

## RAPID SUBMISSION

### ALTERATIONS OF SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR LEVELS IN EARLY ALCOHOL WITHDRAWAL

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(Received 2 August 2007; first review notified 9 October 2007; in revised form 5 October 2007; accepted 16 November 2007;  
advance access publication 7 March 2008)

**Abstract — Aims:** Alcohol withdrawal-enhanced neurotoxicity contributes to the addictive process. Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal plasticity and learning. In this study, we explored the changes of serum BDNF levels in alcoholic patients at baseline and after one-week alcohol withdrawal. **Methods:** Twenty-five alcoholic patients were admitted for alcohol detoxification treatment, and 22 healthy control subjects were also enrolled. We collected blood samples of the patient group on the first and seventh day of alcohol withdrawal, and measured serum BDNF level with sandwich enzyme-linked immunosorbent assay. The severity of withdrawal symptoms was evaluated by the Clinical Institute Withdrawal Assessment-Alcohol, Revised every eight hours. **Results:** Serum BDNF levels did not differ significantly between alcoholic patients and control subjects. But BDNF levels were found to be significantly increased one week after alcohol withdrawal (from  $13.9 \pm 3.8$  ng/ml to  $15.4 \pm 3.8$  ng/ml,  $P = 0.03$ ). A significant positive correlation was found between baseline BDNF level and baseline withdrawal severity ( $r = 0.45$ ,  $P = 0.03$ ). **Conclusions:** The present study suggests that elevated serum BDNF levels were found in early alcohol withdrawal, implying that BDNF may involve in neuroadaptation during the period.

## INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is the most abundant member of the nerve growth factor (NGF)-related family of neurotrophic factors in brain, and responsible for neuronal survival, outgrowth, and differentiation during development (Snider, 1994; Lewin and Barde, 1996). In adults, BDNF has also been found to serve as a neurotransmitter modulator to participate in neuronal plasticity, such as long-term potentiation and learning. (Hyman *et al.*, 1991; Thoenen, 1995; Li *et al.*, 1998; Lyons *et al.*, 1999; Hall *et al.*, 2000; Guillin *et al.*, 2001). Although BDNF is highly concentrated in the nervous system, it is also present in human and rat peripheral blood, and is more concentrated in the serum. Previous studies suggest that BDNF can cross the blood–brain barrier (Pan *et al.*, 1998), that serum BDNF levels may reflect BDNF levels in the brain (Radka *et al.*, 1996; Karege *et al.*, 2002), and that these levels are relatively stable among adult primates including humans (Mori *et al.*, 2003).

Activity-dependent activation of BDNF is associated with the neuroadaptation, which is involved in the development of addiction (Horger *et al.*, 1999; Guillin *et al.*, 2001; Hall *et al.*, 2003; McGough *et al.*, 2004). BDNF is responsible for normal expression of dopamine D3 receptor in nucleus accumbens where it receives mesolimbic projections from ventral tegmental area, involving in reward system (Guillin *et al.*, 2001). Increased BDNF expression has been reported to be

associated with drug abuse, such as morphine (Numan *et al.*, 1998), amphetamine (Meredith *et al.*, 2002), cocaine (Grimm *et al.*, 2003), and delta-tetrahydrocannabinol (Butovsky *et al.*, 2005).

Alcohol dependence is a chronic disease characterized by uncontrolled drinking and a chronically relapsing course. Several lines of evidence suggest that BDNF also plays some role in developing alcohol addiction. The finding from the *in vitro* study showed biphasic changes, that BDNF mRNA expression is increased under acute alcohol exposure, but decreased after continuous ethanol exposure (McGough *et al.*, 2004). In animal studies, BDNF mRNA expression has been found to be reduced following chronic alcohol exposure, but increased after its withdrawal (MacLennan *et al.*, 1995; Tapia-Arancibia *et al.*, 2001). Compared to wild-type mice, heterozygous *BDNF* (+/–) mutant mice can self-administer a larger amount of alcohol (Hensler *et al.*, 2003). The adaptive changes in the brain during alcohol withdrawal which contribute to the progressive nature of alcohol dependence (Koob, 2003) are associated with neurodegeneration (Littleton *et al.*, 2001; Crews *et al.*, 2004). Therefore, neurotrophic factors for those adaptive changes might play an important role during alcohol withdrawal.

To date, only one study exploring neurotrophin during alcohol withdrawal in human showed a significant increase in plasma NGF level (Aloe *et al.*, 1996). We assumed both that alcohol withdrawal and its neurotoxic effect could induce a neuroadaptive response and that the withdrawal symptoms might reflect central excitotoxicity (Tsai and Coyle, 1998; Littleton *et al.*, 2001). Therefore, we carried out this study to test the hypothesis that serum BDNF in alcoholic patients would be increased after alcohol withdrawal and to explore the correlation between BDNF and the severity of alcohol withdrawal symptoms.

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## METHODS

Alcoholic subjects were recruited from an alcohol detoxification treatment unit in Taipei City Psychiatric Center. Patients were invited to participate in the study if they fulfilled the following inclusion criteria of age being between 20 to 60 years, being admitted for alcohol detoxification, and having DSM-IV diagnosis of alcohol dependence. Excluded were those who were co-morbid with other current non-nicotine substance abuse or dependence; had significant physical illnesses, such as ischemic heart disease or poorly controlled diabetes mellitus; had other psychiatric disorders, such as schizophrenia bipolar disorder, or major depressive disorder; had been treated with antipsychotics or antidepressant; and suffered from severe cognitive impairment with difficulty in understanding the study content. We assessed the patients with initial clinical interview to ascertain the DSM-IV diagnoses. Then the subjects received physical examination and urine toxicology test to screen for illicit drugs and to exclude other substance use disorders. Approval was obtained from the institutional review board at Taipei City Psychiatric Center before the study began. After initial assessments, we gave all eligible patients a comprehensive description of the study and then recruited them into the study project after obtaining written informed consent for participation.

We collected subjects' socio-demographic data (age, gender, educational level, and marital status). Then we interviewed patients to gather drinking history (age at first intoxication, age at dependence, and average daily amount of alcohol consumption in the past one month). Alcohol consumption was stopped abruptly and completely at admission and patients' withdrawal symptoms were evaluated using the Clinical Institute Withdrawal Assessment-Alcohol, Revised (CIWA-Ar) (Sullivan *et al.*, 1989) every eight hours by trained nurses. Average of CIWA-Ar score and the highest CIWA-Ar score in the first day were recorded as the baseline withdrawal severity. The patients received fixed-dose schedule of alcohol detoxification treatment with oral lorazepam 2 mg for four doses with gradual tapering thereafter, and the as-needed use of oral trazodone (50 mg) at night for sleeping problems. They also received multivitamins. All smoking patients simultaneously received aided nicotine patches, with dose administration adjusted by individual's average smoking amount, for smoking cessation program.

The control group included healthy subjects without known physical and psychiatric illnesses identified in the interview and the results of routine laboratory tests for liver and renal functions. They did not meet the diagnostic criteria of alcohol abuse or dependence in the past nor drink alcohol during the previous three months.

Serum samples of the study inpatients were collected on the next morning of admission for detoxification treatment (baseline) and on the seventh day following the treatment (one week). The serum samples were stored at  $-80^{\circ}\text{C}$  until used for the assay. Serum BDNF levels were measured using the BDNF Emax Immunoassay System Kit according to the manufacturer's instructions (Promega, USA). We measured serum BDNF levels of all subjects on the same day.

The results are presented as mean and standard deviation (SD). With independent *t*-test, we analyzed the differences between groups. With paired *t*-test, we analyzed the differences

of serum BDNF level collected at baseline and one week after alcohol withdrawal. Pearson's correlation test was used to determine the correlation between BDNF and clinical parameters. The differences between the groups were considered significant, if *P*-values were smaller than 0.05.

## RESULTS

A total of 25 patients (21 male and 4 female) and 22 healthy controls (19 male and 3 female) were enrolled, with mean age  $\pm$  SD being  $41.3 \pm 7.8$  and  $43.6 \pm 6.3$  years, respectively. In the alcoholic patients, the average duration of alcohol dependence was  $8.5 \pm 5.8$  years, and the average daily amount of alcohol consumption in the past one month was  $208.8 \pm 125.4$  grams of pure ethanol. The classical serum biomarkers (mean  $\pm$  SD) of drinking in our patients were: AST  $122.0 \pm 101.2$  U/l, ALT  $54.1 \pm 26.6$  U/l, gamma-glutamyltransferase (GGT)  $693.57 \pm 762.1$  U/l, MCV  $95.0 \pm 9.7$  fl, and uric acid  $7.4 \pm 2.0$  mg/dl ( $n = 22$ ).

Figure 1 showed the serum BDNF levels of alcoholic patients in two different time points and normal controls. Serum BDNF levels of the alcoholic group were significantly increased from  $13.9 \pm 3.8$  ng/ml at baseline to  $15.4 \pm 3.8$  ng/ml after one-week alcohol withdrawal ( $P = 0.03$ ). Despite neither of these values differed significantly from that of the healthy control group,  $15.8 \pm 3.7$  ng/ml, there was a nonsignificant trend that the baseline BDNF levels of alcoholic patients were lower than those of control subjects ( $P = 0.09$ ). The magnitude of one-week BDNF elevation did not significantly correlate with clinical characteristics (age, amount of cigarette consumption, age at first alcohol intoxication, age at alcohol dependence, and duration of alcohol dependence), but had marginally significant positive correlation with average amount of alcohol consumption ( $r = 0.38$ ,  $P = 0.06$ ).

A significant correlation was also found between baseline BDNF levels and the highest first-day CIWA-Ar scores ( $r = 0.44$ ,  $P = 0.03$ ) or average first-day CIWA-Ar scores ( $r = 0.45$ ,  $P = 0.03$ ) (Fig. 2). Even after controlling the amount of cigarettes, the correlation between baseline BDNF levels and average of first-day CIWA-Ar scores was still significant ( $r = 0.46$ ,  $P = 0.02$ ). The other clinical variables (age, age at first alcohol intoxication, age at alcohol dependence, duration of alcohol dependence, average daily amount of alcohol consumption, or biochemical values) were not correlated with their baseline BDNF levels.

## DISCUSSION

This is the first study to show significant increase in serum BDNF levels after one-week alcohol withdrawal in patients with alcohol dependence, and a significant positive correlation between patients' baseline BDNF levels and their baseline withdrawal severity. In addition, the results of this study revealed a trend that baseline BDNF levels of alcoholic patients were lower than those of control subjects, but BDNF levels after one-week alcohol withdrawal were approximately equivalent to those in controls. Despite nicotine can also increase BDNF expression (Le Foll *et al.*, 2005), baseline BDNF level still remained significantly correlated with baseline withdrawal severity even after controlling cigarette amount

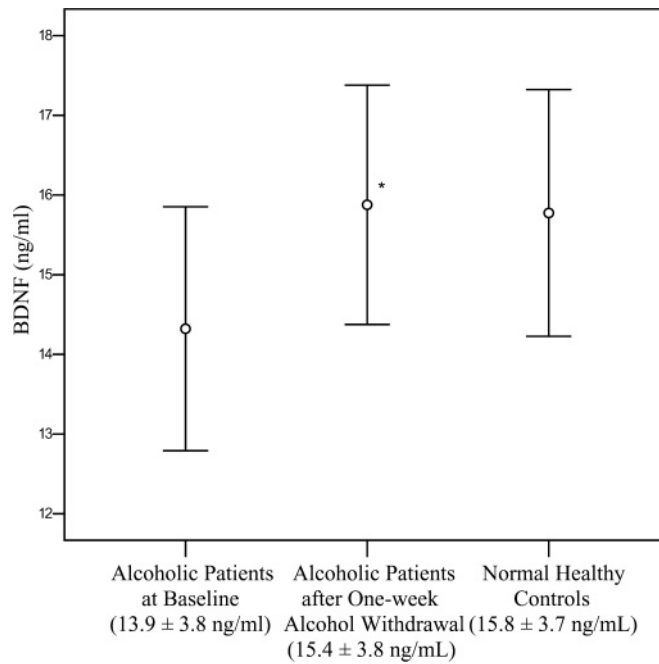


Fig. 1. Serum BDNF levels (mean  $\pm$  SD) of alcohol patients ( $n = 25$ ) at baseline and after one-week alcohol withdrawal as well as of the healthy controls ( $n = 22$ ). \*Significant difference ( $P = 0.03$ ) between BDNF levels after one-week alcohol withdrawal and those at baseline of alcoholic patients.

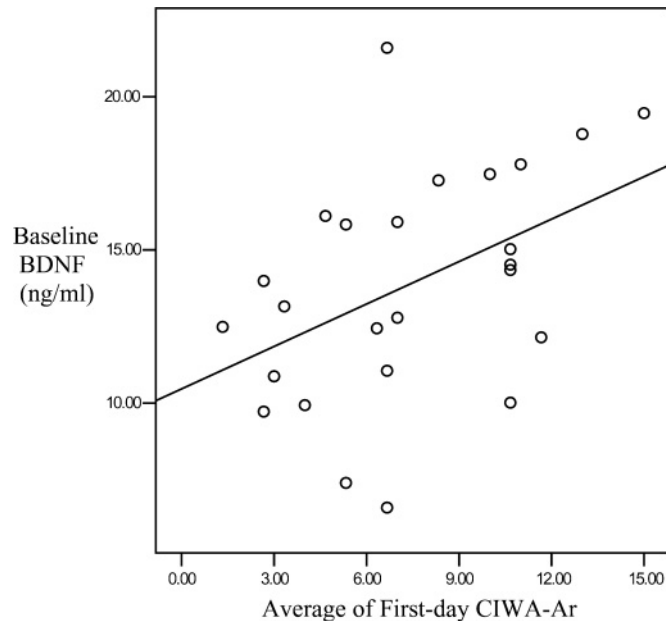


Fig. 2. The correlation between baseline serum BDNF levels and the average of first-day CIWA-Ar scores in alcoholic patients ( $n = 25$ ) ( $r = 0.45$ ,  $P = 0.03$ ).

statistically. Interestingly, a near-significant trend existed between the magnitude of BDNF elevation and the amount of alcohol consumption.

The finding of increased neurotrophin after alcohol withdrawal in our study is consistent with a previous human study showing increased NGF after alcohol withdrawal (Aloe *et al.*, 1996). In animal study, the pattern of alcohol administration might influence BDNF expression. Repeated episodic alcohol exposure, to exhibit withdrawal phenomenon, caused BDNF

and NGF elevation in hippocampus, while chronic alcohol exposure leads to decreased BDNF expression in rats (Miller, 2004). Following alcohol withdrawal after four-weeks alcohol vapor inhalation, a significant increase in BDNF mRNA expression was found in rat hippocampus and hypothalamus (Tapia-Arancibia *et al.*, 2001). The disinhibition of BDNF could exert protective function against neuronal damage and stimulation of sprouting and synaptic reorganization during withdrawal from chronic alcohol ingestion (Tapia-Arancibia

*et al.*, 2001). Phosphorylated cAMP response element binding-protein (CREB) regulates the expression of several downstream cAMP-inducible genes, including BDNF. Phosphorylated CREB protein levels in hippocampus increased after alcohol withdrawal after binge alcohol treatment (Bison and Crews, 2003), possibly contributing to increased neurogenesis during abstinence (Nixon and Crews, 2004).

Sustained stress, kindling-induced seizures, head trauma, or hypoglycemic coma can trigger elevated BDNF expression in the CNS of rats (Lindvall *et al.*, 1994). Glutamate release and calcium influx were postulated to be the key factors responsible for increasing BDNF during the brain insults. Thus, neuronal excitotoxicity mediated by glutamate receptors has long been implicated in the pathophysiology of alcohol withdrawal phenomenon and resulting neuronal damage (Tsai and Coyle, 1998; Littleton *et al.*, 2001). BDNF treatment in neuron culture was shown to prevent NMDA excitotoxicity sensitized by ethanol (Crews *et al.*, 2004). Therefore, BDNF could be involved in promoting the resilience of brain cells and needs to be elevated to cope with aggressive stress, such as NMDA over-excitation during alcohol withdrawal. The result of an animal study showed that compensatory neurogenesis, reflected by increased cell proliferation, was correlated with alcohol withdrawal severity despite the use of diazepam (10 mg/day) (Nixon and Crews, 2004). Consistent with these previous findings, our study showed a significant positive correlation between baseline BDNF level and baseline withdrawal severity, supporting a neuomodulating role of BDNF to oppose withdrawal-enhanced neurotoxicity.

Regarding BDNF gene polymorphism, alcoholic subjects with a history of delirium tremens had a higher frequency of AA genotypes and A allele, which has been speculated to correlate with impaired BDNF secretion (Matsushita *et al.*, 2004). Thus, those with inadequate BDNF expression to counteract the neurotoxicity could experience more severe withdrawal. BDNF levels in mesolimbic dopamine system progressively elevated after cocaine withdrawal. The prolonged BDNF increase was implicated in neuroadaptation of craving (Grimm *et al.*, 2003). Moreover, the volume of alcohol intake was also reported to be associated with craving during alcohol withdrawal (Hillemacher *et al.*, 2006). These findings might, in part, explain our intriguing clinical finding of correlation with near-significant correlation of magnitude of one-week post-withdrawal BDNF increment with the amount of alcohol consumption.

Plasminogen activator inhibitor 1 has been suggested to inhibit the action of tissue-type plasminogen activator (tPA), which cleaves plasminogen and thus, activates plasmin. Plasmin proteolytically cleaves pro-BDNF to yield mature BDNF (Pang *et al.*, 2004). The result of a previous study indicated that PAI-1 has been decreased significantly from day 1 to day 22 after alcohol withdrawal (Delahousse *et al.*, 2001). Similarly, the finding of another study showed that PAI-1 levels were significantly higher in alcoholics at baseline and decreased after one-week alcohol withdrawal (Soardo *et al.*, 2006). Moreover, an animal study showed that tPA activity is upregulated to promote stress-induced neuronal remodeling in mouse brain under acute restraint stress (Pawlak *et al.*, 2003). Thus, we postulate that attenuated PAI-1 after alcohol withdrawal may result in increased tPA activity, which further enhances higher BDNF levels as an adaptation to the physi-

ological stress. The finding of elevated BDNF in our patients after alcohol withdrawal is in agreement with this supposed mechanism.

Generalization of the research data should be cautious because this study had three limitations. First, the sample size was small. The difference between baseline BDNF levels in alcoholic patients and controls was nearly significant ( $P = 0.09$ ). Therefore, larger samples are needed to examine the BDNF downregulation in alcoholic patients. Second, we could not rule out the effects of medications, such as lorazepam on BDNF levels in our alcoholic patients. But the pertinent report still remains paucity. The only indirect clue is that acute or chronic injection of another benzodiazepine, chlordiazepoxide did not protect neuronal damage in animals (Haynes *et al.*, 2004). Thus, the influence of lorazepam on BDNF levels in our patients might have been minimal, but still needs further clarification. Third, the effect of cigarette smoking cessation on BDNF level might confound the results in this study. A recent report indicated that plasma BDNF levels in chronic smokers increased following two-month unaided smoking cessation (Kim *et al.*, 2007). Although we gave aided nicotine patch to minimize the confounding effect of smoking cessation on BDNF elevation during one-week interval, however, we still cannot rule out the potential impact. Adequate subjects to examine the difference of BDNF levels in both smokers and nonsmokers of alcoholic patients during withdrawal are needed.

In conclusion, this study showed that BDNF levels did not differ between alcoholic patients and controls, but increased after one-week alcohol withdrawal in alcoholic patients. Furthermore, the baseline BDNF level was found positively correlated with baseline alcohol withdrawal severity. These findings support that BDNF is involved in the neuroadaptation of alcohol withdrawal, a stress condition or brain insult which requires neurotrophin to promote neuronal resilience or survival.

## FUNDING

This work was supported by grants from Taipei Medical University-Wan Fang Hospital, Taiwan (94TMU-WFH-01), Department of Health, Executive Yuan, Taiwan (DOH94-TD-M-113-048 and DOH95-TD-M-113-041), and National Science Council, Taiwan (NSC94-3414-B-038-067).

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