Hydrogen Gas Inhalation Treatment in Acute Cerebral Infarction: A Randomized Controlled Clinical Study on Safety and Neuroprotection

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Background: Molecular hydrogen (H_2) acts as a therapeutic antioxidant. Inhalation of H_2 gas (1-4%) was effective for the improvement of cerebral infarction in multiple animal experiments. Thus, for actual applications, a randomized controlled clinical study is desired to evaluate the effects of inhalation of H_2 gas. Here, we evaluate the H_2 treatment on acute cerebral infarction. *Methods:* Through this randomized controlled clinical study, we assessed the safety and effectiveness of H_2 treatment in patients with cerebral infarction in an acute stage with mild- to moderate-severity National Institute of Health Stroke Scale (NIHSS) scores (NIHSS = 2-6). We enrolled 50 patients (25 each in the H_2 group and the control group) with a therapeutic time window of 6 to 24 hours. The H_2 group inhaled 3% H_2 gas (1 hour twice a day), and the control group received conventional intravenous medications for the initial 7 days. The evaluations included daily vital signs, NIHSS scores, physical therapy indices, weekly blood chemistry, and brain magnetic resonance imaging (MRI) scans over the 2-week study period. *Results:* The H_2 group showed no significant adverse effects with improvements in oxygen saturation. The following significant effects were found: the relative signal intensity of MRI, which indicated the severity of the infarction site, NIHSS scores for clinically quantifying stroke severity, and physical therapy evaluation, as judged by the Barthel Index. *Conclusions:* H_2 treatment was safe and effective in patients with acute cerebral infarction. These results suggested a potential for widespread and

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Conflict of interest: S. Ohta is a patentee on a medical use of hydrogen gas. He did not contribute to the registration of the patients, data collection, and interpretation. The other authors declare no conflicts of interest.

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general application of H_2 gas. **Key Words:** Hydrogen gas—acute cerebral infarction—randomized controlled clinical study—neuroprotection—National Institute of Health Stroke Scale—MRI—Barthel Index.

Introduction

Molecular hydrogen (H_2) acts as a therapeutic antioxidant, and inhalation of H_2 gas (1-4%) was markedly effective for the improvement of cerebral infarction in a rat model.[1] Moreover, H_2 inhalation during normoxic resuscitation was shown to improve neurological outcomes in a rat model of cardiac arrest.[2] In addition to extensive animal experiments, more than 20 clinical studies examining the efficacy of H_2 have been reported, including double-blinded pilot clinical studies.[3,4] Moreover, the safety check of H_2 inhalation was performed in patients with acute cerebral ischemia or post-cardiac arrest.[5,6] Thus, a randomized controlled clinical study is desired to assess the overall benefit of this treatment.

Here, we performed a randomized controlled clinical study investigating the safety and effectiveness of H_2 treatment in patients with acute cerebral infarction and mild- to moderate-severity National Institute of Health Stroke Scale (NIHSS) scores (NIHSS = 2-6). In this study, we aimed to assess the clinical values of H_2 inhalation by more extensive safety checks and a more objective evaluation of the clinical conditions. Our aim also includes accelerating possible evolvement of the H_2 therapy to a novel tool for the actual treatment.

Materials and Methods

Study Design and Participants

For this randomized controlled study, we enrolled 50 acute cerebral infarction patients, consisting of 25 study patients (H_2 group) and 25 control patients (control group). Their ID numbers were used for randomization. This study was not performed in a completely blinded manner because a placebo machine was not available; however, data collection and analysis were performed in a blinded manner by not disclosing the group name of each patient to nursing staff and other personnel, such as radiologists and physical therapists.

The criteria for inclusion were as follows: (1) therapeutic time window of 6 to 24 hours; (2) neurological deficits (NIHSS scores) of 2-6; and (3) small- to medium-sized magnetic resonance imaging (MRI) lesion (.5-3.0 cm in greatest diameter in any of the diffusion-weighted image slices within the territory of single major cerebral artery perfusion (Fig 1)). Ischemic stroke subtypes were classified according to the classification of Trial of Org 10172 in Acute Stroke Treatment.[7]

Obvious occlusion of major arteries and multiple lesions scattered in multiple cerebral arterial territories were excluded. Other exclusion criteria were as follows: severe uncontrolled diabetes, liver and kidney dysfunction, severe heart disease, particularly with atrial fibrillation, severe lung disease with pneumonia, and asthma and pleural effusion (Fig 1). Patients with any evidence of acute hemorrhage in the brain tissue and patients who received plasminogen activator treatment or who were taking medication for anticoagulation were also excluded.

Treatments

All patients received daily physical therapy and evaluation, starting on the second day (Day 2) after admission.

The H_2 treatment group inhaled 3% H_2 gas for 1 hour twice a day for 7 days through a regular non-rebreathing facial mask. The H_2 gas was provided by a homemade Nishijima hydrogen generator (Numazu, Shizuoka, Japan). The concentration was examined with a gas chromatography. To confirm sufficient inhalation, immediately before the end of the H_2 gas inhalation on Day 2, an additional venous blood sample was withdrawn for the gas chromatography measurement of blood H_2 levels as described previously.[5] In the H_2 group, the physicians regarded H_2 as neuroprotective and antioxidant agent and avoided giving edaravone. They used ozagrel[8] as a grade B medication, starting on the second day.

The control group received no H_2 inhalation, but otherwise, there was no restriction on any other agents including grade B medications. Thus, the attending physicians naturally selected, as in the guidelines, evidence B medications such as edaravone (30 mg intravenously every 12 hours for 14 days), a scavenger of free radicals,[9] for neuroprotection with antioxidant effects, ozagrel (80 mg intravenously every 12 hours for 14 days) as an antiplatelet agent, and argatroban (60 mg intravenously for the first day, then 10 mg every 12 hours for 4 days)[10] as an anticoagulant. In the control group, all patients received edaravone, 76% received ozagrel, and 24% received argatroban concomitantly.

Evaluation

Vital signs such as blood pressure, pulse rate, body temperature, daily amount of food intake, and oxygen saturation by pulse oximeter were checked 3 times a day and more frequently in case of any abnormality. NIHSS scores were blindly recorded every day for 2 weeks. In

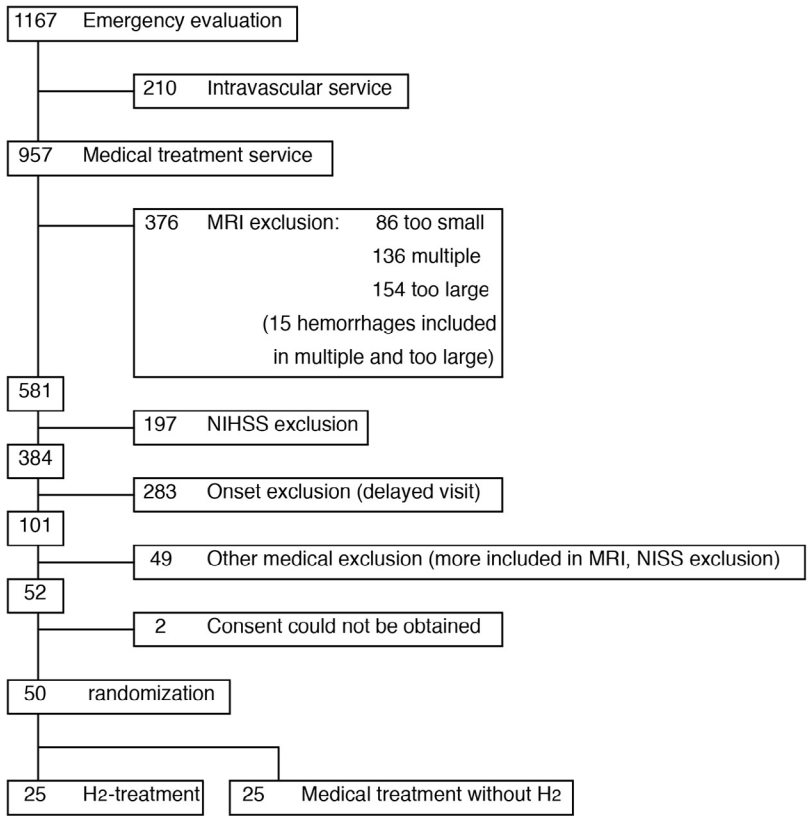

Figure 1. *Trial profile. Fifty patients were enrolled according to the Materials and Methods and randomized.*

the physical therapy department as a part of regular evaluations, scores for the Barthel Index (BI),[11] Brunnstrom Stage (BRS),[12] modified Rankin Score (mRS),[13] and Functional Independence Measure (FIM)[14] were recorded in a blinded manner.

Blood Test

Patients' venous blood samples were withdrawn on Days 1, 7, and 14 for regular blood tests including the liver, kidney, pancreas, cardiac enzymes, and electrolytes, in addition to peripheral blood counts, in a blinded manner. An electrocardiogram was ordered on admission and later as needed.

MRI Evaluation

MRI scans of the brain were repeated on Days 3, 5, 7, 10, and 14 after Day 1 (day of admission). The infarction site was determined as the hyperintensity area in the diffusion weighted image. The abnormality was evaluated with the size (volume) and severity (MRI signal intensity). The size (=A) was obtained by manually surrounding the infarct core using the region of interest (ROI) software of the Digital Imaging and Communication in Medicine (DICOM) (Rosslyn, VA, USA) and by automatic counting. The severity (B and C) was obtained as the average of the MRI signal intensity of pixels in the infarct core ROI (=B) and the contralateral normal brain ROI (=C) of exactly the same size and mirror-image location, also automatically by the DICOM software. The signal intensity ratio between the infarct and the normal brain was calculated as B/C.

Diffusion weighted images in the serial MRI scans were compared using the relative MRI signal intensity (=RSI).[15] The RSI used in this study was a product (A × B/C) of the size of the infarct (A) and the signal intensity ratio (B/C).

The calculation was repeated for all of the slices where the infarction extended with continuity. In case of multiple infarcts, the calculation was obtained in the exact same fashion for all of the infarct sites. The data obtained were reproducible and reliable, as has been reported previously.[16]

Approval of This Study

We received informed consent written by a family member for all patients. The protocol of this clinical study was approved by the Nishijima Hospital Ethics Committee and was pre-registered as follows: *Clinical Trial Registration*–JMACCT ID:JMA-IIA00142 URL: https://dbcentre3.jmacct.med.or.jp/jmactr/default.aspx?JMACCTID=JMA-IIA00142

Statistical Analysis

All statistical analyses were performed with EZR version 1.29 (Saitama Medical Center, Jichi Medical University,

Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). Normality was assessed using the Kolmogorov–Smirnov test, and the statistical significance of differences in sequential data was evaluated with analysis of variance (ANOVA) and Mauchly tests for sphericity and Greenhouse–Geisser and Huynh–Feld corrections. For nonparametric data, the Mann–Whitney U test was used. Throughout this study, we did not consider any adjustment factors for any analyses.

The statistical methods were consulted with and approved by a specialist of Saitama Medical Center, Jichi Medical University, Saitama, Japan.

Results

Patients

Patients were enrolled between October 1, 2014, and January 28, 2016; the study end date (at which the last patient completed the randomized study) was February 28, 2016. Figure 1 shows the trial profile for this randomized study.

The average age of the patients was 76.0 in the H_2 group and 73.3 in the control group. Twelve of the H_2 group and 9 of the control group were above 80 years old. These differences were not statistically significant (Student's *t*-test, $P = .44$). Other baseline characteristics were also similar in the two groups with no significant differences (Table 1).

Table 1. *Baseline characteristics*

	H_2 group (n = 25)	Control (n = 25)
Average age (years old)	76.0	73.3
Sex (male)	12	7
NIHSS (Day 1)	3.28 (2-5)	3.36 (2-5)
MRI RSI (Day 1)	241 (28-855)	260 (18-1875)
Ischemic stroke subtype		
Large-artery atherosclerosis	0	0
Cardioembolism	0	0
Small-vessel occlusion (lacune)	18	19
Other determined etiology	7	6
History		
Ischemic stroke	4	4
Intracranial hemorrhage	2	1
Hypertension	13	14
Myocardial infarction	3	1
Current		
Statin	7	6
Antihypertensive	12	12
Anticoagulant	0	0
Antidiabetic	3	4
Systolic blood pressure, average (range) mmHg	143 (94-208)	147 (96-186)
HgA1c average (range)	5.93 (4.6-10.1)	6.06 (5.1-10.3)
Total cholesterol	194 (91-278)	208 (146-276)
Referred from outside	5	8

Abbreviations: MRI, magnetic resonance imaging; NIHSS, National Institute of Health Stroke Scale; RSI, relative MRI signal intensity.

Vital Signs and Laboratory Tests

Vital signs, checked daily for 14 days, were not significantly different between the H_2 and control groups except the level of oxygen saturation, which showed significant improvement in the H_2 group as compared with the control group ($P = .03$) (Fig 2). Moreover, there was no statistical difference between the two groups on the results of blood tests performed on Days 1, 7, and 14 (Fig 3). These values mean the safety of H_2 in patients with acute cerebral infarction.

Average blood concentration at the end of H_2-gas (3%) inhalation after 1 hour was 24.5 μM. Because the saturated level of H_2 is at ~800 μM at atmospheric pressure, ~ 24 μM (800 μM × .03 = 24 μM) is reasonable for sufficient inhalation.

MRI Improvements

No MRI signs of intracerebral hemorrhage were seen in the infarction sites and other areas in both groups. Regarding the severity of the infarction site, the calculation was done with the RSI. The initial values on Day 1 varied, ranging from 28 to 855 (average 241) in the H_2 group, and from 18 to 1,895 (average 272) in the control group (Fig 4, A). Sequential changes in the RSIs were significant between the H_2 and control groups on Day 7 and Day 14 ($P = .025$ and .028, respectively). Moreover, after a log transformation, the total sequential change between the two groups was statistically significant ($P = .002$) (Fig 4, B) and indicated more improvement in the H_2 group.

The late hike in the H_2 group (Day 10 of the RSI) was small (152% of Day 1), and the RSI on Day 14 decreased (121% of Day 1) to almost a normal range, whereas the RSI on Day 14 of the control group was still very high at 219% of the RSI on Day 1, indicating that the severity of pathological changes at the infarction site was much less and more quickly near-normalized in the H_2 group compared with the control group.

Neurological Improvement

Neurological status, represented by the NIHSS scores, showed improvement in both groups with time, and the improvement was more marked in the H_2 group (Fig 5).

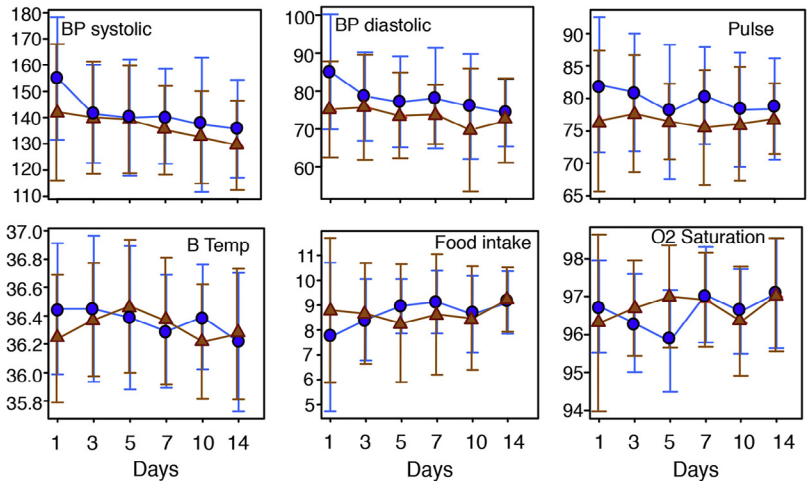

Figure 2. *Vital signs.*
Vital signs (blood pressure (BP systolic, BP diastolic), pulse rate (Pulse), body temperature (B Temp), daily amount of food intake (Food intake), and oxygen saturation (O_2 Saturation) were not significantly different between the 2 groups, except the oxygen saturation which showed improvement in the H_2 group as compared with the data of the control group, and the difference was statistically significant (P = .03). Closed blue circles and dark-red triangles indicate the mean values with standard deviations of the H_2 and control groups, respectively. The P values between H_2 and control groups were obtained by analysis of variance as P = .45 (BP systolic), .40 (BP diastolic), .56 (Pulse), .31 (B Temp), .28 (Food intake) and .03(O_2 Saturation), respectively. (Color version of figure is available online.)

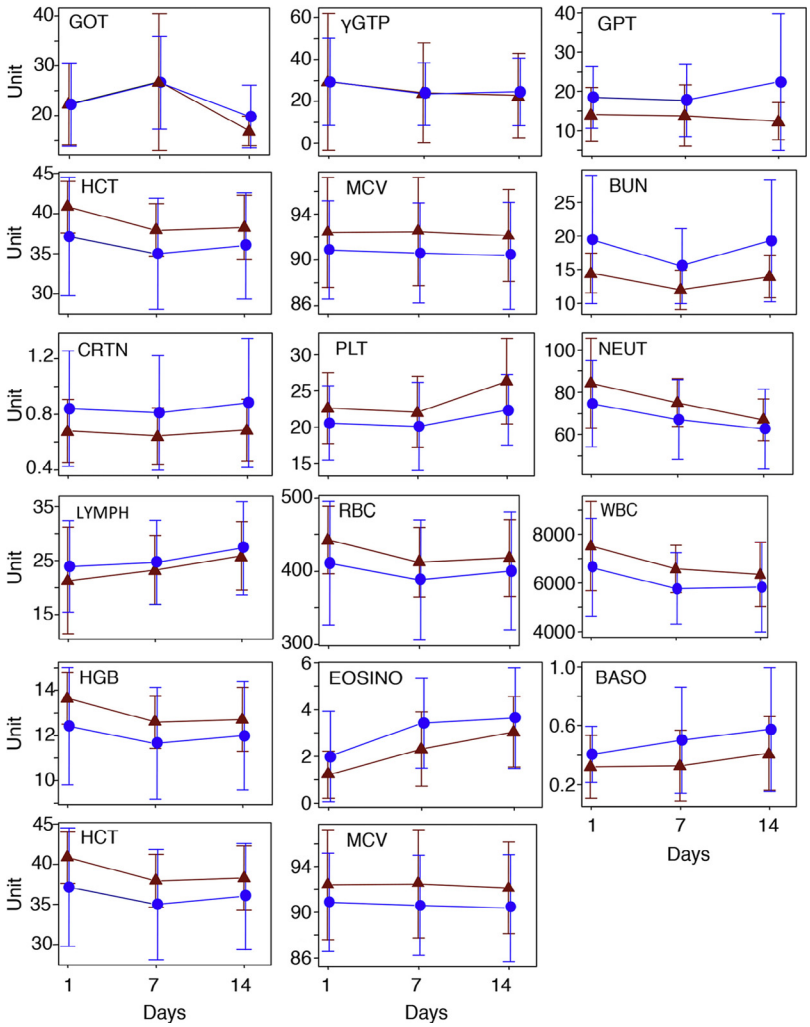

Figure 3. *Laboratory blood tests.*
Patients' venous blood samples were withdrawn on Days 1, 7, and 14 for regular blood tests, including the examination of the liver, kidney, pancreas, cardiac enzymes, and electrolytes, in addition to peripheral blood counts. Abbreviations: ALB, albumin; BASO, basophilic white blood cell; BUN, blood urea nitrogen; CRP, c-reactive protein; CRTN, creatinine; EOSINO, eosinophilic; HCT, hematocrit; HGB, hemoglobin; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γGTP, γ-glutamyl transpeptidase; Lymph, lymphocyte; MCV, quotient of the mean corpuscular volume; NEUT, neutrophil; PLT, platelet; RBC, red blood cell; WBC, white blood cell. Closed blue circles and dark-red triangles indicate the mean values with standard deviations of the H_2 and control groups, respectively. The P values between the H_2 and control groups were obtained by analysis of variance as P = .62 (GOT), .57 (γGTP), .22 (GPT), .15(ALB), .69 (CRP), .67 (BUN), .64 (CRTN), .28 (PLT), .45 (NEUT), .87 (LYMPH), .49 (RBC), .76 (WBC), .30 (HGB), .57 (EOSINO), .53 (BASO), .39 (HCT), and .78 (MCV), respectively. No significant differences were noted between the two groups. These values indicated the safety of H_2 even in elderly patients with cerebral infarction. (Color version of figure is available online.)

From Day 3 to 5 after beginning the admission, the average NIHSS score increased slightly (symptoms got worse) in the control group, whereas no significant increase in the scores after admission was seen in the H_2 group.

Then, the scores began to improve in both groups, and there was a significant difference between the two groups. In the H_2 group, the improvement was more marked on each evaluation day, with all days being statistically

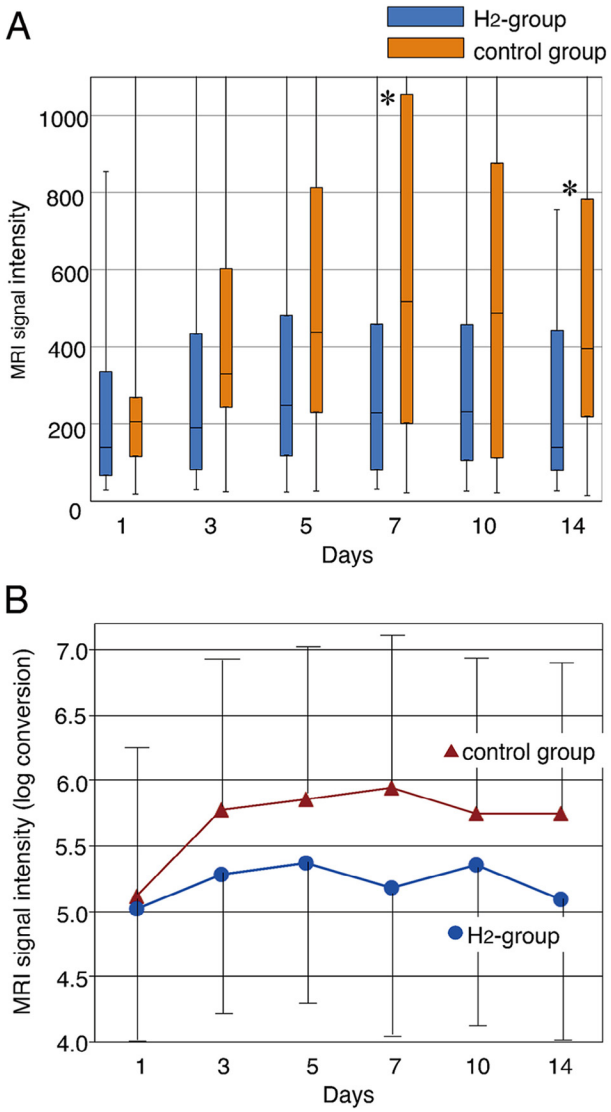

Figure 4. *Changes in magnetic resonance imaging (MRI) signal intensity at cerebral infarction sites.*
*(A) Relative MRI signal intensity (RSI) data on each date are illustrated. Blue and orange boxes indicate the H_2 and control groups, respectively. Bars in the boxes are 25% maximum, median, and 75% minimum values from the top. Vertical bars indicate the standard deviations. * indicates P < .05 between two groups by Student's t-test (P = .025 and .028 on Day 7 and Day 14, respectively).*
(B) The data are transformed on a logarithmic scale; blue and orange dots indicate the H_2 group and the control group, respectively. The data were evaluated statistically as total changes according to analysis of variance with Greenhouse–Geisser and Huynh–Feldt correction, (P = .002).

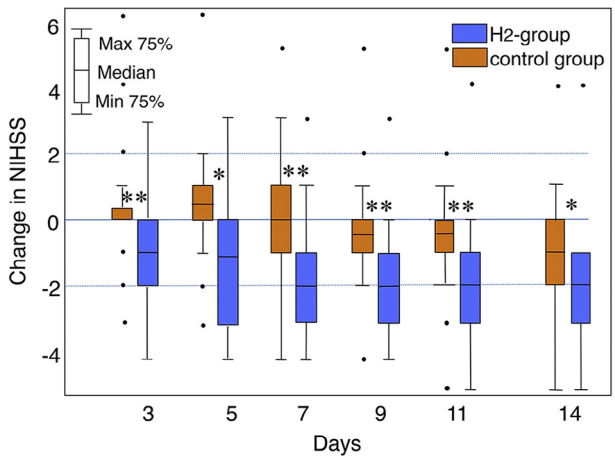

Figure 5. *Changes in neurological improvement.*
*National Institute of Health Stroke Scale (NIHSS) scores were obtained on Days as indicated. Changes in NIHSS scores are illustrated, where blue and orange boxes indicate the H_2 group and the control group, respectively. Horizontal bars in the boxes indicate 25% maximum, median, and 75% minimum values from the top. Vertical bars indicate the standard deviations. Outliers are displayed as closed circles. * and ** indicate P < .01, and P < .001, respectively, according to the Mann–Whitney U test.*

significant ($P < .01$ on Days 5 and 14 and $P < .001$ on Days 3, 7, 9, and 11).

Physical Therapy Improvement

Activities of daily living (ADL) capability of the patients was evaluated by physical therapy data using the BI,[11] BRS,[12] mRS,[13] and FIM[14] for the first 2 weeks (Fig 6). Because 9 patients (3 in the H_2 group and 6 in the control group) could not go to the physical therapy department every day, the data from these patients were excluded from the final assessment.

No complications were noted during physical therapy in either group. The scores in all of the indexes gradually improved in both groups and showed a trend to show more improvement in the H_2 group for the mRS, BRS, and FIM. The difference in BI between the two groups was statistically significant (Fig 6, upper right) ($P < .05$).

Study Limitations

This study has several limitations. The number of patients enrolled for this study is 50. More patients should be tested to obtain a more definitive conclusion. ADL capability of the patients was evaluated from physical therapy data for 2 weeks only. A longer-term evaluation will be required. However, it seems clear that H_2 treatment achieved a qualification as a "first, do no harm" therapy because there were no risks involved with its use.

Discussion

Numerous studies have strongly suggested that H_2 has the potential for therapeutic and preventive applications. There are several methods to ingest or consume H_2: inhaling H_2 gas, drinking H_2 dissolved in water (H_2 water), injecting H_2 dissolved in saline (H_2 saline), taking an H_2 bath, or dropping H_2 saline onto the eyes.[3,4] Because inhaled H_2 gas acts rapidly, it could be suitable for defense against acute oxidative stress in an emergency case by a rapid increase in the H_2 level.[1] In addition, in many acute clinical situations, an excess of fluid is prohibited

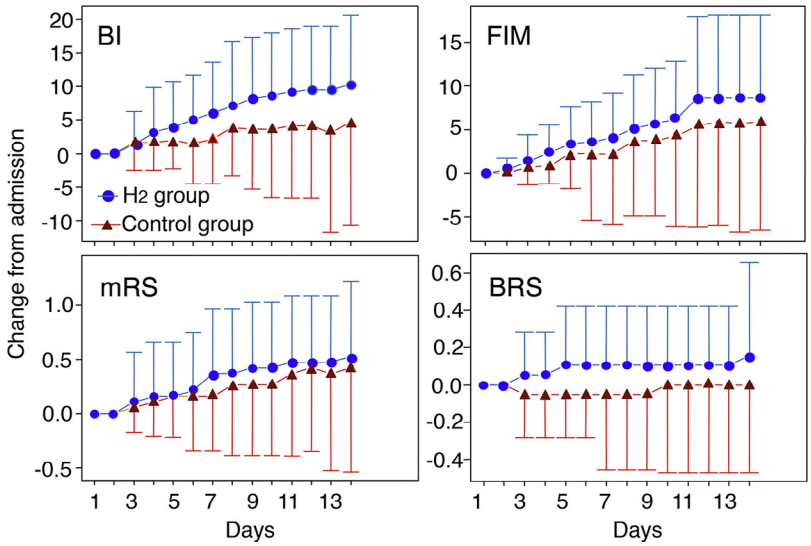

Figure 6. *Changes in the rehabilitation index. As changes of physical therapy indexes, BI, BRS, mRS, and FIM scores were obtained. Blue and red dots indicate the mean values of the H_2 group and the control group, respectively, with vertical lines indicating the standard deviation. Only for the BI evaluation was the difference in improvement between the two groups significant by modified analysis of variance with Mauchly tests for sphericity ($P < .05$). Abbreviations: BI, Barthel Index; BRS, Brunnstrom Stage; FIM, Functional Independence Measure; mRS, modified Rankin Score. (Color version of figure is available online.)*

and oral administration is actually impossible. Thus, inhalation may be the safest way for the H_2 treatment.

A majority of the present patients were above the age of 75 years, including nonagenarians. The treatment with H_2-gas inhalation exhibited no observable adverse effects and no complications, and improved the level in oxygen saturation. These findings indicate the potential for widespread and general use of H_2 even for the aged patients. In this study, because the patients were successfully divided into 2 groups randomly, the baseline characteristics of each group were similar with no significant difference, as described in Table 1. Thus, we did not consider any adjustment factors for any analyses.

In an infarcted brain tissue, various pathological processes occur, such as energy failure, loss of membrane integrity, inflammation, excitotoxicity, oxidative stress, necrosis, apoptosis, and tissue edema from a disrupted blood–brain barrier, and finally the brain tissue becomes irreversibly damaged.[17] In addition to the role of H_2 as an antioxidant, H_2 regulates various signal transduction pathways and the expression of many genes, including genes involved in inflammation. The molecular mechanisms by which H_2 at low concentrations exerts multiple effects on signal transduction are poorly understood. A recent study suggested that increased oxidative stress triggers free-radical chain reactions and subsequently produces mediators derived from phospholipids that could contribute to modifying signal transduction and gene expression.[18]

Both H_2 and edaravone have the ability to scavenge hydroxyl radicals[19]; however, a previous report indicated that H_2 was more effective than edaravone in cerebral infarction in a rat model.[1] Our physicians elected to give edaravone to all of the control patients in addition to argatroban (24% of the control group) and ozagrel (76% of the control group and 100% of the H_2 group) concomitantly. Because the efficacies of argatroban and ozagrel seem to be similar, the apparent advantage of H_2 could not be influenced by these medications.[20] Thus, this study indicates that the effects of the inhalation of H_2 were more beneficial than those of the administration of edaravone.

The present MRI findings suggested that the pathological change occurring in the brain infarction site was milder and recovered more quickly in the H_2 group compared with the control group. On Day 14, the RSI reached an almost normal level with minimal late hike on Day 10, which is usually caused by vasogenic edema with extravasation of water molecules from the blood–brain barrier breakdown due to inflammatory mediators. In the control group, both the maximal and the minimal RSIs were significantly worse than in the H_2 group. These findings may suggest that the recovery of the brain infarction site had already started to occur by Day 5 in the H_2 group, because of minimal vasogenic edema, whereas the trend was not apparent in the control group without having the late hike even on Day 14. These MRI scan data suggest that H_2 worked in the area of the penumbra in addition to the core pathology of the cerebral infarction. Thus, it is possible that H_2 treatment works more efficiently if the treatment is started earlier, even before or during intravascular treatment.

As an evaluation of the effect by H_2 inhalation, the NIHSS score in the H_2 group was significantly better than that in the control group. In particular, on Day 3, a significant improvement in the H_2 group was observed, whereas at the earlier time point, the NIHSS score became worse in the control group.

ADL capability of the patients was evaluated from physical therapy data using the BI, BRS, mRS, and FIM indexes. The BI score in the H_2 group exhibited more significant improvement than that in the control group, whereas the better improvement in the BRS, mRS, and FIM indexes showed only trends in the H_2 group. The BRS evaluates the muscle function of extremities and thus may not be

directly related to the level of ADL. The FIM is probably the most detailed evaluation method.[14] However, the mixture of the evaluation in the motor and cognition parts may dilute the significance of the severity of somatic impairments in our study. The mRS has been adopted for the more chronic and long-term evaluation studies; however, in this study, we evaluated these indexes only for 2 weeks. Because many comparative studies of rehabilitation effects studies require at least months or more before exhibiting significant differences between H_2 and control groups, a significant difference may not be seen by a short-term examination.

The BI is oriented more toward somatic dysfunction or physical function disorders and evaluates ADL-related activities such as eating, dressing, sphincter control, tub or shower transfer, and others. In this sense, the H_2 treatment assessed by the BI score for 2 weeks appears to be a marked achievement.

Conclusions

The inhalation of H_2 gas was safe and effective in patients with acute cerebral infarction. Thus, H_2-gas therapy has a potential for actual application in acute cerebral infarction as a novel and safe therapeutic treatment.

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