Effectiveness of Hydrogen Rich Water on Antioxidant Status of Subjects with Potential Metabolic Syndrome—An Open Label Pilot Study

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Summary **Metabolic syndrome is characterized by cardiometabolic risk factors that include obesity, insulin resistance, hypertension and dyslipidemia. Oxidative stress is known to play a major role in the pathogenesis of metabolic syndrome. The objective of this study was to examine the effectiveness of hydrogen rich water (1.5–2 L/day) in an open label, 8-week study on 20 subjects with potential metabolic syndrome. Hydrogen rich water was produced, by placing a metallic magnesium stick into drinking water (hydrogen concentration; 0.55–0.65 mM), by the following chemical reaction; $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$. The consumption of hydrogen rich water for 8 weeks resulted in a 39% increase ($p<0.05$) in antioxidant enzyme superoxide dismutase (SOD) and a 43% decrease ($p<0.05$) in thiobarbituric acid reactive substances (TBARS) in urine. Further, subjects demonstrated an 8% increase in high density lipoprotein (HDL)-cholesterol and a 13% decrease in total cholesterol/HDL-cholesterol from baseline to week 4. There was no change in fasting glucose levels during the 8 week study. In conclusion, drinking hydrogen rich water represents a potentially novel therapeutic and preventive strategy for metabolic syndrome. The portable magnesium stick was a safe, easy and effective method of delivering hydrogen rich water for daily consumption by participants in the study.**

Effects of Hydrogen-Rich Water on Abnormalities in a SHR.Cg-Leprcp/NDmcr Rat - A Metabolic Syndrome Rat Model

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Abstract

Background: Hydrogen (H_2), a potent free radical scavenger, selectively reduces the hydroxyl radical, which is the most cytotoxic of the reactive oxygen species (ROS). An increase in oxygen free radicals induces oxidative stress, which is known to be involved in the development of metabolic syndrome. Therefore, we investigated whether hydrogen-rich water (HRW) affects metabolic abnormalities in the metabolic syndrome rat model, SHR.Cg-*Lepr*^{cp}/NDmcr (SHR-cp).

Methods: Male SHR-cp rats (5 weeks old) were divided into 2 groups: an HRW group was given oral HRW for 16 weeks, and a control group was given distilled water. At the end of the experiment, each rat was placed in a metabolic cage for 24 h, fasted for 12 h, and anesthetized; the blood and kidneys were then collected.

Results: Sixteen weeks after HRW administration, the water intake and urine flow measured in the metabolic cages were significantly higher in the HRW group than in the control group. The urinary ratio of albumin to creatinine was significantly lower and creatinine clearance was higher in the HRW group than in the control group. After the 12-h fast, plasma urea nitrogen and creatinine in the HRW group were significantly lower than in the control group. The plasma total antioxidant capacity was significantly higher in the HRW group than in the control group. The glomerulosclerosis score for the HRW group was significantly lower than in the control group, and a significantly positive correlation was observed between this score and plasma urea nitrogen levels.

Conclusion: The present findings suggest that HRW conferred significant benefits against abnormalities in the metabolic syndrome model rats, at least by preventing and ameliorating glomerulosclerosis and creatinine clearance.

Key Words: hydrogen-rich water, renal glomerulosclerosis, metabolic syndrome model rats, oxidative stress

RESEARCH ARTICLE Open Access

Hydrogen-rich water inhibits glucose and α,β -dicarbonyl compound-induced reactive oxygen species production in the SHR.Cg-*Lepr*^{cp}/NDmcr rat kidney

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Abstract

Background: Reactive oxygen species (ROS) production induced by α,β-dicarbonyl compounds and advanced glycation end products causes renal dysfunction in patients with type 2 diabetes and metabolic syndrome. Hydrogen-rich water (HRW) increases the H_2 level in blood and tissues, thus reducing oxidative stress in animals as well as humans. In this study, we investigated the effects of HRW on glucose- and α,β-dicarbonyl compound-induced ROS generation in vitro and in vivo.

Methods: Kidney homogenates from Wistar rats were incubated in vitro with glucose and α,β-dicarbonyl compounds containing HRW, following which ROS levels were measured. In vivo animal models of metabolic syndrome, SHR.Cg-*Lepr*^{cp}/NDmcr rats, were treated with HRW for 16 weeks, following which renal ROS production and plasma and renal α,β-dicarbonyl compound levels were measured by liquid chromatograph mass spectrometer.

Results: HRW inhibited glucose- and α,β-dicarbonyl compound-induced ROS production in kidney homogenates from Wistar rats in vitro. Furthermore, SHR.Cg-*Lepr*^{cp}/NDmcr rats treated with HRW showed a 34% decrease in ROS production. Moreover, their renal glyoxal, methylglyoxal, and 3-deoxyglucosone levels decreased by 81%, 77%, and 60%, respectively. Positive correlations were found between renal ROS levels and renal glyoxal ($r = 0.659$, $p = 0.008$) and methylglyoxal ($r = 0.782$, $p = 0.001$) levels.

Conclusion: These results indicate that HRW inhibits the production of α,β-dicarbonyl compounds and ROS in the kidneys of SHR.Cg-*Lepr*^{cp}/NDmcr rats. Therefore, it has therapeutic potential for renal dysfunction in patient with type 2 diabetes and metabolic syndrome.

Keywords: Hydrogen-rich water, α,β-dicarbonyl compounds, Oxidative stress, Metabolic syndrome model, Advanced glycation end products

Hydrogen-rich water decreases serum LDL-cholesterol levels and improves HDL function in patients with potential metabolic syndrome

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Abstract **We have found that hydrogen (dihydrogen; H_2) has beneficial lipid-lowering effects in high-fat diet-fed Syrian golden hamsters. The objective of this study was to characterize the effects of H_2-rich water (0.9–1.0 l/day) on the content, composition, and biological activities of serum lipoproteins on 20 patients with potential metabolic syndrome. Serum analysis showed that consumption of H_2-rich water for 10 weeks resulted in decreased serum total-cholesterol (TC) and LDL-cholesterol (LDL-C) levels. Western blot analysis revealed a marked decrease of apolipoprotein (apo)B100 and apoE in serum. In addition, we found H_2 significantly improved HDL functionality assessed in four independent ways, namely, *i*) protection against LDL oxidation, *ii*) inhibition of tumor necrosis factor (TNF)-α-induced monocyte adhesion to endothelial cells, *iii*) stimulation of cholesterol efflux from macrophage foam cells, and *iv*) protection of endothelial cells from TNF-α-induced apoptosis. Further, we found consumption of H_2-rich water resulted in an increase in antioxidant enzyme superoxide dismutase and a decrease in thiobarbituric acid-reactive substances in whole serum and LDL. In conclusion, supplementation with H_2-rich water seems to decrease serum LDL-C and apoB levels, improve dyslipidemia-injured HDL functions, and reduce oxidative stress, and it may have a beneficial role in prevention of potential metabolic syndrome.**—Song, G., M. Li, H. Sang, L. Zhang, X. Li, S. Yao, Y. Yu, C. Zong, Y. Xue, and S. Qin. **Hydrogen-rich water decreases serum LDL-cholesterol levels and improves HDL function in patients with potential metabolic syndrome.** *J. Lipid Res.* **2013.** 54: **1884–1893.**

Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance

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Abstract

Oxidative stress is recognized widely as being associated with various disorders including diabetes, hypertension, and atherosclerosis. It is well established that hydrogen has a reducing action. We therefore investigated the effects of hydrogen-rich water intake on lipid and glucose metabolism in patients with either type 2 diabetes mellitus (T2DM) or impaired glucose tolerance (IGT). We performed a randomized, double-blind, placebo-controlled, crossover study in 30 patients with T2DM controlled by diet and exercise therapy and 6 patients with IGT. The patients consumed either 900 mL/d of hydrogen-rich pure water or 900 mL of placebo pure water for 8 weeks, with a 12-week washout period. Several biomarkers of oxidative stress, insulin resistance, and glucose metabolism, assessed by an oral glucose tolerance test, were evaluated at baseline and at 8 weeks. Intake of hydrogen-rich water was associated with significant decreases in the levels of modified low-density lipoprotein (LDL) cholesterol (ie, modifications that increase the net negative charge of LDL), small dense LDL, and urinary 8-isoprostanes by 15.5% ($P < .01$), 5.7% ($P < .05$), and 6.6% ($P < .05$), respectively. Hydrogen-rich water intake was also associated with a trend of decreased serum concentrations of oxidized LDL and free fatty acids, and increased plasma levels of adiponectin and extracellular-superoxide dismutase. In 4 of 6 patients with IGT, intake of hydrogen-rich water normalized the oral glucose tolerance test. In conclusion, these results suggest that supplementation with hydrogen-rich water may have a beneficial role in prevention of T2DM and insulin resistance.

Preservative Effect of Electrolyzed Reduced Water on Pancreatic β-Cell Mass in Diabetic *db/db* Mice

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Oxidative stress is produced under diabetic conditions and involved in progression of pancreatic β-cell dysfunction. Both an increase in reactive oxygen free radical species (ROS) and a decrease in the antioxidant defense mechanism lead to the increase in oxidative stress in diabetes. Electrolyzed reduced water (ERW) with ROS scavenging ability may have a potential effect on diabetic animals, a model for high oxidative stress. Therefore, the present study examined the possible anti-diabetic effect of ERW in genetically diabetic mouse strain C57BL/6J-*db/db* (*db/db*). ERW with ROS scavenging ability reduced the blood glucose concentration, increased blood insulin level, improved glucose tolerance and preserved β-cell mass in *db/db* mice. The present data suggest that ERW may protects β-cell damage and would be useful for antidiabetic agent.

"NORDENAU PHENOMENON" - APPLICATION OF NATURAL REDUCED WATER TO THERAPY

FOLLOW UP STUDY UPON 411 DIABETES PATIENTS

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Abstract: This prospective observation study examines changes in the relevant tests parameters of 411 diabetes patients drinking natural reduced water from the "Nordenau Spring", as well as a correlation of these changes with the fluctuation of the reactive oxygen species in their blood. The average age of the test persons is 71.5 years and the daily consumption of reduced water is as much as two liters. The average duration of stay in Nordenau is 6 days. The diagnostic parameters such as blood sugar, HbA1c, cholesterol, LDL, HDL, and serum creatinine concentration are tested twice – at the beginning and at the end of the participants stay in Nordenau. Additionally a random sample of reactive oxygen species in the blood of 136 patients is taken in order to find out its possible causal connections to the diabetes relevant test parameters.HbA1c has been considered as the substantial test parameter in order to break down the whole group into responder and non-responder categories. One hundred and eighty six tested persons or 45% of the total have been assigned to the responder group, meaning that the patients' HbA1c and blood sugar improved significantly. Furthermore we evaluated among the responder group a portion of patients who in the same time significantly improved their cholesterol, LDL, HDL and serum creatinine concentration average value. This stage of our follow up study regarding type II diabetes patients estimates number needed to treat on four patients in order to achieve the significant improvement of all diabetes relevant parameters. This is a very good quotient; moreover it could be achieved entirely without side effects. The significant improvement of diabetes relevant parameters like blood fats and creatinine can be also beneficial to other diseases like high blood pressure, circulatory disturbance, renal insufficiency or atherosclerotic dementia. In addition to our previous tests, we administered to a random sample group of 136 patients a blood free oxygen radicals test (FORT). The test resulted in a decrease of the ROS of 70.6% of the group or 96 patients. Taking account of the fact that the natural reduced water as well as the electrolyzed reduced water obviously improves in a very short time and entirely without side effects very important metabolic parameters, it can be said that the reduced water shall be considered a useful supplement to the usual orthodox medication of ROS-associated diseases.

Anti-diabetic effects of electrolyzed reduced water in streptozotocin-induced and genetic diabetic mice

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Abstract

Oxidative stress is produced under diabetic conditions and is likely involved in progression of pancreatic β-cell dysfunction found in diabetes. Both an increase in reactive oxygen free radical species (ROS) and a decrease in the antioxidant defense mechanism lead to the increase in oxidative stress in diabetes. Electrolyzed reduced water (ERW) with ROS scavenging ability may have a potential effect on diabetic animals, a model for high oxidative stress. Therefore, the present study examined the possible anti-diabetic effect of ERW in two different diabetic animal models. The genetically diabetic mouse strain C57BL/6J-*db/db* (*db/db*) and streptozotocin (STZ)-induced diabetic mouse were used as insulin deficient type 1 and insulin resistant type 2 animal model, respectively. ERW, provided as a drinking water, significantly reduced the blood glucose concentration and improved glucose tolerance in both animal models. However, ERW fail to affect blood insulin levels in STZ-diabetic mice whereas blood insulin level was markedly increased in genetically diabetic *db/db* mice. This improved blood glucose control could result from enhanced insulin sensitivity, as well as increased insulin release. The present data suggest that ERW may function as an orally effective anti-diabetic agent and merit further studies on its precise mechanism.

Suppressive effects of electrolyzed reduced water on alloxan-induced apoptosis and type 1 diabetes mellitus

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Abstract Electrolyzed reduced water, which is capable of scavenging reactive oxygen species, is attracting recent attention because it has shown improved efficacy against several types of diseases including diabetes mellitus. Alloxan produces reactive oxygen species and causes type 1 diabetes mellitus in experimental animals by irreversible oxidative damage to insulin-producing β-cells. Here, we showed that electrolyzed reduced water prevented alloxan-induced DNA fragmentation and the production of cells in sub-G1 phase in HIT-T15 pancreatic β-cells. Blood glucose levels in alloxan-induced type 1 diabetes model mice were also significantly suppressed by feeding the mice with electrolyzed reduced water. These results suggest that electrolyzed reduced water can prevent apoptosis of pancreatic β-cells and the development of symptoms in type 1 diabetes model mice by alleviating the alloxan-derived generation of reactive oxygen species.

Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice

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A B S T R A C T

Oxidative stress is implicated in atherogenesis; however most clinical trials with dietary antioxidants failed to show marked success in preventing atherosclerotic diseases. We have found that hydrogen (dihydrogen; H_2) acts as an effective antioxidant to reduce oxidative stress [I. Ohsawa, M. Ishikawa, K. Takahashi, M. Watanabe, K. Nishimaki, K. Yamagata, K. Katsura, Y. Katayama, S, Asoh, S. Ohta, Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals, Nat. Med. 13 (2007) 688–694]. Here, we investigated whether drinking H_2-dissolved water at a saturated level (H_2-water) *ad libitum* prevents arteriosclerosis using an apolipoprotein E knockout mouse ($apoE^{-/-}$), a model of the spontaneous development of atherosclerosis. $ApoE^{-/-}$ mice drank H_2-water *ad libitum* from 2 to 6 month old throughout the whole period. Atherosclerotic lesions were significantly reduced by *ad libitum* drinking of H_2-water ($p = 0.0069$) as judged by Oil-Red-O staining series of sections of aorta. The oxidative stress level of aorta was decreased. Accumulation of macrophages in atherosclerotic lesions was confirmed. Thus, consumption of H_2-dissolved water has the potential to prevent arteriosclerosis.

Colloidal Platinum in Hydrogen-Rich Water Exhibits Radical-Scavenging Activity and Improves Blood Fluidity

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The 'colloidal platinum' stabilized with polyvinylpyrrolidone (Pt/PVP-colloid) was dispersed in hydrogen-rich water (HW; hydrogen concentration, 0.82 ppm; oxidation-reduction potential, −583 mV) or regular water (RW; < 0.01 ppm, +218 mV). And we evaluated the antioxidant activity of Pt/PVP-colloid in HW or RW on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and improvement of blood fluidity under 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress. When applied with the 0.25–0.5 ppm Pt/PVP-colloid in RW or HW, the level of DPPH radicals decreased to 77.5–59.6% or 16.1–5.6%, in contrast to the level as high as 81.3% for HW alone, respectively, as measured by an electron spin resonance method. The horse blood, which was subjected to AAPH-induced oxidative stress, was incubated for 24 hr with RW or HW, and thereafter required 13.7 sec (100%) or 5.7 sec (42.3%) for passing through the micro-channels in a rheology equipment. When treated with 0.5–1.0 ppm Pt/PVP-colloid in RW or HW, the blood passage time in the micro-channels decreased dose-dependently to 9.7–7.3 sec (71.6–53.8%) or 4.3–1.3 sec (32.8–10.3%), and the rate of micro-channels clogged with erythrocyte aggregates decreased to 23.8–21.0% or 15.8–9.8%, respectively, from 42.8% for no addition of Pt/PVP. By scanning electron microscopy, AAPH-treated erythrocytes lost intact surface morphology on the membrane together with protrusions and without hollows, being indicative of impaired transforming ability, and the rate of erythrocyte agglutination was increased to 46.2%. When treated the horse blood with HW alone significantly decreased the rate of erythrocyte agglutination to 29.6%, whereas 1.0 ppm Pt/PVP-colloid in RW or HW decreased it to 24.1% or 21.1%, respectively. Thus, DPPH-radical-scavenging and erythrocyte-protecting effects of Pt/PVP-colloid in HW were superior to those of Pt/PVP-colloid in RW or Pt/PVP-free HW. The results could be mainly attributed to the enhanced antioxidant activity of Pt/PVP in HW, which may be due to captured-hydrogen on platinum.

Amelioration of rat cardiac cold ischemia/reperfusion injury with inhaled hydrogen or carbon monoxide, or both

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BACKGROUND: Recent advances in novel medical gases, including hydrogen and carbon monoxide (CO), have demonstrated significant opportunities for therapeutic use. This study was designed to evaluate the effects of inhaled hydrogen or CO, or both, on cold ischemia/reperfusion (I/R) injury of the myocardium.
METHODS: Syngeneic heterotopic heart transplantation was performed in rats after 6 or 18 hours of cold ischemia in Celsior solution. Survival, morphology, apoptosis and marker gene expression were assessed in the grafts after in vivo inhalation of hydrogen (1% to 3%), CO (50 to 250 ppm), both or neither. Both donors and recipients were treated for 1 hour before and 1 hour after reperfusion.
RESULTS: After 6-hour cold ischemia, inhalation of hydrogen (>2%) or CO (250 ppm) alone attenuated myocardial injury. Prolonged cold ischemia for 18 hours resulted in severe myocardial injury, and treatment with hydrogen or CO alone failed to demonstrate significant protection. Dual treatment with hydrogen and CO significantly attenuated I/R graft injury, reducing the infarcted area and decreasing in serum troponin I and creatine phosphokinase (CPK). Hydrogen treatment alone significantly reduced malondialdehyde levels and serum high-mobility group box 1 protein levels as compared with air-treated controls. In contrast, CO only marginally prevented lipid peroxidation, but it suppressed I/R-induced mRNA upregulation for several pro-inflammatory mediators and reduced graft apoptosis.
CONCLUSIONS: Combined therapy with hydrogen and CO demonstrated enhanced therapeutic efficacy via both anti-oxidant and anti-inflammatory mechanisms, and may be a clinically feasible approach for preventing cold I/R injury of the myocardium.
J Heart Lung Transplant 2010;29:544–553

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AMELIORATION OF RAT CARDIAC COLD ISCHEMIA/REPERFUSION INJURY WITH INHALED HYDROGEN, CARBON MONOXIDE, OR BOTH

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Background: Recent advances in novel medical gases, including hydrogen and carbon monoxide (CO), have demonstrated significant opportunities for therapeutic use. This study was designed to evaluate the effects of inhaled hydrogen, CO, or both on cold ischemia/reperfusion (I/R) injury of the myocardium.

Methods: Syngeneic heterotopic heart transplantation was performed in rats after 6 or 18 hours of cold ischemia in Celsior. Survival, morphology, apoptosis, and marker gene expression were assessed in the grafts after in vivo inhalation of hydrogen (1-3%), CO (50-250 ppm), both, or neither. Both donors and recipients were treated for 1 hour before and 1 hour after reperfusion.

Results: After 6 hours cold ischemia, inhalation of hydrogen (>2%) or CO (250 ppm) alone attenuated myocardial injury. Prolonged cold ischemia for 18 hours resulted in severe myocardial injury, and treatment with hydrogen or CO alone failed to demonstrate significant protection. Dual treatment with hydrogen and CO significantly attenuated I/R graft injury, reducing the infarcted area and decreasing in serum troponin I and CPK. Hydrogen treatment alone significantly reduced malondialdehyde levels and serum high-mobility group box-1 protein levels as compared with air-treated controls. In contrast, CO only marginally prevented lipid peroxidation, but suppressed I/R-induced mRNA upregulation for several proinflammatory mediators and reduced graft apoptosis.

Conclusions: Combined therapy with hydrogen and CO demonstrated enhanced therapeutic efficacy via both antioxidant and anti-inflammatory mechanisms, and may be potentially a clinically feasible approach for preventing cold I/R injury of the myocardium.

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Improved brain MRI indices in the acute brain stem infarct sites treated with hydroxyl radical scavengers, Edaravone and hydrogen, as compared to Edaravone alone. A non-controlled study.

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Abstract

Background

In acute stage of cerebral infarction, MRI indices (rDWI & rADC) deteriorate during the first 3-7 days after the ictus and then gradually normalize in approximately 10 days (pseudonormalization time), although the tissue is already infarcted. Since effective treatments improve these indices significantly and in less than the natural pseudonormalization time, a combined analysis of these changes provides an opportunity for objective evaluation on the effectiveness of various treatments for cerebral infarction. Hydroxyl radicals are highly destructive to the tissue and aggravate cerebral infarction. We treated brainstem infarction patients in acute stage with hydroxyl radical scavengers (Edaravone and hydrogen) by intravenous administration and evaluated the effects of the treatment by a serial observation and analysis of these MRI indices. The effects of the treatment were evaluated and compared in two groups, an Edaravone alone group and a combined group with Edaravone and hydrogen, in order to assess beneficial effects of addition of hydrogen.

A basic study on molecular hydrogen (H_2) inhalation in acute cerebral ischemia patients for safety check with physiological parameters and measurement of blood H_2 level

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Abstract

Background

In animal experiments, use of molecular hydrogen (H_2) has been regarded as quite safe and effective, showing benefits in multiple pathological conditions such as ischemia-reperfusion injury of the brain, heart, kidney and transplanted tissues, traumatic and surgical injury of the brain and spinal cord, inflammation of intestine and lung , degenerative striatonigral tissue and also in many other situations. However, since cerebral ischemia patients are in old age group, the safety information needs to be confirmed. For the feasibility of H_2 treatment in these patients, delivery of H_2 by inhalation method needs to be checked for consistency.

Methods

Hydrogen concentration (HC) in the arterial and venous blood was measured by gas chromatography on 3 patients, before, during and after 4%(case 1) and 3%(case2,3) H2 gas inhalation with simultaneous monitoring of physiological parameters. For a consistency study, HC in the venous blood of 10 patients were obtained on multiple occasions at the end of 30-min H_2 inhalation treatment.

Results

The HC gradually reached a plateau level in 20 min after H_2 inhalation in the blood, which was equivalent to the level reported by animal experiments. The HC rapidly decreased to 10% of the plateau level in about 6 min and 18 min in arterial and venous blood, respectively after H_2 inhalation was discontinued. Physiological parameters on these 3 patients were essentially unchanged by use of hydrogen. The consistency study of 10 patients showed the HC at the end of 30-min inhalation treatment was quite variable but the inconsistency improved with more attention and encouragement.

Conclusion

H_2 inhalation of at least 3% concentration for 30 min delivered enough HC, equivalent to the animal experiment levels, in the blood without compromising the safety. However, the consistency of H_2 delivery by inhalation needs to be improved.

Safety of intravenous administration of hydrogen-enriched fluid in patients with acute cerebral ischemia: initial clinical studies

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Abstract

Background: Most of the results regarding hydrogen (H_2) therapy for acute cerebral ischemia are derived from *in vitro* studies and animal experiments, with only a few obtained from human trials with a limited number of subjects. Thus, there is a paucity of information regarding both the beneficial therapeutic effects as well as the side effects of H_2 on acute cerebral ischemia in humans. We designed a pilot study to investigate single dose intravenous H_2-administration in combination with edaravone, aiming to provide an initial estimate of the possible risks and benefits in select patients presenting with acute ischemic stroke.

Methods: An open-label, prospective, non-randomized study of intravenous H_2-administration was performed in 38 patients hospitalized for acute ischemic stroke. All patients received an H_2-enriched intravenous solution in addition to edaravone immediately after the diagnosis of acute ischemic stroke. Acute stroke patients within 3 h of onset received intravenous tissue plasminogen activator (t-PA) (0.6 mg/kg) treatment, and patients receiving t-PA had to commence the administration of the H_2-enriched intravenous solution and edaravone before or at the same time as the t-PA was infused.

Results: Complications were observed in 2 patients (5.3%), which consisted of diarrhea in 1 patient (2.6%) and cardiac failure in 1 patient (2.6%). No deterioration in laboratory tests, urinary tests, ECG, or chest X-ray radiograms occurred in any patient in this study. In all patients, the mean National Institutes of Health Stroke Scale (NIHSS) scores at baseline, and 7, 30, and 90 d after admission were 8.2 ± 7.5, 5.6 ± 7.1, 4.9 ± 6.5, and 4.5 ± 6.3, respectively. The early recanalization was identified in 4 of 11 patients (36.4%) who received intravenous t-PA administration. Hemorrhagic transformation was observed in 2 patients (18.2%). None of the patients in this study that were treated with t-PA developed symptomatic intracranial hemorrhage.

Conclusions: Data from the current study indicate that an H_2-enriched intravenous solution is safe for patients with acute cerebral infarction, including patients treated with t-PA.

Keywords: Acute ischemic stroke, Edaravone, Free radical scavenger, Hydrogen, Reactive oxygen species, Safety, Tissue plasminogen activator

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Amelioration of cardio-renal injury with aging in dahl salt-sensitive rats by H_2-enriched electrolyzed water

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Abstract: Recent studies have revealed the biological effects of H_2 in suppressing organ injuries due to acute inflammation and oxidative stress. Dahl salt-sensitive (SS) rats naturally develop elevated blood pressure (BP) and kidney injury with aging. The present study examined the effect of long-term supplementation of H_2 in drinking water on age-related changes.

Four-week-old male Dahl SS rats were fed 3 types of water (n = 30 each) for up to 48 weeks: filtered water (FW), water with a high H_2 content (492.5 ppb) obtained with water electrolysis (EW), or dehydrogenated EW (DW). Animals were subjected to histological analysis at 16, 24, and 48 weeks.

The FW group showed progressive BP elevation and increases in albuminuria and cardiac remodeling during the course of treatment. Histologically, there were significant changes as a function of aging, i.e., glomerular sclerosis with tubulointerstitial fibrosis in the kidney, and increased cardiomyocyte diameter with interstitial fibrosis in the heart at 48 weeks. These changes were related to the enhanced inflammation and oxidative stress in the respective organs. However, there were no striking differences in BP among the groups, despite histological alterations in the EW group being significantly decreased when compared to FW and DW in both organs, with concurrently lower oxidative stress and inflammatory markers at 48 weeks.

Conclusion: Long-term *ad libitum* consumption of H_2-enriched electrolyzed water can ameliorate the processes of kidney injury and cardiac remodeling with aging in Dahl SS rats by suppressing, at least partly, elevated inflammation and oxidative stress.

Keywords: Aging, Cardiac remodeling, Chronic kidney disease, Hydrogen molecule, Electrolyzed water

Molecular Hydrogen Improves Obesity and Diabetes by Inducing Hepatic FGF21 and Stimulating Energy Metabolism in *db/db* Mice

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Recent extensive studies have revealed that molecular hydrogen (H_2) has great potential for improving oxidative stress-related diseases by inhaling H_2 gas, injecting saline with dissolved H_2, or drinking water with dissolved H_2 (H_2-water); however, little is known about the dynamic movement of H_2 in a body. First, we show that hepatic glycogen accumulates H_2 after oral administration of H_2-water, explaining why consumption of even a small amount of H_2 over a short span time efficiently improves various disease models. This finding was supported by an *in vitro* experiment in which glycogen solution maintained H_2. Next, we examined the benefit of *ad libitum* drinking H_2-water to type 2 diabetes using *db/db* obesity model mice lacking the functional leptin receptor. Drinking H_2-water reduced hepatic oxidative stress, and significantly alleviated fatty liver in *db/db* mice as well as high fat-diet-induced fatty liver in wild-type mice. Long-term drinking H_2-water significantly controlled fat and body weights, despite no increase in consumption of diet and water. Moreover, drinking H_2-water decreased levels of plasma glucose, insulin, and triglyceride, the effect of which on hyperglycemia was similar to diet restriction. To examine how drinking H_2-water improves obesity and metabolic parameters at the molecular level, we examined gene-expression profiles, and found enhanced expression of a hepatic hormone, fibroblast growth factor 21 (FGF21), which functions to enhance fatty acid and glucose expenditure. Indeed, H_2 stimulated energy metabolism as measured by oxygen consumption. The present results suggest the potential benefit of H_2 in improving obesity, diabetes, and metabolic syndrome.

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Molecular hydrogen attenuates fatty acid uptake and lipid accumulation through downregulating CD36 expression in HepG2 cells

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Abstract

Background: There is accumulating evidence that obesity is closely associated with an impaired free fatty acid metabolism as well as with insulin resistance and inflammation. Excessive fatty acid uptake mediated by fatty acid translocase CD36 plays an important role in hepatic steatosis. Molecular hydrogen has been shown to attenuate oxidative stress and improve lipid, glucose and energy metabolism in patients and animal models of hepatic steatosis and atherosclerosis, but the underlying molecular mechanisms remain largely unknown.

Methods: Human hepatoma HepG2 cells were exposed to palmitate-BSA complex after treatment with or without hydrogen for 24 h. The fatty acid uptake was measured by using spectrofluorometry and the lipid content was detected by Oil Red O staining. JNK phosphorylation and CD36 expression were analyzed by Western blot and real-time PCR analyses.

Results: Pretreatment with hydrogen reduced fatty acid uptake and lipid accumulation after palmitate overload in HepG2 cells, which was associated with inhibition of JNK activation. Hydrogen treatment did not alter CD36 mRNA expression but reduced CD36 protein expression.

Conclusion: Hydrogen inhibits fatty acid uptake and lipid accumulation through the downregulation of CD36 at the protein level in hepatic cultured cells, providing insights into the molecular mechanism underlying the hydrogen effects *in vivo* on lipid metabolism disorders.

Keywords: Molecular hydrogen, HepG2 cells, Fatty acid, JNK, Phosphorylation, CD36, Hepatic steatosis

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