

# Plasma midkine levels in patients with cocaine use disorder during abstinence

## *Niveles plasmáticos de midkina en pacientes con trastorno por uso de cocaína en abstinencia*

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### Abstract

Preclinical evidence suggests that endogenous midkine could play a key modulatory role on the neurotoxic and addictive effects of different kinds of drugs of abuse, including psychostimulants. However, this hypothesis has not yet been explored in humans. As a first approach to progress in this knowledge, we have comparatively studied plasma midkine levels in 75 patients with cocaine use disorder under abstinence and 26 control subjects matched for sex, age and body mass index. Patients were further segmented into early-abstinent (up to one month of abstinence,  $n = 30$ ) and late-abstinent (more than one month of abstinence,  $n = 45$ ). Midkine levels were quantified in plasma samples of all the participants by enzyme-linked immunosorbent assays. Early-abstinent patients exhibited a 60% increase of midkine plasma concentration in comparison with the controls. This elevation tended to normalize upon the progression of abstinence. The results obtained demonstrate that peripheral midkine levels are closely related to cocaine use and are consistent with the idea that this cytokine could play a protective role by limiting the biological activity of psychostimulants.

**Keywords:** Midkine; cocaine use disorder; cocaine abstinence; neuroprotection; psychostimulants.

### Resumen

Diversos estudios preclínicos han sugerido que la midkina endógena podría jugar un papel modulador clave sobre los efectos neurotóxicos y adictivos de distintas drogas, incluidos los psicoestimulantes. Esta hipótesis no ha sido aún explorada en humanos. Como primer paso en esta dirección, en el presente trabajo hemos medido los niveles plasmáticos de midkina en 75 pacientes con trastorno por uso de cocaína en abstinencia y 26 controles apareados con los anteriores por sexo, edad e índice de masa corporal. Los pacientes fueron además divididos en un grupo de abstinencia temprana (menos de un mes,  $n = 30$ ) y otro de abstinencia tardía (más de un mes,  $n = 45$ ). Se cuantificaron los niveles plasmáticos de midkina de todos los participantes mediante un ensayo por inmunoabsorción ligado a enzimas. Los pacientes en abstinencia temprana mostraron un incremento del 60% en su concentración plasmática de midkina con respecto a los controles que tendió a desaparecer en los pacientes con periodos de abstinencia más prolongados. Los resultados demuestran que los niveles periféricos de midkina están estrechamente relacionados con el uso de cocaína y apoyan la idea de que dicha citoquina podría jugar un papel protector limitando la actividad biológica de los psicoestimulantes.

**Palabras clave:** Midkina; trastorno por abuso de cocaína; abstinencia de cocaína; neuroprotección; psicoestimulantes.

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**M**idkine is a heparin-binding cytokine that promotes the survival and differentiation of different cell types and seems to play an important role in central nervous system development and repair after injury (Muramatsu, 2011). A growing amount of experimental data tend to show that endogenous midkine function could be critical to limit the neurotoxic and addictive properties of different drugs of abuse (Herradón & Pérez-García, 2014; Alguacil & Herradón, 2015). In the particular case of psychostimulants, it has been reported that midkine knockout mice exhibit enhanced amphetamine-induced astrogliosis in the striatum (Gramage, Martín, Ramanah, Pérez-García & Herradón, 2011) and are particularly resistant to extinguish cocaine-induced conditioned place preference (Gramage et al., 2013). Despite these interesting results, there is no data to our knowledge supporting a possible relationship between psychostimulant abuse and midkine function in humans. As a first step to increase this knowledge, we have compared plasma midkine levels between abstinent cocaine abusers and control subjects and have also studied possible correlations between plasma midkine levels and variables related to cocaine use such as years of drug consumption, severity of cocaine addiction and duration of abstinence.

## Method

This study was approved by the Ethical Committees of both the Hospital Regional Universitario de Málaga and Universidad San Pablo-CEU and fulfilled The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, as well as the General Data Protection Regulation of the European Union (2016/679). Informed consent was obtained from the subjects included. The study was conducted in white Caucasian population, and included a sample of patients diagnosed with Cocaine Use Disorder (CUD) currently in abstinence, as well as healthy control subjects. Patients were recruited at the addiction treatment facilities of the Centro Provincial de Drogodependencias (Málaga, Spain). Those subjects who fitted criteria of alcohol or cannabis dependence within the month before the study were excluded. Control participants were included from data bases of healthy subjects willing to participate in medical research projects at the Hospital Regional Universitario de Málaga (Málaga, Spain) and were matched to patients for sex, age and body mass index. CUD was evaluated according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition-Text Revision (DSM-IV-TR) (American Psychiatric Association, 2000) using the Spanish version of the Psychiatric Research Interview for Substance and Mental Diseases (PRISM) (Torrens, Serrano, Astals, Pérez-Domínguez & Martín-Santos, 2004). The group of CUD patients consisted of 75 subjects, 88% males,

with  $34.1 \pm 7.5$  years of age and  $\text{BMI} = 24.9 \pm 4.6 \text{ kg/m}^2$  (means  $\pm$  standard deviations). These subjects had been consuming cocaine for  $5.7 \pm 5.4$  years, reached a score of  $7.1 \pm 3.7$  in the cocaine trait severity scale for cocaine abuse and dependence (which reflects a heavy consumption) and had been abstinent from cocaine for  $13.7 \pm 32.2$  months; 32.4% of them had shown a problematic use of alcohol earlier in life (27% within the last year) and had been abstinent to alcohol a mean of 127.8 days before recruitment. The control group consisted of 26 subjects, 85% males, with  $36.8 \pm 10.1$  years of age and  $\text{BMI} = 24.6 \pm 2.8 \text{ kg/m}^2$ .

Blood samples from the participants were obtained by experienced nurses in the morning after fasting for 8–12 h. Venous blood was extracted into 10 ml EDTA tubes (BD, Franklin Lakes, NJ, USA), immediately centrifuged at  $2,200 \times g$  for 15 min ( $4^\circ\text{C}$ ) and individually assayed to detect infectious diseases by 3 commercial rapid tests for HIV, hepatitis B, and hepatitis C (Strasbourg, Cedex, France). Plasma samples were individually characterized, registered, and stored at  $-80^\circ\text{C}$  until the day of analysis, which was performed by using a sandwich ELISA Midkine kit (MKELISA, Cellmid, Sydney, Australia) according to the instructions of the manufacturer (which include the use of duplicates). This particular kit was selected because it was specifically developed for human samples, provides a high sensitivity (limit of detection =  $8 \text{ pg/ml}$ ), high specificity (0 reactivity to pleiotrophin, a cytokine closely related to midkine) and reliable quantification of the analyte at concentrations up to  $10 \text{ }\mu\text{g/ml}$ .

According to the literature showing that the most prominent phenomenological and neurobiological features of cocaine abstinence happen within the first weeks of cocaine withdrawal (Pathiraja, Marazziti, Cassano, Diamond & Borison, 1995), we split the patients for statistical analysis into an early-abstinent group (up to one month of abstinence,  $n = 30$ ) and a late-abstinent group (more than one month of abstinence,  $n = 45$ ). Midkine levels were comparatively studied in these two groups and control subjects by using one-way ANOVA followed by Bonferroni post-hoc tests. The possible correlations between midkine levels and each of the three variables related to cocaine use (severity score, duration of consumption and length of abstinence) were first studied by using Pearson coefficients. Besides, we also investigated these correlations after segmentation of the former variables into 5 groups according to percentile criteria by applying Spearman coefficients. The level of statistical significance was always established at  $p < 0.05$ .

## Results

CUD patients under early abstinence exhibited a significant, 60% increase of plasma midkine levels with respect to control subjects; this difference was later reduced

and did not achieve statistical significance when abstinence exceeded one month, as shown in the late abstinent group (Figure 1).

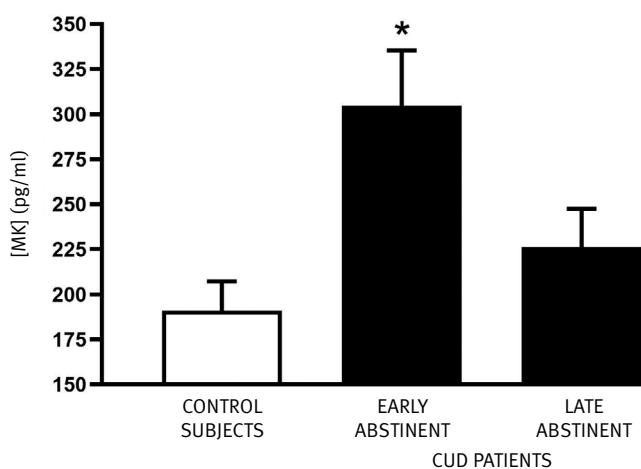


Figure 1. Midkine concentration in the plasma of control subjects and CUD patients. \*  $p < 0.05$  vs control subjects.

Midkine concentration did not correlate with the duration of lifetime cocaine use or the severity of cocaine addiction, but interestingly it was found to be inversely related to the time elapsed from cocaine withdrawal (Table 1, Figure 2).

## Discussion

The results obtained in this study provide the first evidence of a significant relationship between cocaine use

and midkine regulation in humans. Bearing in mind that our patients were abstinent to cocaine in the moment of the collection of the samples, it remains to be established if the elevation of plasmatic midkine was a consequence of the previous use of cocaine or was triggered by cocaine withdrawal; in any case, the levels of the cytokine consistently came back to control values upon the progression of abstinence, hence they seemed to be inversely parallel with cocaine dependence. Up to our knowledge the correlation between central and peripheral midkine levels has been poorly addressed both in health and disease; in spite of this, blood midkine changes have been associated to several neuropsychiatric conditions such as schizophrenia (Shimizu et al., 2003), Alzheimer disease (Salama et al., 2005) or autism (Esnafoglu & Cirrik, 2018), thus suggesting that plasma midkine levels could be sensitive to pathological changes affecting midkine levels or function in the brain. According to this idea, it is possible that our finding of elevated plasma midkine in patients could be secondary to an upregulation of central midkine triggered by cocaine use and/or cocaine withdrawal. Such an effect would be consistent with preclinical data suggesting a neuroprotective role of midkine upregulation in situations involving brain tissue injury, which include exposition to drugs, ischemia and neurodegenerative alterations (Muramatsu, 2011; Herradón & Pérez García, 2014; Alguacil & Herradón, 2015). Obviously, this hypothesis needs further testing since elevations of midkine levels in the periphery could also reflect other alterations related to cocaine use, not necessarily of central origin. Thus, for instance, vascular endothelial cells are known to release

Table 1. Analysis of correlations between midkine levels and variables associated to cocaine abuse.

	UNGROUPED DATA ANALYSIS		SEGMENTED DATA ANALYSIS	
	Correlation Coefficient	p-value	Correlation Coefficient	p-value
CUD severity (DSM score)	-0.061	0.601	-0.072	0.538
Duration of consumption (years)	-0.117	0.318	-0.045	0.699
Length of abstinence (months)	-0.140	0.231	<b>-0.253</b>	<b>0.029</b>

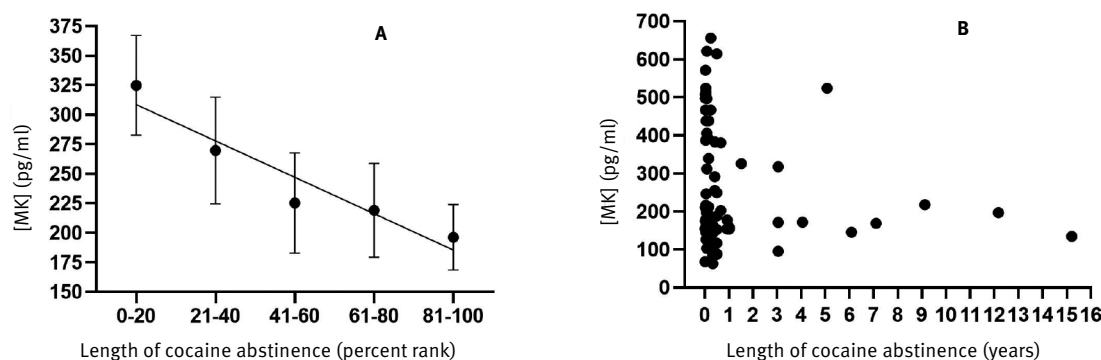


Figure 2. Midkine concentration in the plasma of abstinent CUD patients. Panel A shows the linear regression obtained ( $r^2 = 0.91$ ) after segmenting the length of cocaine abstinence into 5 percentile groups (points represent means  $\pm$  SEM). Panel B shows ungrouped data.

midkine (Fujisawa et al., 1998) and this could be affected by the potent cardiovascular actions of cocaine. Besides, some other conditions with higher incidence among drug addicts could also contribute to an elevation of blood midkine in these subjects, i.e. chronic kidney disease (Campbell et al., 2017), malignancies (Jones, 2014) or immune disorders (Sorrelle, Dominguez & Brekken, 2017). One limitation of this study is the impossibility to rule out any influence of alcohol or tobacco use on the observed changes of plasma midkine levels. Although our patients were not abusing alcohol when the samples were collected, a mild to moderate use cannot be discarded and this may affect midkine expression in the brain (Flatscher-Bader & Wilce, 2008). Smoking should be specifically monitored in future studies, since it has been also shown to increase midkine serum levels in some previous works (Ito et al., 2019), but not in others (but not in others: see Salaru et al., 2014). Accordingly, further work is needed to confirm the present results and provide a better understanding of the precise involvement of midkine function in cocaine use disorder; in this way, cerebrospinal fluid and plasma correlation studies appear to be especially relevant.

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### Conflict of interests

The authors declare they have no conflict of interest.

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