Decreased levels of whole blood glial cell line-derived neurotrophic factor (GDNF) in remitted patients with mood disorders

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Abstract

Recent post-mortem and imaging studies provide evidence for a glial reduction in different brain areas in mood disorders. This study was aimed to test whether glial cell line-derived neurotrophic factor (GDNF), a member of transforming growth factor (TGF)-β superfamily, in blood levels was associated with mood disorders. We measured GDNF and TGF-β levels in whole blood in remitted patients with mood disorders [n = 56; major depressive disorders (MDD) 39, bipolar disorders (BD) 17] and control subjects (n = 56). GDNF and TGF-β were assayed with the sandwich ELISA method. Total GDNF levels were significantly lower in MDD and in BD than in control subjects (MDD, p = 0.0003; BD, p = 0.018), while no significant difference in total TGF-β1 or total TGF-β2 levels was found in these groups. Our study suggests that lower GDNF levels might be involved in the pathophysiology of mood disorders, although this preliminary study has several limitations.

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Key words: GDNF, mood disorders, transforming growth factor (TGF), whole blood.

Introduction

Mood disorders, including major depressive disorders (MDD) and bipolar disorders (BD), are common mental dysfunction with uncertain aetiology. A number of imaging and post-mortem studies in patients with mood disorders have revealed a reduction of particular areas such as the prefrontal cortex, hippocampus and amygdala in total volume and cell density/size, especially glial cells (Manji et al., 2001; Ongur et al., 1998; Rajkowska, 2002). In correspondence with these studies, recent findings showed that brain-derived neurotrophic factor (BDNF), neurotrophin-3 and fibroblast growth factor (FGF) systems, which are potent regulators for neuronal plasticity, survival and development, are altered in different samples such as post-mortem brain, cerebrospinal fluid, and blood from patients with mood disorders (Evans et al., 2004; Hock et al., 2000; Shimizu et al., 2003). It suggests that the dysregulation of multiple neurotrophic/growth factor systems might be involved in the aetiology of mood disorders.

Glial cell line-derived neurotrophic factor (GDNF), a member of transforming growth factor (TGF)-β superfamily, was originally purified from a rat glial cell line supernatant as a trophic factor for midbrain dopamine neurons, and was later found to have pronounced effects on other neuronal populations (Airaksinen and Saarma, 2002). GDNF has been reported to play important roles in higher-ordered brain function such as cognitive abilities and drug addiction (Gerlai et al., 2001; Messer et al., 2000). However, to the best of our knowledge, a relationship between GDNF and mood disorders has not been investigated.

Here, we examine whether blood levels of GDNF in patients with mood disorders would be altered from healthy control subjects.
Method

Fifty-six Japanese patients with mood disorders (37 male, 19 female; mean age ± s.d. = 59.0 ± 12.4 yr; age range = 27–85 yr) were recruited from the Department of Psychiatry at NHO Kure Medical Center and the Department of Neuropsychiatry at Hiroshima University Hospital. According to the DSM-IV (APA, 1994), diagnosis of mood disorders and evaluation of symptoms were determined after a clinical interview and a review of clinical records by two psychiatrists. The fifty-six patients consisted of 39 MDD, a single episode or recurrent, and 17 BD, including 9 bipolar I disorder and 8 bipolar II disorder. All patients were under partial or full remitted state when their blood was drawn, and were treated with psychotropic medication such as antidepressants and mood stabilizers. The following antidepressant drugs were administered: paroxetine (n = 15), amoxapine (n = 13), sulpiride (n = 10), trazodone (n = 7), fluvoxamine (n = 7), mianserin (n = 6), milnacipran (n = 3), imipramine (n = 3), clomipramine (n = 3), maprotiline (n = 2). We estimated 150 mg of imipramine-equivalent dose of each antidepressant as follows; paroxetine, 40 mg; mianserin, 60 mg; milnacipran, 150 mg; fluvoxamine, 150 mg; amitriptyline, 150 mg; clomipramine, 150 mg; amoxapine 150 mg; maprotiline, 150 mg; sulpiride, 300 mg; trazodone, 300 mg. Twenty-three of patients received combined antidepressant treatment with more than two types of antidepressants. Twenty-nine patients received mood stabilizers such as lithium (n = 27, 600 ± 175 mg/d), valproate (n = 2, 400–800 mg/d) and carbamazepine (n = 1, 600 mg/d). Five patients received a monopharmacotherapy of mood stabilizer without any antidepressant.

Fifty-six race- and gender-matched healthy control subjects (17 male, 39 female; mean age ± s.d. = 47.5 ± 9.41 yr, age range = 27–60 yr) were recruited from volunteers in NHO Kure Medical Center and the Hiroshima University Hospital. Subjects with any other diagnosed mental or physical illness were excluded.

The study was approved by the NHO Kure Medical Center Ethics Committee and the Hiroshima University Medical Ethics Committee. Written informed consent was obtained after a full written and verbal explanation of the study.

The blood samples from the patients and the controls were drawn into tubes with ethylenediaminetetraacetic acid around noon and directly transferred to other tubes for storage at −80 °C until assayed. Total and free levels of GDNF, TGF-β1, and TGF-β2 levels were measured by using the enzyme linked immunosorbent assay (ELISA; Emax Immunoassay System kit, Promega, Madison, WI, USA), according to the manufacturer’s instructions. GDNF is likely to interact with its ligands from receptors or binding proteins in blood and many types of tissues; thus, the acid treatment procedure is reported to cause dissociation of the ligands from GDNF and to increase the detectable amount of GDNF (total GDNF) (Okragly and Haak-Frendscho, 1997). Therefore, we measured total GDNF, TGF-β1 and TGF-β2 levels by the acid treatment procedure (the diluted samples were acidified to pH 2.6, followed by neutralization to pH 7.6 and centrifuged) according to the manufacturer’s instructions, and measured free GDNF level without the procedure. All assays were performed in duplicate. For statistical analysis, the data were presented as the mean ± s.d. χ² analysis was performed on binomial data such as gender, and Student’s t test was employed for the continuous variables (Table 1). One-way analysis of variance (ANOVA) was used to check statistical tendencies. When significant tendency was suggested, differences between groups were analysed by Fisher’s protected least significant differences post-hoc test (Figures 1, 2, Table 2). The relationship between two variables was examined using Pearson’s correlation coefficient. p values < 0.05 were considered significant.

Results

One-way ANOVA indicated a significant difference in whole blood levels of total GDNF between the patients with MDD, BD, and the control subjects (F = 7.8, p < 0.01).
we could not statistically estimate these values, although the values of these groups appeared similar. Further, one-way ANOVA revealed that there were no group differences in the levels of total TGF-β1 ($F=0.1$, $p=0.91$) and TGF-β2 ($F=2.7$, $p=0.07$) (Table 2). Table 1 shows the participants’ demographics for which gender was matched, but age differed significantly between the groups, although no significant correlation was detected between levels of any TGF-β superfamily (including GDNF) and age in any of the subjects ($n=112$; total GDNF: $r=-0.126$, $p=0.186$; total TGF-β1: $r=0.22$, $p=0.819$; total TGF-β2: $r=0.009$, $p=0.924$). There was a tendency of gender difference in total GDNF levels in any subject ($n=112$; male = 34, 719 ± 462 pg/ml; female = 78, 907 ± 469 pg/ml, $p=0.052$). Neither age of onset ($n=56$, $r=0.045$, $p=0.744$), number of episodes ($n=56$, $r=0.007$, $p=0.958$), dose of antidepressants (imipramine equivalents/d) ($n=56$, $r=0.09$, $p=0.5156$) or dose of lithium ($n=27$, $r=0.058$, $p=0.778$) correlated significantly with the levels of total GDNF in patients with mood disorders. In addition, there was no difference in total GDNF levels between subgroups of mood disorders treated with and without antidepressants, although the total GDNF levels in each group were significantly lower than that in control subjects (Figure 2a). In the same way, there was no difference in total GDNF levels between subgroups of mood disorders treated with and without lithium (Figure 2b). The total GDNF levels in patients with a family history of mood disorders ($n=10$, 656 ± 330 pg/ml) did not differ from those in patients without one ($n=46$, 706 ± 423 pg/ml, $p=0.73$). There was no difference in total GDNF levels between subgroups of partial and full remitted patients (partial: $n=47$, 717 ± 428 pg/ml; full: $n=9$, 511 ± 152 pg/ml, $p=0.16$), as well as between subgroups of bipolar I and bipolar II disorders (bipolar I: $n=9$, 707 ± 152 pg/ml; bipolar II: $n=8$, 731 ± 173 pg/ml, $p=0.92$).

**Discussion**

We found that total GDNF levels in whole blood in remitted patients with mood disorders were significantly lower than those in healthy control subjects, whereas total TGF-β1 and total TGF-β2 were not altered between the groups. This is the first report showing a possible association of GDNF to mood disorders.

In this study, we could not exclude the effects of antidepressants and mood stabilizers on the total GDNF levels in the patients. In the case of antidepressants, previous animal studies have reported
that chronic treatments with antidepressants did not affect on GDNF mRNA and protein levels in several areas of rat brain (Chen et al., 2001). In contrast, sub-chronic treatment with antidepressants increased GDNF mRNA and protein levels in glial cultured cells (Hisaoka et al., 2001; Mercier et al., 2004). In the case of mood stabilizers including lithium, chronic treatments with lithium and valproate did not affect on GDNF mRNA and protein levels in several areas of rat brain (Fukumoto et al., 2001). In the Finders Resistant Line rats, chronic treatment with lithium increased GDNF protein levels in the frontal cortex and occipital cortex, decreased the GDNF levels in the hippocampus, and did not alter the GDNF levels in the striatum (Angelucci et al., 2003). These previous reports suggest different effects of antidepressants and mood stabilizers on the GDNF expressions by strain, areas of brain, and types of experimental system. In this clinical study, there was no difference in total GDNF levels between subgroups of mood disorders treated with and without antidepressants, as well as between subgroups of mood disorders treated with and without lithium (Figure 2). In addition, no correlation between total GDNF levels in whole blood and dose of antidepressants/mood stabilizers among the patients was observed. Consequently, it is unlikely that medication may decrease the total GDNF levels in the patients. Another important concern is whether the GDNF effect in remitted patients with mood disorders is a state or trait marker. Our study indicates that degree of psychiatric symptom might not affect the GDNF levels because there was no difference in total GDNF

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control subjects</th>
<th>MDD (n=39)</th>
<th>BD (n=17)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Total TGF-β1</td>
<td>4499±2475</td>
<td>4299±1615</td>
<td>4938±2407</td>
<td>F=0.1, p=0.91</td>
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<tr>
<td>Total TGF-β2</td>
<td>4565±2969</td>
<td>3606±2949</td>
<td>5556±3245</td>
<td>F=2.7, p=0.07</td>
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Table 1. Demographics of subjects and controls

<table>
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<tr>
<th></th>
<th>Mood disorders</th>
<th>Control subjects</th>
<th>t or χ²</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>No. of subjects</td>
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<td>56</td>
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<td></td>
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<tr>
<td>Subtype (MDD/BD)</td>
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<td>-</td>
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<tr>
<td>Gender (M/F)</td>
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<td>17/39</td>
<td>χ²=0.548</td>
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<td>MDD</td>
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<tr>
<td>BD</td>
<td>4/13</td>
<td></td>
<td></td>
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<tr>
<td>Mean age (yr)</td>
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<tr>
<td>MDD</td>
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<td>47.5±9.41</td>
<td>t=5.52‡</td>
<td>0.0001</td>
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<tr>
<td>BD</td>
<td>56.9±11.2</td>
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<td>Onset (yr)</td>
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<td>MDD</td>
<td>51.9±12.0</td>
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<td>t=1.59†</td>
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<td>BD</td>
<td>53.6±11.8</td>
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<tr>
<td>No. of episodes</td>
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<tr>
<td>MDD</td>
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<td>t=−2.81‡</td>
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<td>BD</td>
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<td>Imipramine-equivalents (mg/d)</td>
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<tr>
<td>BD</td>
<td>135±84.0</td>
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MDD, Major depressive disorder; BD, bipolar disorder.
Data are shown as mean±s.d.
† Comparisons between two groups of mood disorders and control subjects.
‡ Comparisons between MDD and BD.

Table 2. Total levels of whole blood transforming growth factor (TGF) in patients with mood disorders and control subjects

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levels between subgroups of partial and full remitted patients, although the results are not conclusive. Therefore, additional studies by investigating drug responses of GDNF levels to drug-naive patients with psychiatric symptoms are needed to clarify whether a decrease of GDNF levels is secondary (i.e. effects of the drug medication or psychiatric symptoms) or primary (a genetic vulnerability) in patients with mood disorders.

The source of circulating GDNF in blood is totally unknown, although glia, neuron, kidney and ovary appear to be candidates (Golden et al., 1999). In the case of BDNF, the sources of circulating BDNF are considered to be platelets, brain neurons and vascular endothelial cells (Radka et al., 1996), and a positive correlation between blood and cortical BDNF levels was observed in rats (Karege et al., 2002). This suggests that blood cells could store and release neurotrophic factors. Actually, total GDNF levels in whole blood in healthy subjects were higher than those in plasma and serum were (data not shown), suggesting that an unknown source of blood cells might be involved in the total GDNF blood levels. Therefore, in this study, we measured concentrations of total GDNF in whole blood including the contents of all blood cell types. It is vital to confirm whether blood GDNF levels positively correlate with cerebral GDNF levels, as blood BDNF levels do.

This study is a preliminary report that has a number of limitations such as age difference and medication use. Recent imaging and biochemical studies in mood disorders have revealed a morphological reduction of several brain areas (Manji et al., 2001; Ongur et al., 1998; Rajkowska, 2002) and alternations of multiple neurotrophic/growth factor systems (Evans et al., 2004; Hock et al., 2000; Shimizu et al., 2003), although a direct association among both of them is still unclear. Taken together with our results that total GDNF levels in whole blood were reduced in patients with mood disorders, it is suggested that dysregulation of multiple neurotrophic/growth factor systems such as BDNF, FGF, and GDNF may be involved in the aetiology of the complex disorders. Further in-depth study will be necessary.

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Statement of Interest

None.

References


