Lower Serum Tropomyosin Receptor Kinase B Levels in Patients with Schizophrenia

Yi-Yung Hung, Tiao-Lai Huang

Background: Brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) have previously been found to be reduced in the prefrontal cortex of patients with schizophrenia. In this study, we tried to investigate the protein levels of BDNF and TrkB from peripheral blood in the veins of individuals with schizophrenia and health controls.

Methods: From January 2008 to November 2010, we recruited 40 schizophrenic patients and 56 healthy controls. Serum BDNF and total TrkB protein levels were detected with enzyme-linked immunosorbent assay (ELISA) kits. Outliners of BDNF and TrkB were excluded initially. Analysis of covariance (ANCOVA) with age adjustment was used for group mean differences of different groups.

Results: After using the ANCOVA with age adjustment, the results of this work showed that BDNF presented no significant difference ($F = 0.065$, $p = 0.800$), but the serum TrkB protein level was significantly lower in schizophrenic patients than in healthy controls ($F = 8.34$, $p = 0.005$).

Conclusion: Our findings showed a lower TrkB protein level in serum from schizophrenia patients compared with healthy controls, indicating that the signaling transmission of BDNF/TrkB may be affected in peripheral blood from individuals with schizophrenia.

(Biomed J 2013;36:132-136)

Key words: brain-derived neurotrophic factor, schizophrenia, tropomyosin receptor kinase B

At a Glance Commentary

Scientific background of the subject

Although Brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) have been found to be associated with schizophrenia in central nerve system, there is few data in the published literature regarding the changes in peripheral blood.

What this study adds to the field

Lower TrkB protein level is found in patients with schizophrenia especially in female.

Schizophrenia is thought to be a chronic, severe, and disabling brain disease. However, its neuropathological basis remains unclear.[1] Some evidence has suggested that the pathogenesis of schizophrenia is neuro-developmental impairments, especially in brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family.[2] Besides, BDNF in serum was identified as one of the biological signatures for schizophrenia.[3,4] The role of BDNF in schizophrenia has been extensively investigated in different stages of the disease, including the effect of antipsychotic drug treatment.[5,6] Postmortem studies of the central nervous system have shown that BDNF protein levels, as measured by enzyme-linked immunosorbent assay (ELISA), were increased in cortical areas and decreased in the hippocampus in schizophrenia;[7] however, immunohistochemical studies have shown increased expression of BDNF.[8] Several studies have reported a reduction of BDNF in peripheral blood in chronic and relapsed schizophrenia patients.[2,9,10]

On the other hand, the tropomyosin receptor kinase B (TrkB), a receptor of BDNF on cell membrane, is implicated in the neuropathology of schizophrenia and other psychiatric diseases.[11] The level of expression of TrkB in the brain of subjects with schizophrenia is still inconclusive. In one postmortem study, the expressions of TrkB

From the Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan

Received: Apr. 25, 2012; Accepted: Jul. 16, 2012

Correspondence to: Dr. Tiao-Lai Huang, Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan. 123, Dapi Rd., Niaosong, Kaohsiung 833, Taiwan (R.O.C.). Tel: 886-7-7317123 ext. 8753; Fax: 886-7-7326817; E-mail: a540520@adm.cgmh.org.tw

DOI: 10.4103/2319-4170.113229

receptor were reduced significantly in the hippocampus and the prefrontal cortex.[12] However, other studies have reported increased TrkB-positive neurons in the hippocampus of subjects with schizophrenia.[8] When comparing BDNF and TrkB mRNA levels in the prefrontal cortex of 15 pairs of subjects with schizophrenia and matched control subjects, as assessed by *in situ* hybridization, one study found a significant decrease in the prefrontal cortex of subjects with schizophrenia.[13] In addition, a diminished expression of TrkB mRNA in large neurons, but not in small neurons, was reported in the dorsolateral prefrontal cortex in patients with schizophrenia.[14] A TrkB protein level elevation in peripheral blood was reported previously in patients with major depression,[15] but there is still no data on subjects with schizophrenia. Therefore, we assumed that BDNF/TrkB signal pathway might be involved in schizophrenia and sought to investigate the BDNF/TrkB protein level in peripheral blood of schizophrenia patients.

METHODS

Subjects

Serum BDNF and TrkB protein levels in acute exacerbated schizophrenic outpatients or inpatients and healthy controls were collected from January 2008 to November 2010 at Chang Gung memorial hospital (CGMH)-Kaohsiung medical center, Taiwan. Institutional Review Board (IRB) approval was obtained from the CGMH Ethics Committee. The structured clinical interview for DSM-IV Axis I Disorders (SCID-1) was used by a psychiatrist to diagnose schizophrenia.[16] Psychopathology was assessed with the positive and negative syndrome scale (PANSS) for schizophrenia by the same psychiatrist.[17]

Voluntary healthy controls were enrolled from the medical staff and students. These healthy controls had neither a personal history nor a first-degree family history of psychiatric disorder. The Chinese health questionnaire-12 was used to assess the healthy control group by the same psychiatrist, to exclude psychiatric diseases based on diagnostic and statistical manual of mental disorders, Fourth Edition (DSM-IV) criteria.[18] The questionnaire was derived from a Chinese translation of the general health questionnaire, with the addition of specially designed, culturally relevant items.

None of the participants had an acute or chronic physical illness, including heart, lung, liver, kidney, or metabolic diseases. No medication was taken for at least 1 week before entering the study. After the demographic data was collected, patient group was treated with antipsychotic drugs.

Laboratory data

Venous blood (5 ml) samples were drawn into plain tubes between 08.00 and 10.00 a.m., before the patients had eaten breakfast. The serum samples were separated in a centrifuge at 1500 rpm and stored in a 80 C refrigerator quickly until assayed in 3 months. Serum BDNF and total TrkB protein levels were detected with ELISA kits (BDNF Emax Immunoassay System, Promega Co., Madison, WI, USA; The Human Total TrkB DuoSet IC ELISA, R&D Systems, Inc., Minneapolis, MN, USA). Absorbencies were measured by microtiter plate reader (absorbency at 450 nm). The BDNF assay detects a minimum of 15.6 pg/ml of BDNF and shows less than 3% cross-reactivity with other related neurotrophic factors (Nerve growth factor (NGF), Neurotrophin-3 (NT-3), and Neurotrophin-4 (NT-4)) at 100 ng/ml. The TrkB assay is as sensitive as immunoprecipitation (IP)-Western blot analysis and does not cross-react with TrkA or TrkC.

Statistical analysis

Outliners of BDNF and TrkB were excluded initially. Student's *t*-test was used to compare the continuous demographic data. Chi-square test was used to compare categorical data. BDNF and TrkB analyses were performed by using an analysis of covariance (ANCOVA) with age adjustment

Table 1: Demographics and serum BDNF protein levels in all participants after excluding outliners of BDNF

	Schizophrenia	Healthy controls	F and *p* values
Sex (M/F)	14/21	24/28	*p*=0.570
Age (years)	34.83±1.85	31.15±0.79	*p*=0.075
Male	30.86±2.02	29.58±1.37	*p*=0.593
Female	37.48±2.66	32.50±0.83	*p*=0.087
PANSS	112.60±5.06	-	-
Male	130.00±4.00	-	-
Female	117.67±7.91	-	-
BMI	22.55±0.78	23.26±0.55	*p*=0.447
Male	24.62±1.53	24.08±0.83	*p*=0.736
Female	21.67±0.68	22.55±0.72	*p*=0.183
Education (years)	12.34		
Duration of illness (years)	7.50±0.86	-	-
BDNF (ng/ml)	3.73±0.17	4.67±0.34	F=0.065 *p*=0.800
Male	3.89±0.32	6.11±0.42	F=0.019 *p*=0.89
Female	3.61±0.19	3.43±0.38	F=0.027 *p*=0.871

Data are presented as mean±SD. Student's *t*-test was used to compare age, PANSS, and BMI. Chi-square test was used to compare sex. BDNF and TrkB analysis were performed by using an analysis of covariance (ANCOVA) with age adjustment. Abbreviations: PANSS: Positive and negative syndrome scale; BDNF: Brain-derived neurotrophic factor; BMI: Body mass index

for group mean differences of different groups. All statistical analyses were performed using SPSS, version 12. For each test, $p < 0.05$ was considered significant.

RESULTS

Our sample consisted of 40 schizophrenia patients and 56 healthy control subjects. Table 1 shows the demographic data and serum BDNF after excluding the outliner of BDNF. Table 2 shows the demographic data and serum TrkB after excluding the outliner of TrkB. The mean total PANSS score was 112.60 ± 5.06 in Table 1 and 122.30 ± 4.67 in Table 2. T here was no dif ference between the two groups with regard to demographic data. The medication prescribed before survey was either risperidone 4-6 mg or clozapine 100-300 mg. Using ANCOVA with age adjustment, no significant difference in the mean values of BDNF protein levels in the patients and controls was found (F = 0.065, p = 0.800). Neither males (F = 0.19, p = 0.89) nor females (F = 0.027, p = 0.871) presented significant difference in comparison with controls. The TrkB protein level was significantly lower in schizophrenia patients than in healthy controls (F = 8.34, p = 0.005). When analyses were done in different genders, the TrkB protein level was significantly lower in schizophrenia patients than in healthy controls, in women: 322.50 ± 94.12 pg/ml and 609.80 ± 96.07 pg/ml for patients and controls, respectively (F = 5.219, p = 0.027), but not in men (F = 0.94, p = 0.338).

Table 2: Demographics and serum TrkB protein levels in all participants after excluding outliners of TrkB

	Schizophrenia	Healthy controls	F and p value
Sex (M/F)	17/22	21/32	p=0.703
Age (years)	34.50±1.60	31.33±0.76	p=0.079
Male	32.29±1.86	29.86±1.48	p=0.307
Female	36.29±2.44	32.34±0.75	p=0.076
PANSS	122.30±4.67	-	-
Male	128.29±3.55	-	-
Female	117.33±7.89	-	-
BMI	22.56±0.74	23.00±0.52	p=0.62
Male	24.60±1.30	23.97±0.86	p=0.68
Female	20.91±0.67	22.34±0.64	p=0.14
Education (years)	12.67		
Duration of illness (years)	7.64±0.84	-	-
TrkB (pg/ml)	399.99±80.07	663.56±77.33	F=8.34 p=0.005*
Male	495.71±135.73	741.75±129.35	F=0.94 p=0.338
Female	322.50±94.12	609.80±96.07	F=5.219 p=0.027*

Data are presented as mean±SD. Student's t-test was used to compare age, PANSS, and BMI. Chi-square test was used to compare sex. TrkB analysis is performed by using an analysis of covariance (ANCOVA) with age adjustment. Abbreviations: PANSS: Positive and negative syndrome scale; BDNF: Brain-derived neurotrophic factor; BMI: Body mass index; TrkB: Tropomyosin receptor kinase B; *$p < 0.05$,

DISCUSSION

The present study investigated TrkB levels in the serum and their relationship to psychopathology in schizophrenia patients. The key findings from the study are as follows: 1. Though TrkB was thought to function as a surface protein, it is detectable in serum and its serum levels are significantly different between schizophrenia patients and healthy controls. 2. BDNF protein levels were no different in schizophrenia patients compared to healthy controls, although protein levels of TrkB were low in this group. These data indicate defective serum TrkB protein in schizophrenia patients for the first time.

There is an important implication in the findings of this study for the possible pathology and treatment of schizophrenia. The present study gives direct evidence that changes in BDNF/TrkB signaling are not only affected by the changing levels of surface receptor. Extracellular receptor protein also plays an important role. One study reported a 44% reduction of TrkB in hippocampal extracts from schizophrenia patients.[12] Significant diminished expression of TrkB mRNA in the large neurons of schizophrenia patients as compared to controls was also reported.[14] However, two studies showed no decrease in full-length TrkB expression.[19,20] The inconsistent results with regard to the levels of TrkB lead to the suggestion that further mechanistic studies are needed to understand BDNF/TrkB signaling in schizophrenia.

The negative result in BDNF level difference between schizophrenia patients and healthy controls is inconsistent with some previous findings. Serum BDNF concentration in schizophrenia has controversial data. Some studies reported negative finding in serum BDNF levels in schizophrenia patients compared with healthy volunteers,[2] while other studies found significant decreases in total or truncated BDNF protein levels.[21,22] Reduced BDNF is not specific to schizophrenia. Some confounding factors such as depressive status, antipsychotic drugs, extrapyramidal symptoms, and tardive dyskinesia may affect the level of BDNF.[23,24]

Another interesting finding is the gender difference. A similar finding was reported in our previous study in which it was found that TrkB levels in women were increased significantly during depression.[15] This study presents a significant decrease in TrkB levels in female schizophrenia patients. Sex hormones may play a role in the pathogenesis of schizophrenia.[25,26] The distribution of phosphorylated TrkBs was found to be affected by estradiol levels.[27] However, the real mechanism of estrogen that influences TrkB protein is still unknown.

The mechanism underlying the elevated TrkB serum

protein level in schizophrenia patients compared to controls is not known. There are many factors that affect the level of extracellular protein, including degradation and retro-translocation.[28,29] The reason why TrkB could be found in serum is unknown. The function of translocon or direct interactions between protein and lipid are a possible mechanism.[30,31]

Among the limitations of this work, we should underscore the following. To begin with, truncated TrkB receptors were reported to have dominant inhibitory effects on BDNF signaling.[32] Increased truncated TrkB receptors may contribute to reduced overall BDNF/TrkB signaling and lead to reduced neuronal plasticity.[33] However, we are only investigating total TrkB protein level. Besides, the sample size is too small, therfore analyses with larger sample sizes and better methodology are needed.

Taken together, the findings from the present study give evidence to the importance of the appropriate serum protein level of TrkB in schizophrenia patients. Decreased expression of TrkB in serum indicates the possibility that TrkB signaling impairment in schizophrenia is not at the level of neurotrophin supply, but rather at the level of the TrkB receptor being secreted in the serum. Determination of cell surface and serum TrkB receptor ratios may help in designing novel therapeutic strategies based on BDNF signaling in schizophrenia. In addition, the sample size was small in this study. A larger sample size and proper control groups are necessary to investigate and elucidate the relationships between serum TrkB protein levels and the different stages of schizophrenia in the future.

Acknowledgment

This work was supported by three clinical research grants from Chang Gung Memorial Hospital (NMRPG860231, CMRPG870952, CMRPG870951) in Taiwan.

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