

**Clinical benefits of antioxidative supplement Twendee X  
for mild cognitive impairment  
: a multicenter, randomized, double-blind, and placebo-  
controlled prospective interventional study**

**Koh Tadokoro<sup>a\*</sup>, Ryuta Morihara<sup>a\*</sup>, Yasuyuki Ohta<sup>a</sup>, Nozomi Hishikawa<sup>a</sup>, Satoko Kawano<sup>a</sup>,  
Ryo Sasaki<sup>a</sup>, Namiko Matsumoto<sup>a</sup>, Emi Nomura<sup>a</sup>, Yumiko Nakano<sup>a</sup>, Yoshiaki Takahashi<sup>a</sup>,  
Mami Takemoto<sup>a</sup>, Toru Yamashita<sup>a</sup>, Setsuko Ueno<sup>b</sup>, Yosuke Wakutani<sup>b</sup>, Yoshiki Takao<sup>b</sup>,  
Nobutoshi Morimoto<sup>c</sup>, Yumiko Kutoku<sup>d</sup>, Yoshihide Sunada<sup>d</sup>, Katsushi Taomoto<sup>e</sup>,  
Yasuhiro Manabe<sup>f</sup>, Kentaro Deguchi<sup>g</sup>, Yasuto Higashi<sup>h</sup>, Haruhiko Inufusa<sup>i</sup>, Fukka You<sup>j</sup>,  
Toshikazu Yoshikawa<sup>k</sup>, Markus Matuschka von Greiffenclau<sup>l</sup>, and Koji Abe<sup>a</sup>**

\*Koh Tadokoro and Ryuta Morihara equally contributed to this work.

- [a] Department of Neurology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan.
- [b] Department of Neurology, Kurashiki Heisei Hospital, Kurashiki, Japan.
- [c] Department of Neurology, Kagawa Prefectural Central Hospital, Takamatsu, Japan.
- [d] Department of Neurology, Kawasaki Medical School, Kurashiki, Japan.
- [e] Department of Neurosurgery, Ohnishi Neurological Center, Akashi, Japan.
- [f] Department of Neurology, National Hospital Organization Okayama Medical Center, Okayama, Japan.
- [g] Department of Neurology, Okayama City Hospital, Okayama, Japan.
- [h] Department of Neurology, Himeji Central Hospital, Himeji, Japan.
- [i] Division of Anti-Oxidant Research, Life Science Research Center, Gifu University, Gifu, Japan.
- [j] Division of Anaerobe Research, Life Science Research Center, Gifu University, Gifu, Japan.
- [k] Louis Pasteur Center for Medical Research, Kyoto, Japan.
- [l] European Medical Foundation, TIMA Est., Balzers, Liechtenstein.

**Running title:** Placebo-controlled trial of Twendee X in MCI

Address correspondence and reprint requests to: Prof. Koji Abe

Department of Neurology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,  
Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama, 700-8558, Japan.

Tel: 81-86-235-7365, Fax: 81-86-235-7368, Email: [p86m6fma@okayama-u.ac.jp](mailto:p86m6fma@okayama-u.ac.jp)

## **ABSTRACT**

Oxidative stress involves in a whole pathological process of the development of Alzheimer's disease (AD) including mild cognitive impairment (MCI) stage. Twendee X (TwX) is a strong antioxidative mix supplement containing 8 antioxidants, which showed a clinical and pathological benefit in AD model mice. Here we conducted a multicenter, randomized, double-blind, and placebo-controlled prospective interventional study to evaluate the efficacy of TwX on MCI subjects. The primary outcomes were the differences of the mini-mental state examination (MMSE) and Hasegawa dementia scale-revised (HDS-R) score changes from baseline at 6 month between placebo and TwX groups. 78 MCI subjects underwent randomization for 37 to placebo and 41 to TwX. TwX showed a significant difference of MMSE at 6M compared with placebo ( $p = 0.018$ ), and also a significant improvement of HDS-R score from baseline at 6 M ( $p = 0.025$ ). TwX showed no effect on affective and ADL scores at 6 M. The present study revealed the clinical benefits of strong antioxidative supplement TwX for cognitive functions of MCI subjects.

## **INTRODUCTION**

Dementia is a serious growing problem all over the world, and Alzheimer's disease (AD) is the most common cause of dementia. Although there is a great demand for effective interventions to prevent dementia, any drugs or supplement therapies have not been established.

Oxidative stress contributes to the development of AD through mitochondrial dysfunction[1], neuroinflammation[2], lipid peroxidation[3], and other mechanisms[4, 5] from prodromal stage, namely mild cognitive impairment (MCI)[6]. Therefore, antioxidant therapy is a potential intervention for preventing AD.

Twendee X (TwX) is a patented supplement containing 8 antioxidants. We have reported the clinical and pathological benefits of TwX in animal models of AD and ischemic stroke through strong antioxidant effects[7, 8]. Here, we carried out a clinical trial to evaluate the efficacy of TwX for cognitive functions of MCI subjects.

## **MATERIALS AND METHODS**

### ***Participants***

The present study included native Japanese participants with cognitive impairment (mini-mental state examination (MMSE) score  $\geq 24$  and clinical dementia rating (CDR) score[9] = 0.5), aged from 65 to 85. Patients with neurodegenerative diseases such as Parkinson's disease, dementia with Lewy bodies (DLB), fronto-temporal lobar degeneration (FTLD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and multiple system atrophy (MSA), cognitive declines due to hypothyroidism, vitamin deficiency, idiopathic normal pressure hydrocephalus (iNPH), head trauma, epilepsy, encephalitis, and meningitis, past history of psychiatric disorders including schizophrenia,

depression, alcoholism, and drug addiction, and stroke within 3 months (M), diabetes mellitus with HbA1c  $\geq$  8.0, and metabolic syndrome diagnosed by the Japanese criteria[10] were excluded. Subjects taking anti-dementia drugs or any supplements were excluded. All participants gave written informed consent.

### ***Study supplement***

TIMA Japan Corporation (Osaka, Japan) provided the study supplement, TwX; a tablet containing coenzyme Q10 (10 mg), niacin amid (2 mg), L-cystine (50 mg), ascorbic acid (94 mg), succinic acid (10 mg), fumaric acid (10 mg), L-glutamine (85 mg), and riboflavin (4 mg). Participants orally took placebo or TwX once a day more than 20 min before breakfast every day. Daily tablet dose was determined depending on the body weight (BW) of the subjects; 2 tablets for BW less than 40 kg, 3 tablets for BW between 40 and 60 kg, and 4 tablets for BW more than 60 kg.

### ***Study design***

The present study was a multicenter, randomized, double-blind, and placebo-controlled prospective interventional study. 17 medical institutions in Japan were registered in the study, and the study duration was originally planned from June 2017 to March 2024. An institutional review board at each institution or the representative institution approved the study protocol. The study was

registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) with a registration number UMIN000026268.

The study period was originally designed for 12 M. After checking eligibility, participants were randomized to placebo or TwX group with a stratification by age and sex. Subjects visited hospital once in 3 M, and received medical examination including cognitive and blood tests to monitor adverse events.

### ***Study outcomes***

The primary outcomes were the differences of the mini-mental state examination (MMSE)[11] and Hasegawa dementia scale-revised (HDS-R)[12] score changes from baseline at 6 and 12 M between placebo and TwX groups, indicating the differences of cognitive functional changes.

The secondary outcomes were the differences of geriatric depression scale (GDS)[13], apathy scale (AS)[14], Abe's behavioral and psychological symptoms of dementia (BPSD) score (ABS)[15], and Alzheimer's disease cooperative study-activities of daily living inventory (ADCS-ADL)[16] score changes from baseline at 6 and 12 M between placebo and TwX groups, indicating the differences of affective functional and activity of daily living changes.

### ***Statistical analysis***

According to our previous report[17], we estimated MMSE score at 12 M of 26.5 in placebo group and 28.0 in TwX group with standard deviation of 4.0. Then, we originally calculated that 200 participants for each group would be needed to provide 80 % statistical power.

The analyses for primary and secondary outcomes were based on per-protocol population after excluding discontinuation and trial termination (Fig. 1). Analysis of covariance (ANCOVA) was performed to evaluate the difference of score changes between placebo and TwX groups, with the baseline MMSE score as covariate. Friedman test and Wilcoxon signed rank test were performed to assess the score changes from baseline. Baseline characteristics of each group was evaluated by using Student's t-test for continuous and normally distributed data, Mann-Whitney U test for continuous and not normally distributed data, and Pearson's chi square test for categorical data. A two-sided level of significance of 5 % was chosen for interpretation.

All of the statistical analyses were performed using a statistical software (SPSS 22.0.0.0; IBM, Armonk, New York, USA).

## **RESULTS**

### ***Subject characteristics***

78 MCI subjects underwent randomization for 37 to placebo and 41 to TwX (Fig. 1). The number was smaller than that of original plan owing to the premature trial termination caused by the

Governmental Clinical Trials Act enforced in Japan on April 2018 requiring unacceptable modification of the study. At the time of trial termination (March 2019), 59 subjects (27 in placebo and 32 in TwX groups) reached the evaluation at 6 M (Fig. 1). In each group, 7 subjects discontinued for reasons other than study termination by 6 M. Because of the above legal change, outcomes were analyzed only at 6 M.

Baseline characteristics of subjects who reached the evaluation at 6 M were summarized in Table 1. Between both groups, there was no significant difference in demographic characteristics (sex and age), baseline cognitive (MMSE and HDS-R), affective (GDS, AS and ABS) and ADCS-ADL scores. Although GDS ( $3.4 \pm 2.8$  in placebo and  $4.4 \pm 4.0$  in TwX group) and AS ( $11.3 \pm 5.9$  in placebo and  $10.3 \pm 6.9$  in TwX group) were slightly elevated in both groups, those scores did not reach the criteria for depressive nor apathic states (GDS > 5[13], AS  $\geq$  14[14]). In the complications of present subjects, hypertension was the most frequent (48.1 % in placebo and 25.0 % in TwX groups), followed by dyslipidemia, and diabetes mellitus (3.7 % in placebo and 18.8 % in TwX groups), but ischemic heart disease was rare (Table 1). There was no significant difference in the prevalence of hypertension, diabetes mellitus, and ischemic heart disease, except for dyslipidemia in placebo (40.1 %) than TwX group (Table 1, 15.6 %, \* $p < 0.05$ ).

### ***Endpoints***

The results of primary and secondary endpoints at 6 M were summarized in Table 2. The MMSE score change from baseline at 6M was  $-0.85 \pm 2.48$  in placebo and  $0.66 \pm 2.60$  in TwX groups, and the difference of MMSE score changes between both groups was significant (Table 2, Fig. 2, ANCOVA:  $*p = 0.018$ , placebo vs TwX). The change of MMSE score from baseline to 6 M in each group was not significant (Table 2, Wilcoxon signed rank test:  $p = 0.092$  in placebo,  $p = 0.181$  in TwX).

The difference of HDS-R change from baseline at 6M between both groups was not significant (Table 2,  $0.74 \pm 2.19$  in placebo and  $1.09 \pm 2.62$  in TwX group), but only TwX group showed a significant improvement of HDS-R from baseline to 6 M (Table 2, Fig. 2, Wilcoxon signed rank test:  $\#p = 0.025$ ).

The changes of GDS, AS, ABS and ADCS-ADL scores from baseline to 6 M in each group were not significant (Table 2). The differences between both groups were not significant in GSD, AS, ABS, and ADCS-ADL from baseline at 6 M (Table 2, ANCOVA:  $p = 0.315, 0.356, 0.252, \text{ and } 0.284$ , respectively).

## **DISCUSSION**

Oxidative stress plays an important role in the pathology of MCI and early stage of AD[5, 6]. TwX is a strong antioxidative supplement containing 8 antioxidants, showing a significant amelioration of cognitive decline, amyloid- $\beta$  pathology, neuronal loss, oxidative stress, and



neuroinflammation in the AD model mice with chronic cerebral hypoperfusion and ischemic stroke model mice[7, 8]. Thus, we first conducted the present clinical trial to examine the efficacy for preventing cognitive declines of MCI subjects. In the present study, TwX significantly improved MMSE score change of the MCI subjects at 6 M compared with placebo (Fig. 2, Table 2), and HDS-R score from baseline to 6 M (Fig. 2, Table 2). On the other hand, TwX showed no effect on affective (GDS, AS, ABS) and ADL scores (Table 2).

Dietary supplements have been expected to reduce the risk of dementia[18, 19]. However, systematic reviews concluded that a single supplement such as vitamin B[20-22], vitamin C[20, 22], vitamin D[22], vitamin E[20-22], polyunsaturated fatty acid (PUFA)[21, 22], and their mixtures[20, 21, 23], lacks the evidence of effects on ameliorating cognitive declines, showing no or small benefit [24-27]. Other promising supplements including curcumin[28-30], ferulic acid[31, 32] and rosmarinic acid[33, 34] have yet to establish clear clinical effectiveness for preventing dementia. To our knowledge, TwX is the first supplement that clearly improved MMSE score change of MCI subjects in randomized placebo-controlled prospective multicenter interventional trial. Although antioxidant vitamins C or E did not show therapeutic benefit for dementia[20-22], combined 8 different antioxidants of TwX showed stronger antioxidant and anti-inflammatory effects than single antioxidant vitamins, which clearly improved cognitive functions of MCI subjects (Fig. 2). Stronger antioxidant effects of TwX than vitamin C was also demonstrated in irradiated mice model[35]. On

the other hand, TwX showed no effect on affective (GDS, AS, ABS) and ADL scores (Table 2), although oxidative stress is also associated with pathologies of affective disorders[36, 37]. One possible reason might be that deteriorations of affective and ADL baseline scores was too mild to show significant differences after treatments.

The present study has some limitations. First, the premature trial termination due to Government Clinical Trials Act of Japan shortened the study period from 12 into 6 M. To evaluate longer term effects of TwX, a further study with longer period is required. Next, only per-protocol analysis was originally designed in this study to evaluate the treatment effect of TwX. Most of the data of the subjects after discontinuation was missed, thus an intention-to-treat analysis was not performed. Finally, there are some concerns about the cognitive evaluations using MMSE and HDS-R that are not fully sensitive to detect the cognitive decline of MCIs[38, 39]. However, MMSE and HDS-R are commonly used in both clinical settings and trials [24, 40]. Furthermore, a recent study demonstrated that MMSE could detect the efficacy of treatment in MCI subjects, which was more sensitive compared to ADAS-Cog [41]. Therefore, we designed to use MMSE and HDS-R as primary endpoints in the present clinical trial for MCI subjects

In conclusion, the present study revealed the clinical benefits of TwX for cognitive functions of MCI subjects, suggesting that the strong antioxidative therapy may be useful for MCI subject to convert into AD.

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## CONFLICTS OF INTEREST

TIMA e.s. has patents on Twendee X. Haruhiko Inufusa and Fukka You belong to antioxidant laboratory of Gifu University, sponsored by TIMA e.s.. Toshikazu Yoshikawa is an adviser of TIMA e.s.. Markus Matuschka von Greiffenclau is a chairman of TIMA e.s..

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**Table 1. Characteristics of the subjects who reached evaluation at 6 month**

	<b>placebo</b>	<b>Twendee X</b>	
	<b>(n = 27)</b>	<b>(n = 32)</b>	<b>p value</b>
<b>Demographic characteristics</b>			
Female	44.4 %	56.2 %	0.366



Age (y)	76.3 ± 5.0	75.2 ± 4.4	0.383
<b>Complications</b>			
Hypertension	48.1 %	25.0 %	0.064
Diabetes mellitus	3.7 %	18.8 %	0.075
Dyslipidemia	40.1 %	15.6 %	0.031*
Ischemic heart disease	3.7 %	0.0 %	0.272
<b>Baseline scores</b>			
MMSE (/30)	27.2 ± 2.0	27.2 ± 2.2	0.945
HDS-R (/30)	25.6 ± 3.3	26.5 ± 2.6	0.305
GDS (/15)	3.4 ± 2.8	4.4 ± 4.0	0.616
AS (/42)	11.3 ± 5.9	10.3 ± 6.9	0.493
ABS (/44)	2.1 ± 3.4	1.1 ± 1.7	0.408
ADCS-ADL (/30)	26.1 ± 3.4	27.6 ± 3.0	0.119

MMSE, Mini-mental state examination; HDS-R, Hasegawa dementia scale - revised; GDS, geriatric depression scale; AS, Apathy scale; ABS, Abe's BPSD score; ADCS-ADL, Alzheimer's disease cooperative study - activities of daily living inventory.

\*  $p < 0.05$  (vs placebo)

**Table 2. Cognitive and affective score changes in primary and secondary outcomes from baseline at 6 month**

	<b>placebo</b> (p value for change from baseline)	<b>Twendee X</b> (p value for change from baseline)	<b>p value</b> placebo vs Twendee X
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**Primary outcomes**

MMSE	-0.85 ± 2.48 (0.092)	0.66 ± 2.60 (0.181)	0.018*
HDS-R	0.74 ± 2.19 (0.112)	1.09 ± 2.62 (0.025#)	0.593
<b>Secondary outcomes</b>			
GDS	0.08 ± 2.10 (0.829)	-0.43 ± 3.19 (0.554)	0.315
AS	-0.38 ± 5.05 (0.615)	0.93 ± 5.96 (0.684)	0.356
ABS	-0.38 ± 1.44 (0.228)	-0.26 ± 1.10 (0.294)	0.252
ADCS-ADL	0.59 ± 1.96 (0.210)	-1.11 ± 3.61 (0.379)	0.284

MMSE, Mini-mental state examination; HDS-R, Hasegawa dementia scale - revised; GDS, geriatric depression scale; AS, Apathy scale; ABS, Abe's BPSD score; ADCS-ADL, Alzheimer's disease cooperative study - activities of daily living inventory.

\*  $p < 0.05$  (vs placebo), #  $p < 0.05$  (vs baseline)

Fig. 1 Randomization, assignment and follow-up

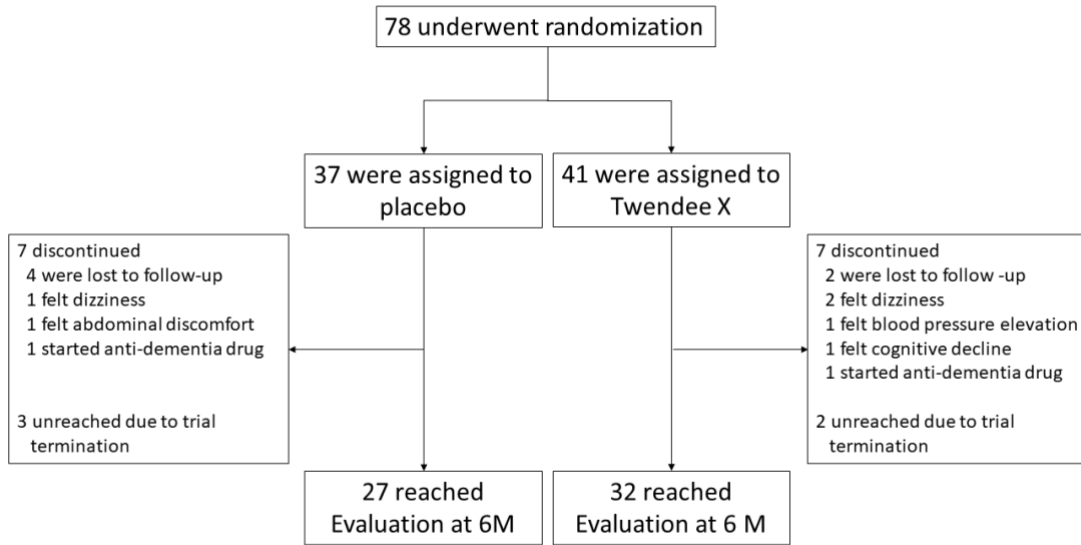
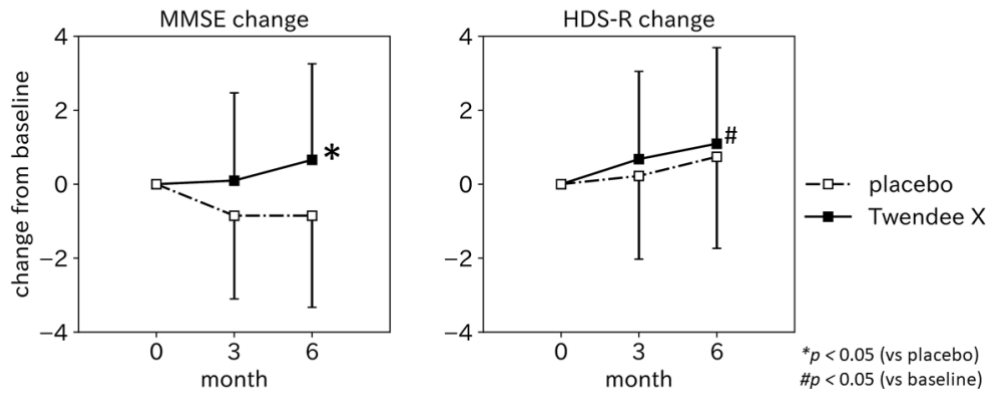


Fig. 2 MMSE and HDS-R change



## FIGURE LEGENDS

Fig. 1) Randomization, assignment and follow-up of the present trial.

Fig. 2) MMSE and HDS-R change in 6 M. Note the significant difference of MMSE score between

Twendee X and placebo at 6 M ( $*p < 0.05$ , left panel), and a significant improvement of HDS-

R from baseline to 6 M only in TwX group ( $\#p < 0.05$ , right panel).