【Original Article】

Single and repeated oral dose toxicity study of hydrogen-rich water in mice

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Summary

Objective: As a part of general toxicity studies of hydrogen-rich water (HRW) prepared using Doctor Hydrogen Water® (DHW), a plastic shelled product that includes metallic magnesium (99.9% pure) and natural stones in polypropylene containers (Doctor SUISOSUI®=DHW, manufactured by FDR Friendear Inc., Tokyo, Japan) in mice, this study examined the toxicity of HRW in single and repeated administrations following the previous report in order to apply this product in preventive medicine.

Methods: The safety of oral ingestion of HRW prepared using the DHW stick was examined in 6-week-old male and female ICR mice with single and 28-day repeated administration by gavage of the maximum acceptable dose of HRW and additional *ad libitum* ingestion of HRW prepared inside water feed bottles during the administration periods. The study was conducted using distilled water as a control following the methods for general toxicity studies described in the"Guidelines for Non-clinical Studies of Pharmaceutical Products 2002". Using distilled water as a control, 1) observation of general conditions, 2) measurement of body weight, 3) determination of food consumption, 4) determination of water consumption, 5) blood test and urinalysis and 6) pathological examination were performed for the administration of HRW. Mice received HRW for 4 weeks and outcomes were compared with those of the control group that received distilled water. Animals were weighed once a week and average body weight was calculated for each group to determine the dose. Blood cell count was performed by collecting blood samples from tail veins at the beginning and end of the study. For urinalysis, samples were collected by forced urination at the end of the study. General symptoms were observed at least once a day. For pathological examination, necropsy was conducted by abdominal section on the last day of the study, followed by measurement of organ weight and gross observation to check for abnormalities.

Results: The results of the above examinations revealed no significant differences between control and HRW groups for both males and females. Thus, no notable toxicity was confirmed with single and repeated oral administrations of HRW.

Discussion: Safety of HRW was examined in 6-week-old male and female ICR mice by single and 4-week repeated oral administration of HRW and distilled water as a control. In both HRW and control groups, water was orally administered in two divided doses in the morning and evening (2 g/kg body weight/day) as well as given *ad libitum* during the day. Oral administration at the above dose did not result in abnormal symptoms or death during the observation period. No abnormalities in blood cell count or organ weights were seen. Without any evidence of toxicity to cells and organs, HRW is speculated to not adversely affect living organisms. The 50% lethal dose of HRW with oral administration in mice is estimated to be greater than 2 g/kg body weight/day (in two administrations by gavage) for both male and female mice.

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Key Words: Hydrogen-rich water, Single and repeated dose toxic study, Blood test, Urine test

INTRODUCTION

Oxidative stress is well known to be associated with various disorders, including life-style related diseases such as type II diabetes and arteriosclerosis and neurodegenerative diseases such as dementia, Alzheimer's disease and Parkinson disease, attracting much attention from the perspective of preventive medicine [1-4]. The hydrogen molecule (H_2) has recently been reported to reduce harmful reactive oxygen species in cells[5] and to be effective for preventing and treating a number of diseases, including ischemia-reperfusion injury, diabetes, arteriosclerosis, Alzheimer's disease and Parkinson disease, leading to rapid progress in research for medical applications of hydrogen[3,4]. Since hydrogen produces effects not only when inhaled as a gas, but also through oral or intravenous administration or local application of aqueous solutions, its efficacy has been reported in many fields. The magnesium stick is a convenient product that allows easy, inexpensive preparation of hydrogen-rich water (HRW) containing a high concentration of hydrogen gas. As a part of general toxicity studies into HRW prepared using Doctor Hydrogen Water (DHW) in mice, this study examined the toxicity of HRW in single and repeated administrations following the previous report[5] in order to apply this product in preventive medicine.

STUDY METHODS AND RESULTS

1. Objective

To examine the toxicity of hydrogen-rich water (HRW) prepared using the DHW stick by single and 4-week repeated oral administration.

2. Materials and Methods

2.1 Test animals and husbandry

Four-week-old male and female ICR mice were purchased from Clea Japan (Tokyo, Japan) and were housed in the laboratory animal room illuminated with 150-300 lux of light at our university. The examiners wore working clothes, head covering, masks, gloves and other protective clothing. Other conventional conditions were employed, including room temperature of 22 ± 3°C and 60% relative humidity. Mice were allowed *ad libitum* access to consume food (EC-2, Clea Japan) and tap water. The study was started after a 2-week acclimation period.

2.2 Assignment and identification of animals

Twenty male and 20 female mice were each divided into a control group (receiving distilled water) and an HRW group (receiving HRW prepared using the DHW stick), forming a total of four groups of 10 animals each.

First, during the acclimation period, animals that were considered healthy were weighed and categorized into the groups divided by body weight at interval of 5 g. Then, from each control group (receiving distilled water) and HRW group, 10 male and 10 female mice that were close to the average weight for each sex were selected. Animals were identified by hair coat dye or identification cards.

2.3 Preparation method, dose and administration of HRW

Each mouse received 2 g/kg body weight/day of water by oral gavage in two divided doses in the morning and evening. In addition, mice in both control and HRW groups were allowed *ad libitum* access to drink the assigned water. The administration period was 4 weeks (28 days), during which time water was given 7 days/week. HRW was prepared using the following method. For administration by gavage, HRW was prepared by placing two magnesium sticks in distilled water in a 500-mL plastic bottle and the water was used after 24 h. For *ad libitum* ingestion, HRW was prepared by placing a magnesium stick in a 200 mL-plastic water feed bottle for mice and filling the bottle with distilled water 12 h before use.

Many bottles were prepared 1 day prior to use and the sticks were later replaced by new ones for the next preparation.

Total daily water consumption in each group was measured every day and average water consumption per mouse was calculated.

The hydrogen molecule concentration in HRW given by gavage was 1.4-1.7 ppm and that of HRW given *ad libitum* was 0.4-0.6 ppm. The concentration of dissolved hydrogen was determined using a dissolved hydrogen densitometer (DHS-001; Able, Tokyo, Japan).

2.4 Method for calculating number of deaths and survival rate

Survival rate was calculated over the 28 days of the administration period with the interval of 4 days using the following formula:

survival rate = (number of surviving animals/ number of reared animals) × 100 (%)

2.5 Observed and examined items

Safety was evaluated following the "Guidelines for Non-clinical Studies of Pharmaceutical Products 2002", using distilled water as a control.

1) Observation of general conditions

Animals were observed at least once daily for general symptoms and mortality from the starting day of administration until Day 28.

After single administration, changes in symptoms were observed in detail for 6 h. After repeated administration, observation and measurement were conducted for the following items.

2) Measurement of body weight

All animals used in the study were weighed before the start of administration and at intervals of 1 week up to Day 28 after the start of administration.

3) Food and water consumption

Food and water consumption were measured daily. Amounts were determined for each group and the average of 10 mice was calculated as food and water consumption for individual mice.

4) Hematological test and blood biochemistry

Blood samples were collected before the study and on the last day of the study (Day 28) and tested for the following items. Specifically, blood was drawn from the fundus and then from the heart for hematological tests (blood samples collected from the fundus and heart were combined and tested).

a) Hematological test

1. Red blood cell count
2. White blood cell count
3. Platelet count
4. Hemoglobin
5. Hematocrit

b) Blood biochemistry

1. Serum (plasma) protein
2. Albumin
3. Albumin/globulin (A/G) ratio
4. Protein fraction
5. Glucose
6. Cholesterol
7. Triglyceride
8. Bilirubin
9. Urea nitrogen
10. Creatinine
11. Transaminases (Alanine transaminase (ALT; Glutamic Pyruvic Transaminase(GPT)), Aspartate Transaminase (AST; Glutamic Oxaloacetic Transaminase (GOT))
12. Alkaline phosphatase

5) Urinalysis

The following items that could be tested by test strips were examined (animals were forced to urinate directly onto test strips without urine collection): pH; protein; glucose; ketone bodies; and bilirubin.

6) Pathological examination

a) Gross observation

All surviving animals were anesthetized with CO^2 gas and sacrificed by abdominal incision followed by exsanguination. The external

surface of the skin, oral cavity and eyes and then all internal organs and tissues were grossly observed.

b) Measurement of organ weight

In addition to actual measured organ weights (absolute weights), ratios compared to body weight (relative weight) were also calculated to clarify the implications of changes in organ weight.

The heart, lungs, liver, spleen, kidneys, adrenal glands, prostate, ovaries, brain, pituitary gland, salivary glands, thymus, thyroid glands, seminal gland and uterus were weighed

When no abnormalities were found in a) or b), histopathological observation was not conducted.

2.6 Methods for statistical analysis

Data were statistically processed using Statistical Analysis System software (Cary, NC) and Labcat module (Innovative Programming Associates, Princeton, NJ).

3. Results

3.1 General conditions

Observation of the general condition of mice in the control groups (receiving distilled water, male and female) and HRW groups (male and female) revealed no evidence of abnormalities in face washing or other movements, fur, skin sensitization or other conditions.

3.2 Changes in body weight among mice in each group

Table 1 and Figure 1 show mean and standard error (SE) values for body weight of male and female mice from control and HRW groups and changes over time. The vertical axis of the plot represents body weight and the horizontal axis elapsed time. No irregular increase or decrease in

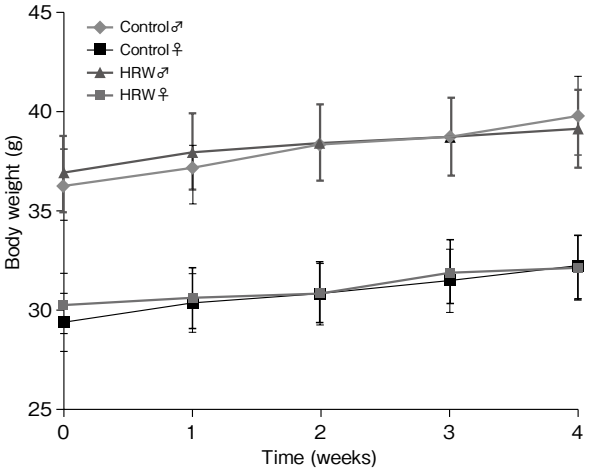

Fig.1. Change in weight for ICR mice. Results represent the mean ± SE (n=10)

Table 1. Body weight changes for each group of mice.

The results represent the mean ± S.E. (n=10).

Male mice		0 week (g)	one week (g)	two weeks (g)	three weeks(g)	four weeks; 28th(g)
Control	mean	36.3	37.2	38.4	38.8	39.8
	S.E.	1.4	2.1	2.4	2.9	2.9
HRW	mean	36.9	38.0	38.4	38.7	39.1
	S.E.	1.5	1.6	2.4	2.5	2.5

Female mice		0 week	one week	two weeks	three weeks	four weeks
Control	mean	29.4	30.4	30.8	31.5	32.2
	S.E.	1.1	1.2	1.6	1.7	1.5
HRW	mean	30.3	30.6	30.9	31.9	32.1
	S.E.	1.3	1.1	0.9	1.6	0.4

body weight was seen in control or HRW groups. No significant differences were identified between male and female animals in either group.

3.3 Changes in food and water consumption

The mean volume of water ingested daily by male and female mice in the control and HRW groups was within the range of 6-7 mg/day/mouse. For both male and female mice, no differences in food consumption were seen between control and HRW groups. Table 2 and Figure 2 show mean and SE values of food consumption for male and female mice from each group and changes over time.

3.4 Blood test results

1) Changes in blood cell count

Table 3 shows changes in blood cell count (white blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet count) in male and female mice in the control and HRW groups, as mean and SE. No significant differences were observed in changes to any item of blood cell count for either male or female animals between control and HRW groups.

WBC, white blood cells (×102/μL); RBC, red blood cells (×104/μL); HGB, hemoglobin concentration (g/dL); HCT, hematocrit (%); MCV, mean corpuscular volume (fL); MCH, mean corpuscular hematocrit(pg); MCHC, mean corpuscular hematocrit concentration(g/dL); PLT, platelets(104/μL)

2) Blood biochemistry results

Table 4 shows mean and SE values of blood biochemistry results in male and female mice in control and HRW groups. For both male and female animals, no significant differences were seen in any items of blood chemistry, comprising serum (plasma) protein, albumin, A/G ratio, protein fraction, glucose, cholesterol, triglyceride, bilirubin, urea nitrogen, creatinine, transaminases (ASAT (GDT), ALAT (GPT)) and alkaline phosphatase between control and HRW groups. TDL, total-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; GOT,

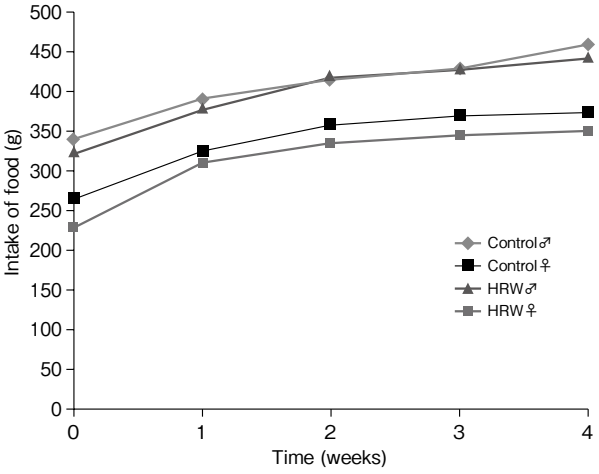

Table 2. Food intake for each group of mice.

The results represent the mean. (n=10).

Male mice		0 week (g)	one week (g)	two weeks (g)	three weeks(g)	four weeks; 28th(g)
Control	mean	340	390	415	430	460
HRW	mean	325	380	420	430	445

Female mice		0 week	one week	two weeks	three weeks	four weeks
Control	mean	265	325	360	370	375
HRW	mean	230	310	335	345	350

Table 3. Blood cell analysis for each group of mice

The results represent the mean ± S.E. (n=10).

Male mice			WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Control	Before administration	Mean	132.9	824.0	15.3	43.5	51.7	17.9	35.1	110.8
		S.E.	43.4	37.4	3.4	9.2	1.2	0.6	1.6	9.8
	After administration	Mean	133.3	862.2	15.4	43.6	50.1	16.9	32.4	121.0
		S.E.	11.8	69.1	1.8	1.9	1.4	0.4	0.9	3.9
HRW	Before administration	Mean	129.4	820.6	14.2	41.3	49.9	17.1	34.2	112.7
		S.E.	28.9	39.5	3.0	7.8	1.6	1.0	1.3	9.5
	After administration	Mean	146.9	860.5	14.9	43.8	44.6	15.4	31.5	121.5
		S.E.	6.2	10.6	0.4	0.5	1.5	0.4	0.8	5.5

Female mice			WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
Control	Before administration	Mean	129.7	842.9	12.6	35.4	51.5	18.3	35.6	108.0
		S.E.	15.3	28.0	1.8	4.1	1.3	1.3	2.9	5.2
	After administration	Mean	132.2	847.7	14.0	32.1	49.0	18.6	35.2	112.3
		S.E.	12.7	92.3	0.7	4.7	1.5	1.4	1.9	12.4
HRW	Before administration	Mean	132.1	830.6	14.0	39.0	50.3	18.1	36.1	107.4
		S.E.	30.4	36.0	2.2	6.2	1.9	0.8	1.1	9.0
	After administration	Mean	147.4	852.2	14.7	34.2	47.5	14.6	34.2	117.3
		S.E.	33.1	11.2	0.5	2.0	0.7	0.4	0.8	5.9

WBC, white blood cells ($\times 10^2/\mu$ L); RBC, red blood cells ($\times 10^4/\mu$ L); HGB, hemoglobin concentration (g/dL); HCT, hematocrit (%); MCV, mean corpuscular volume (fL); MCH, mean corpuscular hematocrit (pg); MCHC, mean corpuscular hematocrit concentration (g/dL); PLT, platelets ($10^4/\mu$ L)

glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase

3.5 Measurement of organ weight

Table 5 shows mean and SE values for organ weight of male and female mice in the control and HRW groups. For both male and female animals, no significant differences were observed in any of the organs weighed, comprising lungs, heart, liver, stomach, kidneys, spleen, intestine, adrenal glands, prostate, brain, pituitary gland, salivary glands, thymus, thyroid gland, seminal glands, testicles, uterus and ovaries between the control and HRW group.

3.6 Urinalysis results

Table 6 summarizes the results of urinalysis for mice in each group. Neither male nor female animals demonstrated any significant difference in protein, glucose, occult blood, urobilinogen, ketone bodies or bilirubin between control and HRW groups. However, pH values indicated that urine tended to be slightly more alkaline in the HRW group compared with that in the control group, although no significant difference was identified.

3.7 Survival rate

Fig.3 shows survival rates of male and female mice in the control and HRW groups. For both male and female animals, survival rates were 100% in both control and HRW groups. No deaths occurred during the study.

4. Discussion

The mice received administration of HRW

Table 4. Blood biochemistry results

Groups	Serum CRP (plasma) protein (mg/dL)	Albumin (g/dL)	Albumin/ globulin ratio (A/G ratio: 1.2 - 2.0)	Bilirubin (mg/dL)	BUN (9-21 mg/ dL)	Creatinine (mg/dL)	ALP (IU/I)	Glucose (ml/dL)
Control male								
Mean	0.2	4.2	1.5	2.7	13.5	0.7	148.6	106.8
SE	0.01	0.10	0.06	0.31	0.65	0.071	8.21	1.65
Control female								
Mean	0.2	4.3	1.4	3.1	13.4	0.6	150.2	106.6
SE	0.009	0.07	0.05	0.36	0.93	0.11	10.23	1.60
HRW male								
Mean	0.2	4.2	1.5	2.7	15.7	0.6	146.1	106.4
SE	0.006	0.06	0.05	0.34	1.13	0.11	9.44	1.55
HRW female								
Mean	0.3	4.2	1.3	2.8	13.6	0.7	147.6	106.6
SE	0.005	0.09	0.05	0.27	0.70	0.10	6.30	0.91

CRP, C-reactive protein; **BUN**, blood urea nitrogen; **ALP**, alkaline phosphatase.

Groups	**Neutral fat (ml/dL)**	**TDL (ml/dL)**	**LDL (ml/dL)**	**HDL (ml/dL)**	**GOT (IU/L)**	**GPT (IU/L)**
Control male						
Mean	138.9	194.4	113.4	68.3	75.7	18.9
SE	2.95	5.04	2.57	4.08	12.6	1.96
Control female						
Mean	141.7	208.3	109.4	66.6	64.9	23.7
SE	2.80	6.14	2.13	2.58	12.2	1.98
HRW male						
Mean	133.3	196.5	117.4	68.9	76.7	26.8
SE	1.96	3.81	3.60	3.19	11.6	1.69
HRW female						
Mean	134.7	191.0	111.8	67.0	65.7	21.0
SE	2.0	4.62	2.58	4.03	12.4	1.51

TDL, total-density lipoprotein; **LDL**, low-density lipoprotein; **HDL**, high-density lipoprotein; **GOT**, glutamic-oxaloacetic transaminase; **GPT**, glutamic-pyruvic transaminase

prepared using DHW by gavage at a dose of 2 g/kg/day and were also allowed to drink HRW *ad libitum*, resulting in additional ingestion of approximately 6-7 g/day/mouse. Each mouse thus ingested approximately 150 g/kg/day or more (calculated assuming a body weight of 40 g) of HRW in total in this study. We examined the toxicity of HRW by single and 28-day-repeated administration and by comparing groups receiving the above amount of HRW and control groups receiving distilled water. The results are discussed below.

4.1 Observation of general conditions

Table 5. Organ weight

Organ/ Groups	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Kidney (g)	Spleen (g)	Intestine (g)	Adrenal gland (g)	Prostate (g)
Control male	0.12	0.21	2.24	0.62	0.75	0.06	4.02	0.058	0.076
± SE	0.087	0.07	0.136	0.132	0.169	0.049	0.261	0.002	0.004
Control female	0.06	0.17	1.57	0.46	0.51	0.05	3.36	0.0579	N
± SE	0.049	0.078	0.11	0.128	0.054	0.05	0.261	0.0017	N
HRW male	0.17	0.22	2.19	0.79	0.67	0.05	3.91	0.0583	0.0848
± SE	0.05	0.06	0.104	0.197	0.046	0.05	0.171	0.0019	0.001
HRW female	0.07	0.15	1.44	0.60	0.46	0.04	3.16	0.0581	N
± SE	0.05	0.05	0.066	0.045	0.049	0.049	0.196	0.0015	N

Organ/ Groups	Brain (g)	Pituitary gland (g)	Salivary gland (g)	Thymus (g)	Thyroid gland (g)	Seminal gland (g)	Testicle (g)	Uterus (g)	Ovary (g)
Control male	0.636	0.001	0.538	0.035	0.0333	0.6366	0.3248	N	N
± SE	0.031	0.0001	0.023	0.001	0.0001	0.030	0.012	N	N
Control female	0.5606	0.0017	0.4927	0.0347	0.0324	N	N	0.34	0.032
± SE	0.035	0.0001	0.017	0.0012	0.0002	N	N	0.012	0.002
HRW male	0.6361	0.0018	0.5383	0.0351	0.0332	0.6421	0.3391	N	N
± SE	0.028	0.0001	0.023	0.002	0.0001	0.04	0.02	N	N
HRW female	0.5618	0.0017	0.4927	0.0345	0.0323	N	N	0.35	0.033
± SE	0.027	0.0003	0.017	0.0011	0.0001	N	N	0.022	0.0004

The results represent mean ± SE (n=10), N=none

Male mice	Protein	Glucose in urine	Occult blood	Urobilinogen	Ketone bodies	Bilirubin	pH
Control	-	-	-	±	-	-	6 ± 2.5
HRW	-	-	-	±	-	-	8 ± 2.8

Female mice	Protein	Glucose in urine	Occult blood	Urobilinogen	Ketone bodies	Bilirubin	pH
Control	-	-	-	±	-	-	6 ± 2.3
HRW	-	-	-	±	-	-	8 ± 2.1

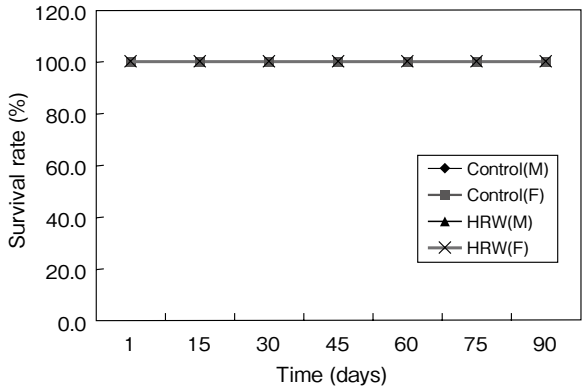

Fig.3. This graph shows the survival rate (%).

For both single and repeated administration and for both male and female animals, no notable differences in general symptoms were seen between HRW and control groups.

4.2 Changes in body weight

Body weight steadily increased in each group without irregular increases or decreases. No significant difference was observed between control and HRW groups, indicating an absence of any influence from HRW on body weight. Based on this result, we speculate that daily ingestion of HRW prepared using Doctor HRW would not affect body weight in humans.

4.3 Influence on food and water consumption

Mean daily water consumption was 6-7 mg/day/mouse in both control and HRW groups when mice were allowed to drink water *ad libitum* in addition to twice-daily administration (2 g/kg body weight/day) in the morning and evening by gavage. For both male and female animals, no difference in food consumption was noted between the control and HRW groups. The HRW group thus did not specifically show any influence of HRW on food or water consumption relative to the control group.

4.4 Changes in blood cell count and blood biochemistry

For all items determined by blood cell counts, no significant differences were observed between control and HRW groups. We therefore speculate that daily ingestion of HRW would not influence blood cells, indicating an absence of cytotoxicity. For both male and female animals, no significant difference was detected between the HRW and control groups when blood biochemistry was examined over time (Table 4). Ingestion of HRW did not result in significant differences in serum (plasma) protein, albumin, A/G ratio, protein fraction, glucose, cholesterol, triglyceride, bilirubin, urea nitrogen, creatinine, transaminases (ASAT (GDT), ALAT (GPT)) or alkaline phosphatase. No toxicity was evident in terms of blood biochemistry.

4.5 Influence on organ weight

None of the organs demonstrated significant differences in weight between control and HRW groups or any macroscopic abnormality. For this reason, no histopathological observation was conducted for any organ. We speculated that daily ingestion of HRW would not be associated with toxicity to any particular organs. Active oxygen is produced in energy production in various organs, including liver and muscle tissue. Hydrogen molecules in HRW are known to be quickly absorbed and distributed throughout the body and have also been reported to selectively eliminate active oxygen.[5] Instead of being toxic to organs, HRW is expected to protect organs from the excessive presence of harmful reactive oxygen species and thus effectively prevent diseases associated with these species.[6,7]

4.6 Influence on urine

Neither control nor HRW groups showed any changes in items of urinalysis, such as protein, glucose in urine, occult blood, urobilinogen, ketone bodies or bilirubin. A tendency toward a difference in pH was seen between control and HRW groups, but was not statistically significant. We considered that this was partially because the HRW prepared

by DHW used in this study was alkaline, with a pH of 7.6 to 8.6.

4.7 Survival rate

Survival rates for male and female animals in both control and HRW groups were 100%, without any evidence of fatal toxicity.

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マウスを利用した水素水（HRW）の経口投与による単回及び反復投与毒性試験

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要　旨

本研究はドクター水素水（HRW）（(株) FDR フレンディア製マグネシウムスティック）の予防医療への応用を目的として当該スティックで作成した水素豊富水のマウスに対する一般毒性試験として単回及び反復投与毒性を前報に引き続き検討した。6 週齢雌雄 ICR マウスを用いてドクター水素水（HRW）スティックで作成した水素豊富水（Hydrogen Rich Water, HRW と略す）の許容できる最高用量を強制経口投与で単回投与及び 4 週間反復投与し、この間更に給水瓶中で作成した水素豊富水を自由摂取させる事により経口摂取による水素豊富水の安全性を検討した。対照群、水素水（HRW）群ともに投与は、朝晩 2 回分与による経口投与（2g/kg body weight/ 日）に加えて終日 *ad lib* でそれぞれの水を自由摂取させた。試験法は「医薬品非臨床試験ガイドライン 2002」の一般毒性試験法に準拠して蒸留水を対照として実施した。体重測定は、1 週間に 1 回行い各群の平均体重を求め、投与量を決定した。血球測定は、尾静脈採血し、研究開始時と研究終了時に行った。尿検査は研究終了時に強制排尿させ採尿した。一般症状観察は 1 日 1 回以上行い、病理学的検査は試験最終日に開腹解剖し臓器重量測定および肉眼的観察を行い異常の有無を検討した。蒸留水を対照として水素水（HRW）の①一般状態の観察、②体重測定、③摂餌量、④摂水量、⑤血液検査、尿検査、⑥病理学的検査を実施した。

これらの用量の水素水を経口投与した結果、観察期間中に異常症状及び死亡例は認められなかった。また、血球測定時、各臓器測定及び観察においても異常は認められなかった。従って、細胞に対する毒性及び臓器に対する毒性もなく、水素水飲水による生体への影響はないと考えられた。これらの結果から、供試スティクで製造した水素水（HRW）のマウスにおける経口投与による LD50 値は、強制投与に加えて自由摂取分を加えると雌雄ともに（2g/kg body weight/ 日（2 回強制投与））以上であると考えられる。

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