Molecular Hydrogen Suppresses Renal Injury in Chronic Kidney Disease Rats

Bo Chen1, Dong Song2, Juan Ma3 and Wanjun Zhu2*

1Lianyungang Traditional Chinese Medicine Hospital, Kangda College, Nanjing Medical University, Lianyungang, Jiangsu Province, China
2Kangda College, Nanjing Medical University, Lianyungang, Jiangsu Province, China
3Nanjing Medical University, Lianyungang, Jiangsu Province, China

*Corresponding author: Wanjun Zhu, Kangda College, Nanjing Medical University, Lianyungang, Jiangsu Province, China, Tel: +86518-80689632; E-mail: zhutohoku@163.com

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Abstract

Background: Cathode side commercial hydrogen dissolved water (HW) exhibits low dissolved oxygen, high dissolved hydrogen and significant negative redox potential. Therapeutic applications of HW have recently been reported. Thus, the present study aimed to examine effects of HW consumption on renal injury in a rat model of chronic kidney disease.

Methods: Twenty Dahl S rats were given HW and tap water (TW), over a 4-week period. Thereafter, they were allocated to either group: non stressed (NS), and oxi-carbonyl stressed (OC: 5% salt diet and 1% methylglyoxal in drinking water) group, respectively (HW, TW n=5 each group). OS groups were subjected to unilateral kidney ischemic reperfusion (IR) in the final week.

Results: No differences were found in blood pressure and urinary parameters between HW and TW. Glomerular adhesion rates in the IR kidney and positive osteopontin of the non-IR kidney were significantly higher in OC rats on HW, respectively. ED-1 staining on HW was significantly lower than TW in both the IR and non-IR kidneys of the OC rats. Plasma MCP-1 was significantly lower on HW after IR.

Conclusion: Drinking HW at least partly suppressed renal damage in rats with combination of oxidative, carbonyl and ischemic stimulus.

Keywords: Commercial hydrogen dissolved water; Dahl salt rat; Chronic kidney disease; Methylglyoxal; Ischemic reperfusion; Oxidative stress; Inflammation

Introduction

Hydrogen molecules are scavengers of hydroxyl radicals. In vitro, H2 can selectively reduce ROS. It only reacts with the strongest oxidants, which means that the use of H2 is mild and has no serious side effects [1]. Molecular hydrogen can inhibit the release of cell adhesion molecules and proinflammatory cytokines. H2 can increase the level of anti-inflammatory cytokines. H2 enhances HO-1 expression and activity, suggesting that H2 can inhibit excessive inflammation and endothelial injury through Nrf2 (nuclear factor RBC 2 p45 related factor 2)/ HO-1 pathway [2]. Water electrolysis gives rise to unique properties in cathode-side water (HW), such as increased alkalinity, low dissolved oxygen, high dissolved hydrogen, and negative oxidation-reduction potential [3]. In the Lipopolysaccharide (LPS) or cecftis group, the serum Cr were much higher than in the sham group. The increases of serum Cr were significantly reduced in the H2 inhalation group [4–7]. But one study found no significant difference in BUN/Cr rates across all groups. Molecular hydrogen therapy may not be as effective as we thought when the BUN/Cr ratio was used to determine whether azotemia or tubular ischemia predated the presence of AKI [7]. In addition, someone suggested that hydrogen molecules have the ability to influence a variety of ways, and help the MPO (myeloperoxidase), MCP, Caspase 3, Caspase-12, TNF (tumor necrosis factor), interleukin, Bcl-2, Bax and cox-2 (as shown in Figure 1) [8] gene regulation or protein expression. In chemical terms, HW is known to suppress generation of superoxide anions and hydrogen peroxide during the oxidative process [3], and decreases oxidative injury to DNA in vitro [3,9]. Biologically, HW protects islet cells from oxidative injury induced by high glucose levels [10].
which can aggravate the condition of septic shock patients and lead to cell injury was improved in the H%

of [4,5]. Acute renal injury (AKI) is a common disease in sepsis patients, and carbonyl stress are characteristic features of chronic kidney disease (CKD) [12,13]. These stressors supposedly play a crucial role in progressive renal deterioration. Kidney injury in the Dahl salt-sensitive (SS) rat, a model of CKD, has been shown to be ameliorated by antioxidants such as vitamins C and E [14], the superoxide dismutase-
mimetic tempol [15], and N-acetylcysteine [16]. However, no promising results have been confirmed in the clinical application of antioxidants [17]. Despite some controversy, molecular hydrogen therapy is considered an effective way to reduce kidney structural damage, protect kidney function, and resist inflammation and oxidation. All studies have shown that molecular hydrogen therapy improves the survival rate of septic animals regardless of the effects of drug administration and sepsis [18]. In this context, the application of HW for CKD could offer an innovative treatment. The present study examined the effects of HW drinking on kidney damage in CKD model rats.

Methods

Animals and protocols

Seven-week-old male Dahl SS rats were housed in a temperature and humidity-controlled room with 12-h light/dark cycles. A 0.5% salt diet was provided, and rats were allocated to HW and tap water (TW) groups (n=10 each) with ad libitum access to water over a 4-week period. Thereafter, rats were reallocated as follows: 10 rats (n=5 each from HW and TW groups) remained in the same groups for an additional 16 weeks (non-stressed group), while the remaining rats (n=5 each from HW and TW groups) were given a 5% salt diet and 1% methylglyoxal (MGO) in drinking water for 16 weeks (oxi-carbonyl stressed rats). Further, rats of the latter group were subjected to a unilateral kidney artery clamp (left kidney, for 45 min), followed by ischemic reperfusion (IR) in week 20. Water was changed twice a day, in the morning and afternoon, and was delivered by metallic straw from a closed bottle.

During the course of the study, blood pressure, body weight, volume of drinking water, 24-h urinary volume, 24-h urinary excretion of protein and thiobarbituric acid reactive substances (TBARS) were measured every 4-weeks regularly. Whole kidneys for histological examination and blood samples from the aortic artery were collected at the end of the study in the non-stressed group, and 3 days after unilateral IR in the oxi-carbonyl stressed group.

During IR, rats were anesthetized using intraperitoneal phenobarbital. All procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals and protocols were approved by the Animal Committee at Tohoku University School of Medicine.

Generation and chemical properties of HW

What we used was one of the commercial products. Most of the hydrogen water's character is pH>8.5, hydrogen concentration>1 ppm, ORP<-600 mV, the properties of HW are shown in Table 1.

| Water type | pH   | Dissolved oxygen (mg/l) | Dissolved hydrogen (mg/l) | Redox potential (mV) |
|------------|------|-------------------------|--------------------------|---------------------|
| TW         | 7.0~7.2 | 2.6~7.0                  | 0                        | +200 ≈ +350         |
| HW         | 10~10.5 | 1.3~3.5                  | 0.3~0.6                  | -200 ≈ -120         |

Table 1: Chemical properties of commercial hydrogen dissolved water (HW).
Measurements

Blood pressure was measured by the tail-cuff method using Blood pressure monitor for mice and rats MK2000A (Muromachi, Tokyo, Japan) in the morning. Urinary protein was measured using a Quick Start bovine serum albumin standard set (Bio-Rad Laboratories, Hercules, and CA). Urinary TBARS were measured by lipid peroxidation assay method. Plasma creatinine and BUN (Blood urea nitrogen) were measured using an auto-analyzer (Beckman Coulter, Fullerton, CA) and MCP-1 was measured using an ELISA (Enzyme linked Immuno Sorbent Assay) kit (Invitrogen, Carlsbad, CA). MGO was measured by a LC/MS (Liquid chromatography-mass spectrometry) method, as previously reported (6) at Trim Medical institute Co. Ltd.

Histological examinations

Kidney sections were stained using the Elastica-Masson method for determining renal injury and cardio-injury, then examined using light microscopy. Glomerular adhesion was determined from the findings of all cortical glomeruli in each rat (>70). Cardio-fibrosis area was measured by using Image J software (NIH public domain for experimental analyze). For immunohistochemical analysis, kidney tissue was immediately fixed with 95% ethanol overnight, then with 100% ethanol overnight. Tissue was embedded in paraffin, and 3-µm-thick sections were cut and mounted on slide glasses. Slides were deparaffinized with xylene and ethanol. Immunohistochemical analysis was performed using monoclonal antibodies against osteopontin (OPN; Santa Cruz Inc, Santa Cruz, CA) and ED-1 (Serotec, Oxford, UK), then incubated overnight at 4°C. Results were analyzed using Image J software.

Statistical analysis

Data are expressed as mean ± standard error of the mean and analyzed using independent t-test or two-way repeated measure ANOVA. Differences between the two groups were considered significant for values of p < 0.05. All analyses were performed using Sigma stat 3.5 software (Systat Software, Chicago, IL).

Results

Changes in body weight, volume of drinking water ingested, mean blood pressure (BP) urinary protein and TBARS are shown in Tables 2 and 3. No differences were found in the change of those parameters between TW and HW subgroups in both non-stressed and oxi-carbonyl stressed groups.

|               | Baseline | 4 weeks | 8 weeks | 16 weeks | 20 weeks | P   |
|---------------|----------|---------|---------|----------|----------|-----|
| Body weight   | TW       | 163 ± 2 | 259 ± 2 | 340 ± 5  | 440 ± 7  | 469 ± 9 |
| (g)           | HW       | 164 ± 1 | 266 ± 1 | 328 ± 3  | 434 ± 7  | 451 ± 6 |
| Water intake  | TW       | 25.2 ± 1.4 | 38.8 ± 1.8 | 30.8 ± 2.6 | 40.4 ± 0.2 | 40.6 ± 12.2 |
| (g/day)       | HW       | 26.8 ± 1.8 | 42.3 ± 1.8 | 33.6 ± 2.8 | 40.3 ± 0.2 | 58.6 ± 4.6 |
| Mean BP       | TW       | 107 ± 2 | 103 ± 4 | 166 ± 5  | 178 ± 11 | 184 ± 14 |
| (mmHg)        | HW       | 110 ± 1 | 116 ± 5 | 162 ± 11 | 161 ± 14 | 190 ± 8 |
| Urine-protein | TW       | 23.7 ± 6.9 | 107.3 ± 12.4 | 158.9 ± 17.4 | 206.9 ± 16.1 | 250.5 ± 43.0 |
| (mg/day)      | HW       | 23.2 ± 6.3 | 110.8 ± 13.7 | 155.3 ± 18.6 | 181.0 ± 40.7 | 267.1 ± 38.4 |
| Urine-TBARS   | TW       | 74.7 ± 12.8 | 170.9 ± 17.7 | 156.1 ± 10.1 | 145.2 ± 9.6 | 139.6 ± 15.1 |
| (μM MDA/day)  | HW       | 79.5 ± 10.6 | 152.9 ± 26.3 | 147.8 ± 5.6 | 101.5 ± 13.0 | 127.9 ± 10.6 |

Table 2: Comparison of parameters between rats on tap water (TW) and commercial hydrogen dissolved water (HW) in the non-stressed group.
Table 3: Comparison of parameters between rats on tap water (TW) and commercial hydrogen dissolved water (HW) in the oxi-carbonyl stressed group.

| Parameter                  | TW           | HW           | P     |
|----------------------------|--------------|--------------|-------|
| Urine-TBARS (μM MDA/day)   | 136.8 ± 14.8 | 171.1 ± 25.3 | NS    |
|                           | 154.5 ± 6.5  | 189.9 ± 9.1  |       |
|                           | 159.3 ± 17.2 |              |       |
|                           | 128.4 ± 7.3  | 145.5 ± 15.4 |       |
|                           | 157.1 ± 9.2  | 167.4 ± 8.5  |       |
|                           | 133.0 ± 14.9 |              |       |

Representative histological findings are shown in Figures 1b, 2b, 3b and 4b. Histologically, no differences were found in glomerular adhesion rate between rats on TW or HW in the non-stressed group and the non-IR kidney of the oxi-carbonyl stressed groups. However, a significantly lower rate was found in the IR kidney of rats on HW (28.5 ± 2.3% in TW vs. 11.7 ± 1.8% in HW per slice; p<0.05) (Figure 1a). No differences were found in OPN staining area between rats on TW and HW in the non-stressed group and the IR kidney of the oxi-carbonyl stressed groups. While a significantly lower level was found in the non-IR kidney of rats on HW of the oxi-carbonyl stressed groups (14.0 ± 3.8 in TW vs 5.0 ± 0.6% in HW; p<0.05) (Figure 2), with ED-1 staining, significantly more positive cells were seen in rats on HW as compared to those on TW in the non-IR and IR kidneys of the oxi-carbonyl stressed group (1.6 ± 0.4 vs. 0.7 ± 0.1 cells/glomerulus; and 1.1 ± 0.2 vs. 0.6 ± 0.0 cells/glomerulus, respectively, p<0.05) (Figure 3a).

With cardio-fibrosis significantly higher fibrosis area was seen in rats on HW as compared to those on TW in oxi-carbonyl stressed groups (5.0 ± 0.63 in TW vs. 3.2 ± 0.30% in HW; p<0.05), not in rats of no stressed groups.

Plasma levels of creatinine, BUN and MCP-1 in the oxi-carbonyl stressed group are shown in Table 4. No significant differences were found in creatinine or BUN levels between TW and HW groups, whereas significantly lower MCP-1 values were found in rats on HW (61.5 ± 12.9 vs. 19.4 ± 1.0 pg/ml; p<0.05).

Table 4: Comparison of blood parameters between rats on TW and HW in the oxi-carbonyl stressed group after IR.

| Parameter                  | TW           | HW           | P     |
|----------------------------|--------------|--------------|-------|
| Creatinine (mg/dl)         | 38.0 ± 2.7   | 29.3 ± 1.4   | NS    |
| Blood urea nitrogen (mg/dl)| 0.6 ± 0.1    | 0.6 ± 0.1    | NS    |
| MCP-1 (pg/ml)              | 61.5 ± 12.9  | 19.4 ± 1.0   | <0.05 |

Figure 2: (a) Osteopontin staining in the outer medulla (%). Data are expressed as mean ± SEM (n=5 each) *p<0.05. (b) Osteopontin staining in the outer medulla of oxi-carbonyl stressed group. (1) non-IR kidney on TW, (2) non-IR kidney on HW, (3) IR kidney on TW, (4) IR kidney on HW. (× 80) TW: rats on tap water; HW: rats on commercial hydrogen dissolved water; IR: ischemic reperfusion.

Figure 3: (a) ED-1 staining in glomeruli in oxi-carbonyl stressed group. More than 70 glomeruli were measured in each sample. Data are expressed as mean ± SEM (n=5 each, *p<0.05. b) Representative ED-1-positive cells. (1) Negative; (2) positive findings; (× 400) TW: rats on tap water; HW: rats on commercial hydrogen dissolved water; IR: ischemic reperfusion.
The present study were exposed to the same level of carbonyl stress. The results may suggest that HW could suppress enhancement of medullary OPN expression in the non-IR kidney of oxi-carbonyl stressed rats. The medulla is highly sensitive to oxidative stress [14], carbonyl stress and ischemic insult [13], and protection of the medulla could play a key role in preserving kidney function. OPN is expressed in the loop of Henle and distal nephron, and expression is known to be closely related to renal fibrosis [19]. Renal ischemia reperfusion induced remote organ damage, like cardio-fibrosis involved oxidative stress and inflammatory system, and drinking commercial hydrogen dissolved water with high concentration hydrogen protect this organ damage. In this context, it seems that HW drinking showed a protective effect on the medullary portion under enhanced oxidative and carbonyl stress. Furthermore, we performed IR in oxi-carbonyl stressed group to test the influence of ischemic insult in CKD condition. As a result, glomerular adhesion rates, ED-1 staining in glomeruli, and plasma MCP-1 levels were significantly lower in rats on HW as compared to those on TW, respectively. Thus, it was indicated that HW drinking inhibits inflammatory conditions leading to glomerular adhesions by suppressing macrophage activations.

Taken together the results, it was indicated that HW drinking showed a protective effect on the medullary as well as glomerular portions against the enhanced oxidative and carbonyl stress and inflammatory condition, and this may indicate a therapeutic potential of HW for CKD management. Nevertheless, no effect of HW was observed in terms of BP or urinary parameters during the course of the study, and the histological differences were limited. Several mechanisms may have contributed to this finding. First, the chemical properties of HW may not have been preserved throughout the day. For example, oxidation-reduction potential level was shown to be elevated up to 60% at 24 h (data not shown). Second, the anti-oxidative capacity of HW administered in the present study, may not have been sufficient to counteract the oxidative stressors in oxi-carbonyl stressed rats. This needs further investigations.

Characteristic chemical features of HW are the high dissolved hydrogen and low dissolved oxygen. The biological mechanism of HW to suppress oxidative stress has not clearly elucidated so far [3]. Recently, a series of studies on inhalation of hydrogen gas have revealed that hydrogen application has suppressive effects on brain infarct lesions by cerebral artery occlusion [1] and liver injury by ischemic-reperfusion [20]. Furthermore, drinking hydrogen-saturated water could ameliorate brain oxidation [21], and recent functional memory disturbance caused by oxidative stress [13]. Drinking HW can suppress oxidative stress and inflammation induced by ischemia reperfusion. Thus, the biological effects of HW may, at least partly, involve dissolved hydrogen levels of water. Those studies employed water with dissolved hydrogen levels >0.4 mM [21], approximately equivalent to the water employed in the present study. The role of hydrogen of HW needs to be addressed in future studies.

In conclusion, HW at least partially suppressed renal damage in rats with the combination of oxidative, carbonyl stress and ischemic insult. The leading mechanism by which HW exerts its effects may involve the inhibition of oxidative-carbonyl- inflammatory conditions. HW consumption may open a novel approach to managing CKD patients.

References

1. Ohswa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, et al. (2007) Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Natu Medici 13: 688-669.
2. Chen H, Xie K, Han H, Yuan L, Liu L (2015) Molecular hydrogen protects mice against polymicrobial sepsis by ameliorating endothelial dysfunction via an Nrf2/HO-1 signaling pathway. Inter Immunopharmaco 28: 643-654.

3. Shirahata S, Kabayama S, Nakano M, Miura T, Kusumoto K, et al. (1997) Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. Biochem Biophysi Res Commun 234: 269.

4. Xie K, Yu Y, Pei Y, Hou L, Chen S (2010) Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. Shock 34: 90-97.

5. Xie K, Fu W, Xing W, Li A, Chen H, et al. (2012) Combination therapy with molecular hydrogen and hyperoxia in a murine model of polymicrobial sepsis. Shock 38: 656-663.

6. Li GM, Ji MH, Sun XJ, Zeng QT, Tian M, et al. (2013) Effects of hydrogen-rich saline treatment on polymicrobial sepsis. J Surgic Res 181: 279-286.

7. Liu W, Dong XS, Sun YQ, Liu Z (2014) A novel fluid resuscitation protocol: provide more protection on acute kidney injury during septic shock in rats. Inter J Clinic Experi Med 7: 919-926.

8. Dixon BJ, Tang J, Zhang JH (2013) The evolution of molecular hydrogen: a noteworthy potential therapy with clinical significance. Med Gas Res 3: 10-11.

9. Lee MY, Kim YK, Ryoo KK, Lee YB, Park EJ (2006) Electrolyzed-reduced water protects against oxidative damage to DNA, RNA, and protein. Applied Biochem Biotech 135: 133-144.

10. Kim M, Jung KH, Uhm YK, Kim KH (2007) Preservative effect of electrolyzed reduced water on pancreatic beta-cell mass in diabetic db/db mice. Biologic Pharmaceu Bulletin 30: 234.

11. Kim MJ, Kim HK (2006) Anti-diabetic effects of electrolyzed reduced water in streptozotocin-induced and genetic diabetic mice. Life Sci 79: 2288-2292.

12. Terawaki H, Yoshimura K, Hasegawa T, Matsuura Y, Negawa T, et al. (2004) Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin. Kidney Inter 66: 1988.

13. Nakayama K, Nakayama M, Iwabuchi M, Terawaki H, Sato T, et al. (2008) Plasma alpha-oxoaldehyde levels in diabetic and nondiabetic chronic kidney disease patients. Americ J Nephrolo 28: 871-878.

14. Taylor NE, Cowely AW (2005) Effect of renal medullary H2O2 on salt-induced hypertension and renal injury. Am J Physiol Regul Integr Comp Physiol 289: 1573-1579.

15. Nishiyama A, Yoshizumi M, Hitomi H, Kagami S, Kondo S, et al. (2004) The SOD mimetic tempol ameliorates glomerular injury and reduces mitogen-activated protein kinase activity in Dahl salt-sensitive rats. Journal of the American Society of Nephrology Jasn 15: 306.

16. Zhang L, Fujii S, Igarashi J, Kosaka H (2004) Effects of thiol antioxidant on reduced nicotinamide adenosine dinucleotide phosphate oxidase in hypertensive Dahl salt-sensitive rats. Free Radic Bio Medici 37: 1813-1820.

17. Manning R, Tian N, Meng S (2005) Oxidative stress and antioxidant treatment in hypertension and the associated renal damage. Americ J Nephrolo 25: 311-317.

18. Zhang J, Wu Q, Song S, Wan Y, Zhang R, et al. (2014) Effect of hydrogen-rich water on acute peritonitis of rat models. International Immunopharmacology 21: 94-101.

19. Yoo KH, Thornhill BA, Forbes MS, Coleman CM, Marcinko ES, et al. (2006) Osteopontin regulates renal apoptosis and interstitial fibrosis in neonatal chronic unilateral ureteral obstruction. Kidney International 70: 1735-1741.

20. Fukuda K, Asoh S, Ishikawa M, Yamamoto Y, Ohbasa I, et al. (2007) Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. Biochem Biophysi Res Communi 361: 670-674.

21. Sato Y, Kaiyama S, Amano A, Kondo Y, Sasaki T, et al. (2008) Hydrogen-rich pure water prevents superoxide formation in brain slices of vitamin C-depleted SMP30/GNL knockout mice. Biochem & Biophy Res Communi 375: 346-350.