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Association between serum levels of glutamate and neurotrophic factors and response to clozapine treatment

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ABSTRACT

Clozapine is the only available therapy for about 30% of schizophrenia patients otherwise refractory to antipsychotics. Unfortunately, the mechanism of action of the drug is still unknown and there are no biomarkers that can predict a positive response to clozapine. We aimed to examine serum neurotrophins and glutamate levels as putative biomarkers for clozapine response based on the hypothesized mode-of-action of the compound. Blood samples of 89 chronic schizophrenia patients maintained on clozapine were analyzed in a cross-sectional design. Serum brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), neurotrophic growth factor (NGF), glial derived neurotrophic factor (GDNF) and glutamate were determined. Differences between responders and non-responders to clozapine and correlation between clinical and biological measures were analyzed. Our sample consisted of 54 (61%) responders and 35 (39%) non-responders. Responders had higher mean BDNF levels than non-responders (2066 ± 814 vs. 1668 ± 820 pg/ml, $p < 0.05$. respectively) and higher serum glutamate levels (1.61 ± 2.2 vs. 0.66 ± 0.9 pg/ml, respectively, $p < 0.05$). Furthermore, there was a significant correlation between serum glutamate levels and positive symptoms among the clozapine-responder group (rho = 0.47, $p < 0.005$). High serum levels of BDNF and glutamate were associated with response to clozapine, while glutamate levels correlated with the psychosis severity in clozapine responders only. Large-scale, prospective longitudinal studies are needed to support these findings and the assumption that serum glutamate and BDNF can discriminate between clozapine responders and non-responders.

1. Introduction

Whereas 50–70% of schizophrenia patients have a sufficient response to standard antipsychotic compounds (typical or atypical), there is still up to a third of this population who remains treatment-resistant (Kane, 1996; Suzuki et al., 2011; Gillespie et al., 2017). For this population, the only recommended approach would be clozapine therapy (Meltzer, 1997; Stroup et al., 2009; Lieberman and Stroup, 2011); however, about half of the clozapine-treated population will not respond even to this "last resort" (Chakos et al., 2001; Buckley et al., 2001). For these clozapine-resistant schizophrenia patients, also referred to as ultra-resistant schizophrenia patients, there is a clear, unmet need for efficient treatment (Suzuki et al., 2011; Sommer et al., 2012).

Candidate biomarkers relevant to clozapine response may derive from the putative pharmacological mechanism of action of clozapine. Clozapine has an extremely complex pharmacological profile, with significant activity at the dopamine receptors D1, D2, D4, glutamate neurotransmission, serotonin receptors 5-HT2A, 5-HT1A, 5-HT3, 5-HT6, 5-HT7, adrenergic receptors α-1, α-2A, α-2B, α-2C, acetylcholine receptors M1, M2, M3, M4 and histamine H1 receptors (Kapur and Seeman, 2001; Miyamoto et al., 2012) and most probably more unknown mechanisms.

1.1. Neurotrophins and clozapine

Neurotrophins, and especially brain-derived neurotrophic factor (BDNF), are abundant in the brain. They are important for neurogenesis, neuronal survival, and normal maturation of neural developmental pathways (Shoval and Weizman, 2005).

Neurotrophic factors, such as nerve growth factor (NGF), BDNF and glial derived neurotrophic factor (GDNF) are also considered relevant

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to the mode of action of antipsychotic drugs (Chlan-Fourney et al., 2002). NGF and BDNF have been implicated in the neuroprotective action of antipsychotics (Angelucci et al., 2004). GDNF is a distantly related member of the transforming growth factor-beta family that was isolated from a glial cell line (Lin et al., 1993).

GDNF enhances the survival of dopaminergic neurons and it has been postulated as the most potent neurotrophic factor for dopaminergic neurons (Choi-Lundberg et al., 1997). A genetic association study found a weak link between a polymorphism in one of the GDNF receptors and response to clozapine (Souza et al., 2010).

Vascular Endothelial Growth Factor (VEGF) is generally accepted as the major factor involved in the process of angiogenesis and blood flow in the brain. A recent study (Pillai et al., 2016) found a significant inverse correlation between serum VEGF and total frontal region volume in patients with schizophrenia or schizoaffective disorder.

BDNF is the neurotrophin most extensively studied as a contributor to the pathophysiology of schizophrenia and response to antipsychotic treatment. Brain BDNF was shown to be associated with schizophrenia (Takahashi et al., 2000), and serum levels seem to correlate with brain BDNF levels (Karege et al., 2002). Some studies have demonstrated decreased (Grillo et al., 2007) while others reported elevated BDNF serum levels (Gama et al., 2007) in patients with schizophrenia compared to controls. A meta-analysis has shown that serum BDNF levels were reduced in schizophrenia, however, there was a considerable heterogeneity in the results (Green et al., 2011). In another meta-analysis (Ahmed et al., 2015) higher peripheral levels of BDNF expression corresponded to a better performance on one task of a neurocognitive assessment.

No difference of serum BDNF levels between clozapine treated patients and healthy controls was found (Yamamori et al., 2013), while others showed BDNF levels of clozapine treated to be lower than healthy control and to correlate with clozapine dose (Grillo et al., 2007; Pedrini et al., 2011). A small-scale longitudinal study (Chen and Huang, 2011) found that BDNF levels remained unchanged relative to study entry after four weeks of atypical antipsychotic treatment. Serum BDNF was significantly increased in a subgroup receiving risperidone compared to the one receiving clozapine, albeit only in the 15 male subjects and not in the 17 females. These results suggest that gender might significantly influence BDNF expression in the serum. It has also been suggested that val66met polymorphism of the BDNF gene might be associated with response to clozapine, however, a recent meta-analysis failed to show evidence for such an association (Cargnin et al., 2016). No study has examined the association of neurotrophin levels and clinical response to clozapine.

1.2. Glutamate and clozapine

It is generally accepted that in patients who respond to typical antipsychotics, dopamine D2 receptor blockade is the key mediator of antipsychotic efficacy (Kapur et al., 2000). However, in patients who do not respond to conventional antipsychotics, increasing D2 occupancy by raising the antipsychotic dose has no additional therapeutic effect. Symptoms and antipsychotic response in these patients may be related to non-dopaminergic mechanisms (Howes and Kapur, 2014). Glutamate is also implicated in the pathophysiology of schizophrenia (Merritt et al., 2016; Egerton and Stone, 2012), and recent work using MR spectroscopy suggests that a poor response to conventional antipsychotics in schizophrenia is associated with glutamatergic, rather than dopaminergic, abnormalities (Demjaha et al., 2014; Mouchlianitis et al., 2016). Patients with long-term clozapine treatment were also shown to have serum tyrosine and glutamic acid concentrations markedly elevated compared to patients on long-term conventional antipsychotic treatment (Melkersson et al., 2015).

There is an unmet need for an empirical basis of choosing the appropriate pharmacological treatment for the individual patient, based on clozapine mechanism of action and biomarkers associated with clozapine response. In this study, we aim to evaluate serum neurotrophin and glutamate levels as putative biomarkers for clinical response to clozapine.

2. Methods

2.1. Population

This is a cross-sectional, observational study including patients diagnosed with schizophrenia based on DSM-IV-TR criteria, as confirmed by the MINI interview (Sheehan et al., 1998) conducted by trained psychiatrists. Patients were recruited from inpatient and outpatient wards at Geha mental health Center (GMHC), a large, regional tertiary referral center covering a catchment area of about 800,000 inhabitants with mixed ethnicity. Patients were included if they were treated with clozapine for at least 18 weeks and had a stable daily dose of clozapine for the last 4 weeks prior to study entry.

The study was approved by the GMHC Review Board and each participant provided written informed consent before commencing the study.

2.2. Procedure

Upon consenting, each patient underwent clinical assessment by a trained psychiatrist. Evaluation included clinical interview to establish diagnosis and to rate the severity of symptoms. Demographic data were retrieved from patients and clinical records. A trained clinician rated the Positive and Negative Symptom Scale (PANSS) score on the day of assessment. Patients were defined as either responsive or resistant to clozapine based on clinical measures of the psychosis domain. Clozapine resistance was defined as a reduction of <20% in pre-clozapine treatment PANSS score (where that data was available, in about 10% of the studied population) or persistent severe psychotic symptomatology as reflected by a score of moderate (4) and above in at least two items of the positive symptoms domain of PANSS, based on the modified version of the Kane criteria for treatment resistance (Conley and Kelly, 2001).

Following that, blood samples were taken (at 08:00–10:00 am) through venipuncture and were transferred to the Felsenstein Medical Research Center (FMRC, Petach-Tikva, Israel) for centrifugation and separation of serum. Sera were stored in a freezer (−80 °C) until assayed.

2.3. Biochemical assays

Samples were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) commercial kits. For levels of neurotrophins: BDNF (cat # DY248,R&D systems, Minneapolis, MN), NGF (cat # HLH-BNGF, RayBiotech, Norcross, GA), GDNF (cat # HLH-GDNF, RayBiotech, GA USA) and VEGF (cat # HLH-VEGF, RayBiotech, Norcross, GA). We used calorimetric commercial kit (cat # K629-100, BioVision, Milpitas, CA) to analyze serum glutamate levels.

2.4. Statistical analysis

We used SPSS version 22 (IBM, Armonk, USA) for descriptive and inferential statistical analysis. Comparisons between clozapine responder and non-responder groups were conducted by Student's t or Mann-Whitney tests for continuous variables and χ^2 test for categorical variables. We used Spearman's Rho for bi-variate correlations. We analyzed correlations between serum trophic factors and glutamate levels, and clinical and demographic parameters first in the total sample and then in each group (responders vs. non-responders). Statistical significance was set at $p < 0.05$. As for correlations, Bonferroni's correction was used for multiple comparisons, and therefore significant p value was set as <0.005.

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