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Levamisole-Contaminated Cocaine Use in HIV-Infected and Uninfected Unstably Housed Women

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Abstract

A growing number of case reports cite serious health complications linked to the cocaine adulterant, levamisole and women are disproportionately affected; however, the clinical effects are not well established. Between April and October of 2010, we conducted a cross-sectional study among 222 homeless and unstably housed women (116 human immunodeficiency virus [HIV]-infected and 106 HIV-uninfected). Immune markers and behavioral factors were compared in separate models by cocaine and levamisole exposure. Overall, 63% of participants were toxicology positive for cocaine/benzoylecgonine, 85% of whom also tested positive for levamisole. Differences in immune markers did not reach levels of significance among HIV-uninfected persons. Compared to HIV-infected persons who were negative for both cocaine and levamisole, the adjusted odds of low white blood cell count were significantly higher among HIV-infected persons positive for both ($p = 0.03$), but not for those positive for cocaine only. Neutrophil count and HIV viral load did not differ by cocaine and levamisole status among HIV-infected persons. In a separate model, the adjusted odds of testing positive for levamisole were higher among African American women compared to Caucasian and Asian women ($p = 0.02$). In the context of high levamisole prevalence, results suggest that decreased immune function as a result of levamisole exposure occurs mainly in individuals who are already immune compromised (*e.g.*, HIV-positive), and race/ethnicity appears to be an important factor in understanding levamisole exposure among cocaine-using women. While larger and geographically diverse studies are needed to elucidate these initial findings, results suggest that levamisole may be one mechanism of immune dysfunction in HIV-infected cocaine-using women.

Introduction

In recent years, there has been a growing number of international reports about the increased presence of levamisole in cocaine obtained during law enforcement drug seizures, and biological samples from cocaine users.[1–4] Used as an immunomodulator for specific conditions in humans, and a veterinary antihelminthic, it has been speculated that one reason for contaminating cocaine with levamisole is to potentiate the effects of cocaine through increased peripheral sympathetic activity and increased central neurotransmission.[5,6]

Case reports and laboratory studies have established that levamisole produces a number of potential harmful effects in humans. Harmful effects include agranulocytosis, necrotizing vasculitis, fixed drug eruptions and pruritic rashes,[7–13] and levamisole-toxicity cases are disproportionately described among women.[14–16] Accurately identifying levamisole-induced health complications is essential for discontinuing exposure, and preventing unnecessary use of other treatments with potentially strong side effects such as immunosuppressive therapy.[17] While the number of case reports appearing in the medical literature has increased, the population-level clinical and socioeconomic correlates of levamisole-contaminated cocaine use are not well established.

In 2010, an unprecedented number of low-income women in San Francisco presented to the county hospital for emergency care with neutropenia and extensive cutaneous necrosis linked to cocaine use.[16] In response, Lynch et al. developed mass spectrometry-based methods for the detection of levamisole in urine[4] to begin investigating the link between cutaneous skin necrosis and levamisole-induced toxicity.[16] The current study leveraged these mass spectrometry methods, and an existing community-based study conducted during the same time period, to assess associations between the presence of levamisole and inflammation, immune and social factors. The objective was to better understand exposures and potential subclinical

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outcomes outside of extreme acute health consequences like necrotizing vasculitis in a population identified as being at disproportionately high risk.

Materials and Methods

Design and sample

Between April and October of 2010, a time when low-income San Francisco women were presenting to the county hospital for emergency care due to extensive cutaneous necrosis, a cohort study of women who reflected the patient profile was already in existence. The analysis presented here represents a cross-sectional sub-study within the ''Shelter, Health and Drug Outcomes among Women'' (SHADOW) study. SHADOW considered the impact of multiple exposures, emphasizing human immunodeficiency virus (HIV) and cocaine, on the health and well-being of homeless and unstably housed women living in San Francisco.[18]

Sampling methods for SHADOW were based on those developed by Burnam and Koegel[19] to recruit a sample population of homeless women that accurately reflected the larger population of San Francisco homeless women. This approach minimized the possibility of a biased sample such as one consisting of a particular or extreme subset of individuals recruited by chance.[19] As described previously, recruitment was accomplished by a mobile outreach team, which approached women at all free meal programs serving over 100 meals per day, all homeless shelters housing women, and low-cost single room occupancy hotels selected with probability proportionate to the number of female residents.[18]

Inclusion criteria were female sex (biological), age >18 and a history of housing instability (slept in a public place, a shelter, or stayed with a series of other people because there was nowhere else to sleep [''couch-surfed'']). HIV testing was conducted on site and HIV-infected women were oversampled on additional recruitment days to ensure statistical power for HIV-based analyses. Participants were reimbursed $20 for each completed interview and urine specimen collection. All study procedures were approved by the Institutional Review Board at the University of California, San Francisco.

This study maintains a focus on women because women are disproportionately represented among individuals presenting for care due to levamisole-contaminated cocaine use.[14–16] Furthermore, women have higher rates of stimulant dependence, more severe forms of stimulant addiction and lower rates of treatment than men,[20–23] which may result in differential levamisole exposure. In addition, sex differences in both the pharmacokinetics of HIV medication[24] and the natural course of HIV disease[25] have been identified.

Data collection

We obtained serum samples among 222 individuals to test for malnutrition and inflammation, which was indicated by low levels of prealbumin (Reference range 17–34 mg/dL; Quest Diagnostics, Sacramento, CA), and compromised immune function, indicated by low neutrophil count (Reference Range: 1500–7800 Cells μL: Quest Diagnostics) and total white blood cell count (Reference Range: $3.8–10.8 \times 10^3/\mu$L; Quest Diagnostics).

We also obtained urine specimens to test for the presence of levamisole, cocaine, and benzoylecgonine, the cocaine metabolite, using a qualitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Individuals who were positive for cocaine and/or benzoylecgonine were considered cocaine positive. Data acquisition and generation of mass spectra took place using an AB Sciex 3200QTRAP® LC-MS/MS system. This targeted method monitored the presence of levamisole, cocaine, and benzoylecgonine using ion transitions specific to each compound. Mass spectra were acquired when ion transitions were present, and then searched against a mass spectral library for positive identification of levamisole, cocaine, and benzoylecgonine. The methodology has proven sensitive and specific for the detection of these compounds in urine.[4]

Interviewer-administered questionnaires regarding behaviors during the prior 6 months accompanied specimen collection. Interview topics included the following: socioeconomic factors (age, race/ethnicity, income, high school diploma), incarceration (any jail or prison time); literal homelessness (proportion of time spent sleeping in a homeless shelter and proportion of time spent sleeping in a public place); at-risk alcohol use (>1 drink/day for women[26] and binge drinking, defined as >5 drinks at any time); and self-reported use of cocaine, heroin, and methamphetamine. HIV-infected individuals also completed questions regarding self-reported use of antiretroviral therapy. CD4 cell count and viral load measures were obtained through electronic medical records available from the San Francisco Department of Public Health's Community Health Network (77% of HIV-infected persons had available data).

Data analysis

To better understand associations between levamisole-contaminated cocaine use and biomarkers of interest, we compared mean and clinically low biomarker levels (low prealbumin defined as <17 mg/dL; low neutrophil count defined as <1500 Cells/μL; low white blood cell count defined as $<3.8 \times 10^3/\mu$L; low lymphocyte count defined as $<1.0 \times 10^3/\mu$L; among HIV-infected persons, low viral load was defined as <50 copies/mL [undetectable]) by cocaine and levamisole status. Cocaine and levamisole categories included (1) persons who were toxicology negative for both cocaine and levamisole (–/–), (2) those who were toxicology positive for cocaine only (+/–), and (3) those who were toxicology positive for both cocaine and levamisole (+/+). No one was cocaine negative and levamisole positive. In this way, potential effects of levamisole were more clearly delineated from cocaine effects. Due to immune deficiencies inherent in HIV-infection, HIV-infected and uninfected persons were evaluated separately.

Based on prior research suggesting the important role of white blood cell count in negative health outcomes linked to levamisole among women, particularly neutrophil count,[15,16] we assessed unadjusted and adjusted odds between clinically low white blood cell count ($<3.8 \times 10^3/\mu$L) and white blood cell differentials (lymphocyte count and neutrophil count) by cocaine/levamisole status.

To better understand potential patterns of levamisole exposure in cocaine users, we considered associations between the presence of levamisole and social factors in the sub-group of individuals who were toxicology positive for cocaine. In this way, estimates of relative odds were restricted to persons who had opportunity to experience the outcome. Social factors were not considered in biomarker comparisons due to dissimilar types of data and over adjustment for factors

within the causal pathway, which could not be addressed in this cross sectional analysis.

All analyses were performed using SAS Version 9.2 (SAS Institute, Cary, NC). Comparisons of means between the three groups (+/+, +/– and –/– for cocaine and levamisole respectively) were conducted in pairs and in the group as a whole using the Kruskal–Wallis test for continuous variables. Pairwise comparisons of binary data and in the group as a whole were made using Fisher's Exact Chi-square test. Odds ratios (OR) resulted from logistic regression using Firth's penalized likelihood method to account for separation due to small and zero cell sizes.

Multivariable logistic regression was used to simultaneously consider lymphocyte count and neutrophil count as components of white blood cell count. Given that few studies have been conducted comparing levamisole exposed to similar but unexposed persons, this area of research is still in a preliminary stage of investigation. To gain a more flexible preliminary understanding of cocaine and levamisole influences, we used an alpha of 0.10. Additionally, in consideration of prior research regarding the inflation of Type II error in small and exploratory studies,[27,28] we did not adjust for multiple comparisons.

Results

Sample characteristics

The study sample included 222 biological women (116 HIV-infected and 106 HIV-uninfected) with complete interview data and lab results. Differences between participants for whom data were available and those for whom data were incomplete ($n = 24$, 10.8%) did not reach levels of statistical significance regarding age, race, unmet subsistence needs, or cocaine use (all $p > 0.30$). Seventy-three percent of participants were of non-Caucasian race/ethnicity, the median age was 48 years and 48% self-reported the use of crack or powdered cocaine (Table 1).

Urine toxicology results indicated that 63% of participants had recently used cocaine. Among participants who were toxicology positive for cocaine, 86% were also positive for levamisole. Among participants who were toxicology negative for cocaine, none tested positive for levamisole.

Inflammation and immune comparisons

Among HIV-negative persons, differences in inflammation and immune markers by cocaine and levamisole status did not reach levels of statistical significance (Table 2). Among HIV-infected persons, inflammation and immune function were significantly worse among persons testing positive for both cocaine and levamisole (+/+) compared to those testing positive for cocaine only (+/–) or negative for both cocaine and levamisole (–/–) (Table 2). Specifically, a higher proportion of +/+ persons had lower prealbumin ($p < 0.05$), white blood cell count ($p < 0.05$), and CD4 cell count ($p = 0.07$).

In analysis restricted to HIV-infected participants, the unadjusted odds of low white blood cell count were significantly higher among +/+ persons compared to –/– persons (OR =

Table 1. Social and Drug Use Factors (Prior 6 Months) Among Homeless and Unstably Housed Women ($N = 222$)

	Total sample prevalence (N = *222*) n *(%)*	*Prevalence among HIV+* (n = *106*) n *(%)*	*Prevalence among HIV–* (n = *116*) n *(%)*	p
Race/Ethnicity				0.24†
African American	105 (47.3)	58 (50.0)	47 (44.3)	
Caucasian	61 (27.5)	29 (25.0)	32 (30.2)	
Latina	12 (5.4)	5 (4.3)	7 (6.6)	
Asian/Pacific Islander	6 (2.7)	1 (0.9)	5 (4.7)	
Multi-racial	32 (14.4)	18 (15.5)	14 (13.2)	
Other	6 (2.7)	5 (4.3)	1 (0.9)	
Age, median	48.2 (IQR = 42.9–53.8)	48.1 (IQR = 42.7–53.4)	48.5 (IQR = 42.9–53.9)	0.94
Incarceration	22 (9.9)	15 (12.9)	7 (6.6)	0.12
Total income/$100	9.3 (IQR = 8.2–11.4)	9.3 (IQR = 8.4–10.9)	9.5 (IQR = 7.0–13.0)	0.50
% Time slept in a shelter				0.23†
0	194 (87.4)	103 (88.8)	91 (85.9)	
1–25	17 (7.7)	10 (8.6)	7 (6.6)	
26–100	11 (5.0)	3 (2.6)	8 (7.6)	
% Time slept in public				0.37†
0	184 (82.9)	98 (84.5)	86 (81.1)	
1–25	29 (13.1)	12 (10.3)	17 (16.0)	
26–100	9 (4.1)	6 (66.7)	3 (3.3)	
>1 alcoholic beverage/day	47 (21.2)	26 (22.4)	21 (19.8)	0.64
Binge drinker	86 (38.7)	46 (40.0)	40 (37.7)	0.77
Self-reported cocaine (crack or powdered) use	106 (47.8)	58 (50.0)	48 (45.3)	0.48
Self-reported marijuana use	116 (52.3)	61 (52.6)	55 (51.9)	0.92
Self-reported meth use	43 (19.4)	17 (14.7)	26 (24.5)	0.06
Self-reported heroin use	27 (12.2)	15 (12.9)	12 (11.3)	0.71
Taking antiretroviral therapy	95 (42.8)	95 (89.6)	—	—

Frequencies were compared using the Chi-square test unless small cell counts required the use of Fisher's Exact test, denoted by † above. Means for continuous age and income were compared using Wilcoxon Rank Sums test.

HIV, human immunodeficiency virus.

TABLE 2. DIFFERENCES IN BIOMARKERS BY COCAINE* AND LEVAMISOLE STATUS AMONG HOMELESS AND UNSTABLY HOUSED WOMEN

	HIV-uninfected				*HIV-infected*			
	Cocaine–/ levamisole– (–/–) (N=*36*)	*Cocaine+/ levamisole– (+/–)* (N=*9*)	*Cocaine+/ levamisole+ (+/+)* (N=*61*)	p	*Cocaine–/ levamisole– (–/–)* (N=*46*)	*Cocaine+/ levamisole– (+/–)* (N=*11*)	*Cocaine+/ levamisole+ (+/+)* (N=*59*)	p
Prealbumin count (mean ± SD [*N*])	24.9 ± 7.64 (*N* = 36)	21.6 ± 8.38 (*N* = 9)	23.1 ± 6.59 (*N* = 60)	Total: 0.33 –/– vs. +/+: 0.48 +/– vs. +/+: 0.74 +/– vs. –/–: 0.46[a]	23.3 ± 5.94 (*N* = 46)	24 ± 9.21 (*N* = 11)	20.6 ± 7.93 (*N* = 58)	Total: 0.01 –/– vs. +/+: 0.01 +/– vs. +/+: 0.46 +/– vs. –/– 0.96[a]
Prealbumin category = clinically low (<17 mg/dL)	5 (13.9%)	3 (33.3%)	11 (18.3%)	Total: 0.41 –/– vs. +/+: 0.78 +/– vs. +/+: 0.37 +/– vs. –/–: 0.33[b]	5 (10.9%)	3 (27.3%)	21 (36.2%)	Total: 0.01 –/– vs. +/+: <0.01 +/– vs. +/+: 0.74 +/– vs. –/–: 0.17[b]
Neutrophil count 10 (mean ± SD [N])	5.88 ± 0.897 (*N* = 32)	5.64 ± 0.998 (*N* = 8)	5.71 ± 1.28 (*N* = 55)	Total: 0.79 –/– vs. +/+: 0.91 +/– vs. +/+: 0.96 +/– vs. –/–: 0.67[a]	5.39 ± 1.27 (*N* = 43)	5.41 ± 1.89 (*N* = 11)	5.26 ± 1.11 (*N* = 55)	Total: 0.71 –/– vs. +/+: 0.68 +/– vs. +/+: 0.94 +/– vs. –/–: 0.97[a]
Neutrophil category = clinically low (<1500 cells μL)	0 (0.0%)	0 (0.0%)	5 (9.1%)	Total: 0.24 –/– vs. +/+: 0.15 +/– vs. +/+: 1.0 +/– vs. –/–: 1.0[b]	7 (16.3%)	2 (18.2%)	6 (10.9%)	Total: 0.65 –/– vs. +/+: 0.55 +/– vs. +/+: 0.61 +/– vs. –/–: 1.0[b]
White cell count (mean ± SD [N])	6.21 ± 1.76 (*N* = 36)	5.92 ± 1.71 (*N* = 9)	6.59 ± 2.32 (*N* = 56)	Total: 0.72 –/– vs. +/+: 0.82 +/– vs. +/+: 0.79 +/– vs. –/–: 0.95[a]	5.62 ± 1.53 (*N* = 45)	5.71 ± 2.03 (*N* = 11)	4.81 ± 1.61 (*N* = 57)	Total: 0.04 –/– vs. +/+: 0.03 +/– vs. +/+: 0.56 +/– vs. –/–: 0.91[a]
White cell count category = clinically low ($<3.8 \times 10^3/\mu$L)	2 (5.6%)	1 (11.1%)	4 (7.1%)	Total: 0.71 –/– vs. +/+: 1.0 +/– vs. +/+: 0.50 +/– vs. –/–: 0.54[b]	4 (8.9%)	0 (0.0%)	15 (26.3%)	Total: 0.02 –/– vs. +/+: 0.04 +/– vs. +/+: 0.11 +/– vs. –/–: 0.58[b]
Lymphocyte (mean ± SD [N])	1894 ± 599 (*N* = 32)	1985 ± 699 (*N* = 8)	2006 ± 779 (*N* = 56)	Total: 0.81 –/– vs. +/+: 0.81 +/– vs. +/+: 1.0 +/– vs. –/–: 0.93[a]	1950 ± 676 (*N* = 43)	2000 ± 1041 (*N* = 11)	1665 ± 735 (*N* = 52)	Total: 0.16 –/– vs. +/+: 0.14 +/– vs. +/+: 0.71 +/– vs. –/–: 0.99[a]
Lymphocyte category = clinically low ($<1.0 \times 10^3/\mu$L)	2 (6.3%)	1 (12.5%)	5 (8.9%)	Total: 0.74 –/– vs. +/+: 1.0 +/– vs. +/+: 0.57 +/– vs. –/–: 0.50[b]	1 (2.3%)	1 (9.1%)	8 (15.4%)	Total: 0.07 –/– vs. +/+: 0.04 +/– vs. +/+: 1.0 +/– vs. –/–: 0.37[b]
CD4/100 (mean ± SD [N])	—	—	—	—	5.85 ± 2.21 (*N* = 27)	6.62 ± 3.48 (*N* = 5)	4.9 ± 4.75 (*N* = 36)	Total: 0.05 –/– vs. +/+: 0.07 +/– vs. +/+: 0.30 +/– vs. –/–: 0.93[a]
Undetectable HIV viral load	—	—	—	—	11 (40.7%)	2 (33.3%)	12 (33.3%)	Total: 0.87 –/– vs. +/+: 0.60 +/– vs. +/+: 1.0 +/– vs. –/–: 1.0[b]

*Toxicology positive for cocaine and/or benzoylecgonine; –/– vs. +/+ compares cocaine–/levamisole– to cocaine+/levamisole+; +/– vs. +/+ compares cocaine+/levamisole– to cocaine+/levamisole+; +/– vs. –/– compares cocaine+/levamisole– to cocaine–/levamisole–.

[a]Based on Kruskal–Wallis test.

[b]Based on Fisher's Exact Chi-square test.

3.36; 95% confidence interval [CI] = 1.16–11.70) and (+/–) persons (OR = 8.39, 95% CI = 0.98–1100.3), and higher for persons with a low lymphocyte count (OR = 14.49; 95% CI = 3.78–66.74); however, estimates did not reach a level of statistical significance for low neutrophil count (Table 3).

Adding lymphocyte count to an adjusted model with cocaine/levamisole status decreased the magnitude of effect between +/+ and –/– persons and increased the effect between +/– and –/– persons, suggesting that lymphocyte count mediates the effect of cocaine/levamisole status on white blood cell count. By contrast, adding neutrophil count to an adjusted model with cocaine and levamisole status did not substantively change the magnitude of effect from cocaine/levamisole on white blood cell count, suggesting no evidence for mediation by neutrophil count. Similarly, in a model adjusting for all three, the odds of low lymphocyte count were significantly higher among +/+ persons compared to +/– persons, but not +/+ persons compared to –/– persons, and significantly higher among those with low lymphpocyte count, and low neutrophil count did not reach a level of significance. These results provide further evidence for mediating influences of lymphocyte count on white blood cell count, but not neutrophil count in this restricted sample of HIV-infected women.

Social and behavioral comparisons

In comparative analyses restricted to persons who tested cocaine positive ($n = 140$), race/ethnicity and self-reported cocaine use were the only social/behavioral factors significantly associated with the presence of levamisole (Table 4). The adjusted odds of testing positive for levamisole were significantly lower among Caucasian (OR = 0.24, 95% CI = 0.07–0.73) and Asian Pacific Islander women (OR = 0.08, 95% CI = 0.01–0.60) compared to African American women; odds were significantly higher among persons who self-reported cocaine use compared to women who did not disclose use (OR = 2.71, 95% CI = 1.01–7.40). Although the numbers were small, it should be noted that all Latina women who tested positive for cocaine also tested positive for levamisole.

Discussion

As one of the first epidemiological studies to contrast levamisole-exposed persons to comparable but unexposed persons within a community-based sample, this study found a high level of levamisole (85%) among individuals who were toxicology positive for cocaine. It is unclear whether individuals who were cocaine positive and levamisole negative used cocaine that was not adulterated with levamisole or, given the shorter half-life of levamisole (5 hours for levamisole compared to 6 hours for benzoylecgonine),[29,30] whether levamisole had already been metabolized and cleared from the system.

Similarly, compared to African American women who tested positive for cocaine, fewer Caucasians and Asian Pacific Islanders who tested positive for cocaine also tested positive for levamisole, and it is unclear whether this was due to the absence of levamisole in the cocaine used or clearance of the drug. These findings suggest that race/ethnicity is associated with either the presence of levamisole in procured drug, higher drug use frequency, or faster drug metabolism. In all of the three possibilities, race/ethnicity appears to be an important factor in understanding the influences of levamisole on the health of cocaine-using women.

Table 3. Associations Between Clinically Low White Blood Cell Count ($<3.8 \times 10^3/\mu L$) and White Blood Cell Differentials (Lymphocyte Count and Neutrophil Count) Among HIV-Infected Homeless and Unstably Housed Women ($N = 116$)

Cocaine[a] *and levamisole status*			*Low lymphocyte count* ($<1.0 \times 10^3/\mu L$)	*Low neutrophil count (<1500 cells/μL)*
Comparison group	*Reference group*	*OR (95% CI)*	*OR (95% CI)*	*OR (95% CI)*
Simple models				
Cocaine+/Levamisole+	Cocaine–/Levamisole–	3.36 (1.16, 11.70)[b]	—	—
Cocaine+/Levamisole+	Cocaine+/Levamisole–	8.39 (0.98, 1100.3)[c]		
Cocaine+/Levamisole–	Cocaine–/Levamisole–	0.40 (0.01, 4.21)		
—			14.49 (3.78, 66.74)[b]	—
—			—	1.32 (0.31, 4.53)
2-Variable models				
—			16.58 (4.18, 79.10)[b]	2.35 (0.53, 8.84)
Cocaine+/Levamisole+	Cocaine–/Levamisole–	2.55 (0.79, 9.42)	12.90 (3.00, 74.84)[b]	—
Cocaine+/Levamisole+	Cocaine+/Levamisole–	12.25 (1.10, 1805.7)[b]		
Cocaine+/Levamisole–	Cocaine–/Levamisole–	0.21 (0.01, 2.81)		
Cocaine+/Levamisole+	Cocaine–/Levamisole–	3.40 (1.17, 11.87)[b]	—	1.62 (0.36, 6.04)
Cocaine+/Levamisole+	Cocaine+/Levamisole–	8.97 (1.05, 1176.7)[b]		
Cocaine+/Levamisole–	Cocaine–/Levamisole–	0.38 (0.01, 3.95)		
Full model				
Cocaine+/Levamisole+	Cocaine–/Levamisole–	2.72 (0.84, 10.17)	15.09 (3.40, 89.62)[b]	2.89 (0.62, 11.90)
Cocaine+/Levamisole+	Cocaine+/Levamisole–	12.88 (1.19, 1864.5)[b]		
Cocaine+/Levamisole–	Cocaine–/Levamisole–	0.21 (0.001, 2.75)		

[a]Toxicology positive for cocaine and/or benzoylecgonine.
[b]$p < 0.05$.
[c]$p < 0.10$.
OR, odds ratio; CI, confidence interval.

Table 4. Associations Between the Presence of Levamisole and Social/Behavioral Factors Among Individuals Testing Toxicology Positive for Cocaine ($N=140$)

	Levamisole prevalence	*Unadjusted OR (95% CI)*	*Adjusted OR (95% CI)*
Race/ethnicity			
African American	69/74 (93.2%)	1.00	
Caucasian	25/34 (73.5%)	0.21 (0.06–0.65)[a]	0.24 (0.07–0.73)[a]
Latina	6/6 (100.0%)	1.03 (0.10–140.67)	
Asian/Pacific Islander	2/4 (50.0%)	0.08 (0.01–0.60)[a]	0.08 (0.01–0.60)[a]
Multiracial	16/19 (84.2%)	0.37 (0.09–1.75)	
Other	2/3 (66.7%)	0.13 (0.01–1.62)	
Age Quartile			
20–42	30/34 (88.2%)	1.00	
43–47	29/31 (93.6%)	1.74 (0.36–10.62)	
48–53	34/43 (79.1%)	0.54 (0.14–1.75)	
54–69	27/32 (84.4%)	0.74 (0.18–2.87)	
Incarceration	14/15 (93.3%)	1.77 (0.40–16.78)	
Total income/$100	Median = $935	1.05 (0.95–1.23)	
Time slept in a shelter (%)			
0	103/120 (85.8%)	1.00	
1–25	9/11 (81.8%)	0.64 (0.16–3.57)	
26–100	8/9 (88.9%)	0.96 (0.20–9.39)	
Time slept in public (%)			
0	92/111 (82.9%)	1.00	
1–25	21/22 (95.5%)	3.02 (0.70–28.28)	
26–100	7/7 (100.0%)	3.16 (0.36–416.72)	
>1 alcoholic beverage/day	31/35 (88.6%)	1.29 (0.45–4.43)	
Binge drinker	58/67 (86.6%)	1.13 (0.45–2.93)	
Self-reported cocaine use (crack or powdered)	90/100 (90.0%)	2.97 (1.14–7.76)[a]	2.71 (1.01–7.40)[a]
Self-reported Marijuana use	73/84 (86.9%)	1.28 (0.49–3.25)	
Self-reported Methamphetamine use	28/33 (84.8%)	0.87 (0.31–2.72)	
Self-reported Heroin use	18/20 (90.0%)	1.34 (0.38–7.09)	
HIV and antiretroviral therapy status			
HIV−	61/70 (87.1%)	1.00	
HIV+/ART−	12/15 (80.0%)	0.55 (0.15–2.46)	
HIV+/ART+	47/55 (85.5%)	0.86 (0.32–2.39)	

Toxicology positive for cocaine and/or benzoylecgonine.
[a]Statistically significant 95% CI, which does not include 1.
ART, antiretroviral therapy.

Biomarkers were consistently lower, indicating worse immune function, among cocaine-positive/levamisole-positive (+/+) persons who were also HIV-infected, but the same significant associations were not observed among HIV-negative women. In a prior study, Baum and colleagues reported that, independent of adherence to highly active antiretroviral therapy (HAART), HIV-infected crack cocaine users were more likely to experience a decline in CD4 cell count and an increase in viral load over 30 months.[31] Similarly, Cook and colleagues found in the Women's Interagency HIV Study cohort of HIV-infected women that crack cocaine-using women showed greater CD4 cell loss and higher HIV-1 RNA levels, even after controlling for use of HAART, other illicit substances used, depression, and hepatitis C virus coinfection.[32] These consistent findings of an association between cocaine use and decline in CD4 cell counts independent of HAART use led to subsequent studies that identified several alternative mechanisms for cocaine-related disease progression, including reduced thymic endocrine function[33] and enhanced permissiveness of quiescent T cells.[34] Results presented here support the hypothesis that levamisole may be an additional mechanism of immune dysfunction among HIV-infected persons. Results did not indicate a significant association between levamisole and HIV viral load, which likely reflects the specificity of levamisole's effects as an immunomodulator.

Compared to HIV-infected women who were negative for both cocaine and levamisole (–/–), HIV-infected women who were +/+ had lower levels of prealbumin ($p<0.01$), white blood cell count ($p=0.03$), and CD4 cell count ($p=0.07$), but those who were positive for cocaine only (+/–) did not (Table 2). These results suggest that associations between levamisole and negative health outcomes are not entirely due to cocaine, rather levamisole is independently associated with immune marker levels. This finding is biologically plausible and consistent with the nature of this immunomodulator. Furthermore, adjusted analysis shows that the odds of low white blood cell count were higher for +/+ individuals compared to +/– (Table 3), again suggesting an association with levamisole that is independent of cocaine.

While neutropenia has been cited as a concern in case reports of persons seeking treatment for conditions linked to levamisole,[9,14,16,35,36] we did not find significant differences in neutrophil count by cocaine and levamisole status (Table 2). Furthermore, adjusted models suggested mediation of cocaine/levamisole effects on white blood cell count by lymphocyte count, but not by neutrophil count (Table 3). Thus, lymphocyte

count appears to be the white blood cell component most affected by levamisole in HIV-infected homeless women, not neutrophil count. Results are consistent with a nonsignificant association between neutrophil count and levamisole found in a study by Chai et al., which is one of the few investigations outside of this study to compare levamisole-positive to levamisole-negative individuals.[37] The lack of a strong association between levamisole and neutrophil count in comparative studies, combined with differential effects by HIV status found in this study, may suggest that patients represented in prior case reports were immune compromised before levamisole exposure. Alternatively, these results may suggest that severe toxicities experienced by patients represented in prior case studies had different (or additional) underlying mechanisms than immune and inflammation effects experienced by this study's participants, none of whom experienced severe toxicities. For instance, persons experiencing severe levamisole toxicities may be genetically predisposed to autoimmune diseases (*e.g.*, HLA-B27).[38–40] While not definitive, these findings suggest immune dysfunction rather than a medication side effect or adverse reaction to levamisole itself, the latter of which would have been supported by a strong association between levamisole and neutrophil count.

Limitations

Certain limitations must be considered in the interpretation of results presented here. First, while the study adds a new population-based perspective to the growing number of case reports regarding levamisole-contaminated cocaine use, the sample is small, which has implications for representativeness and statistical analysis. Regarding representativeness, recruiting a probability sample of unstably housed women from community venues likely reduced the possibility of a biased sample from San Francisco's population of homeless and unstably housed women; however, results may not be generalizable to women from areas outside San Francisco, such as those that are less-resourced, and HIV-specific results may not be specific to all HIV-infected women.

Regarding analysis, stratification by HIV and three levels of cocaine/levamisole status resulted in small cell sizes and wide confidence intervals. This high level of variability should be considered when interpreting results. The fact that statistically significant results were observed in a small unbiased sample suggests true associations; however, there may be additional associations this study was underpowered to detect. In addition, it is possible that associations reported here are confounded by unmeasured factors. For instance, data regarding adherence to antiretroviral therapy and other medications did not exist and it is possible that adherence to medications significantly influenced immune function.

Lastly, the study was cross-sectional and could not assess change over time. This modest study is a first step toward understanding influences of levamisole, but additional studies are needed. Future studies that include larger samples of cocaine users from multiple geographic areas and follow individuals over time will help to further elucidate associations between levamisole and race as well as biomarkers highlighted here. Strengths of the study include a community-based probability sample of HIV-infected and uninfected women with a high prevalence of cocaine use, which made this comparative study possible.

Conclusions

While cases of serious health problems linked to levamisole are increasing in the literature,[7–13] they are still relatively rare considering the high prevalence of levamisole detected in this probability sample of community-recruited homeless and unstably housed women (85% among cocaine-using persons). Our results suggest that decreased immune function as a result of levamisole-contaminated cocaine use may occur mainly in individuals who are already immune compromised (*e.g.*, HIV-infected). Given the dearth of information regarding levamisole and HIV, it is unclear whether previous case reports support these findings. It is unlikely that all previous cases were in HIV-infected individuals, but our data suggest that HIV significantly modifies the effects of levamisole on inflammation and immune function. Whether the apparent effect modification also extends to persons who are genetically predisposed to autoimmune diseases (*e.g.*, HLA-B27)[38–40] cannot be tested with these data, but remains a possibility.

This study extends knowledge from the growing number of case reports regarding levamisole-contaminated cocaine use with a population-level perspective and provides a starting point for future comparative studies of levamisole toxicity in humans. Results indicate that HIV-infected African American women disproportionately test positive for levamisole, and the white blood cell count of HIV-infected persons who also test positive for levamisole are disproportionately low, a phenomenon that appears to be mediated by the influence of levamisole on lymphocyte count. While larger and geographically diverse studies are needed to elucidate these initial findings, results presented here suggest that the cocaine adulterant, levamisole may be an additional mechanism of immune dysfunction among HIV-infected individuals.

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Author Disclosure Statement

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