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Drug-related death: Adulterants from cocaine preparations in lung tissue and blood

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ABSTRACT

The abuse of drugs such as street cocaine is known to cause a variety of toxic effects, some of which involve the lungs and often induce lethal complications. While the toxicity of cocaine itself is reviewed well, the influence of toxic effects of its adulterants on the human body is not thoroughly studied. Therefore, we examined heart blood, femoral vein blood and lung tissue from 11 cases for typically used adulterants in cocaine preparations and check whether if the concentrations in the lung tissue are higher than in the blood. The adulterants were isolated using solid-phase (SPE) and liquid–liquid extraction (LLE) and quantified via high-pressure-liquid-chromatography-time-of-flight–mass spectrometry (LC/TOF–MS). Five adulterants, i.e., phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine, were detected. We found out that the concentration of these substances was often higher in the lung than in the analogous analysed body fluids. It should therefore be considered whether – for the determination in the cause of death – the lung should be examined in addition to heart blood, urine or brain tissue.

1. Introduction

The abuse of drugs such as cocaine or heroin is known to cause a variety of toxic effects that involve the cardiovascular system [1], the brain [2] and the lungs [3]. In cases of fatal intoxication, the cause of death is often attributed to the drug itself. The adulterants, or cutting agents, in drug preparations were assigned only a minor role [4,5], if they were examined at all. Still their role in autonomous effects as well as in addition to cocaine toxicity seems not considered methodically. Adverse side effects or lethal complications may occur when cocaine is found to contain other pharmacologically active compounds. In the past, sugars and sugar alcohols (e.g., mannitol) served as the main adulterants in drug preparations. Currently, cocaine or heroin preparations are adulterated with pharmacologically active substances, such as the antihelminthic levamisole, the analgetics phenacetin and paracetamol and the stimulant caffeine. Occasionally, the local anaesthetics lidocaine, procaine, benzocaine, tetracaine and articaine; the calcium channel blocker diltiazem; the antihistamine hydroxyzine; the analgetics ibuprofen and ketamine; the stimulants pholedrine and phenmetrazine; and atropine are found in cocaine preparations (Table 1). Especially if these drugs are smoked or snorted, apart from the drug itself, all of the adulterants will also be incorporated initially into the lung [6]. Lung damage and lung disease may occur if these substances where ingest systematically [42,43]. For example aminorex a metabolite of levamisole is known to cause pulmonary hypertension and lidocaine may cause pulmonary parenchymal injury [42]. Therefore, the aim of this work was to determine the amounts of several adulterants in lung tissue and to compare these amounts to their concentrations in the heart and in the femoral vein blood. In addition we tried to find out whether the lung tissue could serve as a suitable medium for various forensic-toxicological investigations. The expectation of a longer detection window for some adulterants in the lung and their facile extraction might give additional information in fatal cases even if the concentration of the analyte is below its LOD in the blood.

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Table 1
Typically found adulterants in cocaine preparations with their formula, monoisotopic mass as [M + *n*H] and applications.

Adulterant	Formula	Monoisotopic mass [M + *n*H]	Used as
Ketamine	$C_{13}H_{16}ClNO$	238.0993	Analgetic
Paracetamol	$C_8H_9NO_2$	152.0706	Analgetic
Phenacetin	$C_{10}H_{13}NO_2$	180.1019	Analgetic
Levamisole	$C_{11}H_{12}N_2S$	205.0793	Antihelminthic
Hydroxyzine	$C_{21}H_{27}ClN_2O_2$	375.1833	Antihistamine
Diltiazem	$C_{22}H_{26}N2O_4S$	415.1686	Calcium channel blocker
Atropine	$C_{17}H_{23}NO_3$	290.1750	Drug with a large field of applications
Articaine	$C_{13}H_{20}N_2O_3S$	285.1267	Local anaesthetic
Benzocaine	$C_9H_{11}NO_2$	166.0862	Local anaesthetic
Lidocaine	$C_{14}H_{22}N_2O$	235.1804	Local anaesthetic
Procaine	$C_{13}H_{20}N_2O_2$	237.1597	Local anaesthetic
Tetracaine	$C_{15}H_{24}N_2O_2$	265.1910	Local anaesthetic
Phenmetrazine	$C_{11}H_{15}NO$	178.1226	Stimulant
Pholedrine	$C_{10}H_{15}NO$	166.1226	Stimulant

2. Materials and methods

For determination of adulterants in heart blood, femoral vein blood and lung tissue two extraction procedures were combined because best results were not observed for all adulterants with one method alone.

2.1. Specimen

For the analysis of adulterants, we collected heart blood, femoral vein blood (in only one case was this body fluid not available) and lung tissue from 11 cocaine users (10 males and one female) that were autopsied at the Institute of Legal Medicine of the University Hospital in Duesseldorf. Heart blood was mixed with sodium fluoride (10 mg/ml) before it was stored at −18 °C.

2.2. Chemicals, solutions and materials

The following materials were used for the analysis:

- Levamisole, pholedrine and ketamine were purchased from Sigma-Aldrich (Steinheim, Germany).
- Phenacetin, hydroxyzine, tetracaine and procaine were obtained as reference substances by the State Police of NRW.
- Diltiazem was obtained as reference substances from the Institute of Legal Medicine in Duesseldorf.
- Lidocaine was acquired from Pharma GmbH Stern (Wedel, Germany), benzocaine from Fa. Hoechst (Frankfurt am Main, Germany), cocaine from Merck (Darmstadt, Germany), paracetamol from v.d. Linde Arzneimittel, atropine from Cerilliant Paloma (Round Rock, Texas), phenmetrazine from U.S.P.C., Inc. (Rockville, MD) and articaine from EDQM (Strasbourg, Cedex).
- The certified deuterated standards of cocaine-d_3 (1 mg/ml in acetonitrile), benzoylecgonine-d_3 and methadone-d_9 (each 1 mg/ml in methanol), 7-aminoflunitrazpam-d_7 and 6-monoacetylmorphine-d_3 (each 0.1 mg/ml in acetonitrile) and morphine-d_3, codeine-d_3 and dihydrocodeine-d_6 (each 0.1 mg/ml in methanol) were acquired from LGC Promochem GmbH, Wesel, Germany.
- All other solvents and buffers used in this study were of p.a. quality and were purchased from Merck/VWR (Darmstadt, Germany) and Sigma-Aldrich Chemie GmbH (Steinheim, Germany).
- The Bond Elut Certify 130 mg, 3 ml column for solid-phase extraction (SPE) was obtained from Agilent Technologies (Waldbronn, Germany).

2.3. Instrumentation

Quantitative analysis of the adulterants was carried out using an HPLC system from Agilent (1200 series LC) and a mass spectrometer from Bruker (Bruker Micro TOF MS-Q II) in the ESI mode. A YMC-Pack ODS-AQ column, 150 mm × 2 mm × 3 μm (YMC Europe GmbH), was used at 30 °C oven temperature. The signals were recorded in full scan mode, with a capillary voltage of 4500 V, ranging from 50 to 1000 amu. For the mobile phase, a mixture of acetonitrile (LiChrosolv; Merck KGaA; Darmstadt, Germany) water (containing 0.05% formic acid; Sigma-Aldrich Chemie GmbH; Steinheim, Germany) was used at a flow rate of 0.2 ml/min. The evaluation was performed with the Bruker-Analysis software (Compass version 1.3 Smart Form Manually). For the solid-phase extraction, a RapidTrace SPE Workstation from Biotage AB (Uppsala, Sweden) was used. For the evaporation of the extracts, a heating block (Medax GmbH & Co. KG. Neumuenster, Germany) with nitrogen streamed vial positions (Gebr. Liebisch GmbH & Co. KG; Bielefeld, Germany) was used. For the homogenisation of the lung tissues, an Ultra Turrax from Janke & Kunkel GmBH (IKA-Labortechnik, Staufenheim i. Br., Germany) was used.

2.4. Solid-phase extraction

To 0.6 ml of heart blood (HB), 0.6 ml of femoral vein blood (FVB) or 0.6 g of lung tissue (LT) (homogenised with an Ultra Turrax), 20 μl of deuterated standard mix, 0.1 ml of water, 1 ml of acetonitrile and 0.1 ml of isopropanol were added. The solution was mixed and centrifuged for 10 min at 14,000 rpm (4 °C). Before centrifugation, the lung samples were incubated for 15 min in an ultrasonic bath (Bandelin Electronic; Berlin, Germany). A 0.75 ml aliquot of the supernatant was transferred to a test tube and worked up by automated solid-phase extraction. The extracts were concentrated to dryness under a stream of nitrogen, and 0.05 ml of a water/formic acid mixture was added. Then, 0.005 ml of the final solution was injected into the LC/TOF–MS. The quantifications of phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine were performed with the use of cocaine-d_3 as an internal standard.

2.5. Liquid–liquid extraction

To equal aliquots of the appropriate material (HB, FVB and LT), 20 μl of the deuterated standard mixture (described above 2.4) and 0.1 ml of carbonate buffer (pH 8.6) were added. The specimens were extracted with 1.2 ml of a mixture of dichloromethane/ether (70/30, v/v) and centrifuged. The organic phase was evaporated to dryness, resuspended in 0.05 ml of a water/formic acid mixture and analysed by LC/TOF–MS (see Section 2.4).

2.6. Calibration

Calibrations for Sections 2.4 and 2.5 corresponded to the specimen (blood versus lung tissue) that was used.

Table 2
LoD and LoQ in blood and lung tissue for phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine.

Substance	Blood [ng/ml]		Lung tissue [ng/g]	
	LoD	LoQ	LoD	LoQ
Phenacetin	0.73	2.66	0.48	1.80
Lidocaine	0.53	1.96	0.80	2.89
Diltiazem	0.77	2.79	0.85	3.06
Levamisole	0.57	2.10	0.90	3.20
Hydroxyzine	0.66	2.38	0.48	1.79