Heliyon 9 (2023) e20671

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

The clinical research on the effect of hydrogen-rich water on primary retinitis pigmentosa

Xiaohong Chen [1], Yanjian Chen [1], Xingchui Lin [1], Qian Ye, Sheng Zhang, Yunpeng Wang [***], Meizhu Chen [**], Weiming Yan [*]

Department of Ophthalmology, Fuzong Clinical Medical College of Fujian Medical University (900th Hospital of Joint Logistic Support Force of PLA, Dongfang Hospital Affiliated to Xiamen University), 350025, Fuzhou, China

ARTICLE INFO

Keywords:
Retinitis pigmentosa
Hydrogen-rich water
Retina
Choroid
Electrophysiology

ABSTRACT

Objective: To investigate the feasibility and effectiveness of hydrogen in the treatment of retinitis pigmentosa (RP) patients through the drinking of hydrogen-rich water (HRW).

Methods: RP patients clinically diagnosed in our hospital were selected and given HRW for drinking at 400–500 ml twice a day for four consecutive weeks. Changes in best corrected visual acuity (BCVA), intraocular pressure, the retinal thickness, and choroidal thickness, as well as the amplitude and peak time of visual electrophysiological examinations before and after HRW drinking were observed. Data were statistically analyzed.

Results: In total, 24 eyes of 13 patients with RP (3 males and 10 females aged–27-65 years old, were enrolled in the study. The BCVA after HRW drinking was 0.34 ± 0.25, which was statistically improved compared with that before ($P < 0.05$). There were no significant differences in intraocular pressure, retinal lhickness, or choroidal thickness before and after HRW drinking (all $P > 0.05$). The amplitudes of the b-wave in Dark-adaptation 0.01 response, a and b waves in Dark-adaptation 3.0 response, the Dark-adaptation Ops total wave, a and b waves in Light-adaptation 3.0 response, and the Light-adaptation Flicker response of electroretinogram (ERG) were significantly higher than those before HRW drinking (all $P < 0.05$). The corresponding peak times iwere mproved to some extent compared to those before HRW consumption (all $P < 0.05$). Six patients with RP (11 eyes) had a BCVAm ore than 20/200. The amplitude and peak time of the P100 -ave from the 1°p attern visual evoked potentials (PVEP) were not significantly different from those before HRW drinking ($P > 0.05$), while the data from the 15′ PVEP were statistically different ($P < 0.05$). Seven patients with RP (13 eyes) had a BCVA less than. 20/200 No significant differences were found in the amplitude and peak time of the P2 wave from the 1.0 Hz flash visual evoked potentials (FVEP) and the amplitude from the 12 Hz FVEP compared with those before HRW drinking (all $P > 0.05$).

Conclusion: Short-term HRW drinking slightly improved visual function in patients with primary RP, whereas no significant improvement was found in the thickness of the retina and choroid.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: 34165596@qq.com (Y. Wang), jumychen@126.com (M. Chen), ywming@fjmu.edu.cn (W. Yan).
[1] Co-first authors.

https://doi.org/10.1016/j.heliyon.2023.e20671
Received 6 May 2023; Received in revised form 4 October 2023; Accepted 4 October 2023
Available online 5 October 2023

1. Introduction

Primary retinitis pigmentosa (RP) is a hereditary retinal degenerative disease characterized by progressive loss of photoreceptors, with an incidence of approximately 1/4000 [1]. Night blindness is the primary symptom in the early stages. The main cause of severe visual impairment in RP is progressive apoptosis of retinal photoreceptors, the course of which is irreversible. At present, there is a lack of intervention measures and effective drugs to treat RP [2]. The pathogenesis of RP is complex and exhibits a high genetic heterogeneity [3]. Oxidative stress refers to the imbalance between oxidation and antioxidant levels in the body and leads to the generation of free radicals, which can damage biological macromolecules such as DNA and proteins. Oxidative stress has been reported to be a key factor in the pathogenesis of RP [4]. The cytotoxic effect of elevated oxygen levels caused by oxidative stress is deemed to be a primary cause of progressive retinal photoreceptors loss in RP. Several studies focusing on decreasing oxidative stress have been reported in treatment of RP with varied success [5–7].

Molecular hydrogen (H_2) is an electron donor with reduction capacity and is considered as a new type of antioxidant with an extensive potential application value. The beneficial effects of H_2 on diseases mainly depend on its selective antioxidation, and it also has a high penetrability, anti-inflammatory and anti-apoptosis effects [8]. H_2 has been reported to be involved in the treatment of cardiovascular and cerebrovascular ischemia/reperfusion, Alzheimer's disease, Parkinson's disease, and other degenerative neuropathies[9]. Recently, it has also been used as an adjuvant treatment for COVID-19 [10]. Different from the form of H_2 gas, hydrogen-rich water (HRW) is a kind of drinking-water that contains a saturated concentration H_2. It is easy to carry, easy to use, safe, and can also have antioxidant effects [11]. HRW has been proved to render no side effects in clinical researches [12]. n our previous research, HRW was found to provide some certain protection on the retinal morphology and function in animal models of RP [13,14]. However, the feasibility and effectiveness of H_2 in the treatment of RP patients have not been reported before.

Based on the aforementioned role of oxidative stress in the pathogenesis of RP and the antioxidative effect of H_2, the clinical efficacy of HRW in the treatment of RP patients was further discussed in this study. Our study was aimed to provide preliminary data to extent the clinical application of HRW and to explore new clinical countermeasures for RP.

2. Subjects and methods

2.1. Subjects

Patients clinically diagnosed with RP criteria at our hospital between July and September, 2019 were enrolled in the study. This study was approved by the Ethics Committee of the 900th Hospital of the PLA with the clinical trial registration number of No. #2019006782 at June 1st, 2019. Written informed consent was obtained from all the patients.

The inclusion criterias for RP in this study were as follows:

(1) Meeting any two of the following standards: ① A history of progressive night blindness; ② Decreased visual field and/or increased threshold value of dark adaptation curve; ③ Fundus characteristics, such as retinal pigment epithelium atrophy, typical bone cell-like pigmentation, atrophy of retinal vessels, and waxy yellow or pale color of the optic disc; ④ Abnormal electroretinogram (ERG), which was characterized by decreased rod cell reaction and/or decreased cone cell reaction.
(2) No other diseases causing secondary retinal pigment epithelium degeneration in the fundus;
(3) Age $\leq$65 years;
(4) The best corrected visual acuity (BCVA) of each eye $\geq$20/2000.
(5) No hearing loss, ataxia, neurological or intellectual disorders, cardiac or renal dysfunction, other diseases, or organ dysfunction.
(6) No history of high myopia, keratopathy, glaucoma, other congenital eye diseases, or ocular surgery.

2.2. Preparation of hydrogen-rich water (HRW)

The HRW mainly involves drinking water saturated with concentrated H_2. This was achieved by using the patented hydrodynamic nanobubble atomization technology developed by Beijing Active Hydrogen Drinks Co., Ltd. The resulting HRW had a saturated concentration of H_2 at 1.6 mg/L. Briefly, by utilizing the ultrasonic atomization technology, the energy during the mixing process of H_2 and water could be sufficiently high. Microscopic bubbles (consisting of air and H_2) present in the liquid vibrunderwentation, growth, and continuous aggregation of acoustic energy under the influence of the ultrasonic field. When the energy reach reachedrtain peak, the cavitation bubbles abruptly collapse and dclose. Thed region of cavitation point formation can gcould rate punc temperatures and pressures, reaching astonishing temperatures of over 4000° Celsius and 1800 standard atmospheres. This ensures a sedpersaturation and a stable concentration of H_2.

2.3. Methods

RP patients who met the above inclusion criterias were administered HRW according to a protocol of drinking consuming 400–500 ml of HRW twice a day, within 15 min each time for four weeks. The patients' relevant ocular indicators, including the visual acuity, BCVA, intraocular pressure (IOP), retinal thickness, and choroidal thickness, as well as parameters in the amplitude and peak time from visual evoked potentials (VEP) and ERG, were observed and measured before the study began and after the HRW drinking.

2.3.1. Routine eye examination

The visual acuity of patients was assessed using a standard decimal visual acuity chart. In details, the participants stood at a designated distance of 5 m and read the position of the "E" symbol from the chart. The results were represented in decimal notation when the participant read out the last line of the "E" symbol correctly. After that, the BCVA was further examined by using a computer auto refractometer (NIDEIK, Japan), which could reveal the refractive errors of each participant. The participants were then tested the visual acuity after correction of the refractive errors using lenses. The results were documented as the BCVA.

The IOP was measured through a non-contact tonometer (NIDEIK, Japan) with three consecutive measurements, and the average value was recorded. In greater details, the participants were seated in front of the tonometer with their head stabilized and their chin supported. They were asked to look at a fixed point to ensure that their head and eye position were steady. The tonometer gently touched the cornea with a small air puff, causing a slight indentation. This indentation was sensed by the tonometer, and it measured the force required to produce the indentation, which corresponded to the IOP.

2.3.2. Retinal coherence tomography (OCT)

The Spectralis OCT system (Heidelberg, version 6.0, Germany) was applied to scan the macular fovea and posterior retina using line scanning, whereas the choroidal imaging was performed in the CDI mode of the system. OCT software was used to select sections with a uniform scanning direction for the measurement of retinal and choroidal thickness. In details, the retinal thickness were measured from the inner edge of inner limited membrane to the outer edge of the retinal pigment epithelium (RPE), which included the retinal neuroepithelial and RPE layer. Choroidal thickness measurements were obtained by measuring the distance between the outer edge of the RPE and inner surface of the sclera. The above manual measurements of both the retinal and choroidal thicknesses were taken at the macular fovea, as well as the nasal and temporal sides, at 500 μm intervals. The average of three measurements was obtained to calculate the retinal and choroidal thickness. All measurements were independently performed by an experienced clinician.

2.3.3. VEP

The RETI-port visual electrophysiological examination system (Roland, Germany) was applied to collect VEP data as previously reported [15]. The skin of each participant was cleaned prior to testing to reduce the resistance to less than 5 kΩ. The recording electrode was positioned 2 cm above the occipital tuberosity, and the reference electrode was placed on the forehead below the hairline. A grounding electrode was placed on the skin of the mastoid behind the outer ear. Pattern visual evoked potentials (PVEP) and flash visual evoked potentials (FVEP) were recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards. The peak time and amplitude of the P100-wave from 1° (degree) and 15' (0.25°) PVEP, the amplitude and peak time of the P2-wave from 1 Hz FVEP, and the total amplitude from 12 Hz FVEP were recorded in both eyes. All measurements were collected independently by the same clinician.

2.3.4. Electroretinogram (ERG)

The RETI-port visual electrophysiological examination system (Roland, Germany) was used for ERG detection as previously reported [16]. Prior to testing, the patient underwent sufficient mydriasis and 30 min of dark adaptation. The skin surface was cleaned to

Table 1
Basic information and ophthalmic parameters of the enrolled patients with retinitis pigmentosa (RP) in our study.

Number	Age	Gender	Eye	BCVA	IOP/mmHg
1	59	F	OD	0.25	14
			OS	0.4	13
2	47	F	OD	0.6	13
			OS	0.6	14
3	48	F	OD	0.01	12
			OS	0.4	13
4	48	M	OD	0.5	14
			OS	0.01	12
5	56	F	OD	0.01	16
6	44	F	OD	0.5	15
			OS	0.5	13
7	41	F	OS	0.12	15
8	27	M	OD	0.4	12
			OS	0.5	12
9	59	M	OD	0.8	12
			OS	0.01	15
10	49	F	OD	0.08	15
			OS	0.2	16
11	67	F	OD	0.25	13
			OS	0.4	14
12	49	F	OD	0.01	19
			OS	0.01	20
13	63	F	OD	0.8	13
			OS	0.6	12

Note: BCVA: best corrected visual acuity; IOP: intraocular pressure; OD: the right eye; OS: the left eye; F: female; M: male; RP: retinitis pigmentosa.

ensure a resistance of less than 5 kΩ. A corneal electrode was placed at the center of each eye's cornea, with a reference electrode positioned on the orbital rim, temporal to each eye and a grounding electrode located at the mastoid behind the ear. Following the ISCEV standards, ERG recordings were taken simultaneously with a full-field stimulator. The following measurements were recorded after 30 min of dark adaption: Dark-adapted 0.01 ERG, Dark-adapted 3.0 ERG, Dark-adapted 3.0 oscillatory potentials (OPs). After a 10 min of light adaption, Light-adapted 3.0 ERG and Light-adapted 30 Hz flicker ERG were sequently recorded. The amplitudes and peak times of the main waves were recorded for each response. An experienced clinician independently performed all measurements.

2.3.5. Statistical methods

Statistical analysis was performed using SPSS software (version 26.0). The data obtained in this study were presented as mean ± standard error (mean ± S.E.M.). The Paired *t*-test was conducted to compare the visual acuity, BCVA, IOP, retinal and choroidal thickness, as well as parameters in the amplitude and peak time from VEP and ERG in the RP patients before and after HRW drinking. Difference was considered statistically significant when a *P* value was less than 0.05.

3. Results

3.1. Comparison of routine ophthalmic parameters of RP patients before and after HRW drinking

There were 13 patients with RP enrolled in our study, including 3 male and 10 female. They were aged 27–65 years, with an average age of 50.54 ± 10.26 years. There were 24 eyes included in the study, including 12 right eyes (OD) and 12 left ones (OS). The other 2 eyes were excluded because of a lack of light perception. (Table 1).

The mean BCVA prior to drinking HRW was 0.33 ± 0.26, while the mean BCVA after HRW intake was 0.34 ± 0.25. There was a statistically significant improvement in the mean BCVA following HRW consumption (t = -2.954, $P = 0.007$) (Table 2). Among the included RP patients, the average intraocular pressure (IOP) measured was 14.04 ± 2.12 mmHg before HRW consumption and 13.71 ± 1.92 mmHg after HRW intake. However, no significant difference in mean IOP was observed before and after HRW consumption (t = 1.115, $P = 0.276$) (Table 2).

3.2. Comparison of retinal and choroidal thickness in RP patients before and after HRW drinking

Thirteen RP patients with 24 eyes had an average retinal thickness of 143.54 ± 55.89 μm , including the retinal neuroepithelial and RPE layer, before drinking HRW. After drinking, the average retinal thickness,including the retinal neuroepithelial and RPE layer, was 144.79 ± 55.29 μm. However, statistical analysis indicated no statistically significant difference in the average thickness of the retinal neuroepithelial and RPE layer after HRW drinking compared to that before HRW drinking (t = -1.166, $P = 0.256$) (Table 3).

Additionally, the mean choroidal thickness was 188.88 ± 79.09 μm before HRW drinking in these 13 RP patients with 24 eyes. Following HRW intake, the mean choroidal thickness was 187.67 ± 74.42 μm. Similarly, there was no statistically significant difference in the mean choroidal thickness before and after HRW drinking (t = 0.574, $P = 0.571$) (Table 3).

3.3. omparison of ERG parameters in RP patients before and after HRW drinking

Thirteen RP patients with 24 eyes had an average peak time of 77.95 ± 9.73 ms in the b-wave from Dark-adapted 0.01 ERG, which was not statistically different from that before HRW drinking (78.11 ± 9.82 ms, t = 1.414, $P = 0.171$). However, the average amplitude of the b-wave was 43.63 ± 31.55 μV, which was statistically different from that before HRW drinking (42.83 ± 31.20 μV, t = -3.45, $P = 0.020$).

The average peak time of the a-wave from Dark-adapted 3.0 ERG before HRW drinking was 19.18 ± 6.05 ms. After HRW drinking, the data was 16.86 ± 5.59 ms, which was statistically different from that before HRW drinking (t = 7.53, $P = 0.010$). Similarly, the average amplitude of the a-wave before HRW drinking was 110.19 ± 121.42 μV. The parameter was 112.75 ± 122.92 μV after HRW drinking and it statistically differed from that before HRW drinking (t = -5.71, $P = 0.010$). Before HRW drinking, the average peak time of the b-wave from Dark-adapted 3.0 ERG was 45.75 ± 5.75 ms, and it was 45.27 ± 5.85 ms after drinking, which was statistically significant (t = 3.80, $P = 0.010$). Additionally, the average amplitude of the b-wave before drinking was 106.25 ± 74.79 μV, while it was 110.81 ± 76.93 μV after drinking, which was statistically different compared to that before drinking (t = -3.91, $P = 0.010$).

The average peak time of the O_2-wave from Dark-adapted 3.0 OPs before HRW drinking was 43.01 ± 9.47 ms, and it was 39.34 ± 9.93 ms after drinking, which was statistically different (t = 6.29, $P = 0.010$). The average overall amplitude of Dark-adapted 3.0 OPs

Table 2
Comparison of best corrected visual acuity (BCVA) and intraocular pressure (IOP) in retinitis pigmentosa (RP) patients before and after hydrogen-rich water (HRW) drinking ($\bar{x}$ ±S.E.M. , n = 24).

	Before HRW	After HRW	t	*P*
BCVA	0.33 ± 0.26	0.34 ± 0.25	-2.954	0.007
IOP/mmHg	14.04 ± 2.12	13.71 ± 1.92	1.115	0.276

Note: BCVA: best corrected visual acuity; IOP: intraocular pressure; HRW: hydrogen-rich water; RP: retinitis pigmentosa; Before HRW: before the HRW drinking; After HRW: after the HRW drinking.

Table 3
Comparison of retinal thickness and choroidal thickness in retinitis pigmentosa (RP) patients before and after hydrogen-rich water drinking (HRW) ($\bar{x}$ ±S.E.M, n = 24).

	Before HRW	After HRW	t	*P*
retinal thickness/μm	143.54 ± 55.89	144.79 ± 55.29	-1.166	0.256
choroidal thickness/μm	188.88 ± 79.09	187.67 ± 74.42	0.574	0.571

Note: HRW: hydrogen-rich water; RP: retinitis pigmentosa; Before HRW: before the HRW drinking; After HRW: after the HRW drinking.

before drinking was 184.53 ± 74.97 μV, while it was 186.07 ± 75.28 μV after drinking, which was statistically different compared to that before drinking (t = -9.71, $P = 0.010$).

The average peak time of the a-wave from the Light-adapted 3.0 ERG before HRW drinking was 20.62 ± 8.04 ms, and it was 18.86 ± 7.37 ms after HRW drinking, which was statistically different (t = 8.25, $P = 0.010$). The average amplitude of the a-wave before drinking was 29.44 ± 29.56 μV, and it was 32.32 ± 29.12 μV after drinking, which was statistically different (t = -10.13, $P = 0.010$). Before HRW drinking, the average peak time of the b-wave from the Light-adapted 3.0 ERG was 39.76 ± 14.63 ms. After drinking, it was 37.47 ± 14.48 ms, which was statistically different (t = 12.85, $P = 0.010$). Additionally, the average amplitude of the b-wave before drinking was 63.75 ± 50.02 μV, while it was 65.35 ± 50.05 μV after drinking, which was statistically different (t = -20.35, $P = 0.010$). Before HRW drinking, the average amplitude of the Light-adapted 30 Hz flicker ERG was 61.40 ± 55.93 μV. After drinking, the average amplitude was 62.80 ± 55.87 μV, which was statistically different (t = -14.25, $P = 0.001$). (Tables 4 and 5, respectively).

3.4. Comparison of VEP parametersof RP patients before and after HRW drinking

Six RP patients (11 eyes) with a BCVA of more than 20/200 underwent pattern visual evoked potential (PVEP) before and after HRW drinking. The average amplitude of P100-wave from 1° PVEP before drinking was 8.66 ± 6.91 μV. The parameter was 8.73 ± 6.97 μV after HRW intake, which was not statistically different from that before HRW dinking (t = -1.024, $P = 0.330$). The average peak time of P100-wave from 1° PVEP before drinking was 130.71 ± 14.53 ms, and it was 130.59 ± 14.31 ms after drinking. The difference was not statistically significant (t = 1.115, $P = 0.291$). The average amplitude of P100-wave from 15′ PVEP before drinking was 9.38 ± 9.78 μV, while the parameter after HRW drinking was 9.52 ± 9.79 μV, which not statistically different from before (t = -1.64, $P = 0.132$). The average peak time of P100-wave from 15' PVEP before drinking was 132.78 ± 14.69 ms, while it was 132.49 ± 14.62 ms after HRW drinking, showing a statistical difference compared to that before HRW drinking (t = 2.82, $P = 0.018$). (Table 6).

Additionally, seven RP patients (13 eyes) with a BCVA of less than 20/200 were examined with FVEP before and after HRW drinking. The average amplitude of P2-wave from 1.0 Hz FVEP was 9.30 ± 5.96 μV before HRW drinking. After drinking, it was 9.42 ± 6.11 μV, showing no statistically difference (t = -1.252, $P = 0.235$). Similarly, the average peak time of P2-wave before HRW drinking was 106.89 ± 22.01 ms, and it was 106.59 ± 21.43 ms after drinking, which was not statistically different (t = 1.682, $P = 0.118$). Furthermore, the average amplitude of from 12 Hz FVEP before drinking was 6.52 ± 2.85 μV, while the average amplitude after drinking was 6.72 ± 2.96 μV. The difference was not statistically different compared to that before the HRW intake (t = -1.853, $P = 0.089$). (Table 7).

4. Discussion

As aforementioned, RP is a progressive vision loss disease characterized by the degeneration of photoreceptor cells. Currently, there are no effective clinical intervention measures or drugs available for RP [17]. Antioxidation has been argued as a promise measurement for encountering the photoreceptor apoptosis in RP. H_2 has been previously shown potential as a newly-found antioxidant agent that can reduce cell damage by penetrating the tight biological tissue barrier to reach the target organ [18]. Thus, we aimed to investigate the feasibility and effectiveness of drinking HRW in RP, based on the antioxidation effect of H_2, from various aspects, including routine ophthalmological indicators, retinal function, and the choroid and retinal structures. The HRW in our study was

Table 4
Comparison of parameters in dark-adapted electroretinogram (ERG) from retinitis pigmentosa (RP) before and after hydrogen-rich water (HRW) drinking ($\bar{x}$ ±S.E.M. , n = 24).

	Before HRW	After HRW	t	*P*
0.01 ERG b peak time/ms	78.11 ± 9.82	77.95 ± 9.73	1.414	0.171
0.01 ERG b amplitude/μV	42.83 ± 31.20	43.63 ± 31.55	-3.450	0.020
3.0 ERG b peak time/ms	45.75 ± 5.75	45.27 ± 5.85	3.800	0.010
3.0 ERG b amplitude/μV	106.25 ± 74.79	110.81 ± 76.93	-3.910	0.010
3.0 ERG a peak time/ms	19.18 ± 6.05	16.86 ± 5.59	7.530	0.010
3.0 ERG a amplitude/μV	110.19 ± 121.42	112.75 ± 122.92	-5.710	0.010
OPs O_2 peak time/ms	43.01 ± 9.47	39.34 ± 9.93	6.290	0.010
OPs all amplitude/μV	184.53 ± 74.97	186.07 ± 75.28	-9.710	0.010

Note: HRW: hydrogen-rich water; RP: retinitis pigmentosa; Before HRW: before the HRW drinking; After HRW: after the HRW drinking; ERG: electroretinogram.

Table 5
Comparison of parameters in light-adapted electroretinogram (ERG) from retinitis pigmentosa (RP) patients before and after hydrogen-rich water (HRW) drinking ($\bar{x}$ ±S.E.M. , n = 24).

	Before HRW	After HRW	t	*P*
3.0 ERG a peak time/ms	20.62 ± 8.04	18.86 ± 7.37	8.250	0.010
3.0 ERG a amplitude/μV	29.44 ± 29.56	32.32 ± 29.12	-10.130	0.010
3.0 ERG b peak time/ms	39.76 ± 14.63	37.47 ± 14.48	12.850	0.010
3.0 ERG b amplitude/μV	63.75 ± 50.02	65.35 ± 50.05	-20.350	0.010
30 Hz flicker ERG amplitude/μV	61.40 ± 55.93	62.80 ± 55.87	-14.250	0.010

Note: HRW: hydrogen-rich water; RP: retinitis pigmentosa; Before HRW: before the HRW drinking; After HRW: after the HRW drinking; ERG: electroretinogram.

Table 6
Comparison of parameters in pattern visual evoked potentials (PVEP) from retinitis pigmentosa (RP) patients before and after hydrogen-rich water (HRW) drinking ($\bar{x}$ ±S.E.M. , n = 11).

	Before HRW	After HRW	t	*P*
1°-P100 amplitude/μV	8.66 ± 6.91	8.73 ± 6.97	-1.024	0.330
1°-P100 peak time/ms	130.71 ± 14.53	130.59 ± 14.31	1.115	0.291
15min-P100 amplitude/μV	9.38 ± 9.78	9.52 ± 9.79	-1.640	0.132
15min-P100 peak time/ms	132.78 ± 14.69	132.49 ± 14.62	2.820	0.018

Note: HRW: hydrogen-rich water; RP: retinitis pigmentosa; Before HRW: before the HRW drinking; After HRW: after the HRW drinking; PVEP: pattern visual evoked potential.

Table 7
Comparison of parameters in flash visual evoked potentials (FVEP) from retinitis pigmentosa (RP) patients before and after hydrogen-rich water (HRW) drinking ($\bar{x}$ ±S.E.M. , n = 13).

	Before HRW	After HRW	t	*P*
1.0 Hz P2 amplitude/μV	9.30 ± 5.96	9.42 ± 6.11	-1.252	0.235
1.0 Hz P2 peak time/ms	106.89 ± 22.01	106.59 ± 21.43	1.682	0.118
12 Hz amplitude/μV	6.52 ± 2.85	6.72 ± 2.96	-1.853	0.089

Note: HRW: hydrogen-rich water; RP: retinitis pigmentosa; Before HRW: before the HRW drinking; After HRW: after the HRW drinking; PVEP: flash visual evoked potential.

given to RP patients for drinking, which provided a concentration of 0.8 mM (1.6 mg/L) of H_2 and was convenient for daily use. The HRW drinking has also been implemented in treatment for other clinic diseases and has been revealed to render no side effects[12]. Increased water intake may affect aqueous humor circulation and then affect the IOP. Our data did not present significant differences in IOP in RP patients before and after the HRW drinking. Our results thus indicated that the intake of HRW in our study might not impact the aqueous humor circulation in RP patients.

Assessing retinal and choroidal morphology is crucial for evaluating the progression of RP [19]. RP is characterized by histopathological changes such as shortening of the photoreceptor layer, degeneration of RPE cells, and thinning of the choroidal vessels [20]. Progressive degeneration of the photoreceptor layer and RPE cells can damage the inner retinal layers, which can eventually cause atrophy of the whole retina. In addition, thinning of the choroidal vessels also contributes to histopathological changes in patients with RP. OCT imaging could reveal characteristic morphological changes in RP, such as thinning of the retinal layer and choroid [21]. Previous animal studies have indicated that intraperitoneal injection of HRW could improve retinal morphology in an RP animal model induced by N-methyl-N-nitrosourea (MNU), as evidenced by the examinations of the retinal sections and OCT scanning [22,23]. Due to the limitation of obtaining human retinal sections, we employed OCT to assess the effect of HRW drinking on retinal and choroidal morphology in RP patients in the present study. Our data revealed that HRW consumption did not result in significant improvement or exacerbation of the retinal and choroidal atrophy. This result might indicate that short-term drinking of HRW did not adversely affect the retina and choroid morphology, which in turn reflected the feasibility and safety of HRW.

Evaluation of retinal function is another crucial parameter for assessing RP progression. ERG is a method for assessing retinal function, including the rod and cone systems. In the ERG, the dark-adaption response mainly reflects the retinal function of the rod system, whereas the light-adaption response reflects the cone system. Specifically, the a-wave represents the function of the photoreceptor cells, and the b-wave mainly reflects the function of the bipolar cells. Additionally, the OPs wave mainly indicates the function of retinal vessels, which are surrounded by nondendritic cells [24]. In RP, the rod system, represented by the dark-adaption responses, deteriorates first, followed by a decline in the light-adapted response as the disease progresses. Advanced RP patients often exhibit extinguished or very small ERG waveforms due to the loss of nearly all photoreceptor cells [25]. Our data revealed that HRW drinking did not worsen the ERG parameters and even led to slight improvements, particularly in the cone system. Since the RP patients included in the study had a long-term disease duration, the retinal function of the rod system was more severely affected than that of the cone system. Indeed, the value of amplitude for a, b waves in the rod and cone system from our RP patient was quite low compared

to that of the normal range. Therefore, the worsen retinal function of the rod system might not able to be improved by HRW drinking. Our ERG results were consistent with the morphological data, indicating that HRW consumption did not accelerate RP progression in the retinal function. Additionally, we observed a slight improvement in the BCVA of RP patients after consuming the HRW. The slight improvement of BCVA observed in our study might be in accordance with the function improvement of ERG. However, as is well-known, RP typically leads to a sever deterioration of visual acuity in the late stage. The subjective sensation of light or psychological comfort of the treatment might need to be taken into consideration for the slight improvement of BCVA [26].

VEP reflects the functional integrity of the visual system, specially the optic nerve. In particular, PVEP obtains a waveform with pattern-reversal checkboard stimuli of different degrees (1 or 0.25 degree), and is usually recorded when the patient's BCVA is more than 20/200. The P100-wave is the primary indicator in PVEP and shows minimal variation among subjects. FVEP is usually recorded from flash stimulation at 1 Hz or 12 Hz when BCVA is less than 20/200 due to ocular media opacities or other ocular lesion. The P2-wave is a consistent and robust component of the 1 Hz FVEP [27]. RP predominantly affects photoreceptors, whereas neurons in the inner retina and ganglion cells are also affected in the advanced stage, leading to atrophy of the optic nerve and worsen of visual function [28]. In our study, eyes from patients with RP were tested for PVEP or FVEP according to the BCVA. The data from our study revealed no significant changes in the corresponding parameters of PVEP and FVEP following the HRW consumption. Previous animal studies argued that H_2 had a protective effect on optic nerve injuries based on VEP examination, with a mechanism related to its antioxidation and anti-apoptotic properties[29]. However, our VEP results indicated that short-term HRW consumption did not positively affect the overall visual conduction in RP patients. VEP provides a functional assessment of the entire visual system, including the ocular media, retina, optic nerve, and visual cortex. The deterioration of the retina including the optic nerve in late stage of RP could affect VEP recordings together, which might be out of the intervention effect of H_2. Therefore, HRW drinking may not result in significant changes in the VEP parameters for RP patients.

As far as we know, there have been several clinical trials conducted on the use of nutritional supplements for treating RP, but the results have been controversial. Vitamin A palmitate, at a dosage of 15,000 IU per day, has shown potential in slowing down retinal function changes as detected by ERG. However, it did not appear to have an effect on visual acuity or visual field [30]. DHA supplementation at a dosage of 400 mg per day has shown no impact on visual acuity or visual field in males with x-linked RP, although higher concentrations of DHA in red blood cells were associated with preserved cone ERG function [31]. Lutein supplementation at a dosage of 20 mg per day for six months was found to increase the macular pigment in some individuals with RP or Usher syndrome, but it did not result in any change in central vision [32]. Another study showed that lutein supplementation had a significant effect on visual field but no effect on visual acuity or contrast sensitivity [33]. In addition, the preliminary studies revealed that some medications that were effective in induced RP models failed to rescue RP in inherited genetic models [34,35], indicating that there may be specific mechanisms of photoreceptor apoptosis in different RP models. All the above finding of previous clinical and preliminary researches, including that of ours, suggest that the complexity of pathogenesis exists in RP and further explorations for the medical interventions are warranted.

Our present study had some limitations. Due to the COVID-19 pandemic when our study was conducted, quite a lot of the RP patients could not refer to our department for the follow-up study. The sample size of RP patients included in our study was relatively small, and the duration of HRW drinking was limited to only about one month. Therefore, we conducted our study by comparing the clinical data of patients themselves before and after the HRW drinking. In addition, our study only applied a certain HRW concentration and intake amount adapted from Song et al.'s clinical research report [36]. The analysis of our results might be affected by the relatively small sample size, individual differences among patients, and the short follow-up time of observation. Besides, the potential influence of the diet or the intake of food, normal water during the 4 weeks of HRW intake on the visual function was not assessed, which might impact the outcomes.

5. Conclusion

In summary, our preliminary findings suggested slightly positive effect of short-term HRW consumption on visual function in primary RP patients, and the HRW consumption did not adversely impact the IOP of the RP patients. However, the retinal and choroidal morphological pathologies were not significantly improved. Further research with a larger sample size, a longer observation period and a more detailed assessment of the patients' dietary pattern is warranted to comprehensively assess and validate the positive biological effects of HRW on RP observed in our study.

Ethics statement

This study was reviewed and approved by the Ethics Committee of the 900th Hospital of the PLA, with the approval number: #2019006782.

All patients provided informed consent to participate in the study.

All patients provided informed consent for the publication of their anonymised case details and images.

Funding

This work was supported by grants from the Key Scientific Research Project of the 900th Hospital of Joint Logistic Support Force, PLA (Project number: 2022ZD04), Pilot projects of Fujian Province (Grant numbers: 2020Y0076 and 2020J05282), Postdoctoral Science Foundation of the Fuzhou General Hospital of the Nanjing Military Area Command (Grant number: 48678), and the National

Natural Science Foundation of China (Grant number. 81870255).

Data availability statement

Data will be made available on request by contacting the authors.

CRediT authorship contribution statement

Xiaohong Chen: Writing – original draft, Formal analysis, Data curation. **Yanjian Chen:** Methodology, Investigation, Formal analysis. **Xingchui Lin:** Methodology, Formal analysis, Data curation. **Qian Ye:** Formal analysis. **Sheng Zhang:** Data curation. **Yunpeng Wang:** Supervision, Investigation. **Meizhu Chen:** Visualization, Supervision, Funding acquisition, Conceptualization. **Weiming Yan:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] F. Zheng, J. He, X. Fang, Ultrawide-field swept source-OCT angiography of etinitis pigmentosa, Ophthalmology 130 (1) (2023) 67.
[2] S. Partin, E. Westfall, G. Sanda, K. Branham, K. Muir, C. Bellcross, et al., Readability, content, and accountability assessment of online health information for retinitis pigmentosa & retinitis pigmentosa treatment options, Ophthalmic Genet. 44 (1) (2023) 43–48.
[3] L. Kong, G. Chu, W. Ma, J. Liang, D. Liu, Q. Liu, et al., Mutations in VWA8 cause autosomal-dominant retinitis pigmentosa via aberrant mitophagy activation, J. Med. Genet. (2023).
[4] Y. Kanan, S.F. Hackett, H.T. Hsueh, M. Khan, L.M. Ensign, P.A. Campochiaro, Reduced inspired oxygen decreases retinal superoxide radicals and promotes cone function and survival in a model of retinitis pigmentosa, Free Radic. Biol. Med. 198 (2023) 118–122.
[5] R. Garcia-Arroyo, E.B. Domenech, C. Herrera-Ubeda, M.A. Asensi, D.A.C. Nunez, J.M. Cuezva, et al., Exacerbated response to oxidative stress in the Retinitis Pigmentosa Cerkl(KD/KO) mouse model triggers retinal degeneration pathways upon acute light stress, Redox Biol. 66 (2023), 102862.
[6] Y. Kanan, S.F. Hackett, K. Taneja, M. Khan, P.A. Campochiaro, Oxidative stress-induced alterations in retinal glucose metabolism in Retinitis Pigmentosa, Free Radic. Biol. Med. 181 (2022) 143–153.
[7] E.M. Vingolo, L. Casillo, L. Contento, F. Toja, A. Florido, Retinitis pigmentosa (RP): the role of oxidative stress in the degenerative process progression, Biomedicines 10 (3) (2022).
[8] M.Y. Artamonov, A.K. Martusevich, F.A. Pyatakovich, I.A. Minenko, S.V. Dlin, T.W. Lebaron, Molecular hydrogen: from molecular effects to stem cells management and tissue regeneration, Antioxidants 12 (3) (2023).
[9] C. Wu, P. Zou, S. Feng, L. Zhu, F. Li, T.C. Liu, et al., Molecular hydrogen: an emerging therapeutic medical gas for brain disorders, Mol. Neurobiol. 60 (4) (2023) 1749–1765.
[10] D. Alwazeer, F.F. Liu, X.Y. Wu, T.W. Lebaron, Combating oxidative stress and inflammation in COVID-19 by molecular hydrogen therapy: mechanisms and perspectives, Oxid. Med. Cell. Longev. 2021 (2021), 5513868.
[11] F. Xie, X. Jiang, Y. Yi, Z.J. Liu, C. Ma, J. He, et al., Different effects of hydrogen-rich water intake and hydrogen gas inhalation on gut microbiome and plasma metabolites of rats in health status, Sci. Rep. 12 (1) (2022) 7231.
[12] M Sim, CS Kim, WJ Shon, Y.K. Lee, E.Y. Choi, D.M. Shin, Hydrogen-rich water reduces inflammatory responses and prevents apoptosis of peripheral blood cells in healthy adults: a randomized, double-blind, controlled trial, Sci. Rep. 10 (1) (2020), 12130.
[13] W.M. Yan, T. Chen, X.C. Wang, L.S. Qi, G.H. Zhao, G.Q. Yang, et al., The reason for the amelioration of N-methyl-N-nitrosourea-induced retinitis pigmentosa in rats by hydrogen-rich saline, Int. J. Ophthalmol. 10 (10) (2017) 1495–1503.
[14] W.M. Yan, L. Zhang, T. Chen, G.H. Zhao, P. Long, J. An, et al., Effects of hydrogen-rich saline on endotoxin-induced uveitis, Med. Gas Res. 7 (1) (2017) 9–18.
[15] W. Yan, Y. Chen, X. Chen, Q. Ye, Y. Wang, C. Jiang, et al., Diagnosis of keratoconus in a young male by electrophysiological test findings: a case report, Medicine (Baltim.) 101 (27) (2022), e29351.
[16] W. Yan, Y. Wang, Q. Ye, X. Chen, Y. Chen, C. Jiang, et al., The electrophysiological features of X-linked juvenile retinoschisis in a young male: a case report, J. Int. Med. Res. 50 (1) (2022), 675866797.
[17] K.Y. Wu, M. Kulbay, D. Toameh, A.Q. Xu, A. Kalevar, S.D. Tran, Retinitis pigmentosa: novel therapeutic targets and drug development, Pharmaceutics 15 (2) (2023).
[18] D. Ramanathan, L. Huang, T. Wilson, W. Boling, Molecular hydrogen therapy for neurological diseases: a review of current evidence, Med. Gas Res. 13 (3) (2023) 94–98.
[19] C. Li, C. Peng, C. Zhang, N. Li, Thicknesses of the retina and choroid in children with retinitis pigmentosa, BMC Ophthalmol. 23 (1) (2023) 25.
[20] D.J. Song, X.L. Bao, B. Fan, G.Y. Li, Mechanism of cone degeneration in retinitis pigmentosa, Cell. Mol. Neurobiol. 43 (3) (2023) 1037–1048.
[21] A. Arrigo, E. Aragona, C. Perra, L. Bianco, A. Antropoli, A. Saladino, et al., Characterizing macular edema in retinitis pigmentosa through a combined structural and microvascular optical coherence tomography investigation, Sci. Rep. 13 (1) (2023) 800.
[22] W.M. Yan, T. Chen, X.C. Wang, L.S. Qi, G.H. Zhao, G.Q. Yang, et al., The reason for the amelioration of N-methyl-N-nitrosourea-induced retinitis pigmentosa in rats by hydrogen-rich saline, Int. J. Ophthalmol. 10 (10) (2017) 1495–1503.
[23] T. Chen, Y. Tao, W. Yan, G. Yang, X. Chen, R. Cao, et al., Protective effects of hydrogen-rich saline against N-methyl-N-nitrosourea-induced photoreceptor degeneration, Exp. Eye Res. 148 (2016) 65–73.
[24] A.G. Robson, L.J. Frishman, J. Grigg, R. Hamilton, B.G. Jeffrey, M. Kondo, et al., ISCEV Standard for full-field clinical electroretinography (2022 update), Doc. Ophthalmol. 144 (3) (2022) 165–177.
[25] W.C. Huang, P.K. Liu, N.K. Wang, Electroretinogram (ERG) to evaluate the retina in cases of retinitis pigmentosa (RP), Methods Mol. Biol. 2560 (2023) 111–122.
[26] M. Mateos-Olivares, E.M. Sobas, K. Puertas-Neyra, M.I. Peralta-Ramirez, R. Gonzalez-Perez, J. Martin-Vallejo, et al., Hair cortisol level as a molecular biomarker in retinitis pigmentosa patients, Exp. Eye Res. 219 (2022), 109019.
[27] J.V. Odom, M. Bach, M. Brigell, G.E. Holder, D.L. Mcculloch, A. Mizota, et al., ISCEV standard for clinical visual evoked potentials: (2016 update), Doc. Ophthalmol. 133 (1) (2016) 1–9.
[28] K. Nakajima, T. Inoue, M. Maruyama-Inoue, Y. Yanagi, K. Kadonosono, A. Ogawa, et al., Relationship between the vessel density around the optic nerve head and visual field deterioration in eyes with retinitis pigmentosa, Graefes Arch. Clin. Exp. Ophthalmol. 260 (4) (2022) 1097–1103.
[29] J.C. Sun, T. Xu, Q. Zuo, R.B. Wang, A.Q. Qi, W.L. Cao, et al., Hydrogen-rich saline promotes survival of retinal ganglion cells in a rat model of optic nerve crush, PLoS One 9 (6) (2014), e99299.

[30] E.L. Berson, B. Rosner, M.A. Sandberg, K.C. Hayes, B.W. Nicholson, C. Weigel-Difranco, et al., A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa, Arch. Ophthalmol. 111 (6) (1993) 761–772.
[31] D.R. Hoffman, K.G. Locke, D.H. Wheaton, G.E. Fish, R. Spencer, D.G. Birch, A randomized, placebo-controlled clinical trial of docosahexaenoic acid supplementation for X-linked retinitis pigmentosa, Am. J. Ophthalmol. 137 (4) (2004) 704–718.
[32] T.S. Aleman, J.L. Duncan, M.L. Bieber, E. de Castro, D.A. Marks, L.M. Gardner, et al., Macular pigment and lutein supplementation in retinitis pigmentosa and Usher syndrome, Invest. Ophthalmol. Vis. Sci. 42 (8) (2001) 1873–1881.
[33] H. Bahrami, M. Melia, G. Dagnelie, Lutein supplementation in retinitis pigmentosa: PC-based vision assessment in a randomized double-masked placebo-controlled clinical trial [NCT00029289], BMC Ophthalmol. 6 (2006) 23.
[34] C. Wei, Y. Li, X. Feng, Z. Hu, F. Paquet-Durand, K. Jiao, RNA biological characteristics at the peak of cell death in different hereditary retinal degeneration mutants, Front. Genet. 12 (2021), 728791.
[35] C. Grimm, A. Wenzel, D. Stanescu, M. Samardzija, S. Hotop, M. Groszer, et al., Constitutive overexpression of human erythropoietin protects the mouse retina against induced but not inherited retinal degeneration, J. Neurosci. 24 (25) (2004) 5651–5658.
[36] G. Song, M. Li, H. Sang, L. Zhang, X. Li, S. Yao, et al., Hydrogen-rich water decreases serum LDL-cholesterol levels and improves HDL function in patients with potential metabolic syndrome, J. Lipid Res. 54 (7) (2013) 1884–1893.