Drug and Alcohol Dependence 183 (2018) 19–24

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

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

Full length article

Inflammatory chemokine eotaxin-1 is correlated with age in heroin dependent patients under methadone maintenance therapy

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ARTICLE INFO

Keywords:
Amyloid beta
CCL11
FGF-2
Insomnia
Methadone

ABSTRACT

Background: Degeneration of central neurons and fibers has been observed in postmortem brains of heroin dependent patients. However, there are no biomarkers to predict the severity of neurodegeneration related to heroin dependence. A correlation has been reported between inflammatory C-C motif chemokine ligand 11 (CCL11, or eotaxin-1) and neurodegeneration in Alzheimer's disease.

Methods: Three-hundred-forty-four heroin dependent, Taiwanese patients under methadone maintenance treatment (MMT) were included with clinical assessment and genomics information. Eighty-seven normal control subjects were also recruited for comparison.

Results: Using receiver operating characteristics curve analyses, CCL11 showed the strongest sensitivity and specificity in correlation with age by a cut-off at 45 years (AUC = 0.69, $P < 0.0001$) in MMT patients, but not normal controls. Patients 45 years of age or older had significantly higher plasma levels of CCL11, fibroblast growth factor 2 (FGF-2), nicotine metabolite cotinine, and a longer duration of addiction. Plasma level of CCL11 was correlated with that of FGF-2 (partial $r^2 = 0.24$, $P < 0.0001$). Carriers with the mutant allele of rs1129844, a functional single nucleotide polymorphism (Ala23Thr) in the *CCL11* gene, showed a higher plasma level of Aß42, ratio of Aß42/Aß40, and insomnia side effect symptom score than the GG genotype carriers among MMT responders with morphine-negative urine results.

Conclusions: The results suggest possible novel mechanisms mediated through CCL11 involving neurotoxicity in heroin dependent patients.

1. Introduction

Heroin dependence is a severe mental illness. The prevalence was increased in males in Taiwan (Chiang et al., 2007; Lin et al., 2013). Neuronal pathological changes in the brain have been reported in these patients (Li et al., 2005). This neuronal change is especially obvious in young heroin dependent patients (Kovacs et al., 2015). The present study is aimed to identify plasma biomarkers of the neuronal changes in heroin dependent patients. We screened several plasma cytokines and chemokines correlated to age in an attempt to uncover age-related biomarkers, which can potentially be used to identify the severity of neuronal changes in patients who are long-term heroin users.

Correlations between the C-C motif chemokine ligand 11 (CCL11, eotaxin-1), a chemokine, and cigarette smoking (Krisiukeniene et al., 2009; Shiels et al., 2014), asthma (Nakamura et al., 1999), and age-related neurodegenerative diseases have been reported in literature (Guerreiro and Bras, 2015; Lalli et al., 2015; Villeda et al., 2011). It has been suggested that the mechanism of action is primarily though binding to the CCR3 receptors distributed across different tissues (Owen, 2001; Xia and Hyman, 1999). In the central nervous system, the CCL11 may cross the blood-brain barrier through the transporter protein (Erickson et al., 2014), then bind to the CCR3 on the macrophage to exert the release of reactive oxygen species (Parajuli et al., 2015), which mediates the cytotoxic effect in the brain neurons.

Although several neuroimaging and postmortem studies provided evidence that drug abusers show signs of accelerated brain aging

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https://doi.org/10.1016/j.drugalcdep.2017.10.014
Received 12 July 2017; Received in revised form 3 October 2017; Accepted 7 October 2017
Available online 28 November 2017
0376-8716/

(Cheng et al., 2013; Kovacs et al., 2015), there are no biomarkers in the peripheral blood that can be used to indicate the severity of neurodegeneration. Significant elevation of level of plasma CCL11 has been reported in cannabis users (Fernandez-Egea et al., 2013). In this study, plasma C-C motif chemokine ligand 2 (CCL2; also called monocyte chemoattractant protein-1, MCP-1), ligand 11 (CCL11; eotaxin-1), ligand 22 (CCL22; also called macrophage-derived chemokine, MDC), and fibroblast growth factor 2 (FGF-2) were screened. These chemokine ligands possess specific chemoattractant activity for different blood circulatory cells: CCL2 chemotactic for monocytes and basophil (Bischoff et al., 1992; Deshmane et al., 2009), CCL11 for eosinophils (Williams, 2015), and CCL22 for primary activated T lymphocytes (Chang et al., 1997). FGF-2 has been implicated in diverse biological process including neuronal development (Raballo et al., 2000). In this study, these factors were measured for their roles in the aging process of heroin dependent patients under methadone maintenance treatment.

Amyloid beta (Aß) peptides result from the amyloid precursor protein (APP) after the cleavage by ß and γ secretases (O'Brien and Wong, 2011). Aß40 and 42 are the most common peptide isoforms derived from APP where the numbers 40 and 42 represent the amino acid residues. Increased Aß42/Aß40 ratio has been reported in patients with early-onset type Alzheimer's disease (Kim et al., 2015). As this study is aimed to search for the inflammatory chemokines and FGF-2 by age correlation statistical analyses in a cohort of methadone maintenance treatment (MMT) heroin dependent patients, we also examined the roles of plasma Aß40 and 42 in this cohort.

2. Methods

2.1. Subjects

Three-hundred-forty-four patients in MMT participated in this study. This study was approved by the institutional review boards of the National Health Research Institutes (Zhunan, Taiwan) and the six participating hospitals. Written informed consent was obtained from all participants. The project has also been registered with the National Institutes of Health Clinical Trial (http://www.clinicaltrial.gov/ct/show/NCT01059747). Normal control subjects were recruited from referrals, advertisements and posted notice, and they met the following criteria: 1) no lifetime DSM-IV diagnosis of opioid abuse or dependence; and 2) no any illicit drug use or alcohol intoxication in the past 28 days.

2.2. Clinical assessments

Clinical characteristics and methadone treatment courses were obtained from the medical records. Lists of medications other than methadone taken in the previous week were obtained from the medical records or self-report. Clinical assessments including urine morphine tests, which served as one of treatment outcomes profile (TOP), and treatment emergent symptoms scale (TESS) including insomnia symptom scores, were conducted by research nurses before administering methadone to patients. The higher the symptom score, the more severe clinical symptoms would be.

2.3. ECG measurement

The electrocardiogram (ECG) was performed in each participating hospital according to the regular standard operation procedure (SOP) as described previously (Wang et al., 2013). The QT interval, corrected for heart rate according to the Bazzet formula (QTc), was used for subsequent analysis (Bazett, 1920). The QTc change represents the difference between the baseline and current QTc intervals for patients with a complete set of baseline and current ECG measurement data.

2.4. Plasma cotinine assay

Plasma concentrations of nicotine metabolite, cotinine, were measured by enzyme-linked immunosorbent assay (ELISA) kit (Neogen Corporation, Lansing, MI) (Chen et al., 2013).

2.5. Plasma methadone and metabolite measurement

Twelve ml, whole blood samples were collected with ethylenediaminetetraacetic acid (EDTA) as anticoagulant before the next methadone dose was given, when the plasma concentration of methadone is likely to be at its lowest level. Plasma concentrations of racemic methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were measured using high-performance liquid chromatography (HPLC) with the settings described in our previous report (Wang et al., 2010).

2.6. Plasma CCL2, CCL11, CCL22 and FGF-2 assays

Plasma was obtained from the supernatant of whole blood after centrifugation at 2000 × *g* in a Kubota 2800 centrifuge (Kubota Co., Osaka, Japan) for 20 min at 4 °C, then dispensed into 1 mL/microcentrifuge tube and frozen at −80 °C until use.

Plasma chemokine ligands CCL2, CCL11, CCL22 and cytokine FGF-2 were determined using the Milliplex MAP human cytokine/chemokine magnetic bead panel kit (Millipore, Billerica, MA). The analyses were performed according to the protocol of the manufacturer. All sample data were acquired from a MAGPIX Multiplex Reader (Luminex Corp., Austin, TX).

2.7. Plasma amyloid β protein Aβ40 and Aβ42 assays

The plasma β amyloids, Aβ40 and Aβ42, analysis is performed using the High Sensitivity Human Amyloid β40 and 42 enzyme-linked immunosorbent assay (ELISA) kit (Merck Millipore, EZHS40/EZHS42, Darmstadt, Germany). The ELISA analysis is performed according to the manufacturer's instructions. Briefly, 50 µL of the antibody conjugate working solution is added to each well in the ELISA plate, followed by the addition of 50 µL of calibration samples, QC samples, and plasma samples. The plate is covered with plate sealer, mixed for 5 min on an orbital shaker (800 rpm/min) and incubated without shaking over night at 2–8 °C. Each well is washed with 5 × 300 µL wash buffer followed by the addition of 100 µL of Enzyme Conjugate Working Solution. The plate is covered and incubated for 30 min at room temperature on an orbital shaker (800 rpm/min). After washing with 5 × 300 µL wash buffer, 100 µL Substrate Solution is added to each well. The plate is covered and incubated on a plate shaker for 5–20 min. The reaction is stopped by adding 100 µL Stop Solution. The absorbance is read at 450 nm (590 nm as reference) on a SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA).

2.8. CCL11 functional single nucleotide polymorphism genotyping

Genomic DNAs of the 344 MMT patients were isolated from the buffy coat of the whole blood lymphocyte pellets using Gentra Puregene Blood kit (QIAGEN Sciences, Germantown, Maryland). *CCL11* SNP rs1129844 genotyping was performed using probe C_7449808_10 of Applied Biosystems TaqMan SNP genotyping assay and analyzed by the StepOnePlus Real-time PCR System (Applied Biosystems, Foster City, USA) according to the manufacturer's protocol.

2.9. Statistics

Association analyses between age and chemokines/cytokines were calculated by Spearman's rank correlation analysis. The general linear model (GLM) and univariate regression analyses with permutation test