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The cocaine cutting agent levamisole is frequently detected in cocaine users

JEFFREY D. POPE[1,2], OLAF H. DRUMMER[2], HANS G. SCHNEIDER[1,3]

[1]*Clinical Biochemistry, Alfred Pathology Service, Alfred Health, Melbourne, Vic, Australia;* [2]*Department of Forensic Medicine, Monash University, Melbourne, Vic, Australia;* [3]*Central Clinical School, Monash University, Melbourne, Vic, Australia*

Summary

Cocaine use in Australia is increasing, with approximately 2.5% of the surveyed population having used cocaine. In the USA, levamisole, a widely used anti-helminthic veterinary drug has been increasingly detected as a cutting agent in cocaine seizures. Levamisole is known to cause agranulocytosis in humans. We ascertained the prevalence of levamisole-adulterated cocaine, detectable in the urine from patients that had undergone a pathology request for a urine drug screen.

We assayed routinely requested urines that were positive for cocaine on immunoassay with liquid chromatography high resolution quadrupole time of flight mass spectrometry (LC-QToF). We investigated available urine samples from a period of 2 years that had a positive result for cocaine. In addition, we examined samples that were below the cut-off for cocaine on immunoassay. Specimens were analysed for the presence of levamisole and other 'unknown' drugs.

In the period under investigation the laboratory examined 3665 urine samples for cocaine: 1.4% (n = 51) of the samples were positive for cocaine by immunoassay and half of these (n = 26, 51%) were further examined by LC-QToF. In addition, we examined 10 samples that were negative by immunoassay (as defined by AS/NZS 4308:2008). Levamisole was detected in the urine of cocaine users in approximately 75% of cases. Other illicit drugs were also frequently found in this cohort. The most common illicit drugs detected were methamphetamine, ecstasy and cannabis.

Australian cocaine is widely adulterated with levamisole. Cocaine users are at risk of levamisole related health problems in addition to the problems related to cocaine.

Key words: Levamisole; cocaine; urine drug screen; toxicology; high resolution mass spectrometry.

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INTRODUCTION

Cocaine is a powerful central nervous stimulant and a widely abused illicit drug. Cocaine is mostly produced in South America and trafficked internationally. It is the second (after cannabis) most widely abused illicit drug globally.[1] In Australia, cocaine use has doubled over the past decade. It is estimated that 2.5% of the surveyed population (>14 years old) has used cocaine in the past year (compared with about 1% in 2004, 2.1% in 2010 and 2013, and 2.5% in 2016).[1] The use of cocaine has been increasing since 2004 and is currently at the highest levels yet seen.[2] The United Nations Office on Drugs and Crime (UNODC) World Drug Report 2016 report put cocaine use at about 2.5% of the Australian population.[2]

In 2003, the USA's Drug Enforcement Agency (DEA) first reported the presence of levamisole in a cocaine-containing drug seizure. In 2010 the DEA Intelligence Production Unit reported that 73% of cocaine-containing drug seizures contained levamisole.[3] Cutting agents are used for economic reasons, but also to enhance or mimic the target substance and to aid in the administration of the drug.[4,5] It is reported that incorporating levamisole into cocaine might make the cocaine crystals more visually pleasing and augment its effects. It is known that levamisole is metabolised to aminorex and some have postulated that this compound acts as an amphetamine-like stimulant.[6] Aminorex was an over the counter anorectic, widely consumed in German speaking countries. It was withdrawn from the market in 1972 after it was linked to a high incidence of primary pulmonary hypertension.[7] There have also been case reports in the literature that have associated aminorex poisoning in cocaine users.[8,9]

Currently levamisole is still used in veterinary medicine as an anti-helminthic drug. It is cheap and widely available. It was once used in humans as an immunomodulator in rheumatoid arthritis and a chemotherapy adjuvant in colorectal cancer treatment, however due to the risk of agranulocytosis and neutropenia it was withdrawn.[10]

Coincidently, the first few cases of unexplained infections in cocaine users occurred around the same time as levamisole was incorporated into cocaine. Since then a number of medical conditions have been linked to levamisole-adulterated cocaine including vasculitis, glomerulonephritis, haemorrhagic skin necrosis, neutropenia and agranulocytosis.[11] More recently, there have been several published fatal case reports likely attributable to levamisole.[12,13]

Confirmation of cocaine positive immunoassay screens is not a requirement for clinical samples and not all pathology laboratories will utilise mass spectrometry methods. We are unaware of any pathology laboratories in Australia that currently test for levamisole.

METHODS

Urine samples with clinical requests for urinary drug screens were tested in the routine laboratory using a CEDIA cocaine metabolites immunoassay on an

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Abbott Architect ci16000 (Abbott Australasia, Australia) according to the manufacturer's instructions. A positive cut-off level of 300 µg/L was employed, consistent with the Australian standard ASNZ4308:2008. Selected urine samples were further tested to confirm the presumptive immunoassay screen results. Cocaine and metabolites benzoylecgonine, ecgonine methyl ester, cocaethylene, levamisole and other unknown drugs were analysed by a validated liquid chromatography-quadrupole time of flight mass spectrometry (LC-QToF) urine drug screen method. Chromatographic separation was achieved using an iClass Acquity Ultra High-Performance Liquid Chromatography (UHPLC) coupled to a Xevo G2-XS Quadrupole Time of Flight (QToF) mass spectrometer (Waters Corporation, USA). The QToF was operated in positive and negative electrospray ionisation (ESI) modes. Mass correction was performed with leucine enkephalin at 20 second intervals. Detailed UHPLC and QToF method details have been published elsewhere.[14,15] The toxicology library used contains over 1300 drugs and metabolites. Strict positive criteria, typical for high resolution mass spectrometry methods, were followed to determine a positive finding. Drugs needed to have an accurate mass (<5 ppm), retention time (RT) match to a commercial standard and the presence of at least one high energy fragment at the same RT as the precursor mass. Levamisole, cocaine and metabolite detection data are outlined in Table 1.

Sample selection

Over the period of approximately two years, 26 urine samples were selected by the first author where samples were available. These samples were originally sent to the hospital laboratory for a urinary drug screen (UDS) and had tested positive for the cocaine immunoassay screen above the 300 µg/L cut-off. In addition, 10 samples which were positive by immunoassay but fell under the ASNZ4308:2008 cut-off were also analysed. Figure 1 outlines the sample selection process. All samples received were from the greater Melbourne area. This study was approved by the Alfred Heath Ethics committee (Alfred Health HREC 35/17).

RESULTS

Between the period of 15 June 2014 to 19 September 2016, 3665 urine specimens were tested for cocaine by immunoassay screen. Of these, 51 samples (1.3%) tested positive to the immunoassay screen above 300 µg/L. Twenty-six of these samples (51%) were available to the authors for further LC-QToF analysis. An additional ten samples that were positive in the immunoassay screen but fell below the cut-off (immunoassay levels were between 17 and 224 µg/L) were also analysed.

Twenty-three of the tested urine specimens were obtained from male patients (age 19–54 years, median 38), and 13 from women (age 16–45 years, median 26). The majority of requests for urine drug screens in these patients came from the emergency department (77%), followed by requests from psychiatry and outside referrals.

Levamisole was present in 27 of the 36 samples tested (75%). Levamisole was present in 7 of 10 samples with negative immunoassay (70%) and in 20 of 26 samples (77%) with positive immunoassay for cocaine.

In addition, we detected a number of other illegal drugs in the urine specimens of these patients. The most common illicit drugs detected were either methamphetamine or MDMA (ecstasy) in 23 patient samples (both were detected in 9), cannabis metabolites (carboxy-THC detected in 12 samples) and heroin or metabolite (as 6-acetylmorphine) in two samples. One patient also tested positive to the cathinone ethylone. Four patients were receiving pharmacotherapy for opiate dependency and several patients tested positive for psychiatric drugs. Patient demographics and urine drug screen findings are summarised in Table 2.

We reviewed the patients' medical records and searched for known side effects of levamisole as described in the literature. In one patient we found an unexplained low neutrophil count (29-year-old female, neutrophils 1.5×10^9/L) at the time of emergency department review (reference interval 1.9–8.0). At an attendance some months later, the same patient showed a normal neutrophil count. The skin necrosis described in the literature was not observed in any of the 36 patients.

DISCUSSION

Over the 2 year study period the frequency of positive cocaine samples at our hospital was 1.3%, slightly lower than the estimate of cocaine users according to data from UNODC[2] and the Australian Institute of Health and Welfare (AIHW)[1] reports. All 26 cocaine screen positive samples were also positive for cocaine by the LC-QToF method confirming the immunoassay screen results. A further ten samples that were suspicious for the presence of cocaine, but were below the AS/NZS 4308:2008 cut-off, were also confirmed positive by the LC-QToF method. The application of the cut-off as described in the standard will lower frequency of cocaine containing urine samples in our population.

We detected levamisole in 75% of the cocaine positive samples. Even in the ten samples that were below the cocaine cut-off, 70% were positive for levamisole. The absence of levamisole in the remaining 25% is likely due to the preparation or 'cutting' of cocaine or levamisole's relatively short half-life of 4 hours. Since the routine laboratory use of the LC-QToF for urine drug screens (March 2017), we have never detected levamisole in urine specimens that were negative for cocaine by LC-QToF. This is consistent with the source being the cocaine preparations.

In 2014, a French Hospital showed levamisole was widely detected in the urine of cocaine users.[16] Our study confirms that levamisole can be readily detected in the urine of cocaine users, and thus is the first evidence that levamisole is

Table 1 LC-QToF detection requirements for cocaine, cocaine metabolites and levamisole

Drug/metabolite	Retention time (min)	Precursor mass[a] $[M+H]^+$	High energy fragments[b] $[M+H]^+$
Cocaine	4.41	304.1543	182.1176, 82.0652, 105.0335, 150.0914
Benzoylecgonine	2.84	290.1387	168.1020, 105.0335, 82.0652
Ecgonine methyl ester	0.88	200.1281	182.1176, 82.0652, 150.0914
Cocaethylene	5.45	318.1700	196.1333, 82.0652, 150.0914, 168.1020
Levamisole	2.09	205.0794	178.0685, 144.0808

[a] Measured in MS function 1 at 6 eV.
[b] Measured in MS function 2 with collision energy, 10–40 eV ramp.

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