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Plasma levels of mature brain-derived neurotrophic factor (BDNF) and matrix metalloproteinase-9 (MMP-9) in treatment-resistant schizophrenia treated with clozapine☆

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HIGHLIGHTS

- Plasma levels of mature BDNF in schizophrenia were measured for the first time.
- No significant difference was observed in mature BDNF levels in schizophrenia.
- MMP-9 plasma levels were significantly increased in patients with schizophrenia.
- Plasma mature BDNF levels were significantly correlated with plasma MMP-9 levels.

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) regulates the survival and growth of neurons, and influences synaptic efficiency and plasticity. Peripheral BDNF levels in patients with schizophrenia have been widely reported in the literature. However, it is still controversial whether peripheral levels of BDNF are altered in patients with schizophrenia. The peripheral BDNF levels previously reported in patients with schizophrenia were total BDNF (proBDNF and mature BDNF) as it was unable to specifically measure mature BDNF due to limited BDNF antibody specificity. In this study, we examined whether peripheral levels of mature BDNF were altered in patients with treatment-resistant schizophrenia. Matrix metalloproteinase-9 (MMP-9) levels were also measured, as MMP-9 plays a role in the conversion of proBDNF to mature BDNF. Twenty-two patients with treatment-resistant schizophrenia treated with clozapine and 22 age- and sex-matched healthy controls were enrolled. The plasma levels of mature BDNF and MMP-9 were measured using ELISA kits. No significant difference was observed for mature BDNF however, MMP-9 was significantly increased in patients with schizophrenia. The significant correlation was observed between mature BDNF and MMP-9 plasma levels. Neither mature BDNF nor MMP-9 plasma levels were associated clinical variables. Our results do not support the view that peripheral BDNF levels are associated with schizophrenia. MMP-9 may play a role in the pathophysiology of schizophrenia and serve as a biomarker for schizophrenia.

Abbreviations: BDNF, brain-derived neurotrophic factor; MMP-9, matrix metalloproteinase-9; MDD, major depressive disorder; DSM-IV, diagnostic and statistical manual of mental disorders, fourth edition; PANSS, positive and negative syndrome scale.

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1. Introduction

Schizophrenia is a severe psychiatric disease characterized by delusions, hallucinations, impairment of cognitive function and incoherent behavior. It affects approximately 1% of the general population worldwide. Mounting evidence suggests that a deficit in neurotrophin supply to cortical neurons may be an underlying factor in the pathophysiology of schizophrenia as adequate neurotrophic support is required for normal brain development, maturation and function [3,4].

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that regulates neuronal survival, differentiation and growth during brain development, with important effects on neurogenesis and neuroplasticity. It is also important for hippocampal-related learning and memory [17]. A common single nucleotide polymorphism (SNP) of the BDNF gene has impact on episodic memory, hippocampal morphology and memory-related hippocampal activity in human [9,16]. Mature BDNF is initially synthesized as a precursor protein, preproBDNF. Following cleavage of the signal peptide, proBDNF is converted to mature BDNF by extracellular proteases, such as matrix metalloproteinase-9 (MMP-9). Mature BDNF and poBDNF each plays important roles in several physiological functions. Recent studies show that mature BDNF and pro BDNF elicit opposing effects via the TrkB and $p75^{NTR}$ receptors respectively. Mature BDNF preferentially binds to the TrkB receptor and plays an important role through BDNF-TrkB signaling which fulfills wide variety of functions such as cell survival, migration, outgrowth of neuritis and synaptic plasticity. In contrast, pro BDNF preferentially binds to the $p75^{NTR}$ receptors and elicit apoptosis rather than cell survival [8,11]. Considering the important roles of mature BDNF, it would be informative to specifically measure mature BDNF. Although BDNF levels in human blood can be measured using commercially available human BDNF ELISA kits, due to the limited specificity of the BDNF antibody, it has not been possible to distinguish between proBDNF and mature BDNF. Recently, peripheral levels of mature BDNF have been reported to be measurable using newly available human BDNF ELISA kits [23].

It is of great interest to assess the potential contribution of BDNF to the pathophysiology of schizophrenia. Several studies report altered BDNF mRNA and protein in prefrontal cortical regions and hippocampus of post-mortem brain tissues [13,21,22]. Peripheral BDNF levels in patients with schizophrenia have also been widely reported in the literature. However, there is no widespread agreement on the degree of peripheral BDNF levels in patients with schizophrenia, as measured in blood serum or plasma. A recent meta-analysis reported that peripheral BDNF levels were reduced in schizophrenia. However, there was considerable heterogeneity in the results [5]. Considering the important roles of mature BDNF such as cell survival, migration, outgrowth of neuritis and synaptic plasticity, it would be informative to specifically measure mature BDNF in patients with schizophrenia because dysfunction of these mature BDNF roles might be an underlying factor in the pathophysiology of schizophrenia. The peripheral BDNF levels previously reported in patients with schizophrenia were total BDNF (proBDNF and mature BDNF); peripheral levels of mature BDNF specifically have not been investigated in patients with schizophrenia. This study aimed to determine whether peripheral levels of mature BDNF were altered in patients with treatment-resistant schizophrenia. We also investigated Matrix metalloproteinase-9 (MMP-9) levels, as MMP-9 plays a role in the conversion of proBDNF to mature BDNF [8].

Table 1
Demographic variables for subjects.

Variables	Control $n=22$	Patients with schizophrenia $n=22$
Age (years)	38.1 ± 12.9	38.1 ± 13.2
Gender (male/female)	(12/10)	(12/10)
Age at onset	–	21.9 ± 8.4
Duration of illness	–	17.2 ± 11.1
PANSS positive	–	23.0 ± 4.6
PANSS negative	–	25.5 ± 5.5
PANSS general	–	52.9 ± 9.6
Clozapine dose (mg)	–	448.6 ± 130.0

Means ± SD are shown.

2. Materials and methods

2.1. Subjects

Twenty-two patients with treatment-resistant schizophrenia who were treated with clozapine were included in this study. Twenty-two age- and sex-matched healthy controls also participated in this study (Table 1). Cases were recruited at Osaka University hospitals. Each subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on structured clinical interview. Treatment-resistant schizophrenia was defined according to the following criteria mentioned in clozapine drug information in Japan: (1) Non- or little response to treatment from at least two adequately dosed antipsychotic trials for at least 4 weeks (including at least one second-generation antipsychotic, >600 mg/day of chlorpromazine equivalent) and patients never had the Global Assessment of Functioning (GAF) scores that were higher than 40. (2) Intolerance to at least two second-generation antipsychotics because of uncontrolled extrapyramidal symptoms. All subjects included in this study met the criteria of non- or little response. Symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Cases of schizophrenia with the comorbidities of substance-related disorders or mental retardation were excluded. Controls were recruited through local advertisements. Psychiatrically, medically and neurologically healthy controls were evaluated using the DSM-IV structured clinical interview, non-patient version. Subjects were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active stage cancer, cerebrovascular disease, epilepsy or seizures. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University and Chiba University.

2.2. Measurement of mature BDNF and MMP-9

Plasma levels of mature BDNF and MMP-9 were measured using the human BDNF ELISA Kit (Adipo Bioscience, Santa Clara, CA, USA), and the human MMP-9 ELISA Kit (R&D Systems, Minneapolis, MN, USA), respectively. To minimize assay variance, plasma levels of mature BDNF and MMP-9 were measured in each subject on the same day. All experiments were performed in duplicate. Protocols were performed according to the manufacturer's instructions. The optical density of each well was measured using an automated microplate reader (Emax; Molecular Devices, Sunnyvale, CA, USA). As plasma levels of proBDNF are not measurable by the newly available proBDNF ELISA kit due to low sensitivity, we measured only mature BDNF.

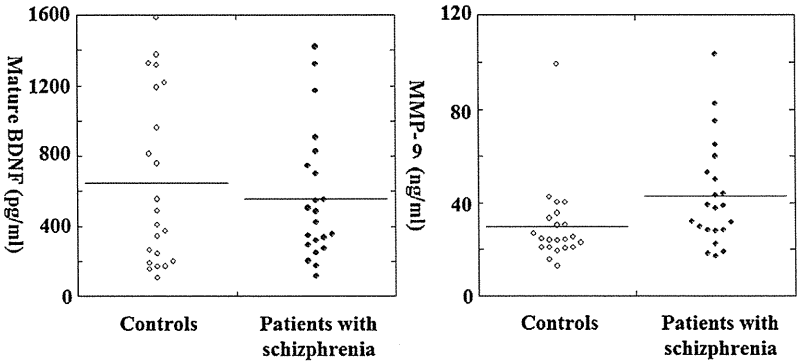

Fig. 1. Plasma levels of mature BDNF and MMP-9 in treatment-resistant schizophrenia treated with clozapine. The plasma levels of mature BDNF and MMP-9 in the controls and treatment-resistant patients with schizophrenia who were treated with clozapine (control, $n=22$, schizophrenia, $n=22$).

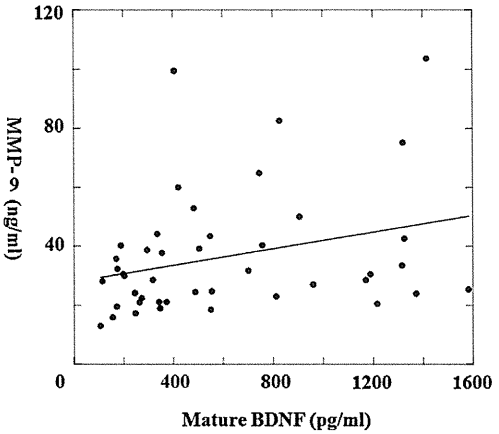

Fig. 2. Correlation between plasma levels of mature BDNF and MMP-9. Positive correlation was observed between plasma levels of mature BDNF and MMP-9 (patients with schizophrenia and controls, $n=44$, $r=0.333$, $p=0.027$).

2.3. Statistical analysis

Statistical analyses were performed using SPSS 20.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were analyzed using χ^2 tests for categorical variables. The groups did not differ with respect to age or gender (Table 1). Kolmogrov–Smirnov test was used to test the normality of data distribution. Mature BDNF did not normally distribute in both patients with schizophrenia and controls (patients with schizophrenia; $p=0.041$, controls; $p=0.042$). MMP-9 distributed normally in patients with schizophrenia, however did not distribute normally in controls (patients with schizophrenia; $p=0.130$, controls; $p=0.012$). And differences between patients and controls were analyzed using the Mann–Whitney U-test for continuous variables. Homogeneity of variance was assessed by Levene's test. The assumption of homogeneity of group variance was not violated in both mature BDNF and MMP-9 levels (mature BDNF; $p=0.052$, MMP-9; $p=0.112$). Test of rejection of Smirnoff–Grubbs was performed. Spearman rank order correlation test was performed to assess the possible correlation between plasma levels of mature BDNF and MMP-9 and clinical characteristics. The significant level for statistical tests was set at $p<0.05$.

3. Results

The plasma levels of mature BDNF and MMP-9 were compared between patients with treatment-resistant schizophrenia who were treated with clozapine and controls, and no significant difference was observed for mature BDNF (Fig. 1, Mann–Whitney test; $U=238$, $p=0.925$). However, MMP-9 was significantly increased in patients with schizophrenia (Fig. 1, Mann–Whitney test; $U=139$, $p=0.016$). When we exclude each one sample in both groups by test of rejection of Smirnoff–Grubbs, MMP-9 was still significantly increased in patients with schizophrenia (Mann–Whitney test; $U=118$, $p=0.010$). As MMP-9 plays a role in the conversion of proBDNF to mature BDNF, the correlation between the levels of mature BDNF and MMP-9 was examined. There were significant correlation between the levels of mature BDNF and MMP-9 in (Fig. 2, patients with schizophrenia and controls, $n=44$, $r=0.333$, $p=0.027$). When we investigate this correlation in patients and controls groups separately, significant correlation was observed in patients with schizophrenia ($n=22$, $r=0.585$, $p=0.004$) but not in controls ($n=22$, $r=0.322$, $p=0.143$). To determine the effect of clozapine on mature BDNF and MMP-9 levels, we also examined the correlation between the plasma levels of mature BDNF or MMP-9 and clozapine dosage. No significant correlation was observed between the plasma levels of mature BDNF or MMP-9 and clozapine dosage (Table 2, BDNF and clozapine dosage; $n=22$, $r=0.028$, $p=0.901$, MMP-9 and clozapine dosage; $n=22$, $r=0.131$, $p=0.562$). The correlations between the plasma levels of mature BDNF or MMP-9 and positive and negative symptom scores on the PANSS were also investigated; no significant correlations were observed (Table 2, BDNF and PANSS positive; $n=22$, $r=-0.014$, $p=0.952$, BDNF and PANSS negative; $n=22$, $r=-0.079$, $p=0.726$, MMP-9 and PANSS positive; $n=22$, $r=0.306$, $p=0.167$, BDNF and PANSS negative; $n=22$, $r=0.127$, $p=0.574$). The correlations between the plasma levels of mature BDNF or MMP-9 and duration of illness were also investigated; no significant correlations were observed (Table 2, BDNF and duration of illness; $n=22$, $r=0.121$, $p=0.592$, MMP-9 and duration of illness; $n=22$, $r=0.087$, $p=0.699$).

Table 2
Correlation analysis.

	Clozapine dosage	PANSS positive	PANSS negative	PANSS general	Age at onset	Duration of illness
Mature BDNF	0.901	0.952	0.726	0.865	0.332	0.592
MMP-9	0.562	0.167	0.574	0.454	0.685	0.699

p values are shown.

4. Discussion

In this study, for the first time, we measured the plasma levels of mature BDNF in patients with schizophrenia. The plasma levels of mature BDNF were decreased in treatment-resistant schizophrenia, however the difference did not reach statistical significance. Our result was consistent with some previous studies that investigated the serum levels of total BDNF in patients with schizophrenia [10,20]. Treatment-resistant schizophrenia patients treated with clozapine were enrolled because some studies suggest that peripheral BDNF levels increase in association with antipsychotics treatment including clozapine which is used for the treatment of poorly responsive patients with schizophrenia [6,10] and serum BDNF levels were reported to be significantly correlated with clozapine daily dose but not with typical antipsychotics [15]. However, we found no effect of clozapine treatment on the plasma levels of mature BDNF. A possible explanation would be the difference in race. This is the first study investigating the effect of clozapine treatment on the plasma levels of mature BDNF in Japanese population. Accumulating evidence suggests that BDNF plays a key role in the pathophysiology of major depressive disorder (MDD). It was reported that BDNF serum levels in patients with MDD were significantly lower than those of healthy controls, and that there was a negative correlation between BDNF serum levels and the severity of depression in patients [19]. Furthermore, decreased serum levels of BDNF in antidepressant naive patients with MDD, recovered to levels associated with amelioration of depressive symptoms, after antidepressant treatment. Three meta-analyses and a study using a large sample size confirmed these findings [7]. Recently, peripheral levels of mature BDNF have been reported to be decreased in MDD [23]. Further study using larger samples is needed to see whether peripheral levels of mature BNDF are not altered in schizophrenia and mature BDNF levels are not associated with clozapine.

We also investigated MMP-9 plasma levels, as MMP-9 plays a role in the conversion of proBDNF to mature BDNF [8]. The significant correlation was observed between mature BDNF and MMP-9 plasma levels, suggesting that MMP-9 plays a role in the conversion of proBDNF to mature BDNF in the samples of this study. The serum levels of MMP-9 have been reported to be increased in patients with schizophrenia [2]. A higher frequency of positive MMP-9 activity in serum from patients with schizophrenia has also been reported [1]. We confirmed the presence of elevated plasma MMP-9 levels in patients with treatment-resistant schizophrenia. In patients with schizophrenia, MMP9 might be induced to recover the decreased mature BDNF. The finding that significant correlation between mature BDNF and MMP-9 was observed only in patients with schizophrenia but not in controls supports this idea. Plasma levels of MMP-9 have been proposed to be a useful biomarker for assessing pathological event in brain. It was reported that levels of MMP-9 in plasma and brain were significantly correlated after cerebral ischemia in rats [14]. MMP-9 is an enzyme implicated in a number of pathological conditions including neuropsychiatric disorders [18]. A role of MMP-9 in the plasticity of the central nervous system has been investigated in experimental studies and MMP-9 is reported to be required for hippocampal long-term potentiation and memory [12]. MMP-9 may have some roles in pathophysiology of schizophrenia.

Our study must be interpreted in lights of its limitations. Firstly, the sample size of this study is small. Secondly, only treatment-resistant schizophrenia patients treated with clozapine were included and patients treated with other antipsychotics or patients without antipsychotics treatment were not included in this study. Further studies are needed to evaluate the relationship between plasma levels of mature BDNF and schizophrenia and clozapine treatment.

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