DL-3-n-butylphthalide Delays Cognitive Decline in Patients With Mild to Moderate Alzheimer's Disease Already Receiving Donepezil: A Multicenter, Prospective Cohort Study

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Research

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Abstract

Background: Vascular factors and mitochondria dysfunction contributeto thepathogenesis of Alzheimer's Disease (AD).DL-3-n-butylphthalide (NBP)has an effect in protecting mitochondria and improving microcirculation. We investigated the effect of NBP in patients with mild-moderate AD already receiving donepezil.

Methods: It was a prospective cohort study. 92 mild-moderate AD patients were classified into the donepezil alone group (n=43) or the donepezil combined NBP group (n=49) for 48 weeks. The primary outcome was change of Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog) from baseline after treatment 48 weeks. All patients were also evaluated with clinician's interview-based impression of change plus caregiver input (CIBIC-plus), Alzheimer's disease cooperative study-activities of daily living (ADCS-ADL) and neuropsychiatric inventory (NPI) every 12 weeks. All patients were monitored for adverse events (AEs). The efficacy was analyzed using logistic regression analysis.

Results: The univariate analysis showed that age wasolder in donepezil alone group(*P*=0.005), prevalence of hypertension was higher in donepezil alone group(*P*=0.026).The ADAS-cog score change from baseline in thedonepezil alone group was significant than that in the donepezil combined NBP group at 48 weeks(1.82±5.20 vs -0.38±4.46, *P*=0.048). The multivariate logistic regression analysis showed that between the 2 groups, there were significant differencesin changes on the ADAS-cog(OR=0.879,95% CI:[0.785,0.984],*P*=0.026),MMSE(OR=1.270,95% CI:[1.036,1.557],*P*=0.021), and ADCS-ADL(OR=1.067,95% CI:[1.002,1.136],*P*=0.042) but no significant differences for changes on the NPI(OR=0.955,95% CI:[0.901 1.013],*P*=0.125)and CIBIC-plus (OR=0.356,95% CI:[0.093,1.364],*P*=0.132). The occurrence of AEs was similar in the 2 groups.

Conclusions: Over the 48-week treatment period, donepezil combined NBP group had slower cognitive decline and better activities of daily living in patients with mild to moderate AD. These indicated that the multi-target therapeutic effect of NBP may be a new choice for AD treatment.

Trial registration:

Clinical trial registration URL:

https://clinicaltrials.gov/ct2/show/NCT02711683?term=NCT02711683&draw=2&rank=1

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1. Background

Alzheimer's disease (AD) is the most common cause of dementia. At present, there are no drugs that can cure AD, and the drugs currently used can only temporarily improve the symptoms of AD patients. Although the pathogenesis of AD had not been fully understood, recent studies have shown that there are extensive vascular lesions in the brain of AD patients, including asymptomatic infarction and

demyelination of the white matter. The blood flow of the cerebral hemispheres, especially the temporal parietal lobe, decreases; risk factors for vascular diseases, such as hypertension, diabetes, hyperlipidemia, and hyperhomocysteine, are closely related to the pathogenesis of AD; and vascular risk factor intervention is currently the main method to prevent AD. These findings suggest that vascular factors play an important role in the occurrence and development of AD [1–4]. Unlike other tissues and organs in the body, brain tissue consumes a large amount of sugar and oxygen but has no sugar and oxygen reserves. Therefore, brain tissue is particularly sensitive to ischemia and hypoxia [5]. Mitochondria are the main source of energy metabolism in brain tissue, and reduced blood flow in brain tissue will first affect mitochondrial energy metabolism. Therefore, brain microcirculatory disorders and mitochondrial dysfunction may play important roles in the pathogenesis of AD and are hotspots in the current study of the pathogenesis of AD.

DL-3-n-butylphthalide (NBP), a new drug developed in China, is extracted from celery seeds and has a unique effect in protecting mitochondria and improving microcirculation. It is wildly used in acute ischemic stroke patients and can significantly improve neurological damage. In vitro and animal experiments, NBP alleviated the neurotoxicity of Aβ, improved the learning and memory function of rats through inhibiting the NF-κB pathway [6, 7], downregulated autophagy to increase cell viability [8]. The potential mechanisms of NBP regarding its neuroprotective effects include anti-oxidant and anti-inflammatory effects and the stimulation of the proliferation, migration, and differentiation of hippocampal neural stem cells [9]. In clinic trail,NBP was shown to effectively improving cognitive and global functioning in patients with subcortical vascular cognitive impairment without dementia over a 6-month treatment period[10]. However, the effects of NBP on AD is not studied. Here, we investigated the effect of NBP in patients with mild to moderate AD patients already receiving donepezil.

2. Methods

2.1 Study design

This was a prospective cohort study. The patients were enrolled from the Department of Neurology or Geriatrics of 5 hospitals in Xi'an, China. The experimental protocol was reviewed by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (approval number: XJTU1AF2015LSL-066). All subjects signed an informed consent form. The study was registered in Clinical Trials (registration number: NCT02711683) and was funded by the clinical research project of the First Affiliated Hospital of Xi'an Jiaotong University.

2.2 Participants And Eligibility Criteria

Each patient was examined by a doctor in the clinic and was given a diagnosis of AD according to their clinical history, laboratory examination and brain MRI.

All patient met the following inclusion criteria: 1) age of 50–85 years old (including those who were 50 and 85 years old), either sex; 2) met the diagnostic criteria for suspected AD by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer‘s Disease and Related Disorders Association (NINCDS-ADRDA) (1984)[11]; 3) mild to moderate AD patients, that is, patients with 11 points ≤ MMSE total score ≤ 26 points (or patients with an elementary school education level: 11 points ≤ MMSE total score ≤ 22 points); [12, 13] 4) total Hachinski ischemic scale (HIS) [14] score ≤ 4 points; 5) memory loss for at least 12 months, with a tendency of progressive deterioration; 6) brain magnetic resonance imaging (MRI) scan suggest a significant possibility of AD (medial temporal lobe atrophy (MTA) visual rating scale[15] grade 2 or higher, Fazekas scale[16] ≤ 2); 7) no obvious physical signs during nervous system examination; 8) no prior history of treatment with donepezil or with treatment history of donepezil and a relatively stable disease status; 9) stable and reliable caregivers, with the ability to contact the caregivers frequently (at least 4 days a week, and at least 2 hours a day); the caregivers helped the patients participate throughout trial; 10) elementary school or higher education level and the ability to complete the cognitive ability measurement and other tests specified in the protocol; and 11) signed an informed consent form.

Exclusion criteria were 1) previous nervous system diseases (including stroke, optic neuromyelitis, Parkinson's disease, epilepsy, etc.); 2) mental illness, according to the *Diagnostic and* Statistical Manual *of* Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria[17], including schizophrenia and other mental illness, bipolar disorder, and severe depression or paralysis; 3) unstable or severe heart, lung, liver, kidney, or hematopoietic diseases; 4) uncorrectable visual and auditory disorders that affected completing neuropsychological tests and scale assessments; and 5) simultaneous use of other cholinesterase inhibitors or Memantine.

2.3 Interventions

According to the patient's condition, family income, compliance and other factors, the doctor consults with the patients’ caregivers to formulate the treatment plan. Patients with mild to moderate AD were divided into the donepezil alone group and donepezil combined NBP group according to different treatment regimens: donepezil alone group - donepezil 5 mg, once daily; donepezil combined NBP group: donepezil 5 mg daily plus NBP 200 mg, three times a day for 48weeks.

2.4 Trial End Point And Evaluation Indexes

The primary end point index was the Alzheimer's disease assessment scale-cognitive subscale (ADAS-Cog/12) score [18]. Secondary end point indexes were the clinician's interview-based impression of change plus caregiver input (CIBIC-Plus) scale [19], Alzheimer's disease cooperative study ADL scale (ADCS-ADL) [20], and neuropsychiatric inventory (NPI) scale [21]. The ADAS-Cog can assess 6 cognitive fields, including memory, language, orientation, logic, social cognition, and attention. The lower the score is, the lighter the cognitive damage. The CIBIC-Plus reflects the improvement of an individual’s clinical

symptoms by interviewing the individual and his/her caregiver. The score ranges from 1 to 7 points, where 1 represents maximum improvement, 4 represents no change, and 7 represents maximum deterioration. The clinician's impression of the severity of the disease based on the interview with the subject at baseline served as the reference for CIBIC-Plus scoring in follow-ups. Clinicians evaluating the CIBIC-Plus were not aware of the scores of other scales. The ADCS-ADL is a clinician assessment standardized questionnaire composed of 23 items that assesses the actual performance of specific actions and behaviors of the individual observed by the caregiver in the past 4 weeks. The score ranges from 0 to 78 points, and the lower the score is, the more severe the disorder. The NPI includes 10 items regarding behavior and 2 items regarding the autonomic nervous system. The total score ranges from 0 to 144, and the higher the score is, the more severe the damage.

Safety assessments included physical examinations, vital signs, and adverse event (AE) reports. Each patient attended 5 visits, including baseline, 12 weeks, 24 weeks, 36 weeks, and 48 weeks. At each follow-up, the above scales were evaluated, and AEs were recorded.

To make ensure the reliable of all assessment, a blind method was used. Neuropsychological assessors didn’t know what treatment each patient received. Each center at least had two neuropsychological assessors, one responsible for the assessment of the MMSE and ADAS-cog, another responsible for the evaluation of ADL, NPI, CIBIC-plus. The scale assessors and doctors received uniform training in scale testing and disease diagnosis. The reliability of the cognitive tests and diagnoses between assessors was greater than 0.90. All trainees passed a scale consistency test before participating in the trial.

2.5 Statistical Analysis

The difference between the scale scores at 48 weeks (ADAS-cog, NPI, ADCS-ADL, and CIBIC-plus) and the scale scores at baseline was used as an indicator of changes in cognitive function, mental behavior, daily living ability, and overall efficacy. All data were used to build a database in EpiData and were analyzed using SPSS 18.0 statistical software. Variables related to the study participants that conformed to an approximate normal distribution are expressed as the mean ± standard deviation (x ± s), variables that had a severely skewed distribution are expressed in quartiles, and categorical variables are expressed as value (%). The x^2 test, *t* test or rank sum test was used for comparisons between groups according to the different types of data, and multivariate logistic regression was used for the multivariate analysis. $P < 0.05$ (double sided) represented statistical significance for all tests.

3. Results

3.1 General information of patients

One hundred twenty-six outpatients with dementia were selected from the Department of Neurology of 5 hospitals in Xi'an, China, from January 2016 to June 2018, among whom 92 patients with mild to

moderate AD met the inclusion criteria. Figure 1 shows the screening and grouping strategy in more detail [see Fig. 1]

At baseline, age in the donepezil combined NBP group was younger than that in the donepezil alone group ($P = 0.005$). The remaining demographic information and clinical characteristics had no significantly difference between the 2 groups (Table 1). Because both groups had patients who failed to complete the experiment, the patients in two groups who completed the 48week follow-up were 37 and 41 respectively.

Table 1
Baseline characteristics of the participants

	Total (n = 92)	**Donepezil (n = 43)**	**Donepezil combined NBP (n = 49)**	***P***
Age, mean ± SD, y	69.36 ± 8.37	71.93 ± 8.36	67.10 ± 7.79	0.005
Male, n(%)	46(50.0)	23(53.5)	23(46.9)	0.531
Education, n(%)				
≤6y	12(13.0)	5(11.6)	7(14.3)	0.706
6y	80(87.0)	38(88.4)	42(85.7)	
Duration, y	3[2, 4]	3[2, 5]	3[2, 3]	0.494
Medical history, n(%)				
Hypertension	28(30.4)	13(30.2)	15(30.6)	0.901
Diabetes mellitus	13(14.1)	7(16.3)	6(12.2)	0.579
MRI Fazekas score, n(%)				
0	75(81.5)	31(72.1)	44(89.8)	0.062
1	15(16.3)	10(23.3)	5(10.2)	
2	2(2.2)	2(4.7)	0(0.0)	
MRI MTA score				
1	5(5.4)	3(7.0)	2(4.1)	0.477
2	81(88.0)	36(83.7)	45(91.8)	
3	6(6.5)	4(9.3)	2(4.1)	
Psychometric scores, mean ± SD				
MMSE	18.78 ± 4.06	18.84 ± 4.09	18.74 ± 4.07	0.905
ADAS-cog	21.63 ± 9.03	21.84 ± 9.73	21.44 ± 8.48	0.832
ADCS-ADL	63.97 ± 9.84	61.98 ± 12.37	65.59 ± 6.32	0.076
NPI	10.01 ± 11.48	9.83 ± 12.21	10.17 ± 10.93	0.892

Abbreviations: NBP, dl-3-n-butylphthalide; SD, standard deviation; MRI, magnetic resonance imaging; MTA, medial temporal lobe atrophy; MMSE, mini-mental state examination; ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; ADCS-ADL, Alzheimer's disease cooperative study activities of daily living scale; NPI, neuropsychiatric inventory; HAMD, Hamilton depression scale.

	Total (n = 92)	Donepezil (n = 43)	Donepezil combined NBP (n = 49)	*P*
HAMD	2.23 ± 2.65	2.16 ± 2.48	2.29 ± 2.82	0.826

Abbreviations: NBP, dl-3-n-butylphthalide; SD, standard deviation; MRI, magnetic resonance imaging; MTA, medial temporal lobe atrophy; MMSE, mini-mental state examination; ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; ADCS-ADL, Alzheimer's disease cooperative study activities of daily living scale; NPI, neuropsychiatric inventory; HAMD, Hamilton depression scale.

3.2 Changes of the neuropsychological scores.

The ADAS-cog score change from baseline to week 48 was significantly different between the two groups (1.82 ± 5.20 vs. -0.38 ± 4.46, *P* = 0.048). However,there was no difference between the two groups at other follow-up time points. The MMSE score change from baseline to week 24 was significantly different between the two groups (-0.909 ± 2.429 vs. 0 ± 2.536, *P* = 0.020), while there was no difference between the two groups at other follow-up time points. The ADCS-ADL, NPI, CIBIC-plus scores change from baseline to each follow-up time point also had no difference between the two groups (shown in Table 2). Figure 2 shows this in more detail [see Fig. 2]

Table 2
Univariate analysis of psychometric score changes from baseline to week 48.

	Donepezil (n = 37)	Donepezil combined NBP (n = 41)	*P*
ADAS-cog	1.82 ± 5.20	-0.38 ± 4.46	0.048
MMSE	-1.16 ± 3.07	-0.02 ± 2.34	0.068
ADCS-ADL	-6.87 ± 9.55	-4.73 ± 7.77	0.286
NPI	1.54 ± 11.05	-2.68 ± 10.33	0.085
CIBIC-plus	4.19 ± 0.57	4.00 ± 0.55	0.139
AE	2(5.4)	2(4.9)	0.916

Abbreviations: NBP, dl-3-n-butylphthalide; ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; MMSE, mini-mental state examination; ADCS-ADL, Alzheimer's disease cooperative study activities of daily living scale; NPI, neuropsychiatric inventory; CIBIC-plus, clinician's interview-based impression of change plus caregiver input; AE, adverse event.

Table 3
Multivariate logistic regression analysis of psychometric score changes from baseline to week 48.

variables	B	S.E.	Wald	OR	95%CI	*p*
ADAS-cog	-0.129	0.058	4.978	0.879	0.785–0.984	0.026
MMSE	0.239	0.104	5.311	1.270	1.036–1.557	0.021
ADCS-ADL	0.065	0.032	4.130	1.067	1.002–1.136	0.042
NPI	-0.046	0.030	2.356	0.955	0.901–1.013	0.125
CIBIC-plus	-1.033	0.686	2.272	0.356	0.093–1.364	0.132

Abbreviations: ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; MMSE, mini-mental state examination; ADCS-ADL, Alzheimer's disease cooperative study activities of daily living scale; NPI, neuropsychiatric inventory; CIBIC-plus, clinician's interview-based impression of change plus caregiver input.

The multivariate logistic regression analysis showed that there was a significant difference for change from baseline to week 48 for the ADAS-cog (OR = 0.879, 95% CI: [0.785, 0.984], $P = 0.026$), MMSE (OR = 1.270, 95% CI: [1.036, 1.557], $P = 0.021$), ADCS-ADL (OR = 1.067, 95% CI: [1.002, 1.136], $P = 0.042$) scores and no significant difference for change from baseline for the NPI (OR = 0.955, 95% CI: [0.901, 1.013], $P = 0.125$) and CIBIC-plus (OR = 0.356, 95% CI: [0.093, 1.364], $P = 0.132$) scores between the 2 groups (Table 3).

3.3 Safety

The occurrence of AEs was similar in the 2 groups (Table 2). The main adverse reactions are gastrointestinal reactions, such as nausea, vomiting, anorexia. In general, patients with good tolerance, do not affect the continued use of drugs.

4. Discussion

Currently, AD is still mainly treated with cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, which can only delay the progression of the disease [22–26]. All treatments targeting the pathological changes of AD are still in the testing phase [27–30]. Because the pathogenesis of AD involves a variety of mechanisms, multi-target treatment may be a new direction for AD treatment. As the first clinical trial using NBP for the treatment of AD, our trial is an exploratory study. From an ethical perspective, we designed the study for all AD patients already undergoing treatment with donepezil, and then based on this treatment, NBP was administered in combination with donepezil, or donepezil was administered alone. The design of the trial, patient selection, outcome analysis, sample size estimation, and experimental observation time were determined based on previous relevant clinical studies of AD. The study results also provide new ideas for AD treatment.

The cascade hypothesis of Aβ represents the core pathogenesis of AD, while inflammatory reactions, oxidative stress, free radical damage, and mitochondrial dysfunction are also possible mechanisms that lead to the onset of AD [31–33]. Previous animal experiments have shown that NBP treatment can reduce oxidative stress and soluble Aβ and Aβ oligomers in the brain of APP/PS1 rats, improve synapse plasticity, and reduce learning and memory defects [34]. Some studies also found that in 3xTg-AD mice, NBP could promote the metabolism of APP to non-amyloid formation and reduce the production of Aβ [35], protecting the synapse function of aged APP Tg mice by inhibiting the deposition of Aβ senile plaques and neuroinflammatory reactions [36]. Therefore, DBP shows promising preclinical potential as a multi-target drug for the prevention and treatment of AD.

This trial was an observational study, and there was a significant difference in age between the 2 groups, mainly because the family members of patients in different age ranges had different opinions regarding treatment: family members of younger patients were more inclined to active treatment, and therefore, patients who opted for donepezil combined NBP were younger than patients who opted for donepezil alone. However, age was corrected for when analyzing differences in neuropsychiatric scales between groups. After the common covariates that could affect cognitive function were corrected, the 2 groups demonstrated significant differences in ADAS-cog, MMSE, and ADCS-ADL scores when compared with baseline. Therefore, the application of donepezil combined NBP, compared with donepezil treatment alone, can delay the deterioration of cognitive function in patients with mild to moderate AD and improve their daily living ability. The major adverse reaction in the 2 groups was side effects in the gastrointestinal tract, which were related to the drug itself. However, the incidence of adverse reactions was low, and there was no significant difference between the 2 groups.

There are some limitations to this trial. First, this was a prospective cohort study rather than a randomized controlled trial (RCT), and there was a certain bias in patient grouping. Second, the sample size was too small. Third, we did not detect the biomarkers of AD, such as brain glucose metabolism and Aβ deposition. It is necessary to design a RCT and use subjective measure to further clarify the effects of NBP on AD progression.

5. Conclusions

Over the 48-week treatment period, donepezil combined NBP was effectively delayed cognitive decline and improved activities of daily living in patients with mild to moderate AD. These indicated that the multi-target therapeutic effect of NBP may be a new choice for AD treatment.

Declarations

Availability of data and materials

The data that support the findings of this study are available on reasonable request from the corresponding author Qiumin Qu. The data are not publicly available due to protection of personal

information of participants.

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Authors' contributions

JW made substantial contributions to the analysis and interpretation of the data and was involved in drafting and revising the manuscript. XG, WL□JL□HZ□QQ□HS and LM took part in the recruitmentand assessment of patients with AD. JWand QQ designed the prospective cohort study.FG contributed to the analysis of data. QQ was involved in revising the manuscript critically. All authors have read and approved the final article.

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Ethics declarations

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (No: XJTU1AF2015LSL-066), and written informed consent was obtained from every participant, or their caregivers.The study was registered in Clinical Trials (registration number: NCT02711683).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

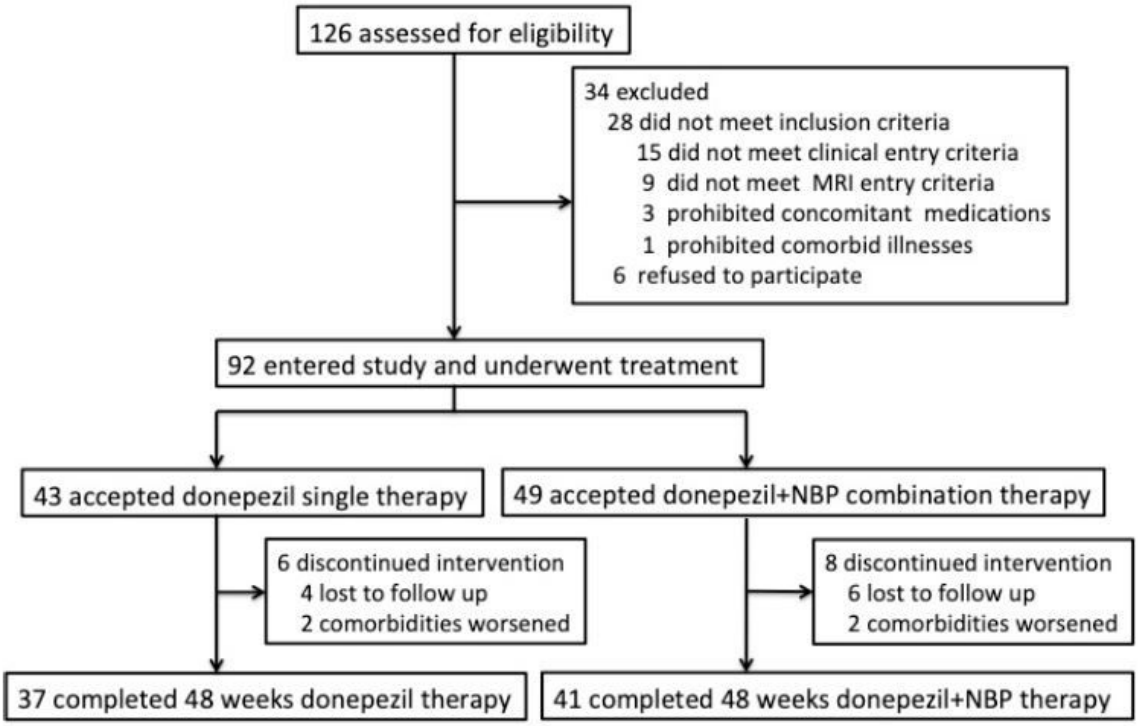

Figure 1

Trial profile.

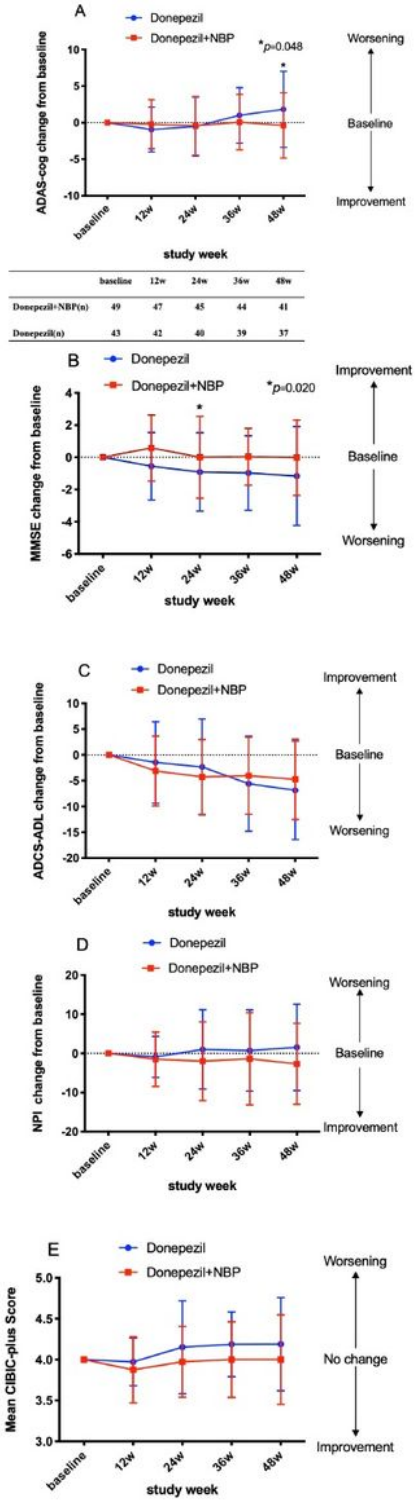

Figure 2

Comparison of psychometric score changes from baseline to different follow-up periods between the two groups. A. Alzheimer’s disease assessment scale-cognitive subscale (ADAS-cog) change from baseline; B. mini-mental state examination (MMSE) change from baseline; C. Alzheimer's disease cooperative study activities of daily living scale (ADCS-ADL) change from baseline; D. neuropsychiatric inventory (NPI)

change from baseline; E. clinician's interview-based impression of change plus caregiver input (CIBIC-plus).