

ARCHIVAL REPORT

Benzoate, a D-Amino Acid Oxidase Inhibitor, for the Treatment of Early-Phase Alzheimer Disease: A Randomized, Double-Blind, Placebo-Controlled Trial

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Background: *N*-methyl-D-aspartate receptor (NMDAR)-mediated neurotransmission is vital for learning and memory. Hypofunction of NMDAR has been reported to play a role in the pathophysiology of Alzheimer disease (AD), particularly in the early phase. Enhancing NMDAR activation might be a novel treatment approach. One of the methods to enhance NMDAR activity is to raise the levels of NMDA coagonists by blocking their metabolism. This study examined the efficacy and safety of sodium benzoate, a D-amino acid oxidase inhibitor, for the treatment of amnesic mild cognitive impairment and mild AD.

Methods: We conducted a randomized, double-blind, placebo-controlled trial in four major medical centers in Taiwan. Sixty patients with amnesic mild cognitive impairment or mild AD were treated with 250–750 mg/day of sodium benzoate or placebo for 24 weeks. Alzheimer's Disease Assessment Scale-cognitive subscale (the primary outcome) and global function (assessed by Clinician Interview Based Impression of Change plus Caregiver Input) were measured every 8 weeks. Additional cognition composite was measured at baseline and endpoint.

Results: Sodium benzoate produced a better improvement than placebo in Alzheimer's Disease Assessment Scale-cognitive subscale ($p = .0021, .0116, \text{ and } .0031$ at week 16, week 24, and endpoint, respectively), additional cognition composite ($p = .007$ at endpoint) and Clinician Interview Based Impression of Change plus Caregiver Input ($p = .015, .016, \text{ and } .012$ at week 16, week 24, and endpoint, respectively). Sodium benzoate was well-tolerated without evident side-effects.

Conclusions: Sodium benzoate substantially improved cognitive and overall functions in patients with early-phase AD. The preliminary results show promise for D-amino acid oxidase inhibition as a novel approach for early dementing processes.

Key Words: Alzheimer disease, clinical trial, D-amino acids oxidase (DAAO) inhibitor, mild cognitive impairment, *N*-methyl-D-aspartate, sodium benzoate

The prevalence of dementia in elderly persons is increasing rapidly in the aging society, of which the deteriorating clinical course is a heavy burden to both the patients and their family. Early detection and intervention of Alzheimer disease (AD) is pivotal for the outcome (1). Mild cognitive impairment (MCI), particularly amnesic mild cognitive impairment (aMCI), is a risk factor and might be a prodromal stage of AD. The mainstream treatment for mild and moderate AD is acetylcholine esterase inhibitor (AChEI). However, its efficacy and tolerability are unsatisfactory. Furthermore, AChEI does not show convincing efficacy for MCI (2–4), implying that other mechanism(s) might underlie the pathogenesis of early-phase AD.

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Excessive glutamatergic neurotransmission, particularly through the *N*-methyl-D-aspartate receptor (NMDAR), leads to neurotoxicity (5,6), which is implicated in the pathophysiology of AD, especially in the late phase. The NMDAR antagonists are developed for the treatment of AD, on the basis of the hypothesis of NMDAR overactivation (7). Memantine is an uncompetitive NMDAR partial antagonist of low affinity, which supposedly can block NMDAR overactivation by preventing excessive influx of calcium (8–10). Memantine is approved as an antidementia medication for moderate–severe AD (11); however, it has limited efficacy at the early phase, including MCI and mild AD (12). The NMDAR antagonists such as MK-801 also induce apoptosis and neurodegeneration in both in vitro and in vivo studies (13). Ketamine, another NMDAR antagonist, impaired spatial learning and verbal information ability in healthy humans in a double-blind, randomized, placebo-controlled trial (14). These findings raise concern that NMDA antagonist might impair cognition and memory in early AD.

Conversely, optimal NMDAR activation is pivotal for synaptic plasticity (15), memory, and cognitive function (16). Attenuation of NMDAR-mediated neurotransmission can result in loss of neuronal plasticity and cognitive deficits in the aging brain, which might account for clinical deterioration and brain atrophy (17). Age-related decrease in the density of NMDAR in cerebral cortex and hippocampus was observed in humans (18). Earlier studies also found a decrease of glycine-dependent radioligand binding to the NMDAR in cerebral cortices from postmortem and neurosurgical tissues in patients with AD (19,20). D-cycloserine, a partial agonist at the glycine site of NMDAR, was reported in some clinical studies to activate the NMDAR in brains of AD patients (21) and improve their score on the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) (22). Recently, 1000 mg/day of sodium benzoate, an inhibitor of

D-amino acids oxidase (DAAO), was also found to be beneficial for neurocognitive function in patients with schizophrenia (23). Because of the supporting evidence, we propose that NMDA-enhancing agents might be beneficial for the early declining process of AD, due to their role in learning and memory as well as neurogenesis and neuroplasticity.

There are several avenues to enhance NMDA activation. One of them is inhibiting the activity of DAAO, a flavoenzyme of peroxisomes responsible for degrading D-serine and D-alanine (24–26), and thereby raising levels of the D-amino acids that are the neurotransmitters for the coagonist site of the NMDAR. Recent data indicate that aging is related to reduced D-serine levels and thereby impaired NMDAR transmission, and D-serine treatment significantly decreases the extent of neuron death, suggesting that D-serine has neuroprotective effect against apoptosis (27). In addition, neural stem cells from postnatal mouse forebrain can synthesize D-serine and thereby stimulate proliferation and neuronal differentiation of the stem cells (28).

Enhancing NMDAR through DAAO inhibition might be a safe way to reduce nephrotoxicity of D-serine (29), particularly in the elderly population. Sodium benzoate is a DAAO inhibitor. Benzoic acid exists in many plants and is a natural constituent of food, including milk products (30). Benzoic acid and its salts, including sodium benzoate, which are generally recognized as safe, are also food preservatives widely used in manufacturing fruit jelly, buffer, soy-bean sauce, processed meat, and the like (31).

There are several other preclinical studies supporting the central nervous system (CNS) effects of DAAO inhibitors, although the memory effect was not examined (32–34). Sodium benzoate is effective in NMDAR models such as pain relief (35,36) and partially prevented cell death in glial cells (37). The CNS bioavailability of benzoate is good (38). To test the hypothesis that DAAO inhibition is beneficial for the early phase of dementia, we conducted this trial to examine the efficacy and safety of sodium benzoate in patients with aMCI or mild AD.

Methods and Materials

Participants

Patients were recruited from the outpatient clinics at the Department of Psychiatry and Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung; Department of Psychiatry, China Medical University Hospital, Taichung; Department of Psychiatry, Taichung Veterans General Hospital, Taichung; and Department of Neurology, Lin-Shin Hospital, Taichung, which are four major medical centers in Taiwan. The study was approved by the institutional review board at four sites and conducted in accordance with the current revision of the Declaration of Helsinki. Patients were evaluated by research psychiatrists and neurologists after a thorough medical and neurological workup.

Patients were enrolled into this study if they: 1) satisfied National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (39) criteria for probable AD and had a Clinical Dementia Rating (CDR) (40) score of 1 or criteria for an aMCI (41) of a presumably degenerative nature defined as subjective memory complaint corroborated by an informant and insufficient global cognitive and functional impairment to meet National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria and had a CDR score of .5; 2) 50–90 years of age; 3) were physically healthy and had all laboratory assessments

(including urine/blood routine, biochemical tests, and electrocardiograph) within normal limits; 4) had a Mini-Mental State Examination (42) score of 17–26; 5) had sufficient education to communicate effectively and were capable of completing the assessments of the study; and 6) agreed to participate in the study and provided informed consent. For patients who had already been on a regimen of AChE therapy, AChE had to be continued for at least 3 months before enrollment. The AChE dose had to be kept unchanged during the study duration. For patients who had not yet been on a regimen of AChE therapy, AChE or other antedementia medication was forbidden during the study duration.

Exclusion criteria included history of significant cerebrovascular disease; Hachinski Ischemic Score >4; major neurological, psychiatric, or medical conditions other than AD; substance (including alcohol) abuse or dependence; delusion, hallucination, or delirium symptoms; severe visual or hearing loss; and inability to follow protocol.

Study Design

All patients were randomly assigned to receive a 24-week treatment of sodium benzoate or placebo in a double-blind manner. Efficacy and safety were evaluated at baseline and at the ends of weeks 8, 16, and 24. Two hundred fifty milligrams of sodium benzoate or placebo were packed with identical capsules provided in coded containers. The dose was started at 250–500 mg/day (250 mg once or twice daily) in the first 8 weeks, then increased by 250–500 mg/day from the 9th week, and further increased by another 250–500 mg/day from the 17th week of the study if clinically indicated. On the basis of an earlier study in which sodium benzoate given at the dose of 1000 mg/day improved a variety of symptom domains and neurocognition in patients with chronic schizophrenia without obvious side effects (23), we decided to apply 250–750 mg/day, considering the older age of the subjects in the present study. Patients were randomized in a cluster of six subjects to receive sodium benzoate or placebo in a 1:1 ratio by an independent investigational pharmacist.

Patients, caregivers, and investigators, except the investigational pharmacist, were all blind to the assignment. Patient medical adherence and safety were closely monitored by caregivers and research physicians, and pill-counting was monitored by the study staff.

Assessments

The primary outcome was the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) (43) measured at weeks 0, 8, 16, and 24. The ADAS-cog is the most popular cognitive assessment instrument used in AD clinical trials. It consists of 11 tasks, including word recall, naming, commands, constructional praxis, ideational praxis, orientation, word recognition, instructions remembering, spoken language ability, word-finding difficulty, and comprehension. Its scores range from 0 (best) to 70 (worst).

The secondary outcome measurements included the Clinician's Interview-Based Impression of Change plus Caregiver Input (CIBIC-plus) (44) measured at weeks 8, 16, and 24 and the additional cognition composite measured at baseline and endpoint.

The CIBIC-plus is a global assessment of change based on a comprehensive, semi-structured interview that includes caregiver-supplied information. It is a 7-point rating scale ranging from 1 to 7, where 1 represents markedly improved; 4 represents no change; and 7 represents markedly worse.

The additional cognition composite was calculated by the average of the T scores of speed of processing (Category Fluency), working memory (Wechsler Memory Scale-Third Edition, Spatial Span) (45), and verbal learning and memory tests (Wechsler Memory

Scale–Third Edition, Word Listing) (45). The raw score of speed of processing, working memory, and verbal learning and memory tests was standardized to a T score with a mean of 50 and an SD of 10 for making each test comparative. The additional cognition composite was applied in combination with ADAS-cog to make the cognitive assessment more thorough. Decrease in processing speed has been found to be associated with aging (46,47). Working memory (48) and verbal learning/memory (49) also decline in patients with AD.

Systemic side effects of treatments were evaluated by means of physical and neurological examinations and laboratory tests including CBC and biochemistry and reviewed by applying the Udvalg for Kliniske Undersogelser Side-effects Rating Scale (50) at baseline and weeks 8, 16, and 24.

Clinical ratings were performed by the research psychiatrists and neurologists who were trained and experienced in the rating scales. Inter-rater reliability was analyzed with the analysis of variance test. Only raters reaching the intra-class correlation coefficients of $\geq .90$ during prestudy training were allowed to rate the study patients. To maintain high inter-rater reliability and to prevent rater drift, raters met at least once/quarter for training and reliability retesting. To minimize inter-rater variability, each individual patient was assessed by the same research psychiatrist or neurologist throughout the trial.

Data Analysis

Chi-square test (or Fisher's exact test) was used to compare differences of categorical variables and Student two-sample *t* test (or Mann-Whitney *U* test if the distribution was not normal) was used for continuous variables between two treatment groups. Mean changes from baseline in repeated-measure assessments (ADAS-cog) were assessed with the generalized estimating equation (GEE) method with treatment, visit, and treatment-visit interaction as fixed effects and intercept as the only random effect and baseline value as the covariance. The GEE analyses were performed with the SAS/STAT (SAS Institute, Cary, North Carolina) "PROC GENMOD" procedure with first-order autoregressive working correlation structure with the marginal model instead of the mixed effect model. Therapeutic effect sizes (Cohen's *d*) were used to determine the magnitude of improvement for the continuous variables (51) resulting from sodium benzoate treatment compared with placebo.

Finally, all of the 60 randomized patients completed at least one follow-up, and 50 (90%) of them completed the 24-week trial (Figure 1). No imputation for the incomplete data was used for the GEE analysis.

There were no baseline scores for the CIBIC-plus, because this is scored as a judgment of change from baseline. Differences in CIBIC-plus scores at weeks 8, 16, and 24 and endpoint between groups

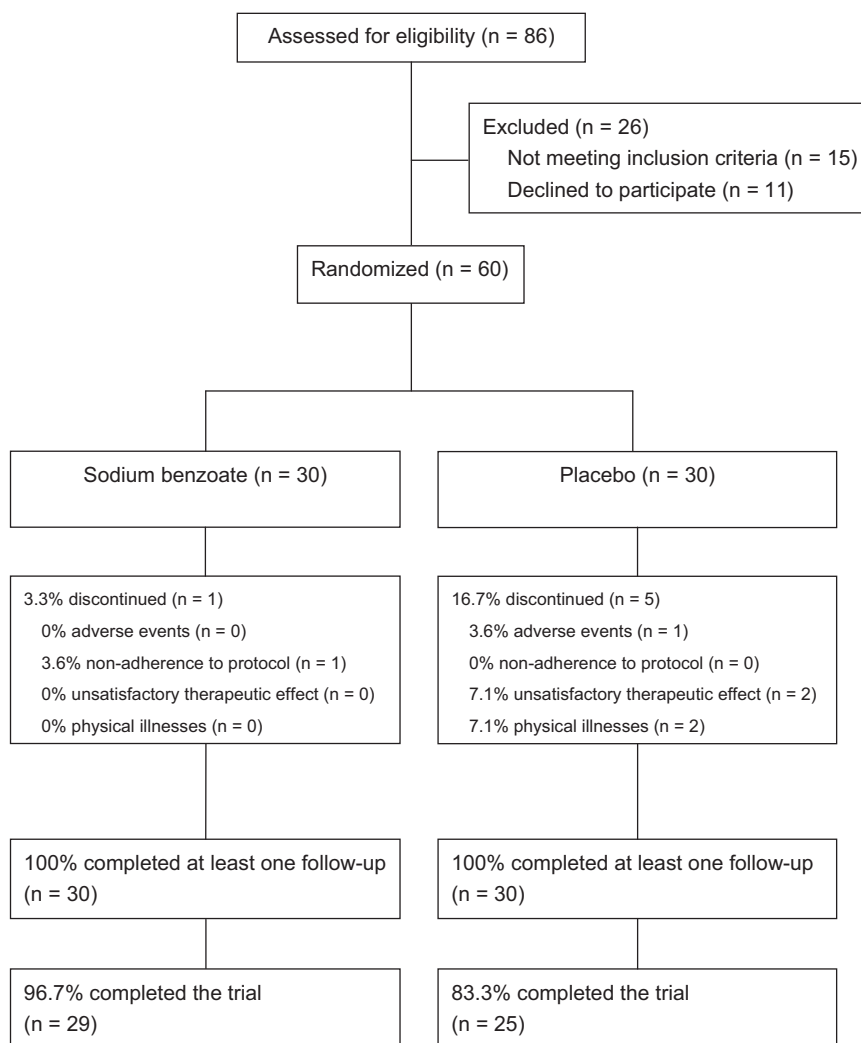


Figure 1. Flow diagram and disposition of the two treatment groups.

Table 1. Baseline Demographic Characteristics of the Placebo or Sodium Benzoate Treatment Group

	Treatment Groups		<i>p</i>
	Benzoate (<i>n</i> = 30)	Placebo (<i>n</i> = 30)	
Demographic Data			
Female, <i>n</i> (%)	18 (60.0)	19 (63.3)	1.0 ^a
Age, yrs, mean (SD)	70.7 (7.9)	69.7 (9.0)	.64 ^b
Age at illness onset, yrs, mean (SD)	69.8 (7.1)	68.5 (8.9)	.54 ^b
Illness duration, months, mean (SD)	14.2 (15.6)	13.6 (17.9)	.47 ^c
CDR at baseline, <i>n</i> (%)			1.0 ^a
CDR .5	15 (50.0)	16 (53.3)	
CDR 1	15 (50.0)	14 (46.7)	
Education, yrs, mean (SD)	5.9 (4.7)	7.5 (5.2)	.36 ^c
BMI, mean (SD)	24.6 (4.1)	23.9 (3.4)	.51 ^b
Patients using AChEIs, <i>n</i>			
Total	9	9	1.0 ^a
Donepezil (dose, mean ± SD)	7 (7.9 ± 2.7)	5 (8.0 ± 2.7)	1.0 ^c
Rivastigmine (dose, mean ± SD)	0 (0 ± .0)	3 (7.5 ± 2.6)	NA
Galantamine (dose, mean ± SD)	2 (16.0 ± .0)	1 (16.0 ± .0)	1.0 ^c

AChEI, acetylcholine esterase inhibitor; BMI, body mass index; CDR, Clinical Dementia Rating; NA, not associated.

^aFisher's exact test.

^bIndependent *t* test.

^cMann-Whitney *U* test.

were assessed by Student two-sample *t* test (or Mann-Whitney *U* test if the distribution was not normal).

Fisher's exact test was used to compare differences in the dropout rates between the two groups. Cohen's *w* was applied for

determining the effect size of categorical variables (52). All data were analyzed by IBM SPSS Statistics (version 18.0; SPSS, Chicago, Illinois) or SAS version 9.3. All *p* values for clinical measures were based on two-tailed tests with a significance level of .05.

Table 2. Mean ± SD Scores of Both Primary and Secondary Outcomes

Scale	Benzoate, Mean ± SD (<i>n</i>)	Placebo, Mean ± SD (<i>n</i>)	Estimate ^a	SEM	<i>Z</i>	<i>p</i>
Primary Outcome						
ADAS-cog						
Baseline	15.6 ± 7.6 (30)	15.0 ± 7.3 (30)				
Week 8	11.6 ± 6.5 (30)	11.7 ± 8.5 (30)	−2.8819	.9592	−3.00	.0027
Week 16	9.8 ± 6.2 (29)	12.3 ± 9.1 (26)	−1.7582	1.2270	−1.34	.1519
Week 24	9.7 ± 6.4 (28)	11.3 ± 9.2 (25)	−2.7456	1.1845	−2.32	.0205
Endpoint	9.6 ± 6.2 (30)	12.4 ± 9.1 (30)	−1.8067	1.1255	−1.61	.1084
Drug			−2.1860	2.2676	−.96	.3351
Week 8 × drug			−1.0236	1.1491	−.89	.3730
Week 16 × drug			−4.1835	1.3608	−3.07	.0021
Week 24 × drug			−3.3543	1.3294	−2.52	.0116
Endpoint × drug			−3.9648	1.3424	−2.95	.0031
Secondary Outcome						
Additional cognition composite (<i>T</i> score)			Cohen's <i>d</i>		<i>t</i>	
Baseline	48.9 ± 6.6 (26)	51.2 ± 8.4 (27)			−1.111	.272 ^b
Endpoint	50.4 ± 6.6 (26)	49.6 ± 8.7 (27)			.404	.688 ^b
Difference	1.5 ± 3.1 (26)	−1.6 ± 4.8 (27)	.7826		2.837	.007 ^b
CIBIC-plus			Cohen's <i>d</i>		<i>Z</i>	<i>p</i>
Week 8	3.4 ± .5 (30)	3.5 ± .6 (30)	.2441		−.869	.385 ^c
Week 16	3.3 ± .6 (30)	3.7 ± .7 (26)	.6637		−2.445	.015 ^c
Week 24	3.2 ± .7 (28)	3.7 ± .7 (27)	.6973		−2.416	.016 ^c
Endpoint	3.2 ± .7 (30)	3.7 ± .8 (30)	.7290		−2.520	.012 ^c

Results of measures of Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog), additional cognitive tests, and Clinician Interview Based Impression of Change plus Caregiver Input (CIBIC-plus) over the 24-week treatment with generalized estimating equations (GEE) method. Additional cognition composite, the composite test score of speed of processing, working memory, and verbal learning, and memory.

^aEstimate is the coefficient of treatment-visit interaction term in the GEE method multiple linear regression model. A first-order autoregressive covariance matrix was fit to the within-patient repeated measures. The *p* values were based on two-tailed tests.

^bIndependent *t* test.

^cMann-Whitney *U* test was used, because the distribution of CIBIC-plus score was not normal.

Table 3. Results of Measures of ADAS-cog Over 24-Week Treatment With GEE Method in Subgroups

Scale	Benzoate Mean \pm SD (n)	Placebo Mean \pm SD (n)	Estimate ^a	SEM	Z	p
CDR .5						
Baseline	13.8 \pm 6.1 (15)	11.9 \pm 5.4 (16)	Reference			.3560
Week 8 \times drug	10.4 \pm 6.0 (15)	8.3 \pm 4.0 (16)	.3213	.9690	.33	.7402
Week 16 \times drug	9.1 \pm 6.1 (15)	10.2 \pm 5.6 (15)	–3.2694	1.9222	–1.70	.0890
Week 24 \times drug	9.5 \pm 6.2 (14)	8.7 \pm 5.0 (14)	–1.8480	1.7834	–1.04	.3001
Endpoint \times drug	9.2 \pm 6.1 (15)	9.8 \pm 5.9 (16)	–2.3444	1.9664	–1.19	.2332
CDR 1						
Baseline	17.1 \pm 8.6 (15)	16.7 \pm 8.2 (14)	Reference			.8910
Week 8 \times drug	13.0 \pm 7.1 (15)	15.6 \pm 10.6 (14)	–2.5727	2.0872	–1.23	.2177
Week 16 \times drug	10.6 \pm 6.4 (14)	15.2 \pm 12.2 (11)	–5.0788	2.0897	–2.43	.0151
Week 24 \times drug	9.9 \pm 6.8 (14)	14.5 \pm 12.3 (11)	–4.6262	2.2375	–2.07	.0387
Endpoint \times drug	10.0 \pm 6.6 (15)	15.4 \pm 11.3 (14)	–5.4755	2.1031	–2.60	.0092

Abbreviations as in Tables 1 and 2.

^aEstimate is the coefficient of treatment–visit interaction term in the GEE method multiple linear regression model. A first-order autoregressive covariance matrix was fit to the within-patient repeated measures. The *p* values were based on two-tailed tests.

Results

Sixty patients were eligible and randomized (Figure 1). Demographic data, education level, age at illness onset, illness duration, CDR, body mass index, and AChEI use at baseline were similar between the benzoate group (*n* = 30) and the placebo group (*n* = 30) (*p* > .05) (Table 1). The AChEI doses were within the therapeutic range and similar between two groups (Table 1). Mean dose of sodium benzoate at weeks 8, 16, and 24 were 275.0 \pm 76.3 mg/day, 525.0 \pm 100.6 mg/day, and 716.7 \pm 182.6 mg/day, respectively.

Outcome Measures

The mean \pm SD scores of both primary and secondary outcomes—including ADAS-cog, additional cognition composite, and CIBIC-plus—of the two groups of patients are shown in Table 2. At week 0 (baseline), there were no significant differences between the two groups in ADAS-cog and additional cognition composite (*p* = .75 and *p* = .27, respectively).

For the primary outcome, sodium benzoate produced greater improvement in ADAS-cog score than the placebo therapy throughout the study (mean differences from baseline were 3.8, 5.4, 5.9, and 5.9 in the benzoate group and 2.4, 1.7, 2.7, and 1.7 in the placebo group, at weeks 8, 16, 24, and endpoint; *p* = .3730, *p* = .0021, *p* = .0116, and *p* = .0031, respectively), with effect size of .86 at the end of the study (Table 2). The results were similar when the baseline ADAS-cog score was controlled in the GEE model (Table S1 in Supplement 1).

Table 4. Results of Measures of Additional Cognition Composite Over 24-Week Treatment with Independent *t* Test in Subgroups

Scale	Benzoate		Placebo		Cohen's <i>d</i>	<i>t</i>	<i>p</i>
	Mean \pm SD (n)	Mean \pm SD (n)	Mean \pm SD (n)	Mean \pm SD (n)			
CDR .5							
Baseline	49.0 \pm 6.9 (14)	52.8 \pm 7.6 (15)				–1.395	.174
Endpoint	50.5 \pm 7.1 (14)	50.8 \pm 8.5 (15)				–.108	.915
Difference	1.5 \pm 3.5 (14)	–1.9 \pm 5.8 (15)			.7098	1.939	.063
CDR 1							
Baseline	48.8 \pm 6.6 (12)	49.3 \pm 9.3 (12)				–.150	.882
Endpoint	50.3 \pm 6.1 (12)	48.0 \pm 9.0 (12)				.742	.466
Difference	1.6 \pm 2.8 (12)	–1.3 \pm 3.5 (12)			.9150	2.176	.041

The *p* values were based on two-tailed tests. Additional cognition composite, the composite test score of speed of processing, working memory, and verbal learning and memory.

CDR, Clinical Dementia Rating.

For the secondary outcomes, sodium benzoate was better than placebo in the additional cognition composite at endpoint (*p* = .007, effect size = .78). Benzoate treatment also produced greater improvement in CIBIC-plus score than placebo therapy at week 16 (*p* = .015), week 24 (*p* = .016), and endpoint (*p* = .012, effect size = .73 at endpoint) (Table 2).

The dropout rate (3.3%) of the sodium benzoate group tended to be lower than that (16.7%) of the placebo group, yet insignificantly (*p* = .195).

For subgroup analysis, we further examined efficacy of sodium benzoate versus placebo in CDR .5 and CDR 1 subgroups. For ADAS-cog, sodium benzoate produced greater improvement than placebo therapy at weeks 16 and 24 and endpoint (*p* = .0151, *p* = .0387, and *p* = .0092, respectively) in the CDR 1 subgroup. However, sodium benzoate was not superior to the placebo therapy in the CDR .5 subgroup throughout the study (*p* > .05) (Table 3).

Sodium benzoate showed better efficacy in the CDR 1 subgroup (*p* = .041) and borderline significance in the CDR .5 subgroup (*p* = .063) in improving the additional cognition composite (Table 4). For CIBIC-plus, sodium benzoate produced greater improvement than placebo therapy at week 24 and endpoint (*p* = .040 and *p* = .018, respectively) in the CDR 1 subgroup but not in the CDR .5 subgroup (Table 5).

Adverse Effects

Both sodium benzoate and placebo were well-tolerated. Only one patient in the placebo group reported dizziness at week 16. The side effect was mild and did not warrant medical treatment. There was no reported side effect in the sodium benzoate group assessed by the Uvalg for Kliniske Undersogelser Side-effects Rating Scale at all visits. No dropout was due to side effect.

The routine blood cell count and chemistry were all within the normal ranges and remained unchanged after treatment (data not shown).

Discussion

It is critical to identify and treat AD as early as possible, potentially to arrest its progression (53). The current study is the first to apply a DAAO inhibitor, sodium benzoate herein, as a novel treatment for the early stage of cognitive decline. The result showed that sodium benzoate had better efficacy than placebo in improving ADAS-cog score, additional cognition composite (consisting of speed of processing, working memory, and verbal learning and memory),

Table 5. Results of Clinical Measures of CIBIC-Plus Over 24-Week Treatment with Mann-Whitney *U* Test in Subgroups

Scale	Benzoate		Placebo		Cohen's <i>d</i>	<i>Z</i>	<i>p</i>
	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)			
CDR .5							
Week 8	3.5 ± .5 (15)	3.4 ± .5 (16)	.1771	−.508	.611		
Week 16	3.3 ± .5 (15)	3.7 ± .8 (15)	−.6042	−1.720	.085		
Week 24	3.3 ± .5 (14)	3.6 ± .6 (16)	−.4867	−1.260	.208		
Endpoint	3.3 ± .5 (15)	3.6 ± .6 (16)	−.4086	−1.029	.303		
CDR 1							
Week 8	3.3 ± .5 (15)	3.6 ± .6 (14)	−.6697	−1.719	.086		
Week 16	3.2 ± .7 (15)	3.6 ± .5 (11)	−.7374	−1.678	.093		
Week 24	3.1 ± .8 (14)	3.8 ± .9 (11)	−.8805	−2.052	.040		
Endpoint	3.1 ± .8 (15)	3.9 ± .9 (14)	−.9494	−2.370	.018		

The *p* values were based on two-tailed tests. Mann-Whitney *U* test was used, because the distribution of CIBIC-plus score was not normal.

CDR, Clinical Dementia Rating; CIBIC-plus, Clinician Interview Based Impression of Change plus Caregiver Input.

and global function in all subjects as a whole. Subgroup comparisons found that benzoate was beneficial for all outcome measures among patients with mild AD. In the aMCI subgroup, sodium benzoate showed borderline significance in improving the cognition composite, but not in ADAS-cog. This is probably due to small sample size lacking the power to detect a smaller effect than mild AD. Moreover, sodium benzoate also demonstrated favorable safety profiles.

Although NMDAR activity is essential for cognitive function, its role in AD is still not fully understood. The NMDAR overactivation by glutamate results in cell death. The excitotoxicity is one of the theories of AD, particularly in the late stage (54). Memantine, a low-affinity, voltage-dependent uncompetitive NMDA antagonist, has been used for the treatment of moderate–severe AD. The current study suggests that NMDAR enhancement is beneficial for early and mild dementia. There is an age-related decrease of glutamate content and synthesis in human cerebral cortex and hippocampus (18,55), of which the most significant and consistent finding is decreased density of NMDAR in elderly persons and in patients with AD (18). Lower levels of D-serine and higher levels of L-serine in the serum were also observed in patients with AD (56). Therefore, in addition to the cholinergic system, dysfunction of NMDA neurotransmission might also play an important role in the pathophysiology of AD.

With regard to the dosing strategy, sodium benzoate provided better efficacy than placebo at week 16 and week 24, with the mean dose of 525 mg/day and 716 mg/day respectively, possibly implying that sodium benzoate at 500–750 mg/day is more effective than 250 mg/day. Another possibility is that longer sodium benzoate treatment duration yields better treatment response. Further studies comparing different doses of sodium benzoate with a fixed-dose design are required for finding the time to response and the optimal dose for the treatment of mild AD or MCI.

The AChEIs are commonly used for the treatment of AD (57,58) but not recommended for the treatment of MCI, due to weak beneficial effects and risk of side effects (59,60). The consensus statement from the British Association for Psychopharmacology concludes that neither AChEIs nor memantine is effective in treating MCI (61). Other compounds commonly used for the treatment of MCI, such as vitamin E (62), folic acid (63), omega-3 fatty acid (64), piracetam (65), and ginkgo biloba (66), also failed to show convincing evidence for a cognitive enhancing effect. Sodium benzoate is generally safe; however, its efficacy for aMCI

did not reach statistical significance in the current small-sized study either, although it suggested a trend of improvement.

Although ADAS-cog is widely used in AD clinical trials, it might be less sensitive for MCI (67). One of the strategies to improve the detection of responsiveness for MCI is to add additional cognitive tests. People with MCI have been found to be impaired in neuropsychological functions (68) such as speed of processing (69), working memory (70), and verbal learning and memory (71). In the aMCI subgroup of the present study, sodium benzoate showed borderline significance in improving the additional cognition composite, consisting of speed of processing, working memory, and verbal learning/memory, but not in ADAS-cog score. Our result echoes the suggestion that additional neuropsychological tests that are more sensitive to subtle deficits should also be applied in the trials for MCI.

In addition, sodium benzoate also did not improve CIBIC-plus score in the aMCI subgroup. A possible explanation is a ceiling effect that functional impairment is minimal in the MCI individuals, thereby restricting the space for further improvement. More sensitive and specific measurements for the function of MCI individuals, such as Clinical Dementia Rating Sum of Boxes (72) or Alzheimer's disease Cooperative Study scale for ADL in MCI (73), can be applied in the future studies for MCI.

This study is limited by its small sample size, which led to underpowered results particularly in the subgroup analysis of aMCI and a lack of MCI-specific functional assessments. Furthermore, whether the finding in Han Chinese can be extrapolated to other populations is unclear. We have found that benzoate can increase the brain mass by magnetic resonance imaging study (74) and is beneficial for neurocognitive function in patients with schizophrenia (23). Biomarkers such as neurocognitive, cerebrospinal fluid, and neuroimaging data are also important for future studies to strengthen the methodology of detection.

Very high levels of DAAO are detected in the cerebellum of adult brain, whereas the activity of DAAO is low in the forebrain, such as prefrontal cortex and hippocampus, despite robust expression (75,76). The cellular localization and function of DAAO are likely different between forebrain and cerebellum: it is glial in the cerebellum but mainly neuronal in the cerebral cortex. However, the effect of DAAO inhibitors on forebrain D-serine level is inconsistent. Most DAAO inhibitors can cause a measurable increase in D-serine in the forebrain as observed in the cerebellum (77), whereas some inhibitors might not. Nevertheless, cerebellum is involved in cognition. Sodium benzoate might exert its procognitive effects by not only cerebral but also cerebellar mechanism.

Despite the aforementioned limitations, this study suggests that sodium benzoate, a DAAO inhibitor, is beneficial for cognitive and overall function in patients with early-phase AD. If the finding is confirmed in future larger-sized studies, this approach of applying NMDA-enhancing agents for early AD will bring hope for the growing aging population with cognitive decline. Because of the findings that cognitive deficits (the core symptoms of schizophrenia) can be improved by sodium benzoate in patients with schizophrenia (23), the potential of NMDA-enhancing agents in improving cognitive function for patients with other CNS disorders or for general populations deserves further investigation.

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ClinicalTrials.gov: NMDA-enhancing Agent for Treatment of Mild Cognitive Impairment and Mild Alzheimer's Disease; <http://clinicaltrials.gov/show/NCT01600469>; 01600469.

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