行政院國家科學委員會專題研究計畫 成果報告

重鬱症患者抗鬱劑治療前後血清 BDNF 及 T 淋巴球 CREB 磷酸化的變化

研究成果報告(精簡版)

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中 華 民 國 96 年 10 月 23 日

附件一

行政院國家科學委員會補助專題研究計畫 V 成果報告 □期中進度報告

（計畫名稱）

重鬱症患者抗鬱劑治療前後血清 BDNF 及 T 淋巴球 CREB 磷酸化的變化

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成果報告類型(依經費核定清單規定繳交)：v□精簡報告　□完整報告

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□赴國外出差或研習心得報告一份
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□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份
處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
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執行單位：

中　華　民　國　96　年　10　月　23　日

中文摘要

本研究目的在研究未服藥之重度憂鬱症患者血清之腦部滋養因子濃度與健康人之差異,同時探討血清之腦部滋養因子濃度與憂鬱症狀及壓力感受嚴重度之相關.

本研究在台北醫學大學-萬芳醫院執行;共收集 21 位未服藥之重度憂鬱症患者及 17 位健康人.以 17-item Hamilton Depression Rating Scale (HAMD-17) 評估憂鬱症狀嚴重度 14-item 壓力感受量表評估壓力感受程度.血中腦部滋養因子濃度以 ELISA 測定.

結果發現血清之腦部滋養因子濃度在未服藥之重鬱症患者(mean = 26.7 ± 7.0 ng/ml) 與健康控制組並無顯著差異 (mean = 26.8 ± 6.5 ng/ml). 且血清之腦部滋養因子濃度與憂鬱症狀之嚴重度及壓力感受程度並無顯著相關(r = 0.01 and 0.27).

本研究結果發現重鬱症患者之血清之腦部滋養因子濃度與常人並無差異;但受限於樣本數太小,並無法做確切推論.但血清之腦部滋養因子濃度在壓力相關疾病仍然值得進一步探討.

英文摘要

Backgrounds: The study is aimed to demonstrate serum brain-derive neurotrophic factor (BDNF) levels in drug-free depressive patients compared to those in health controls and to investigate the correlation of the perceived stress level and serum BDNF levels in Taiwan.

Methods: The study was conducted in Taipei Medical University-Wan Fang Hospital. Twenty one drug-free patients with major depressive disorder, defined by DSM-IV criteria, and 17 health control were included. The severity of depression was assessed with 17-item Hamilton Depression Rating Scale (HAMD-17) and the severity of perceived stress was assessed with 14-item version of the Perceived Stress Scale (PSS). Serum BDNF was assayed with an enzyme-linked immunosorbent assay.

Results: There is no difference in serum BDNF levels between drug-free depressive patients (mean = 26.7 ± 7.0 ng/ml) and health control (mean = 26.8 ± 6.5 ng/ml). No significant correlation between serum BDNF level and HAMD-17 and PSS scores was found. (r = 0.01 and 0.27, respectively).

Conclusions: The negative finding is contrary to previous studies. Though the reasons for the conflicting results are not clear, BDNF remains an interesting molecule for further research of stress-related disorders.

Introduction

Major depressive disorder (MDD), representing a collection of psychological, behavioral, and physiological symptoms [1], is one of major causes of morbidity, disability, and suicide throughout the world [2].Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor family, is recognized as playing an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and in adulthood [3, 4].

Accumulating evidence suggests that BDNF might be a candidate molecule involved in the pathophysiology of depression [5, 6]. BDNF mRNA levels were significantly depressed in animal models of depression [7, 8]. In addition, antidepressant treatment and long-term electroconvulsive seizures increased the expression of BDNF and neurogenesis in the hippocampal regions [9-12] and infusion of BDNF into the hippocampus produces antidepressant effects in two behavioral models of depression [13]. Such results support that the upregulation of BDNF expression may be important in the clinical response to antidepressant treatment.

In postmortem study, increased BDNF expression in hippocampal regions was found in subjects treated with antidepressant medications at the time of death, compared with untreated subjects [14]. Decreased levels of BDNF in the serum of antidepressant-free or antidepressant-naïve patients with major depressive disorder have been reported [15-18] and the reduced BDNF levels normalized after antidepressant treatment [15, 16].

Though the significant association between BDNF polymorphism and MDD in Caucasian has been reported [19], there is no significant difference in the genotype and/or allele frequency of the BDNF 196G/A polymorphism between major depressive patients and controls in Hans [20]. So we wonder whether lower serum BDNF levels will be found in major depressive Hans. The primary study goal is to investigate the serum BDNF levels in drug-free depressive patients compared to those in health controls. Because the life stress events have been demonstrated to be one of major risk factors triggering or inducing depressive episodes [21, 22]. The animal models of depression are based on the assumption that stress may induce depression-like behaviors. However, the relationship between perceived stress and serum BDNF levels in human has not been studied. The second study goal is to investigate the correlation of the perceived stress and serum BDNF levels.

Subjects and Methods

The study was conducted in psychiatric outpatient clinics of Taipei Medical University-Wan Fang Hospital and was approved by Institutional Review Board.

Subjects

Patients, who aged from 18 to 65 year-old, meeting the DSM-IV criteria of MDD, and did not take any medication including antidepressants in at least 3 months, were invited to participate in the study. Comorbidity with other Axis-I diagnosis and major physical illnesses was not allowed. Health controls were volunteers who had no known physical illnesses or psychiatric illness after being interviewed by CHC. After explaining the study process, written informed consent was obtained from both groups of participants.

Clinical measurements

The severity of depression was rated by senior psychiatrists (CHC and MLL) using Hamilton's Depression Rating Scale-17 items (HAMD-17) [23]. The questionnaire rates the severity of symptoms such as depressed mood, feelings of guilt, suicide, insomnia, agitation, anxiety, and weight loss. The rater must choose the possible responses to each question by interviewing the patient and observing his or her symptoms. The stress is determined by 14-item version of the Perceived Stress Scale (PSS) [24]. It was used to express how unpredictable, uncontrollable, and overloaded respondents found their lives to be, for example, subjects were asked "In the last week, how often have you felt that you were unable to control the important

things in your life?" The Taiwanese version of PSS has adequate test-retest reliability and construct validity [25].

BDNF assay

The blood was collected at 10:00-12:00 AM. A total of 20 ml of blood was withdrawal from antecubital vein for the analyses. Serum was stored at -80 ℃ before testing, and assayed within 6 months. Serum BDNF levels were measured using the BDNF ELISA kit (Promega Co, Madison, WI) according to the manufacturer's instructions. Briefly, 96-well plates were coated with 100 µL of coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6) containing 1 µg/mL anti-BDNF monoclonal antibody for 1 hour at room temperature. Plates were washed and blocked for 1 hour at room temperature with Block & Sample Buffer. Patient sera and BDNF standards were incubated at room temperature for 2 hours. After wash, the plates were incubated with anti-human BDNF polyclonal antibody (pAb) at room temperature for 2 hours, washed, and the amount of specifically bound pAb is then detected with anti-Ig Y antibody conjugated to horseradish peroxidase for 1 hour incubation at room temperature. Finally, plates were washed and incubated in peroxidase substrate and tetramethylbenzidine solution at room temperature, and the optic density (OD) absorbance at 450 nm was read using an Emax Microplate Reader (Molecular Devices, Sunnyvale, CA) to determine serum BDNF values.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Chi-squared test was used for categorical variables and Student's t test was used to for continuous variables between drug-free major depressive patients and health controls. Analysis of variance was used to compare difference of continuous variables between more than two groups. Pearson's correlation was used to test the relationship between serum BDNF level and clinical variables, i.e. severity of depression and perceived stress. A p value $< .05$ was considered to have statistically significant.

Results

Table 1 shows the demographic characteristics and clinical variables of MDD patient and control groups. There is no significant difference in demographic characteristics between MDD patients and control subjects. However, depressed patients perceived more stress than did health controls. The HAMD-17 score in depressed group ranged from 10-29, with a mean of 19.0 ± 5.9.

Figure 1 displays the serum BDNF level of depressed and control groups. There is no difference in serum BDNF levels between drug-free depressive patients and health control. Mean serum levels of depressed and control groups were 26.7 ± 7.0 ng/ml and 26.8 ± 6.5 ng/ml, respectively. Among the depressed group, we stratified them into mild and moderate-severe depression at HAMD-17 cutoff of 18. The serum BDNF of mild depression group ($n = 10$), with mean of 27.3 ± 7.1 ng/ml, was similar to that of moderate-severe depression group ($n = 11$), with mean of 26.2 ± 7.2 ng/ml. There is no significant difference on serum BDNF levels between mild depression, moderate-severe depression, and healthy control groups ($F = 0.07$, d.f. = 2, 35, $p = 0.93$). No significant correlation between serum BDNF level and HAMD-17 and PSS scores was found. ($r = 0.01$ and 0.27, respectively)

Discussion

Our results showed that there was no significant difference in serum BDNF levels between drug-free MDD patients and health control subjects. We also could not fine the correlation between serum BDNF levels and severity of depression or perceived stress. These results were

not compatible with previous studies, which showed low serum BDNF level in major depressive patients and negative correlation between serum BDNF levels and HAMD scores [17, 18].

To date, BDNF role in major depression has been widely discussed and all published studies reported lower serum BDNF levels in major depressive patients. Though BDNF can penetrate the blood-brain barrier and serum BDNF levels reflect cortical BDNF [26, 27], the effect of BDNF in brain might be site-specific. Most research studying the relationship of brain BDNF expression and depression focused on hippocampus BDNF expression. However, BDNF's action in the ventral tegmental area-nucleus accumbens pathway induced a depression-like phenotype, which is opposite of BDNF's proposed role in the hippocampus [28]. Furthermore, candidate sources of circulating BDNF include not only brain origin, but also platelets [29] and vascular endothelial cells [30]. So the role of peripheral BDNF in depression might be more complicated than we think. In addition, from the genetic studies, the association between BDNF gene polymorphism (mainly Val66Met) and major depression was inconsistent [19, 20]. In fact, the association between BDNF gene polymorphism and major depression was not supported in one recent meta-analysis report [31]. On the other hand, even more strong evidence about the association of BDNF Val66Met to schizophrenia has been reported [31], the serum BDNF levels in schizophrenia were inconsistent. Some reported the low serum BDNF levels among schizophrenia [32-34]. However, some did not find difference in serum BDNF levels between treated or drug-naïve schizophrenia patients and control [35, 36], and some found higher BDNF level in treated schizophrenia patients [37]. So the inconsistent finding about serum BDNF levels in MDD patients as in our study might be plausible.

We can not fine the correlation between serum BDNF levels and severity of depression in this study. The correlation between serum BDNF level and severity of depression was inconsistent in past study. Some reported negative correlation between serum BDNF levels and severity of depression [17, 18], but some did not find such correlation [38]. Regarding the relationship between BDNF and stress, Smith et al. demonstrated that stress decreases the expression of BDNF in hippocampus [8]. Various animal models of depression were based on the stress-induced depression-like behavior changes in animals. No study evaluated the severity of perceived stress in human and its correlation with serum BDNF. In our study, we can not find the relationship between perceived stress and serum BDNF level.

There are some limitations in this study. First, the sample is too small to detect the difference between drug-free MDD patients and control. However, when we initiated the study, the sample size of drug-naïve MDD patients in previous studies were 16 and 25 [17, 18]. So we suggest that the sample size is adequate to detect the difference between drug-free MDD patients and control. However, if the difference between drug-free MDD patients and control is less obvious in Hans, we can not detect the difference using small sample size. Second, compared with Shimizu's and Aydemir's studies (mean HAMD-17 = 27.8 $\pm$ 10.2 and 23.2 $\pm$ 4.6, respectively), the severity of depression was less severe with mean HAMD-17 of 18.9 $\pm$ 5.8 in our MDD subjects. One recent study reported that BDNF level in dysthymic disorder, a mild but chronic form of depression, was not different from that in control [38]. It might account for the negative finding in our study. However, in our further analysis among control subjects, mild and moderate-severe depression groups, the negative results remained unchanged. So difference in severity of depression seems not to explain the negative results in our study.

In conclusion, though the reasons for the conflicting results are not clear, BDNF remains an interesting molecule for further research of stress-related disorders. Role of peripheral BDNF might be more complicated than we knew, further studies need to elucidate its function in mental disorders.

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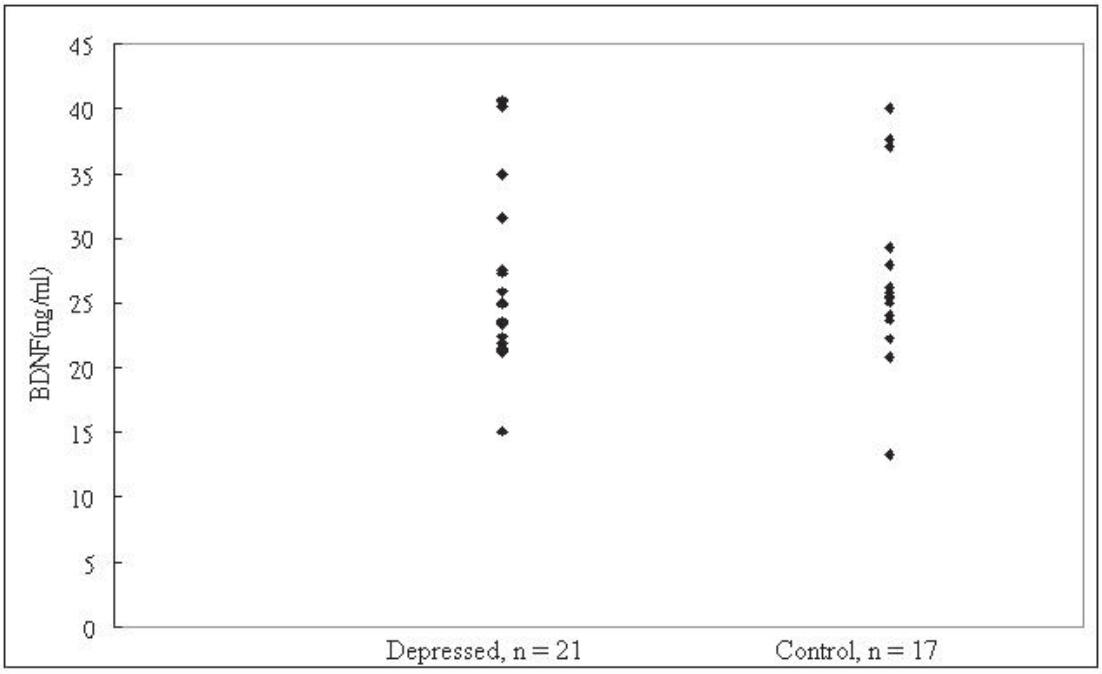
Table 1

Characteristics of drug-free major depressive patients and control subjects

	MDD (n = 21)	Control (n = 17)
Mean age ± SD	33.7 ± 12.9	32.8 ± 12.6
Gender		
Male	5	8
Female	16	9
Onset Age (years)	32.3 ± 12.4	
Duration of Current Episode (weeks)	19.0 ± 15.6	
Past Major Depressive Episodes	1.3 ± 0.6	
Perceived Stress Scale*	25.6 ± 5.9	17.7 ± 3.8
HAMD-17	19.0 ± 5.9	
BDNF (ng/ml)	26.7 ± 7.0	26.8 ± 6.5

*$p < 0.01$

Fig. 1. Serum BDNF levels of the drug-free major depressive patient and control groups



(六)計畫成果自評部份

本研究內容與原計畫內容有如下之差異.

1. 原計畫內容欲探討用藥前後之 BDNF 改變,但是因爲門診重鬱症患者初診願意參加研究者就少,且在門診追蹤個案之返診率過低(事實上只有 3 位完成 12 週之追蹤,所以並未進行治療前後之分析。

2. T 淋巴球 CREB 磷酸化之程度，由於 BDNF 初步結果爲 negative findings 故實驗室未再分析此部分。

本研究發現台灣人之重鬱症患者之血清 BDNF 濃度與常人並無不同，雖然受限於樣本數，可能不易解釋此結果，但是在報告中也嚐試探討 negative finding 可能之原因。我們也將研究結果投稿到國外期刊，目前正在 review 中。