Detection of Levamisole Exposure in Cocaine Users by Liquid Chromatography–Tandem Mass Spectrometry

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Abstract
Levamisole, a veterinary antihelminthic, was recently recognized as an adulterant in cocaine and is known to cause severe adverse reactions in some cocaine users. Because of the health concerns involving levamisole-adulterated cocaine, we developed a liquid chromatography–tandem mass spectrometry (LC–MS–MS) method for the detection of levamisole in urine. This method was used to determine the prevalence of levamisole in cocaine-positive patient samples. All cocaine-positive urine samples that were sent to the San Francisco General Hospital Clinical Laboratory were tested for levamisole for one month. For LC, an Agilent 1200 series was used with a C18 column and a gradient of mobile phase A (0.05% formic acid) and B (acetonitrile/methanol). Detection was carried out with an Applied Biosystems QTRAP® LC–MS–MS. The levamisole LC–MS–MS method was linear over the range of 5–2500 ng/mL (r > 0.996). Interassay and intraassay CVs were < 6%. The lower limit of detection for levamisole was 0.5 ng/mL. Out of 949 total urine drug screens, 20% were positive for benzoylecgonine, and of those, 88% were positive for levamisole. The high prevalence of levamisole-adulterated cocaine and potential toxicity in cocaine users is a serious public health concern. These findings validate the utility of an LC–MS–MS method for the detection of levamisole.

Introduction
Cocaine is frequently diluted throughout the chain of distribution with myriad less expensive cutting agents and adulterants. These substances are mainly used to increase the weight of the product, resulting in a higher profit. Some adulterants are added in attempts to potentiate or mimic the effects of cocaine or, in some cases, attenuate the deleterious side effects associated with cocaine use. Since 2005, there have been multiple reports of the presence of levamisole, a veterinary antihelminthic, in seized cocaine (1–3). Levamisole was previously used in humans as adjuvant therapy in the treatment of colorectal cancer and nephrotic syndrome. It was also used in the treatment of rheumatoid arthritis because of its immunomodulatory effects. It is no longer available for human use in North America because of its potential serious adverse side effects. There has been a series of case reports describing adverse side effects associated with levamisole-adulterated cocaine over the past year (4–9). These side effects include, but are not limited to, agranulocytosis, ANCA-associated vasculitis, and retiform purpura. Cases often present with symptoms and laboratory results that resemble an autoimmune disorder.

According to the United States Drug Enforcement Administration (DEA) and state testing laboratories, the percentage of cocaine specimens containing levamisole has increased steadily over the past eight years. Levamisole was identified in 30% of the cocaine seized by the DEA from July to September 2008. In July 2009, that number increased to 70% (6). The Wayne County Medical Examiner’s Office has reported that, in 2008, levamisole was present in 36.6% of all samples that contained cocaine and/or its metabolites; however, no information about the analytical method was provided (10). To date, there are no published liquid chromatography–mass spectrometry (LC–MS) methods specific for the detection of levamisole in biological samples, and the prevalence of levamisole in cocaine-positive samples has not been reported since 2008. Because of the potential public health concern related to levamisole-adulterated cocaine, we developed an LC–MS–MS assay for screening cocaine-positive urine samples. This method was then used to determine the prevalence of levamisole in cocaine-positive patient samples in San Francisco.

Materials and Methods

All cocaine-positive urine samples that were sent to the San Francisco General Hospital Clinical Laboratory for drug screening in October 2009 were tested for levamisole. Assay results were not revealed to patients or health care providers. This work was approved by the University of California San Francisco Institutional Review Board, which determined that...
Cocaine screening was performed using the CEDIA® Cocaine Assay (Microgenics, Fremont, CA). The assay was performed on a Siemens Advia® 1800 Chemistry System (Deerfield, IL) following manufacturer’s instructions with a cutoff value of 300 ng/mL. Confirmatory testing was conducted using an LC–MS–MS method. For levamisole testing, urine was diluted 1:10 with 0.05% formic acid and spiked with aminorex, the internal standard. All organic solvents and reagents were of analytical grade and were purchased from Fisher Scientific (Fair Lawn, NJ). The levamisole standard was purchased from Sigma-Aldrich (St. Louis, MO), and aminorex was obtained from Cerilliant (Round Rock, TX). For LC, an Agilent 1200 series was used with a Phenomenex (Torrance, CA) Kinetex™ C18 2.6-µm (50 × 2.1 mm) column, maintained at 25°C and a gradient of mobile phase A (0.05% formic acid) and mobile phase B (acetonitrile/methanol, 50:50, v/v). The flow rate was 400 µL/min, and the LC program was 0–0.5 min, 10% B; 0.5–1.5 min, 10% to 40% B; 1.5–1.75 min, 40%; 1.75–2.25 min, 40% to 10% B; 2.25–5 min, re-equilibration with 10% B. Levamisole and the internal standard eluted at 1.1 and 1.0 min, respectively (Figure 1A).

Detection was carried out with an Applied Biosystems QTRAP LC–MS–MS system equipped with a TurboIon Spray™ ionization source, controlled by Analyst 1.5 software (Life Technologies/Applied Biosystems, Foster City, CA). The ion transitions used were 205.2/178.1 (levamisole) and 163.1/120.1 (internal standard). Positive ionization was performed, and the following parameters were used: ion spray voltage, 5000 V; curtain gas, 15 psi; ion source gas 1, 50 psi; ion source gas 2, 50 psi; CAD gas, low; and temperature, 600°C. The compound dependent parameters for levamisole were as follows: declustering potential 55 V, entrance potential 7 V, and collision energy 33 V. The data acquisition was performed using an information-dependent acquisition (IDA) method. The precursor/product ion transitions were first monitored by a selected reaction monitoring survey scan. This was followed by the generation of mass spectra by way of a product ion scan in Q3, functioning as a linear ion trap, when IDA criteria were met (detection of a peak > 1000 cps). With every batch of samples, a blank (drug-free urine), two quality control samples (20 and 200 ng/mL), and a calibration curve from 5 to 1000 ng/mL were run. In addition to identification of a peak at the correct retention time, a library search of the acquired product ion spectra was performed and a match factor (purity) > 80% between the unknown and the levamisole library product ion spectra was required to report levamisole as positive. Figure 1B shows a representative product ion spectrum for levamisole.

Results and Discussion

The levamisole assay was linear from 5 to 2500 ng/mL ($r > 0.996$). The interassay imprecision was < 3% ($n = 10$) and the intraassay imprecision was < 6% ($n = 20$) (controls at 20 and 200 ng/mL). The accuracy and recovery of controls at 20 and 200 ng/mL were > 95.8% based on the weigh-in value. Carry-over was not detected at 30,000 ng/mL. Matrix effect experiments were conducted as described previously (11). The ion suppression was 19.7% ($n = 20$) for levamisole and was compensated for by use of an internal standard. The limit of detection, defined as the concentration that provides a signal-to-noise ratio of 3, was 0.5 ng/mL.

There were a total of 949 urine drug screens ordered through the San Francisco General Hospital Clinical Laboratory during the levamisole screen. The cocaine immunoassay, which targets benzoylecgonine, was used to establish if these patients were positive for cocaine. It was determined that 191 (20%) of all the urine drug screens were cocaine positive. These results were confirmed by LC–MS–MS. Levamisole was detected in 169 (88%) of the cocaine-positive samples. The levamisole concentrations in these samples ranged from 5 to 32,720 ng/mL, with a mean and standard deviation of 1887 ± 4238 ng/mL. The corresponding benzoylecgonine concentrations ranged from 4 to 588,000 ng/mL, with a mean and standard deviation of 44,451 ± 76,100 ng/mL. There were no statistically significant differences ($p < 0.05$) in the prevalence of levamisole by any of the demographic or drug use variables (Table I), including sex, race/ethnicity, age, or drug use.

This study identified that the prevalence of levamisole in cocaine-positive patient samples at San Francisco General Hospital was 88%. This suggests that the majority of cocaine used in San Francisco is adulterated with levamisole. The half-life of levamisole is on average 5.6 h and approximately 2–5%
is excreted unchanged in the urine (12,13). It is possible that the prevalence of levamisole-adulterated cocaine is greater than 88%. A percentage of the urine samples could have been collected past the window of detection for levamisole, while still being within the window of detection for benzoylcegonine. Further studies are required to determine the rate of levamisole elimination in the urine. Some of the metabolites of levamisole are not available for purchase commercially, making it difficult to develop targeted analytical methods for the detection of these compounds in addition to levamisole. One limitation of this study is that levamisole is the l-isomer of tetramisole, and the method described here does not distinguish between the l- and d-isomers of tetramisole. Therefore, this study cannot rule out the possibility that the d-isomer of tetramisole is also present in the patient samples. Another limitation is that the sampling in this study was not conducted at random; therefore, it is not possible to generalize these results to the whole population of cocaine users in San Francisco.

This study suggests that clinicians should consider levamisole-adulterated cocaine in users with unexplained atypical symptoms, such as idiopathic agranulocytosis. Further studies are required to determine why only a subset of cocaine users exposed to levamisole develops adverse reactions that mimic an autoimmune disorder. Some patients may be genetically predisposed to developing these symptoms. Previous studies suggest that levamisole-induced agranulocytosis may be associated with the HLA-B27 genotype (7,14,15).

This is the first published study to date that determines the prevalence and concentration of levamisole in cocaine-positive patient samples in a hospital setting. These findings validate the utility of an LC–MS–MS method for the detection of levamisole. Given the high prevalence and underappreciated risks associated with exposure to levamisole, this is a serious public health concern in geographical regions with a high incidence of cocaine use, such as San Francisco.

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References


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