Psychoneuroendocrinology 97 (2018) 37–46

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Differential effect of the *DRD3* genotype on inflammatory cytokine responses during abstinence in amphetamine-dependent women

Shin-Chang Kuo[a,b], Yi-Wei Yeh[b], Chun-Yen Chen[a,b], Chang-Chih Huang[a,c], Pei-Shen Ho[d], Chih-Sung Liang[a,e], Chun-Long Lin[a,f], Ta-Chuan Yeh[b], Chang-Chih Tsou[b], Bao-Zhu Yang[g], Ru-Band Lu[b], San-Yuan Huang[a,b,*]

[a] *Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan, ROC*
[b] *Department of Psychiatry, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC*
[c] *Department of Psychiatry, Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, Taiwan, ROC*
[d] *Department of Psychiatry, Taichung Armed Forces General Hospital, Taichung, Taiwan, ROC*
[e] *Department of Psychiatry, Beitou Branch, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC*
[f] *Department of Psychiatry, Hsinchu Branch, Taoyuan Armed Forces General Hospital, Hsinchu, Taiwan, ROC*
[g] *Department of Psychiatry, Division of Human Genetics, Yale University School of Medicine, New Haven, CT, 06511, USA*

ARTICLE INFO

Keywords:
Cytokine
Immune balance
DRD3 gene
Amphetamine dependence

ABSTRACT

Amphetamine exposure impacts on innate and adaptive immunity and *DRD3* may modulate the effect of amphetamine on the immune response. We assessed the immune-cytokine markers in 72 female patients with amphetamine dependence (AD) at baseline and after 4-week drug abstinence and in 51 healthy women. Multiplex magnetic bead assay was used to measure the plasma cytokine expression level simultaneously in all participants and *DRD3* rs6280 polymorphism was genotyped in patients. We demonstrated an increase of the T helper 1 (Th1) cytokines (IL-2), Th2 cytokines (IL-4, IL-5, IL-6 and IL-10) and other cytokines (IL-1β) in the entire AD cohort. A similar cytokine pattern, along with a significantly decreased IL-8 and IL-10 levels was observed after 4-week abstinence. Among AD patients with *DRD3* rs6280 TT genotype, the cytokine expression profile was consistent with total AD cohort at baseline and revealed a significant down-regulated plasma level of the Th1, Th2, and other cytokines except for IL-6 after 4-week abstinence. In AD group with *DRD3* rs6280 C allele carrier, we found IL-2 level was significantly higher than healthy controls at baseline and remained higher, accompanied with a borderline increase in IL-4, IL-6 and IL-1β levels after 4-week abstinence. Our results suggest that chronic use of amphetamine increased both pro- and anti-inflammatory cytokines in AD patients, indicating the immune imbalance that may persist for 4 weeks or more. Besides, *DRD3* rs6280 TT genotype may be associated with favorable recovery in general inflammatory cytokines during period of abstinence.

1. Introduction

Amphetamine dependence (AD) is a chronic, relapsing brain disorder with no effective pharmacological treatment. AD is a significant public health epidemic and societal burden because of its negative consequences on patients (Courtney and Ray, 2014; Gonzales et al., 2010), including heightened risk to various infectious diseases and rapid progression in severity. In *in vitro* experimental (House et al., 1994; Iwasa et al., 1996) and animal studies (Harms et al., 2012; In et al., 2005; Peerzada et al., 2013) have revealed that methamphetamine (MA) administration modulates innate and adaptive immunity, supporting a possible link between MA and immune dysfunction in adult mammals. MA exposure causes hyperactivation of the innate immune response in the brain (Wisor et al., 2011; Yamamoto et al., 2010) and alters the expression of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-6, after lipopolysaccharide (LPS) stimulation (Buchanan et al., 2010). Furthermore, central immune signaling affects on amphetamine addictive behaviors (Coller and Hutchinson, 2012; Stolyarova et al., 2015), and human studies also suggested that alterations in peripheral immune factor expression are associated with neuropsychiatric symptoms in patients with AD (Huckans et al., 2015). Peripheral cytokines can cross the blood brain barrier (BBB) and central immune changes are reflected in peripheral circulation (Banks, 2005). Thus, peripheral cytokine levels

* Corresponding author at: Department of Psychiatry, Tri-Service General Hospital, National Defense Medical Center, No. 325, Sec. 2, Chenggong Road, Neihu District, Taipei, 114, Taiwan, ROC.
E-mail address: hsy@ndmctsgh.edu.tw (S.-Y. Huang).

https://doi.org/10.1016/j.psyneuen.2018.06.023
Received 7 April 2018; Received in revised form 25 May 2018; Accepted 29 June 2018

may be useful biomarkers for the clinical evaluation of the effects of amphetamine on immune function in patients with AD, and a better understanding of the psychoneuroimmunology of amphetamine dependence may provide the possible therapeutic perspectives in addiction treatment.

Dopamine (DA), a neurotransmitter, functions via five DA receptors, termed DRD1–DRD5, which are all expressed on human CD4 + T cells (Cosentino et al., 2007). In the thymus and spleen of mice, DA is involved in the maturation and selection of lymphocytes and activation of immune responses (Mignini et al., 2009). Moreover, the modulatory effects of DA on LPS-induced blood cytokines are mediated by dopamine receptors expressed on immune cells (Matalka et al., 2011). Thus, DA is an important regulator of the immune response (Pinoli et al., 2017).

Moreover, *DRD3* is associated with MA addiction. One study found that DRD3 may be upregulated in the brains of MA abusers (Boileau et al., 2012). DRD3 has also been shown to modulate locomotor responses to both acute and repeated MA exposure (Zhu et al., 2012), and the inhibition of the DRD3 attenuates the self-administration, conditioned place preference, behavioral sensitization, and reinstatement of drug-seeking effects of MA (Chen et al., 2014; Higley et al., 2011; Sun et al., 2016), suggesting that DRD3 may be a potential therapeutic target for MA addiction (Le Foll et al., 2014; Paterson et al., 2014). Intriguingly, DRD3 has been implicated in the alternation of immune responses in activated T cells (Ilani et al., 2004), and the migration and homing of naïve CD8 + T cells (Watanabe et al., 2006). Moreover, genetic deficiency of DRD3 in CD4 + T cells reduces neuroinflammation and subsequent neurodegeneration in a mouse model of Parkinson's disease (PD) (Gonzalez et al., 2013), and DRD3 may favors both T-cell activation and acquisition of the Th1 inflammatory phenotype (Contreras et al., 2016). The effects of DRD3 on Toll-like receptor (TLR4) signaling may be involved in the regulation of MA-mediated mast cell activation induced by LPS (Xue et al., 2016). Taken together, these studies support the possible role of DRD3 in modulation of the immune response after exposure to or abstinence from MA. Furthermore, the *DRD3* rs6280 is a well-known functional polymorphism that causes a serine to glycine (Ser9Gly) change in the extracellular N terminus of DRD3, followed by a thymine (T) to cytosine (C) substitution. Diverse rs6280 genotypes are associated with different dopamine affinities and differential downstream signal transduction mechanisms (Hellstrand et al., 2004). Thus, we hypothesized that the *DRD3* genotype may regulate the effects of MA on the immune response. However, studies of the changes in immune cytokines in patients with AD have been limited due to their cross-sectional design (Huckans et al., 2015; Loftis et al., 2011).

In this study, we aimed to compare the plasma cytokines levels at baseline and after 4-week abstinence in patients with AD and to determine whether changes in plasma cytokine levels were related to different *DRD3* genotypes. We also examined cytokine ratios to help clarify whether the *DRD3* genotype altered the balance toward the Th1 or Th2 response.

2. Materials and methods

2.1. Participants

This study was performed in accordance with the 1994 Declaration of Helsinki (ethical laws pertaining to the medical profession). The research protocol was approved by the Institutional Review Board for the Protection of Human Subjects (TSGHIRB 096-05-073 & 1-106-05-095) at the Tri-Service General Hospital (TSGH; a medical teaching hospital belonging to the National Defense Medical Center in Taipei, Taiwan). Considering that use of amphetamines is illegal in Taiwan, we emphasized that their legal status would not be affected by participation in the study. All participants gave their written informed consent after having received a detailed description of the study procedures and were free to withdraw their participation at any time. Each participant was screened for psychiatric conditions by a well-trained psychologist using a Chinese version of the modified Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L) (Endicott and Spitzer, 1978; Merikangas et al., 1998) after initially evaluated by an attending psychiatrist.

A total of 72 female patients with AD were recruited from drug rehabilitation clinic of a detention center in Northern Taiwan, where women are detained for correction treatment of amphetamine related problems. The 4-week demanded/compulsory rehabilitation program included forced drug abstinence, psychiatric assessment for addiction severity and relapse tendency, individual and group counseling, psychosocial intervention, integrated moralization & recreational activities, and legal & life education, under regular schedules for course and rest. The patients received urine toxicology test on the day of admission. They were not allowed to use any kind of psychoactive substance during the retention center stay with strict security check. To date, MA is used predominantly in the crystal form among those AD patients in Taiwan and all of the patients inhaled or smoked MA in this study. The participants would be investigated and blood sampling at the fist day of registration (baseline; less than 3 days after their last drug use) and the day before discharge from detention center (after 4-week abstinence). The principal diagnosis of AD was confirmed on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text-Revision (DSM-IV-TR, American Psychiatric Association, 2000). All patients were enrolled voluntarily in this study, and met the DSM-IV-TR criteria for AD based on interviews and all available information with the help of (1) physicians' medical records and hospital data and (2) a positive urine toxicology test for methamphetamine, analyzed by gas chromatography coupled with mass spectrometry (GC/MS), on the day of admission. The exclusion criteria were as follows: (1) dependence on another substance, except nicotine; (2) major psychiatric disorders such as schizophrenia, bipolar disorder, and major depressive disorder; (3) recent use of medications such as psychotropics, steroids, antibiotics, non-steroidal anti-inflammatory drugs or immune-modulatory drugs, either during the course of the study or within the month prior to enrollment; (4) general medical conditions associated with immune imbalances such as liver diseases, inflammatory or rheumatologic diseases, cardiovascular disease, respiratory diseases, or recent infections; (5) if women of childbearing age were pregnant or in the post-partum period. The control group consisted of 51 physically and psychiatrically healthy female volunteers enrolled from the community. In addition, there was no family history of psychiatric disorder or substance use disorder in the first-degree relatives of the control subjects.

2.2. Blood collection and cytokine measurements

Peripheral blood samples were collected within 24 h after recruitment from both healthy controls and the patients with AD. In week 4 of abstinence from drug abuse, blood samples were collected from patients only. Venous blood samples were obtained by venipuncture and collected in EDTA-containing tubes between 0730 and 1000 A M after a night of fasting and bed rest. The same conditions were applied to all of the samples. The blood was placed on ice and centrifuged (3500 rpm for 15 min, at 4 °C) within 1.5 h. The plasma was collected and stored at −80 °C freezer until the cytokine levels were measured. The samples had not been thawed before the cytokine analysis.

Plasma levels of Th1-related cytokines – interferon gamma (IFN-γ), TNF-α and IL-2, Th2-related cytokines – IL-4, IL-5, IL-6 and IL-10, and other cytokines – IL-1β, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) – were simultaneously determined by multiplex immunoassay using the human ultrasensitive cytokine magnetic 10-Plex panel (Novex® by Life Technologies™) with a Luminex analyzer (MAGPIX® systems), according to the manufacturer's instructions,. All analyses were performed in one batch using kits from the same production lot (Catalog#: LHC6004 M). The sensitivity of detection (pg/