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ORIGINAL RESEARCH

Efficacy and Safety of Hydrogen Therapy in Patients with Early-Stage Interstitial Lung Disease: A Single-Center, Randomized, Parallel-Group Controlled Trial

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Purpose: Several in vivo experiments have shown that molecular hydrogen is a promising therapeutic agent for interstitial lung diseases (ILD). In this study, hydrogen therapy was investigated to determine whether it is superior to N-Acetylcysteine (NAC) for the treatment of patients with early-stage ILD.

Patients and Methods: A prospective, single-center, randomized, controlled clinical trial was conducted in 87 patients with early-stage ILD. Hydrogen or NAC therapy was randomly assigned (1:1 ratio) to the eligible patients. The primary endpoint was the change in the high-resolution computed tomography (HRCT) and composite physiologic index (CPI) scores from baseline to week 48. Pulmonary function was evaluated as a secondary endpoint, and adverse events were recorded for safety analysis.

Results: The rate of HRCT image improvement from the baseline in the HW group (63.6%) was higher than that in the NAC group (39.5%). A significant decrease in CPI and improvement in D_LCO-sb were observed in the hydrogen group compared with those in the control group. Changes in other pulmonary function parameters, including FVC, FEV_1, FEV_1/FVC%, and TLC, were not significantly different between the two groups. Adverse events were reported in 7 (15.9%) patients in the HW group and 10 (23.3%) patients in the NAC group, but the difference was not significant (P=0.706).

Conclusion: Hydrogen therapy exhibits superior efficacy and acceptable safety compared with NAC therapy in patients with early-stage ILD.

Keywords: hydrogen, N-acetylcysteine, interstitial lung disease, therapeutic effects

Introduction

Interstitial lung disease (ILD) is a heterogeneous group of non-neoplastic and non-infectious diseases that affect the lung parenchyma. It is characterized by an initial inflammation of the pulmonary alveoli that extends to the interstitium and beyond, leading to diffuse pulmonary fibrosis.[1] These lesions could cause a decrease in lung capacity, diffusing function, and disturbance of the ventilation–perfusion ratio, ultimately resulting in hypoxemia and respiratory failure. ILD can be divided into known-causes ILD (including pneumoconiosis and connective tissue disease-associated ILD) and unknown causes (including idiopathic pulmonary fibrosis).[2]

It is well established that oxidative stress plays a key role in ILD pathogenesis. The increase in reactive oxygen species (ROS) in the lung could induce an inflammatory response, which in turn activates the proliferation of lung

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fibroblasts to produce collagen deposition and ultimately leads to pulmonary fibrosis.[3] Since pulmonary fibrosis is irreversible, effective intervention in the early inflammatory stage of ILD is considered an important treatment modality. N-Acetylcysteine (NAC) is a precursor of reduced glutathione (GSH), which can scavenge oxygen free radicals in the body.[4] As a commonly used antioxidant, NAC monotherapy is effective for stopping cognitive decline in forced vital capacity (FVC) in patients with early-stage ILD.[5–8] However, oral administration of NAC exhibits poor bioavailability in lung tissue, even when given in high doses.[9,10] Without high bioavailability, NAC could not increase the antioxidant capacity of the lungs associated with the inability to maintain steady state of GSH levels. Unlike specific binding drugs, NAC nonspecifically binds to oxygen free radicals, which may remove ROS with important signaling effects, resulting in an imbalance in the redox status in the body and limiting the therapeutic effect. Recently, two randomized controlled trials did not observe a significant survival benefit with NAC monotherapy.[11,12]

Molecular hydrogen (H_2), which is formed by two hydrogen atoms tightly connected by covalent bonds, is the lightest and smallest gas molecule. Benefiting from its low density, strong permeability, and fast diffusion speed, H_2 can directly reach the vicinity of the mitochondria responsible for producing ROS in cells and selectively reduce the levels of the most cytotoxic ROS, such as hydroxyl radicals (·OH) and peroxynitrite ($ONOO^-$), without interfering with metabolic redox reactions.[13] Consequently, it has been accepted to treat many diseases as an efficient and harmless antioxidant.[14,15] Several in vivo experiments have validated the protective effects of H_2 in ILD.[16–18] By establishing a mouse model of rheumatoid arthritis (RA)-associated ILD, Yasuhiro demonstrated that H_2 can reduce oxidative stress and the level of lung inflammation and fibrosis in RA-ILD mice.[19] Here, we propose that H_2 may be a more effective treatment for early-stage ILD owing to its special advantages. Thus, we conducted a randomized controlled trial to compare the efficacy and safety of hydrogen therapy and NAC in patients with early-stage ILD.

Materials and Methods

Trial Design

A prospective, single-center, randomized, controlled trial comparing hydrogen therapy and NAC in patients with early-stage ILD. Patients were recruited from January 1, 2019, to December 31, 2021, in the *Department of Dermatology* and *Department of Allergy & Immunology*, Huashan Hospital Affiliated to Fudan University in Shanghai, China. The trial was approved by the local ethics committee and was registered as ChiCTR-ONC-17013055. Informed consent was obtained from all recruited patients in accordance with the Declaration of Helsinki.

Participants Eligibility

Eligible patients met the following criteria: patients aged 30–85 years fulfill the diagnosis of ILD.[1] The diagnosis of ILD requires multidisciplinary cooperation and evaluation by clinicians and radiologists. High-resolution computed tomography (HRCT), which was evaluated by two professors of radiology, revealed ground glass, fine mesh, and honeycombing. Pulmonary function, which can be used as an auxiliary test, is characterized by restrictive ventilatory dysfunction and decreased diffusion. ILD is divided into early-stage ILD and advanced-stage ILD.[2] Those with more than one of the following will be classified as advanced-stage ILD: shortness of breath, pulmonary hypertension, diffusing capacity of lung for carbon monoxide (D_LCO) less than 40% of the expected, oxygen saturation of the six-minute walk test less than 88%, and high-resolution CT showing honeycomb pneumonia.[2] All eligible patients with early-stage ILD belonged to GAP stage I.[20]

Patients were ineligible if they had primary lung diseases, including 1) lung diseases caused by various microbial infections, such as community-acquired pneumonia and tuberculosis; 2) previous history of lung diseases, such as COPD; 3) silicon lung and silicon work contact history; and 4) long-term use of pulmonary toxic drugs.

Intervention

The patients were randomly assigned to receive either hydrogen or NAC therapy in a 1:1 ratio. For the hydrogen therapy group, patients received an oral administration of 350 mL 1.6 ppm hydrogen-rich water (HW, provided by Shanghai

Yiqingquan Health Technology Co., Ltd.) twice a day. In the control group, the patients received oral administration of 600 mg NAC three times a day. The follow-up period was 48 weeks.

Outcomes and Assessment

Change from baseline in HRCT images was the primary efficacy endpoint, including improvement, stability, and worsening, which were defined as reduction, no change, and progression in the extent of fibrosis (honeycombing and reticular opacity), and ground-glass opacity compared with baseline, respectively. The composite physiologic index (CPI) is another primary indicator. The calculation formula of CPI: CPI = 91-(0.65 × D_LCO%predicted) − (0.53 × FVC% predicted) + (0.34 × FEV_1%predicted). The secondary endpoints were the change in pulmonary function from baseline, which included forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC%, total lung capacity (TLC), and D_LCO single-breath method (D_LCO-sb). Any adverse events (AEs) were recorded during the trial period in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation, and two independent samples *t*-test was used to compare continuous variables between groups. Classification data were presented as numbers (percentages) and analyzed by χ^2 test. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using IBM SPSS Statistics (RRID:SCR_019096), and all figures were mapped using GraphPad Prism (RRID:SCR_002798). ITT analysis was performed on all patients who were randomly assigned, and PP analysis was performed on patients who were treated according to the regimen and completed follow-up.

Results

Patient Characteristics

A total of 139 subjects were screened and 87 were randomly assigned to the treatment group (Figure 1). Hydrogen therapy was administered to 44 patients, and NAC therapy was administered to 43 patients. A total of 87 patients were included in the ITT analysis as full analysis set (FAS) population. Furthermore, 75 patients completed the study and were included in the per-protocol set (PPS) analysis (HW group: 37; NAC group: 38). The two groups were well balanced in terms of baseline characteristics including age, sex, smoking, comorbidities, CPI, and pulmonary function parameters (Table 1).

Primary Endpoint

Although no significant difference was observed in the primary endpoint in the FAS population, the rate of improvement in the HW group (63.6%) was higher than that in the NAC group (39.5%), whereas the rate of worsening in each group was similar (Table 2). Notably, in the PPS population, patients who received hydrogen therapy showed a more significant improvement in HRCT images than those who received NAC ($P = 0.008$). More importantly, in both the FAS ($P < 0.05$, Figure 2a) and PPS populations ($P < 0.01$, Figure 2b), a significant reduction in CPI was observed in the HW group compared to that in the NAC group.

Secondary Endpoints

With regard to pulmonary function parameters, changes in FVC (Figure 3), FEV_1 (Figure 4), FEV_1/FVC% (Figure 5), and TLC (Figure 6) from the baseline did not differ significantly between the HW and NAC groups. These pulmonary function parameters were consistent between the FAS and PPS groups. However, a significant improvement in DLCO-sb was observed when hydrogen therapy was compared with NAC in the FAS ($P < 0.01$, Figure 7a) and PPS populations ($P < 0.001$, Figure 7b).

Safety Analysis

As summarized in Table 3, 7 (15.9%) adverse events were reported in the HW group and 10 (23.3%) in the NAC group, with no significant difference ($P = 0.706$). Common AEs in the two groups included cough (4.5% vs 4.7%), upper

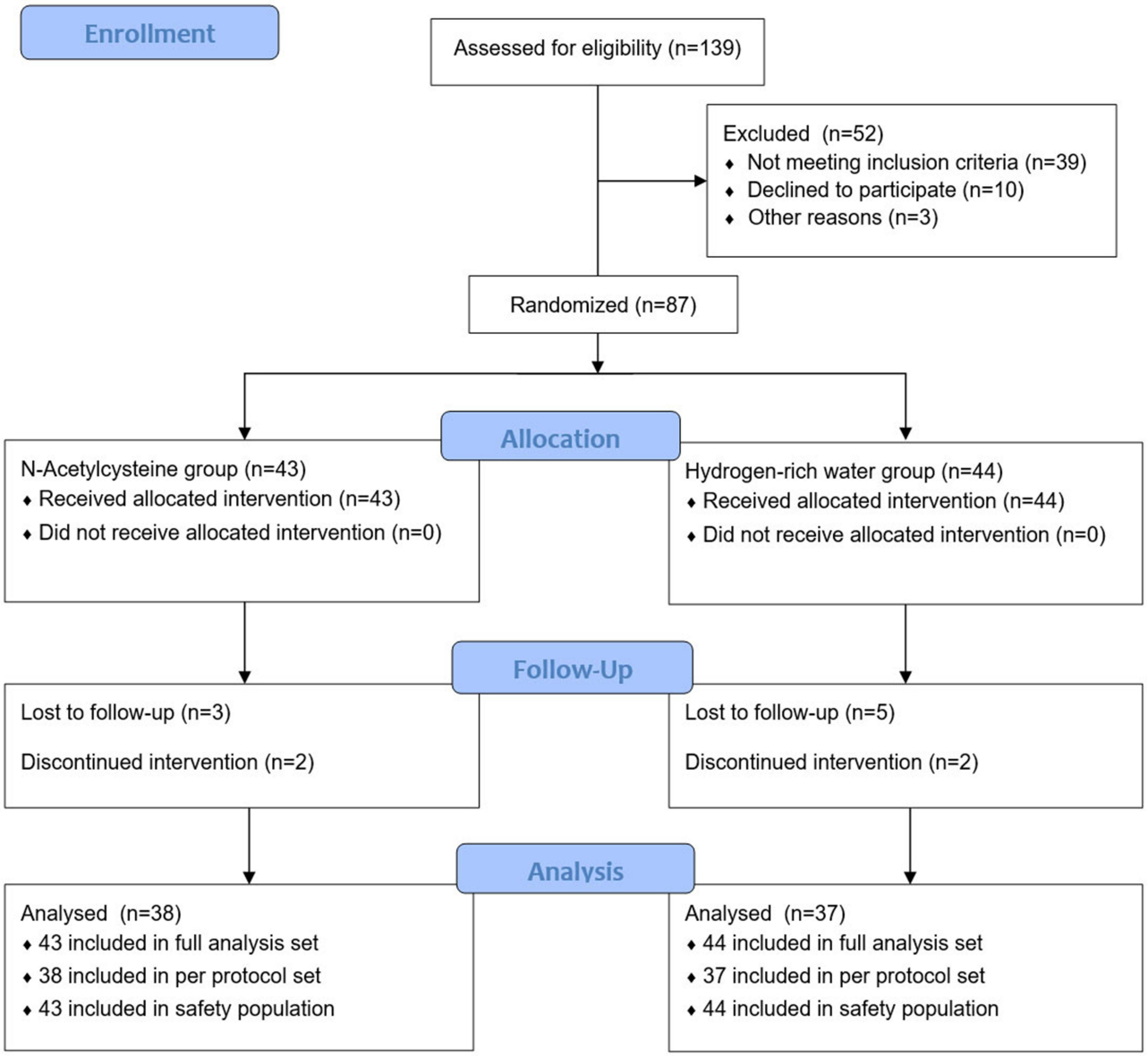

Figure 1 Patient flow diagram.

respiratory tract infection (4.5% vs 7.0%), and diarrhea (6.8% vs 4.7%). Elevated blood pressure, arrhythmias, and deep venous thrombosis were observed in the NAC group. No deaths occurred during the study.

Discussion

An HRCT scan of the chest was performed to determine whether the patient had ILD and to identify the progress of the disease.[21] The primary endpoint was divided into improving, stable, and worsening based on the comparison of HRCT images before and after treatment. Consequently, the improvement in HRCT images from baseline was greater in patients who received HW than in those who received NAC. More exhilaratingly, >60% of patients receiving hydrogen therapy could avoid developing irreversible pulmonary fibrosis. In 2003, Wells et al developed CPI that combines multiple lung function parameters to assess the severity of ILD, and confirmed that it is superior to a single lung function index in assessing the degree of pulmonary fibrosis and predicting survival.[22] Here, our study showed a greater degree of decline in CPI in the HW group. Both HRCT and CPI results may indicate a better prognosis in patients receiving hydrogen therapy.

Table 1 Baseline Variables of Patients in FAS Population

Characteristic	NAC Group (n=43)	HW Group (n=44)	Statistical Value	*P* value
Age-year, mean (SD)	56.0 (12.2)	50.0 (16.2)	−1.956	0.054
Male sex-n (%)	21 (48.8%)	16 (36.4%)	1.384	0.282
Smoking-n (%)	13 (30.2%)	11 (25.0%)	0.298	0.585
Disease-n (%)			7.073	0.215
Pemphigus	27 (62.8%)	21 (47.7%)		
Bullous pemphigoid	7 (16.3%)	7 (15.9%)		
Scleroderma	4 (9.3%)	8 (18.2%)		
Lupus erythematosus	3 (7.0%)	6 (13.6%)		
Vasculitis	0 (0.0%)	2 (4.5%)		
Other connective tissue disease	2 (4.7%)	0 (0.0%)		
CPI, mean (SD)	25.6 (15.6)	30.2 (9.8)	1.580	0.119
FVC % predicted, mean (SD)	86.8 (18.4)	84.9 (14.8)	−0.525	0.601
FEV_1% predicted, mean (SD)	87.4 (20.1)	87.0 (14.9)	−0.114	0.909
FEV_1/ FVC%, mean (SD)	83.9 (14.9)	89.2 (10.2)	1.401	0.168
TLC % predicted, mean (SD)	77.7 (15.1)	82.6 (10.5)	1.547	0.127
D_LCO-sb % predicted, mean (SD)	76.1 (19.0)	70.0 (10.5)	−1.828	0.072

Abbreviations: FAS, full analysis set; NAC, N-Acetylcysteine; HW, hydrogen-rich water; SD, standard deviation; CPI, composite physiologic index; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; D_LCO-sb, diffusing capacity of lung for carbon monoxide single-breath method.

Table 2 Primary Endpoint in FAS and PPS Population

Primary Endpoint	NAC Group	HW Group	Statistical Value	*P* value
FAS population-n (%)			5.619	0.060
Improving	17 (39.5%)	28 (63.6%)		
Stable	22 (51.2%)	12 (27.3%)		
Worsening	4 (9.3%)	4 (9.1%)		
PPS population-n (%)			9.676	0.008
Improving	15 (39.5%)	27 (73.0%)		
Stable	20 (52.6%)	7 (18.9%)		
Worsening	3 (7.9%)	3 (8.1%)		

Abbreviations: FAS, full analysis set; PPS, per protocol set; NAC, N-Acetylcysteine; HW, hydrogen-rich water.

With regard to the secondary endpoints, FVC, FEV_1, FEV_1/FVC%, and TLC were comparable between the two groups during the treatment period. However, regarding D_LCO-sb, our study showed a statistically significant increase in the HW group compared to the NAC group in both FAS and PPS populations. Among ILDs, D_LCO-sb correlates better with the extent of disease on HRCT scans than lung volumes or spirometry.[23–25] More importantly, several investigative groups have identified a baseline decrease in D_LCO-sb to be highly predictive of mortality in ILDs.[26–28] The better improvement in D_LCO-sb after oral administration of HW supported our primary outcomes, indicating the superiority of hydrogen therapy. A previous study in RA-ILD found that drinking H_2-rich water reduces inflammation and fibrosis of the lungs, further supporting the findings of this study[19] Actually, a growing number of studies have shown that hydrogen may protect the lungs from various diseases, including acute lung injury, chronic obstructive pulmonary disease, asthma, lung cancer, pulmonary arterial hypertension, and COVID-19.[29] In conclusion, hydrogen therapy appears to have the potential to be a novel and effective treatment for patients with early-stage ILD.

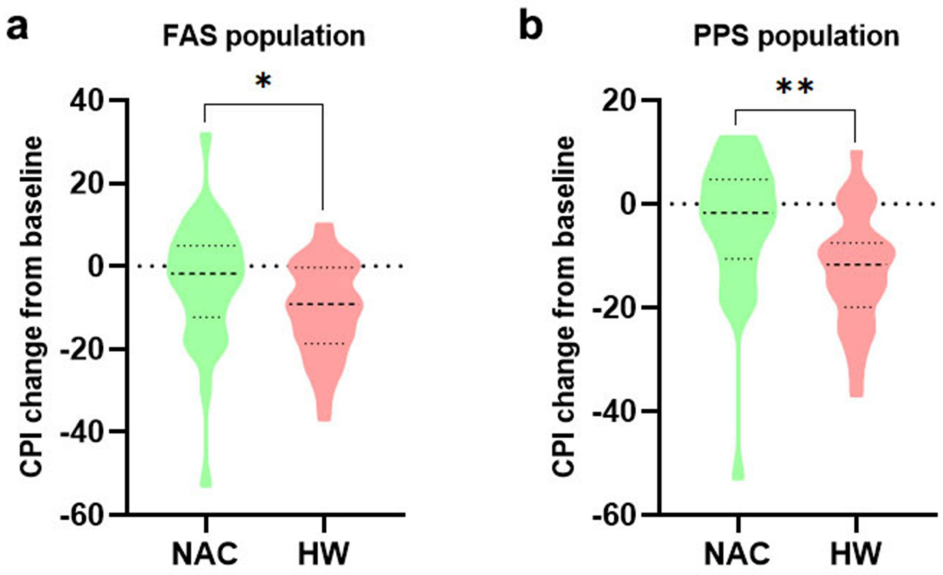

Figure 2 Changes from baseline in CPI in FAS (**a**) and PPS (**b**) population. *CPI* composite physiologic index, *FAS* full analysis set, *PPS* per-protocol set, **P<0.05*, ***P<0.01*.

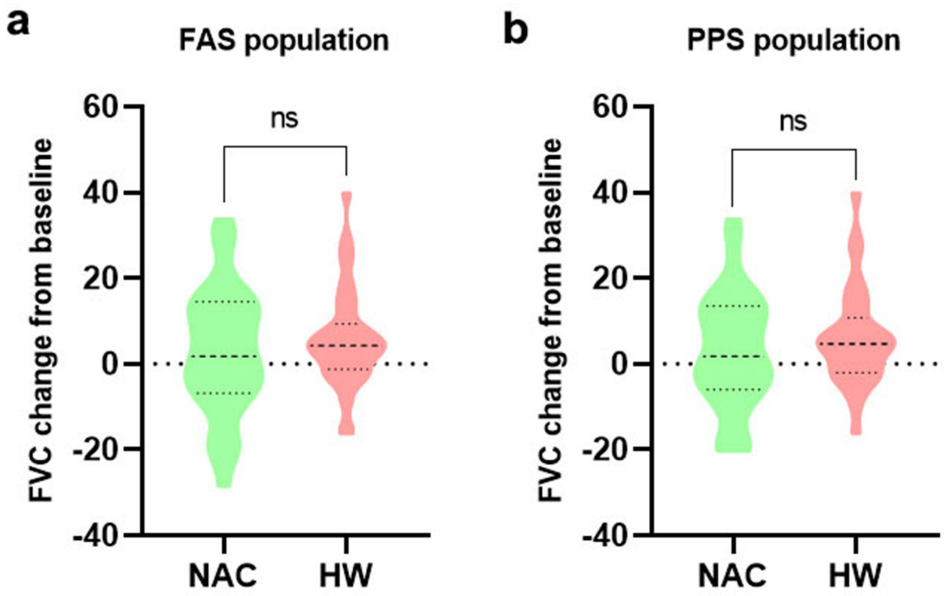

Figure 3 Changes from baseline in FVC in FAS (**a**) and PPS (**b**) population. *FVC* forced vital capacity, *FAS* full analysis set, *PPS* per-protocol set, *ns* no significance.

Inhalation of hydrogen gas is a typical method for treating pulmonary diseases. This often requires a hydrogen manufacturing machine and inhaler, which is inconvenient and expensive. In addition, there is a risk of an explosion that limits the hydrogen concentration in the device.[14] Compared with inhalation, the oral intake of hydrogen-rich water is cheaper, safer, and more portable. A study investigating the pharmacokinetics of H_2 in pigs proved that drinking a highly concentrated H_2-rich solution within a short time is also an effective way to supply H_2 directly to the lung.[30]

The pathology of ILD includes chronic lung inflammation in the early stage and irreversible alveolar fibrosis in the late stage.[1] ROS in the inflammatory stage of ILD can enhance transforming growth factor-β1 (TGF-β1) production and modulate extracellular matrix (ECM) deposition, both of which induce epithelial–mesenchymal transition (EMT).[31–34] EMT can activate myofibroblasts, which play a central role in the pathogenesis of pulmonary fibrosis.[35] H_2 effectively

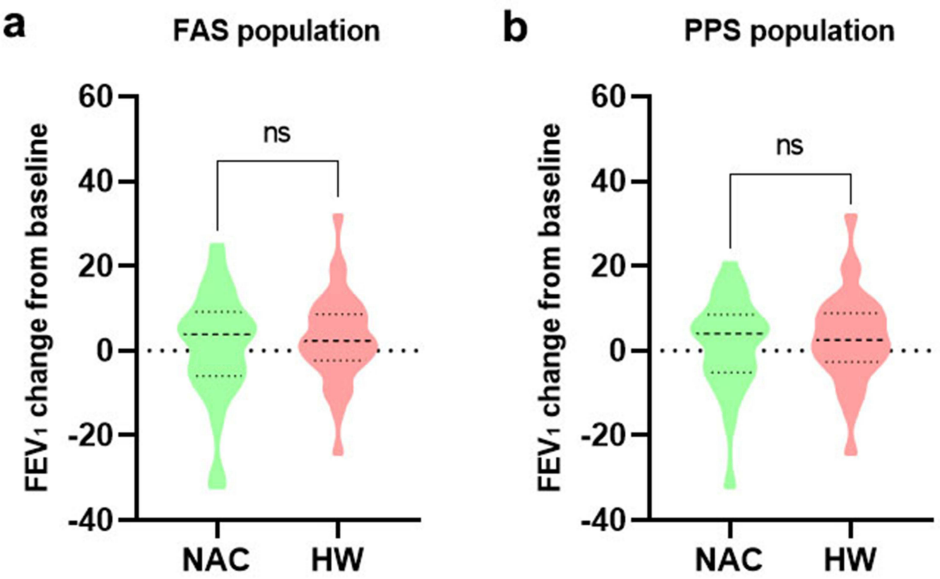

Figure 4 Changes from baseline in FEV_1 in FAS (**a**) and PPS (**b**) population. *FEV_1* forced expiratory volume in one second, *FAS* full analysis set, *PPS* per-protocol set, *ns* no significance.

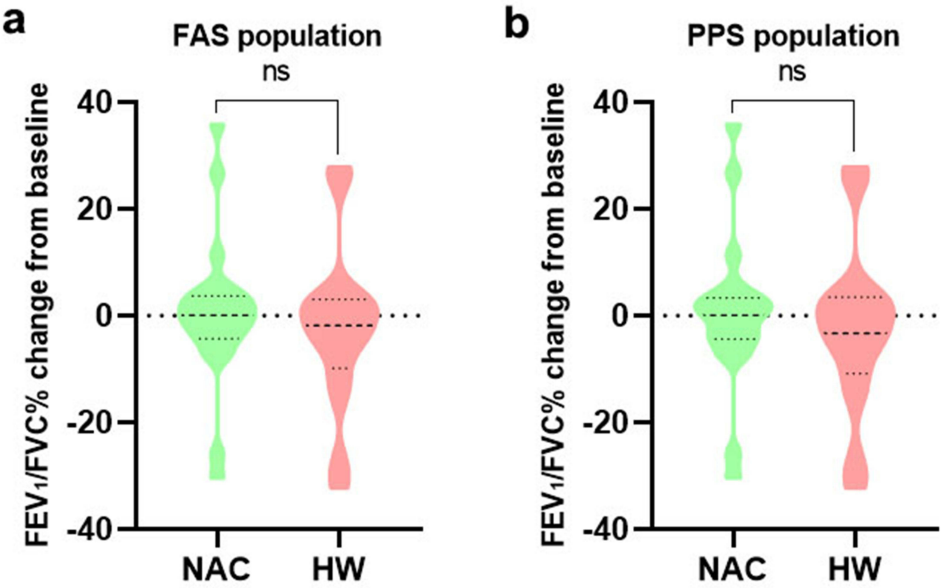

Figure 5 Changes from baseline in FEV_1/FVC% in FAS (**a**) and PPS (**b**) population. *FAS* full analysis set, *PPS* per-protocol set, *ns* no significance.

suppresses oxidative stress by inhibiting TGF-β1, increasing E-cadherin (an epithelial marker), and decreasing vimentin (a mesenchymal marker) in lung tissues, thereby blocking alveolar EMT and fibrosis.[16,17]

Safety is a critical requirement in the development of new treatments. In this trial, approximately 20% of the patients experienced treatment-related AEs. All AEs were mild or moderate in severity and have been reported in other publications.[8,14] Overall, both hydrogen and NAC therapy have acceptable safety and tolerability. Additionally, fewer AEs occurred in the hydrogen group, indicating that hydrogen therapy has a more favorable safety profile than NAC. We speculate that hydrogen therapy may be an alternative to NAC in treating ILD in early-stage ILD.

Despite the significant findings of this study, it has several limitations. First, it lacks blindness owing to the differences in pharmaceutical formulations. Second, all patients in this trial were diagnosed with early-stage ILD after

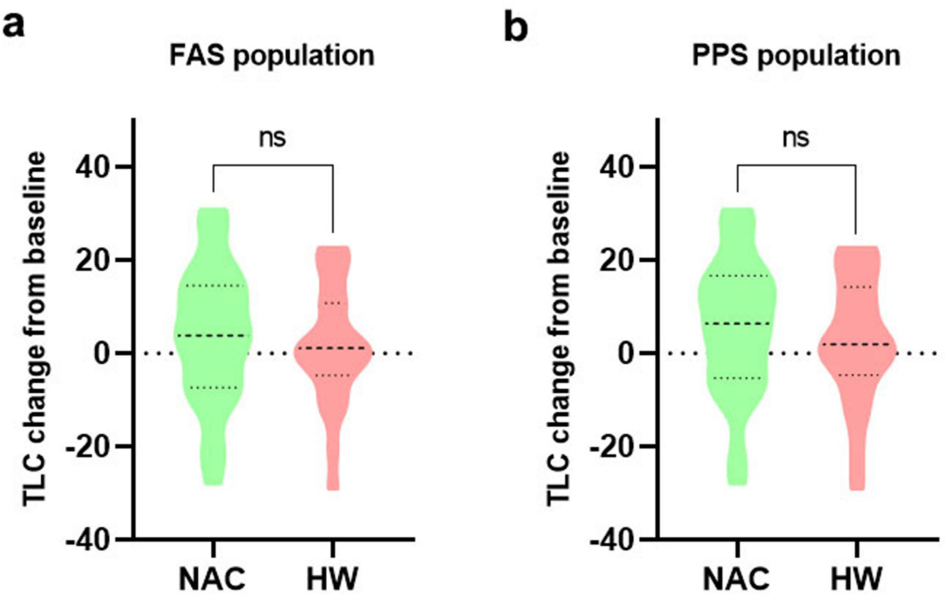

Figure 6 Changes from baseline in TLC in FAS (**a**) and PPS (**b**) population. *TLC* total lung capacity, *FAS* full analysis set, *PPS* per-protocol set, *ns* no significance.

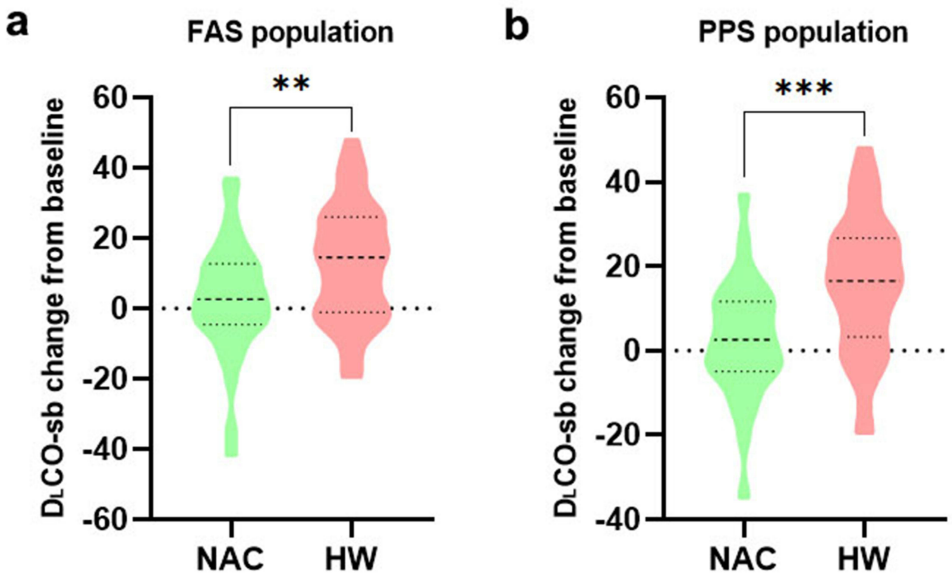

Figure 7 Changes from baseline in D_LCO-sb in FAS (**a**) and PPS (**b**) population. *D_LCO-sb* D_LCO single-breath method, *FAS* full analysis set, *PPS* per-protocol set, ***P<0.01*, ****P<0.001*.

HRCT and pulmonary function tests due to autoimmune skin diseases. Therefore, differences in etiology may need to be considered when hydrogen therapy is administered to patients with unknown causes of ILD. Finally, the relatively small sample size of this trial may have decreased the power of the conclusions. A larger-scale clinical trial is needed to validate this conclusion based on the findings of this study.

Conclusion

For the first time, we verified the efficacy and safety of hydrogen molecules in the treatment of early-stage ILD in a randomized controlled clinical trial. This provides a new perspective for the treatment of ILD, as an effective block in

Table 3 Summary of Adverse Events

Events-n (%)	NAC Group (n=43)	HW Group (n=44)
Any adverse events	10 (23.3%)	7 (15.9%)
Cough	2 (4.7%)	2 (4.5%)
Upper respiratory infection	3 (7.0%)	2 (4.5%)
Diarrhea	2 (4.7%)	3 (6.8%)
Elevated blood pressure	1 (2.3%)	0 (0.0%)
Arrhythmias	1 (2.3%)	0 (0.0%)
Deep venous thrombosis	1 (2.3%)	0 (0.0%)

Abbreviations: N-Acetylcysteine; HW, hydrogen-rich water.

the early inflammatory stage, where hydrogen molecules can prevent irreversible pulmonary fibrosis and improve the prognosis.

Abbreviations

ILD, interstitial lung disease; ROS, reactive oxygen species; NAC, N-acetylcysteine; GSH, glutathione; FVC, forced vital capacity; H_2, molecular hydrogen; OH, hydroxyl radicals; $ONOO^-$, peroxynitrite; RA, rheumatoid arthritis; D_LCO, diffusing capacity of the lung for carbon monoxide; HW, hydrogen-rich water; HRCT, high-resolution computed tomography; CPI, composite physiological index; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; D_LCO-sb, D_LCO single-breath method; AEs, adverse events; PP, Per protocol; ITT, intention-to-treat; FAS, full analysis set; PPS, per protocol set; TGF-β1, transforming growth factor-β1; ECM, modulate extracellular matrix; EMT, epithelial–mesenchymal transition.

Data Sharing Statement

Data in this study can be made available upon request to the corresponding author.

Ethics Approval and Informed Consent

The trial was approved by the Ethics Committee of Huashan Hospital affiliated with Fudan University and registered as ChiCTR-ONC-17013055. Informed consent was obtained from all the recruited patients in accordance with the Declaration of Helsinki (revised in 2013).

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Disclosure

The authors report no conflicts of interest in this work.

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