A Confirmed Case of Agranulocytosis after Use of Cocaine Contaminated with Levamisole

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Abstract More than 2 million Americans use cocaine each month (National Survey on Drug Use and Health, Department of Health and Human Services: Substance Abuse and Mental Health Services Administration (SAMHSA) & Office of Applied Studies (OAS), Rockville, MD 2007). Starting in early 2003, South American cocaine cartels began to add levamisole, a pharmaceutical agent, to bulk cocaine prior to shipment to the USA (Valentino and Fuentecilla 2005). A dramatic increase in the prevalence of levamisole in cocaine was noted in early 2008. By October, 30% of cocaine

bricks analyzed by the United States Drug Enforcement Administration contained levamisole (Casale et al. 2008). Exposure to levamisole can cause agranulocytosis (Amery and Bruynseels 1992). We report the first confirmed case of agranulocytosis associated with consumption of levamisole-contaminated cocaine in the USA. A previously healthy adult male presented to the emergency department with 5 days of mouth pain. He admitted to chronic active ethanol and crack cocaine abuse. Laboratory studies revealed severe neutropenia, with an absolute neutrophil count of 19 cells/mm³ (normal=1,500–8,000 cells/mm³). A urine screen for drugs of abuse was positive for cocaine metabolites and opiates. Evaluation of a peripheral blood smear showed leukopenia with severe absolute neutropenia. A bone marrow biopsy revealed recently injured bone marrow showing early recovery. While in the hospital, the patient had little spontaneous bone marrow recovery. He received granulocyte colony-stimulating factor with improvement in peripheral white blood cell counts. The residue in the patient's crack pipe contained 10% levamisole. Subsequently, levamisole was detected in the patient's urine. Levamisole-associated agranulocytosis should be considered in the diagnosis of patients who present with neutropenia and a history or evidence of cocaine use.

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Introduction

According to the National Survey on Drug Use and Health, approximately 2.1 million people in the USA use cocaine each month [1]. Data from the Monitoring the Future study show that 7.8% of US high school seniors have tried cocaine, and 2% are current users [5].

In early 2003, the United States Drug Enforcement Administration detected levamisole in some cocaine bricks analyzed in its laboratories [2]. Levamisole-containing cocaine was relatively uncommon until April 2008 when the prevalence rose dramatically. The most recent data from October 2008 show that 30% of cocaine bulk shipments entering the USA are adulterated with levamisole [3]. More than half of cocaine samples analyzed after street “busts” in Denver, Colorado now contain levamisole.

Levamisole, the *levo* enantiomer of tetramisole, was originally developed as an antihelminthic agent [4]. Levamisole is approved by the United States Food and Drug Administration as adjuvant chemotherapy, with 5-fluorouracil, for the treatment of colon cancer. Because it has immunostimulant and immunomodulatory properties, levamisole has also been used for treatment of various autoimmune disorders (rheumatoid arthritis, juvenile rheumatoid arthritis, and nephrotic syndrome) and cancers (breast and lung). Although levamisole is no longer widely utilized in humans, it is a common veterinary pharmaceutical used to treat worm infestations in sheep, cattle, and pigs [4]. Levamisole can be readily purchased worldwide as oral tablets, pastes, gels, soluble powder, feed premixes, topical solutions, and injectable solutions [6].

Levamisole stimulates the formation of antibodies to various antigens, enhances T-cell activation and proliferation, and increases neutrophil mobility, adherence, and chemotaxis [7]. Between 0.4% and 20% of patients treated with levamisole form isoimmune antibodies to antigens on their neutrophil cell walls, leading to neutrophil destruction [4, 8]. Agranulocytosis, a profound depletion of circulating neutrophils, is a rare but life-threatening condition. In one study, the incidence of agranulocytosis was five cases per million population per year, with a case-fatality rate of 9.1% [9]. Like any profoundly neutropenic patient, those with levamisole-associated agranulocytosis are susceptible to fulminant and opportunistic infections.

In mid-2008, public health authorities in New Mexico and Alberta, Canada, began investigating case clusters of unexplained agranulocytosis [10, 11]. Epidemiologic investigations revealed a strong association between agranulocytosis and cocaine use, with confirmation of urinary levamisole in some patients from New Mexico and Canada [10, 11].

We report the first case of levamisole-induced agranulocytosis in the USA, confirmed by laboratory analysis of patient urine and source material.

Case Report

A previously healthy adult male presented to the emergency department in February 2009, with 5 days of mouth pain. Other symptoms included fever, chills, night sweats, mild headache, dark urine, and intermittent tinnitus. He took no chronic medications, but self-medicated with aspirin, *Echinacea*, *Arnica montana* (wolf's bane), vitamin C, and an unknown over-the-counter analgesic intermittently in a therapeutic fashion over the prior 5 days. He admitted to chronic active ethanol and crack cocaine abuse. He also had occupational exposure to unknown inhaled solvents 7 years prior when he worked as a carpenter and machinist. He had no personal or family history of malignancy.

The patient's initial blood pressure was 143/72 mmHg, oral temperature was 37.2°C, pulse was 92 beats per minute, respiratory rate was 18 breaths per minute, and oxygen saturation was 95% while breathing ambient air at altitude. Physical exam revealed a thin man in moderate discomfort, with poor dentition, white exudates on his lateral gum edges, and moderate oropharyngeal erythema. His exam was otherwise unremarkable except for mild right inguinal lymphadenopathy.

Laboratory studies revealed severe neutropenia, with an absolute neutrophil count of 19 cells/mm³ (normal=1,500–8,000 cells/mm³) (Table 1). In addition, he had mild total

Table 1 Peripheral white blood cell counts

Day	1	2	3	4	5	10	24
Total leukocyte count (cells/mm ³)	1,900	2,300	3,000	3,800	8,500	10,800	10,100
Absolute neutrophil count (cells/mm ³)	19	92	90	1,710	4,505	6,588	5,858
White blood cell differential (%)							
Neutrophils	1	3	3	20	26	61	58
Band forms	0	1	0	25	27	0	0
Metamyelocytes	0	0	0	3	1	0	0
Lymphocytes	87	69	58	36	29	31	36
Atypical lymphocytes	5	8	24	2	4	0	0
Monocytes	7	17	15	14	13	4	3
Basophils	0	2	0	0	0	0	2
Eosinophils	0	0	0	0	0	1	1

leukopenia (white blood cell count=1,900 cells/mm³; normal=4,500–10,000 cells/mm³). Serum electrolyte, renal function, glucose, hemoglobin, platelet count, liver enzymes, and coagulation studies were within normal range. A chest radiograph and electrocardiogram were normal, and a serum HIV antibody test was negative. A urine screen for drugs of abuse was positive for cocaine metabolites and opiates, and a serum acetaminophen level was supratherapeutic [33 µg/mL (219 µmol/L); therapeutic range=10–20 µg/mL (66–132 µmol/L)]. Serum salicylate was not detected.

The patient was admitted to the hospital with neutropenic precautions and treated with clindamycin and acyclovir for oral infection and N-acetylcysteine for possible acetaminophen toxicity. Shortly thereafter, he developed a fever to 38.8°C. Blood, urine, and stool cultures were obtained, and antibiotic coverage was broadened to include cefipime, vancomycin, and piperacillin/tazobactam.

Evaluation of a peripheral blood smear showed leukopenia with severe absolute neutropenia, 1+ toxic granulation, reactive lymphocytes, and immature reactive monocytes (Fig. 1). A bone marrow biopsy, performed the morning of the third day of hospitalization prior to the administration of bone marrow stimulating agents, showed a hypercellular bone marrow. There was a relative myeloid hypoplasia with a marked left shift: only 0.5% of the cellular marrow was composed of mature neutrophils. A marked megakaryocyte hyperplasia also was identified (Fig. 2). Taken together, the findings in the bone marrow were consistent with recently injured bone marrow showing early recovery. Testing for causes of neutropenia included a

Fig. 1 This image shows the absence of leukocytes in a typical field

Fig. 2 Bone marrow core biopsy, hematoxylin and eosin staining, ×400 magnification, obtained on day 3, prior to the first dose of filgrastim. This biopsy shows hypercellular marrow with relative myeloid hypoplasia, megakaryocyte hyperplasia, and a marked left shift in the neutrophil series. Only 0.5% of the cellular marrow was composed of mature neutrophils. *Thick arrows* point to immature myeloid precursors. *Thin arrows* point to foci of megakaryocytic hyperplasia

weakly detectable c-antineutrophil cytoplasmic antibodies (c-ANCA; detectable at 1:20 dilution). Perinuclear-ANCA (p-ANCA) levels and cardiolipin IgA, IgM, and IgG antibodies were negative. HLA typing was not performed.

While in the hospital, the patient had little spontaneous bone marrow recovery. He received granulocyte colony-stimulating factor (filgrastim, 300 µg subcutaneously) on the third, fourth, and fifth days of hospitalization, with brisk improvement in peripheral white blood cell counts (Table 1).

Fig. 3 Crack pipe from this patient. Residue in the pipe contained 10% levamisole by weight

Because of clinical suspicion of levamisole-induced agranulocytosis and after receiving legal assurance of non-prosecution from the Denver District Attorney, the patient submitted his crack pipe for testing by the Denver Police Department Crime Lab. The residue in the crack pipe contained 10% levamisole by weight (Fig. 3). Subsequently, levamisole was detected in the patient's urine by gas chromatography/mass spectrometry by a specialty laboratory at the New Mexico Department of Health.

All cultures were negative, and the patient was discharged after a 5-day hospitalization. At outpatient follow-up 10 and 24 days after initial presentation, the patient remained asymptomatic, with normal total white blood cell and neutrophil counts. He remained drug free by personal communication at follow-up.

Discussion

We consider this patient the first confirmed case of agranulocytosis associated with cocaine adulterated with levamisole in the USA. This patient used cocaine adulterated with levamisole and his urine contained both cocaine metabolites and levamisole. An extensive workup revealed no other cause of agranulocytosis.

Adulteration of cocaine with levamisole has the potential to become a major public health problem. Levamisole poisoning can occur with both powder and crack cocaine abuse [10, 12]. Approximately 500,000 Americans consume levamisole-contaminated cocaine each month [1, 3]. Preliminary data suggest that other cases have occurred. Immediately after learning of the suspected cases in New Mexico, we searched the Denver Health and Hospital Authority data warehouse to identify patients treated in the past 3 years who had cocaine abuse and neutropenia, but who were not under active treatment for cancer. To date, we have identified at least 10 recent cases of severe neutropenia for which the cause remained elusive after extensive diagnostic workup. Seven of these patients were first diagnosed in 2008 or 2009, and one additional patient first diagnosed in 2007 has ongoing neutropenia. Denver Health Hospital Authority is attempting to contact these patients and their physicians to arrange for re-evaluation. Active public health investigations are underway in New Mexico, Colorado, and the Canadian provinces of Alberta and British Columbia.

Levamisole is thought to cause agranulocytosis by inducing autoimmunity to antigens on neutrophil cell walls. Although it is not clear why only certain patients develop anti-granulocyte antibodies, rheumatoid arthritis and the presence of HLA-B27 antigens appear to confer increased risk [8, 13]. Other isoantibodies, including c-ANCA, p-ANCA, anti-phospholipid, and IgM anti-cardiolipin antibodies, have also been reported in association with

levamisole use [9]. In a recently published Canadian letter, all patients with levamisole-associated agranulocytosis had detectable lupus anticoagulant [10].

It is unknown why cartels utilize levamisole to cut cocaine. In general, adulterants are added to illicit drugs either to modify the effect of the drug or to increase the weight or volume of product ("cut" the cocaine) prior to sale [14, 15]. Since adding an inert ingredient to illicit cocaine prior to shipment into the USA makes smuggling more difficult, the possibility that levamisole is an effect modifier must be carefully considered. Recent research shows that cocaine addiction is modulated by the dopamine- and melanin-concentrating hormone systems of the mesolimbic system and nucleus accumbens of the brain [16]. In animal models, levamisole increases dopamine levels in the hypothalamus, striatum, and midbrain [17]. Although levamisole is not inherently addictive, it is plausible that levamisole increases the addictive properties of cocaine.

There are many known toxic and infectious causes of agranulocytosis [18, 19]. In the suspected cases, we reviewed at our institution, we found several in which, after an exhaustive negative workup, agranulocytosis was attributed to unspecified viral illness, atypical presentations of autoimmune diseases such as systemic lupus, or to a common medication, such as trimethoprim/sulfamethoxazole or risperidol [18, 19]. In some cases, agranulocytosis persisted or recurred after exposure to the ostensible offending agent was discontinued and cocaine use continued. It seems prudent to re-evaluate patients diagnosed with agranulocytosis since 2003, and case reports of unusual causes of agranulocytosis published in this time frame, for the possibility of unrecognized levamisole exposure.

Levamisole testing of urine is currently available from the Colorado and New Mexico Departments of Health. Based on the pharmacokinetics of levamisole, such testing is likely to be positive only if a specimen is collected within 24–48 h of the last use of adulterated cocaine [8]. Anti-neutrophil antibody testing is available from some major commercial laboratories.

Levamisole-associated agranulocytosis should be considered in the diagnosis of patients who present with neutropenia and a history or evidence of cocaine use.

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