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Inflammatory response in heroin addicts undergoing methadone maintenance treatment

Yuan-Yu Chan [a,b], Szu-Nian Yang [a], Jyh-Chyang Lin [a], Junn-Liang Chang [b], Jaung-Geng Lin [c], Wan-Yu Lo [d,e,*]

[a] Department of Psychiatry, Taoyuan Armed Forces General Hospital, Taoyuan County, Taiwan
[b] Department of Pathology and Laboratory Medicine, Taoyuan Armed Forces General Hospital, Taoyuan County, Taiwan
[c] Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan
[d] Graduate Institute of Integrated Medicine, China Medical University, Taichung County, Taiwan
[e] Department of Life Science, National Chung Hsing University, Taichung County, Taiwan

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ABSTRACT

Opioid addiction influences many physiological functions including reactions of the immune system. The objective of this study was to investigate the immune system function in heroin addicted patients undergoing methadone maintenance treatment (MMT) compared to healthy controls. We tested the cytokine production of IL-1β, IL-6, IL-8, IL-10 and tumor necrosis factor (TNF)-α from a group of heroin addicts ($n=34$) and healthy controls ($n=20$). The results show that production of IL-1β, IL-6 and IL-8 was significantly higher in the group of methadone-maintained patients than in the healthy control group. Plasma TNF-α and IL-6 levels were significantly correlated with the dairy methadone dosage administered, and the IL-1β level was significantly correlated with the duration of methadone maintenance treatment. These findings suggest that methadone maintenance treatment influences the immune system functions of opioid-dependent patients and may also induce long-term systemic inflammation.

1. Introduction

Opiates addiction, both physiological and psychological, is a chronic medical illness and a major public health concern worldwide. Heroin is the most common opiate worldwide with several million addicts worldwide. The social, medical, and economic problems resulting from heroin dependence are severe and current efforts to wean addicts off heroin have often yielded limited results because of a high relapse rate and troublesome subjective symptoms. For managing heroin dependence, a methadone maintenance treatment (MMT) was introduced in the mid-1960s (McLachlan et al., 1993), although methadone has been effective in heroin maintenance treatment as a long-acting opioid receptor agonist that can increase adherence to treatment, and reduce illicit drug use and mortality. The opioid relapse rate after methadone discontinuation still remains high. Some studies have revealed that opioid drugs, such as morphine and heroin, exert negative effects on the immune system (Sacerdote, 2006; Vallejo et al., 2004). Heroin can affect various physiological functions of the body, including immune system reactions, by directly acting on the opioid receptors present on lymphocytes and macrophages (Nelson et al., 2000; Stefano et al., 1996) or by indirectly acting on the central nervous system (CNS) (Fecho et al., 1996; McCarthy et al., 2001; Peterson et al., 1993, 1998).

Cytokines are small molecules that function as immune system messengers. Several in vitro studies have reported that acute morphine treatment alters the secretion of various cytokines, including tumor necrosis factor (TNF)-α (Kapasi et al., 2000; Zubelewicz et al., 2000). In a neuropathic rat model, transected lumbar spinal cords that were chronically treated with morphine revealed increased proinflammatory cytokine secretion and glial cell overactivation (Raghavendra et al., 2002). Another rat study revealed that a combination of heroin and cocaine caused marked neurotoxicity (Cunha-Oliveira et al., 2010). Chronic opioid treatment markedly increased the plasma and drug addiction related cytokine expression in the brain (Chen et al., 2012b), a phenomenon suggesting that chronic opioid use can cause inflammation, neuronal degeneration, and neuronal damage.

Various studies on heroin-dependent patients have repeatedly demonstrated that opioids consistently cause immunosuppression (Donahoe and Vlahov, 1998; Friedman et al., 2003; Neri et al., 2005).

* Correspondence to: Graduate Institute of Integrated Medicine, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan.
E-mail addresses: xaviorchan@gmail.com (Y.-Y. Chan), snyang@aftygh.gov.tw (S.-N. Yang), chyangjyhlin@aftygh.gov.tw (J.-C. Lin), junn9liang@yahoo.com.tw (J.-L. Chang), jglin@mail.cmu.edu.tw (J.-G. Lin), drwanyu@gmail.com (W.-Y. Lo).

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In addition, a study reported that chronic intrathecal morphine administration upregulated IL-6 expression in the cerebrospinal fluid (Zin et al., 2010). Moreover, inducible nitric oxide synthase (iNOS) and cytokine expression were detected in noradrenergic locus coeruleus cells in the brains of heroin-dependent patients (Dyuizen and Lamash, 2009). Chronic morphine use substantially decreases the number of dopaminergic neurons in the ventral tegmental area (Chu et al., 2008; Sklair-Tavron et al., 1996) and elevates serum IL-6 levels by activating the hypothalamic–pituitary–adrenal and autonomic nervous systems (Bertolucci et al., 1996; Houghtling and Bayer, 2002). In the peripheral nervous system, opioids indirectly affect peripheral cytokine expression that can cross the blood–brain barrier (Simard and Rivest, 2005), and high peripheral cytokine levels induce sustained neuronal inflammation, damage, and degeneration in the central nervous system (Qin et al., 2007).

Therefore, the present study aimed to investigate the primary inflammatory cytokine expressions in heroin-dependent patients receiving MMT, and compared with the corresponding expressions in healthy controls. We monitored peripheral plasma cytokine (IL-1β, IL-6, IL-8, IL-10 and TNF-α) levels because monitoring these levels in the human brain is unethical and challenging. The hypothesis was that long-term MMT and higher methadone dosage treatment might affect immune system functions.

2. Methods

2.1. Subjects

The study was conducted at the outpatient clinic of the Department of Psychiatry at Taoyuan Armed Forces General Hospital, a regional teaching hospital in Taiwan. Subjects were recruited from January 2014 to November 2014 through referrals from psychiatrists and advertisements at the clinic. Only male participants older than 20 years who satisfied the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria for opiate dependence and who had been receiving MMT for more than 1 month were included in the study. Participants with a history of antidepressant or neuroleptic medication use or who were HIV positive or had any severe physical illness, bleeding disorders, or were taking anticoagulant drugs were excluded. According to the aforementioned criteria, 34 heroin addicts and 20 healthy blood donors (controls) were enrolled in this study for assessment. The study design, consent forms and procedures were approved by the Institutional Review Board of Tri-Service General Hospital National Defense Medical Center in Taiwan (IRB no. TY102-03).

2.2. Data collection

The patient inclusion form requested the following information: patient identification, heroin and amphetamine abuse history (years), daily consumption of methadone, duration of methadone maintained (months), legal problem, marital status, Beck Anxiety Inventory (BAI) score, Beck Depression Inventory II (BDI-II) score, Pittsburgh Sleep Quality Index (PSQI) score and opioid urine screen.

The anxiety symptom severity was measured using the BAI (Beck et al., 1988), which lists 21 symptoms of anxiety, such as feeling hot, scared, or nervous. Each item was rated on a four-point Likert scale, ranging from 0 (*not bothered*) to 3 (*severely bothered*), yielding a maximal total score of 63 points. According to the BAI manual, total scores ranging from 0 to 7 indicated minimal anxiety, those ranging from 8 to 15 indicated mild anxiety, those ranging from 16 to 25 indicated moderate anxiety, and those ranging from 26 to 63 indicated severe anxiety.

The symptoms of depression were measured using the BDI-II, which comprised 13 items, rated from 0 to 3, to evaluate depression according to the degree to which it reflected the patient's state during the previous week. The BDI-II has a high reliability and concurrent validity (Beck and Steer, 1984). According to the BDI-II manual, total scores indicated four levels of depression: minimal depression (0–13), mild depression (14–19), moderate depression (20–28), and severe depression (29–63).

Sleep quality was assessed using the Taiwanese version of the PSQI (Buysse et al., 1989), which has demonstrated reliability and validity (Tsai et al., 2005); this system was used to evaluate sleep disturbances by using seven subscales. Each subscale was rated on a four-point scoring scale (0–3 points; 3 indicates a greater effect), and all the subscale scores were summed together to yield a global score (0–21). Higher scores indicated severe sleep disturbance, whereas a global score exceeding 5 indicated poor sleep (Buysse et al., 1989).

2.3. Determination of inflammatory cytokine

We collected the blood samples (6 ml) from all patients between 13:00 and 17:00 p.m., after their daily methadone intake. Blood samples were collected and centrifuged at 500*g* for 5 min at 4 °C to extract the serum. The serum samples from both the patients and healthy donors were stored at −80 °C until analysis. A panel of cytokines was quantified using the Human Inflammatory Cytokines Kit (BD Biosciences Pharmingen, San Diego, CA, USA) by flow cytometry (BD FACSCanto™ System; Becton Dickinson Corp., San Jose, CA, USA). In the analysis, six bead populations with distinct fluorescence intensities were coated with six type specific antibodies to capture and simultaneously determine the various cytokines. The cytokine-captured beads were incubated with the secondary antibodies to form the sandwich complexes. After incubation, washing, and acquisition, the results were determined by BD array software (FCAP Array V 3.0).

2.4. Statistical analysis

Statistical analyses were performed using SPSS 18.0 statistical software for Windows. All data are presented as the mean ± standard error of the mean (SEM). Student's *t* test was used for the statistical evaluations between the methadone-maintained patients (patient group) and the healthy controls (control group). The correlation among the different parameters in the patient group was evaluated using the mean of the Pearson correlation test; $p < 0.05$ was considered statistically significant for all tests.

3. Result

Table 1 summarizes the sociodemographic characteristics of all participants. No significant differences were observed regarding age, employment status, and marital status between the control and the patient groups. In the patient group, 2.9% of the patients reported legal problems. The mean methadone dose was 59.1 ± 5.6 mg. The mean duration of MMT was 23.56 ± 5.6 months. On average, the reported history of heroin and amphetamine abuse was 8.6 ± 1.1 and 4.2 ± 1.0 years, respectively. In the 30 days before baseline assessment, 50.0% and 17.65% of the patients reported occasional heroin and amphetamine use, respectively. In addition, the average BAI score was 9.65 ± 1.25, which indicated mild anxiety. The average BDI-II score was 15.56 ± 2.36, indicating mild depression, and the average PSQI score was 10.79 ± 1.24, indicating poor sleep.

The leukocytes obtained from the control group participants were cultivated alone, and the levels of the cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α were determined using ELISA. The mean IL-1β, IL-6 and IL-8 were significantly higher in the patient group than those in the control group; in addition, similar finding were observed regarding the corresponding median levels in both groups. The median number of IL-1β, IL-6 and IL-8 levels was higher in the patient group (IL-1β:1131.56 fg/ml, IL-6:472.27 fg/ml, and IL-8:15,998.41 fg/ml) than that in the control group

Table 1
Characteristics of the study participants.

	Methadone-maintained patients ($n=34$)	Health controls ($n=20$)
Age (years)	40.12 ± 1.20	37.85 ± 2.07
Employed (%)	73.3	70
Married (%)	41.1	65
IL-1β (fg/m)	2822.83 ± 580.58**	51.08 ± 12.33
IL-6 (fg/m)	1834.64 ± 603.55*	499.9 ± 207.41
IL-8 (fg/m)	57,849.56 ± 14,377.92**	2315.48 ± 292.44
IL-10 (fg/m)	631.75 ± 260.97	320.35 ± 120.68
TNF-α (fg/m)	912.82 ± 465.72	38.51 ± 14.24

Values are mean ± SEM.

* $p < 0.05$.

** $p < 0.01$.