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Research

Pilot Study on Therapeutic Inhalation of Hydrogen Gas for Improving Patients with Alzheimer's Disease Assessed by Cognitive Subscale Scores and Magnetic Resonance Diffusion Tensor Imaging

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Abstract

Reason

Hydrogen gas (H_2) has emerged as a therapeutic and preventive medical gas with multiple functions. H_2-inhalation was safe and efficiently improved patients with various diseases including cerebral infarction. Moreover, recent clinical study revealed that drinking H_2-infused water improved mild cognitive impairment in subjects with the apolipoprotein E4 genotype. Administration of Lithium (Li_2CO_3) was evaluated for therapeutic use against neurodegenerative diseases as well as bipolar disorder. Then, we examined the effect on Alzheimer's disease (AD) patients by inhalation of H_2 gas accompanied with oral administration of Li_2CO_3.

Methods

Eleven patients with AD inhaled 3% H_2 gas for 1 hr twice per day and received oral Li_2CO_3 (one 200 mg tablet) twice per day for 4 - 7 months. The patients were clinically evaluated using the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog). To evaluate the integrity of the neurons, the neuronal bundles that pass through the hippocampus were visualized by a modified diffusion tensor imaging (DTI) technique using advanced magnetic-resonance imaging (MRI) at various fractional anisotropy (FA) values.

Results

The ADAS-cog profile suggested that the effects were caused by H_2-inhalation. The mean ADAS-cog score change (n=11) was significantly improved to –2.7 after ~5 months treatment in comparison with that in non-treated patients (n=5) which worsened to +7. When two patients with an ADAS-cog score of more than 50 at the baseline were excluded, the mean ADAS-cog score change was –4.1. By objective DTI evaluation, inhalation of H_2 significantly increased the hippocampal neuronal tract size at the higher FA values. The DTI change was correlated with the clinical improvement assessed by ADAS-cog scores.

Conclusion

The DTI evaluation as well as a clinical test showed that inhalation of H_2 gas improved AD patients. It will need to confirm whether this treatment provides symptomatic relief only or disease modifying effects.

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Keywords: ADAS-Cog; Alzheimer's Disease; Diffusion Tensor Imaging; DTI; H_2; Hydrogen Gas; Molecular Hydrogen; and Lithium Carbonate

Introduction

Alzheimer's Disease (AD) is a fatal neurodegenerative disorder, having a complex etiology with numerous possible drug targets [1]. Oxidative stress is a physiological age-related brain process, dramatically over expressed in neurodegenerative disorders including AD [2,3]. Nevertheless, the pathophysiological role of oxidative stress in AD pathology has not been clarified yet. Single modality of a specific target strategy for treating AD may have failed, and no effective treatment is currently available because complicated multiple factors should be involved in pathophysiology and severity of AD. Future therapies on multiple-targets strategy will need to address multiple aspects to block the progression of pathogenesis of AD [4,5].

Molecular hydrogen (H_2) was initially reported as a therapeutic antioxidant by one of the present authors [6]. Recent extensive studies revealed that H_2 exerts multiple functions, including anti-inflammation and stimulation of energy metabolism, to exhibit efficiency against a variety of disease models [7,8]. We reported that inhalation of H_2 gas provided various benefits in elderly patients with cerebral infarction [9]. A large-scale clinical trial is ongoing in order to protect the brain for patients with post-cardiac arrest syndrome as an advanced therapy that the Japanese government had approved [10]. For dementia, we have recently reported that H_2 actually improved mild cognitive impairment (MCI) subjects with the apolipoprotain E4 (APOE4) genotype by a subgroup analysis involving 13 subjects [11]. Thus, H_2 alone should function as a multi-functional drug.

Another candidate multi-functional drug, lithium (Li_2CO_3) has been approved as an effective psychotropic agent and evaluated recently for repurposing use in neurodegenerative diseases [12] (ClinicalTrials.gov. NCT 03185208 & TNCT02862210). For AD pathologies, lithium inhibits the production of amyloid β by suppressing sequential cleavages of amyloid precursor protein and phosphorylation of Tau, and the blood concentration of phosphorylated Tau decreased in AD model mice receiving lithium [13]; however, lithium has a high incidence of side effects when the blood level goes beyond a safe level, particularly when used in elderly patients [14]. Thus, the Japanese government has issued special warnings for the use of lithium (http://pmda.go.jp/files/000145551.pdf). On the other hand, H_2 mitigated side effects caused by toxic drugs [15]. Moreover, H_2 is clinically safe with no reported side effects and we have recently reported on H_2 use in elderly acute cerebral infarction patients [9]. Additionally, lithium and H_2 should be able to reach the brain neuron via voltage-dependent Na^+ channels and by rapid gaseous diffusion [6], respectively.

Thus, we tried to examine the efficacy of the combination treatment of lithium and H_2 in AD patients. The Alzheimer's Disease Assessment Scale-cognition sub-scale (ADAS-cog) score is the most widely used general cognitive measurement in clinical trials of AD [16, 17].

As a more objective evaluation, a modified diffusion tensor imaging (DTI) method, as an advanced magnetic resonance imaging (MRI) technique, was applied to detect early microstructural alterations in AD patients before gross anatomic alterations became visible [18,19]. In the present study, we show that inhalation of H_2 gas exhibited a marked effect on AD patients.

Methods

Approval for this Study

This study was carried out in accordance with "The Code of Ethics of the World Medical Association (Declaration of Helsinki)". The protocol of this clinical study was approved by the Nishijima Hospital Ethics Committee, and was pre-registered at URL: http://www.jmacct.med.or.jp. Clinical Trial Registration–JMACCT ID: JMAIIA00308. We received written informed consents from a family member for all patients.

Initially, we would have liked to examine the effects of H_2-inhalation on AD patients; however, the committee members suggested a combination of H_2-inhalation and one more drug because there was no experience of inhalation of H_2 gas in AD patients even as a pilot study. Finally, this pilot study was approved with combination treatment with H_2-inhalation and oral administration of lithium.

The period of the study protocol was 6 months; however, in accordance with requests from family members, we could alter each individual period. The administration of lithium ceased when side effects were suspected, regardless of the approved protocol. On the other hand, when a patient's family member requested to continue the administration lithium or inhalation of H_2 gas at the end of this clinical trial, this was allowed with the full understanding that this was at his or her own responsibility for any consequences.

Patient Selection

The criteria for inclusion were as follows: (1) diagnosis of AD in accordance with the recommendations by the National Institute on Aging-Alzheimer's Association group (NIA/AA) [20]; (2) routine treatment in Neurology Dementia Clinic with multiple ADAS-cog /MMSE tests at least every 6 months for the last 2-3 years with recent worsening; (3) an ADAS-cog score of more than 10 or a corresponding score converted from the MMSE using the formula [70-(MMSEx2.33)] [21] and the score was getting worse; (4) treatment with at least one of anti-cholinesterase drugs and/or an NMDA receptor antagonist had already been attempted, and yet the ADAS scores were worsening; and (5) no significant airway disease such as Chronic Obstructive Pulmonary Disease (COPD), pneumonia, bronchitis, or asthma that might interfere with adequate inhalation of H_2.

Prior to study entry, the patients were given 400 mg/day of Li_2CO_3 for 1 week, and kidney and liver functions were confirmed to be within the normal ranges for safe oral intake of lithium. At least one brain MRI had been examined and other causes of dementia were ruled out in our neurology clinic. For all patients who satisfied the inclusion criteria, entry into the treatment group was offered first with 1 week of test medication and test inhalation.

After the test week, only patients and patient's family/care givers were included in this study, if they could manage the generator as instructed, including checking the water level of the generator, complete 1 hour inhalation 14 times as ordered and also showed no symptoms or signs of lithium side effects, with a blood level remaining less than 0.8 mEq/dL, and no kidney or liver dysfunction. As regular Alzheimer's medications (donepezil, galantamine, rivastigmine, memantine), the patients continued to receive at least one of these medicines throughout the study.

Supplementary Figure 1 shows the profile on the recruitment, elimination, and follow-up post treatment. The "putative control" patients accepted occasional ADAS-cog tests and MRI examinations for 6 months, but inhaled neither placebo gas nor received lithium during the study period. In this paper, we described them as non-treated patients.

Treatments

Because the complication rate increases with higher lithium concentration in the blood, we planned this clinical study with reduced-dose lithium [22]. The patients in the treatment group received daily oral lithium carbonate (Li_2CO_3: one 200 mg tablet twice a day) (Tanabe-Mitsubishi, Pharma Corporation, Tokyo, Japan) and inhalation of 3% H_2 for 1 hour twice daily through a regular facial mask in their own home or in the nursing home where they resided. The hydrogen generator was taken to their residence and used as instructed for 1 hour, twice daily and family members were asked to stay with the patient and watch the facial mask and inhalation continuously for 1 hour because AD patients tended to take off the facial mask frequently.

H_2 gas (3%) with 21% oxygen was generated using a portable H_2 generator (Nishijima/Enoa gas hydrogen generator) as described previously [9]. The blood concentration was confirmed to increase as described previously [9]. The gas generator was brought back every month to check for adequate gas production.

Blinded Evaluation

Clinical effectiveness was assessed by monitoring ADAS-cog scores. The ADAS-cog scores were independently obtained in the physical therapy department. As an objective assessment, brain MRI was examined in the radiology department. The staffs of these departments had no information on whether subjects were participants in this study or common outpatients. These results were reported to doctors via an electronic chart system in a blinded manner.

Measurement of Hippocampal and Parahippocampal Volume

Starting at 9 mm behind the frontal end of the hippocampus, 5 coronal slices were selected 5 mm apart. The area of each coronal slice was calculated using DICOM ROI software, which automatically records the size of the area in mm^2 and the volume of the hippocampal and parahippocampal regions, which includes the subiculum, entorhinal area, and parahippocampal gyrus, were calculated by multiplying the area in mm^2 by 5 mm (=distance between the control slices) to convert to mm^3. Therefore, our volume measurement was done on the major part of the hippocampus from 9 mm from the tip to 34 mm into the body as shown in Supplementary Figure 2. All of the processing and calculation were done in a blinded manner by a radiology technician.

Measurement of Hippocampal and Parahippocampal Tract Sizes by Diffusion Tensor Imaging

Five seed points were set at the volumetric measurement sites where the neuronal bundles passed through the entire hippocampus or parahippocampus as shown in Supplementary Figure 2. Digital tractography imaging was performed using Neuro3D with the GRAPPA technique in order to shorten the examination time. DTI were obtained with fractional anisotropy (FA) values of 0.10, 0.15, and 0.2. The tract size was calculated from the pixel number of the tract images, and the number of the pixels in the tract was calculated using the Image J software.

Statistical analysis

Statistical analysis was 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 Foundation,Vienna, Austria) for analyzing the change of ADAS-cog score. Statistical analyses were performed by an academic biostatistician using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) by Student's t-test with two tails for DTI analyses.

Results

ADAS-cog scores in most patients were improved by H_2 therapy

Figure 1 shows the profiles of changes in ADAS-cog scores in each patient by the combination of H_2 and lithium therapy. When the combination therapy ceased, the score soon worsened (patients **b** and **d**). On the other hand, the scores continued to be improved with H_2 even without lithium (patients **e** and **h**). In contrast, when H_2-treatment was discontinued, the scores of patients, **b**, **d**, **f**, **g**, **i**, and **j** worsened (continued to increase). Thus, the major effect of the combination therapy might be due to H_2-inhalation alone.

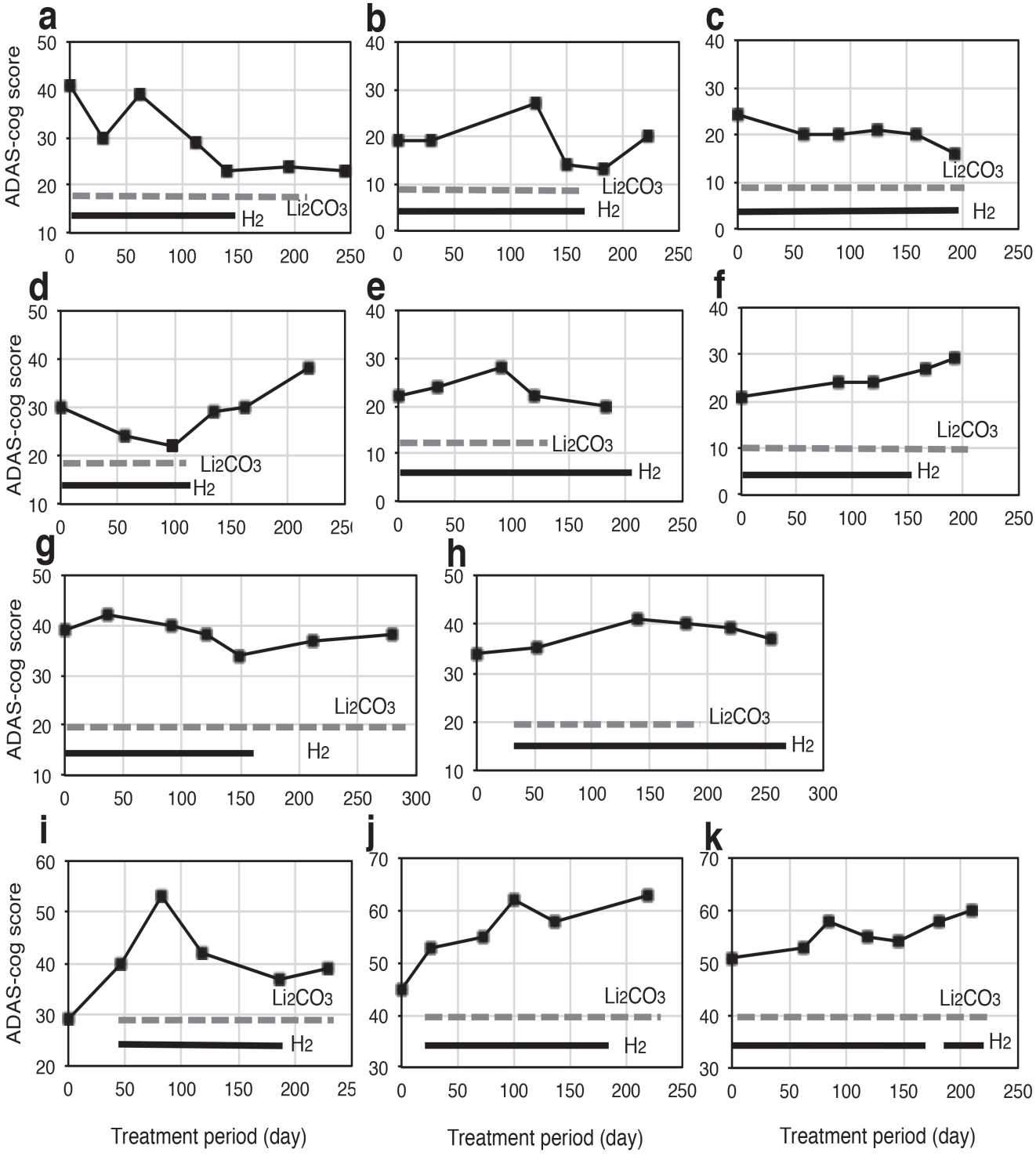

Figure 1. Profiles of ADAS-cog scores in the 11 patents.

Panels a - k indicate the time course of changes in ADAS-cog scores of each patient. Straight and dotted lines indicate the periods of H_2 and Li_2CO_3 treatments, respectively.

Table 1. Baseline characteristics and ADAS-cog scores in treated patients.

patient	sex	age	diabetes	lipidemia	baseline	follow-up	change	change
a	F	80	N	N	41	23	−18	−18
b	F	70	N	moderate	19	14	−5	−5
c	F	71	N	N	23	20	−3	−3
d	F	77	N	mild	30	22*	−8	−8
e	F	85	N	N	21	20	−1	−1
f	F	70	N	N	22	24	2	2
g	F	83	N	N	40	34	−6	−6
h	M	84	N	N	35	39	4	4
i	F	85	mild	N	40	38	−2	−2
j	F	68	N	moderate	53	58	5	excluded
k	F	73	N	moderate	51	53	2	excluded
mean		76.9			34.1	31.4	−2.7	−4.1
SEM		1.9			3.5	4.1	1.9	2

The baselines indicate scores at the starting time of the H2/lithium treatment The folllow-up peroods are approximately 5 months, but * indicates the follow-up perood is 4 months.

Table 2. Baseline characteristics and ADAS-cog scores in non-treated patients.

patient	sex	age	diabetes	lipidemia	baseline	follow-up	change
l	F	77	N	moderate	20	34	14
m	F	89	N	moderate	21	28	7
n	F	76	mild	N	58	61	3
o	F	79	N	mild	12	21	9
p	M	81	N	N	27	29	2
mean		80.4			27.6	34.6	7
SEM		1.8			6.5	5.6	1.8

The baseline indicate scores at each participant time. The follow-up periods are approximately 5 months with no treatment.

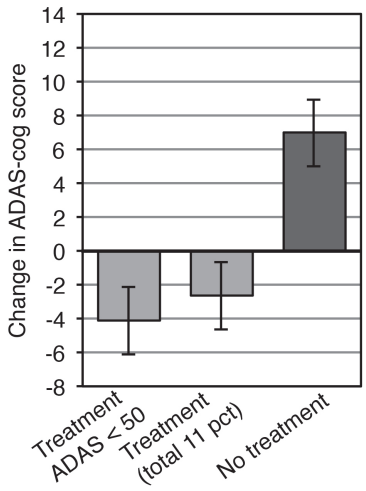

Figure 2. Change in ADAS-cog score from baseline to follow-up time.

The mean ADAS-cog score change from baseline after ~5 months treatment was obtained in 9 treated patients with an ADAS-cog score of less than 50 in (Treatment ADAS < 50), 11 treated patients in total [Treatment (total 11 pct)], and 5 non-treated patients (No treatment). Error bars indicate SEM.

Two sample t-test indicated t = −3.35, df = 12, p-value = 0.0058, 95% confidence interval: −18.34 to −3.88 between (Treatment ADAS < 50) and (No treatment), and t = -2.95, df = 14, p-value = 0.011, 95% confidence interval: -16.81 to -2.65 between [(Treatment (total 11 pct)] and (No treatment).

As shown in **Table 1 and Figure 2**, the mean changes from baseline after ~5 months treatment was improved to -2.7 in 11 treated patients, whereas all of the non-treated patients worsened, and the mean change was +7 (**Table 2, Fig. 2**). The difference in change between treated and non-treated patients was significant (by paired samples t-test of the difference, p=0.011, 95% confidence interval = −16.81 to −2.65) **(Fig. 2)**. Two patients with an ADAS-cog score of more than 50 did not improve (**Table 1**). When these patients were excluded, the mean change was maintained at −4.1 from baseline to the follow-up time, and the difference was significant between treated and non-treated groups (by paired samples t-test of the difference, p=0.0058, 95% confidence interval = −18.34 to −3.88) (**Fig. 2**). This finding suggested that H_2-inhalation was marked effective in patients with moderate AD patients, but not in patients with severer AD.

Volumes of Hippocampal and Parahippocampal Regions did not Change During Treatment.

The volumes were measured at least 3 times during the treatment period as described in Methods. The observation period continued for 8 months in the case of g during the treatment period, while ADAS-cog scores were significantly changed (Figure. 1g); however, no increase in the size of the hippocampus occurred in other cases during the 6- month treatment period (Supplementary Figure 2).

Hydrogen Treatment Increased DTI Tracts and Correlated With ADAS-Cog Scores.

DTI is useful to evaluate neuronal integrity as a useful method to evaluate AD [18,19]. We focused our attention on the neuron bundles that passed through the entire hippocampus or parahippocampus. For this purpose, we fixed 5 seed positions through which the neuronal bundles passed

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Figure 3AB demonstrate representative DTI at the FA values of 1.0, 1.5, and 2.0 for a side of the brain of patient g who was treated with lithium and H_2 for 2, 4 and 6 months, and then with only lithium for 4 months. The tracts with high FA values (FA=0.2) appeared to be the most sensitive to change. The DTI tracts were unchanged in the parahippocampal region by the H_2 and lithium treatment. The DTI tracts with FA=0.15 and 0.2 in the hippocampus region appeared to increase after H_2 and lithium treatment, and the effect of the treatment was strongest with FA=0.2 (Figure. 3A). Moreover, when treatment with H_2 was discontinued, the DTI tracts with FA=0.15 and 0.2 tract were decreased. Thus, in this case, the increase in the DTI tract was not due to lithium treatment, suggesting that the improvement of the neuron integrity was due to H_2-inhalation.

This profile was in a good agreement with ADAS-cog scores as shown in Figure. 1g. In contrast, parahippocampal DTI tracts were unchanged (Figure. 3B). Figure 3CD shows semi-quantitative analysis of the DTI tract at FA=1.0, 1.5 and 2.0. These data indicated that the representative hippocampal DTI correlated with ADAS-cog score especially at higher FA values.

Figure 4 shows the mean values with SEM of DTI by averaging 4 images; with right and left hemispheres from lateral and axial views. Figure 4A shows the comparison at FA=0.2 between the baseline and follow-up status after intra-normalization with FA=0.1. Figure 4B shows the comparison after intra-normalization with parahippocampal DTI. The tracts in DTI visualized at FA=0.2 in the hippocampus were significantly increased by H_2 -treatment (Figure. 4 left), whereas it was decreased in the non-treated group after the corresponding follow-up period (Figure. 4 right). Moreover, the DTI tracts were decreased in the patients with a ADAS-cog score of more than 50 with a good correlation with clinical decline (Figure. 4 middle).

Thus, the changes in DTI reflected the effect of H_2-treatment on hippocampal neuron integrity.

Discussion

With the current combination treatment with H_2 and lithium, most patients seemed to experience benefits of treatment as assessed using ADAS-cog scores. The ADAS-cog score [16] is the most widely used general cognitive measure in clinical trials of AD [25,26], and it assesses multiple cognitive domains, including memory, language, praxis, and orientation.

Because there was no experience of H_2-inhalation treatment in AD patients, combination treatment with H_2 and lithium was applied in this pilot study. In the present combination treatment, the number of the patients was too small to provide a conclusive interpretation; however, it is suggested that the current treatment efficacies were due solely to H_2-inhalation because the scores continued to improve with H_2 even without lithium (in patients e and h), whereas the scores of patients b, d, f, g, i, j, and k worsened (continued to increase) by discontinuous H_2-treatment. Moreover, the increase in DTI tracts at FA=0.2 in the hippocampus depended upon only H_2-treament, but not upon lithium treatment (in patient g).

These improvements associated with H_2-inhalation were marked when the effect was compared with that of donepezil; donepezil decreased ADAS-cog score by 3 points after 6 weeks; however, after 6 months, the scores had returned to the initial level although the placebo group had worsened [27]. With the current H_2-treatment, the mean change, including patients with an ADAS-cog score of more than 50 was maintained at −2.7 from baseline after ~5 months treatment. Because two patients with an ADAS-cog score of more than 50 in at baseline did not improve, H_2-inhalation treatment may not be effective in patients with severer AD. When these patients with severe AD were excluded, the mean value was maintained at −4.1 from baseline.

Moreover, DTI showed significant recovery of the tract size, particularly at of high FA values with the treatment. Cerebral white matter bundles are arranged in a highly directional and packed manner. The water diffusivity is much higher along the direction of the bundle compared with other directions. The difference between diffusivity along and across bundles increases with axonal density. FA values reflect the density of axons within bundles, with lower values corresponding to lower axonal density. The mean neuron tract size visualized at FA=0.2 in the treated group increased, whereas that in the non-treated group decreased. Furthermore, the DTI tract decreased in the non-responders with more in ADAS-cog score of more than 50 (Figure.4). The present DTI findings were in good agreement with the clinical improvement in AD patients that were assessed using ADAS-cog.

H_2 acts as an efficient antioxidant inside cells owing to its ability to diffuse rapidly across membranes [6]. Moreover, as a secondary anti-oxidative function, H_2 seems to activate NF-E2-related factor 2 (Nrf2) [7], which

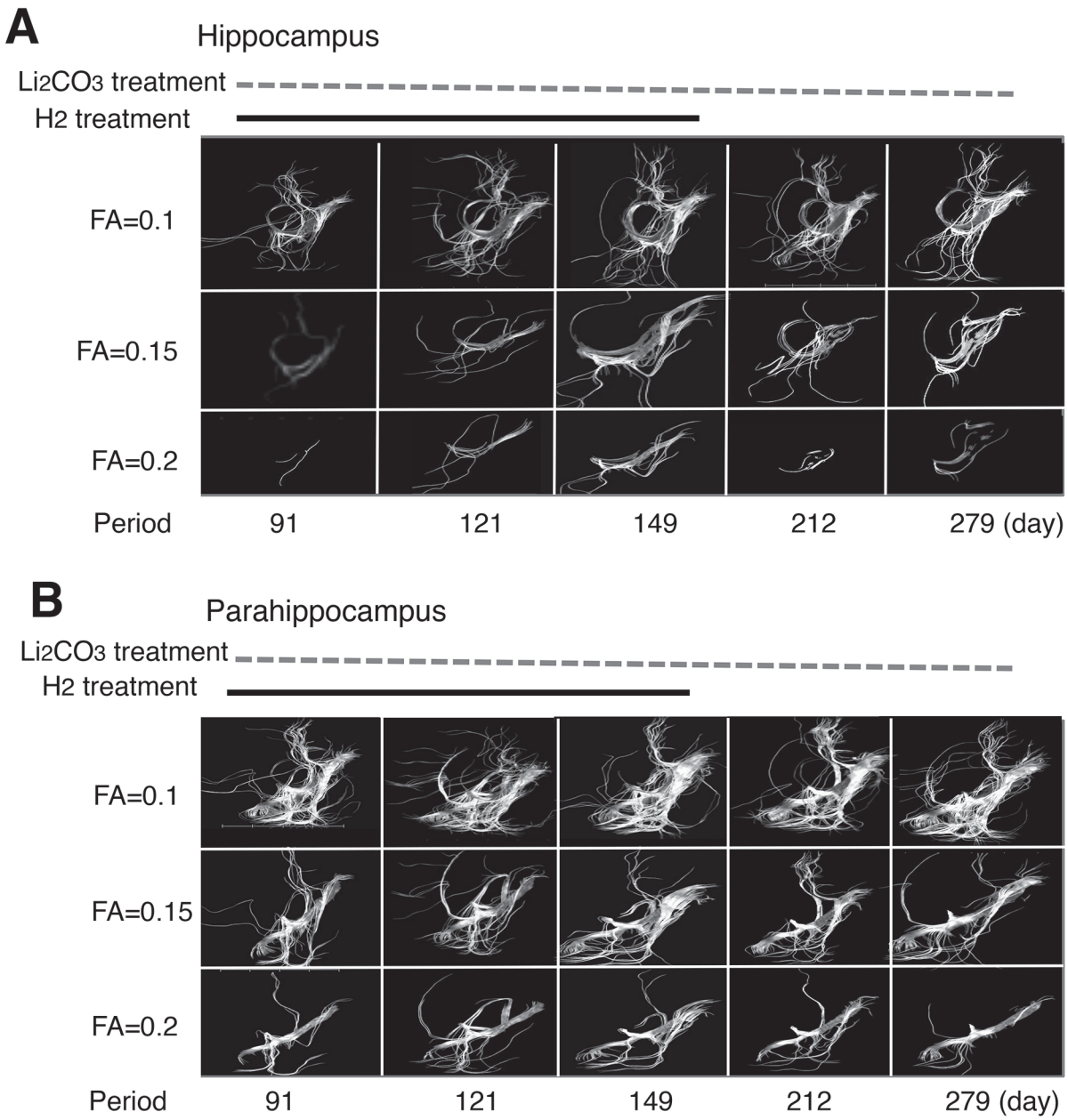
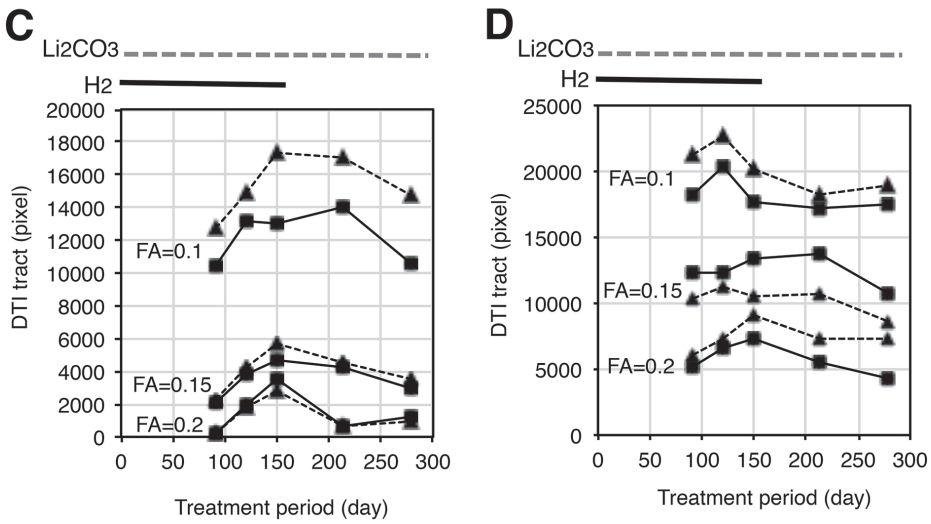


Figure 3. Representative presentations of diffusion tensor imaging on the effect of inhalation of H_2 gas in case d.

The neuron bundles were selected that passed through the hippocampal or parahippocampal region on 5 seed points, as described in **Methods**.

Straight and dotted lines indicate the periods of H_2 and Li_2CO_3 treatments, respectively. Representative diffusion tensor images of MRI of the hippocampus (**A**) and parahippocampus (**B**) regions of patient **d** at FA=0.1, 0.15, or 0.2. The period of treatment is shown in days under the pictures. **C** and **D** show semi-quantitative analyses of DTI tracts in hippocampal and parahippocampal regions, respectively. Squares and triangles indicate pictures observed from the left side and the front, respectively.

Figure 3 C & D



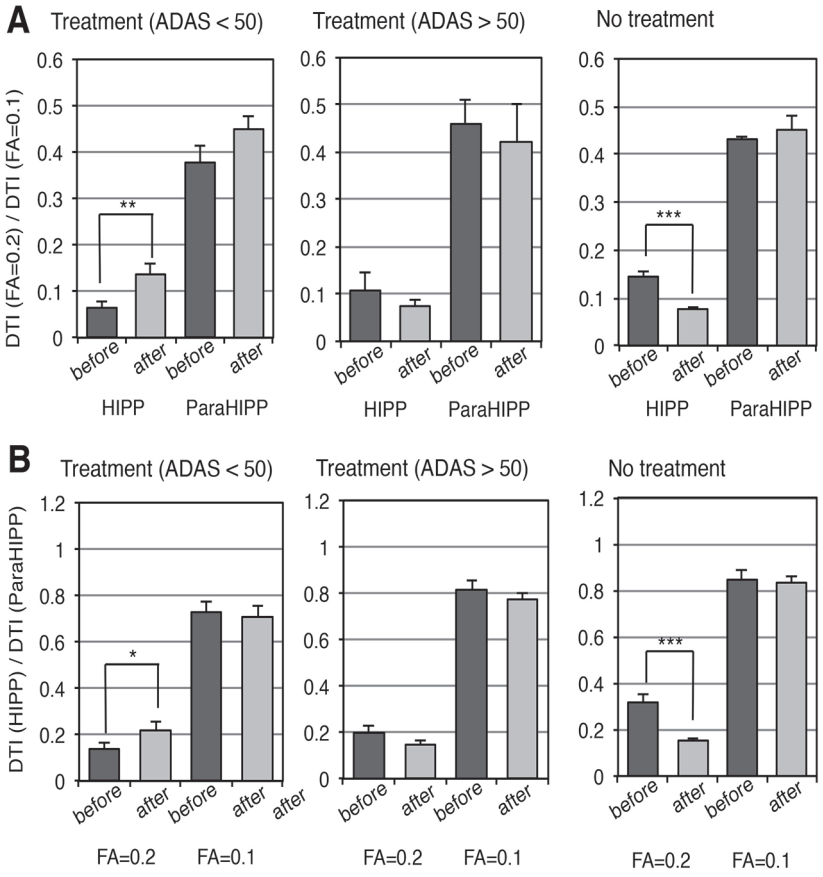


Figure 4. Diffusion tensor image tracts

The relative tracts of the neuronal bundle that passed through 5 seed points that had been fixed in the hippocampal or parahippocampal region were evaluated after intra-normalization. The mean was obtained by averaging 4 images; the right and left hemispheres from lateral and axial views. (**A**) The pixel numbers of the corresponding neuronal bundle tracts in FA=0.2 were normalized with those in FA=0.1 at baseline (before) and follow-up time (after) in the hippocampal (HIPP) and parahippocampal (ParaHIPP) regions. Left, middle, and right panels show the DTI tracts at FA=0.2 normalized with those at FA=0.1 in 9 treated patients with an ADAS-cog score of less than 50 [Treatment (ADAS < 50)], 2 treated patients with an ADAS-cog score of more than 50 [(Treatment (ADAS > 50)) at baseline, and 5 non-treated patients (No treatment), respectively. (**B**) The tracts of hippocampal DTI [DTI (HIPP)] bundles were normalized with parahippocampal DTI [DTI (ParaHIPP)] bundles at baseline (before) and follow-up time (after) at FA=0.2 and FA=0.1.

Error bars indicate SEM. *, ** , and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, by Student's t-test with two tails.

reduces oxidative stress by expression of a variety of anti-oxidant enzymes [28]. We reported that drinking H_2-water prevented arteriosclerosis using APOE knockout mice, a model of the spontaneous development of atherosclerosis accompanying a decrease in oxidative stress [29]. Thus, it is possible that inhalation of H_2 improves vascular damage by decreasing oxidative stress as a direct or indirect antioxidant, leading to the improvement of AD.

H_2 exhibits multiple functions by a decrease in the levels of pro-inflammatory cytokines and an increase in energy metabolism in addition to anti-oxidative roles. To exert multiple functions, H_2 regulates various signal transduction pathways and the expression of many genes [7]. For example, H_2 protects neural cells and stimulates energy metabolism by stimulating the hormonal expression of ghrelin [30] and fibroblast growth factor 21 [31], respectively. In contrast, H_2 relieves inflammation by decreasing pro-inflammatory cytokines [32]. Thus, the combination of these functions of H_2 on anti-inflammation and energy metabolism-stimulation might prevent the decline in brain function [7]. Therefore, it is possible that the multiple functions of H_2, including energy metabolism-stimulation and anti-inflammation, may contribute to the improvement of AD.

As an alternative aspect, H_2 suppresses the nuclear factor of activated T cell (NFAT) transcription pathway to regulate various gene expression patterns [33]. NFAT signaling is altered in AD and plays an important role in driving amyloid β-mediated neurodegeneration [34]. Moreover, the NFAT transcriptional cascade contributes to amyloid β synaptotoxicity [35]. Therefore, the beneficial effects of H_2 on AD may be explained by the suppression of NFAT transcriptional regulation.

Limitations of the study

This study needs to confirm whether the combination of lithium and H_2 is essential with a crossover observation although the current treatment effects were suggested to be due solely to H_2-inhalation. The current method of H_2-inhalation and requirement for continuous observation by a family/caregiver needs to be reevaluated because this was significant burden, and the resulting number of patients in the study was rather small. We compared clinical data and DTI between treated and no-treated groups; however, further study requires a placebo-controlled group to clarify the effect by H_2-inhalation. The study period also needed to be longer because the recurrence of the symptoms occurred soon after the treatment ended.

Conclusion

Inhalation of H_2 gas provided marked improvements in AD symptoms. No serious side effects were noted. However, the symptoms recurred soon after the treatment ended. This may warrant another study with a longer treatment period in order to determine whether this treatment provides symptomatic relief only or disease-modifying effects.

Conflict of Interest

H.O., Yo.Ni., and S.O. are inventors of a pending related patent. The other authors declare that there is no conflict of interest on this study.

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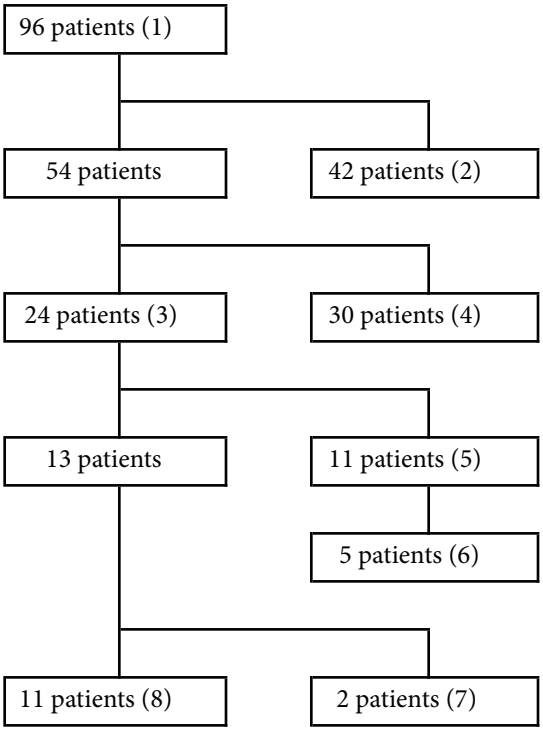
Supplementary Figure 1.



Supplementary Figure 1 Study profile

(1) 96 patients who were routinely treated in the Neurology Dementia Clinic with multiple ADAS-cog/MMSE tests at least every 6 months for the last 2-3 years with recent worsening.

(2) 42 patients were excluded by initial ADAS-cog score or MMSE converted ADAS-cog scores of more than 10 and recent worsening of more than 6.

(3) 24 patients or their family members agreed to be evaluated.

(4) 30 patients were not interested in the study due to other medical conditions, family/care-giver situation, and fear for hydrogen etc.

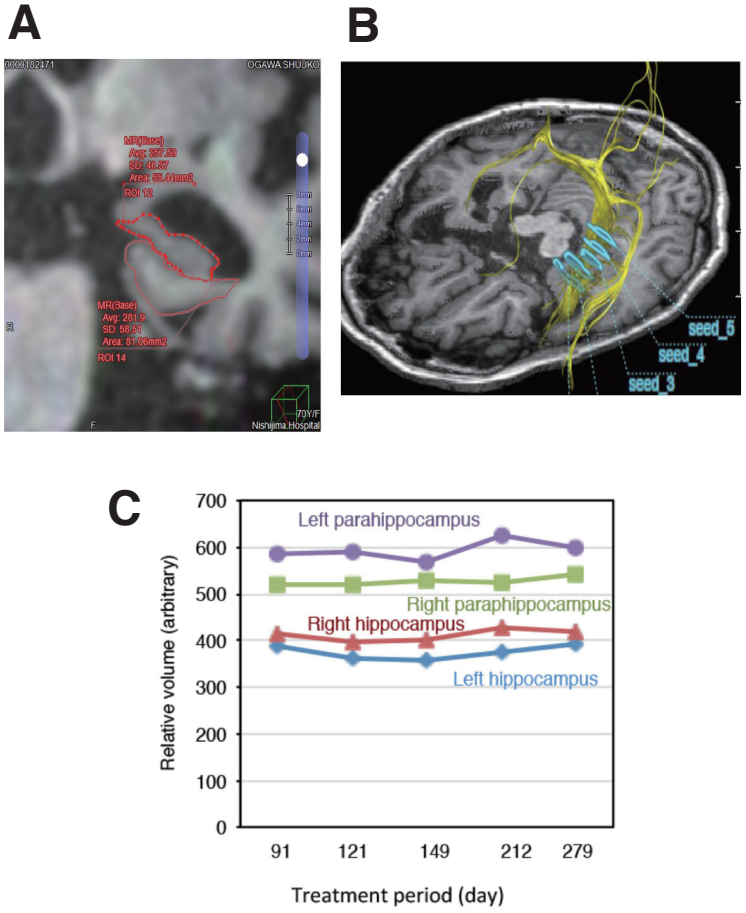
(5) 11 patients refused to enter the study, due to difficulty in meeting the requirements of the study (home care of the portable H_2 gas generator, required presence of the family member/caregiver, side by side with the patient twice per day for 1 hr for 6 months).

(6) 5 patients agreed to be examined for ADAS-cog and MRI as non-treated patients. They neither inhaled a placebo gas, and nor received lithium.

(7) 2 patients were excluded from the study because of illness of a family member/caregiver, violation of the study protocol

(8) 11 patients were selected for the treatment group.

Supplementary Figure 2



A: Hippocampal and parahippocampal regions were visualized by MRI.

B: Five seed points were set at the volumetric measurement sites where the neuronal bundles passed through the entire hippocampus or parahippocampus. Digital tractography imaging was performed using Neuro3D with the GRAPPA technique. Blue gates indicate the five seed points for the selection of the neuron bundles that passed through. Yellow indicates the neuron bundles.

C: Changes in volume of hippocampal and parahippocampal regions in patient d during treatment. The area of the each coronal slice was calculated using DICOM ROI software, which automatically records the size of the area in mm^2 and the volume of the hippocampal and parahippocampal region, which includes the subiculum, entorhinal area and parahippocampal gyrus, were calculated by multiplying the area in mm^2 by 5 mm (=distance between the control slices) to convert to mm^3.