Possible Role of Nerve Growth Factor in the Pathogenesis of Alcohol Dependence

Su-Jung Yoon*, Sungwon Roh*, Heejin Lee, Ji-Youl Lee, Bun-Hee Lee, Yong-Ku Kim, and Dai-Jin Kim

Background: Recent studies have raised the possibility that nerve growth factor (NGF) is abnormally regulated in the central nervous system (CNS) of animal models of chronic ethanol treatment. The goals of this study were to determine whether prolonged alcohol consumption is associated with the plasma NGF levels and to assess the effect of a positive family history of alcohol dependence on plasma NGF levels in the alcohol-dependent patients.

Methods: We used the enzyme-linked immunosorbent assay (ELISA) to determine the concentrations of peripheral NGF in patients with alcohol dependence and in a control group.

Results: The plasma NGF concentrations in the alcohol-dependent patients were significantly lower than in the controls (71.9 vs 110.5 pg/mL, respectively). Moreover, the alcohol-dependent patients with positive family histories showed a greater decrease in their NGF levels than those subjects with negative family histories (64.7 vs 83.3 pg/mL, respectively).

Conclusions: Our study suggests that the NGF levels may be a trait marker for the development of alcohol dependence.

Key Words: Alcohol Dependence, Neurotrophin, Nerve Growth Factor, Family History.

PROLONGED ALCOHOL CONSUMPTION and withdrawal following chronic alcohol intake causes various kinds of neuronal damage; this results from increased cell death or decreased cell proliferation in several regions of the brain (Hamre and West, 1993; Miller, 1996; Nixon and Crews, 2002; Pierce et al., 1993). Recent anatomical studies of the alcoholic brain indicate that pathology in the hippocampus, extrahippocampal cortex, and basal forebrain can produce memory dysfunction (Alvarez et al., 1995; Bohbot et al., 2000; Casamenti et al., 1993; Fischer et al., 1991). Moreover, it has been reported that the developing brain is more susceptible to alcohol exposure (Crews et al., 2000; Livy et al., 2001; Maier et al., 1997). Clear and consistent evidence has suggested that the children of alcoholic individuals are more likely to have alcohol use problems and greater deficits in cognitive functioning (Berman et al., 1993; Drejer et al., 1985; Finn et al., 1997; Knop et al., 1985; Sher et al., 1991). In addition, heavy drinkers with a positive family history of problem drinking may have reduced brain volume compared with heavy drinkers without a family history of problem drinking (Hill et al., 2001). However, scientific proof of identified mechanisms explaining these characteristics of alcoholic individuals with a family history of drinking has not yet been established.

Although the mechanisms involved in chronic alcohol treatment–induced neurotoxic actions have not yet been clearly identified, recent studies have hypothesized that prolonged alcohol intake may affect the synthesis of neurotrophins, which are a family of proteins that play a crucial role in cognitive function, including learning and memory (Chao, 2000; Keyvani and Schallert, 2002; Levine, 2001). Neurotrophins appear to be particularly important in many aspects of CNS development and neuronal survival (Moore et al., 2004a, 2004b).

Nerve growth factor (NGF) was the first identified member of the neurotrophin family. It is one of the best-characterized members (Connor and Dragunow, 1998). In the central nervous system (CNS), NGF plays a critical role in the development and maintenance of cholinergic neurons, and these neurons have been implicated in the memory and learning process (Hefti et al., 1984; Rylett and Williams, 1994). Moreover, NGF has been implicated in the alteration of cognitive function following brain damage and during the aging process, and it has been reported to have a crucial role in neuroplasticity of CNS neurons (Connor and Dragunow, 1998). Therefore, the possible alteration of NGF by prolonged alcohol intake

From the Department of Psychiatry, College of Medicine, Catholic University of Korea, Seoul, Korea (SJY, HL, DJK); the Department of Neuropsychiatry, College of Medicine, Hanyang University, Seoul, Korea (SR); the Department of Urology, College of Medicine, Catholic University of Korea, Seoul, Korea (JYL); and the Department of Psychiatry, College of Medicine, Korea University, Seoul, Korea (BHL, YKK).

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**Co-first authors.*

Reprint requests: Dai-Jin Kim, Department of Psychiatry, Holy Family Hospital, College of Medicine, The Catholic University of Korea, 2 Sosa-Dong, Buchon-City, Kunggi, 420-717 South Korea. Fax: +82-32-340-2670; E-mail: kdj922@chollian.net

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may play an important role in alcohol-induced neurotoxicity. Recent studies have raised the possibility that NGF is abnormally regulated in the CNS of animal models after chronic ethanol treatment (Aloe et al., 1993; Aloe and Tirassa, 1992; Heaton et al., 1993, 1994, 2000; Seabold et al., 1998; Walker et al., 1993). However, the data on the interaction of neurotrophins with chronic exposure to alcohol are controversial (Baek et al., 1994; MacLennan et al., 1995); only a few studies comparing the plasma NGF concentrations in alcoholic patients and control patients have been conducted.

Therefore, we hypothesized that prolonged alcohol consumption is associated with alteration of the plasma NGF levels and that alcoholic individuals having a family history of alcohol dependence are more susceptible to the alterations in neurotrophin levels of the brain due to the effects of alcohol because of their limited reserve capacity.

To test this hypothesis, we examined the plasma NGF levels in the patients with alcohol dependence and also in healthy controls. In addition, we investigated the effect of a family history of alcohol dependence on the concentration of NGF in human plasma.

MATERIALS AND METHODS

Subjects

We recruited 69 males (mean age 46.9 ± 10.1 years) with alcohol dependence to participate in this study. Consensus on the diagnosis was achieved by two board-certified psychiatrists (S.J.Y., D.J.K.), according to the *Diagnostic and Statistical Manual of Mental Disorders-IV* (DSM-IV, American Psychiatric Association, 1994) criteria for alcohol dependence and a modified form of a semi structured interview that was used in our previous study (Kim et al., 2005) was administrated to all the patients. All the subjects were inpatients and abstinent from alcohol consumption for 30 days or longer before the start of the study. The mean duration of the abstinence period was 96.8 ± 60.2 days. All the subjects underwent the 7-day inpatient-detoxification program that includes oral benzodiazepine usage with a clinically guided gradual tapering. The use of benzodiazepines was permitted during the abstinence period for protracted alcohol withdrawal symptoms or behavior control. Individuals were excluded if they were seropositive for HIV or if they had any history of comorbid psychiatric illness, any neurological disorder, current medical/surgical illnesses, addiction to drugs other than alcohol or nicotine, or any head trauma with loss of consciousness for more than 30 minutes. Sixty-seven subjects had past and/or current nicotine-use history.

Our nonalcoholic control population consisted of 83 randomly selected healthy males (mean age 35.1 ± 11.6 years) who visited the Korea University Ansan Hospital for regular health screening. Before collecting the blood samples, all the volunteers were evaluated for a past history or a family history of a psychiatric disease by performing a nonstructured interview that was conducted by trained psychiatrists.

The family histories of alcohol dependence were assessed using a family tree questionnaire (Mann et al., 1985). The participants were classified as family history–positive (FHP) if either the paternal or maternal first-degree relatives (parents and siblings) or second-degree relatives (grandparents, uncles, and aunts) were identified as being alcohol-dependent.

All the subjects were asked to sign informed consent forms after receiving a complete description of the study. This study was approved by the hospital ethics committee of Holy Family Hospital at the Catholic University of Korea in Seoul. All the procedures were in accordance with the Helsinki Declaration of 1975 (1983 revision).

Enzyme-Linked Immunosorbent Assay

All participants were requested to fast for a period of 12 hours before giving 10 mL of blood between 8 AM and 9 AM. The samples were collected in sodium heparin vacuum tubes and they were immediately centrifuged at 3,800 r.p.m. (2,660×g) for 10 minutes. The plasma was stored at −70 °C until thawed for further testing. The human β-NGF was assayed using the DuoSet enzyme-linked immunosorbent assay (ELISA) Development System (DY256, R&D Systems, UK). All the assays were performed in duplicate using the manufacturer's recommended buffers, diluents, and substrates. The assay detection ranges were from 3 to 1,000 pg/mL. The intraassay and interassay variation coefficients were 6.7 and 9.6%, respectively. The sample concentrations in each plate were calculated according to standard curves and dilution factors.

Statistical analysis

The Kolmogorov–Smirnov test showed a skew of the data from normality; therefore, a nonparametric Mann–Whitney test and descriptive statistics were performed for the analysis of the clinical characteristics where appropriate. An analysis of covariance (ANCOVA) with age as a covariate was used to compare the NGF levels between the alcoholic group and the control group. For the alcohol-dependent patients, we examined the categorical effect of a family history of alcohol dependence on the plasma NGF levels using ANCOVA; this covered for age, the first age of alcohol treatment and the duration of illnesses, and it accounted for the possible differences in drinking severity among the alcoholic subjects. In addition, within the alcoholic group, we used multiple regression analysis to examine the relationship between continuous measures of the drinking severity (e.g., onset age of drinking alcohol, duration of illness, frequency of alcohol consumption, daily amount of alcohol consumed, and the first age of alcohol treatment) on the plasma NGF levels. The plasma NGF level was the dependent variable and the effects of several drinking severity measures were then examined. All the statistics were carried out using the Statistical Package for the Social Science (SPSS) 12.0 for Windows. The statistical significance level was set at $p < 0.05$. All the data are presented as means ± SD.

RESULTS

Data on the demographic characteristics and the alcohol use history of the patients with alcohol dependence are shown in Table 1. The groups were similar in height ($p = 0.53$) and weight ($p = 0.36$), but they differed in age ($p < 0.001$). Table 2 shows the alcohol-related clinical characteristics for the patients with or without a family history. The results showed that the FHP alcoholic patients had earlier treatment ($p = 0.007$) and several more ($p = 0.041$) treatments; this reflected that the FHP alcoholic individual had a more severe clinical course compared with patients with family history–negative (FHN). However, the FHP alcoholic subjects had a shorter duration of illness ($p = 0.011$) than the FHN alcoholic subjects. This might have been due to the relatively younger age ($p = 0.023$).

Table 1. Characteristics of the Alcohol-Dependent Patients and the Controls

	Alcoholics ($n = 69$), mean ± SD	Normal controls ($n = 83$), mean ± SD
Demographic data		
Age (years)	46.9 ± 10.1	35.1 ± 11.6
Height (cm)	168.0 ± 5.6	169.7 ± 5.2
Weight (kg)	64.6 ± 9.1	66.0 ± 9.3
	mean ± SD	Range
Alcohol-dependent patients		
Onset age of drinking (years)	18.6 ± 3.0	8–27
Duration of illness (months)	337.2 ± 121.9	108–672
Frequency of alcohol consumption (per week)	4.6 ± 1.6	1–7
Daily amount of alcohol consumed (standard drink)	7.9 ± 3.8	3–24
First age of alcohol treatment	38.5 ± 11.2	21–71
Number of prior detoxifications	4.5 ± 5.8	0–40
Abstinence period (days)	96.8 ± 60.3	30–361

As shown in Fig. 1, the mean plasma NGF levels in the patients with alcohol dependence (71.9 ± 37.4 pg/mL) were lower than those in the control subjects (110.5 ± 95.1 pg/mL, $p = 0.001$). Our ANCOVA results when covaried with age confirmed that the lower NGF levels in the alcoholic group were not due to differences across the age groups ($F = 8.677$, $p = 0.004$). Further, no correlation was found between NGF and age in patients and control group ($r = 0.016$, $p = 0.897$; $r = 0.037$, $p = 0.741$, respectively).

Figure 2 shows that the mean plasma NGF levels of the alcoholic subjects with a family history of alcohol dependence (64.7 ± 34.6 pg/mL) were lower than those without a family history of alcohol dependence (83.3 ± 38.7 pg/mL, $p = 0.031$). Our ANCOVA results confirmed that the lower NGF levels in the alcohol-dependent participants with family histories of alcoholism were not due to differences across the groups for age and disease severity ($F = 5.249$, $p = 0.025$).

The plasma NGF levels were not found to be significantly associated with any measures of drinking severity (onset age of drinking alcohol, $p = 0.890$; duration of illness, $p = 0.692$; daily amount of alcohol consumed, $p = 0.158$; frequency of alcohol consumption, $p = 0.463$; number of prior detoxification, $p = 0.328$; and the first age of alcohol treatment, $p = 0.863$).

DISCUSSION

The results of this study suggest that chronic alcohol exposure results in the down-regulation of human plasma NGF. This finding is consistent with previous studies that the level of NGF in the brain is reduced following chronic ethanol treatment in animals (Aloe et al., 1993; Aloe and Tirassa, 1992; Heaton et al., 1993, 1994, 2000; Seabold et al., 1998; Walker et al., 1993). There is considerable evidence showing that alcohol decreases the synthesis, availability, delivery, and biological activity of neurotrophin and that it possibly alters the capacity of target neurons to respond to these factors (Walker et al., 1993). The most commonly reported consequences of chronic alcohol treatment–induced decrement of neurotrophins are as follows: blunted nerve outgrowth, reduced neuronal survival, reduced ability to promote neuronal regeneration, and changes in the neurochemical phenotypes of specific neuronal populations (Heaton et al., 1993; Seabold et al., 1998; Walker et al., 1993). Among the neurotrophins, NGF has been implicated in these alterations because of its capability of protecting neuronal cultures against alcohol toxicity (Seabold et al., 1998). In studies of chronic alcohol intake in animal models, researchers have reported that alcohol induces a sharp decrease in NGF activity in rat hippocampus and hypo-

Table 2. Clinical Characteristics of the Subjects with Positive or Negative Family Histories (FHP and FHN Patients)

Clinical characteristics	FHP ($n = 40$)	FHN ($n = 29$)	p value
Age (years)	44.3 ± 8.8	50.4 ± 10.8	0.023
Height (cm)	168.4 ± 4.6	167.4 ± 6.8	0.918
Weight (kg)	65.2 ± 9.9	63.8 ± 8.1	0.480
Onset age of drinking alcohol (years)	18.8 ± 3.5	18.4 ± 2.4	0.396
Duration of illness (months)	303.6 ± 106.5	383.6 ± 128.3	0.011
Frequency of alcohol consumption (per week)	4.7 ± 1.5	4.5 ± 1.6	0.463
Daily amount of alcohol consumed (standard drink)	8.6 ± 3.8	7.0 ± 3.7	0.076
First age of alcohol treatment	35.1 ± 8.7	43.1 ± 12.6	0.007
Number of prior detoxification	5.5 ± 6.9	3.1 ± 3.5	0.041

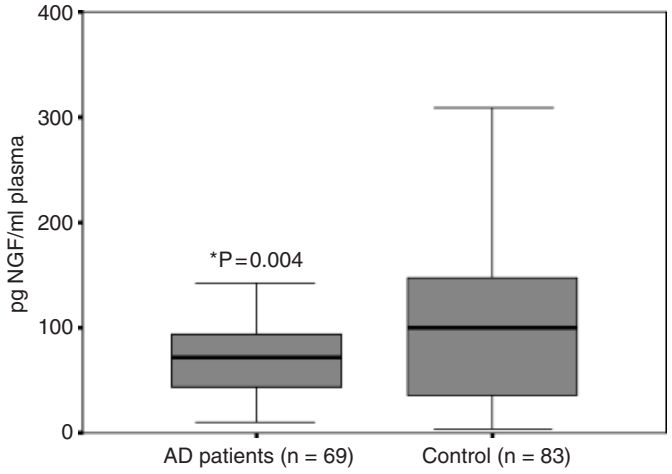

Fig. 1. Comparison of the plasma nerve growth factor (NGF) concentration between the patients with alcohol dependence and the controls. *Significant difference between the groups.

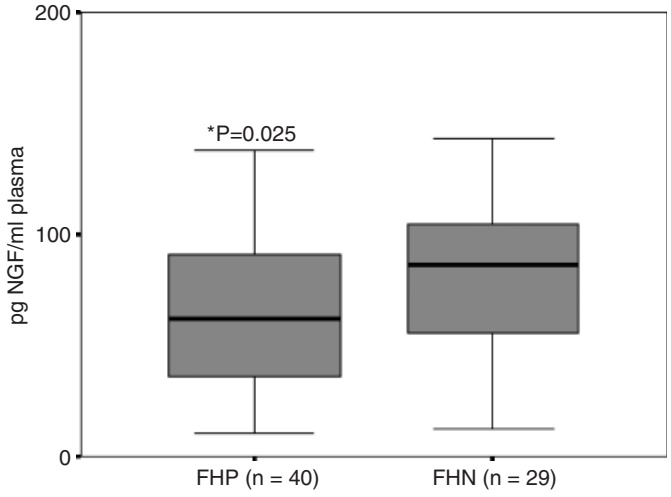

Fig. 2. Comparison of the plasma nerve growth factor (NGF) concentrations between the family history–positive (FHP) alcohol-dependent patients and those subjects with family history–negative (FHN). *Significant difference between the groups.

thalamus (Miller et al., 2002). Preclinical studies have shown that long-term alcohol intake causes reduction in the levels of brain NGF and NGF-regulated cholinergic enzymes (Aloe et al., 1993). Therefore, it seems likely that plasma NGF alteration could play a role in chronic alcohol consumption–induced neurotoxicity.

There was a published study on elevated NGF levels in human plasma after alcohol withdrawal (Aloe et al., 1996). This study hypothesized that withdrawal from chronic alcohol consumption causes a significant increase in anxiety levels, and this condition triggers the release of the NGF. However, our finding of lowered NGF levels in alcohol-dependent patients was somewhat divergent from those of Aloe et al. (1996). There is a potential explanation for these disparate findings. In our study, we included alcoholic patients who were abstinent for 30 days or longer and so we ruled out the alcohol withdrawal effects such as anxiety, while Aloe et al. (1996) studied alcoholic patients who were in the withdrawal stage. Therefore, our results reflect a trait marker of alcohol dependence rather than the state marker.

Another finding from the present study was that the alcohol-dependent patients having positive family histories showed greater decreases in their NGF levels than those subjects with negative family histories. The lower NGF levels in the FHP alcoholic subjects compared with the FHN alcoholic subjects may suggest the effect of their genetic burden. As stated in the introductory paragraph, NGF plays a critical role in the development of hippocampal cholinergic neurons, which have been implicated in memory and the learning (Calamandrei and Alleva, 1995; Kang and Schuman, 1996). The selective alteration of NGF and their receptors in the hippocampus and basal forebrain has been reported in an animal study of chronic alcohol intake (Miller et al., 2002). Furthermore, developmental alcohol exposure has an adverse effect on the synthesis of neurotropic factors and/or its receptors (Moore et al., 2004a, 2004b). Hence, our results that reported the decreased plasma levels of NGF in alcoholic patients compared with the controls and that these levels were also lowered in the FHP alcoholic subjects than in the FHN indicate that NGF either could play a role either in the neuropathological process or it can serve as a trait marker for the development of alcohol dependence. However, we did not have genotypic data on these patient groups. Therefore, further investigations should focus on the classical linkage and association genetic studies and on the screening of mutations in the genes encoding the neurotrophins, including NGF and its receptors.

Unfortunately, there were no statistically significant associations between any of the clinical severity characteristics and the NGF levels. For the onset age of drinking alcohol, Cloninger subtyped alcoholism into type 1 or ''milieu-limited'' and type 2 or ''male-limited'' on the basis of these personality dimensions (Cloninger, 1987). Type 2 alcohol dependence is characterized by onset at an early age and a socially disruptive set of behaviors (Cloninger, 1987). As the age of onset plays a critical role in the prognosis and clinical characteristics of alcohol dependence, we included the onset age of drinking as an independent variable. However, the plasma NGF levels were not significantly associated with this variable. Although we performed careful semistructured interviews, there are always inaccuracies in any retrospective report of alcohol use by patients. Thus, we could not define the exact amount of the alcohol that was consumed. Therefore, further prospective investigations will be required to draw more reliable conclusions on the causal effect relating NGF with alcohol-induced neurotoxicity.

There are several limitations in this study. First, we measured the NGF levels in plasma. However, observations that intracerebroventricular NGF injections can affect the functional activities of peripheral cells (Jonha-

gen, 2000), that peripherally administrated NGF can reach brain cells (Poduslo et al., 1994), and that some members of the neurotrophin family such as NGF can cross the blood–brain barrier (Pan et al., 1998; Poduslo et al., 1994) do not exclude the possibility that peripheral changes in NGF may reflect the NGF levels in the brain.

Second, because nicotine has been known to have neuroprotective effects via increasing the expression of NGF (Garrido et al., 2003; Hernandez and Terry, 2005), we cannot entirely exclude the possibility that nicotine-use history in the individuals with chronic alcoholism contributed to the findings. Our patients group showed lower NGF levels even though all ($n = 67$) had past and/or current nicotine-use history. This would be another mechanism of change in NGF other than nicotine-use history in chronic alcoholic patients. However, further studies will be undertaken to more precisely define the role of nicotine and to examine its effects on the neurotrophins of patients with alcohol dependence.

Third, our sample consisted of only male subjects. Gender differences in alcohol responsiveness have been reported; for instance, the changes in neurotrophin receptor expression are generally more prevalent in males than in females (Moore et al., 2004a, 2004b). Therefore, there is a clear need to investigate female alcohol-dependent subjects and controls to determine whether our findings also apply to females.

Fourth, as the recent studies suggest an association of BDNF gene with alcohol dependence (Matsushita et al., 2004; Tsai et al., 2005), it would be of interest to provide data on the other neurotrophins, such as brain-derived neurotrophic factor (BDNF), or neurotrophic-3 to discuss the specificity of our findings. Therefore, further studies for other neurotropins in alcohol dependence are necessary.

In conclusion, in this study, we found that the plasma NGF levels were decreased in patients with alcohol dependence and that alcohol-dependent patients with positive family histories showed a greater decrease in their NGF levels compared with subjects with negative family histories. These findings might provide further evidence that NGF plays a role as a trait marker in the development of alcohol dependence. However, further studies, including genetic studies, will more precisely define the role of the NGF and its effects on the brain and the psychopathology of alcohol dependence.

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