Amisulpride Augmentation Therapy Improves Cognitive Performance and Psychopathology in Clozapine-resistant Treatment-refractory Schizophrenia (CTRS): a 12-week Randomized, Double-blind, Placebo-controlled Trial

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Abstract

Background

Although clozapine is an effective option for treatment-resistant schizophrenia (TRS), there are still 1/3 to 1/2 of TRS patients who do not respond to clozapine. The main purposes of this randomized, double-blind, placebo-controlled trial was to explore the amisulpride augmentation efficacy on the psychopathological symptoms and cognitive function of CTRS patients.

Methods

A total of 80 patients were recruited and randomly assigned to receive an initial clozapine plus amisulpride or clozapine plus placebo. Positive and Negative Syndrome Scale (PANSS), Scale for the Assessment of Negative Symptoms (SANS), Clinical Global Impression (CGI) scale scores, Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), Treatment Emergent Symptom Scale (TESS), laboratory measurements and electrocardiograms (ECG) were performed at baseline, week 6, and week 12.

Results

Compared with clozapine plus placebo group, clozapine plus amisulpride had lower PANSS total score, positive subscore and general psychopathology subscore at week 6 and week 12 (all $p_{\text{Bonferroni}} < 0.01$). Furthermore, compared with clozapine plus placebo group, clozapine plus amisulpride showed improved RBANS language score at week 12 ($p_{\text{Bonferroni}} < 0.001$). Clozapine plus amisulpride group had a higher treatment response rate ($p = 0.04$), lower scores of CGI severity (CGI-S) and CGI efficacy (CGI-E) at week 6 and week 12 than clozapine plus placebo (all $p_{\text{Bonferroni}} < 0.05$). There were no differences in BMI, QT intervals or laboratory measurements between the groups. Our results demonstrate that amisulpride augmentation therapy can safely improve the psychiatric symptoms and cognitive performance of CTRS patients.

Conclusions

Our study indicates that amisulpride augmentation therapy has important clinical significance for the treatment of CTRS to improve clinical symptoms and cognitive function with tolerability and safety.

Trial registration

Clinicaltrials.gov identifier- NCT03652974. Registered 31 August 2018, <https://clinicaltrials.gov/ct2/show/NCT03652974>

Background

Schizophrenia is a serious psychiatric disorder characterized by positive symptoms, negative symptoms and cognitive deficits^[1,2]. Despite a wide variety of available antipsychotic drugs, there are still many schizophrenia patients (about 1/5 to 1/3) who are resistant to two or more antipsychotic treatments, defined as “treatment-resistant schizophrenia (TRS)” or “treatment-refractory schizophrenia”^[3,4].

Clozapine is the only evidence-based antipsychotic drug for the treatment of TRS patients^[5,6]. However, even with sufficient clozapine levels in the blood, about 1/3 to half of TRS patients are still resistant to clozapine^[7,8]. According to the definition of TRS proposed by Kane^[9] and Honer et al.^[10], TRS patients who do not respond well to clozapine monotherapy are known as having clozapine-resistant treatment-refractory schizophrenia (CTRS). According to the National Institute for Health and Care Excellence (NICE) guidelines for the treatment of TRS^[11], augmentation therapies may have potential benefits for TRS patients who do not respond to clozapine monotherapy^[12]. Previous studies have shown that when dopamine D2 receptors are 70% or more occupied, antipsychotics achieve their maximum efficacy^[13]. Clozapine is an antipsychotic drug with multi-receptor blocking effects, and its affinity for dopamine D2 receptors is low (less than 60%)^[14,15]. Amisulpride has highly selective blocking effects on dopamine D2 and dopamine D3 receptors^[16], and has no affinity for any other known receptors^[17,18]. The unique dopamine receptor blocking effects of amisulpride can selectively enhance the limited dopamine D2 receptor blocking effects of clozapine^[15,19], making it suitable drug for combination with clozapine^[20]. In addition, a previous meta-analysis has shown that the efficacy of amisulpride is second only to clozapine, and that its treatment interruption rate is the lowest among the 15 antipsychotics commonly used for schizophrenia treatment^[21]. Previous randomized, double-blind, placebo-controlled trials using amisulpride augmented with clozapine in TRS patients showed no statistical advantages^[20,22]. However, these studies had relatively small sample sizes, and their patients may not have met CTRS criteria for the following reasons: (1) patients participating in the study may not have actually received two antipsychotic agents with different mechanisms of action in the past five years, or patients may not have taken the appropriate dose for a sufficient period of time before clozapine treatment; and (2) the studies stipulated that clozapine monotherapy should be administered for at least 3 months instead of 6 months. Previous studies have shown that some patients may have a delayed response to clozapine. Among these patients 30% respond after 6 weeks, 20% respond after 3 months, and 10% to 20% respond after 6 months^[23,24], suggesting that clozapine resistance should ideally be measured after 6 months. The evidence for amisulpride combined with clozapine in the treatment of CTRS patients remains poor. Most importantly, to the best of our knowledge, no studies have investigated the effects of amisulpride augmentation on cognitive function of CTRS patients. It has been reported that 98% of patients with schizophrenia have cognitive impairment, including first-onset or chronic episode patients^[25,26]. The recovery of cognitive function is considered to be one of the main goals of clinical treatment of schizophrenia^[27-29].

Thus, this 12-week, randomized, double-blind, placebo-controlled study is the first to investigate the efficacy and safety of amisulpride augmentation therapy in CTRS patients who have received at least two appropriate doses of antipsychotics with different chemical structures within a sufficient period of time, and

have recently received a stable dose of clozapine (i.e., at least 400 mg or more per day) for at least 6 months. The main purpose of this study was to investigate whether amisulpride augmentation therapy improved the psychopathological symptoms and cognitive performance of these CTRS patients.

Methods

Participants

The protocol was approved by the Institutional Review Board of Shanghai Pudong New Area Mental Health Center and each written informed consent was signed. The clinical trial registration number is NCT03652974. All participants were recruited from the Shanghai Pudong New Area Mental Health Center between September 6, 2018 and August 1, 2021.

The inclusion criteria were: (1) Han Chinese ethnicity; (2) between 18 and 65 years old; (3) satisfied the diagnostic criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), using the Structured Clinical Interview for DSM-IV (SCID-I/P); (4) the patient had received at least two antipsychotic agents with different mechanisms of action, at appropriate doses for sufficient course of treatment, and had recently received a stable dose of clozapine (i.e., at least 400 mg/d or more for at least 6 months) in order to ensure a reasonable response to clozapine monotherapy; (5) a review of the patient's past medical history revealed that the patient had stubborn psychotic symptoms and had never been effectively controlled; and (6) the patient had a baseline PANSS score > 60 before entering the study^[30]. Patients with (1) any other major Axis I disorder; (2) serious physical diseases; (3) substance abuse/dependence; or (4) pregnant women.

Clinical assessments

The Positive and Negative Syndrome Scale (PANSS) was applied to assess psychiatric symptoms. Negative symptoms were evaluated using the Scale for the Assessment of Negative Symptoms (SANS). The Assessment of Neuropsychological Status (RBANS) was applied to assess the cognitive performance. The Clinical Global Impression severity (CGI-S), CGI improvement (CGI-I) and CGI efficacy (CGI-E) were applied to assess the symptom severity, treatment responses and treatment effects. The Treatment Emergent Symptom Scale (TESS) was applied to assess adverse events related to treatment. All psychiatrists were trained in the administrations of the assessments, and inter-rater correlation coefficients were all above 0.8.

Intervention procedures

After the enrollment was completed, all eligible CTRS patients continued to take clozapine (doses from 400mg to 550mg) and were randomly assigned to receive clozapine plus amisulpride or clozapine plus placebo on a 1:1 basis. Randomization was carried out according to computer-generated random identification. The titration started with amisulpride 200 mg/d or one placebo tablet in the first week, amisulpride 400 mg/d or two placebo tablets in the second week, and up to 800 mg of amisulpride or four placebo tablets for the remaining 10 weeks.

Patients suffering from severe anxiety or insomnia were treated with benzodiazepines over a short time. Diphenylethyl hydrochloride was applied for a limited time in the patients who had extrapyramidal symptoms (EPS). No other antipsychotics and antidepressants were allowed during this study. The amisulpride and placebo tablets were identical in appearance. All researchers and participants were masked for treatment randomization and assessments.

The assessments of PANSS, SANS, RBANS, CGI and TESS were evaluated at baseline, week 6 and week 12. The primary outcome was the PANSS scores at week 6 and week 12. The secondary outcome was the responder rate, SANS, RBANS and CGI scores at week 6 and week 12. The treatment response was determined by a reduction of more than 25% in the PANSS total score^[31, 32]. The PANSS reduction rate was calculated using the following formula: (baseline PANSS total score - follow-up PANSS total score)/(baseline PANSS total score - 30)^[33].

Laboratory measurement, physical examination and electrocardiogram during clinical trial

Laboratory measurements, electrocardiogram (ECG) and physical examinations were performed at baseline, at the 6th week timepoint, and at the 12th week timepoint. After an overnight fast, blood samples were collected to detect serum clozapine levels, carry out routine blood analysis, obtain a lipid profile, and measure glucose, liver, and renal function.

Statistical analysis

The distribution of the data was detected through the Kolmogorov-Smirnov one-sample test. The balance of baseline demographic and clinical characteristics between groups was compared using the chi-square test and analysis of variance (ANOVA). An intent-to-treat (ITT) analysis was used for sensitivity purposes, and the principle of last-observation-carrying-forward (LOCF) was used to deal with missing data.

In the beginning, a repeated measure multivariate analysis of variance (RM MANOVA) was applied to obtain the overall p value of PANSS scores and RBANS scores, respectively. Then, a repeated measures analysis of variance (RM ANOVA) was used to examine each score of PANSS and RBANS, respectively, setting between-group factors (amisulpride and placebo) and within-group factors (baseline, week 6 and week 12), while also adjusting for confounding covariates. An RM ANOVA was conducted to measure every item of PANSS subscale in the amisulpride group. An RM ANOVA was conducted to measure SANS scores, CGI scores, TESS total scores, BMI, QT interval and each laboratory measurement index, respectively. After performing an RM ANOVA, a follow-up significant

multivariate omnibus test was performed, and each univariate effect was detected using an ANCOVA. If the group $\times$ time interaction was not significant, no statistical testing was further needed. If the group $\times$ time interaction had significance, an ANCOVA was used to analyze the group differences at the week 6 and week 12, setting baseline score, BMI, age, sex, disease course, and baseline clozapine serum level applied as covariates. Bonferroni corrections were applied to correct for multiple tests. Based on the power and sample size calculation at the 2-tailed 5% significance level, a sample size of 34 per group (total $n = 68$) yielded 80% of the power to detect significant differences in the primary outcome. In this study, we assumed that the dropout rate was less than 15%. PASW Statistics, version 23.0 (SPSS, Inc., Chicago) was applied for statistical analyses.

Results

Demographic and Baseline Information

Among the 113 participants assessed for eligibility, 80 were recruited and randomly assigned to one of the groups (Figure 1). Among these patients, 78 completed the 6-week trial, and 71 completed the 12-week trial. At week 6, one patient (2.5%) in the amisulpride group, and one patient in the placebo group dropped out. At week 12, three patients (7.5%) in the amisulpride group and four patients (10.0%) in the placebo group dropped out. As shown in Table 1, at baseline, except for BMI ($F = 4.85$, $p = 0.03$), there was no significant difference in any demographic or clinical characteristics (PANSS, RBANS, SANS and CGI scores) between the two groups (all $p > 0.05$). Therefore, BMI was adjusted for in subsequent statistical analysis. There was no difference in the clozapine dose or serum clozapine levels at baseline between the amisulpride and the placebo groups ($p > 0.05$). Furthermore, after adjusting BMI and baseline clozapine dose, RM ANOVA showed no group $\times$ time effect, time effect, or group effect on serum clozapine levels (all $p > 0.05$), indicating that there was no difference in the change of serum clozapine levels after 12 weeks of treatment. In addition, there was no difference in demographic or clinical characteristics between dropouts and completers (all $p > 0.05$).

Effect of amisulpride augmentation therapy on PANSS scores

RM MANOVA was first conducted using PANSS subscales and total score as the outcome measurement and BMI as the covariate, and showed a significant group $\times$ time effect (Wilks' lambda $F = 10.50$, $p < 0.0001$). Then, RM ANOVA showed group $\times$ time effects on PANSS total score (Wilks' lambda $F = 11.75$, $p < 0.0001$), positive symptom subscore (Wilks' lambda $F = 3.66$, $p = 0.03$) and general psychopathology subscore (Wilks' lambda $F = 9.03$, $p < 0.0001$) (Table 2). Next, after adjusting for BMI, age, sex, disease course, baseline PANSS scores and baseline clozapine serum levels, an ANCOVA was used to examine the group difference in PANSS total and subscale scores, at week 6 and week 12, respectively. As shown in Figure 2A-2D, at week 12, amisulpride group displayed lower PANSS total score, positive symptom subscore, and general psychopathology subscore compared with placebo group ($p_{\text{Bonferroni}} = 0.004$, Cohen's $d = 0.45$; $p_{\text{Bonferroni}} < 0.0001$, Cohen's $d = 0.97$; $p_{\text{Bonferroni}} < 0.001$, Cohen's $d = 0.92$; respectively). At week 6, amisulpride group displayed lower PANSS total score, positive symptom subscore and general psychopathology subscore compared with placebo group ($p_{\text{Bonferroni}} = 0.004$, Cohen's $d = 0.88$; $p_{\text{Bonferroni}} < 0.0001$, Cohen's $d = 0.99$; $p_{\text{Bonferroni}} = 0.004$, Cohen's $d = 0.89$; respectively).

Effect of amisulpride augmentation therapy on treatment response rate

ITT analysis showed that, at week 12, the response rate of amisulpride group (10 patients, 25%) was higher than that of placebo group (2 patients, 5%) ($\chi^2 = 6.28$, $p = 0.01$, OR = 6.33, 95% CI: 1.29-31.12). After adjusting for BMI, age, sex, disease course, and baseline clozapine serum levels, significant between-group differences ($B = 1.82$, Wald's statistics = 4.06, $p = 0.04$, OR = 6.15, 95% CI: 1.06-36.03).

Effect of amisulpride augmentation therapy on cognitive function

RM MANOVA showed a significant group $\times$ time effect (Wilks' lambda $F = 4.64$; $p = 0.01$) on RBANS scores. After adjusting for BMI, an RM ANOVA was applied for RBANS total and on subscale scores, respectively. As shown in Table 2, there was a group $\times$ time effect of RBANS total score and language score (Wilks' lambda $F = 3.54$, $p = 0.03$; Wilks' lambda $F = 5.49$, $p = 0.006$).

Then, after adjusting the baseline score and other clinical covariates, an ANCOVA was applied to examine the group differences in the RBANS total score and language score at week 6 and week 12, respectively. As shown in Figure 3A-3F, at week 12, the amisulpride group displayed higher RBANS total and language scores compared with placebo group ($p = 0.01$, Cohen's $d = 0.41$; $p < 0.0001$, Cohen's $d = 0.77$). However, only the difference in language score remained significant after Bonferroni correction ($p_{\text{Bonferroni}} < 0.001$). At week 6, there were no between-group differences in RBANS total score or language score ($p = 0.12$; $p = 0.08$).

Effect of amisulpride augmentation therapy on SANS and CGI scores

An RM ANOVA was performed on SANS, CGI-S, CGI-I and CGI-E scores, after controlling for BMI as a covariate. As shown in Table 2, there were group $\times$ time effects on CGI-S (Wilks' lambda $F = 10.85$; $p < 0.0001$), CGI-I (Wilks' lambda $F = 4.16$; $p = 0.02$) and CGI-E (Wilks' lambda $F = 12.17$; $p < 0.0001$) scores. However, there was no significant group $\times$ time effect on SANS score (Figure 4A).

Next, an ANCOVA was conducted to examine the group differences in the CGI-S, CGI-I and CGI-E scores at week 6 and week 12, respectively, after adjusting for baseline scores and other clinical covariates. As shown in Figure 4B-4D, at week 12, the amisulpride group had lower CGI-S, CGI-I and CGI-E scores than the

placebo group ($p < 0.0001$, Cohen's $d = 0.91$; $p = 0.02$, Cohen's $d = 0.80$; $p < 0.0001$, Cohen's $d = 1.06$; respectively). However, after Bonferroni correction, only CGI-S and CGI-E scores still showed significant between-group differences (both $p_{\text{Bonferroni}} < 0.0001$). At week 6, the amisulpride group had lower CGI-S and CGI-E scores than the placebo group ($p_{\text{Bonferroni}} = 0.003$, Cohen's $d = 0.72$; $p_{\text{Bonferroni}} = 0.01$, Cohen's $d = 0.73$).

Treatment side effects and Safety

As shown in Table 3, after adjusting for BMI as a covariate, RM ANOVA showed no significant group $\times$ time effect, main time effect, or group effect on the TESS total score (all $p > 0.05$). In addition, after Bonferroni correction, there were no significant group $\times$ time effects on BMI, QT intervals, or laboratory measurements ($p_{\text{Bonferroni}} > 0.05$).

At week 12, the most common adverse effects were mild in both groups, including dry mouth, constipation, extrapyramidal symptoms, gastrointestinal reactions, saliva, hypersomnia, insomnia and headache. There was no significant difference in the incidence of side effects between the amisulpride group and the placebo group ($p > 0.05$).

Discussion

The main findings of this RCT study were: (1) Compared with the placebo group, amisulpride augmentation therapy improved psychiatric symptoms (especially positive and general psychopathology symptoms), and reduced CGI-S and CGI-E scores, while also increased treatment response rate and RBANS language score in CTRS patients. (2) There was no difference in the effects of amisulpride vs. the placebo on TESS scores, BMI, QT intervals, or laboratory measurements. Our findings suggest that amisulpride augmentation therapy can safely improve the clinical symptoms and cognitive function of CTRS patients. Compared with the placebo group, the positive and general psychopathological symptoms of CTRS patients in the amisulpride augmentation group continued to improve at week 6 and week 12. Moreover, the 12-week amisulpride augmentation therapy increased the response rate compared to placebo. Also, compared with the placebo, the CGI-S and CGI-E scores of CTRS patients with amisulpride augmentation therapy were significantly reduced. Our results are partially consistent with a previous open-label non-randomized study, which had a relatively small sample size ($n=16$), and found that amisulpride augmentation therapy improved the positive symptoms of patients with schizophrenia who partially responded to clozapine^[34].

The theory that amisulpride enhances the efficacy of clozapine is based on the fact that the receptor profiles of these two drugs are complementary. Among those who do not respond to clozapine, clozapine monotherapy may not reach the level of D2 receptor blockade^[35, 36], because the level of D2 receptor blockade needs to be about 80% to produce a significant response^[37]. In patients who do not respond to clozapine monotherapy, the selective effects of amisulpride in the mesolimbic system may cause D2 receptors to be blocked at therapeutic level. In addition, amisulpride appears to have an effect on 5HT-7 receptors^[38] and presynaptic autoreceptors, which may affect the regulation of endogenous dopamine production^[39]. Furthermore, D3 receptors are located in the nucleus accumbens and cerebral cortex, and are associated with neural circuits implicated in schizophrenia^[40, 41]. A meta-analysis involving more than 2500 patients showed that there is a slight but significant correlation between D3 receptor coding sequence polymorphisms and susceptibility to schizophrenia^[42]. Previous studies have shown that selective D3 antagonists may be effective antipsychotic agents for the treatment of schizophrenia. Because of their anatomical distribution in the ventral striatum^[43], their locomotor adverse effects including extrapyramidal side effects and catalepsy may be negligible^[44-46]. For example, a 6-week RCT trial confirmed the efficacy and safety of D3 antagonists for improving acute exacerbations of schizophrenia^[47]. It has also been reported that high D2 antagonism or higher doses of antipsychotic drugs which are more likely to over-occupy D2 receptor, may increase the risk of secondary negative symptoms^[48]. However, our study did not find any effects of amisulpride augmentation therapy on the negative symptoms of CTRS patients based on the PANSS negative subscale and the SANS assessment.

Previous evidence suggests that amisulpride can improve the cognitive function of schizophrenia patients^[49, 50]. However, few studies have examined the effects of amisulpride combined with clozapine on the cognitive function of schizophrenia patients. Park et al.^[51] previously reported amisulpride augmentation therapy improved working memory of schizophrenia patients who treated with aripiprazole. Recently, Molina et al.^[52] revealed that the combined use of amisulpride and quetiapine improved both clinical symptoms and cognitive function, especially the executive function of TRS. In this study, we found that amisulpride augmentation therapy also improved the cognitive performance of CTRS patients, particularly language function.

Items that RBANS measure language domain in our study include picture naming and semantic fluency tasks, while a previous study used image naming and semantic fluency tasks to measure language functions of PD patients^[53]. Language and perception disorders are the core cognitive impairment symptoms in schizophrenia^[54]. The underlying mechanism may be related to the antagonistic effects of amisulpride on D2, D3 and 5-HT7 receptors. For example, previous evidence showed that resting blood flow in the hippocampus of patients with schizophrenia was abnormally increased^[55, 56], indicating an increase in resting metabolism in this region^[57, 58]. Tregellas et al. found that the resting hyperactivity of the hippocampus strongly relates to cognitive deficits in schizophrenia patients^[59]. Interestingly, dopamine D2 antagonists have been shown to reverse the abnormal increase in hippocampal blood flow in patients with schizophrenia^[60, 61]. In addition, Shin et al. found D2 receptor antagonism may improve the working memory function of schizophrenia patients^[62]. Previous evidences suggest that 5-HT7 receptor antagonists may affect neuronal morphology^[63, 64] and stimulate hippocampal neurogenesis^[65, 66], which is related to schizophrenia and cognitive function. In addition, preclinical studies using a rat model of schizophrenia-like cognitive impairment have demonstrated that 5-HT7 receptor antagonists can improve pro-cognitive function, and that amisulpride can improve stress-related frontal lobe cognitive impairment^[67]. Preclinical evidence shows that D3 antagonists can reverse the deficiency of dopamine tension in the prefrontal cortex^[68], which may improve cognition^[69-71].

In this study, there were no differences in side effects or safety between patients receiving amisulpride augmentation therapy and the placebo, which was partially consistent with a previous open-label non-randomized study^[34]. This outcome shows that amisulpride augmentation therapy improves positive

symptoms of CTRS patients without exacerbating side effects. It is well established that long-term disease courses and antipsychotics, especially atypical antipsychotics, increase the prevalence of metabolic disorders ^[72]. In this study, a comparison between patients treated with clozapine alone and patients treated with clozapine plus amisulpride for 12 weeks showed that both groups had similar metabolic outcomes, including BMI, blood lipids, and fasting blood glucose. As for cardiac side effects, an overdose of amisulpride increases the risk of prolonged QTc, but the risk is low at therapeutic doses ^[73]. Our results indicate that the therapeutic doses of amisulpride augmentation therapy did not increase the risk of QTc interval prolongation in CTRS patients.

Some limitations of our study should be noted. First, the sample size is relatively small, and our findings should be verified in a larger sample that is drawn from multiple centers. Second, the patients included in this study had chronic conditions, so the results of this study cannot be generalized to other settings. Third, The follow-up time for cognitive function improvement is relatively short.

Conclusions

In summary, our findings demonstrate that amisulpride augmentation therapy can safely improve clinical symptoms and cognitive function in CTRS patients. Amisulpride augmentation therapy has important clinical significance for the treatment of CTRS. Although the results of this study are promising, further multiple-center studies with larger sample sizes should be conducted to confirm the efficacy and safety of this treatment in different clinical settings.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author Zezhi Li upon reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Zezhi Li, Minghuan Zhu, Zhenghui Yi and Fengchun Wu designed the study. Minghuan Zhu, Ying Jin, Na Zhu, Ying Huang, Dianhong Shi, Minjia Liu, and Jiayu Yang were responsible for recruiting the patients, performing the clinical ratings, and collecting the clinical data. Zhenghui Yi, QinyuLv, Zhenjing Liu and Qiongyue Hu collected literatures and cleaned the data. Zezhi Li and Minghuan Zhu carried out the statistical analyses. Lei Zhao coordinated the study. Zezhi Li, Minghuan Zhu and Zhenjing Liu wrote the manuscript.

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Tables

Table 1. Demographic and clinical data of amisulpride and placebo groups at baseline

	Amisulpride (n=40)	Placebo (n=40)	χ^2 or $F(p)$	Dropouts (n=9)	Completers (n=71)	χ^2 or $F(p)$
Age (years)	46.60±9.36	47.95±7.20	0.52 (0.48)	45.33±4.80	47.52±8.67	0.54 (0.46)
Sex (male/female)	19 / 21	22 / 18	0.45 (0.50)	5 / 4	36 / 35	0.08 (0.78)
BMI	24.76±3.47	23.06±2.84	4.85 (0.03)	23.00±0.99	24.02±3.43	1.05 (0.31)
Education (years)	11.48±2.46	11.10±2.26	0.27 (0.61)	10.78±1.48	11.35±2.44	0.75 (0.39)
Age of onset (years)	22.70±5.32	23.45±6.26	0.26 (0.60)	23.89±4.37	22.97±5.95	0.38 (0.54)
Illness duration (years)	23.05±5.65	23.73±6.45	0.19 (0.66)	24.38±4.52	23.26±6.22	0.39 (0.54)
CLZ dose (mg/d)	438.75±32.99	447.50±37.47	1.21 (0.28)	441.67±30.62	443.31±36.10	0.07 (0.80)
CLZ plasma concentration	478.05±81.17	491.05±80.17	0.56 (0.46)	484.11±61.17	484.61±82.90	0.01 (0.91)
PANSS total score	82.28±8.54	79.53±6.71	2.59 (0.11)	79.44±4.33	81.08±8.09	0.41 (0.53)
P subscore	19.40±6.54	20.73±3.90	1.44 (0.23)	19.11±4.11	20.18±5.54	0.30 (0.59)
N subscore	21.15±7.45	18.98±4.69	2.86 (0.10)	21.78±4.21	19.85±6.49	0.94 (0.34)
G subscore	41.50±6.33	39.83±3.55	2.17 (0.15)	38.67±3.71	40.92±5.29	1.91 (0.17)
RBANS total score	59.38±7.71	60.28±10.14	0.23 (0.64)	59.44±5.73	59.87±9.32	0.02 (0.89)
Immediate memory	59.43±9.65	59.83±10.68	0.05 (0.83)	60.33±3.35	59.54±11.25	0.04 (0.83)
Visuospatial/construction	67.50±12.23	68.65±11.97	0.11 (0.74)	68.11±5.18	68.07±12.67	0.001 (0.98)
Language	71.15±14.02	71.63±13.86	0.08 (0.78)	70.22±5.87	71.54±14.58	0.07 (0.79)
Attention	73.65±9.68	73.75±11.52	0.26 (0.61)	72.89±5.13	73.80±11.41	0.04 (0.84)
Delayed memory	57.93±7.71	61.03±10.14	1.52 (0.22)	59.00±6.93	59.54±10.11	0.02 (0.89)
CGI-S	5.03±0.70	4.90±0.71	0.49 (0.49)	5.11±0.78	4.94±0.70	0.42 (0.52)
CGI-H	4.06±0.22	4.03±0.16	0.17 (0.68)	4.11±0.33	4.03±0.17	1.30 (0.26)
CGI-E	13.60±0.50	13.58±0.50	0.34 (0.56)	13.44±0.53	13.61±0.49	0.59 (0.45)

Note: CLZ, Clozapine; PANSS, Positive and Negative Syndrome Scale; P, positive symptom; N, negative symptom; G, General psychopathology.

Immediate memory score; VC: Visuospatial/construction score; DM: Delayed memory score.

Table 2. The scores of PANSS, RBANS, SANS and CGI at baseline, week 6 and week 12 follow-up in amisulpride and placebo groups

	Baseline		Week 6		Week 12		Group F(p)	Time F(p)	Gro F(p)
	Amisulpride	Placebo	Amisulpride	Placebo	Amisulpride	Placebo			
PANSS total score	82.28±8.54	79.53±6.71	70.75±9.47	77.75±5.99	73.68±10.35	77.63±6.86	3.51 (0.07)	1.25 (0.29)	11.7 (<0.001)
P subscore	19.40±6.54	20.73±3.90	16.33±3.94	20.35±4.15	15.93±4.36	20.25±4.56	10.99 (0.001)	0.09 (0.92)	3.66 (0.06)
N subscore	21.15±7.45	18.98±4.69	20.27±5.95	18.93±3.74	19.95±6.63	18.38±4.16	3.08 (0.08)	0.73 (0.49)	0.91 (0.34)
G subscore	41.48±6.33	39.83±3.55	34.40±5.38	38.48±3.59	34.38±6.01	39.00±3.76	5.43 (0.02)	1.27 (0.29)	9.03 (<0.001)
RBANS total score	59.38±7.71	60.28±10.14	63.20±8.88	61.75±10.72	65.03±8.31	61.25±9.98	0.48 (0.49)	1.49 (0.23)	3.54 (0.06)
Immediate memory	59.43±9.65	59.83±11.68	61.83±9.26	59.50±10.66	63.50±8.68	60.63±9.83	0.52 (0.47)	0.07 (0.93)	2.11 (0.15)
Visuospatial/construction	67.50±12.23	68.65±11.97	73.30±13.93	70.33±14.29	72.43±12.02	68.35±12.22	0.29 (0.60)	1.06 (0.35)	1.63 (0.21)
Language	71.15±14.02	71.63±13.86	78.05±11.66	74.55±12.99	81.20±10.20	72.38±12.68	2.35 (0.13)	0.92 (0.40)	5.49 (0.02)
Attention	73.65±11.58	73.75±13.94	76.25±11.68	72.55±11.89	74.25±10.90	73.33±11.57	0.16 (0.69)	2.33 (0.10)	2.37 (0.12)
Delayed memory	57.93±9.68	61.03±11.52	62.15±10.14	63.38±13.06	61.48±9.72	63.05±13.37	0.60 (0.44)	0.47 (0.63)	0.86 (0.41)
SANS	45.18±15.38	43.13±14.36	44.78±14.88	44.13±13.56	43.88±13.14	44.68±14.78	0.20 (0.66)	1.03 (0.36)	2.83 (0.09)
CGI-S	5.03±0.70	4.90±0.71	4.20±0.61	4.63±0.59	4.20±0.61	4.78±0.66	3.50 (0.07)	1.26 (0.29)	10.8 (<0.001)
CGI-H	4.05±0.22	4.03±0.16	3.48±0.92	3.90±0.55	3.48±0.96	4.03±0.16	4.41 (0.04)	0.17 (0.85)	4.16 (0.04)
CGI-E	13.60±0.50	13.58±0.50	10.88±3.58	12.98±1.92	10.88±3.58	13.58±0.50	15.38(<0.001)	0.52 (0.60)	12.1 (<0.001)

Note: PANSS, Positive and Negative Syndrome Scale; P, positive symptom; N, negative symptom; G, General psychopathology.

Table 3. TESS score, BMI, QT interval and laboratory parameters at baseline, week 6 and week 12 in amisulpride and placebo groups

	Baseline		Week 6		Week 12		Group F(p)	Time F(p)	Group×Time F(p)
	Amisulpride	Placebo	Amisulpride	Placebo	Amisulpride	Placebo			
TESS score ^a	4.83±3.81	4.93±3.50	6.40±4.82	5.40±3.59	6.30±4.76	5.55±3.63	2.98 (0.42)	0.04 (0.96)	0.79 (0.38)
BMI	24.76±3.47	23.06±2.84	24.82±3.39	23.10±2.81	24.96±3.45	23.19±2.95	5.24 (0.03)	1.50 (0.23)	0.55 (0.58)
WBC ^a ×10 ⁹	6.00±1.21	6.53±1.91	6.11±1.45	6.59±1.52	6.06±1.49	7.06±1.71	2.96 (0.09)	0.08 (0.92)	1.14 (0.33)
Neutrophils ^a	3.77±0.81	4.15±1.62	3.57±1.22	4.12±1.23	3.66±1.00	4.56±1.60	4.45 (0.04)	0.74 (0.48)	1.40 (0.25)
RBC ^a	4.11±0.53	4.26±0.51	4.14±0.55	4.24±0.50	4.20±0.53	4.18±0.46	0.68 (0.41)	0.04 (0.96)	1.81 (0.17)
Hb ^a	121.43±13.60	128.85±13.51	123.00±14.33	129.62±11.06	123.54±14.16	129.62±12.54	4.67 (0.03)	2.67 (0.08)	0.35 (0.70)
PLT ^a	240.63±57.46	261.26±77.67	239.54±63.58	261.59±68.07	248.97±69.62	252.59±73.09	1.31 (0.26)	1.28 (0.28)	1.84 (0.17)
ALT ^a	16.80±10.57	19.97±12.87	18.09±13.33	19.97±12.98	18.46±11.64	21.88±11.60	1.84 (0.18)	0.19 (0.83)	0.25 (0.78)
AST ^a	20.46±6.35	23.38±10.05	21.37±9.81	19.85±5.68	18.29±4.96	21.85±7.37	1.99 (0.16)	0.14 (0.87)	4.52 (0.02)
BUN ^a	4.49±1.20	4.03±1.27	4.31±0.90	4.35±1.20	4.34±1.39	4.18±1.09	1.25 (0.27)	0.18 (0.84)	1.93 (0.15)
Cr ^a	62.74±11.52	73.68±15.22	63.34±11.14	69.91±19.03	62.17±11.27	70.32±16.36	6.31 (0.01)	0.29 (0.75)	2.01 (0.14)
Ur ^a	292.86±91.53	352.26±122.22	283.91±90.78	359.24±105.86	281.57±98.38	333.00±90.51	6.90 (0.01)	0.74 (0.48)	2.05 (0.14)
Glucose ^a	5.11±0.93	5.59±2.02	5.29±0.89	5.26±0.83	5.34±1.00	5.47±1.02	0.45 (0.51)	1.09 (0.34)	0.93 (0.40)
TG ^a	1.31±0.53	1.59±0.66	1.54±0.74	1.74±0.67	1.31±0.68	1.62±0.95	3.21 (0.08)	0.50 (0.61)	0.42 (0.66)
TC ^a	4.03±0.71	4.18±0.94	4.03±0.79	4.24±0.86	4.03±0.86	4.06±0.65	0.11(0.74)	3.14 (0.05)	1.93 (0.15)
HDLC ^a	1.23±0.43	1.18±0.39	1.34±0.48	1.18±0.39	1.34±0.48	1.12±0.33	3.19 (0.08)	0.87 (0.43)	2.00 (0.14)
LDLC ^a	2.11±0.76	2.32±0.95	2.23±0.69	2.38±0.74	2.17±0.71	2.38±0.70	0.81 (0.37)	0.31 (0.74)	0.10 (0.90)
QT interval ^a	384.80±30.67	411.79±31.73	381.03±29.58	413.24±32.76	389.14±31.77	408.41±40.47	10.75 (0.002)	1.18 (0.31)	3.04 (0.06)

^a Adjusting for BMI

Figures

Figure 1

Treatment study flowchart.

Figure 2

Effect of amisulpride augmentation therapy on PANSS scores.

A-D. PANSS total score, positive symptom subscore, negative symptom subscore and general psychopathology subscore.

At week 12, the amisulpride group displayed lower PANSS total score, positive symptom subscore, and general psychopathology subscore compared with placebo group ($F = 11.34$, $p = 0.001$, $p_{\text{Bonferroni}} = 0.004$, Cohen's $d = 0.45$; $F = 17.10$, $p < 0.0001$, $p_{\text{Bonferroni}} < 0.0001$, Cohen's $d = 0.97$; $F = 14.00$, $p < 0.0001$, $p_{\text{Bonferroni}} < 0.001$, Cohen's $d = 0.92$; respectively). At week 6, the amisulpride group displayed lower PANSS total score, positive symptom subscore and general psychopathology subscore compared with placebo group ($F = 11.18$, $p = 0.001$, $p_{\text{Bonferroni}} = 0.004$, Cohen's $d = 0.88$; $F = 16.63$, $p < 0.0001$, $p_{\text{Bonferroni}} < 0.0001$, Cohen's $d = 0.99$; $F = 12.56$, $p = 0.001$, $p_{\text{Bonferroni}} = 0.004$, Cohen's $d = 0.89$; respectively).

Figure 3

Effect of amisulpride augmentation therapy on RBANS scores.

A-F. RBANS total score and the scores of immediate memory, visuospatial/construction, language, attention and delayed memory.

At week 12, the amisulpride group displayed higher RBANS total and language scores compared with placebo group ($F = 6.14$; $p = 0.01$, Cohen's $d = 0.41$; $F = 14.82$; $p < 0.0001$, Cohen's $d = 0.77$). However, only the difference in language score remained significant after Bonferroni correction ($p_{\text{Bonferroni}} < 0.001$). At week 6, there were no between-group differences in RBANS total score or language score ($F = 2.52$; $p = 0.12$; $F = 3.14$; $p = 0.08$).

Figure 4

Effect of amisulpride augmentation therapy on the scores of SANS and CGI.

A. SANS score. B-D. CGI-S, CGI-I, and CGI-E scores.

SANS score showed no difference between two groups at week 12 or week 6 (3A). At week 12, the amisulpride group had lower CGI-S, CGI-I and CGI-E scores than the placebo group ($F = 20.37$; $p < 0.0001$, Cohen's $d = 0.91$; $F = 5.75$; $p = 0.02$, Cohen's $d = 0.80$; $F = 19.02$; $p < 0.0001$, Cohen's $d = 1.06$; respectively). However, after Bonferroni correction, only CGI-S and CGI-E scores still showed significant between-group differences (both $p_{\text{Bonferroni}} < 0.0001$) (3B-3D). At week 6, the amisulpride group had lower CGI-S and CGI-E scores than the placebo group ($F = 10.93$; $p = 0.001$, $p_{\text{Bonferroni}} = 0.003$, Cohen's $d = 0.72$; $F = 8.98$; $p = 0.004$, $p_{\text{Bonferroni}} = 0.01$; Cohen's $d = 0.73$) (3B-3D).