H}_2 \) in HUVECs depended on inhibition of AhR. However, even when \( \text{H}_2 \) concentrations in the medium were highest, there was no inhibition of AhR. Thus, the antioxidant effects of \( \text{H}_2 \) do not depend on inhibition of AhR activity.

The transcription factor, Nrff2, elicits antioxidant genes such as HO-1 and NQO-1 in response to oxidative stress, and therefore has a very important role as a regulatory factor in preventing oxidative stress.\(^{22}\) The antioxidative effects of \( \text{H}_2 \) appear
to be partly attributable to induction of Nrf2. In a previous study, we reported that HO-1 suppresses cellular damage resulting from oxidative stress, which is important in atherosclerosis progression. We therefore examined the relationship between the antioxidative activity of H2 and activation of Nrf2/HO-1 and observed increased expression of phospho-Nrf2 and its related antioxidant enzymes, HO-1 and NQO-1, when the H2 concentration was maximal and at 24 h, when H2 was undetectable in the medium. At 48 h, increased expression of phospho-Nrf2 and its associated antioxidant enzyme group was diminished. Furthermore, pharmacological inhibition abolished hydrogen-induced HO-1 and NQO-1 expression. Taken together, these findings suggest that the sustained antioxidative effects of H2 are at least partly attributable to the induction of the antioxidant enzyme group associated with Nrf2 activation.

Cellular senescence induced by enhanced oxidative stress is important in accelerated atherosclerosis. Furthermore, activation of p53 by acetylation was found to be associated with cellular senescence caused by oxidative stress. To examine further the effects of H2 on preventing cellular senescence, we assessed induction of acetyl-p53 in HUVECs treated with hydrogen-rich water. H2 significantly decreased induction of acetyl-p53 at 24 h after TCDD incubation. Sirt1, the closest homologue of silent information regulator (Sir2), is an NAD+-dependent histone deacetylase. This longevity gene is associated with cellular apoptosis and the stress response implicated in the mechanism of cellular senescence. We previously reported that a decrease in cellular NAD+ caused by ROS production suppressed Sirt1 activity, which resulted in accelerated cellular senescence. Other studies reported that oxidative stress and cellular senescence were ameliorated in endothelial cells that overexpressed Sirt1. In the present study, we examined if NAD+-Sirt1 activation had a role in the H2-induced decrease in acetyl-p53 expression in HUVECs incubated with TCDD. We found that the NAD+/NADH ratio and Sirt1 activity in TCDD-treated HUVECs incubated with H2 were comparable to those in control HUVECs until 24 h; that is, after H2 was undetectable in the medium, a period during which the percentage of SA-β-gal-positive cells declined. However, at 48 h after TCDD exposure, neither of these hydrogen-induced changes was observed. Furthermore, chrysin blocked the anti-senescence effects of hydrogen on TCDD-treated HUVECs. These findings suggest that the anti-senescence effects of hydrogen are mediated by sustained activation of Nrf2 and its associated antioxidant enzyme group.

Conclusions

H2 clearly suppressed oxidative stress and cellular senescence in HUVECs through sustained activation of Nrf2, even after H2 concentrations were undetectable. Regular intake of hydrogen-rich water may thus yield sustained H2 effects in humans.

Acknowledgments

We thank TED Co, Ltd and Amuru Technica Co, Ltd for help with the hydrogen-rich medium preparation.

Conflicts of Interest

None.

References


