**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 30657**

**Manuscript Type:** **ORIGINAL ARTICLE**

***Basic Study***

**Hydrogen-rich water protects against inﬂammatory bowel disease in mice by inhibiting endoplasmic reticulum stress and promoting heme oxygenase-1**

Shen NY *et al.* HRW alleviates DSS-induced IBD

Nai-Ying Shen, Jian-Bin Bi, Jing-Yao Zhang, Si-Min Zhang, Jing-Xian Gu, Kai Qu, Chang Liu

**Nai-Ying Shen, Jian-Bin Bi, Jing-Yao Zhang, Si-Min Zhang, Jing-Xian Gu, Kai Qu, Chang Liu,** Department of Hepatobiliary Surgery, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an 710061, Shaanxi Province, China

**Nai-Ying Shen,** Department of General Surgery, Shaanxi Nuclear Geology 215 Hospital, Xianyang 712000, Shaanxi Province, China

**Author contributions:** Shen NY, Bi JB, Zhang JY contribute equally to the paper; Shen NY, Bi JB and Zhang JY participated in research design and in the writing of the paper, they contribute equally to the paper; Zhang SM participated in literature collection and WB performance; Gu JX participated in statistical analysis and IHC performance; Qu K participated in statistical analysis; Liu C gave many advices in designing this research, assist in guiding writing of the paper.

**Supported by** the Project of Innovative Research Team for Key Science and Technology in Shaanxi Province, No. 2013KCJ-23; the Fundamental Research Funds for the Central Universities, No. 1191320114; and the National Nature Science Foundation of China, No. 81601672.

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of Xi’an Jiaotong University. IACUC protocol number: No. XJTULAC2014-207.

**Conflict-of-interest** **statement:** We declare that there is no conflict of interest regarding the publication of this article.

**Data sharing statement:** The authors declare no competing financial interests.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to:** **Chang Liu, MD, PhD,** Department of Hepatobiliary Surgery, The First Affiliated Hospital of Xi’an Jiaotong University, No. 277 Yanta West Road, Xi’an 710061, Shaanxi Province, China. [liuchangdoctor@163.com](mailto:liuchangdoctor@163.com)

**Telephone:** +86-29-85323900

**Fax**: +86-29-85324642

**Received:** October 11, 2016

**Peer-review started:** October 13, 2016

**First decision:** December 2, 2016

**Revised:** December 20, 2016

**Accepted:** January 17, 2017

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To investigate the therapeutic effect and the potential mechanisms of hydrogen-rich water (HRW) on inﬂammatory bowel disease (IBD).

***METHODS***

Male mice were randomly divided into the following four groups: the control group, in which the mice received equivalent volumes of normal saline (NS) intraperitoneally (ip); the dextran sulfate sodium (DSS) group, in which the mice received NS ip (5 mL/kg body weight twice per day at 8 am and 5 pm) for 7 consecutive days after IBD modeling; the DSS + HRW group, in which the mice received HRW (in the same volume as the NS treatment) for 7 consecutive days after IBD modeling; and the DSS + HRW + ZnPP group, in which the mice received HRW (in the same volume as the NS treatment) and ZnPP [heme oxygenase-1 (HO-1) inhibitor, 25 mg/kg] for 7 consecutive days after IBD modeling. IBD was induced by feeding dextran sulfate sodium (DSS) to the mice, and blood and colon tissues were collected on the 7th day after IBD modeling to determine clinical symptoms, colonic inﬂammation and potential mechanisms.

***RESULTS***

The DSS + HRW group exhibited significantly attenuated weight loss and exhibited a lower extent of disease activity index compared with the DSS group on the 7th day (*P <* 0.05). The HRW exerted protective effects against colon shortening and colonic wall thickening in contrast to the DSS group (*P <* 0.05). In addition, the histological study exhibited milder inflammation in the DSS + HRW group, which was similar to normal inflammatory levels, and the macroscopic and microcosmic damage scores were lower in this group than in the DSS group (*P <* 0.05). The oxidative stress parameters, including MDA and MPO in the colon, were significantly decreased in the DSS + HRW group compared with the DSS group (*P <* 0.05). Simultaneously, the protective indicators, SOD and GSH, were markedly increased with the use of HRW. Inflammatory factors were assessed, and the results showed that the DSS + HRW group exhibited significantly reduced levels of TNF-α, IL-6 and IL-1β compared with the DSS group (*P <* 0.05). In addition, the pivotal proteins involving ER stress, including p-eIF2α, ATF4, XBP1s and CHOP, were dramatically reduced after HRW treatment in contrast to the control group (*P <* 0.05). Furthermore, HRW treatment markedly up-regulated HO-1expression, and the use of ZnPP, the HO-1 inhibitor, obviously reversed the protective role of HRW. In the DSS + HRW + ZnPP group, colon shortening and colonic wall thickening were significantly aggravated, and the macroscopic damage scores were similar to those of the DSS + HRW group (*P <* 0.05). The histological study also showed more serious colonic damage that was similar to the DSS group.

***CONCLUSION***

HRW has significant therapeutic potential in IBD by inhibiting inflammatory factors, oxidative stress and ER stress and by up-regulating HO-1 expression.

**Key words:** Hydrogen; Inﬂammatory bowel disease; Oxidative stress; Endoplasmic reticulum stress; Heme oxygenase-1

**© The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Inﬂammatory bowel disease (IBD) is a chronic and relapsing disease primarily caused by the production of pro-inﬂammatory cytokines and leukocyte inﬁltration, resulting in structural and functional damage to the bowel. Hydrogen has obvious anti-oxidative and anti-inflammatory effects. We launched a study to investigate the protective role of hydrogen-rich water on IBD in mice. The present study found that hydrogen-rich water has significant therapeutic potential in IBD by inhibiting inflammatory factors, oxidative stress and endoplasmic reticulum stress and by up-regulating heme oxygenase-1 expression.

Shen NY, Bi JB, Zhang JY, Zhang SM, Gu JX, Qu K, Liu C. Hydrogen-rich water protects against inﬂammatory bowel disease in mice by inhibiting endoplasmic reticulum stress and promoting heme oxygenase-1. *World J Gastroenterol* 2017; In press

**INTRODUCTION**

Inﬂammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), is a chronic and relapsing disease primarily caused by the production of pro-inﬂammatory cytokinesand leukocyte inﬁltration, resulting in structural and functional damage to the bowel. It is associated with environmental factors, genetics, microbial factors, *etc*[1-3]. The major symptoms of IBD include inﬂammation of the colon and abdominal pain, altered visceral sensation, diarrhea, rectal bleeding, weakness and weight loss[4]. CD is often located in the terminal ileum and/or colon and characterized by formation of non-caseating granulomas, which are involved with transmural and discontinuous inﬂammation in the mucosa. In contrast, UC is a colon disorder in which inﬂammation is restricted to the mucosal and submucosal areas, initially affecting the rectum, but it may extend continuously and diffusely throughout the colon[5].

The major therapeutic goals in IBD patients are the alleviation of inﬂammation and the attenuation of IBD symptoms, mainly abdominal pain and altered bowel movements. The current range of treatments for IBD covers both conventional and biological therapies. Conventional therapy includes the use of anti-inﬂammatory drugs, immunosuppressive agents, antibiotics, and probiotics; biological therapies mainly include the use of different anti-TNF-α agents, and a plethora of other novel biological agents[6]. Dextran sulfate sodium (DSS)-induced IBD in mice is a classical mouse IBD model that is accepted worldwide. The mechanism of DSS-induced colitis is mainly due to the direct toxicity to the colonic epithelial cells, subsequently increasing the permeability of the intestinal mucosa and allowing the transport of luminal bacterial products from the bowel lumen to the submucosal tissue[7,8].

Molecular hydrogen, which has been explored as a new medical gas over the last ten years, is a potent anti-oxidative, anti-apoptotic, and anti-inﬂammatory agent and an ideal therapy for many diseases[9]. The benefit of hydrogen as a novel anti-oxidant is that it can penetrate cell membranes, diffuse into the cytosol and target organelles easily, and selectively reduce hydroxide radicals and peroxynitrite without affecting physiological reactive oxygen species (ROS) involved in normal cell signaling[10]. Moreover, hydrogen therapy has been proven to be safe and effective in many clinical trials[11,12]. With respect to intestinal diseases, previous studies have shown that hydrogen may alleviate intestinal ischemia-reperfusion injury, ulcerative colitis, colon inﬂammation, *etc*[13-15]. However, the detailed mechanism responsible for this effect is not yet well illustrated. Hydrogen-rich water (HRW) is an effective, convenient way to deliver molecular hydrogen, which has the same effectiveness as inhaled hydrogen gas and is more suitable for clinical applications. Therefore, the main aim of our study was to assess the protective role and detailed mechanisms of HRW on inﬂammatory bowel disease in mice.

**MATERIALS AND METHODS**

***Experimental animals and preparation of HRW***

This study was conducted using male C57BL/6J mice (4–5 wk old, 21–26 g) (Animal Feeding Center of Xi’an Jiaotong University Medical School). The animals were acclimatized to laboratory conditions (23 °C, 12 h/12 h light/dark cycle, 50% humidity, and ad libitum access to food and water) for one week prior to experimentation. All mice were housed (5 per cage) in clear, pathogen-free polycarbonate cages in the animal care facility, and they were fed a standard animal diet (NO.120161128007, Jiangsu Xietong Pharmaceutical Bio-technology Co., Ltd.) and water *ad libitum* under controlled temperature conditions with 12-h light-dark cycles. They were cared for in accordance with the Ethical Committee, Xi’an Jiaotong University Health Science Center. The study was reviewed and approved by the Xi’an Jiaotong University Health Science Center Institutional Review Board. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Xi’an Jiaotong University Health Science Center. The animal protocol was designed to minimize pain and discomfort to the animals. All animals were euthanized by isoflurane gas for tissue collection. The HRW was produced by Naturally Plus Japan International Co, Ltd and was stored under atmospheric pressure at 4 °C in an aluminum bag with no dead volume, as performed in our previous studies[16-18].

***Induction of inﬂammatory bowel disease***

Inﬂammatory bowel disease was induced by DSS feeding. Male C57BL/6J mice were provided with drinking water containing 5% (wt/vol) DSS (35–50 kDa, Sigma–Aldrich, Steinheim, Germany) ad libitum from day 0 to day 5. On days 6 to 7, the animals received tap water (without DSS). Control animals received tap water throughout the entire experiment.

***Study design***

Mice in the present study were divided into the following four groups: (1) the control group, in which the mice received equivalent volumes of normal saline (NS) intraperitoneally (ip); (2) the DSS group, in which the mice received NS i.p (5 ml/kg body weight twice per day at 8 a.m. and 5 p.m.) for 7 consecutive days after IBD modeling; (3) the DSS+HRW group, in which the mice received HRW (in the same volume as the NS treatment) for 7 consecutive days after IBD modeling；and (4) the DSS + HRW + ZnPP group, in which the mice received HRW (in the same volume as the NS treatment) and ZnPP [heme oxygenase-1 (HO-1) inhibitor, 25 mg/kg] for 7 consecutive days after IBD modeling. Six mice were used per group in this study. The weight, presence of blood, and gross stool consistency of all mice were monitored daily. Each score was determined as follows: (1) change in weight (0: < 1%, 1: 1%-5%, 2: 5%-10%, 3: 10%-15%, 4: > 15%); (2) stool blood (0: negative, 1: negative, 2: hemoccult positive, 3. hemoccult positive, 4: gross bleeding); and (3) stool consistency (0: normal, 1. loose stools, 2: loose stools, 3: diarrhea, 4: diarrhea). The disease activity index was determined by combining the scores from these 3 categories and dividing that number by 3 (Supplementary Table 1)[19].

***Euthanasia***

Mice were sacrificed after being anesthetized with isoflurane gas on the 7th day after IBD modeling, and blood samples were collected from periorbital plexus. The serum was separated by centrifugation at 4 °Cand 3000 × *g* for 15 min. The colon without the cecum was removed immediately from each mouse and stored at -80 °C until further analysis.

***Macroscopic and microscopic scoring and*** ***histological studies***

After the mice in all groups were sacriﬁced, the colon from each mouse was rapidly isolated and weighed with fecal content. The colon was then opened along the mesenteric border, and the fecal material removed. The total macroscopic damage score was calculated for each animal based on the following parameters: fecal blood (0: absence, 1: presence), presence of diarrhea (0: no diarrhea, 1: loosely shaped moist pellets, 2: amorphous, moist, sticky pellets, 3: diarrhea), The extent of colon damage(0: no inﬂammation, 1: reddening, mild inﬂammation, 2: moderate inﬂammation or more widely distributed, 3: severe inﬂammation and/or extensively distributed), colon length(0: < 5% shortening, 1: 5%–14% shortening, 2:15–24% shortening, 3:25–35% shortening, 4:>35%shortening) and weight (0: < 5% weight loss, 1: 5%–14% weight loss, 2: 15%–24% weight loss, 3: 25%–35% weight loss, 4: > 35% weight loss) (Supplementary Table 2)[20].

Samples from the distal colon sections were stapled ﬂat, mucosal side up, onto cardboard and fixed in 10% formalin solution for 24 h. Then samples were dehydrated and embedded in paraffin. Serial sections of 5-μm thickness were obtained and stained with hematoxylin and eosin (HE) to evaluate the morphology. Two researchers examined the results in a blinded fashion. The microscopic total damage score was assessed using the following parameters: the depletion of goblet cells (0: absence, 1: presence), crypt abscesses (0: absence, 1: presence), the destruction of mucosal architecture (1: normal, 2: moderate, 3: extensive), the extent of muscle thickening (1: normal, 2: moderate, 3: extensive), and the presence and degree of cellular inﬁltration (1: normal, 2: moderate, 3: transmural) (Supplementary Table 3)[21].

***Cytokine measurements in murine serum***

The levels of serum TNF-α, IL-6 and IL-1β were measured with commercial ELISA kits according to the instructions from the manufacturer (Dakewe, Shenzhen, China).

***Measurement of colonic oxidative stress***

The concentrations of malonaldehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) in colon tissue were measured as markers of oxidative stress of colon tissue. Colon tissues were homogenized on ice in 10 volumes (w/v) of normal saline. The homogenates were centrifuged at 4000 r/min at 4 °C for 15 min for MDA, SOD and GSH detection by using assay kits purchased from Nanjing Jiancheng Corp., China. MDA levels in the supernatants were determined by measurement of thiobarbituric acid (TBA)-reactive substance levels using MDA assay kit according to the manufacturer’s instructions. The samples were heated with TBA under acidic conditions and the pink color formed was read at 532 nm. The results were calculated as nmol/mg protein. SOD activity in the supernatants of colon tissue was evaluated by inhibition of nitroblue tetrazolium (NBT) reduction by O2- generated by the xanthine/xanthine oxidase system in accordance with the manufacturer’s instructions. The rate of NBT reduction was measured at 560 nm. The results were expressed as U/mg protein. For GSH assay, 5, 5’-Dithiobis-2-nitrobenzoicacid (DTNB) was used to develop color. The development of yellow color was monitored at 412 nm on a spectrophotometer. The results were expressed as mg/g protein. For MPO assay, colon samples were homogenized in 5 volumes (w/v) of phosphate buffered saline containing 0.5% hexadecyltrimethylammonium hydroxide. Samples were measured on a spectrophotometry at 460 nm absorbance. One unit of MPO activity is defined as degrading 1 lmol of hydrogen peroxide at 37 °C, and MPO activity of tissue was expressed as U/g protein.

***Western blot analysis***

Proteins were extracted from the colon according to the manufacturer’s instruction. BCA protein assay kit was used to detect the concentration of extracted proteins. Equal amounts of protein were loaded and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then the gel electrophoresis was transferred onto poly-vinylidene difluoride (PVDF) membranes, which were immunoblotted with the appropriate primary antibody at 4 °C overnight. Then the membranes were incubated with antibodies. The anti-p-eIF2α, ATF4, XBP1s, CHOP, HO-1 and β-actin monoclonal antibodies were purchased from Beijing Biosynthesis Biotechnology Co., Ltd.. The protein concentration was determined by the BCA method. Western blot analysis was performed as previously described[22].

***Statistical analysis***

The measurement data are expressed as the mean ± standard error of mean (SEM). Differences between the experimental and control groups were assessed by either the analysis of variance (ANOVA) or *t* test, as applicable, using SPSS 18.0 (SPSS, 165 Inc.). A *P* value of less than 0.05 was considered to be statistically signiﬁcant.

**RESULTS**

***Treatment with HRW significantly alleviated the symptoms of*** ***DSS-induced*** ***IBD in mice***

To investigate the effects of HRW treatment on IBD, the weight change and disease activity index were assessed on the 7th day after IBD modeling (Figure1). The results showed that the weights of the mice showed a downward trend 7 d after DSS-induced IBD. However, the DSS + HRW group had significantly less weight loss compared with the DSS group on the 7th day (*P <* 0.05). Considering the change in weight, stool blood and stool consistency, the disease activity index was calculated. The disease activity index observably increased after DSS treatment, and the DSS + HRW group exhibited a lower extent of disease than DSS group (*P <* 0.05).

***Treatment with HRW markedly ameliorated colonic damage in DSS-induced IBD***

Mice were sacrificed, and the colons were assessed on the 7th day after IBD modeling. We discovered the average length of the colons exhibited a significant reduction after DSS administration. More importantly, the HRW exerted a protective effect against the shortening of the colons in the DSS + HRW group, which were markedly longer than in the DSS group (*P <* 0.05). In addition, the colonic wall thickening was alleviated in the DSS + HRW group in contrast to the DSS group (*P <* 0.05). The macroscopic damage score assessed by diarrhea, colon damage and colon length showed that the DSS + HRW group also received a lower score than the DSS group (*P <* 0.05) (Figure 2).

For the further study of the alterations of the colons, we conducted the experiments in microcosmic aspect. The histological study revealed that the mice in the DSS group developed severe colonic inflammation including mucosal hyperemia, [inflammatory](javascript:void(0);) [cell](javascript:void(0);) [infiltration](javascript:void(0);), formation of crypt abscesses, destruction of the mucosal architecture, and the depletion of goblet cells. Conversely, the DSS + HRW group exhibited mild inflammation that was much closer to normal. The microcosmic scores based on the goblet cell depletion, crypt abscesses, destruction of the mucosal architecture, muscle thickening, and cellular inﬁltration in the DSS + HRW group were also lower than those in the DSS group (*P <* 0.05) (Figure 3). This evidence indicated that HRW could improve colonic damage in DSS-induced IBD.

***HRW inhibited*** ***oxidative stress and*** ***inflammatory factors in DSS-induced IBD***

Oxidative stress and inflammation played an initial and crucial role in the process of IBD. The oxidative stress parameters in the colon, including MDA and MPO were significantly decreased in the DSS + HRW group compared with the DSS group (*P <* 0.05). The protective indicator, SOD, was markedly increased with the use of HRW. Additionally, HRW also reversed the depletion of GSH caused by DSS administration (Figure 4). These facts demonstrated that HRW could indeed inhibit oxidative stress.

To explore the anti-inflammatory mechanism of HRW, inflammatory factors were assessed on the 7th day after IBD modeling by determining the plasma levels of TNF-α, IL-6 and IL-1β. A marked increase in TNF-α, IL-6 and IL-1β secretion was observed after DSS treatment. Moreover, the DSS + HRW group had significantly reduced levels of TNF-α, IL-6 and IL-1β compared with the DSS group (*P <* 0.05) (Figure 5).

***HRW inhibited*** ***ER stress in DSS-induced IBD***

ER stress participating in a cellular process triggered by a variety of conditions that disturb the folding of proteins in the ER also aggravates the progress of IBD. The pivotal proteins involved in ER stress, including p-eIF2α, ATF4, XBP1s and CHOP, were detected to assess the effects of HRW on ER stress in DSS-induced IBD (Figure 6). The results demonstrated that the expression of these proteins were significantly increased after DSS administration. Moreover, the p-eIF2α, ATF4, XBP1s and CHOP proteins were dramatically reduced after HRW treatment in contrast to the control group. These findings indicated that HRW may ameliorate the manifestation of IBD by inhibiting the process of ER stress.

***HRW up-regulated*** ***HO-1 expression to alleviate IBD***

HO-1 performs anti-inflammatory and anti-oxidative effects protecting against many diseases. On the 7th day after IBD modeling, the mice were sacriﬁced, and the colon tissues were obtained to detect the HO-1 expression (Figure 7). The results revealed that HRW treatment markedly accelerated HO-1expression compared with the DSS group. For the further study of the role that HO-1 played in IBD, ZnPP, the HO-1 inhibitor, was used. We discovered that colon length was visibly reduced in the DSS + HRW + ZnPP group compared with the DSS + HRW group (*P <* 0.05). The colonic wall in the DSS + HRW + ZnPP group was also thicker than in the DSS + HRW group (*P <* 0.05). In addition, the DSS + HRW + ZnPP group got a higher macroscopic damage score in contrast to the DSS + HRW group (*P <* 0.05). The histological study showed that the DSS + HRW + ZnPP group exhibited more serious colonic damage that was similar to that observed in the DSS group, which was described previously (Figure 8). Based on these findings, we confirmed that HO-1 played a key role in the HRW mechanisms that alleviated the IBD.

**DISCUSSION**

With its anti-oxidant, anti-inflammatory, anti-apoptotic and other protective effects, great progress has been achieved in the research of hydrogen therapy on diseases such as metabolic disorders, tissue ischemia reperfusion injury, myocardial injury, and hepatic injury[23-26].In this study, a model of IBD was established in mice by DSS feeding, and the therapeutic role of HRW was assessed. We demonstrated that treatment with HRW significantly alleviated the symptoms and colonic damage in DSS-induced IBD. The mechanisms by which HRW alleviates DSS-induced IBD may include the following: (1) inhibiting the secretion of inflammatory factors, such as TNF-α, IL-6 and IL-1β to ameliorate the inflammatory response; (2) inhibitingoxidative stress, such as reducing MPO and ROS as well as increasing SOD and GSH; (3) inhibitingER stress such as decreasing the expression of p-eIF2α, ATF4, XBP1s and CHOP; and (4) up-regulating HO-1 expression to ease oxidative stress and decrease inflammation. All the evidence revealed that HRW was a potential new method for the treatment of IBD.

IBD is an enteric disorder characterized by acute and chronic intestinal inflammation. The etiology and precise pathogenesis of IBD are still unclear. However, several possible causes, including genetic, infectious, immunological factors and dysfunction of the adaptive and innate immune systems in response to the fecal microbiome, have been recognized[27-30]. DSS is a physical agent with an intrinsic capacity to disrupt the epithelial barrier and activates macrophages, causing inflammation and tissue damage[8]. The related histological changes include ulceration and inflammation of the intestinal mucosa with leukocyte infiltration. The clinical presentation includes weight loss, stool blood and diarrhea. In the present study, we chose to evaluate changes in weight, the disease activity index, colon shortening, colonic wall thickening, histological study, and macroscopic and microcosmic scores to assess the severity of the DSS-induced IBD.

Oxidative stress is an imbalance of oxidation and anti-oxidation systems in the body and may be caused by excessive detrimental ROS, depletion of GSH, *etc*. In IBD, the production of ROS and MPO exceeds anti-oxidant defenses and leads to a state of oxidative stress that fuels inflammation and causes direct mitochondrial damage[31,32]. Hydrogen selectively quenches detrimental ROS, such as hydroxyl radicals and peroxynitrite but it does not damage physiological ROS, such as superoxide anion radicals, hydrogen peroxide, and nitric oxide[33]. In this study, we found that HRW significantly reduced the levels of MDA and MPO and facilitated the protective indicators SOD and GSH. Furthermore, we measured the levels of inflammatory factors, and the results revealed that HRW markedly inhibited the release of TNF-α, IL-6 and IL-1β. TNF-α is one of the most important pro-inflammatory cytokines, which stimulates the production of downstream cytokines such as IL-6 and IL-8 and plays a significant role in activating the cytokine cascade[34,35]. The researchers reported that anti-TNF-α monoclonal antibodies and other drugs had dramatically improved the treatment of IBD[36,37]. In summary, our study revealed that HRW could quench detrimental oxidative stress and exert an anti-TNF-αrole to alleviate IBD.

ER stress participates in a cellular process triggered by a variety of conditions that disturb the folding of proteins in the ER. ER stress further triggers the unfolded protein response (UPR) by activating the PKR and PERK signals and phosphorylating eIF2α, which is required by the initiation phase of polypeptide chain synthesis[38,39]. ATF4 is a UPR-dependent transcriptional factor, and its sustained expression may up-regulate CHOP expression, inducing apoptosis[40]. XBP1s is also a UPR-dependent transcriptional factor induced by AFT6[41]. ER stress exerts important roles in many diseases such as [ischemia/reperfusion injury in the liver,](http://www.ncbi.nlm.nih.gov/pubmed/26711306) diabetes, and [cardiac myocyte injury](http://www.ncbi.nlm.nih.gov/pubmed/26137860)[42-44]. The previous study also proved that [epithelial ER stress participated in Crohn’s disease and ulcerative colitis](http://www.ncbi.nlm.nih.gov/pubmed/26950312)[45]. Moreover, studies have found that hydrogen has anti-apoptotic and anti-inflammatory functions[46,47]. In this study, we discovered that HRW dramatically reduced the expression of p-eIF2α, ATF4, XBP1s and CHOP proteins and conclude that HRW protects against IBD by inhibiting ER stress.

To determine the deeper mechanism of the protective effect of HRW against IBD, we focused on the effect of hydrogen on HO-1 expression. Heme oxygenase (HO) catalyzes the rate-limiting step in heme degradation, which can produce bilirubin, iron, and carbon monoxide (CO). HO-1, increased by stimuli that induce cellular stress, reduces the secretion of inflammatory cytokines in many diseases, such as sepsis, and LPS-stimulated macrophages[48,49]. Additionally, HO-1 conferred its cytoprotective effects by increasing anti-oxidative capacity and inhibiting oxidative stress[50,51]. In addition, recent studies have shown that HO-1 was involved in the downstream effect of Treg cells[52]. Based on these facts, we speculated that hydrogen may confer its cytoprotective role by up-regulating HO-1. We measured the level of HO-1 and used ZnPP, the HO-1 inhibitor for the further study. Not surprisingly, HRW treatment markedly up-regulated HO-1expression, and the use of ZnPP clearly reversed the protective role of HRW. We verified that HO-1 indeed played a key role in the mechanisms by which HRW alleviated the IBD. The detailedmechanism may be that HO-1 inhibited the secretion of inflammatory cytokines and oxidative stress to alleviate the IBD.

In this study, we have proven that HRW has significant therapeutic potential in the treatment of IBD by inhibiting inflammatory factors and oxidative stress. More importantly, we discovered that HRW could inhibit ER stress toprevent apoptosis and up-regulate HO-1 expression. The high level of HO-1 further exerted anti-oxidative and anti-inﬂammatory functions in the process of IBD. Additionally, due to its advantageous distribution characteristics, hydrogen can penetrate biomembranes and diffuse into the cytosol, mitochondria, and nucleus, successfully targeting the organelles. All of these effects make HRW a potential new treatment method against DSS-induced IBD. However, our study is based on animal experiments, and prospective clinical studies are needed to evaluate whether HRW is fit for the clinical treatment of IBD.

In conclusion, the results of the present study demonstrate that HRW can alleviate the symptoms and colonic damage in DSS-induced IBD, most likely due to its unique cytoprotective properties such as its anti-oxidant and anti-inflammatory activities. More importantly, HRW can inhibit ER stress and up-regulate HO-1 expression. All of these ﬁndings indicate that HRW can be a potential therapy for DSS-induced IBD.

**COMMENTS**

***Background***

Inﬂammatory bowel disease (IBD) is a chronic and relapsing disease with therapeutic goals that include controlling inﬂammation and ameliorating clinical symptoms. Hydrogen-rich water (HRW) is a potent anti-oxidative, anti-apoptotic, and anti-inﬂammatory agent and an ideal therapy formany diseases.

***Research frontiers***

Effective therapeutic schemes for IBD are lacking. Research of mechanisms and new therapeutic approaches for IBD have received increasing attention from scientists and clinicians. Hydrogen therapy is a new medical approach that has recently gained much appreciation. HRW exerts considerable anti-oxidative, anti-apoptotic, and anti-inﬂammatory effects. More importantly, drinking HRW is very convenient in the course of daily life. To explore the effect of HRW on different types of diseases and to promote its clinical usage is currently an important goal in hydrogen medicine.

***Innovations and breakthroughs***

The present study concluded that HRW can significantly prevent IBD in mice by inhibiting inflammatory factors, oxidative stress and ER stress and by up-regulating HO-1 expression. Moreover, based on the use of the pharmaceutical inhibition of HO-1, we can conclude that HO-1 may be a key effective protein in HRW function.

***Applications***

Hydrogen therapy may be a safe and effective treatment for IBD. Moreover, the application of drinking HRW is very convenient and acceptable for usage.

***Terminology***

Hydrogen is the lightest gas in nature, which has powerful anti-oxidant and anti-inflammatory effects. It has therapeutic effects in many diseases, which is proven by many basic research and clinical studies. HRW is produced by forcing hydrogen gas into water by a specific device under high pressure.

***Peer-review***

Congratulations. It is a very well designed work with very interesting results. I just wanted to ask: Was there any examination or histological study of the puncture site? It would have been interesting to know if there is any reaction in that place.

**REFERENCES**

1 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMra020831]

2 **Shanahan F**. Crohn's disease. *Lancet* 2002; **359**: 62-69 [PMID: 11809204 DOI: 10.1016/S0140-6736(02)07284-7]

3 **Strober W**, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007; **117**: 514-521 [PMID: 17332878 DOI: 10.1172/JCI30587]

4 **Beaugerie L**, Sokol H. Clinical, serological and genetic predictors of inflammatory bowel disease course. *World J Gastroenterol* 2012; **18**: 3806-3813 [PMID: 22876031 DOI: 10.3748/wjg.v18.i29.3806]

5 **Wallace KL**, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 6-21 [PMID: 24415853 DOI: 10.3748/wjg.v20.i1.6]

6 **Triantafillidis JK**, Merikas E, Georgopoulos F. Current and emerging drugs for the treatment of inflammatory bowel disease. *Drug Des Devel Ther* 2011; **5**: 185-210 [PMID: 21552489 DOI: 10.2147/DDDT.S11290]

7 **Randhawa PK**, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. *Korean J Physiol Pharmacol* 2014; **18**: 279-288 [PMID: 25177159 DOI: 10.4196/kjpp.2014.18.4.279]

8 **Perše M**, Cerar A. Dextran sodium sulphate colitis mouse model: traps and tricks. *J Biomed Biotechnol* 2012; **2012**: 718617 [PMID: 22665990 DOI: 10.1155/2012/718617]

9 **Xia C**, Liu W, Zeng D, Zhu L, Sun X, Sun X. Effect of hydrogen-rich water on oxidative stress, liver function, and viral load in patients with chronic hepatitis B. *Clin Transl Sci* 2013; **6**: 372-375 [PMID: 24127924 DOI: 10.1111/cts.12076]

10 **Ostojic SM**. Targeting molecular hydrogen to mitochondria: barriers and gateways. *Pharmacol Res* 2015; **94**: 51-53 [PMID: 25720951 DOI: 10.1016/j.phrs.2015.02.004]

11 **McCarty MF**. Potential ghrelin-mediated benefits and risks of hydrogen water. *Med Hypotheses* 2015; **84**: 350-355 [PMID: 25649854 DOI: 10.1016/j.mehy.2015.01.018]

12 **Ohta S**. [Initiation, development and potential of hydrogen medicine: Toward therapeutic and preventive applications of molecular hydrogen against a variety of diseases]. *Seikagaku* 2015; **87**: 82-90 [PMID: 26571560]

13 **Abe T**, Li XK, Yazawa K, Hatayama N, Xie L, Sato B, Kakuta Y, Tsutahara K, Okumi M, Tsuda H, Kaimori JY, Isaka Y, Natori M, Takahara S, Nonomura N. Hydrogen-rich University of Wisconsin solution attenuates renal cold ischemia-reperfusion injury. *Transplantation* 2012; **94**: 14-21 [PMID: 22683850 DOI: 10.1097/TP.0b013e318255f8be]

14 **He J**, Xiong S, Zhang J, Wang J, Sun A, Mei X, Sun X, Zhang C, Wang Q. Protective effects of hydrogen-rich saline on ulcerative colitis rat model. *J Surg Res* 2013; **185**: 174-181 [PMID: 23773716 DOI: 10.1016/j.jss.2013.05.047]

15 **Medani M**, Collins D, Docherty NG, Baird AW, O'Connell PR, Winter DC. Emerging role of hydrogen sulfide in colonic physiology and pathophysiology. *Inflamm Bowel Dis* 2011; **17**: 1620-1625 [PMID: 21674719 DOI: 10.1002/ibd.21528]

16 **Zhang JY**, Song SD, Pang Q, Zhang RY, Wan Y, Yuan DW, Wu QF, Liu C. Hydrogen-rich water protects against acetaminophen-induced hepatotoxicity in mice. *World J Gastroenterol* 2015; **21**: 4195-4209 [PMID: 25892869 DOI: 10.3748/wjg.v21.i14.4195]

17 **Zhang JY**, Wu QF, Wan Y, Song SD, Xu J, Xu XS, Chang HL, Tai MH, Dong YF, Liu C. Protective role of hydrogen-rich water on aspirin-induced gastric mucosal damage in rats. *World J Gastroenterol* 2014; **20**: 1614-1622 [PMID: 24587639 DOI: 10.3748/wjg.v20.i6.1614]

18 **Zhang J**, Wu Q, Song S, Wan Y, Zhang R, Tai M, Liu C. Effect of hydrogen-rich water on acute peritonitis of rat models. *Int Immunopharmacol* 2014; **21**: 94-101 [PMID: 24793096 DOI: 10.1016/j.intimp.2014.04.011]

19 **Simeoli R**, Mattace Raso G, Lama A, Pirozzi C, Santoro A, Di Guida F, Sanges M, Aksoy E, Calignano A, D'Arienzo A, Meli R. Preventive and therapeutic effects of Lactobacillus paracasei B21060-based synbiotic treatment on gut inflammation and barrier integrity in colitic mice. *J Nutr* 2015; **145**: 1202-1210 [PMID: 25926411 DOI: 10.3945/jn.114.205989]

20 **Fichna J**, Dicay M, Lewellyn K, Janecka A, Zjawiony JK, MacNaughton WK, Storr MA. Salvinorin A has antiinflammatory and antinociceptive effects in experimental models of colitis in mice mediated by KOR and CB1 receptors. *Inflamm Bowel Dis* 2012; **18**: 1137-1145 [PMID: 21953882 DOI: 10.1002/ibd.21873]

21 **Sałaga M**, Polepally PR, Zakrzewski PK, Cygankiewicz A, Sobczak M, Kordek R, Zjawiony JK, Krajewska WM, Fichna J. Novel orally available salvinorin A analog PR-38 protects against experimental colitis and reduces abdominal pain in mice by interaction with opioid and cannabinoid receptors. *Biochem Pharmacol* 2014; **92**: 618-626 [PMID: 25265540 DOI: 10.1016/j.bcp.2014.09.018]

22 **Qu K**, Xu X, Liu C, Wu Q, Wei J, Meng F, Zhou L, Wang Z, Lei L, Liu P. Negative regulation of transcription factor FoxM1 by p53 enhances oxaliplatin-induced senescence in hepatocellular carcinoma. *Cancer Lett* 2013; **331**: 105-114 [PMID: 23262037 DOI: 10.1016/j.canlet.2012.12.008]

23 **Heinzelmann SM**, Villanueva L, Sinke-Schoen D, Sinninghe Damsté JS, Schouten S, van der Meer MT. Impact of metabolism and growth phase on the hydrogen isotopic composition of microbial fatty acids. *Front Microbiol* 2015; **6**: 408 [PMID: 26005437 DOI: 10.3389/fmicb.2015.00408]

24 **Zhao Y**, Tang Y, Suo C, Liu D, Li S, Li H. [Effects of hydrogen-rich saline on endoplasmic reticulum stress during myocardial ischemia-reperfusion in rats]. *Zhonghua Yi Xue Za Zhi* 2014; **94**: 3024-3028 [PMID: 25547710]

25 **Tanabe H**, Sasaki Y, Yamamoto T, Kiriyama S, Nishimura N. Suppressive Effect of High Hydrogen Generating High Amylose Cornstarch on Subacute Hepatic Ischemia-reperfusion Injury in Rats. *Biosci Microbiota Food Health* 2012; **31**: 103-108 [PMID: 24936356 DOI: 10.12938/bmfh.31.103]

26 **Qu J**, Lü X. Hydrogen: a promising novel treatment for hepatic encephalopathy? *Free Radic Biol Med* 2013; **63**: 457-458 [PMID: 23743290 DOI: 10.1016/j.freeradbiomed.2013.05.032]

27 **Zanello G**, Kevans D, Goethel A, Silverberg M, Tyler A, Croitoru K. Genetics and innate and adaptive immunity in IBD. *Nestle Nutr Inst Workshop Ser* 2014; **79**: 41-55 [PMID: 25227294 DOI: 10.1159/000360676]

28 **Targownik LE**, Bernstein CN. Infectious and malignant complications of TNF inhibitor therapy in IBD. *Am J Gastroenterol* 2013; **108**: 1835-142, quiz 1843 [PMID: 24042192 DOI: 10.1038/ajg.2013.294]

29 **Lee JW**, Im JP, Cheon JH, Kim YS, Kim JS, Han DS. Inflammatory Bowel Disease Cohort Studies in Korea: Present and Future. *Intest Res* 2015; **13**: 213-218 [PMID: 26130995 DOI: 10.5217/ir.2015.13.3.213]

30 **Liang J**, Sha SM, Wu KC. Role of the intestinal microbiota and fecal transplantation in inflammatory bowel diseases. *J Dig Dis* 2014; **15**: 641-646 [PMID: 25389085 DOI: 10.1111/1751-2980.12211]

31 **Thomson A**, Hemphill D, Jeejeebhoy KN. Oxidative stress and antioxidants in intestinal disease. *Dig Dis* 1998; **16**: 152-158 [PMID: 9618134]

32 **Piechota-Polanczyk A**, Fichna J. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. *Naunyn Schmiedebergs Arch Pharmacol* 2014; **387**: 605-620 [PMID: 24798211 DOI: 10.1007/s00210-014-0985-1]

33 **Hossain MA**, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran LS. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Front Plant Sci* 2015; **6**: 420 [PMID: 26136756 DOI: 10.3389/fpls.2015.00420]

34 **Scharl M**, Vavricka SR, Rogler G. Review: new anti-cytokines for IBD: what is in the pipeline? *Curr Drug Targets* 2013; **14**: 1405-1420 [PMID: 23621511]

35 **Vermeire S**, Van Assche G, Rutgeerts P. Serum sickness, encephalitis and other complications of anti-cytokine therapy. *Best Pract Res Clin Gastroenterol* 2009; **23**: 101-112 [PMID: 19258190 DOI: 10.1016/j.bpg.2008.12.005]

36 **Blotière PO**, Rudant J, Barré A, Racine A, Weill A, Peyrin-Biroulet L, Carbonnel F, Alla F. Conditions of prescription of anti-TNF agents in newly treated patients with inflammatory bowel disease in France (2011-2013). *Dig Liver Dis* 2016; **48**: 620-625 [PMID: 27017107 DOI: 10.1016/j.dld.2016.02.022]

37 **Komaki Y**, Komaki F, Sakuraba A, Cohen R. Approach to Optimize Anti-TNF-α Therapy in Patients With IBD. *Curr Treat Options Gastroenterol* 2016; **14**: 83-90 [PMID: 26872815 DOI: 10.1007/s11938-016-0079-x]

38 **Sano R**, Reed JC. ER stress-induced cell death mechanisms. *Biochim Biophys Acta* 2013; **1833**: 3460-3470 [PMID: 23850759 DOI: 10.1016/j.bbamcr.2013.06.028]

39 **Sovolyova N**, Healy S, Samali A, Logue SE. Stressed to death - mechanisms of ER stress-induced cell death. *Biol Chem* 2014; **395**: 1-13 [PMID: 24002662 DOI: 10.1515/hsz-2013-0174]

40 **Khan I**, Tang E, Arany P. Molecular pathway of near-infrared laser phototoxicity involves ATF-4 orchestrated ER stress. *Sci Rep* 2015; **5**: 10581 [PMID: 26030745 DOI: 10.1038/srep10581]

41 **Cubillos-Ruiz JR**, Bettigole SE, Glimcher LH. Molecular Pathways: Immunosuppressive Roles of IRE1α-XBP1 Signaling in Dendritic Cells of the Tumor Microenvironment. *Clin Cancer Res* 2016; **22**: 2121-2126 [PMID: 26979393 DOI: 10.1158/1078-0432.CCR-15-1570]

42 **Liu D**, Liu X, Zhou T, Yao W, Zhao J, Zheng Z, Jiang W, Wang F, Aikhionbare FO, Hill DL, Emmett N, Guo Z, Wang D, Yao X, Chen Y. IRE1-RACK1 axis orchestrates ER stress preconditioning-elicited cytoprotection from ischemia/reperfusion injury in liver. *J Mol Cell Biol* 2016; **8**: 144-156 [PMID: 26711306 DOI: 10.1093/jmcb/mjv066]

43 **Choi SK**, Lim M, Yeon SI, Lee YH. Inhibition of endoplasmic reticulum stress improves coronary artery function in type 2 diabetic mice. *Exp Physiol* 2016; **101**: 768-777 [PMID: 26990483 DOI: 10.1113/EP085508]

44 **Liu Z**, Zhao N, Zhu H, Zhu S, Pan S, Xu J, Zhang X, Zhang Y, Wang J. Circulating interleukin-1β promotes endoplasmic reticulum stress-induced myocytes apoptosis in diabetic cardiomyopathy via interleukin-1 receptor-associated kinase-2. *Cardiovasc Diabetol* 2015; **14**: 125 [PMID: 26394923 DOI: 10.1186/s12933-015-0288-y]

45 **Cao SS**. Epithelial ER Stress in Crohn's Disease and Ulcerative Colitis. *Inflamm Bowel Dis* 2016; **22**: 984-993 [PMID: 26950312 DOI: 10.1097/MIB.0000000000000660]

46 **Lee D**, Park S, Bae S, Jeong D, Park M, Kang C, Yoo W, Samad MA, Ke Q, Khang G, Kang PM. Hydrogen peroxide-activatable antioxidant prodrug as a targeted therapeutic agent for ischemia-reperfusion injury. *Sci Rep* 2015; **5**: 16592 [PMID: 26563741 DOI: 10.1038/srep16592]

47 **Yang R**, Jia Q, Guo X, Liu X, Ma S, Gao Q, Guan S. [Protective effects of hydrogen sulfide on diaphragmatic muscle of Type 1 diabetic rats and its anti-apoptotic mechanisms]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2015; **40**: 1173-1178 [PMID: 26643418 DOI: 10.11817/j.issn.1672-7347.2015.11.002]

48 **Araujo JA**, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. *Front Pharmacol* 2012; **3**: 119 [PMID: 22833723 DOI: 10.3389/fphar.2012.00119]

49 **Durante W**. Protective role of heme oxygenase-1 against inflammation in atherosclerosis. *Front Biosci (Landmark Ed)* 2011; **16**: 2372-2388 [PMID: 21622183]

50 **Gao Z**, Han Y, Hu Y, Wu X, Wang Y, Zhang X, Fu J, Zou X, Zhang J, Chen X, Jose PA, Lu X, Zeng C. Targeting HO-1 by Epigallocatechin-3-Gallate Reduces Contrast-Induced Renal Injury via Anti-Oxidative Stress and Anti-Inflammation Pathways. *PLoS One* 2016; **11**: e0149032 [PMID: 26866373 DOI: 10.1371/journal.pone.0149032]

51 **Cheng HT**, Yen CJ, Chang CC, Huang KT, Chen KH, Zhang RY, Lee PY, Miaw SC, Huang JW, Chiang CK, Wu KD, Hung KY. Ferritin heavy chain mediates the protective effect of heme oxygenase-1 against oxidative stress. *Biochim Biophys Acta* 2015; **1850**: 2506-2517 [PMID: 26423448 DOI: 10.1016/j.bbagen.2015.09.018]

52 **Schumacher A**, Wafula PO, Teles A, El-Mousleh T, Linzke N, Zenclussen ML, Langwisch S, Heinze K, Wollenberg I, Casalis PA, Volk HD, Fest S, Zenclussen AC. Blockage of heme oxygenase-1 abrogates the protective effect of regulatory T cells on murine pregnancy and promotes the maturation of dendritic cells. *PLoS One* 2012; **7**: e42301 [PMID: 22900010 DOI: 10.1371/journal.pone.0042301]

**P-Reviewer:** Madrid AM, Perse M **S-Editor:** Yu J **L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): A, A

Grade B (Very good): 0

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 1.wmf**

**Figure 1 Hydrogen-rich water decreases the weight loss and achieves a lower disease activity index score in DSS induced inﬂammatory bowel disease.** The changes in weight and disease activity index were assessed on the 7th day after inﬂammatory bowel disease (IBD) modeling. *n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 2.wmf

**Figure 2 Hydrogen-rich water alleviates the changes in colon length, thickness and macroscopic score in DSS induced IBD.** Mice were sacrificed, and the colons were rapidly removed and processed for analysis on the 7th day after inﬂammatory bowel disease (IBD) modeling. A: The representative pictures of the colons in each group; B-D:The macroscopic score, length, and the thickness of the colons from each group. *n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 3.tif

**Figure 3 Hydrogen-rich water alleviates the changes in histological studies and microscopic score in DSS induced inﬂammatory bowel disease.** Mice were sacrificed, and the colons were rapidly removed and processed for analysis on the 7th day after inﬂammatory bowel disease (IBD) modeling. A: Hematoxylin-eosin staining of colon tissues (magnification × 100, 200); B: The microscopic score of the colon in each group. *n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 4.wmf

**Figure 4 Hydrogen-rich water decreases the oxidative stress in DSS induced inﬂammatory bowel disease.**On the 7th day after inﬂammatory bowel disease (IBD) modeling, the colon tissues were harvested to evaluate the oxidative stress. The levels of MDA, MPO, SOD and GSH in the colon tissues were measured. *n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

**C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 5.wmf**

**Figure 5 Hydrogen-rich water** **decreases inflammatory factors in DSS induced inﬂammatory bowel disease.** On the 7th day after inﬂammatory bowel disease (IBD) modeling, the blood samples were harvested to evaluate the oxidative stress. HRW reduced the serum TNF-α, IL-6 and IL-1β concentrations. *n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 6.wmf

**Figure 6 Hydrogen-rich water inhibits ER stress in DSS induced inﬂammatory bowel disease.** On the 7th day after inﬂammatory bowel disease (IBD) modeling, the colon tissues were harvested to evaluate the ER stress.Western blot analysis was conducted to evaluate the protein content of p-eIF2α, ATF4, XBP1s and CHOP proteins. β-actin was used as an internal control. *n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 7.tif

**Figure 7 Hydrogen-rich water up-regulates expression of HO-1 in DSS induced inﬂammatory bowel disease.** On the 7th day after inﬂammatory bowel disease (IBD) modeling, the colon tissues were harvested and Western blot analysis was conducted to evaluate the level of heme oxygenase-1 (HO-1).*n =* 6, mean ± SEM, a*P* < 0.05 *vs* control group; b*P* < 0.05 *vs* dextran sulfate sodium (DSS) group.

**C:\Users\baishideng-2014\Desktop\revised-jyu\30657\30657-Figures\Figure 8.tif**

**Figure 8 Lack of HO-1 reverses the protective role of hydrogen-rich water in DSS induced inﬂammatory bowel disease.** Mice were sacrificed, and the colons were rapidly removed and processed for analysis on the 7th day after inﬂammatory bowel disease (IBD) modeling. A: The representative pictures of the colon in each group; B:The macroscopic score of the colon in each group; C: The length of the colon in each group; D: The thickness of the colon in each group; E: Hematoxylin-eosin staining of colon tissues (magnification ×100). *n =* 6, mean ± SEM, a*P* < 0.05 *vs* dextran sulfate sodium (DSS) group; b*P* < 0.05 *vs* DSS+HRW group. HO-1: Heme oxygenase-1.