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Analysis of cocaine adulterants in human brain in cases of drug-related death

Marianne Knuth*, Oliver Temme, Thomas Daldrup, Evelyn Pawlik

Institute of Legal Medicine, University Hospital Duesseldorf, Moorenstraße 5, 40225 Duesseldorf, Germany

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ABSTRACT

For different reasons, street cocaine is often diluted with pharmacologically active substances, the so-called adulterants such as levamisole or hydroxyzine. A controversial debate exists currently on the uptake of adulterants from cocaine preparations and drug-related death. Previous research convincingly argues that serious adverse side effects that affect the central nervous and cardiovascular systems can be a consequence of adulterated cocaine.

Aims: Having identified the presence of adulterants in lung tissue and blood, the concentrations of these substances in brain, an important target location, was of interest. This provides an opportunity to assess their role in cases of drug-related deaths.

Materials and methods: We developed and validated a method for the analysis of cocaine, two cocaine metabolites and six adulterants, which can typically be found in cocaine preparations, and one adulterant metabolite in brain tissue by gas chromatography–mass spectrometry (GC–MS)[1]. Ten brain samples which were tested positive for cocaine were analyzed. The homogenized brain tissue was embedded into drying paper for protein precipitation. During a subsequent solid-phase extraction (SPE), the eluate and one of the wash fractions were collected. After derivatization with *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) in pyridine and isooctane, the extracts were analyzed by GC–MS.

Results and discussion: The method was fully validated for cocaine (COC), benzoylecgonine (BZE), ecgonine methyl ester (EME), diltiazem (DIL), hydroxyzine (HYD), and levamisole (LEV) and partly validated for cetirizine (CET), lidocaine (LID), phenacetin (PHE), and procaine (PRO) in brain material. By analyzing post-mortem brain tissue of ten cocaine users, LEV, LID, and HYD as well as PHE were identified in contrast to DIL, PRO, and the HYD metabolite CET. HYD and LEV were found in moderate to high concentrations in some cases. Therefore, it cannot be excluded that they have caused adverse side effects.

Conclusion: Because adulterants can potentially affect the central nervous and cardiac systems, it is likely that they enhance COC toxicity.

1. Introduction

According to the report of the European Monitoring Centre of Drugs and Drug Addiction, cocaine (COC) is the most common illegal stimulant. Over three million people in Europe have consumed this drug in 2015 [1]. COC can cause various toxic effects (e.g., seizures, hyperthermia, vasoconstriction, and hypertonia) especially on the central nervous and cardiovascular systems [2]. It was found that the purity of COC preparations, which were sold on the street, ranged between 20% and 75% [1]. The drug was diluted with either the so-called cutting agents or adulterants. Cutting agents are defined as substances that bear no pharmacological activity, such as glucose or mannose [3,4]. By mixing COC with these very cheap agents, a dealer can increase

* Corresponding author.
E-mail address: Marianne.Knuth@med.uni-duesseldorf.de (M. Knuth).

[1] Abbreviations: GC–MS, gas chromatography–mass spectrometry; SPE, solid-phase extraction; MSTFA, *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide; COC, cocaine; BZE, benzoylecgonine; EME, ecgonine methyl ester; DIL, diltiazem; HYD, hydroxyzine; LEV, levamisole; CET, cetirizine; LID, lidocaine; PHE, phenacetin; PRO, procaine; COC-d3, cocaine-d_3; BZE-d_3, benzoylecgonine-d_3; HYD-d_8, hydroxyzine-d_8; LEV-d_5, levamisole-d_5; EME-d_3, ecgonine methyl ester-d_3; SIM, selected ion monitoring; T, target; Q, qualifier ion; GTFCh, German Society of Toxicological and Forensic Chemistry; LoD, limit of detection; LoQ, limit of quantification; ULoQ, upper limit of quantification; RSD, relative standard deviation; ALC, alcohol; COD, codeine; FLU, fluoxetine; IBU, ibuprofen; 6-MAM, 6-monoacetylmorphine; MID, midazolam; MOR, morphine; NOS, noscapine; NRD, nordazepam; NRT, nortilidine; PAR, paracetamol; SIL, sildenafil; TIL, tilidine; THC, tetrahydrocannabinol; ZOP, zopiclone.

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his profit [4]. Adulterants that are pharmacologically active might be used to potentiate the effect of COC or to minimize adverse side effects that occur because of COC abuse [3]. In COC preparations, substances such as hydroxyzine (an antihistaminic [5]), levamisole (an anthelmintic [6]), or diltiazem (a calcium channel blocker [7]) or local anesthetics (benzocaine, lidocaine, or procaine [8]) have been identified as adulterants. It was suggested that the protective effects of calcium channel blockers against the cardiac toxicity might be the reason for adding them. Contrary to these assumptions, Derlet and Albertson [9] showed that diltiazem also potentiates COC toxicity. Local anesthetics induce the effect of oral numbness of COC and lead consumers to assume high quality when they taste the drug [3,4]. Moreover, COC is adulterated with analgesics such as phenacetin [4,10] or paracetamol [5]. It has been suggested they ease pain that may occur after the use of COC [11]. Because of these hypotheses, Broséus et al. [33] suggested that adulteration is more strategic than random or unpredictable. Furthermore, they reported that COC adulteration is very dynamic concerning the number of cutting agents, the variation in frequencies of appearance and concentration. For example, today the main COC adulterants are PHE, LEV, DIL, HYD, LID and caffeine but in 1990, only LID and sugars were used for dilution [33].

At present, a controversial debate on the status of these adulterants in post-mortem toxicology exists. On the one hand, it is asserted that adulterants do not increase the toxicity of COC. On the other hand, a study by Brunt et al. [3] convincingly argues that serious adverse side effects are a consequence of adulterated COC. In particular, hydroxyzine and diltiazem are associated with a significantly higher likelihood of cardiac effects (arrhythmia) or hallucinations. Because of these effects and numerous other adverse side effects, such as headaches, tremor, or allergic reactions linked to adulterants, it could not be excluded that they play a considerable role in fatal COC intoxications [3]. To better understand the central side effects of certain COC preparations, it is of interest to know to what extent these adulterants can pass the blood–brain barrier. Therefore, the purpose of the present study was to develop and validate a gas chromatography–mass spectrometry (GC–MS) method for the analysis of COC, the COC metabolites benzoylecgonine (BZE) and ecgonine methyl ester (EME), six adulterants (diltiazem (DIL), hydroxyzine (HYD), levamisole (LEV), lidocaine (LID), phenacetin (PHE), and procaine (PRO)), and the HYD metabolite cetirizine (CET) (see Fig. 1) in brain tissue. These adulterants were quantified in ten human brain samples to assess their role in cases of drug-related death.

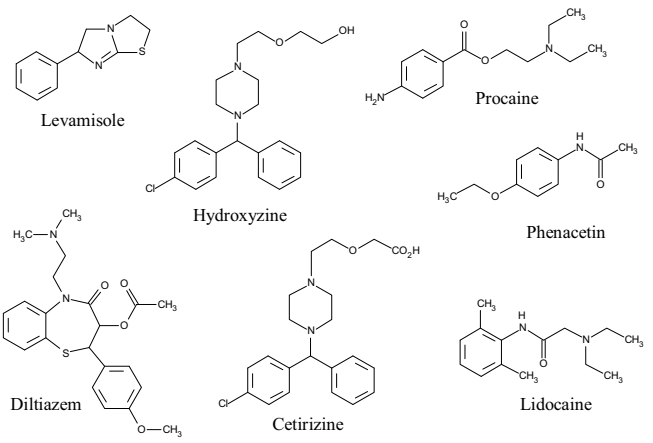

Fig. 1. Typically found adulterants (levamisole, phenacetin, hydroxyzine, diltiazem, lidocaine, and procaine) in cocaine preparations and the hydroxyzine metabolite cetirizine.

2. Materials and methods

For the GC–MS analysis of COC, two COC metabolites (BZE and EME), six adulterants (DIL, HYD, LEV, LID, PRO, and PHE), and CET in brain tissue, a solid-phase extraction (SPE) was performed. Besides the regular extraction fraction, a wash fraction (H_2O) was collected.

2.1. Chemicals and reagents

Reference standards of LEV and PHE were given by the State Bureau of Criminal Investigation NRW (Duesseldorf, Germany). BZE, CET, COC, DIL, EME, HYD, and PRO were inventory of the Institute of Legal Medicine (Duesseldorf, Germany), and LID was purchased from Sigma Aldrich (Steinheim, Germany). Certified deuterated standards of cocaine-d_3 (COC-d_3), benzoylecgonine-d_3 (BZE-d_3), hydroxyzine-d_8 (HYD-d_8), and levamisole-d_5 (LEV-d_5) were purchased from LGC standards (Wesel, Germany), and ecgonine methyl ester-d_3 (EME-d_3) was purchased from Radian Corporation (Austin, USA). Acetanilide was provided by the Institute of Organic Chemistry (Duesseldorf, Germany). All other solvents and sodium hydrogen phosphate dihydrate and potassium dihydrogen phosphate (p.a. quality) were supplied by either Merck/VWR (Darmstadt, Germany) or Macherey Nagel (Dueren, Germany).

2.2. Materials

Bond Elut Certify columns (3 ml) were purchased from Agilent Technologies (Waldbronn, Germany) and used in a RapidTrace SPE Workstation from Biotage AB (Uppsala, Sweden). An Ultra Turrax Tube Drive and the tubes were obtained from IKA (Staufen, Germany).

2.3. Specimens

Ten post-mortem brain samples were collected during autopsy at the Institute of Legal Medicine. The samples were stored at −18 °C.

2.4. SPE and GC–MS analysis of brain samples

The brain samples were extracted from 300 mg homogenized brain tissue, which was embedded in drying paper and centrifuged (14,000 rpm for 10 min). After the addition of 100 μl isopropanol, 20 μl of an internal standard mix (acetanilide, BZE-d_3, COC-d_3, EME-d_3, HYD-d_8, and LEV-d_5), and 1000 μl acetonitrile, the samples were mixed and centrifuged again. Subsequently, 750 μl of the supernatant was used for SPE with Bond Elut Certify columns. Besides the regular extraction fraction, a wash fraction (H_2O) was collected for the analysis of PHE. This fraction was extracted with a mixture of 2 ml dichloromethane/isopropanol/ammonia (80/20/4 v/v/v). The extracts of both fractions were then evaporated to dryness under a stream of nitrogen. Derivatization was accomplished by the addition of 200 μl of a mixture containing isooctane, MSTFA, and pyridine (100/50/50 v/v/v). The samples were incubated at 90 °C for 30 min. One microliter of the final solution was injected on a 30 m, 0.25 μm HP-5-phenylmethylpolysiloxane column. An Agilent 7890A gas chromatograph combined with an Agilent 5975C mass spectrometer was used. The following GC conditions were adjusted: splitless injection at 270 °C with a subsequent temperature gradient (60 °C for 2 min, 40 °C/min to 110 °C, and 13 °C/min to 282 °C for 8 min) with helium as carrier gas. A selected ion monitoring (SIM) acquisition method with three or four masses per analyte was used for detection (Table 1). Data were interpreted on a MSD-Chemstation. For quantification, CET and DIL as well as HYD were analyzed using HYD-d_8 as the internal standard. LEV-d_5 served as the reference for LID and LEV, and PHE was related to acetanilide.