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Serum level of vascular endothelial growth factor decreased in chronic ketamine abusers

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Abstract

Aims—To evaluate the serum level of vascular endothelial growth factor (VEGF) in a group of chronic ketamine abusers in comparison to healthy controls.

Methods—Eighty-one ketamine abusers who were hospitalized for the treatment of ketamine dependence and 39 healthy controls were recruited. Serum VEGF level was measured by enzyme linked immunosorbent assay (ELISA). Psychopathological symptoms were assessed using Positive and Negative Syndrome Scale (PANSS), Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI).

Results—Serum level of VEGF was significantly lower in chronic ketamine abusers compared to healthy controls (64.6 ± 42.1 vs. 92.4 ± 59.4 pg/ml, $F = 7.243$, $p = 0.008$).

Conclusions—Serum level of VEGF decreased in chronic ketamine abusers compared to healthy controls.

Keywords

Ketamine; Vascular endothelial growth factor; Enzyme-linked immunosorbent assay

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Contributors

Ni Fan and Hongbo He designed the study and wrote the protocol. Ni Fan wrote the manuscript. Minling Zhang conducted ELISA experiments and data analysis. Xiaoyin Ke, Yi Ding, Xuefeng Deng, Daping Wang and Yuping Liu recruited the subjects. Ke Xu and Yuping Ning helped on data analysis and manuscript revision. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

1. Introduction

Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist was used widely in medicine, pain management and anesthesia. Its use as a recreational drug of abuse has become widespread after its hallucinogenic effects were discovered in 1970s. Ketamine provokes distinctive pathopsychological symptoms similar to schizophrenia, as well as cognitive impairments in humans and rodents (Becker et al., 2003; Aalto et al., 2005). A single intravenous dose of ketamine given to healthy volunteers produced acute psychotic symptoms, as well as impairment of memory (Krystal et al., 1994; Morgan et al., 2004). Recreational ketamine use has also been reported to produce psychotic symptoms and memory deficits (Morgan et al., 2004). Intriguingly, an acute, single intravenous, subanesthetic dose of ketamine has a rapid-acting antidepressant effect in depressed patients (Berman et al., 2000).

Vascular endothelial growth factor (VEGF), a potent growth factor secreted by endothelial cells, brain cells and other cells, is involved in the regulation of vasculogenesis and vascular function (Takahashi and Shibuya, 2005). Importantly, VEGF was also acknowledged as a key signaling protein in the central nervous system functioning in neuroprotection, neuronal survival, axonal outgrowth, long-term potentiation, and learning (Ruiz de Almodovar et al., 2009). VEGF exerts its effects mainly via binding to specific tyrosine kinase receptors VEGF1 and VEGF2 (Brockington et al., 2004). The alterations of VEGF in multiple mental disorders were identified. It was reported that the VEGF mRNA expression was decreased in the dorsolateral prefrontal cortex of schizophrenia patients (Fulzele and Pillai, 2009). Also serum level of VEGF and its receptor was altered in patients with severe autism (Emanuele et al., 2010).

Even though studies about ketamine are burgeoning, little is known about the alteration of serum level of VEGF in ketamine abusers. Previously, we reported the decrease of another neurotrophic factor, brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in chronic ketamine abusers (Ke et al., 2014). Considering the role of VEGF in the central nervous system and the reported effects related to ketamine use, as well as the possibly entwined regulation of BDNF and VEGF expression (Lin et al., 2014), we investigated the serum level of VEGF in a group of chronic ketamine abusers to test the hypothesis that the chronic ketamine abuse will alter the serum level of VEGF. The associations between the serum VEGF level and ketamine use characteristics were also analyzed.

2. Methods

2.1. Subjects

The recruitment of ketamine abusers and healthy controls was as described previously (Ke et al., 2014). Briefly, ketamine abusers were recruited from two hospitals in Guangzhou, China. Healthy controls were recruited through local advertisements. Subjects' socio-demographic characteristics, psychopathological status, and substance use characteristics were documented during a semi-structured interview. Among ketamine users, the average interval between the interview and last ketamine use was 8.22 ± 4.88 days. The inclusion

criteria for ketamine abusers were: (1) subjects met the criteria of substance dependence according to DSM-IV-TR; (2) no other substance dependence other than tobacco; (3) no other substance use other than alcohol and tobacco for at least 6 months; and (4) age between 16 and 45 years old. Inclusion criteria for healthy subjects included: (1) no axis I diagnosis according to DSM-IV-TR criteria; (2) no familial history (including first- or second-degree relatives) of psychiatric disorders; and (3) age range match with the ketamine group (16–45 years old). Participants were excluded if they had: (1) any known organic diseases or (2) history of head trauma with loss of consciousness, or (3) any unstable physical illnesses, or (4) impairments of color vision or hearing. The study was approved by the Institutional Ethics Committee and signed informed consent was obtained from each participant after giving information about the study.

2.2. Assessment of psychotic symptoms

Clinical symptoms among ketamine users were evaluated with the Positive and Negative Syndrome Scale (Kay et al., 1987), administered by two trained psychiatrists with 3 or more years of clinical experience. The intraclass correlation coefficient (ICC) between raters on the PANSS was 0.954. In addition, ketamine users were asked to complete Beck Depression Inventory (BDI; Beck and Beamesderfer, 1974) and the Beck Anxiety Inventory (BAI; Beck et al., 1988), which assessed their depressive and anxiety symptoms during the two weeks before they were hospitalized.

2.3. Blood sampling

A venous blood sample was drawn from each subject using standard venipuncture technique. The average days of blood drawing since last ketamine use were $8.22 \text{ days} \pm 4.88 \text{ days}$. Serum was obtained by centrifuge at 4000 rpm for 15 min, then aliquoted and stored at -80°C until assay.

2.4. Measurement of serum VEGF level

Serum VEGF level was measured by enzyme linked immunosorbent (ELISA) kit (VEGF Emax Immunoassay System, Boster, Wuhan, Hubei, China) according to the manufacturer's instructions. The minimal detection limits was 31.2 pg/ml for VEGF. No cross-reactivity was observed. All samples were assayed in duplicate. VEGF levels were determined by absorbance at 450 nm wave length (BIO-RAD iMark™) using optical density values against standard curves calibrated with known amounts of proteins.

2.5. Statistical analysis

All statistical analyses were performed using SPSS version 13.0 for windows. A Chi-square test was used to examine the difference of sex, alcohol consumption and tobacco smoking variables between ketamine users and healthy controls. Spearman's rank order correlation was applied for non-normally distributed data to investigate the correlations between serum VEGF level and ketamine use characteristics. A covariance analysis, with smoking as covariate in univariate general linear model, was implemented to compare the serum VEGF levels across the two groups. Differences at $p < 0.05$ level (two tailed) were considered significant.

3. Results

3.1. Clinical and demographic data

Eighty-one ketamine abusers and 39 healthy subjects were enrolled in the study.

Demographic and clinic characteristics of ketamine abusers and healthy subjects are shown in Table 1. There were no significant differences in age and gender between the two groups, although smoking was higher in ketamine users ($p < 0.01$).

In the ketamine group, the score on PANSS was 45.14 ± 9.54 for total score, 7.95 ± 1.87 for positive symptom subscale, 12.91 ± 4.13 for negative symptom subscale and 24.28 ± 5.56 for general psychopathology subscale. The BDI score was 12.13 ± 6.07 . The BAI score was 14.75 ± 8.79 .

3.2. Serum level of VEGF

VEGF serum levels in ketamine users and healthy subjects were 64.6 ± 42.1 vs. 92.4 ± 59.4 pg/ml respectively. Serum VEGF level in ketamine abusers was significantly lower than that in control ($p < 0.05$; Fig. 1). When smoking was added as a covariate into the analysis, the differences of serum VEGF level between the ketamine abusers and the control group were still significant ($F = 7.243$, $p = 0.008$).

3.3. Correlation of serum VEGF with demographic, drug use characteristics and psychotic assessments

The correlation of serum VEGF with demographic and drug use characteristics were analyzed. The average age (years) for ketamine users and healthy control were 25.7 ± 5.16 and 25.1 ± 4.8 respectively. Among the 81 ketamine users, 77 were male, 4 were female; in the healthy control group, 34 were male, 5 were female. For the ketamine users, age of first ketamine use (years) was 19.8 ± 5.12 . Duration of ketamine use (months) was 71.3 ± 36.88 . Duration of dependence (months) was 34.9 ± 24.54 . Current frequency of ketamine use (days per week) was 6.28 ± 1.45 . Current average daily ketamine use (g) was 3.25 ± 3.09 . No significant correlations were found between serum VEGF level and subjects demographic or drug use characteristics ($p > 0.05$), except that serum VEGF level was positively correlated with the days since last use of ketamine which indicated that the longer time interval after cease of ketamine use, the higher the serum VEGF level (Table 2). The serum VEGF was marginally negatively correlated to the age of first ketamine use ($p = 0.06$). The serum VEGF level were also negatively correlated with BAI score and positively correlated with PANSS negative subscale score (Table 2).

4. Discussion

We examined the serum level of VEGF in chronic ketamine users and healthy subjects. We found that the serum level of VEGF significantly decreased in chronic ketamine abusers compared to the control group. Consistent with our previous findings of lower serum BDNF and NGF level in the ketamine users, our findings in VEGF further suggest significant physiological changes following chronic severe ketamine use.

Little is known about the alteration of serum VEGF in chronic ketamine users and the mechanisms responsible for the alteration is unclear. The expression of VEGF is extensively regulated by many different mechanisms. Hypoxia, hypoglycemia, low pH, oxidative stress can activate VEGF expression (Zhang et al., 2000; Harms et al., 2010). NMDA pretreatment elevated the expression level of hypoxia induced factor1 α (HIF1 α), whereas it decreased the expression of VEGF-A. NMDA induced excitotoxicity in part via VEGF signaling between astrocytes and hippocampal neurons (Inada et al., 2014). It was reported that a single, acute application of NMDA receptor antagonists MK801 and AP-5 increased secretion of VEGF from Müller cells, and this effect was absent after AMPA/kainate receptor blockade (Cervantes-Villagrana et al., 2010). The VEGF gene contains a cAMP response element (CRE) and its expression can be stimulated by activation of the CRE-binding protein (CREB; Impey et al., 2004). It is possible that the chronic administration of NMDA receptor antagonist may exert the effect on VEGF expression through these signaling pathways, which down-regulates the expression of VEGF.

Intriguingly, an acute, single intravenous, subanesthetic dose of ketamine has a rapid-acting antidepressant effect in depressed patients (Berman et al., 2000). But the effects of long-term use of ketamine in the general population is unclear. Even though decreased VEGF is not required for depressive behavior in a chronic unexpected stress (CUS) model, experiments in animal models of depression have demonstrated that VEGF signaling is involved in the cellular and behavioral response to antidepressant drugs (Warner-Schmidt and Duman, 2007). In this group of chronic ketamine users we found mild to severe depressive symptoms. As we did not measure the depressive symptoms before ketamine use, it is possible that some ketamine abusers may have had depressive symptoms before ketamine abuse. Even though no significant correlation between serum VEGF and BDI score was found, the relationship between the depressive symptoms and the decreased serum VEGF is worth further study.

In a previous study, we reported the decrease of BDNF and NGF in chronic ketamine users (Ke et al., 2014). BDNF can increase VEGF expression and enhances angiogenesis through signal transduction pathways that involves the TrkB receptor, PLC γ , PKC α , and the HIF-1 α . Knockdown of BDNF decreased VEGF expression in the chick chorioallantoic membrane and Matrigel plug nude mouse models (Lin et al., 2014). Whether or how the alterations of BDNF influences the VEGF expression and the entwined regulation of these proteins deserves further study.

Even though our study shows for the first time, to our knowledge, the decrease of the serum VEGF level in the chronic ketamine abusers, there are several limitations that need to be addressed. First, we did not measure the level of serum VEGF receptors. It was reported that serum VEGF level was lower and sVEGFR-1 higher in patients with severe autism (Emanuele et al., 2010). Second, the subjects in this study were also on antipsychotics, which may have a compound impact on the VEGF level. Finally, because of the co-use of other substances, the effect of other substances cannot be totally ruled out. But the inclusion criteria limited entrants to those with no other reported substance dependencies other than tobacco, and no other substance use other than alcohol and tobacco for at least 6 months.

Thus, confounding is likely to be limited. Nonetheless, our findings provided evidence for the ketamine related alteration in serum VEGF.

The regression analysis showed that serum VEGF level was positively correlated with the days since last use of ketamine which indicated that the longer time interval after cease of ketamine use, the higher the serum VEGF level. It is possible that the decreased VEGF rebounded or returned after stop use ketamine. We also found that the serum VEGF levels were negatively correlated with BAI score and positively correlated with PANSS negative subscale score. Whether or how VEGF played a role in the pathogenesis of psychotic symptoms needs further study. We did not find significant correlation between serum VEGF level and ketamine use characteristics. It is possibly because most of the ketamine abusers enrolled in this study used ketamine for a relatively long time and used it frequently.

In conclusion, our study demonstrated that the serum level of VEGF was decreased in chronic ketamine abusers. The role VEGF played in the clinical symptoms reported within ketamine abusers such as psychotic, depressive and cognitive impairments need further illustration.

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Fig. 1. Comparisons of VEGF serum level in ketamine abusers and healthy subjects: VEGF serum level in ketamine abusers was significantly lower than that in healthy subjects ($p = 0.008$).

Table 1

Clinical and demographic characteristics of ketamine abusers and healthy controls.

	Ketamine users (<i>N</i> = 81)	Healthy subjects (<i>N</i> = 39)	<i>t</i> / χ^2
Age (years)	25.7 ± 5.16	25.1 ± 4.8	<i>t</i> = 0.626
Gender (male/female)	77/4	34/5	χ^2 = 2.358
Years of education [*]	10.6 ± 2.72	11.7 ± 1.7	<i>t</i> = -2.205
Age of first ketamine use (years)	19.8 ± 5.12		
Duration of ketamine use (months) ^a	71.3 ± 36.88		
Duration of dependence (months) ^b	34.9 ± 24.54		
Current frequency of ketamine use (days per week) ^c	6.28 ± 1.45		
Current average daily ketamine use (g)	3.25 ± 3.09		
PANSS			
Positive symptom subscale	7.95 ± 1.87		
Negative symptom subscale	12.91 ± 4.13		
General psychopathology subscale	24.28 ± 5.56		
Total	45.14 ± 9.54		
BDI	12.13 ± 6.07		
BAI	14.75 ± 8.79		
Use of other psychoactive compounds ^d			
Never use	16 (19.8.0%)		
Methamphetamine	26 (32.1%)		
MDMA	60 (74.07%)		
Codeine hydrochloride	11 (13.6%)		
Heroin	2 (2.5%)		
Cannabis	23 (28.4%)		
Cocaine	1 (1.2%)		
Triazolam	1 (1.2%)		
Alcohol			
Drink frequently ^e	21 (25.9%)	5 (12.8%)	χ^2 = 2.664
Not drink frequently ^f	60 (74.1%)	34 (87.2%)	
Smoking ^{**}			
Smoke frequently ^g	74 (91.4%)	19 (48.7%)	χ^2 = 27.449
Not smoke frequently ^h	7 (8.6%)	20 (51.3%)	

Data are the mean ± SD.

Data about use of other psychoactive compounds, alcohol consumption and tobacco smoking are the number of cases (%). MDMA: 3,4-methylenedioxymethamphetamine.

^aTotal months of ketamine use from the first time till now.^bTotal months of ketamine use from dependent till now.^cFrequency of ketamine use in recent 1 month.

^dUse of other psychoactive compounds in one's life span, alcohol and tobacco excluded.

^eDrink no less than once a week.

^fIncluding abstinence, drinking less than once a week and never drinking.

^gSmoke no less than 3 days per week.

^hIncluding cessation, smoking less than 3 days per week and never smoking.

*
 $p < 0.05$.

**
 $p < 0.01$.

Table 2

Correlations of serum VEGF with demographic, drug use characteristics and psychotic assessments.

	VEGF (pg/ml)	
	<i>r</i>	<i>p</i>
Gender	−0.068	0.545
Age	−0.156	0.164
Education	0.005	0.961
Age of first ketamine use	−0.211	0.059
Duration of ketamine use ^a	0.141	0.208
Duration of dependence ^b	0.144	0.200
Current frequency of ketamine use ^c	−0.161	0.151
Current average doses of ketamine consumption	−0.154	0.171
Days since last use ^d	0.252 *	0.023
Alcohol consumption	−0.025	0.823
Tobacco smoking	−0.133	0.235
PANSS		
Positive symptom subscale	0.168	0.136
Negative symptom subscale	0.261 *	0.019
General psychopathology subscale	0.012	0.917
Total score	0.16	0.156
BDI	−0.042	0.708
BAI	−0.219 *	0.05

^aTotal months of ketamine use from the first time till now.^bTotal months of ketamine use from dependent till now.^cFrequency of ketamine use in recent 1 month.^dDays of blood drawing since last ketamine use.* $p < 0.05$.