Prediction of the ability of clozapine to treat negative symptoms from plasma glycine and serine levels in schizophrenia

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Abstract

We previously reported that plasma levels of glycine, a co-agonist at N-methyl-D-aspartate (NMDA)-type glutamate receptors, are decreased in patients with schizophrenia, and that glycine levels are negatively correlated with negative symptoms. The aim of the present study was to determine if glycine, or its ratio to serine, a precursor of glycine, predicts change in negative symptoms in subjects with schizophrenia during treatment with clozapine, an atypical antipsychotic drug with multiple effects on glutamatergic activity. Plasma levels of glycine, serine, and their ratio, were measured in 44 patients with schizophrenia who were subsequently treated with clozapine. Baseline glycine levels or glycine/serine ratios predicted the Scale for the Assessment of Negative Symptoms – Sum of the Global Scales and Avolition–Apathy after 6 wk of clozapine treatment. These results indicate the association of these amino acid measures with response to clozapine in terms of negative symptoms in patients with schizophrenia.

Received 19 September 2004; Reviewed 1 November 2004; Revised 4 November 2004; Accepted 14 November 2004

Key words: Clozapine, glycine, negative symptoms, schizophrenia, serine.

Introduction

The new-generation antipsychotic drugs, or atypical antipsychotic drugs, have been reported to be efficacious in treating negative symptoms and cognitive dysfunction while causing fewer extrapyramidal side-effects at clinically effective doses (Ellenbroek and Cools, 2000). On the other hand, these drugs produce side-effects, such as new-onset diabetes mellitus and weight gain (Sumiyoshi et al., 2004b,c). Therefore, efforts to identify patients who are more likely to benefit from treatment with atypical antipsychotic drugs are worthwhile. Particularly, because of the myelotoxic effect of clozapine, attempts to search for peripheral markers to predict response to it would directly improve risk–benefit ratios in clinical practice. In this context, we have demonstrated that baseline plasma levels of homovanillic acid, a metabolite of dopamine, predict the ability of clozapine to treat positive symptoms (Sumiyoshi et al., 1997a) and improve verbal memory (Sumiyoshi et al., 2004d) in patients with schizophrenia.

We recently reported that plasma levels of glycine, a co-agonist at N-methyl-D-aspartate (NMDA)-type glutamate receptors, as well as its ratio to serine, a precursor of glycine, are decreased in patients with schizophrenia, and that glycine levels are negatively correlated with negative symptoms (Sumiyoshi et al., 2004a). These findings have been replicated by an independent group of investigators (Ermilov et al., 2004). The decreased glycine availability is consistent with the hypoglutamatergic hypothesis of schizophrenia (Farber et al., 1999; Goff and Coyle, 2001; Goff and Wine, 1997; Heresco-Levy and Javitt, 2004; Jentsch and Roth, 1999; Sumiyoshi et al., 2004a). A recent demonstration of decreased serum levels of D-serine, a co-agonist at the glycine site of NMDA receptors, in subjects with schizophrenia (Hashimoto et al., 2003) is further support of the role for the decreased function of the NMDA receptor in the pathophysiology of negative symptoms.
There are a number of studies investigating the effects of adjunctive treatment with glycine site agonists, including full agonists such as glycine itself (Evins et al., 1997; Heresco-Levy et al., 1996, 1999, 2004; Heresco-Levy and Javitt, 2004; Javitt et al., 1994; Leiderman et al., 1996; Potkin et al., 1999) and δ-serine (Tsai et al., 1998, 1999), as well as a partial agonist δ-cycloserine (Goff et al., 1996, 1999; Heresco-Levy and Javitt, 2004), on psychopathology in patients with schizophrenia. Overall, these previous studies suggest efficacy of the glycine agonists for selectively ameliorating negative symptoms. In several of these studies (Evins et al., 1997; Goff et al., 1996; Heresco-Levy et al., 1996, 1999; Leiderman et al., 1996; Tsai et al., 1998), blood levels of amino acids, including glycine and serine, have been related to clinical response to treatment with typical and atypical antipsychotic drugs. Specifically, since the mechanisms underlying clinical efficacy of the atypical antipsychotic drug clozapine may involve partial agonist activity at glycine sites of NMDA receptors (Tsai et al., 1999), some investigators have examined the relationship between plasma glycine levels and response to clozapine, with or without adjunctive treatment with a glycine agonist, in patients with schizophrenia (Evins et al., 1997; Goff et al., 1996).

So far, there is only limited information (Evins et al., 1997) on the ability of glycine levels to predict response to treatment with clozapine in subjects with schizophrenia. Thus, Evins et al. (1997) reported that lower baseline plasma glycine levels were associated with improvement in negative symptoms in a small number of patients treated with clozapine. In this respect, it is assumed that subjects with higher baseline glycine levels, in which even a modest degree of enhanced stimulation at glycine sites of NMDA receptors is sufficient to attain valid levels of glutamatergic activity, would benefit more from treatment with clozapine. Therefore, we hypothesized that higher plasma glycine levels or glycine/serine ratios would be associated with better response in terms of negative symptoms to treatment with clozapine. We were particularly interested in decreased volition and spontaneity among the measures of negative symptoms, since some previous studies (Altamura et al., 1993, 1995) report altered plasma levels of glycine or glycine/serine ratios in subjects with major depression, a disease sharing these symptoms with schizophrenia.

The aim of this study, therefore, was to determine if baseline plasma glycine levels or glycine/serine ratios predict response of negative symptoms, particularly avolition and apathy, to clozapine treatment in patients with schizophrenia.

### Materials and methods

Forty-eight patients meeting DSM-III-R criteria for schizophrenia (APA, 1987) participated in the study. They were a subgroup of patients with schizophrenia in a larger study of baseline plasma glycine levels and negative symptoms (Sumiyoshi et al., 2004a), and were subsequently treated with clozapine for 6 wk. Subjects were interviewed with the Schedule for Affective Disorders and Schizophrenia – Lifetime and Change (SADS-C) version (Endicott and Spitzer, 1978). A psychiatric and treatment history was obtained from the subject, informants, and medical records. Subjects with current history of substance abuse or dependence, seizure or head injury were excluded from the study. Eligible patients had a complete physical examination. Standard laboratory testing was normal. Clinical staff explained the nature of the study to the subjects, the risks and benefits, and the option not to participate in research. If the mental status of a subject was impaired to the point where s/he could not understand the nature of the study, its risks and benefits, or the option not to participate, the subject was not approached to be in the research. This protocol was approved by the Institutional Review Board of University Hospitals of Cleveland. After complete description of the study to the subjects, written informed consent was obtained.

All patients were withdrawn from psychotropic medication except for an occasional dose of benzodiazepines or chloral hydrate for a minimum of 7 d before drawing blood based on the protocol reported elsewhere (Sumiyoshi et al., 1997a,b, 2004a,d).

The Scale for the Assessment of Negative Symptoms (SANS; the Global Scales; Andreasen, 1983) and the Brief Psychiatric Rating Scale (BPRS), 18-item version (Overall and Gorham, 1962) (0–6 scale) were assessed by trained research assistants with intra-class correlation coefficients of > 0.8 (Sumiyoshi et al., 1997a,b, 2004a). The sum of the SANS – Global Ratings was obtained using the all

### Table 1. Clinical profiles of subjects

<table>
<thead>
<tr>
<th>Values represent mean (S.D.)</th>
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<tbody>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Duration of illness (yr)</td>
</tr>
<tr>
<td>SANS – Sum of the Global Scales</td>
</tr>
<tr>
<td>Clozapine dose at 6 wk (mg/d)</td>
</tr>
<tr>
<td>Baseline glycine levels (μmol/l)</td>
</tr>
<tr>
<td>Baseline serine levels (μmol/l)</td>
</tr>
</tbody>
</table>

SANS, Scale for the Assessment of Negative Symptoms.
domains of the SANS (Affective Flattening, Alogia, Anhedonia–Associality, Avolition–Apathy, Attention).

After the baseline assessment, the subjects received treatment with clozapine according to a regimen previously reported (Sumiyoshi et al., 1997a, 2004d). Patients received clozapine at a dose of 25 mg/d initially; this was usually increased by 25–50 mg every few days. The treating psychiatrists adjusted the dose to optimize improvement in psychopathology, while attempting to keep the side-effects of the drug tolerable. SANS was administered again at 6 wk following initiation of clozapine treatment.

Blood sampling procedure was as described in previous reports (Sumiyoshi et al., 1997a,b, 2004a,d). Briefly, blood samples were obtained between 09:30 and 10:00 hours, 60 min after insertion of an indwelling venous catheter following an overnight fast. The overnight fasting for patients was supervised. Physical exercise, alcohol, and caffeine were restricted for all the subjects during the study period.

Plasma levels of glycine and serine were measured using a high performance liquid chromatography with a Waters Pico-Tag column with the intra-assay and inter-assay coefficients of variation of 5% or less, based on an established method (Altamura et al., 1995; Sumiyoshi et al., 2004a; Waters Associates, 1984).

Multiple regression analyses were conducted to predict the psychopathology scores at 6 wk of clozapine treatment from age, glycine levels or glycine/serine ratio, and relevant psychopathology scores at baseline. Values are expressed as mean (S.D.) unless specified otherwise.

Results

Forty-four patients completed the study. Clinical data of these subjects are presented in Table 1. For these patients, time effect of clozapine on SANS – Sum of the Global Scales was not significant [11.9 (4.4) at baseline to 11.3 (4.1) at 6 wk; \( F(1, 35) = 0.60, p = 0.45 \)]. Time effects were also analysed for patients with the high negative symptoms (\( n = 18 \)), i.e. those who showed a BPRS Withdrawal–Retardation subscale score of \( \geq 5 \) as well as a score of \( \geq 3 \) in any item of the BPRS subscales for negative symptoms (Flattened Affect, Social Withdrawal, Motor Retardation) (Meltzer, 1995; Meltzer et al., 2000) at baseline. For this group, clozapine significantly reduced SANS – Sum of the Global Scales [14.3 (4.1) to 11.2 (4.3); \( F(1, 13) = 8.19, p = 0.01 \)].

Negative symptoms at 6 wk in the clozapine-treated patients were predicted from the baseline glycine levels or the glycine/serine ratio adjusted for the relevant psychopathology scores at baseline (Table 2). Thus, higher baseline plasma glycine levels were associated with fewer negative symptoms at 6 wk, as measured by the SANS – Sum of the Global Scales, while higher baseline glycine/serine ratios were predictive of lower scores of SANS Avolition–Apathy at 6 wk. No such associations were found for plasma serine levels (data not presented). Likewise, lower SANS Avolition–Apathy ratings at 6 wk were predicted by higher baseline plasma glycine levels in the high negative symptom patients (Table 2). There was no such association between the amino-acid measures and BPRS Total or Positive scores (data not shown).

Discussion

This study provides data indicating baseline plasma glycine levels or glycine/serine ratios predict some measures of negative symptoms, particularly avolition and apathy, in patients treated with clozapine.

### Table 2. Prediction of negative symptoms at 6 wk from baseline plasma amino-acid levels in clozapine-treated patients with schizophrenia

<table>
<thead>
<tr>
<th>SANS measure</th>
<th>Glycine</th>
<th>Glycine/serine ratio</th>
<th>Regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>( p )</td>
<td>( \beta )</td>
</tr>
<tr>
<td>Sum of all Global scales</td>
<td>-0.03</td>
<td>0.05</td>
<td>-0.47</td>
</tr>
<tr>
<td>Avolition–Apathy</td>
<td>-0.32</td>
<td>0.02</td>
<td>-0.46</td>
</tr>
<tr>
<td>Avolition–Apathy*</td>
<td>-0.03</td>
<td>0.03</td>
<td>-0.81</td>
</tr>
</tbody>
</table>

SANS, Scale for the Assessment of Negative Symptoms.

* Results from patients with high negative symptoms.
The ability of baseline plasma levels of amino acids, such as glycine, to predict response to adjunctive treatment with glycine agonists may depend on the concurrent antipsychotic drug and the type of glycine agonists added to the treatment. Thus, Heresco-Levy et al. (1999) found lower baseline serum glycine was related to better response to peroral glycine in a mixed population of 19 patients with treatment-resistant schizophrenia receiving either typical antipsychotic drugs or clozapine, and suggested that low baseline glycine levels may serve as a predictor of responsiveness to glycine. The same authors also reported an association between high post-treatment serum levels of glycine and improvement in negative symptoms in patients who received augmentation treatment with glycine (Heresco-Levy et al., 2004). However, in treatment-resistant patients administered d-serine, low baseline glycine or change in glycine levels did not predict improvement in negative symptoms (Tsai et al., 1998). Changes in serum glycine concentrations during treatment with clozapine and adjunctive d-cycloserine was shown to correlate with change in negative symptoms in patients with deficit schizophrenia (Goff et al., 1996), although the same group of investigators failed to replicate the finding in patients treated with typical antipsychotic drugs and adjunctive d-cycloserine (Goff et al., 1999).

There is limited information on the ability of baseline glycine levels to predict response to subsequent treatment with clozapine. Thus, lower baseline glycine levels in seven patients who had received typical antipsychotic drugs were associated with more robust improvement in negative symptoms after subsequent treatment with clozapine for 3–12 months (Evins et al., 1997). The results of this previous study do not appear to be in agreement with our results from a larger number of patients, which indicate association of high baseline glycine levels and glycine/serine ratios with better outcome in terms of negative symptoms in patients treated with clozapine. Both studies, however, demonstrated a relationship between baseline glycine levels or glycine/serine ratios with outcome of negative symptoms of schizophrenia when receiving clozapine. One possible explanation for the discrepancy would be that the previous study (Evins et al., 1997) did not adjust for baseline levels of negative symptoms, unlike the present study.

The lack of correlation between plasma serine levels and psychopathology scores after treatment with clozapine is in line with the results of a few previous studies of glycine or d-cycloserine (Goff et al., 1999; Heresco-Levy et al., 1999). Although we measured combined plasma levels of the d- and l-isomers of serine, investigations of d-serine levels, which were found to be decreased in schizophrenia (Hashimoto et al., 2003), would have conveyed more information on this issue. Further controlled studies with a larger number of subjects are warranted to determine the usefulness of plasma levels of glycine, serine and other amino acids as a predictor of response to clozapine and other atypical antipsychotic drugs.

Acknowledgements

Supported, in part, by grants from the William K. Warren Foundation, the Ritter Foundation, and Mr Donald Test to Herbert Y. Meltzer, M.D., as well as a Young Investigator Award from NARSAD, a Pharmacopsychiatry Research Grant from the Mitsubishi Pharma Research Foundation, a Fellowship from the Ministry of Education and Science of Japan, and a Grant-in-Aid for Scientific Research (no. 16591126) from Japan Society for the Promotion of Science to Tomiki Sumiyoshi, M.D., Ph.D. We are grateful to Mr Carlo Altamura, M.D. for helpful suggestions.

Statement of Interest

None.

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