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Clozapine treatment increases serum glutamate and aspartate compared to conventional neuroleptics

Short Communication

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Summary. To examine whether serum excitatory amino acid concentrations change with clozapine treatment and whether these changes correlate with improvement in negative symptoms, serum excitatory amino acids were measured and clinical scales administered in seven subjects with schizophrenia before and after switching from conventional neuroleptics to clozapine. Clozapine treatment was associated with increased serum glutamate and aspartate concentrations. Clinical improvement was negatively correlated with baseline glycine concentrations. These results support the hypothesis that clozapine acts at least in part by increasing glutamatergic activity.

Keywords: Schizophrenia, negative symptoms, clozapine, glutamate, aspartate, glycine, amino acid, psychopharmacology.

Introduction

In previous studies, our group found that patients treated with clozapine had significantly higher serum glutamate concentrations than did patients treated with conventional neuroleptics (Goff et al., 1996). In addition, D-cycloserine, a partial agonist at the glycine receptor of the NMDA receptor complex (Watson et al., 1990), significantly improved negative symptoms when added to conventional neuroleptics (Goff et al., 1995) but exacerbated negative symptoms when added to clozapine (Goff et al., 1996). Based on these findings, we speculate that the action of clozapine to improve negative symptoms of schizophrenia is mediated at least in part via glutamatergic mechanisms. The aim of this prospective study was to assess the effects of switching from conventional neuroleptics to clozapine on serum glutamate, aspartate and glycine concentrations and to examine relationships between changes in these amino acid concentrations and changes in clinical measures of negative

symptoms of schizophrenia. We hypothesized that clozapine treatment would increase glutamatergic activity which would correlate with clinical improvement.

Methods and materials

After obtaining informed consent, patients on conventional neuroleptics who were being considered for clozapine treatment were identified, and clinical diagnosis of schizophrenia was confirmed by a research psychiatrist. Blood was drawn from seven patients who met DSM-IV criteria for schizophrenia, (6 male, 1 female; mean age 46 ± 6 years), prior to switching from conventional neuroleptics (mean dose 629 mg ± 474 mg CPZ equiv./d) to clozapine (mean dose 393 ± 93 mg/d) and again 3–12 months (mean 8 ± 5 mos) later. The decision to switch to clozapine, the titration schedule and the final clozapine dose were based solely on the clinical judgment of the non-research psychiatrist who treated each patient. The second blood sample was taken after a given subject was both on a stable dose of clozapine and on no other neuroleptic for at least one month. Patients were switched from conventional neuroleptics to clozapine for treatment resistant positive (n = 4) or negative symptoms (n = 1) or for tardive dyskinesia (n = 2). Serum samples were taken at approximately the same time of day for individual patients to minimize within subject variability of serum amino acid concentration due to diet and exercise.

The Brief Psychiatric Rating Scale (BPRS) and Scale for Assessment of Negative Symptoms (SANS) were administered by the same rater thoughout the study.

Serum samples were stored at −80°C. Samples were stored 5–29 months (avg. 15 mos) from conventional neuroleptic treatment phase and 1–16 months (avg. 7 mos) from the clozapine treatment phase. Prior to assay, samples were deproteinated with 3 volumes 3% salicylic acid. Assays of glutamate, aspartate and glycine were performed using a quantitative ion exchange column on a Beckman 6300 Amino Acid Analyzer.

Data are presented as mean ± standard deviation. Statistical comparisons were made using paired t-tests or linear regression techniques where appropriate.

Results

Serum glutamate concentrations were significantly higher during treatment with clozapine (mean 71 ± 29 mol/L) compared to conventional neuroleptics (49 ± 27 μmol/L) ($df = 6$, $t = 2.34$, $p = 0.03$, one-tailed) (Table 1). Higher serum glutamate levels were observed in 6 out of 7 subjects after switching to clozapine treatment. Serum aspartate concentrations were also significantly higher in patients on clozapine (22 ± 8 μmol/L) compared to conventional neuroleptics (15 ± 8 μmol/L) ($df = 6$, $t = -2.16$, $p = 0.03$, one-tailed). Higher serum aspartate levels were observed in 5 out of 7 subjects after switching to clozapine treatment. In contrast, glycine levels did not change significantly (Table 1).

SANS scores were not significantly improved with clozapine treatment ($df = 6$, $t = 1.47$, $p = 0.2$, paired two tailed t-test), however SANS scores were significantly improved in paired comparisons when 4 of 5 subscales were used, with exclusion of the Attention Subscale ($df = 6$, $t = 2.2$, $p = 0.04$) (Table 1). Mean scores on the Negative Symptom/Withdrawal Items of the BPRS (affective flattening, emotional withdrawal and motor retardation items) improved significantly on clozapine ($df = 6$, $t = 2.3$, $p = 0.03$); this change correlated positively with change in aspartate concentrations ($df = 5$, $r = 0.80$, $p = 0.03$)

Table 1. BPRS Total and Negative Symptom/withdrawal items and serum amino acid concentrations in seven subjects with schizophrenia before and after conversion from conventional neuroleptics to clozapine

Subject	Clozapine dose	BPRS total		BPRS Neg Sx's		glycine		glutamate		aspartate		SANS		SANS 4/5		Pre/Post interval in months
		pre	post	pre	post	**pre	post	pre	post	pre	post	pre	post	pre	post	
1	500	56	46	15	12	513	356	27	31	10	6	12	12	10	10	12
2	250	46	26	7	6	263	270	47	99	9	28	16	9	12	7	11
3	400	30	31	10	10	230	328	20	58	5	20	9	8	8	7	10
4	500	44	39	10	8	243	230	88	68	26	22	11	9	11	9	16
5	400	38	35	13	13	218	272	71	112	14	29	12	12	10	10	3
6	400	41	38	11	10	273	355	69	83	27	30	12	12	11	10	3
7	300	48	42	7	7	261	255	23	47	14	24	12	12	11	10	3

** Serum amino acid concentrations are expressed as micromoles per liter

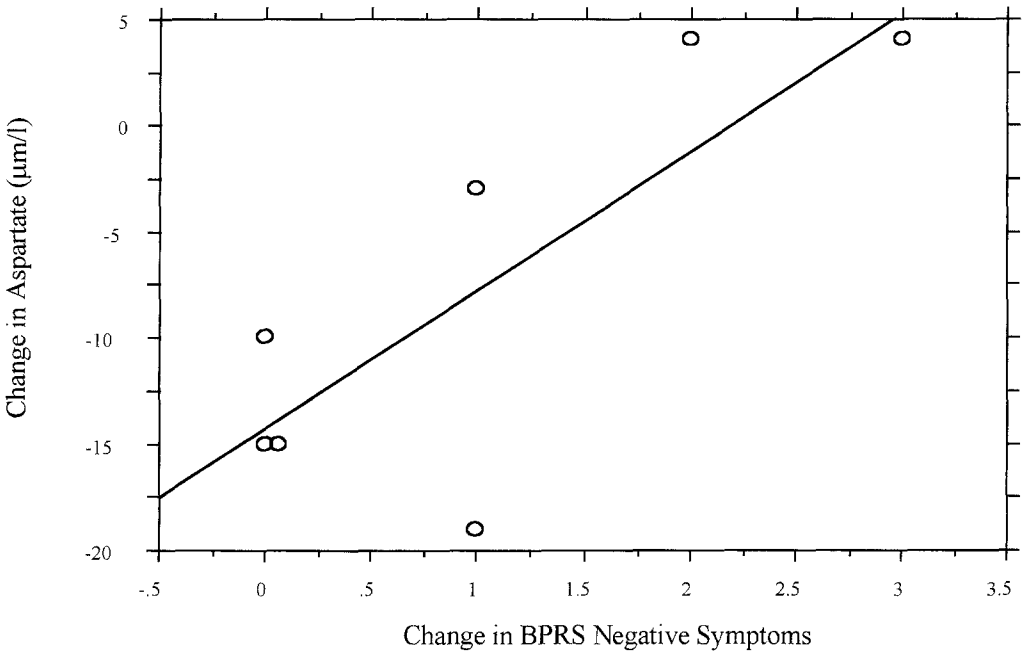

Fig. 1. Correlation between change in aspartate concentrations and change in BPRS negative symptom subscale (change in aspartate: serum aspartate concentration pre clozapine – aspartate concentration post clozapine; change in negative symptoms: BPRS negative symptoms pre clozapine – BPRS negative symptoms post clozapine)

(Fig. 1), showed a trend toward positive correlation with change in serum glutamate concentration ($df = 5$, $r = 0.67$, $p = 0.1$) and correlated negatively with baseline concentrations of glycine ($df = 5$, $r = -0.79$, $p = 0.03$). The clozapine dose correlated inversely with the change in aspartate ($df = 5$, $r = 0.8$, $p = 0.02$) and glutamate ($df = 5$, $r = 0.77$, $p = 0.04$) concentrations.

Discussion

The major aim of this study was to determine whether excitatory serum amino acid concentrations increase with clozapine treatment and to investigate the usefulness of excitatory serum amino acid concentrations as markers for treatment response to clozapine in patients with schizophrenia. While the overall effect of clozapine was to elevate serum concentrations of glutamate and aspartate, the inverse correlation between this change and both clozapine dose and improvement in negative symptoms suggests a curvilinear relationship. Previous studies seeking to determine whether a correlation between serum and CSF glutamate concentrations exists have yielded mixed results. Significant positive correlations between CSF and serum or plasma levels of glutamate have been reported in most (McGale et al., 1977; Alfredsson et al., 1988), but not all (Kim et al., 1980) studies of normal volunteers.

Enzymatic conversion of glutamine to glutamate can occur in vitro with prolonged storage and has been cited as a possible reason for inconsistent results between different laboratories (Alfredsson et al., 1988). In the present study, the samples from the conventional neuroleptic treatment phase were

stored an average of 8 months longer than those from the clozapine treatment phase. If any significant enzymatic conversion from glutamine to glutamate occurred that conversion would have decreased the magnitude of the apparent effect of clozapine upon glutamate concentration that we measured.

Because serum samples were obtained in a non-fasting state, diet may have affected serum amino acid concentrations. However, an effect of clozapine on these values cannot be excluded.

The amino acids measured were chosen according to the hypothesis that clozapine acts in schizophrenic patients at least in part by directly or indirectly increasing glutamatergic activity. Other lines of investigation have implicated a glutamatergic mechanism of action for clozapine. Animal studies have shown that acute administration of clozapine, but not haloperidol, increases medial prefrontal cortical glutamate and aspartate concentrations (Daly and Moghaddam, 1993; Yamamoto et al., 1994), suggesting that clozapine may have selective actions on cortical excitatory amino acid systems. There is also growing evidence that clozapine may act in part by increasing activity at the NMDA receptor, as clozapine has been reported to be the most potent of the antipsychotic agents tested in blocking NMDA receptor antagonist-induced neurotoxicity (Farber et al., 1993; Olney and Farber, 1994), stereotypy (Tiedtke et al., 1990), social isolation (Corbett et al., 1995), and deficits in sensorimotor gating of the startle response (Bakshi et al., 1994; Lang et al., 1992).

While clozapine appears to increase serum concentrations of excitatory amino acids, the effects of conventional neuroleptics is not clear. Alfredsson and colleagues reported that during sulpiride treatment, serum glutamate levels increased in responders while they were decreased in non-responders (Alfredsson and Wiesel, 1990). Korpi and colleagues reported no difference in glutamate concentrations between untreated and haloperidol treated schizophrenic patients (Korpi et al., 1987). In this study, the one patient whose glutamate and aspartate concentrations were decreased on clozapine was on haloperidol 15 mg/d and lorazepam 1 mg/d, and the other patient whose aspartate concentration was decreased on clozapine was on thioridazine 800 mg/d. Otherwise, the seven patients in the study were on 6 different neuroleptics, and some were on SSRI antidepressants and benzodiazepines as well.

This small study prospectively corroborates our previous finding that glutamate concentrations were elevated in patients treated with clozapine compared to patients treated with conventional neuroleptics and further suggests that serum aspartate concentrations also increase with clozapine treatment. In addition, lower glycine levels pre-clozapine were associated with more robust improvement in negative symptoms on clozapine. These results are interesting in the context of the model which proposes that glutamatergic hypoactivity may play a primary role in the pathophysiology of schizophrenia (Olney and Farber, 1995; Coyle, 1996), but a larger study is necessary to corroborate these findings.

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