（醫學系 精神學科）

111 年度國防/醫療事業基金醫學研究計畫
成果報告

中文： 由多元性別差異分析安非他命成癮者之生物因子、免疫體質、人格特質 、生活品質及環境等相關危險因子(第一年)

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中英文摘要及成果運用

中文摘要

目標：

安非他命使用障礙症與先天/後天性免疫功能和遺傳因子有顯著影響。本研究第一年目的在評估和比較安非他命使用障礙症（AUD）（有或沒有精神病症）與健康對照組間的細胞激素血漿濃度之差異。

方法：

本研究合計收了85名個案（其中60名安非他命成癮個案以及25名健康對照組），所有個案皆進行免疫細胞激素實驗分析，以其分析安非他命成癮個案與健康受試者間是否有所差異。另外，安非他命疾病組中將其是否合併精神症狀區分出兩組，一組為合併精神疾病之個案（AUD-P，n = 19），另一組為無合併精神疾病之個案（AUD-NP，n = 41）。在有關細胞激素實驗分析中， 本實驗使用多重磁珠測定法測量所有受試者的血漿細胞激素濃度。

結果：

我們研究初步發現，在整個安非他命組以及其亞型（安非他命無合併精神症狀），輔助型T細胞（Th1）和Th2相關細胞激素濃度含量皆比健康受試者高，安非他命合併精神病症狀亞型中其Th1和Th2相關細胞激素濃度含量也有類似的增加趨勢。然而，只有三種細胞激素 - 白細胞介素（IL）-5，IL-10和腫瘤壞死因子（TNF）-α顯示出顯著差異。除此之外，與安非他命無合併精神症狀亞型相比，合併精神症狀的亞型其相關細胞激素顯示促炎性細胞因子（IL-1β，IL-6，IL-8，IFN-γ）濃度降低，並且向Th2細胞反應轉變。

結論：

研究初步結果表示，安非他命使用障礙症個案顯示出其某些免疫因子濃度比健康對照組高、促炎性細胞激素(Pro-inflammatory cytokines)和抗炎性細胞激素(anti-inflammatory cytokines)的不平衡，以及在安非他命成癮個案早期戒癮期間藥物引起的精神病症其相關免疫表型的存在。但這些初步研究發現，仍需日後類似的研究進行驗證，期待有關安非他命的病因及相關議題能逐一獲得驗證。

關鍵字：安非他命使用障礙、成癮、精神病症、細胞激素

成果運用

國防醫學研究計畫

本次研究成果在計畫所屬範疇為臨床醫學及創新科技研究)之運用及效益

本研究原先申請進行三年計畫，預計分析安非他命成癮者之生物因子、免疫體質、人格特質、生活品質及環境等相關危險因子，並加以區分性別差異、性向等。但研究僅核可一年，且經費不足，收案數不足，僅能完成初步資料分析。將努力爭取日後相關年度計畫繼續完成研究。

研究初步結果表示，安非他命使用障礙症個案顯示出其某些免疫因子濃度比健康對照組高、促炎性細胞激素(Pro-inflammatory cytokines）和抗炎性細胞激素（anti-inflammatory cytokines）的不平衡，以及在安非他命成癮個案早期戒癮期間藥物引起的精神病症其相關免疫表型的存在。但這些初步研究發現，仍需日後類似的研究進行驗證，期待安非他命的病因及相關議題能逐一獲得驗證。**期待找出**有關**興奮劑(安非他命**）的病因及相關議題，進而降低國軍使用毒品機率，防止國軍人員使用毒品，傷害國軍戰力，進而增強國防戰力。

壹 4.中英文關鍵詞

中文關鍵字：安非他命使用障礙、成癮、精神病症、細胞激素

Keywords: amphetamine use disorder; psychosis; cytokine

研究動機及目的

(1) 分析性別/同性戀者飲酒障礙 (AUD) 發展中的生物學因素：聚焦於性激素和免疫因素。

雖然最近的研究表明，免疫功能和卵巢激素是 AUD 發展的重要因素，但這些結果還有限且不清楚，未來需要克服一些方法論問題。此外，目前沒有可用的數據討論性別差異，並缺乏在 AUD 患者中的細胞因子-環境-基因-個性全球特徵。因此，本研究使用相同的評估工具、樣本的採集時間和來源，探討幾種選擇性細胞因子 (例如，IL-1β、IL-2、IL-6、IL-8、IL-10、IF-r 和 TNF-α) 和性激素 (雌二醇、孕酮、泌乳素、睾酮) 在 AUD 患者和健康對照組中的關係，然後我們也將研究性別/同性戀者差異和發展 AUD 的生物學因素之間的關係。

(2)分析不同性別腦中DAT可用性與性別差異是否影響AUD發展的關係。

儘管安非他命使用可能損害腦多巴胺神經傳遞，但關於AUD患者中DAT密度的神經影像學文獻有限且不一致，因為神經影像可能會受到MDMA或酒精（Yen等人，2016）或海洛因（Liang等人，2016）或抑鬱症（Sordo等人，2012）或不同性別（Becker等人，2017）的干擾。 因此，AUD組重點招募目前未使用酒精、海洛因或患有抑鬱症或未濫用其他非法藥物的安非他命使用者，這些納入/排除標準可以更好地窺探紋狀體DAT密度和反覆安非他命暴露之間的關聯。

研究結果

1. Demographic data and clinical characteristics of the study groups

Demographic and clinical characteristics of the AUD and healthy control groups are shown in Table 1. There was no significant difference in mean age and BMI between patients with AUD and controls ($p > 0.05$). Likewise, there were no significant differences in age or BMI in a three-way comparison among healthy controls, AUD-NP, and AUD-P groups ($p = 0.143$ for age; $p = 0.795$ for BMI). Patients with AUD also had significantly lower education attainment (years) than controls (9.73 ± 2.92 versus 14.54 ± 2.74, $p < 0.001$), consistent with epidemiological features of patients with AUD. Furthermore, there were no significant differences in the age, BMI, education level, age at onset, duration of AUD, or severity of dependence scale (SDS) (Gossop et al., 1995) between the AUD-NP and AUD-P groups. Among the patients with AUD, there were no significant associations between cytokine expression levels and clinical parameters such as age, BMI, age of onset, duration of AUD, or severity of dependence scale ($p \geq 0.05$ for all cytokines, as shown in Table 2). Furthermore, IL-8 was positively correlated with psychotic symptoms severity ($p = 0.03$) in AUD-P group, but the correlation became insignificant after Bonferroni correction ($p = 0.005$ was considered as significant, Table 2).

2. Comparison of plasma inflammatory cytokines between the AUD and control groups

Plasma cytokine levels in the AUD and healthy control groups are shown in Table 3. After controlling for age and BMI, the total AUD group had elevated levels of Th1-related cytokines and Th2-related cytokines. Similar cytokine level changes were also noted in the AUD-NP and AUD-P subgroups (Table 3 and Fig 1-3). Moreover, in comparison to the AUD-NP subgroup, the AUD-P subgroup had lower levels of IL-1β ($p = 0.003$), IL-4 ($p = 0.040$), IL-6 ($p = 0.013$) , IL-8 ($p = 0.021$), and IFN-γ($p = 0.003$).

3. Difference in Th2/Th1 cytokine ratios between the AUD and control groups

We further analyzed Th2/Th1 cytokine ratios after controlling for age and BMI. Table 4 reveals the changes in the ratios of Th1 and Th2 cytokine levels among patients with AUD and controls. In comparison to the control group, the total AUD cohort had significantly lower Th2/Th1 ratios of IL-4/IFN-γ($p = 0.02$), IL-4/TNF-α($p = 0.001$), and IL-5/IFN-γ($p = 0.011$) but significantly higher Th2/Th1 ratios of IL-5/IL-2 ($p < 0.001$), IL-5/ TNF-α($p = 0.006$), IL-6/IL-2 ($p < 0.001$), IL-10/IL-2 ($p < 0.001$), IL-10/ TNF-α($p = 0.002$), and IL-10/IFN-γ($p = 0.005$). AUD subgroups also had similar cytokines expression patterns in addition to only a decreasing trend in the ratio of IL-4/IFN-γand IL-5/IFN-γ. However, the ratios of IL-4/IFN-γ($p = 0.049$), IL-5/IFN-γ($p = 0.010$) and IL-6/IFN-γ($p = 0.004$) were significantly higher in the AUD-P subgroup compared to the AUD-NP subgroup.

結論與建議

結論

This study aimed to examine the immune-cytokine effect of amphetamine by simultaneous analysis of 10 cytokines, providing an extended scope with which to elucidate the target cytokines of amphetamine. The first major finding of this study was that chronic exposure to amphetamine had a significant stimulating effect on several peripheral cytokine levels. Out of the entire AUD cohort, when compared to healthy controls, the pro-inflammatory cytokines showing elevated levels were IL-6, IFN-γ,TNF-α, and GM-CSF, while the only anti-inflammatory cytokines showing elevated levels was IL-5 and IL-10 in the view of innate immunity. A similar cytokine pattern, along with a significantly increased level of IL-1βand IL-4 was also observed in the AUD-NP patients. Findings from our study revealed greater elevated levels across all the studied pro- and anti-inflammatory cytokines in the AUD cohort when compared to healthy controls. To our knowledge, this is the first demonstration of a generalized peripheral inflammatory state with compensatory anti-inflammatory activation in AUD. However, further studies are needed to determine the cellular source of the pro- and anti-inflammatory cytokines and the underlying mechanisms that drive these changes.

The second major finding of this study was that subgroup analysis revealed a significant down-regulated plasma level of IFN-γ, IL-1β, IL-4, IL-6, and IL-8 in the AUD-P subgroup compared with the AUD-NP subgroup. Two possible hypotheses to explain the distinct difference in immune profiles when being compared between the two subgroups, with regard to the role of dopamine (DA) in the immune system, are discussed as follows. (A) DA regulates T cells directly; amphetamine acts to increase the release of and sustain extracellular concentrations of neurotransmitters including dopamine (DA) (Sulzer, Sonders, Poulsen, & Galli, 2005). DA is an important regulator linking the nervous and immune systems (Basu & Dasgupta, 2000). Sarkar,

Basu, Chakroborty, Dasgupta, and Basu (2010), in their review of focusing on DA mediated regulation of T cell functions, indicated the unique interactions between DA and T cells, where DA activates naïve or resting T cells, but inhibits activated T cells. For example, DA is reported to induce cytokine secretion in resting T cells (Besser, Ganor, & Levite, 2005). Stimulation of DA D2 and D1/D5 receptors induces IL-10 secretion, while stimulation of DA D3 and D1/D5 receptors increases the secretion of TNF-α(Besser et al., 2005). In contrast to activating resting T cells, stimulation of DA D2/D3 receptors in activated T cells has also been shown to inhibit secretion of IL-2, IFN-γ, and IL-4 (M. C. Ghosh et al., 2003). In line with this evidence, our result, which show increased levels of IL-10 and TNF-αin both the entire AUD cohort and the AUD-NP subgroup, may indicate the immune state as being the a result of DA activating the resting T cells. On the other hand, the AUD-P subgroup had a lower level of IFN-γand IL-4 than the AUD-NP subgroup, which suggests that hyperactive DA transmission was involved in the pathology of psychosis (Meltzer & Stahl, 1976), and may further inhibit activated T cells. The latter model may be also supported by the finding that patients with schizophrenia show increased amphetamine-induced synaptic DA concentration in the mesolimbic areas (Breier et al., 1997). In addition, changes in the status of DA concentrations and/or receptors, particularly in the T cells are associated with dysregulated immune functions in patients with schizophrenia (Boneberg et al., 2006; Ilani, Strous, & Fuchs, 2004). Taken together, these findings suggest that the DA-mediated changes in the immune system are also linked to the etiology of amphetamine-induced psychosis. (B) Neurotransmitters regulate peripheral cytokines through "cortisol" levels; central DA neurotransmission plays a key role in the pathogenesis of schizophrenia (Howes & Kapur, 2009). Moreover, DA promotes the secretion of corticotrophin-releasing hormone (CRH) in the hypothalamus (Calogero, Gallucci, Chrousos, & Gold, 1988), resulting in downstream secretion of cortisol. Higher cortisol levels provide negative feedback to the peripheral immune system to suppress the production of pro-inflammatory cytokines (Watkins, Nguyen, Lee, & Maier, 1999), and may also play an important role in causing a shift from cellular (Th1) to

humoral (Th2) immune responses (Elenkov, 2004). Taken together, this evidence supports our finding that decreased plasma levels of pro-inflammatory cytokines (IL-1β, IL-6, and IFN-γ; Table 3) and higher Th2/Th1 ratio (IL-4/IFN-γ, p=0.049; IL-5/IFN-γ, p=0.026; IL-6/IFN-γ, p=0.003; Table 4) in the AUD-P group, when compared to the AUD-NP group, indicates that excessive brain DA levels in patients with psychosis, which subsequently activates the hypothalamic–pituitary–adrenal (HPA) axis to regulate peripheral cytokine levels through cortisol levels.

Our study supports preliminary evidence of both activated innate and adaptive immune systems in patients with AUD. Moreover, the distinct cytokine profiles between AUD-P and AUD-NP subgroups suggest the existence of an immunological phenotype associated with drug-related psychosis during early abstinence in AUD. Replication of our results is warranted to verify these findings, and further longitudinal studies are required to measure the alteration in cytokines levels in AUD subjects with a long period of drug abstinence.

建議

This study was originally applied for as a three-year research proposal to analyze risk factors of Amphetamine use disorder (AUD) such as biological factors, immune system, personality traits, quality of life, and environment with additional identification made between differences in gender and sexual orientation. However, the initial proposal was only approved for one year and thus both the study funds and number of participants were insufficient. Therefore, only the preliminary data analysis could be completed. Efforts will be maintained in order to continue the research study forthe upcoming year.