Effect of Hydrogen-Rich Water on Oxidative Stress, Liver Function, and Viral Load in Patients with Chronic Hepatitis B

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Abstract

Objective: To investigate effects of hydrogen-rich water (HRW) on oxidative stress, liver function and HBV DNA in patients with chronic hepatitis B (CHB).

Methods: Sixty patients with CHB were randomly assigned into routine treatment group or hydrogen treatment group in which patients received routine treatment alone or additional oral HRW (1200–1800 mL/day, twice daily), respectively, for 6 consecutive weeks. Serum oxidative stress, liver function, and HBV DNA level were detected before and after treatment. Thirty healthy subjects served as controls.

Results: When compared with controls, oxidative stress was obvious in CHB patients, and the liver function also significantly impaired. After treatment, the oxidative stress remained unchanged in routine treatment group, but markedly improved in hydrogen treatment group. The liver function was improved significantly and the HBV DNA reduced markedly after corresponding treatments. Although a significant difference was noted in the oxidative stress between two groups after treatment, the liver function and HBV DNA level were comparable after treatment and both had improved tendencies.

Conclusion: HRW significantly attenuates oxidative stress in CHB patients, but further study with long-term treatment is required to confirm the effect of HRW on liver function and HBV DNA level. Clin Trans Sci 2013; Volume 6: 372–375

Keywords: hydrogen-rich water, oxidative stress, liver function, chronic hepatitis B, viral load

Introduction

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV) and a major global health problem and the most serious type of viral hepatitis. According to the statistics from World Health Organization, an estimated two billion people have been infected with the HBV worldwide, and more than 240 million have chronic liver infections. About 600,000 people die every year due to the acute or chronic consequences of hepatitis B.[1] Hepatitis B is endemic in China and other parts of Asia.[1] There are about 93 million patients with chronic HBV infection of whom 20 million present with chronic hepatitis B (CHB) in China.[2] However, the pathogenesis of hepatitis is still poorly understood. To date, oxidative stress has been found to play an important role in the pathogenesis of hepatitis.[3,4] The reactive oxygen species (ROS) and the ROS induced lipid peroxidation (LPO) involve in the pathology of viral hepatitis and have been regarded as a major cause of liver injury.[3–5] Studies have confirmed the redox imbalance in the CHB patients.[6]

In 2007, Ohsawa et al. found that hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals including hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^{-}$).[7] In previous studies, inhalation of hydrogen and intraperitoneal and oral administration of hydrogen-rich water have been found to exert protective effects in numerous animal models including ischemia/reperfusion injury and inflammation in many organs.[7–9] However, the protective effect of hydrogen on virus induced inflammation including hepatitis has not been reported so far.

This study aimed to investigate the effect of hydrogen-rich water on the oxidative stress (superoxide dismutase [SOD]; glutathione S transferase [GST]; xanthine oxidase [XOD]; malondialdehyde [MDA]), liver function (alanine aminotransferase [ALT]; total biliary acid [TbiL]; cholinesterase [ChE]), and HBV load in patients with CHB. This study may provide evidence for the clinical application of hydrogen-rich water in the treatment of CHB.

Subjects and Methods

Collection of subjects

Thirty healthy subjects (25 males; 5 females) for routine physical examination were recruited as controls. They had no system diseases affect the important organs including heart, liver, kidney, and lung. The liver and kidney function was normal, and they had no viral hepatitis or were not the carriers of viral hepatitis. The mean age was 35.8 ± 12.2 years (range: 20–55 years).

Inclusion criteria: Moderate CHB inpatients (n = 60) were recruited from August 2010 to September 2010. There were 49 males and 11 females. These patients were randomly assigned into hydrogen treatment group or routine treatment group (n = 30 per group). The diagnosis of CHB was based on the Guideline for Prevention and Treatment of Chronic Hepatitis B.[2]

Exclusion criteria: Hepatitis A, C, D, and E, alcoholic hepatitis, drug-induced hepatitis, and autoimmune hepatitis were excluded. Tumors, hematological diseases, heart diseases, hypertension, hyperthyroidism, diabetes, pregnancy, hepatic cirrhosis, and neuropsychiatric disorders were excluded. In the routine treatment group, there were 24 males and 6 females with a mean age of 36.8 ± 15.2 years; in the hydrogen treatment group, there were 25 males and 5 females with a mean age of 35.3 ± 11.1 years. This was a controlled, randomized, blind study. The whole study protocol was approved by the Ethics Committee of Forth People's Hospital of Huai'an, and written informed consent was obtained before study.

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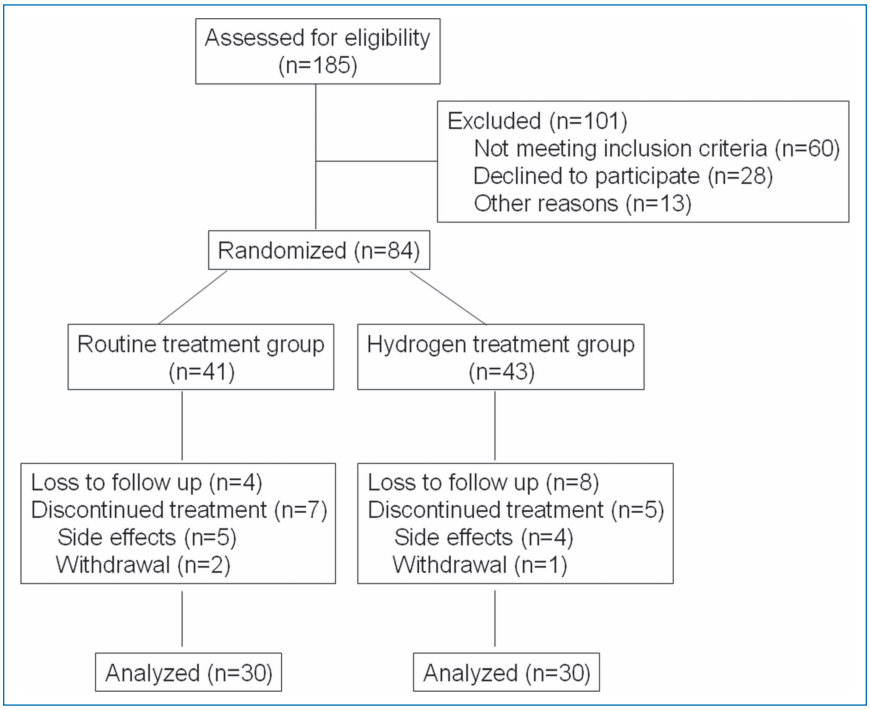

Figure 1. Flow chart of patients' recruitment.

Treatment

In the routine treatment group, patients received routine treatment, and hydrogen-free water was administered to patients at designed time points. In the hydrogen treatment group, besides routine treatment, hydrogen-rich water was orally given thrice daily (8–9 AM; 14–15 PM; 18–19 PM) for consecutive 6 weeks (1,200–1,800 mL daily).[9]

All subjects were not treated with other antioxidants within 1 week before study. The fasting venous blood (4 mL) was collected in the morning before and after 6-week treatment. Following centrifugation at 3,000 rpm for 5 minutes, the serum was collected and stored at –70°C for further use.

Preparation of hydrogen-rich water

Hydrogen-rich water was produced by placing a metallic magnesium stick into drinking water ($Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$); final hydrogen concentration: 0.55~0.65 mM). The magnesium stick contained 99.9% pure metallic magnesium and natural stones in a polypropylene and ceramic container.[9,10]

Detections

The XOD method was used to detect the SOD activity, chemical colorimetric detection was done to detect the GST and XOD, and thiobarbituric acid colorimetric analysis was employed for detection of MDA. Reagents were purchased from the Nanjing Jiancheng Biotech Co., Ltd (Nanjing, China), and detections were done according to the manufacturer's instructions.

Real-time fluorescence quantitative PCR

Real-time fluorescence quantitative PCR was done to measure the HBV DNA (20120114; Da'an Gene Biotech Co., Ltd, Guangzhou, China) with a thermal cycler (ABI 5700; Applied Biosystems, Foster City, CA, USA). The lower limit of detection was 10^3 copies/mL.

Detection of liver function

ALT, TbiL, and ChM were measured with an automatic biochemical analyzer (TBA-120R; Toshiba, Tokyo, Japan), and reagents (No: 12125) were purchased from Whiteman Biotech Co., Ltd. (Nanjing, China). Detection was done according to manufacturer's instructions.

Statistical analysis

Statistical analysis was done with SPSS version 16.0 (SPSS, Chicago, IL, USA) for Windows. Quantitative data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons among the three groups were done with analysis of variance, followed by post hoc test with Newman-Keuls test. However, comparisons between hydrogen treatment group and routine treatment group were done with *t*-test. Qualitative data were expressed as percentage and tested with chi-square test. A value of $p < 0.05$ was considered statistically significant.

Results

Characteristics of patients at baseline in two groups

A total of 185 CHB patients were recruited of whom 101 met the inclusion criteria. Then, 84 patients were randomized into routine treatment group (n = 41) and hydrogen treatment group (n = 43). At the end of study, 30 patients in the routine treatment group and 30 patients in the hydrogen treatment group were included for final analysis (*Figure 1*). The characteristics of CHB patients are shown in *Table 1*. There were no marked differences in the gender, age, HBeAg positive rate, ALT, and HBV DNA level at baseline between routine treatment group and hydrogen treatment group. In addition, there were no marked differences in the demographics at baseline between controls and CHB patients.

Group	Gender (M/F)	Age (year)	HBeAg (%)	ALT (U/L)	HBV DNA (lg copies/mL)
Routine treatment	24/6	36.8 ± 15.2	60.0%	210.1 ± 90.0	5.55 ± 2.08
Hydrogen treatment	25/5	35.3 ± 11.1	63.3%	220.5 ± 95.0	5.07 ± 1.93
p	0.738	0.5435	0.790	0.633	0.260

Table 1. Characteristics of CHB patients at baseline in routine treatment group and hydrogen treatment group.

Group		SOD (U/mL)	GST (U/mL)	MDA (nmol/mL)	XOD (U/L)
Control		98.1 ± 11.8	99.4 ± 7.8	4.4 ± 1.1	12.9 ± 2.1
Routine treatment	Before	84.6 ± 6.5[a,b]	93.4 ± 9.2[a,b]	7.4 ± 2.6[a,b]	16.4 ± 2.2[a,b]
	After	84.9 ± 11.1[a,b]	94.6 ± 9.2[a,b]	6.6 ± 1.4[a,b]	16.8 ± 1.8[a,b]
Hydrogen treatment	Before	84.9 ± 8.2[a,b]	93.6 ± 9.3[a,b]	7.2 ± 2.6[a,b]	16.9 ± 2.6[a,b]
	After	106.8 ± 10.7[a]	109.6 ± 9.6[a]	4.8 ± 2.7	15.3 ± 1.7[a]

Note.[a]$p < 0.05$ versus control group
[b]$p < 0.05$ versus after hydrogen treatment

Table 2. Oxidative stress parameters before and after treatment in different groups ($\bar{x} \pm s$).

Group		ALT (U/L)	TbiL (μmol/L)	ChE (KU/L)	HBV DNA (lg copies /mL)
Control		24.4 ± 23.2	12.3 ± 4.8	7.8 ± 1.4	Undetectable
Routine treatment	Before	210.1 ± 90.0	45.5 ± 21.0	6.7 ± 1.5	5.55 ± 2.08
	After	68.0 ± 38.6[a]	22.5 ± 25.3[a]	7.2 ± 1.4[a]	4.88 ± 2.60
Hydrogen treatment	Before	220.5 ± 95.0	34.9 ± 33.9	6.7 ± 1.5	5.07 ± 1.93
	After	54.8 ± 34.6[a]	16.7 ± 9.6[a]	7.7 ± 1.4[a]	3.89 ± 1.52[a]

Note: [a]$p < 0.05$ versus before treatment.

Table 3. Liver function and HBV DNA before and after treatment in different groups ($\bar{x} \pm s$).

Changes in oxidative stress related parameters in different groups

As shown in *Table 2*, significant differences were found in the SOD, GST, MDA, and XOD between healthy subjects and CHB patients before and after treatment ($p < 0.01$). These parameters remained unchanged in the healthy subjects and routine treatment group. Moreover, the SOD and GST activities increased significantly and the MDA and XOD activity decreased dramatically after hydrogen treatment when compared with those before treatment ($p < 0.01$). In addition, after treatment, the SOD and GST activities increased significantly and the MDA and XOD decreased markedly in the hydrogen treatment group when compared with the routine treatment group ($p < 0.01$). Of note, the SOD and GST activities after hydrogen-rich water treatment were higher than those in the controls.

Liver function and HBV DNA before and after treatment in two groups

As shown in *Table 3*, the ALT, TBiL, and ChE in the CHB patients were significantly different from those in the healthy subjects before treatment.

In CHB patients, the ALT and TBiL reduced and ChE increased significantly after treatments ($p < 0.01$ or 0.05). When compared with routine treatment group, the liver function remained relatively unchanged in the hydrogen treatment group ($p > 0.05$) although the ALT and TBiL slightly reduced and the ChE increased slightly.

After treatment, the HBV DNA in the hydrogen treatment group was comparable to that in the routine treatment group ($p > 0.05$) although the HBV DNA reduced to a certain extent in the hydrogen treatment group.

Discussion

Our results showed that hydrogen-rich water treatment could improve the oxidative stress in CHB patients, and the liver function as well as HBV DNA load had improved tendencies after hydrogen containing comprehensive therapy.

In CHB, the mechanisms underlying the hepatocyte injury are complicated and involve inflammation and immune-induced injury in which ROS play an important role.[11,12] ROS include superoxide anion, singlet oxygen, hydroxyl radicals, and others. ROS and reactive nitrogen species (RNS) have been found to attribute to the liver injury in a variety of liver diseases including viral hepatitis.[4] Studies have shown that the oxidative stress (ROS, RNS) increased but the antioxidative ability was compromised in patients with hepatitis C.[4,6] In addition, patients with hepatitis C have increased LPO in the serum, liver, and peripheral mononuclear cells, and the GSH reduces but the GSSG elevates in the serum and liver.[5,6,13] At different pathological stages, the extent of LPO is also different and has been found to be related to the pathogenesis of hepatitis B.[14,15] These findings confirm that oxidative stress is a major contributor in the pathogenesis of viral hepatitis, which was also confirmed in this study as increases in XOD and MDA (a product of LPO) and decreases in SOD and GST activities. As there is oxidative/antioxidative imbalance in patients with viral hepatitis, to improve the oxidative stress may become a promising strategy for the treatment of viral hepatitis.

Although numerous antioxidants (including natural and synthetic antioxidants have been identified), there is no consensus in the application of antioxidants in the treatment of hepatitis B,[16,17] and some of antioxidants have possible harmful effects.[18] In addition, in animals with liver diseases or liver injury, the poor bioavailability of oral agents is still a challenge.[17] Thus, it is imperative to develop antioxidants without or with minimal side effects for the clinical treatment of hepatitis B.

Recently, hydrogen has been confirmed to possess potent antioxidative capability in numerous diseases related to oxidative stress.[19] In 2007, Ohsawa et al.[7] for the first time found that

hydrogen could selectively neutralize the $^{\cdot}OH$ and $ONOO^{-}$, two free radicals critical for tissue injury, and had no influence on other free radicals. As an ideal antioxidant, hydrogen has some advantages: it can penetrate biomembranes and diffuse into the cytosol, mitochondria and nucleus, and thus it is able to protect nuclear DNA and mitochondria; the product of hydrogen is water and use of hydrogen should not have serious unwanted side effects; hydrogen is mild enough not to disturb the metabolic oxidation/reduction reactions or to disrupt ROS involved in cell signaling. In addition, the collection of hydrogen is relatively easy which makes the hydrogen treatment inexpensive.

In addition, patients receiving hydrogen treatment had improved tendencies in the liver function and HBV DNA level when compared with patients undergoing routine treatment although significant differences were absent. This might be attributed to the short-term treatment with hydrogen. Thus, further study with long-term treatment with hydrogen-rich water is required to confirm the protective effect of hydrogen on the liver function and its suppressive effect on viral replication.

Taken together, hydrogen-rich water may attenuate the oxidative stress and have the potential to improve the liver function and reduce the HBV DNA level in CHB patients. Further studies with long-term treatment of hydrogen-rich water and large sample size are required to confirm the hepatoprotective effect of hydrogen in patients with hepatitis.

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