Short communication

Cocaine abuse and effects in the serum levels of cytokines IL-6 and IL-10

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ABSTRACT

Background: Cocaine abuse is capable of activating the innate immune system in the CNS resulting in deregulation of homeostasis between pro and antiinflammatory cytokines. The aim of this study was to investigate serum levels of pro and antiinflammatory cytokines, IL-6 and IL-10 respectively, in cocaine users from a young population-based sample.
Methods: This is a case–control study nested in a cross-sectional population-based survey, with individuals of 18 and 35 years old. Two groups were selected: 24 healthy controls and 12 subjects who reported cocaine use. Serum IL-6 and IL-10 were measured by ELISA using a commercial kit.
Results: There was a statistically significant increase in IL-6 ($p = 0.037$) and decrease in IL-10 ($p = 0.007$) serum levels, between cocaine users and the control group. There was also an increase in the ratio IL-6/IL-10 ($p = 0.013$) among cocaine users individuals, when compared to the control group.
Conclusions: Our results suggest that cocaine users showed an activation of the immune system when compared a control group, demonstrating a disruption in the balance of pro and antiinflammatory cytokines. Thus, peripheral cytokines may represent a putative biomarkers for cocaine users, contributing to the development of diagnosis and effective treatments.

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1. Introduction

Cocaine abuse is an important public health problem, with a high prevalence in young adults. In 2010, cocaine use on a global level in the population between 15 and 64 years old was 0.3–0.5% (UNODC, 2014). Psychostimulant use in general, including cocaine, has been associated with behavioral and cognitive alterations, such as impaired decision making, negative social,

financial, psychological and physical consequences (Goldstein et al., 2007; Kalivas and Volkow, 2005; Porter et al., 2011). Recent studies have suggested that the chronic and acute use of cocaine is able to activate specific innate immune response components (Yamamoto et al., 2010), which include alterations in the cellular homeostasis of the liver, heart and brain, leading to cellular toxicity processes (Hanisch and Kettenmann, 2007; Narvaez et al., 2013; Streit, 2010). Regarding this cellular damage, several studies there demonstrated that cocaine promotes oxidative stress in these organs, increasing the production of reactive oxygen species (ROS; Hermida-Ameijeiras et al., 2004; Smythies and Galzigna, 1998). In the brain, the cocaine oxidative stress-induced inflammation stimulates the hypothalamus–pituitary–adrenal (HPA) axis, changing the levels of cytokines (Narvaez et al., 2013; Rivest, 2001).

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Cytokines have the function of stimulating cellular immune response and play a fundamental role in the control and modulation of local and systemic inflammatory responses, causing a constant balance between proinflammatory and anti-inflammatory cytokines (Bromander et al., 2012). Interleukin-6 (IL-6) is the main proinflammatory cytokine activated in the innate immune process, resulting in microglial activation (Porrino and Lyons, 2000). In turn, Interleukin-10 (IL-10) is known as a factor that inhibits the cytokine's synthesis, thus being one of the most important endogenous anti-inflammatory agents produced by the organism (Bromander et al., 2012; Fox et al., 2012).

Assessing different peripheral biomarkers in cocaine users, like inflammatory cytokines, can be relevant for a better understanding of the pathophysiological burdens associated with this drug use. There are few studies concerning the effects of cocaine use and the possible changes in the immune system in a population-based sample. Clinical and pre-clinical studies have shown that the use of cocaine incurs in the increase of proinflammatory cytokines and decrease of anti-inflammatory ones (Piechota et al., 2010). The aim of the present study is to evaluate the serum levels of proinflammatory cytokines, IL-6, and anti-inflammatory, IL-10, in cocaine users from a young population-based sample.

2. Methods

This is a case-control study nested in a population-based one, of people aged 18–35, involving 2348 participants living in the city of Pelotas, (Brasil), between June, 2011 and October, 2012. Sample selection was performed by clusters, considering the census division of the city (Pelotas) in 2010 (IBGE—*Instituto Brasileiro de Geografia e Estatística*; http://www.ibge.gov.br).

After identifying the subjects, they were invited to participate in the study and signed the informed consent form. This study was approved by the committee of ethics in research from the Catholic University of Pelotas (UCPEL), under Protocol number 15/2010.

Sociodemographic issues and psychoactive substance use data were collected through a self-administered questionnaire. To evaluate alcohol use disorder, the participants also answered the CAGE questionnaire (Buchsbaum et al., 1992). Socioeconomic evaluation was carried out using the IEN criteria (National Economic Index—*Índice Econômico Nacional*), which is based on the accumulation of material assets and on the schooling of the head of the household. These criteria generate a continuous variable which was presented in tertiles (Barros and Victora, 2005).

From the interview, all individuals who reported cocaine use were selected (n = 26 individuals). However, those individuals diagnosed with mood disorder, or who used alcohol concomitantly, were excluded from the sample (n = 14 individuals), totaling 12 individuals. From these, 24 other individuals who reported no use of licit and illicit substances and no psychiatric disorder were matched by sex and age, constituting a control group.

For the biochemical analysis, 10 mL of blood were withdrawn from each subject after the interview by means of venipuncture into an anticoagulant-free *vacuum* tube, between 8:00 and 11:00 am. The blood was immediately centrifuged at 4000 × g for 10 min, and serum was kept frozen at −80 °C until analysis. Serum IL-6 and IL-10 levels were measured using a commercial immunoassay kit (DuoSet ELISA Development, R&D Systems, Inc., USA). Serum IL-6 and IL-10 levels were expressed in pg/mL.

Statistical analysis was performed with the Statistical Program for Social Sciences (SPSS) 21.0 and *Graph Pad Prism* 6.0. Serum IL-6 and IL-10 levels had non-Gaussian distributions. For nonparametric data, we utilized the Mann–Whitney test, the Spearman correlation test, and the Kruskal–Wallis. Serum cytokines levels were presented as median and interquartile range. A linear regression analysis was applied to control for possible confounding factors with a p value ≤0.2 in the bivariate analysis. Results with p values ≤0.05 were considered statistically significant.

3. Results

Among the 36 analyzed subjects, 12 (33.3%) cocaine users and 8 (22.2%) cannabis sativa users were identified. Table 1 shows socio-demographic characteristics of the subjects according to cocaine use. Our results reveal no difference between control and the cocaine users groups for sociodemographic characteristics. However, among cocaine users, 8 (66.7%) subjects are cannabis users, and show a significant difference between the cocaine and control groups (p ≤ 0.001; Table 1). Table 2 shows the serum cytokines levels (IL-6, IL-10) and socio-demographic characteristics and cannabis sativa users, between cocaine users and controls groups.

There was a statistically significant difference between cocaine users and the control group IL-6 (p = 0.037) and IL-10 (p = 0.007) serum levels. IL-6 levels were higher in the cocaine users group with a median of 19.35 (13.37–34.57) pg/mL in relation to the control group with a median of 13.13 (12.05–15.88) pg/mL, while the levels of IL-10 were lower in the cocaine users group, where the median was 40.37 (33.56–57.50) pg/mL in relation to the control group with a median of 56.68 (48.09–92.23) pg/mL (Fig. 1).

In order to evaluate the influence of possible confounding factors interfering in the results, we performed an adjusted analysis according to gender, socio-economic index and cannabis use for serum cytokines levels and the results remained significant (IL-6: p = 0.039; IL-10: p = 0.045).

For verifying, the relationship between the pro-inflammatory cytokines (IL-6) with the anti-inflammatory cytokines (IL-10) was made as a ratio of these cytokines levels for each subject. There was a statistically significant difference between the two groups in IL-6/IL-10 (p = 0.013). The median ratios were 0.22 (0.15–0.31) in the control group and 0.53 (0.32–0.98) in cocaine user to IL-6/IL-10. After adjustment for potential confounders, both ratios remained statistically significant (IL-6/IL-10: p ≤ 0.001; Fig. 1).

Table 1
Socio-demographic and clinical characteristics of the sample according with cocaine use.

	Total sample	Controls	Cocaine users	p Value
Gender[a]				
Female	9 (25.0)	6 (25.0)	3 (25.0)	1.000
Male	27 (75.0)	18 (75.0)	9 (75.0)	
Ethnicity[a]				
Caucasian	27 (75.0)	19 (79.2)	8 (66.7)	0.414
No caucasian	9 (25.0)	5 (20.8)	4 (33.3)	
Age (years)[b]	25.83 ± 4.59	26.29 ± 4.52	24.92 ± 4.80	0.405
Years of study[b]	12.25 ± 3.04	12.92 ± 2.31	10.92 ± 3.92	0.302
Brazilian Economic Index[a]				0.325
1 (minor)	12 (33.3)	9 (37.5)	3 (25.0)	
2 (intermediate)	12 (33.3)	6 (25.0)	6 (50.0)	
3 (highest)	12 (33.3)	9 (37.5)	3 (25.0)	
Cannabis use[a,c]				
Yes	8 (22.2)	0 (0.0)	8 (66.7)	0.001
No	28 (77.8)	24 (100.0)	4 (33.3)	
Total	**36 (100.0)**	**24 (66.7%)**	**12 (33.3%)**	

[a] χ2 test, displayed as *n* (%).
[b] Student *t* test, displayed as mean ± standard deviation.
[c] Our controls are healthy and did not cannabis use.