Biological Psychiatry

Archival Report

Sodium Benzoate, a D-Amino Acid Oxidase Inhibitor, Added to Clozapine for the Treatment of Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

BACKGROUND: Clozapine is the last-line antipsychotic agent for refractory schizophrenia. To date, there is no convincing evidence for augmentation on clozapine. Activation of *N*-methyl-D-aspartate receptors, including inhibition of D-amino acid oxidase that may metabolize D-amino acids, has been reported to be beneficial for patients receiving antipsychotics other than clozapine. This study aimed to examine the efficacy and safety of a D-amino acid oxidase inhibitor, sodium benzoate, for schizophrenia patients who had poor response to clozapine.

METHODS: We conducted a randomized, double-blind, placebo-controlled trial. Sixty schizophrenia inpatients that had been stabilized with clozapine were allocated into three groups for 6 weeks' add-on treatment of 1 g/day sodium benzoate, 2 g/day sodium benzoate, or placebo. The primary outcome measures were Positive and Negative Syndrome Scale (PANSS) total score, Scale for the Assessment of Negative Symptoms, Quality of Life Scale, and Global Assessment of Functioning. Side effects and cognitive functions were also measured.

RESULTS: Both doses of sodium benzoate produced better improvement than placebo in the Scale for the Assessment of Negative Symptoms. The 2 g/day sodium benzoate also produced better improvement than placebo in PANSS-total score, PANSS-positive score, and Quality of Life Scale. Sodium benzoate was well tolerated without evident side effects. The changes of catalase, an antioxidant, were different among the three groups and correlated with the improvement of PANSS-total score and PANSS-positive score in the sodium benzoate group.

CONCLUSIONS: Sodium benzoate adjuvant therapy improved symptomatology of patients with clozapine-resistant schizophrenia. Further studies are warranted to elucidate the optimal dose and treatment duration as well as the mechanisms of sodium benzoate for clozapine-resistant schizophrenia.

Keywords: Antioxidant, Clinical trial, D-amino acid oxidase (DAAO) inhibitor, *N*-methyl-D-aspartate, Refractory schizophrenia, Sodium benzoate

https://doi.org/10.1016/j.biopsych.2017.12.006

While second-generation antipsychotics are increasingly used (1), treatment for refractory schizophrenia remains a great challenge and an unachieved goal (2). Clinical manifestations of schizophrenia include positive symptoms, negative symptoms, and cognitive deficits (3). Above all, negative symptoms have been considered as the main predictor of the outcome for schizophrenia (4). Clozapine has been regarded as the last-line antipsychotic agent for refractory schizophrenia (5,6), though whether it is truly more efficacious than other competitors requires more rigorous studies (7). A meta-analysis study suggested that clozapine was the most efficacious antipsychotic drug among 15 commonly prescribed antipsychotic drugs for schizophrenia (8). Nevertheless, a substantial portion of patients still suffer from severe psychotic symptoms despite clozapine treatment; moreover, there is no convincing evidence for the efficacy of any augmentation strategy on clozapine (9–11). New pieces of evidence suggest that glutamate receptors may be a candidate target for novel therapy for refractory schizophrenia (12).

The mechanism of clozapine for treating schizophrenia is still unclear. It has been reported to interact with metabotropic glutamate receptor 5 and glycine/*N*-methyl-D-aspartate (NMDA) receptors, and exert its efficacy via NMDA receptor regulation (13–15). Glutamate signaling, particularly through the NMDA receptor, plays an important role in the pathophysiology of schizophrenia (16–20). A series of clinical trials found that currently available NMDA-enhancing agents including glycine, D-cycloserine, D-serine, and sarcosine were efficacious in improving the overall psychopathology of schizophrenia without side effect or safety concern (16–19). Another NMDA receptor agonist, D-aspartate, has also shown the potential to be beneficial for the treatment of schizophrenia

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 ISSN: 0006-3223

(21). However, there is no convincing evidence to support the efficacy of glutamate modulators for clozapine-resistant patients to date (9–12). In addition, oxidative stress may also be implicated in the pathophysiology of schizophrenia (22).

One of new avenues to enhance NMDA activation is inhibiting the activity of D-amino acid oxidase (DAAO), a flavoenzyme of peroxisomes responsible for degrading D-serine and D-alanine (23–25), and thereby raising levels of the D-amino acids, which are the neurotransmitters for the coagonist site of the NMDA receptor (26). Enhancing NMDA receptors through DAAO inhibition may be a safer way to reduce nephrotoxicity of D-serine (27). Sodium benzoate is a DAAO inhibitor (28) and can inhibit reactive oxygen species (29). Sodium benzoate is generally recognized as safe (30) with good central nervous system bioavailability (31) and is revealed to be safe in a 10-year retrospective study in urea cycle disorders (32). Compared with previous studies on NMDA-enhancing agents for schizophrenia (16), sodium benzoate had the greatest effect size for clinical symptoms (33). Sodium benzoate adjuvant therapy at 1 g/day has shown efficacy in improving both the clinical and cognitive symptoms of patients with chronic schizophrenia (33).

However, whether sodium benzoate can improve clozapine-resistant schizophrenia is unknown. Its optimal dose also requires exploration. NMDA receptor activation is pivotal for synaptic plasticity (34), memory, and cognitive function (35); however, excessive glutamatergic neurotransmission, particularly through the NMDA receptor, leads to neurotoxicity (36,37). This study aimed to testify whether sodium benzoate adjuvant therapy is beneficial for patients with clozapine-resistant schizophrenia, and whether 2 g/day is better than 1 g/day.

METHODS AND MATERIALS

Participants

Patients with refractory schizophrenia were recruited from the inpatient units of the Department of Psychiatry, China Medical University Hospital; Department of Adult Psychiatry, Kaohsiung Municipal Kai-Syuan Psychiatric Hospital; Department of Psychiatry, Taichung Chin-Ho Hospital; and Sunshine Psychiatric Hospital, which are four major psychiatric centers in Taiwan. The study was conducted while the patients were in the inpatient unit for the whole study. All patients had received similar environmental and supportive therapy from the four major psychiatric centers under the same coverage of National Health Insurance in Taiwan. The study was approved by institutional review boards and conducted in accordance with the current revision of the Declaration of Helsinki.

Patients were evaluated by research psychiatrists and enrolled into this study if they 1) satisfied DSM-IV criteria for schizophrenia confirmed by the Structured Clinical Interview for DSM (38,39); 2) were 18 to 65 years of age; 3) were physically healthy and all laboratory assessments (including blood routine, biochemical tests, and electrocardiograph) were within normal limits; 4) were resistant to standard treatments of at least two specific antipsychotics before clozapine treatment; 5) were receiving adequate trials of clozapine for more than 12 weeks but without satisfactory response, with a minimum baseline total score of 70 on the Positive and Negative Syndrome Scale (PANSS) (40) and a minimum baseline total score of 40 on Scale for the Assessment of Negative Symptoms (SANS) (41); 6) had sufficient education to communicate effectively and were capable of completing the assessments of the study; and 7) agreed to participate in the study and provided written informed consent after complete description of the study.

Exclusion criteria included DSM-IV diagnosis of schizoaffective disorder or mood disorder; mental retardation; substance (including alcohol) abuse or dependence; history of epilepsy, head trauma, or central nervous system diseases other than schizophrenia; pregnancy or lactation; or inability to follow protocol.

Study Design

All patients were randomly assigned to receive a 6-week, add-on treatment of 1 g sodium benzoate, 2 g sodium benzoate, or placebo in a double-blind manner. Efficacy and safety were evaluated at baseline and at the ends of weeks 2, 4, and 6. Study medications were given twice daily and were provided in coded containers with identical-appearing capsules of placebo or sodium benzoate. Patients were randomized to receive sodium benzoate 1 g/day, benzoate 2 g/day, or placebo in a 1:1:1 ratio by an independent investigational pharmacist. All subjects had been receiving a balanced hospital diet before and during the trial.

Patients, caregivers, and investigators were blinded to the assignment. Patients' medical adherence and safety were closely monitored by nurses and research physicians, and pill counting by the study staff.

The trial was registered on the ClinicalTrials.gov website (NCT01390376).

Assessments

The primary outcome measures were the total score of PANSS (40), SANS (41), Quality of Life Scale (QOLS) (42), and Global Assessment of Functioning (GAF) (39). The secondary outcome measurements included the subscales of PANSS, Hamilton Depression Rating Scale-17 items (HAMD-17) (43), and cognitive function.

For the assessment of negative symptoms, we a priori chose the SANS rather than PANSS-negative score as a primary outcome measure, because the SANS appeared to be more sensitive in earlier studies (4,44). Nevertheless, we also chose the PANSS-negative score as a comparison and a secondary outcome measure.

Cognitive function was assessed using a battery of tests (Supplement) that were the same as or the analogs of (due to lack of Chinese versions for some tests while we designed the study) the tests included in the Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery (MCCB) (45). These tests can be considered similar to the Chinese MCCB and have been successfully applied in our previous study (33), where one of the developers of the MCCB (Dr. M.F. Green) reviewed the tests.

Clinical ratings were performed by the research psychiatrists who were experienced in the rating scales. Interrater reliability was analyzed with the intraclass correlation coefficient (46). Only raters reaching the intraclass correlation

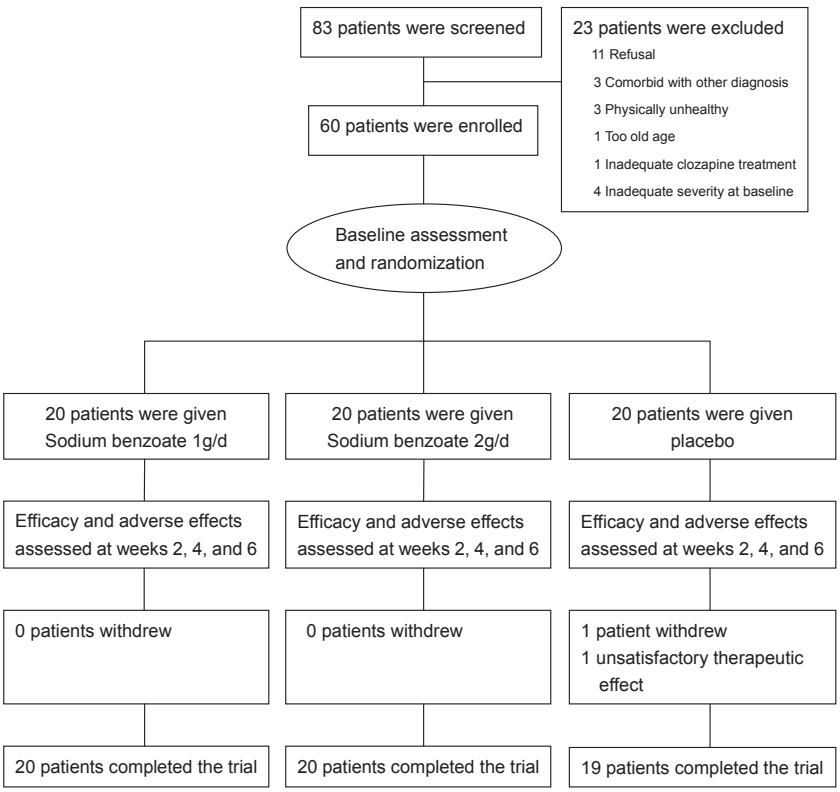

Figure 1. Flow diagram and disposition of the three treatment groups.

coefficient of ≥0.90 during prestudy training were allowed to rate the study patients. To minimize interrater variability, each individual patient was assessed by the same rater throughout the trial. The same raters rated individuals from different groups were blinded to the treatment assignment.

Adverse effect assessments included Simpson-Angus Scale for extrapyramidal side effects (47), Abnormal Involuntary Movement Scale for dyskinesia, and Barnes Akathisia Scale. Systemic adverse effects were evaluated biweekly by physical and neurological examinations and the Udvalg for Kliniske Undersogelser Side Effect Rating Scale (48). Laboratory tests including complete blood count and biochemistry were performed at baseline and week 6. Other laboratory measurements are shown in the Supplement.

Data Analysis

χ^2 (or Fisher's exact test) was used to compare differences of categorical variables and one-way analysis of variance (or Kruskal-Wallis test if the distribution was not normal) for continuous variables among three treatment groups. To compare the changes from baseline in repeated-measure assessments, we used the generalized estimating equation method's multiple linear regression model with treatment, visit, and treatment-visit interaction terms after adjusting for the effects of baseline severity. The working correlation matrix was specified as autoregressive of order 1. Therapeutic effect sizes (Cohen's *d*) were used to determine the magnitude of improvement for the continuous variables (49) resulting from sodium benzoate 1 g/day, sodium benzoate 2 g/day, and placebo. Cohen's *d* was calculated for change from baseline to end point in each treatment group compared with placebo.

Fisher's exact test was used to compare differences in the dropout rates between the two groups. All data were analyzed by SPSS version 22.0 (IBM Corp., Armonk, NY). All *p* values for clinical measures were based on two-tailed tests with a significance level of .05.

RESULTS

Eighty-three inpatients were screened. Twenty-three of the patients were excluded due to screening failure (Figure 1). Sixty patients were eligible and randomly allocated into three groups (Figure 1). Finally, all of the 60 patients completed at least one follow-up, and 59 (98.3%) of them completed the 6-week trial. One patient in the placebo group dropped out at week 2 due to worsening of clinical symptoms. The dropout rates of the 1 g group (0.0%) and the 2 g group (0.0%) tended to be lower than that (5.0%) of the placebo group, yet insignificantly ($p = .37$). No imputation for the incomplete data was used for the generalized estimating equation analysis.

Demographic data, education level, age at illness onset, illness duration, age at first hospitalization, number of hospitalizations, body mass index, and combination antipsychotics use at baseline were similar among the 1 g sodium benzoate group ($n = 20$), 2 g sodium benzoate group ($n = 20$), and placebo group ($n = 20$; $p > .05$) (Table 1). Nine patients (4 in the 2 g group; 4 in the 1 g group; 1 in the placebo group) were

Table 1. Baseline Demographic Characteristics of the Placebo or BE Treatment Groups

	Treatment Group			
	BE 1 g (n = 20)	BE 2 g (n = 20)	Placebo (n = 20)	p Value
Demographics				
Sex, female, n (%)	6 (30.0)	7 (35.0)	6 (30.0)	.996[a]
Age, years, mean (SD)	44.3 (7.2)	44.8 (8.1)	47.0 (11.9)	.626[b]
Age at illness onset, years, mean (SD)	22.5 (6.1)	23.6 (6.1)	21.7 (6.5)	.521[c]
Illness duration, months, mean (SD)	261.0 (85.8)	252.3 (94.2)	301.2 (115.4)	.259[b]
Age at first hospitalization, years, mean (SD)	24.3 (6.1)	25.1 (7.5)	25.8 (8.1)	.904[c]
Hospitalizations, mean (SD)	5.8 (5.1)	5.5 (3.5)	4.3 (4.6)	.186[c]
Education, Years, Mean (SD)	10.5 (1.7)	11.0 (2.1)	10.4 (2.2)	.599[c]
Body Mass Index, Mean (SD)	23.1 (4.2)	23.7 (3.8)	24.4 (3.5)	.588[b]
Patients Using Combination Antipsychotics, n (%)	4 (20.0)	4 (20.0)	1 (5.0)	.360[d]
Haloperidol	4 (20.0)	4 (20.0)	0 (0.0)	
Zotepine	0 (0.0)	0 (0.0)	1 (5.0)	
Clozapine Dose, mg/day, Mean (SD)	275.0 (124.1)	265.0 (89.0)	242.5 (90.0)	.592[b]
Chlorpromazine Equivalent Dose of All Antipsychotics, mg/day, Mean (SD)[e]	463.5 (219.2)	475.5 (195.0)	368.8 (140.1)	.196[c]

BE, sodium benzoate.
[a]χ^2 test.
[b]Analysis of variance.
[c]Kruskal-Wallis test.
[d]Fisher's exact test.
[e]Gardner *et al*. (61).

receiving clozapine plus another antipsychotic agent. Clozapine doses and chlorpromazine equivalent doses of all antipsychotics were similar among three groups (Table 1).

Outcome Measures

For the primary outcomes, there were no significant differences of the mean ± SD scores of PANSS-total score, SANS, QOLS, and GAF among the three groups of patients at week 0 (baseline) (Table 2). After treatment, both doses of sodium benzoate produced greater improvement in SANS scores than the placebo therapy throughout the study (mean differences from baseline to week 6 were 5.8 ± 7.5, 5.0 ± 6.5, and 1.7 ± 2.8 for sodium benzoate 1 g, sodium benzoate 2 g, and placebo, respectively), with Cohen's ds of 0.72 for sodium benzoate 1 g and 0.65 for sodium benzoate 2 g at week 6, respectively (Table 2). Sodium benzoate 2 g also produced greater improvement in PANSS-total score (mean differences from baseline to week 6 were 6.9 ± 4.7 and 3.6 ± 3.2 for sodium benzoate 2 g and placebo, respectively, with Cohen's d of 0.80) and QOLS (mean differences from baseline to week 6 were 3.6 ± 3.6 and 1.1 ± 2.5 for sodium benzoate 2 g and placebo, respectively, with Cohen's d of 0.82) than the placebo therapy. Neither dosage of sodium benzoate was better than placebo in improving GAF at week 6.

For secondary outcomes, there were no significant differences of the mean ± SD scores of PANSS-negative score, PANSS-general psychopathology score, HAMD-17, and cognitive function (global composite score) among the three groups at week 0 (baseline). The mean PANSS-positive score in the placebo group was slightly higher than that in the sodium benzoate 1 g group (Table 3). After treatment, sodium benzoate at 2 g was better than placebo in decreasing the PANSS-positive score throughout the study (p = .005 at end point, Cohen's d = 0.86) (Table 3). For other secondary outcomes, both groups of sodium benzoate were not better than placebo at week 6, though the 1 g or 2 g group revealed brief efficacy at week 2 or week 4 (Table 3). We further excluded patients on two antipsychotics and analyzed again (n = 51; data shown in Supplemental Tables S2 and S3). The trends were similar as the original data.

Multiple outcomes analyses need to be adjusted for type I error. We tried to adjust type I error by dividing the cutoff p value (.05) into four for the primary outcomes (cutoff p value became .0125) and into five for the secondary outcomes (cutoff p value became .01). After the adjustment, sodium benzoate at 2 g still produced significantly greater improvement in PANSS-total score, QOLS, and PANSS-positive score than the placebo therapy.

There were seven domains and two composite scores in the cognitive function measure. Among all the domains and composite scores, neither dosage of sodium benzoate was better than placebo (Supplemental Table S1).

Adverse Effects

The three treatment groups had minimal extrapyramidal syndrome at the beginning of the study. There were no significant differences among the three groups in the Simpson-Angus Scale, Abnormal Involuntary Movement Scale, and Barnes Akathisia Score (Table 4). At the end point, the severity of extrapyramidal syndrome remained minimal and did not reveal significant differences among the three groups.

Treatment-emergent adverse events other than extrapyramidal syndrome were assessed by the Udvalg for Kliniske Undersogelser Side Effect Rating Scale. Only 1 patient in the 2 g benzoate group reported difficulty in concentration at week 2, and 1 patient in the placebo group reported palpitation at

Table 2. Results of Measures of Primary Outcomes Over the 6-Week Treatment Using the Generalized Estimating Equations Method, Which Simultaneously Compared the Three Treatment Groups Using a Single Analysis

Scale	BE 1 g Mean ± SD (*n*)	BE 2 g Mean ± SD (*n*)	Placebo Mean ± SD (*n*)	BE 1 g vs. Placebo Estimate, SE, χ^2 (*p* Value)	BE 2 g vs. Placebo Estimate, SE, χ^2 (*p* Value)
Positive and Negative Syndrome Scale, Total Score					
Baseline	89.4 ± 10.3 (20)	92.3 ± 10.0 (20)	92.9 ± 8.7 (20)	−3.45, 2.94, 1.38 (.241)[a]	−0.55, 2.90, 0.04 (.850)[a]
Week 2	85.7 ± 8.4 (20)	89.9 ± 9.7 (20)	92.5 ± 9.2 (20)	−3.30, 1.19, 7.66 (.006)[b]	−2.05, 0.81, 6.47 (.011)[b]
Week 4	83.2 ± 8.9 (20)	87.4 ± 8.1 (20)	90.5 ± 9.4 (19)	−4.10, 1.53, 7.20 (.007)[b]	−2.85, 1.20, 5.59 (.018)[b]
Week 6	83.1 ± 10.9 (19)	85.5 ± 8.0 (20)	89.4 ± 10.1 (19)	−3.18, 2.41, 1.74 (.187)[b]	−3.62, 1.31, 7.68 (.006)[b]
End point	83.0 ± 10.6 (20)	85.5 ± 8.0 (20)	89.8 ± 9.9 (20)	−3.35, 2.38, 1.99 (.159)[b]	−3.80, 1.35, 7.90 (.005)[b]
Scale for the Assessment of Negative Symptoms-20					
Baseline	59.4 ± 10.5 (20)	63.2 ± 12.0 (20)	55.8 ± 14.8 (20)	3.60, 3.96, 0.83 (.363)[a]	7.45, 4.16, 3.21 (.073)[a]
Week 2	56.9 ± 9.5 (20)	61.9 ± 11.0 (20)	55.9 ± 14.5 (20)	−2.60, 0.88, 8.68 (.003)[b]	−1.50, 0.75, 3.96 (.047)[b]
Week 4	53.7 ± 10.9 (20)	59.0 ± 9.4 (20)	55.6 ± 14.8 (19)	−5.08, 1.59, 10.17 (.001)[b]	−3.63, 1.50, 5.87 (.015)[b]
Week 6	53.9 ± 10.8 (19)	58.2 ± 9.9 (20)	54.5 ± 15.0 (19)	−3.83, 1.75, 4.78 (.029)[b]	−3.34, 1.55, 4.64 (.031)[b]
End point	53.9 ± 10.5 (20)	58.2 ± 9.9 (20)	54.2 ± 14.7 (20)	−3.90, 1.73, 5.11 (.024)[b]	−3.40, 1.54, 4.87 (.027)[b]
Quality of Life Scale					
Baseline	19.8 ± 6.8 (20)	19.9 ± 6.9 (20)	18.3 ± 7.9 (20)	1.50, 2.27, 0.44 (.508)[a]	1.55, 2.28, 0.46 (.497)[a]
Week 2	22.0 ± 6.5 (20)	20.7 ± 6.6 (20)	18.5 ± 7.7 (20)	2.00, 0.81, 6.08 (.014)[b]	0.65, 0.46, 1.97 (.160)[b]
Week 4	23.3 ± 6.8 (20)	22.4 ± 6.1 (20)	19.2 ± 8.3 (19)	2.61, 1.12, 5.44 (.020)[b]	1.61, 0.79, 4.20 (.040)[b]
Week 6	23.6 ± 7.0 (19)	23.5 ± 6.1 (20)	19.4 ± 8.5 (19)	2.67, 1.56, 2.92 (.087)[b]	2.45, 0.94, 6.85 (.009)[b]
End point	23.6 ± 6.8 (20)	23.5 ± 6.1 (20)	19.4 ± 8.3 (20)	2.70, 1.53, 3.11 (.078)[b]	2.50, 0.94, 7.09 (.008)[b]
Global Assessment of Functioning					
Baseline	45.1 ± 10.0 (20)	48.2 ± 8.3 (20)	45.6 ± 10.6 (20)	−0.50, 3.17, 0.03 (.875)[a]	2.55, 2.93, 0.76 (.384)[a]
Week 2	47.1 ± 8.6 (20)	49.3 ± 7.9 (20)	46.1 ± 10.8 (20)	1.50, 0.71, 4.43 (.035)[b]	0.60, 0.32, 3.53 (.060)[b]
Week 4	48.7 ± 8.8 (20)	50.7 ± 7.2 (20)	47.5 ± 11.3 (19)	1.89, 0.84, 5.09 (.024)[b]	0.84, 0.59, 2.00 (.157)[b]
Week 6	48.9 ± 8.9 (19)	52.2 ± 7.4 (20)	48.5 ± 11.5 (19)	1.41, 1.05, 1.82 (.178)[b]	1.44, 0.83, 3.03 (.082)[b]
End point	48.1 ± 8.7 (20)	52.2 ± 7.4 (20)	48.1 ± 11.3 (20)	1.50, 1.03, 2.11 (.147)[b]	1.55, 0.82, 3.56 (.059)[b]

BE, sodium benzoate.

[a]Comparison was based on the average of the total score.

[b]Comparisons were based on the changes from the baseline in average of total score. Estimate is the coefficient of treatment and treatment-visit interaction term in the generalized estimating equations method's multiple linear regression model by specifying the working correlation matrix as autoregressive of order 1. The *p* values were based on two-tailed tests. All degrees of freedom were 1.

weeks 2, 4, and 6. The side effect was mild and did not warrant medical treatment.

The routine blood cell count including absolute neutrophil count (Table 4), chemistry, and electrocardiography were all within the normal ranges and remained unchanged after treatment. No dropout was due to side effect.

DAAO, Amino Acids, and Antioxidants

DAAO levels were measured at baseline and end point. The DAAO changes from baseline to end point were significantly different among the three groups (Table 5). Post hoc analysis revealed that the DAAO declines were greater in the 2 g sodium benzoate group than the placebo group ($p = .029$).

For the five amino acids that we measured, there was no significant change from baseline to end point among the three groups (Table 5). There was no significant correlation between DAAO levels and amino acids levels (data not shown).

Superoxide dismutase (SOD) levels and catalase (CAT) activity were measured at baseline and end point. The SOD changes from baseline to end point were similar among the three groups. The CAT changes from baseline to end point were significantly different among the three groups (Table 5). We further analyzed the correlations between SOD/CAT and clinical symptoms. In the sodium benzoate 1 g and 2 g groups, baseline SOD level was significantly correlated with baseline PANSS-total score and SANS score ($p < .01$). There were significantly negative correlations between CAT change and changes in PANSS-total score and PANSS-positive score ($p < .01$). In the placebo group, there was no significant correlation between SOD/CAT and clinical symptoms (Supplemental Table S4).

DISCUSSION

Treating patients with refractory schizophrenia is always challenging; those patients who fail to improve with clozapine are even harder to treat. To our knowledge, the current study is the first to demonstrate that applying an NMDA-enhancing agent, herein sodium benzoate, can help clozapine-resistant schizophrenia patients. The results showed that both 1 g/day and 2 g/day of sodium benzoate had better efficacy than placebo in improving negative symptoms with favorable safety profiles. Sodium benzoate at 2 g/day also produced better improvement than placebo in overall symptomatology (PANSS-total score) and QOLS. Although neither dosage could improve the cognitive function, the present study has shown greater efficacy than previous studies using

Table 3. Results of Measures of Secondary Outcomes Over the 6-Week Treatment Using Generalized Estimating Equations Method, Which Simultaneously Compared the Three Treatment Groups Using a Single Analysis

Scale	BE 1 g Mean ± SD (*n*)	BE 2 g Mean ± SD (*n*)	Placebo Mean ± SD (*n*)	BE 1 g vs. Placebo Estimate, SE, χ^2 (*p* Value)	BE 2 g vs. Placebo Estimate, SE, χ^2 (*p* Value)
Positive and Negative Syndrome Scale–Positive					
Baseline	21.7 ± 3.3 (20)	22.2 ± 3.5 (20)	23.7 ± 2.9 (20)	−1.95, 0.95, 4.17 (.041)[a]	−1.50, 1.00, 2.26 (.133)[a]
Week 2	20.7 ± 3.0 (20)	21.4 ± 3.4 (20)	23.7 ± 3.2 (20)	−1.00, 0.44, 5.13 (.024)[b]	−0.75, 0.35, 4.71 (.030)[b]
Week 4	20.1 ± 2.9 (20)	20.6 ± 2.9 (20)	22.8 ± 3.2 (19)	−1.01, 0.55, 3.30 (.069)[b]	−0.96, 0.42, 5.28 (.022)[b]
Week 6	20.0 ± 3.1 (19)	19.8 ± 2.4 (20)	22.5 ± 3.4 (19)	−0.59, 0.70, 0.71 (.401)[b]	−1.43, 0.52, 7.63 (.006)[b]
End point	20.2 ± 3.1 (20)	19.8 ± 2.4 (20)	22.8 ± 3.4 (20)	−0.65, 0.70, 0.86 (.354)[b]	−1.50, 0.54, 7.72 (.005)[b]
Positive and Negative Syndrome Scale–Negative					
Baseline	25.4 ± 4.3 (20)	26.3 ± 4.0 (20)	25.5 ± 4.6 (20)	−0.05, 1.37, 0.00 (.971)[a]	0.80, 1.32, 0.37 (.545)[a]
Week 2	24.2 ± 3.7 (20)	25.5 ± 3.6 (20)	25.2 ± 4.5 (20)	−1.00, 0.38, 6.96 (.008)[b]	−0.50, 0.28, 3.18 (.075)[b]
Week 4	23.3 ± 3.5 (20)	24.7 ± 3.3 (20)	24.8 ± 4.6 (19)	−1.34, 0.58, 5.24 (.022)[b]	−0.84, 0.55, 2.35 (.126)[b]
Week 6	23.3 ± 4.3 (19)	24.5 ± 3.7 (20)	24.8 ± 4.9 (19)	−1.45, 0.91, 2.55 (.110)[b]	−1.05, 0.60, 3.11 (.078)[b]
End point	23.2 ± 4.2 (20)	24.5 ± 3.7 (20)	24.7 ± 4.8 (20)	−1.45, 0.88, 2.74 (.098)[b]	−1.05, 0.59, 3.22 (.073)[b]
Positive and Negative Syndrome Scale–General					
Baseline	42.3 ± 5.6 (20)	43.9 ± 4.8 (20)	43.8 ± 4.5 (20)	−1.45, 1.56, 0.86 (.353)[a]	0.15, 1.44, 0.01 (.917)[a]
Week 2	40.9 ± 4.8 (20)	43.0 ± 4.7 (20)	43.6 ± 4.7 (20)	−1.30, 0.56, 5.30 (.021)[b]	−0.80, 0.43, 3.39 (.066)[b]
Week 4	39.9 ± 4.9 (20)	42.2 ± 4.1 (20)	42.8 ± 4.6 (19)	−1.76, 0.70, 6.38 (.012)[b]	−1.06, 0.50, 4.45 (.035)[b]
Week 6	39.7 ± 5.6 (19)	41.3 ± 3.9 (20)	42.1 ± 5.0 (19)	−1.17, 1.05, 1.23 (.268)[b]	−1.16, 0.64, 3.33 (.068)[b]
End point	39.7 ± 5.5 (20)	41.3 ± 3.9 (20)	42.4 ± 5.0 (20)	−1.25, 1.04, 1.44 (.231)[b]	−1.25, 0.65, 3.69 (.055)[b]
Hamilton Rating Scale for Depression-17					
Baseline	6.0 ± 3.5 (20)	8.5 ± 4.4 (20)	6.8 ± 2.8 (20)	−0.80, 0.98, 0.67 (.412)[a]	1.75, 1.13, 2.38 (.123)[a]
Week 2	5.3 ± 3.3 (20)	7.6 ± 4.1 (20)	6.4 ± 2.8 (20)	−0.30, 0.39, 0.59 (.443)[a]	−0.55, 0.48, 1.34 (.247)[b]
Week 4	5.2 ± 3.3 (20)	6.8 ± 3.2 (20)	6.2 ± 2.9 (19)	−0.29, 0.50, 0.33 (.567)[b]	−1.24, 0.62, 3.98 (.046)[b]
Week 6	5.3 ± 3.9 (19)	6.6 ± 3.2 (20)	5.8 ± 2.7 (19)	0.24, 0.69, 0.12 (.729)[b]	−1.01, 0.64, 2.50 (.114)[b]
End point	5.3 ± 3.9 (20)	6.6 ± 3.2 (20)	5.9 ± 2.7 (20)	0.20, 0.69, 0.08 (.772)[b]	−1.05, 0.64, 2.65 (.103)[b]
Cognitive Function[c]					
Baseline	50.7 ± 6.4 (20)	48.7 ± 6.0 (19)	50.6 ± 5.6 (19)	0.06, 1.87, 0.00 (.973)[a]	−1.95, 1.83, 1.13 (.287)[a]
End point	49.8 ± 7.3 (18)	49.0 ± 6.9 (19)	51.5 ± 7.0 (18)	−1.35, 1.02, 1.76 (.185)[b]	−0.09, 0.97, 0.01 (.930)[b]

BE, sodium benzoate.

[a]Comparison was based on the average of the total score.

[b]Comparisons was based on the changes from the baseline in average of total score. Estimate is the coefficient of treatment and treatment-visit interaction term in the generalized estimating equations method's multiple linear regression model by specifying the working correlation matrix as autoregressive of order 1. The *p* values were based on two-tailed tests. All degrees of freedom were 1.

[c]Global composite score. For assessing the global composite, an overall composite *t* score that included all seven domains was calculated by standardizing the sum of *t* scores.

NMDA-enhancing agents in the treatment of clozapine-resistant schizophrenia (9).

The patients in the present study had more severe overall symptomatology (mean PANSS-total score = 91.5) and longer duration of illness (271.5 months) than those in the previous studies on treatment-refractory schizophrenia (9). Although pharmacotherapy augmentations of clozapine showed some effects on cognitive function in refractory schizophrenia, the findings need further confirmation (50). Compared with the previous study that demonstrated that sodium benzoate at 1 g/day improved the cognitive function of patients receiving mainly typical antipsychotics and risperidone (33), both 1 g/day and 2 g/day of sodium benzoate failed to improve cognitive function in the current study focusing on clozapine recipients. It is likely that 6-week treatment might have been too short to help cognitive function of schizophrenia patients who are resistant to clozapine. Future studies with longer duration would be of help to elucidate this issue. In addition, patients in the present study were older and had more numerous hospitalizations than those in the previous one (37.3 years of age in average, hospitalization numbers 3.2 in average) (33), perhaps further hampering the chance to improve in the cognitive function. Another previous study that combined sarcosine with sodium benzoate in a 12-week trial for patients who had been stabilized on antipsychotics showed that adjunctive sarcosine with sodium benzoate improved the cognitive and global functioning of schizophrenia patients (51). Without a group of only add-on sodium benzoate in that study, it remains unclear whether sodium benzoate per se has beneficial effect. That is, the 3 studies enrolled different kinds of patients who received different antipsychotics, and used different study drugs for different treatment durations. To our knowledge, other NMDA-enhancing agents failed to show significant efficacy for refractory schizophrenia. Sarcosine did not show efficacy for schizophrenia patients who received clozapine (52). Clozapine-treated patients exhibited improvement with neither D-serine (53) nor glycine (54,55). D-cycloserine even

Table 4. Baseline and End Point Extrapyramidal Symptom Scores and Absolute Neutrophil Count

	Treatment Group			
	BE 1 g (n = 20)	BE 2 g (n = 20)	Placebo (n = 20)	p Value
Simpson-Angus Scale				
Baseline, mean (SD)	0.4 (0.8)	0.6 (0.9)	0.1 (0.4)	.16[a]
End point, mean (SD)	0.4 (0.8)	0.6 (0.9)	0.1 (0.4)	.16[a]
Abnormal Involuntary Movement Scale				
Baseline, mean (SD)	0.1 (0.4)	0.1 (0.2)	0.1 (0.4)	.90[a]
End point, mean (SD)	0.1 (0.4)	0.1 (0.4)	0.1 (0.4)	1.00[a]
Barnes Akathisia Scale				
Baseline, mean (SD)	0.1 (0.2)	0.1 (0.3)	0.0 (0.0)	.36[a]
End point, mean (SD)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.00[a]
Absolute Neutrophil Count				
Baseline, mean (SD)	5072.8 (3085.9)	4422.0 (1731.5)	4477.3 (1036.6)	.73[b]
End point, mean (SD)	4623.8 (2223.0)	4982.0 (2070.7)	7895.9 (15964.3)	.70[b]
Difference, mean (SD)	−322.6 (2396.6)	531.0 (2131.3)	3796.6 (15899.7)	.80[b]

BE, sodium benzoate.
[a]Analysis of variance.
[b]Kruskal-Wallis test.

worsened negative symptoms in schizophrenia patients treated with clozapine (56).

An unanswered question is the optimal dose of sodium benzoate for those patients. For safety concern, we used sodium benzoate 2 g/day as the highest dose in this study. Sodium benzoate at 2 g/day tends to be more beneficial in improving the overall symptomatology and QOLS compared with sodium benzoate 1 g/day, but insignificantly. Further study is needed to investigate whether higher doses could be beneficial for cognitive function, although the risk of seizure or other potential side effects may be increased when combining higher doses of sodium benzoate with clozapine (57).

Sodium benzoate 1 g/day produced greater improvement in SANS score than PANSS-total score (Table 2). For chronically stable schizophrenia patients, negative symptoms seem to have more influence than positive symptoms on the functional outcome (4). In contrast, there was only a trend for improvement in the PANSS-negative score. While the SANS and PANSS-negative score totals were highly correlated, the individual items from the SANS contained information independent of the PANSS-negative score, suggesting that the two instruments reflected the various dimensions of the negative syndrome (58). There was no significant change in GAF at end point among the three groups in the present study. Although the GAF has a good reliability and predictive validity (59), it is likely that GAF is not sensitive enough to detect subtle functionality change in such a short-term trial. More sensitive tools for the measurement of functional change, e.g., Personal and Social Performance Scale (60), of which a Taiwanese Mandarin version came out after the initiation of the current study, should be considered in future studies. The reduction in depressive symptoms also appeared similar among three groups; the very mild baseline severity (the average HAMD-17 score of all subjects = 7.1) might have limited the space for further improvement.

Although the doses of clozapine in the present study appeared relatively lower than those in the studies of Western countries (61), they were similar to those of Asian patients (62). The dose of clozapine in the current study had been titrated to its optimal dose according to the clinical evaluation for both the efficacy and safety. In clinical practice, 40% to 70% of patients with refractory schizophrenia are resistant to clozapine treatment despite using it with adequate dosage and duration (9,63). Meta-analyses showed that the effect sizes of combining clozapine with a second antipsychotic drug were around 0.1 to 0.2 (11,64). Augmentation of clozapine with other psychotropic agents, brain stimulations, and psychosocial intervention were also tested for their efficacy on cognitive function (9). Among all the glutamatergic drugs examined earlier (10), only CX516, an alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic receptor–positive modulator, showed efficacy for attention and memory in a pilot study (65); however, it failed to improve cognition or symptoms of schizophrenia in a later study (66). A partial NMDA receptor antagonist reduced PANSS score in a study with a small sample size (67), while glycine showed inconsistent results in four different studies (54,55,68,69). Although the PANSS and SANS score reductions in this study were less than a typical improvement (20%–25% reduction for significant clinical improvement), the present study appears to be the first one to demonstrate the efficacy of a DAAO inhibitor on symptomatology of clozapine-resistant schizophrenia.

In contrast with the DAAO declines in the 2 g sodium benzoate group, there was no significant change in amino acids levels among the three groups. There was no significant difference of DAAO change between the 1 g sodium benzoate group and placebo, suggesting that higher doses of sodium benzoate may be required for modulating the DAAO activity. A previous study found that a single oral dose of sodium benzoate induced antipsychotic effects in the phencyclidine model of schizophrenia but caused no changes to D-serine levels in plasma or brain of mice (70). Longer treatment of sodium benzoate might be needed to alter D-amino acid levels. Sodium benzoate also regulates many immune signaling pathways and ameliorates the disease process of an animal model of multiple sclerosis (71). It is interesting to investigate

Table 5. Baseline and End Point DAAO and Amino Acid Levels

	Treatment Group			
	BE 1 g (n = 20)	BE 2 g (n = 20)	Placebo (n = 19)	p Value
DAAO, ng/mL				
Baseline, mean (SD)	46.4 (5.8)	49.9 (8.9)	41.9 (6.2)	.003[a]
End point, mean (SD)	47.5 (7.4)	46.8 (11.8)	45.0 (9.1)	.693[a]
Difference, mean (SD)	1.1 (4.7)	−3.1 (9.2)	3.0 (6.6)	.029[a]
L-Serine, ng/mL				
Baseline, mean (SD)	2937.3 (521.6)	3248.9 (896.8)	3521.6 (1056.8)	.106[a]
End point, mean (SD)	3277.4 (809.5)	3434.5 (1438.7)	3586.9 (1030.4)	.693[a]
Difference, mean (SD)	340.1 (663.1)	185.6 (1350.5)	102.6 (1599.5)	.852[a]
D-Serine, ng/mL				
Baseline, mean (SD)	33.8 (8.6)	37.2 (12.4)	44.8 (29.2)	.179[a]
End point, mean (SD)	38.1 (14.2)	36.7 (13.4)	50.2 (48.8)	.312[a]
Difference, mean (SD)	4.4 (8.9)	−0.5 (15.6)	5.2 (24.6)	.543[a]
Glycine, ng/mL				
Baseline, mean (SD)	3740.0 (701.5)	3958.5 (789.1)	4133.1 (856.7)	.292[a]
End point, mean (SD)	4094.3 (962.4)	4167.9 (989.4)	4259.5 (1277.5)	.893[a]
Difference, mean (SD)	354.3 (846.0)	209.5 (845.0)	179.2 (1573.9)	.874[a]
L-Alanine, ng/mL				
Baseline, mean (SD)	11572.1 (3103.2)	13759.0 (3849.5)	12588.7 (3103.2)	.131[a]
End point, mean (SD)	12452.9 (4940.7)	12860.4 (4070.6)	12913.5 (3381.5)	.931[a]
Difference, mean (SD)	880.8 (3448.3)	−898.5 (3855.7)	311.2 (3855.3)	.312[a]
D-Alanine, ng/mL				
Baseline, mean (SD)	11.7 (12.5)	12.6 (23.9)	10.6 (13.8)	.934[a]
End point, mean (SD)	11.4 (11.0)	18.0 (34.6)	14.9 (21.9)	.696[a]
Difference, mean (SD)	−0.3 (7.8)	5.5 (31.4)	4.1 (10.9)	.632[a]
SOD, U/mL				
Baseline, mean (SD)	.005 (.035)	.021 (.029)	.003 (.031)	.153[b]
End point, mean (SD)	−.002 (.017)	.015 (.028)	.012 (.033)	.101[b]
Difference, mean (SD)	−.007 (.030)	−.006 (.025)	.010 (.038)	.459[b]
CAT, nmol/min/mL				
Baseline, mean (SD)	46.1 (23.0)	34.4 (23.4)	39.1 (21.7)	.093[b]
End point, mean (SD)	44.0 (22.6)	42.7 (21.2)	41.6 (22.6)	.965[b]
Difference, mean (SD)	−2.1 (19.1)	8.4 (16.7)	1.0 (18.5)	.033[b]

BE, sodium benzoate; CAT, catalase; DAAO, D-amino acid oxidase; SOD, superoxide dismutase.
[a]Analysis of variance.
[b]Kruskal-Wallis test.

whether sodium benzoate exerts its antipsychotic effects through mechanisms other than D-serine modulation, e.g., immunomodulation, anti-inflammation, antioxidation, or metabolomic changes (72). In addition to the regulatory role on D-amino acids, evidence suggests that DAAO itself also involves in the process of oxidative stress (73,74). A recent meta-analysis showed that the C-allele of *DAAO* rs4623951 and the T-allele of DAAO activator (*DAOA*) (or named *G72*) rs3916971 was associated with schizophrenia (75). Another meta-analysis also suggested a small effect of *DAOA* on cognitive ability (76). Although genetic and biochemical evidences support the involvement of DAAO in schizophrenia, the processes are not yet clear (77). Previous studies found that DAOA expression was higher in schizophrenia (78,79); however, the function of DAOA is also debated (80).

Baseline SOD level was significantly correlated with baseline PANSS-total score and SANS score, suggesting that SOD may reflect a trait for schizophrenia. The CAT change was negatively correlated with changes in PANSS-total score and PANSS-positive score in the sodium benzoate treatment groups but not in the placebo group, suggesting that CAT may be a state marker for schizophrenia. In consistence, a meta-analysis study supported that CAT may be a state marker for acute exacerbations of psychosis and SOD may be a trait marker for schizophrenia (81). An in vitro study also found that sodium benzoate increased the CAT activity in a cell model of neurotoxicity (82).

This study was limited by its small sample size, which might have led to underpowered results. However, the sample size of the present study was similar to those of previous studies on glutamatergic agents for clozapine-resistant schizophrenia (10,11). In addition, the effect sizes (0.65–0.86) of outcome measures were moderate to good in the current study. Clozapine level was not measured in this study due to limited

sample amount and laboratory limitation (clozapine level was not routinely measured in clinical practice or such studies, nor in our laboratory), although all subjects had been stabilized by clozapine treatment with optimal dose and adequate duration. Another limitation is the short duration of treatment. It is likely that clozapine-resistant schizophrenia patients need longer treatment to improve their cognition. Furthermore, whether the finding in Han Chinese can be extrapolated to other populations is unclear. In addition, the DAAO activity in serum was not steadily detectable. It is important to develop stable measurement for the DAAO activity instead of its level in future studies. Last, a standard adjustment for multiple outcomes analyses should be developed and applied in future studies.

Despite the aforementioned limitations, this study suggests that sodium benzoate may be beneficial for clinical symptoms and QOLS in patients with clozapine-resistant schizophrenia with favorable safety profile. If the finding can be confirmed in the future larger-sized studies, this approach of applying a DAAO inhibitor will bring hope for the patients who are resistant to clozapine treatment. For future studies and potentially clinical use, measurement of benzoic acid levels in peripheral blood may be helpful for determining the optimal dose of treatment.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by Taiwan Department of Health Grant No. DOH99-TD-I-111-TM001 (to HYL), Ministry of Science and Technology Grant No. MOST 104-2325-B-039-004 (to HYL), Welfare Clinical Trial and Research Center of Excellence Grant No. MOHW105-TDU-B-212133019 (to HYL), and China Medical University Hospital, Taiwan Grant No. DMR-104-100 (to HYL).

The sponsors were not involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

All authors report no biomedical financial interests or potential conflicts of interest.

ClinicalTrials.gov: DAAOI-1 Treatment for Treatment-resistant Schizophrenia; https://clinicaltrials.gov/ct2/show/NCT01390376; NCT01390376.

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Received Jul 6, 2017; revised Dec 6, 2017; accepted Dec 8, 2017.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2017.12.006.

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