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Effects of hydrogen-rich water and ascorbic acid treatment on spontaneously hypertensive rats

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Abstract: Hydrogen-rich water (HW) has been suggested to possess antioxidant properties of value in treatments of lifestyle diseases and for prevention of latent pathologies. To date, the potential benefits of HW against the deleterious effects of excessive salt intake and hypertension have not been investigated. Here, we first examined the effects of HW or HW supplemented with 0.1% ascorbic acid (HWA) on spontaneously hypertensive rats (SHR) that had been fed a normal diet. In comparison to control rats given distilled water (DW), we found that HW did not significantly influence systolic blood pressure (SBP) or diastolic blood pressure (DBP) in SHR; however, the increase in SBP and DBP were inhibited in the HWA group. Next, four groups of SHR were given DW, 0.1% ascorbic acid-added DW (DWA), HW, or HWA in combination with a 4% NaCl-added diet. SHR fed the 4% NaCl-added diet showed increased hypertension; HWA treatment resulted in a significant reduction in blood pressure. The HWA group tended to have lower plasma angiotensin II levels than the DW group. In addition, urinary volumes and urinary sodium levels were significantly lower in the HWA group than the DW group. Urinary isoprostane, an oxidative stress marker, was also significantly lower in the HWA group, suggesting that the inhibitory effect of HWA on blood pressure elevation was caused by a reduction in oxidative stress. These findings suggest a synergistic interaction between HW and ascorbic acid, and also suggest that HWA ingestion has potential for prevention of hypertension.
Key words: ascorbic acid, hydrogen water, hypertension, oxidative stress, spontaneously hypertensive rats (SHR)

Introduction

According to the guidelines for the management of hypertension (JSH2019), approximately 43 million people in the Japanese population are hypertensive; blood pressure management is poor in 31 million of these people and preventative treatments are strongly desired [1]. In general, treatment of hypertension is based on drug-mediated symptomatic therapy; however, from the viewpoint of the long-term prevention of hypertension, improvement in everyday eating habits may be important. The intake of salt is still high in the Japanese population. Since high salt intake is considered a risk factor in hypertension, reduction in this intake should result in an overall reduction in blood pressure levels.

Essential hypertension, which accounts for more than 90% of cases, is a multifactorial disease that develops through genetic predisposition and environmental factors such as eating habits, lack of exercise, and stress. Spontaneously hypertensive rats (SHR) are widely recognized as an animal model of essential hypertension. High blood pressure in these rats is in part due to oxidative stress and interstitial nephritis [2]; the rats produce large amounts of reactive oxygen species in their vascular

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endothelial cells [3], suggesting that hypertension might be prevented by reducing oxidative stress.

Another approach to prevent hypertension is the use of functional foods that may have beneficial physiological effects in addition to basic nutrition [4–6]. We have previously investigated the effects of functional foods on blood pressure (BP) and oxidative stress reduction and reported that 5% γ-aminobutyric acid lowered BP in SHR [7]. We subsequently showed that flavonoids and coumarins in citrus fruit waste might mitigate BP elevation in SHR [8].

Recently, functional water (water enhanced by the addition of supplements) has attracted attention. One type of functional water is hydrogen-rich water produced by electrolysis. After electrolysis, the water around the anode and the cathode differs. At the anode, the pH is acidic and oxygen concentration is increased; at the cathode, the pH is alkaline and H_2 is characteristically present although absent in normal water. The water on the cathode side is termed drinkable electrolyzed reduced water or hydrogen-rich water (HW) and is widely used as drinking water by Japanese families. Previous studies have reported that HW has anti-inflammatory activity [9] and that it may be of value for treatment of Alzheimer's disease [10, 11], myocardial infarction [12], arteriosclerosis [13], and diabetes [14, 15].

To date, no study has investigated whether HW can ameliorate the deleterious effects of excessive salt intake and hypertension. To this end, we induced hypertension in SHR by feeding with a high salt load diet (4% NaCl) and then examined the effects of HW treatment. In addition, the possible antihypertensive action of vitamin C (ascorbic acid) in combination with HW was also investigated.

Materials and Methods

Animals and rearing conditions

Four-week-old male SHR/Izm rats (Disease Model Cooperative Research Association, Kyoto, Japan) were used in the study. The animal room was maintained at a constant temperature (23 ± 2°C) and humidity (55 ± 10%), with a mean ventilation frequency of 10–13/h and a 12 h light/dark cycle (light: 7:00–19:00 h). The rats were housed in TPX cages (W260 × D330 × H170 mm, Natsume Seisakusho Co., Ltd., Tokyo, Japan) containing woodchips (Clean Chip, Shimizu Laboratory Supplies, Co., Ltd., Kyoto, Japan). They were fed a standard commercial diet (MF, Orient Shimane University al Yeast Co., Ltd., Tokyo, Japan) ad libitum. Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at (Approval number: IZ27-119).

Hydrogen-rich water and distilled water

HW was produced using an HW generator (HWPA-2000, Tech Corporation Co., Ltd., Tokyo, Japan) and used to fill drinking water bottles for the cages. Any impurities in the water were removed by filtration through a reverse osmosis membrane filter before generation of HW by electrolysis. The normal drinking water (DW) used in the experiments was obtained from the HW generator without hydrogen gas generation; this water was used to fill the bottles in the cages containing control rats.

Preliminary experiments

Three preliminary experiments were performed before commencing the main study. First, we evaluated the use of two different types of water bottle: a 200 ml plastic bottle (CK-200, CLEA Japan, Inc., Tokyo, Japan) and a 200 ml aluminum pouch-made bottle (DP16-TA200, Cowpack Co., Ltd., Aichi, Japan). Time-course changes in dissolved hydrogen concentrations in the HW were measured in both types of bottle. Three bottles of each type were filled with HW generated as described above, sealed with a lid, and kept at room temperature. The dissolved hydrogen concentration was measured using a DH METERDH-35A (Dissolved hydrogen electrode E-532102, DKK-TOA Corporation Co., Ltd., Tokyo, Japan) meter on days 0, 1, 2, and 3. Second, we evaluated the effect of different ascorbic acid concentrations. To determine an appropriate concentration of ascorbic acid (L (+)-ascorbic acid, Nacalai tesque, Inc., Kyoto, Japan) to be added to the HW, rats were provided with HW to which ascorbic acid had been added at different concentrations and their water intake was measured. Six groups of five-week-old SHR were established: DW, HW, HW + 0.05, 0.1, 0.2, or 0.25% ascorbic acid. Each group had 4 animals. Aluminum bottles were used in this experiment and water intake was measured for 2 days. Third, we measured the changes in dissolved hydrogen concentrations in HW and 0.1% ascorbic acid-added HW. Aluminum bottles were filled with HW or 0.1% ascorbic acid-added HW (HWA), sealed with a lid, and kept at room temperature; five bottles of each type were prepared. Dissolved hydrogen concentrations were measured on days 0, 1, 2, 3, 4, and 5 using a DH METERDH-35A meter.

Experimental protocol

Two experiments were performed in the main study: first, the antihypertensive effects of HW and HWA were

evaluated in SHR fed a standard diet; second, the antihypertensive effects of HW and HWA on SHR fed a 4% NaCl-added diet were evaluated. In the first experiment, SHR were allocated to different treatment groups so as to have uniform means and standard deviations of body weight and BP. Three groups of 10 rats were used: DW (control) group, HW group, and HWA group. To maintain the hydrogen concentration in the water bottles, HW and HWA were replaced daily. Rats in each group were given free access to the experimental water. They also had free access to a standard commercial diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The experiment was performed for 12 weeks. Body weights, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured once every 2 weeks during the experimental period. SBP and DBP were measured using an automatic sphygmomanometer apparatus (BP-98A, Softron Co., Ltd., Tokyo, Japan) at the tail vein, employing the tail-cuff method without anesthesia after warming the rat at 38°C for 8 min. BP was measured 5 times for each rat and the mean was calculated.

In the second experiment, four groups of SHR were used (10 animals in each): DW (control) group, 0.1% ascorbic acid-added DW (DWA) group, HW group, and HWA group. The experiment was performed for 12 weeks; the rats in each group had ad libitum access to the experimental water. The rats were fed with a standard commercial diet (MF, Oriental Yeast Co., Ltd.) for the first four weeks of the experiment; the diet was then changed to a 4% NaCl-added diet (prepared by adding 4% NaCl to the standard MF commercial diet). Body weight, heart rate, SBP, and DBP were measured once every 2 weeks during the experiment. BP was measured as described above. Food and water consumption was measured at initiation (0 weeks) and completion (12 weeks) of the experiment. In addition, urine was collected for 24 h at 12 weeks after initiation of the experiment using a metabolic cage (CT-10S, CLEA Japan, Inc.). The collected urine samples were cryopreserved at −80°C until measurement. At 12 weeks, at completion of the experiment, rats were fasted for 16 h and then euthanized by isoflurane anesthesia (Escain, Mylan Seiyaku, Tokyo, Japan) using an anesthesia delivery system (KN-1071-1, Natsume Seisakusho, Co., Ltd.). Blood was collected from the abdominal vena cava, transferred to a micro blood-sampling tube (Capiject, ethylenediaminetetraacetic acid (EDTA)-2Na, Terumo Co., Ltd., Tokyo, Japan), and centrifuged at 860 × g for 15 min at 4°C. The supernatant was used as the plasma sample and subjected to blood biochemistry analyses to determine the levels of the following components using a benchtop chemistry analyzer (SPOTCHEM™ EZ SP-4430, ARKRAY, Inc., Kyoto, Japan): aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), total cholesterol (T-Cho), high-density lipoprotein cholesterol (HDL-c), triglyceride (TG), total protein (T-Pro), albumin (ALB), urea nitrogen (BUN), uric acid (UA), and creatinine (Cre). Urine total protein (U-TP) and urine-sodium (U-Na) in the samples were measured by the Nagahama Life Science Laboratory (Oriental Yeast Co., Ltd., Shiga, Japan). In addition, plasma angiotensin II (Ang II) was measured using a commercial enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd., Tokyo, Japan). After thawing to room temperature, urine samples were centrifuged at 860 × g for 15 min, and the supernatant, excluding precipitate, was used for measurement: 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured using a commercial enzyme-linked immunosorbent assay kit (8-OHdG Check kit, Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd.); urinary isoprostane was measured using a commercial enzyme-linked immunosorbent assay kit (urinary isoprostane kit, Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd.).

Statistical analysis

All data are expressed as the mean ± SEM. ANOVA was used to assess differences in the means among groups, followed by a post hoc Scheffe's PLSD test; $P<0.05$ was considered significant. Analyses were performed using StatView (SAS Institute Inc., Cary, NC, USA).

Results

Preliminary experiments

Two types of water bottle were evaluated (Fig. 1A). In both bottles, a concentration of 0.8 ppm of hydrogen gas was initially present. After 1 day, the concentration fell to 0.55 ppm in the plastic bottle and 0.75 ppm in the aluminum bottle. At 2 days and thereafter, it dropped to 0 ppm in the plastic bottle, but was maintained at 0.65 ppm in the aluminum bottle. The aluminum bottle was therefore better at retaining dissolved hydrogen in the treated water.

In a second preliminary experiment, the water intake of rats given different concentrations of ascorbic acid was compared (Fig. 1B). Rats given either 0.05 or 0.1% ascorbic acid-added HW had higher rates of water consumption than those given HW. On the other hand, water consumption was significantly lower in rats given either 0.2% or 0.25% ascorbic acid-added HW than in those given DW ($P<0.01$). Consequently, as the intake

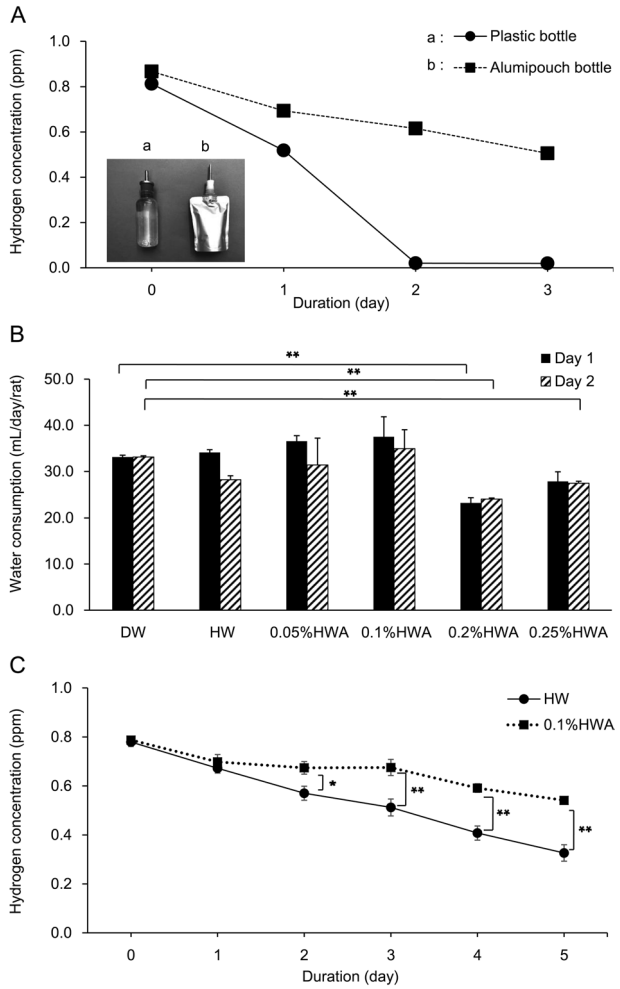

Fig. 1. Preliminary experiments. (A) Dissolved hydrogen concentrations in hydrogen water (HW) after storage in either plastic bottles or aluminum pouch (Alumipouch) bottles. Three bottles were used in each group; these were closed with a lid, and kept at room temperature. (B) Water intake in rats given water supplemented with different concentrations of ascorbic acid. Ascorbic acid (0.05, 0.1, 0.2, and 0.25%) was added to distilled water (DW) and HW. Six groups of 4 animals were treated. The 200 ml aluminum pouch bottle was filled with the experimental water and the water intake of the rats was measured for 2 days. Each value represents the mean ± SEM. ***P*<0.01 versus DW group. (C) Dissolved hydrogen concentrations in HW and 0.1% ascorbic acid-added HW (HWA) were measured on days 0, 1, 2, 3, 4, and 5. Five bottles were used for each group. Each value represents the mean ± SEM. **P*<0.05, ***P*<0.01.

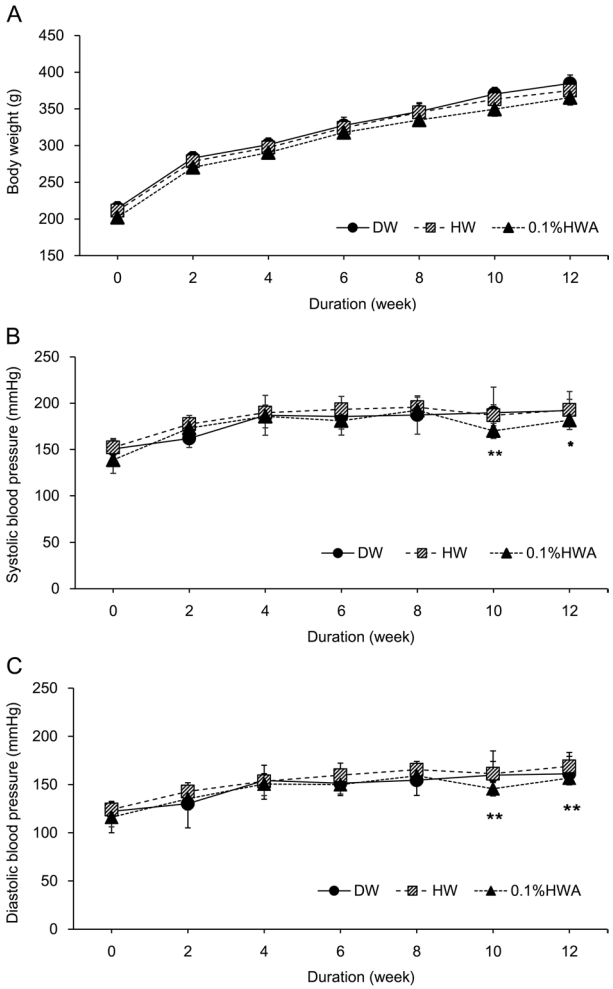

Fig. 2. Changes in blood pressure in spontaneously hypertensive rats (SHR) fed with a standard diet and given HW or HWA. (A) Body weight, (B) systolic blood pressure, (C) diastolic blood pressure. Three groups of 10 animals were used: control group (distilled water, DW), hydrogen water group (HW), and 0.1% ascorbic acid-added HW group (HWA). Each value represents the mean ± SEM. **P*<0.05, ***P*<0.01 versus DW group.

of 0.1% ascorbic acid-added HW was superior to that of DW and HW, we used this concentration in subsequent experiments.

In the third preliminary experiment, changes in dissolved hydrogen concentrations in HW and HWA were evaluated (Fig. 1C). The initial concentration was set as 0.8 ppm in both groups. After 1 day, the concentration fell to 0.7 ppm in both groups. Thereafter, in the HW group, the hydrogen concentration decreased gradually to 0.37 ppm after 5 days. In the HWA group, the concentration remained at 0.7 ppm until day 3 and was significantly higher than in the HW group (P<0.01). The addition of 0.1% ascorbic acid in the HWA group appeared to prevent the decline in hydrogen concentration.

Experiment 1: Antihypertensive effects of HW and HWA in rats fed a standard diet

Measurement of body weight is a useful method for early detection of the onset of disease or debilitation in animals. Here, we found that body weights increased during the experimental period for all groups; no significant differences were found among the groups (Fig. 2A). No significant differences were found for SBP among the groups from the start of experiment to 8 weeks (Fig. 2B). However, SBP was significantly lower in the 0.1% HWA group compared with the DW group at 10

and 12 weeks (P<0.01 and P<0.05, respectively). Similarly, DBP was significantly lower in the HWA group than the DW group at 10 and 12 weeks (Fig. 2C; P<0.01). These results indicate that HWA exerts an antihypertensive effect.

Experiment 2: Antihypertensive effects of HW and HWA in rats fed with 4% NaCl

Water and food consumption by the rats in the different treatment groups are shown in Figs. 3A and 3B, respectively. The animals were fed with the standard diet at the start of experiment and no significant difference was noted in the water or food consumption among the groups. After 4 weeks, the animals were fed the 4% NaCl-added diet. Water and food consumption increased compared with those at the start of experiment, but the intakes were comparable among the groups, with no significant differences. These results suggest that HWA does not affect water and food consumption.

The body weights of rats fed the 4% NaCl-added diet increased in each group (Fig. 4A). There were no significant differences in body weights among the four groups. Heart rates ranged from 300 to 400 bpm with no significant differences among the groups (Fig. 4B). Changes in SBP over the duration of the experiment are shown in Fig. 4C. SBP rose in all groups; however, the increase was significantly lower at 10 and 12 weeks in the HWA group than the DW group (P<0.01 and P<0.05, respectively). SBP in the HWA group was significantly lower than in the DWA group at 12 weeks (P<0.05). DBP increased in all four groups of rats; the different treatments did not result in any significant differences among groups (Fig. 4D). These results indicate that HWA exerts an antihypertensive effect.

We measured plasma Ang II levels during the 12-week period (Fig. 5A). Ang II levels tended to decrease more in the HWA than the DW group. Additionally, the HWA group had a lower Ang II concentration than the DWA group (P<0.01). We also examined changes in oxidative stress markers, namely, urinary 8-OHdG (Fig. 5B) and urinary isoprostane (Fig. 5C). Urinary 8-OHdG levels in the HWA group did not differ significantly from those in the DW group. Urinary 8-OHdG levels in the HWA and DWA groups were lower than in the HW group (P<0.05, respectively). Urinary isoprostane levels in the HWA group were significantly lower than in the DW and HW groups (P<0.01 and P<0.05, respectively). Urinary isoprostane levels in the DWA group were lower than in the DW and HW groups (P<0.05, respectively). These results suggest that the antihypertensive effect of HWA only influenced SBP when NaCl intake was high.

The results of the plasma biochemistry analyses and measurements of U-TP and U-Na levels in the 12-week period are shown in Table 1. These analyses indicated that liver and renal functions were within the normal range in all groups: urinary volume in the HWA and DWA groups were significantly lower than the DW group (P<0.01, respectively); U-TP levels were similar among the groups; U-Na levels were significantly lower in the HWA group compared with the DW group (P<0.05).

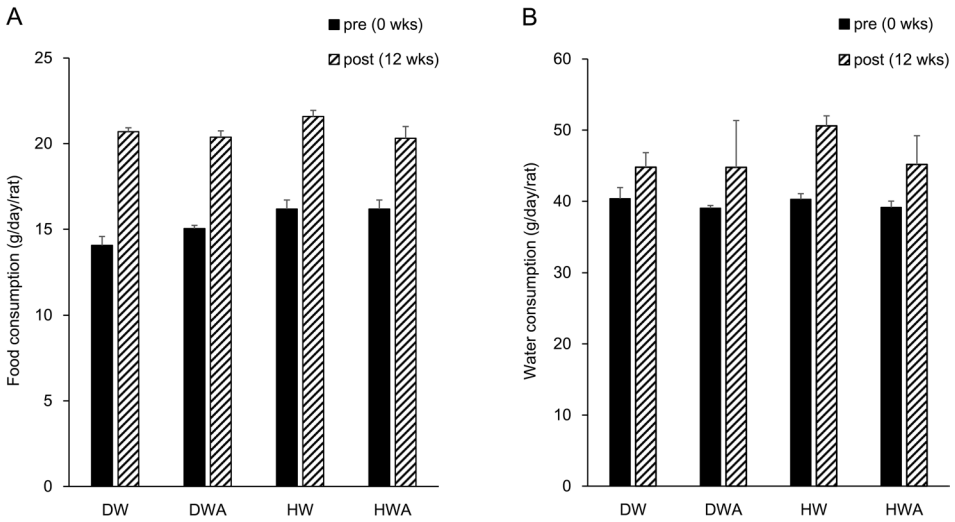

Fig. 3. Changes in (A) food and (B) water consumption in SHR. Rats were fed with the standard diet for 4 weeks before being given the 4% NaCl-added diet until the end of the experiment (12 weeks). Four experimental groups (10 animals in each) were set up: control group (distilled water, DW), 0.1% ascorbic acid-added DW group (DWA), hydrogen water group (HW), and 0.1% ascorbic acid-added HW group (HWA). Each value represents mean ± SEM.

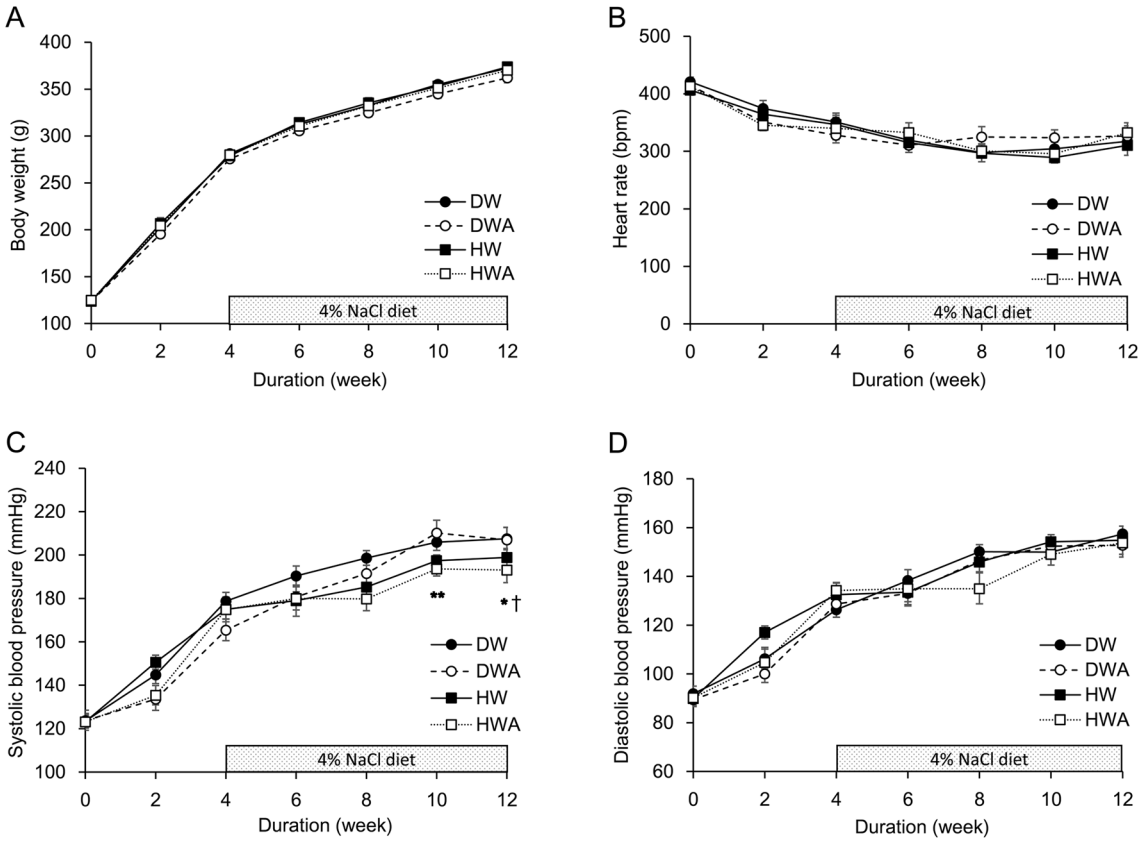

Fig. 4. Changes in blood pressure in SHR fed a 4% NaCl-added diet and provided with DWA, HW or HWA. (A) Body weight, (B) heart rate, (C) systolic blood pressure, (D) diastolic blood pressure. Rats were divided into 4 groups (10 animals in each): control group (distilled water, DW), 0.1% ascorbic acid-added DW group (DWA), hydrogen water group (HW), and 0.1% ascorbic acid-added HW group (HWA). Each value represents the mean ± SEM. **P*<0.05, ***P*<0.01 HWA versus DW group. †*P*<0.05 HWA versus DWA group.

Table 1. Plasma biochemistry analyses, and levels of urinary protein and urinary sodium in SHR fed with a 4% NaCl-added diet

Rat number		DW 10	DWA 10	HW 10	HWA 10
Plasma	AST (IU/L)	69.7 ± 4.53	68.9 ± 3.04	66.8 ± 5.4	83.33 ± 13.42
	ALT (IU/L)	34.1 ± 4.38	31.8 ± 5.89	32.8 ± 4.93	45.56 ± 19.12
	GGT (IU/L)	9.1 ± 0.1	9.7 ± 0.21	9.5 ± 0.17	9.44 ± 0.18
	T-Cho (mg/dL)	64.4 ± 1.4	61.8 ± 1.27	63.3 ± 1.22	60.89 ± 1.03
	HDL-c (mg/dL)	15.9 ± 0.94	16.3 ± 0.84	16.5 ± 0.65	15.22 ± 0.76
	TG (mg/L)	31.6 ± 2.27	29 ± 1.52	26.8 ± 1.25	22.44 ± 2.28
	T-Pro (g/dL)	6.58 ± 0.14	6.72 ± 0.06	6.57 ± 0.06	6.47 ± 0.11
	ALB (g/dL)	3.9 ± 0.06	3.94 ± 0.03	3.93 ± 0.03	3.92 ± 0.04
	BUN (mg/dL)	22.3 ± 0.75	23.3 ± 0.45	23.9 ± 0.84	22.89 ± 0.82
	UA (mg/dL)	3.69 ± 0.46	4.28 ± 0.3	3.53 ± 0.34	3.43 ± 0.24
	Cre (mg/dL)	0.66 ± 0.03	0.66 ± 0.02	0.63 ± 0.03	0.62 ± 0.02
Urine	Urin volume (mL/day)	28.21 ± 1.79	21 ± 1.03**	26.61 ± 1.32	20.54 ± 1.61**
	U-TP (mg/day)	13.7 ± 0.91	13.59 ± 2.36	13.83 ± 0.87	14.11 ± 1.19
	U-Na (Eq/day)	10.66 ± 0.55	7.7 ± 0.74**	10.15 ± 0.42	8.37 ± 0.81*

Each value represents mean ± SEM. **: *P*<0.01 vs. DW, *: *P*<0.05 vs. DW. Control group (distilled water, DW), 0.1% ascorbic acid-added DW group (DWA), hydrogen water group (HW), and 0.1% ascorbic acid-added HW group (HWA). Blood biochemical analyses; Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), total cholesterol (T-Cho), high-density lipoprotein cholesterol (HDL-c), triglyceride (TG), total protein (T-Pro), albumin (ALB), urea nitrogen (BUN), uric acid (UA), and creatinine (Cre), Urine total protein (U-TP), and urine-sodium (U-Na).

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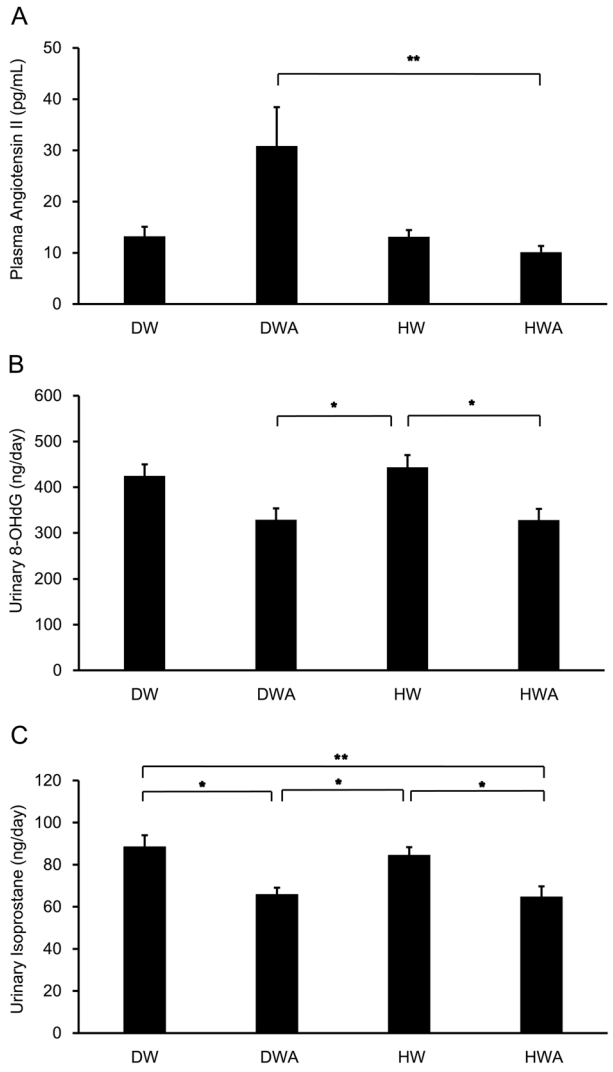

Fig. 5. Comparison of (A) plasma angiotensin II, (B) urinary 8-OHdG, and (C) urinary isoprostane concentrations in SHR fed a 4% NaCl-added diet and given DWA, HW or HWA for 12 weeks. Rats were divided into 4 groups (10 animals in each): control group (distilled water, DW), 0.1% ascorbic acid-added DW group (DWA), hydrogen water group (HW), and 0.1% ascorbic acid-added HW group (HWA). Each value represents the mean ± SEM. **P*<0.05, ***P*<0.01.

Discussion

Our investigation of the potential antihypertensive effects of HW and HWA on rats fed a standard diet or a 4% NaCl-added diet showed a significant lowering of BP in HWA-treated rats compared to the DW and DWA treatments.

Ohsawa *et al.* [16] reported that inhalation of hydrogen gas decreases cerebral infarction volume by reducing oxidative stress in rats; similar effects have since been reported for HW [17–19]. A review of hydrogen as a selective reactive oxygen scavenger suggested that it has clear potential as an antioxidative therapy [20]. Due to its small molecular weight and simple structure, hydrogen can rapidly diffuse through cell membranes into the cytoplasm, mitochondria, and nucleus to exert its antioxidant effect [21]. It has been suggested that hydrogen might not only be effective for the treatment of many diseases and lifestyle diseases, but also valuable for preventive medicine [22].

HW can be produced by injecting hydrogen gas [23, 24], by generating hydrogen through electrolysis [14], and by generating hydrogen through the reaction of metal magnesium and water [25, 26]. The solubility of hydrogen is approximately 1.6 ppm [27], and hydrogen concentrations of 0.8 ppm [28] and 0.3–0.4 ppm [25] have been used experimentally. We used electrolysis to produce HW in this study and a dissolved hydrogen concentration of 0.8 ppm was maintained. In this study, the addition of ascorbic acid maintained the dissolved hydrogen concentration at 0.7 ppm for 3 days (Fig. 1C).

SHR freely ingested the 4% NaCl-added diet. No differences were present in food or water consumption among the DW, DWA, HW, and HWA groups (Fig. 3A). When the salt intake in the experimental period was calculated, the rats in all groups had ingested approximately 2.3 g NaCl per kg body weight per day. High salt loading is believed to increase extracellular fluid volume due to postprandial thirst-drinking and to promote urinary Na excretion [29]. Sodium excretion increases when there is an acute increase in BP. Hypertension induces important functional and structural alterations in the kidney, resulting in proteinuria, glomerular sclerosis, urine Na excretion, and other morphological changes, eventually leading to end-stage renal disease [30]. In the present study, we found that U-Na levels were significantly lower in the HWA and DWA groups. We have no definitive explanation for this at present. However, it is possible that there is a relationship between urine volume and U-Na levels. Indeed, treatment with antioxidants such as vitamin C improves renal dysfunction and lessens renal injury in rats [31].

The renin-angiotensin system (RAS) plays an important role in the development of hypertension in SHR. RAS is implicated in the pathogenesis of hypertension and endothelial damage by reactive oxygen species, such as superoxide anions, hydrogen peroxide, hydroxyl radicals, nitric oxide, and peroxynitrite [32]. Ang II induces oxidative stress in endothelial cells through the activation of NADH/NADPH oxidase [33–35]. Our measurements of the levels of plasma Ang II in the four groups showed that these were slightly lower in the HWA group than the DW group. Unexpectedly, however, Ang II levels in the DWA group tended to be higher than in the other groups. At present, the reason why Ang II lev-

els in the DWA group was higher remains to be resolved. In future work, we shall investigate the molecular mechanisms through which ascorbic acid raises plasma Ang II levels.

The oxidative stress marker urinary 8-OHdG tended to show lower levels in the HWA group than the DW group. Urinary isoprostane levels in the HWA and DWA groups were significantly lower than in the DW and HW groups. Oxidative stress damages cell membranes and tissues; excessive production of reactive oxygen species during metabolism is often associated with the induction of disease. Hydrogen molecules are oxygen scavengers that are capable of specifically and directly removing hydroxyl radicals and peroxynitrite radicals cause of oxidative stress [16]. It has been shown that treatment with antioxidants improves vascular function and reduces BP in animal models [36–39]. Ascorbic acid may have beneficial effects on vascular dilation, possibly through its antioxidant effects on nitric oxide [40–42]. Nevertheless, SBP in the DWA group tended to be higher than in the HWA group. To date, several clinical trials on the effect of vitamin C supplements on BP have yielded inconsistent findings with respect to hypertension in humans [43, 44]. The lack of an antihypertensive effect in studies using supplementation with vitamin C alone could be due to the decreased bioavailability of nitric oxide under conditions of oxidative stress [36].

Although the effect of HW alone was not clear-cut, the addition of ascorbic acid showed synergistic antihypertensive and antioxidant effects. Hydrogen does not exhibit antioxidative actions or effects in the absence of oxidative stress, and its effects are exhibited strongly when a chain reaction involving free radicals is promoted [45]. Excess hydrogen is discharged through exhaled breath via the lungs [46] without influencing physiological parameters such as body temperature and pH [47]. Also, since the hydrogen molecule is very small, it can pass into cells regardless of solubility [11]. Thus, hydrogen is an effective antioxidant through rapid gaseous diffusion into tissues and cells [16]. Moreover, hydrogen is a safe molecule that is mainly produced by intestinal bacteria in rodents and humans, and no adverse effects have been documented [48].

In summary, HWA was found to inhibit BP elevation in SHR that ingested excessive salt, whereas no clear-cut antihypertensive effects were detected in rats that drank HW. This suggests that the antioxidative components of HW and ascorbic acid exhibited synergistic antihypertensive effects. Therefore, HWA ingestion might have beneficial effects on the prevention or treatment of hypertension.

Conflict of Interest

This study was funded to KK by Tech Corporation. DS, and MN are employees of Tech Corporation. HM, TY, and KM have no conflicts of interest.

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