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CASE REPORT

Stella C. Wong,[1] *D.O.; John A. Curtis,*[1] *M.D.; and William E. Wingert,*[2] *Ph.D.*

Concurrent Detection of Heroin, Fentanyl, and Xylazine in Seven Drug-related Deaths Reported from the Philadelphia Medical Examiner's Office

ABSTRACT: Recreational drugs, such as cocaine and heroin, are often adulterated with other pharmacological agents to either enhance or diminish the drug effects. Between April 21, 2006 and August 8, 2006, the Philadelphia Medical Examiner's Office detected xylazine (a veterinary sedative) and fentanyl (a synthetic opioid) in specimens taken from seven cases. Initial immunoassay screening was performed on urine and blood for fentanyl, opiate, cocaine, phencyclidine (PCP), and benzodiazepines. All tests reported positive were confirmed by gas chromatography-mass spectrometry. All seven xylazine positive cases tested positive for fentanyl and six cases tested positive for 6-acetylmorphine (a metabolite and definitive marker for heroin). The seventh case was positive for morphine and had a history of heroin abuse. Xylazine was present in urine in all seven cases and blood levels were detected in three cases. The blood concentrations ranged from trace to 130 ng/mL. Fentanyl was present in the blood and urine in each case and blood concentrations ranged from 4.7 to 47 ng/mL. Adulteration of illicit drugs has become an epidemic health concern for drug users. Healthcare professionals need to be aware of this issue, so the patients can be treated in an effective, timely manner.

KEYWORDS: forensic science, xylazine, fentanyl, heroin adulterants

Recreational drugs, such as cocaine and heroin, are often adulterated with other agents. Adulterants are added to illicit drugs to either increase or decrease the drug effects as well as to increase the value of the drug when selling on the street. For example, scopolamine was reported as an adulterant in heroin with both drugs having adverse clinical effects (1). Between April 21, 2006 and August 8, 2006, the Philadelphia Medical Examiner's Office detected xylazine and fentanyl in drug-related postmortem samples. We are presenting a case series of drug-related deaths in which xylazine and fentanyl were detected by the Philadelphia Medical Examiner's Office.

Xylazine is similar to clonidine (2) in that it acts as a central alpha-2 agonist on the brainstem and may cause bradycardia and transient hypertension followed by hypotension (3,4). It exerts its autonomic effects by blocking the release of norepinephrine which may cause bradycardia, reduced cardiac output and hypotension. It also inhibits the release of acetylcholine causing xerostomia. Xylazine has also been reported to have affinity to H_2-histaminergic, serotonergic, cholinergic, dopaminergic, and possibly opioid receptors (3,5–7).

Fentanyl is a synthetic opioid with higher potency than morphine and heroin, and was first used in the United States in 1968 as an intravenous anesthetic/analgesic medication (8). It binds to mu-receptors producing analgesia and sedation. Other opioid effects include euphoria, respiratory depression, hypotension, and bradycardia. Due to fentanyl's high potency, a larger dose of naloxone (an opioid antagonist) may be required to reverse its effects following overdose.

[1]Department of Emergency Medicine, Nazareth Hospital, Philadelphia, PA.
[2]The Philadelphia Medical Examiner's Office, Philadelphia, PA.

We have not presented this article in other meetings or literature. However, we presented a poster of our findings at the North American Congress of Clinical Toxicology (NACCT) meeting in October 2007

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Materials and Methods

Specimens were taken during autopsies performed at the Philadelphia Medical Examiner's Office. A broad-spectrum drug screen was performed using either urine (when available) or fluoride-preserved blood obtained from the cardiac area. Samples were extracted by an in-house solid phase extraction (SPE) method using CSDAU303 columns and a modified procedure from United Chemical Technologies, Inc. (9). Acid/neutral and basic elutes from the SPE columns were collected separately and analyzed by electron impact gas chromatograph mass spectrometry (EI GC-MS) in the scan mode.

Immunoassays of samples from suspected drug-related deaths were performed using fentanyl, opiate, cocaine, phencyclidine (PCP) or benzodiazepine Micro-plate EIA kits and methods obtained from OraSure Technologies, Inc. (10).

Cases positive for fentanyl by gas chromatography-mass spectrometry (GC/MS) or immunoassay methods described above were further tested and quantified separately for fentanyl, cocaine, methadone, opiates, and PCP when indicated by the previous test results. For each in-house procedure, used on a routine basis, 2 mL of fluoridated blood was extracted by SPE. The cocaine metabolite, benzoylecgonine, and opiates were derivatized to their trimethylsilane (TMS) derivatives before analysis by GC-MS (11). Fentanyl, methadone and PCP were analyzed without derivatizing. Cocaine and benzoylecgonine, opiates, PCP, and fentanyl were analyzed in the SIM mode. Methadone was analyzed in the scan mode. Limits of detection were cocaine, 20 ng/mL; benzoylecgonine, 50 ng/mL; morphine, 50 ng/mL; PCP, 10 ng/mL; fentanyl, 2.5 ng/mL; and methadone, 150 ng/mL.

Cases positive for both fentanyl and xylazine were subsequently quantified for xylazine. Basic fractions from in-house SPE procedure were collected and quantified versus a series of methanolic

TABLE 1—*Case series of postmortem samples positive for xylazine and fentanyl.*

	History	Drugs	Specimen concentration	
			Blood	Urine
1	The decedent was found by his wife in cardiac arrest. She called 911 and the decedent was taken to an emergency room (ER). He was pronounced dead on arrival to ER. Past medical history was significant for hypertension and heroin use. Adverse effect of drugs was the cause of death.	Xylazine	Trace	Present
		Morphine		Present
		Fentanyl	8.5 ng/mL	Present
		Cocaine		Negative
		Benzoylecgonine		Present
		Ibuprofen		Present
		Naproxen		Present
2	The decedent was sitting at home and had a witnessed collapse. When EMS arrived, ACLS was started and the decedent was taken to an ER. The decedent was pronounced dead in ER. Past medical history was significant for diabetes and heroin use. Adverse effect of drugs was the cause of death.	Xylazine	Negative	Present
		6-acetylmorphine	Negative	Present
		Morphine	204 ng/mL	
		Fentanyl	27 ng/mL	Present
		Cocaine	47 ng/mL	Present
		Benzoylecgonine	290 ng/mL	
		Cocaethylene		Present
		Codeine	Negative	Present
		Lidocaine		Present
		Methadone	<150 ng/mL	Present
		Phencyclidine	21 ng/mL	Present
		Procaine		Present
		Quinine/quinidine		Present
3	Decedent's wife called 911 because she found him unresponsive at home. She claimed that the decedent looked intoxicated the night before. Past medical history was significant for heroin use. Adverse effect of drugs was the cause of death.	Xylazine	Negative	Present
		6-acetylmorphine		Present
		Fentanyl	4.9 ng/mL	Present
		Alprazolam	27 ng/mL	
		Codeine		Present
		Diphenhydramine		Present
		Lidocaine		Present
4	Decedent was found decomposed in the backyard bleeding from the head. A needle was found near decedent and three bags of heroin were found in the kitchen. Adverse effect of drugs was the cause of death.	Xylazine	130 ng/mL	Present
		6-acetylmorphine		Present
		Morphine		Present
		Fentanyl	27 ng/mL	Present
		Cocaine		Present
		Cocaethylene		Present
		Ecgonine methyl ester (EME)		Present
		Codeine		Present
		Procaine		Present
		Quinine/quinidine		Present
5	Decedent was found unresponsive in the shower by his mother. Past medical history was significant for alcohol and depression.	Xylazine	Negative	Present
		6-acetylmorphine	Urine	Present
		Morphine	Urine	Present
		Fentanyl	41 ng/mL	Present
		Cocaine	51 ng/mL	Present
		Benzoylecgoninr	2300 ng/mL	Present
		Cocaethylene	Negative	Present
		EME		Present
		Alprazolam		Present
		Citalopram		Present
		Diltiazem		Present
		Mirtazapine		Present
		Procaine		Present
		Quinine/quinidine		Present
6	Decedent's sister called 911 because she found him unconscious on the floor at home. The decedent was pronounced dead when EMS arrived. A syringe and drug packets were found in decedent's hand. Adverse effect of drugs was the cause of death.	Xylazine	80 ng/mL	Present
		6-acetylmorphine	Negative	Present
		Morphine	118 ng/mL	
		Fentanyl	47 ng/mL	Present
		Codeine	Negative	Present
		Naproxen		Present

TABLE 1—*Continued*

	History	Drugs	Specimen concentration	
			Blood	Urine
7	Decedent's mother called 911 because she found him unconscious. The decedent was pronounced dead when EMS arrived. A packet of powdery substance was found in decedent's pants' pocket. Past medical history was significant for drug abuse. Adverse effect of drugs was the cause of death.	Xylazine	Negative	Present
		6-acetylmorphine		Present
		Morphine		Present
		Fentanyl	4.7 ng/mL	Present
		Cocaine	<20 ng/mL	Present
		Benzoylecgonine	1900 ng/mL	Present
		EME		Present
		Codeine	Present	Negative
		Diltiazem		Present
		Hydroxyzine		Present
		Ibuprofen		Present
		Lidocaine		Present
		Procaine		Present

standards (50–5000 ng/mL) prepared from an aqueous, veterinary-grade xylazine standard (20 mg/mL) obtained from Bridgewater Veterinary Hospital (Bensalem, PA) and analyzed by EI GC-MS in the scan mode. Principle ions detected were at m/z 205 and 220 (parent ion) with other significant ions at 177 and 145. Retention times of standards and postmortem samples were 10.2 min. PCP-D_5 was used as the internal standard. The limit of detection for xylazine was 50 ng/mL.

Evidence was available and tested on cases 4 and 7. Syringes were rinsed with 200 μL of methanol, washings collected and 1–2 μL of the sample was analyzed by GC/MS in the scan mode. A small amount of powder was removed from packets, dissolved in 200 μL of methanol, and analyzed by GC/MS in the scan mode.

GC-MS analysis was done with HP 5890 GC with 7673 autosampler and 5971 MS or HP 6890 GC with 7693 autosampler and 5973 MS. Chromatography was accomplished with an HP-5MS capillary column (Agilent Technologies, Santa Clara, CA).

Results

Table 1 describes the seven cases that demonstrated heroin use and that also tested positive for xylazine and fentanyl. Six of seven cases were positive for 6-acetylmorphine (6-AM), a metabolite and a definitive marker for heroin. 6-AM was not detected in case 1; however, morphine was detected in the urine and the decedent had a history of heroin abuse. Xylazine was present in urine in all seven cases and blood levels were detected in cases 1, 4, and 7. The blood concentrations ranged from trace to 130 ng/mL. Fentanyl was present in the blood and urine in each case and blood concentrations ranged from 4.7 to 47 ng/mL. Other drugs of abuse and potential adulterants were detected as well, including: cocaine and/or its metabolites (5/7 cases); codeine (4/7); procaine (4/7); and lidocaine (3/7). All decedents were males between 24 and 51 years of age and were clearly multi-drug users.

Evidence was available for testing in only cases 4 and 7. One syringe and two packets from case 4 were tested as previously described and no drugs were detected. Three syringes and one packet from case 7 were tested; no drugs were detected in methanolic washings from the three syringes. However, lidocaine, procaine, heroin, 6-AM, fentanyl, and xylazine were present in the methanolic washings from the packets.

Discussion

Additives are substances added after the initial synthesis of a drug (12). In addition to adulterants, diluents are often added to illicit drugs as "bulking-agents" to increase drug sample size. Sugars are the most common compounds added to cocaine powder. They have very similar appearance to cocaine powder and they are much less expensive than pure cocaine (13). Lidocaine, procaine, codeine, heroin, cocaine, quinine, naproxen, and many other substances have been reported as either cocaine or heroin additives (12). In addition to common additives reported in the literature, fentanyl has recently been described in the Philadelphia press as a heroin additive sold under the brand "Assassin" (14). Concurrently with fentanyl, we detected xylazine which has not been reported previously as a heroin additive.

Table 1 indicates that all the cases tested positive for heroin metabolites (6-AM and/or morphine). Drug samples were available for testing from cases 4 and 7. Case 7 tested positive for lidocaine, procaine, heroin, 6-AM, fentanyl, and xylazine. We have postulated that xylazine and fentanyl might be used as adulterant*s* in heroin and the results show that this combination was present along with other drugs in at least one of the cases (case 7). We are not aware of other reported cases of xylazine used as a drug adulterant in Philadelphia before April 2006. Toxicology testing showed that the decedents were all multi-drug users and the medical examiner concluded that adverse effect of drugs was the cause of death in all of these cases. In these instances normally it is not possible to determine the individual contributions of each drug. This is true in the series of cases in this study as well, although the blood concentrations of fentanyl (range 4.9–47 ng/mL) all significantly exceed the mean maximal concentration of 2.5 ng/mL reported by the manufacturer of the largest, 100 μg/h fentanyl patch (15). The highest xylazine blood level in our cases was 130 ng/mL. Blood levels of 540 and 570 ng/mL were reported to cause severe intoxication and impaired driving (7,16). In addition, interpretation of individual drug involvement was further complicated by the limited medical histories provided.

The definite reason why xylazine, fentanyl, and other substances are specifically chosen as adulterants for illicit drugs, especially heroin, is still unknown. Financial concerns, e.g., increased street sale value, and enhanced drug effects may be some of the driving forces. Another question that remains is the source(s) of the adulterants. In 2006, the U.S. Department of Justice National Drug Intelligence Center reported that hundreds of nonfatal and fatal overdose cases were related to clandestinely produced fentanyl. There were at least nine U.S. clandestine fentanyl laboratories seized between 1990 and 2005. Seven of these laboratories were found in California, Kansas, and Pennsylvania. Mexico was also reported to be another source of clandestinely produced fentanyl and the U.S. intelligence believed that this might have contributed to the recent rise of fentanyl overdoses in the United States (17,18). It is possible that clandestine

fentanyl laboratories may be the source of fentanyl in our cases; however, the source of xylazine is unknown in our cases.

A major challenge for investigating xylazine and its effects in humans is that it is a veterinary drug and is not approved for human use. At the present time, there is no known antidote for xylazine. Unfortunately, its use in humans can be fatal, especially in overdose cases. Fentanyl is an extremely potent synthetic opioid that requires high doses of opioid antagonist to reverse its effects. However, if it is not recognized or suspected early, it also may cause a fatal outcome in overdose cases. Adulteration in illicit drugs has become an epidemic health concern for drug users. Healthcare professionals need to be aware of this issue, so the patients can be treated in an effective, timely manner.

Official Disclaimers

None.

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Additional information and reprint requests:
Stella C. Wong, D.O.
Department of Emergency Medicine
Nazareth Hospital
2601 Holme Avenue
Philadelphia, PA
E-mail: scw101@gmail.com