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Hermansen, Simon Kjær; Christoffersen, Dorte Jensen

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Common opioids and stimulants in autopsy and DUID cases: A comparison of measured concentrations

Simon Kjær Hermansen*, Dorte Jensen Christoffersen

Section of Forensic Toxicology, Department of Forensic Medicine, Faculty of Health Sciences at the University of Southern Denmark, Odense, Denmark

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ABSTRACT

Quantitative results from toxicological analyses of autopsy material are widely compared to ranges in reference works to determine if drug concentrations are in relevant levels for establishing intoxication. This study compares concentrations of commonly used opioids and stimulants from drug addict autopsies and driving under the influence of drugs (DUID) cases to supplement current knowledge of the possible span and overlaps of measured concentrations. The study included whole-blood results from forensic autopsies of drug addicts performed from 2015 to 2020 (n = 220) and DUID cases from 2015 to 2019 (n = 7088). The focus was on heroin/morphine, methadone, cocaine, amphetamine and MDMA concentrations because these drugs are commonly encountered in both fatal intoxications and DUID cases and the potential for abuse is well known. In the DUID group, the opioids heroin/morphine and methadone and the stimulants amphetamine and MDMA were often seen in concentrations above the reported lower comatose-fatal level whereas cocaine was almost always below. Thus, based on our data, the potential for false assessment of intoxication cases when comparing to reported comatose-fatal limits appears greatest on lower end concentrations of heroin/morphine, methadone, amphetamine and MDMA, whereas false assessment of cocaine appears less likely because most control cases are below reported comatose-fatal levels.

1. Introduction

Interpretation of toxicological findings from autopsy material is inherently difficult as several factors potentially influence results. These factors include age, health status, drug-intaking history, circumstances of death, drug redistribution etc. Still, quantitative results from toxicological analyses are widely compared to ranges in existing reference works to determine if drugs are in relevant range for proving intoxication. Several fatal intoxication reference-works exist [1–5], however, this study aims to supplement current knowledge of the possible span and potential overlaps of recently measured concentrations of commonly used opioids and stimulants in own cases and controls and discuss the consequences hereof.

We present results from fatal intoxications of drug addicts and compare them to drug addicts whose death was not drug-related and results from individuals driving under the influence of drugs (DUID), knowing that these individuals were alive during sampling. A similar case-control course of action has previously been applied for establishing widely recognized postmortem reference values [2,6–8]. We focused on the drugs heroin/morphine, methadone, cocaine, amphetamine and MDMA and discuss obtained concentrations in DUID controls compared to drug addict findings. Comparing post-mortem concentrations with DUID samples will not take post-mortem drug redistribution into account. Caution should be made when comparing post- and antemortem samples.

Compared to the rest of the Nordic countries, Denmark has a high number of fatal methadone intoxications and deaths from CNS stimulants like cocaine, amphetamine and MDMA is increasing across the Nordic countries thus making a study like this of relevance [9]. After methadone, heroin/morphine accounts for most fatal poisonings.

The same blood sampling system was used on both the autopsy and the control groups facilitating optimal basis for comparison without distortion by different blood preparation techniques such as plasma versus whole blood or serum or using different conservation additives which could impact results [10,11]. Furthermore, samples were all analyzed in the same laboratory and on the same type of analytical equipment.

The forensic data originates from police districts of Southern Denmark and Funen, covering approximately 1 million inhabitants resulting in approximately a thousand DUID cases and one hundred

* Corresponding author.
E-mail addresses: shermansen@health.sdu.dk (S.K. Hermansen), dchristoffersen@health.sdu.dk (D.J. Christoffersen).

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and fifty autopsies per year. The districts of Funen and Southern Jutland has the highest prevalence of heroin/morphine in DUID cases in the country and the second highest prevalence of methadone [12], thereby creating good basis for establishing representable concentrations.

2. Material and methods

2.1. Sample material

The present study involved whole-blood results from forensic autopsies of drug addicts performed from 2015 to 2020 (n = 220) and DUID cases from 2015 to 2019 (n = 7088).

Included drug addict cases were deaths, where it has been concluded that the subjects at or before time of death were misusers of illegal drugs of abuse, i.e. drugs included in the Danish Ministry of Health announcement number 698 of 31. august 1993 or later revisions concerning illegal drugs of abuse (excluding cannabis and khat). The information of drug abuse might be obtained from police reports, autopsy reports, forensic toxicology reports, information from witnesses or from other institutions.

The cause of death was reviewed on a case-to-case basis and agreed upon by examining final forensic reports establishing cause of death and categorizing them for subsequent analysis. Drug addicts were grouped into fatal mono-intoxications (A), fatal combination-intoxications (mixed cases including ethanol) (B) and death by other causes than intoxication (C). Death by other causes could be hanging, drowning, stabbing etc. Cases involving pronounced putrefaction, use of different matrices (heart blood, liver blood, muscle tissue, urine etc.) or history of hospital treatment were excluded from the study. Average time between time of death and laboratory sample receival was 8 days (range 2–19 days). In cases where time of death remained unknown, the finding date of the body was used instead. DUID cases (D) consisted of routine cases found positive for one or more drugs above the Danish legal limit (limits listed in [13]). Group D can in some rare cases consist of injured drivers, but no post-mortem samples were in the group. To enable comparison between groups, DUID concentrations were in this case unadjusted for analytical uncertainty (not minimum values). In Denmark, DUID cases are normally subtracted by a 33% safety margin to compensate for analytical uncertainty (reporting of minimum values).

2.2. Analytical methods

Samples were screened using liquid chromatography coupled with a quadropole time-of-flight mass spectrometer (LC-QTOF MS). The LC was a Dionex UltiMate 3000 Rapid Separation ultra-high-performance LC (UHPLC) (Thermo Fisher Scientific, MA, USA) with a VanGuard Pre-Column C18, 1.7 µm,2.1 × 5 mm (Waters Corporation, MA, USA) and an Acclaim RSLC 120 C18. 2.2 µm, 120 Å,2.1 × 100 mm column (Thermo Fisher Scientific, MA, USA). The MS was an Impact II or HD QTOF (Bruker, Billerica, MA, USA).

Screening was performed in both positive and negative ionization mode. For positive ionization mode mobile phases were H_2O/MeOH 9:1 (v/v) with 5 mM ammonium formate and 0,01% formic acid (mobile phase A) and MeOH with 5 mM ammonium formate and 0,01% formic acid (mobile phase B). For negative ionization mode mobile phases were H_2O/MeOH 9:1 (v/v) with 5 mM ammonium acetate (mobile phase C) and MeOH with 5 mM ammonium acetate (mobile phase D). All reagents were LC-MS grade.

Quantitative analysis was performed using an LC coupled with a triple quadropole mass spectrometer (LC-MS/MS). The LC was a Dionex UltiMate 3000 Rapid Separation ultra-high-performance LC (UHPLC) (Thermo Fisher Scientific, MA, USA) with a VanGuard Pre-Column C18, 1.7 µm, 2.1 × 5 mm (Waters Corporation, MA, USA) and an Acquity UPLC BEH C18 Column, 130 Å, 1.7 µm, 2.1 mm × 50 mm (Waters Corporation, MA, USA). The MS was a TSQ Endura Triple Quadropole Mass spectrometer (Thermo Fisher Scientific, MA, USA). Mobile phase A and B (defined above) was used.

The laboratory is accredited under DS/EN ISO/IEC 17025:2017 and participates in proficiency scheme LGC “Forensic Blood Toxicology” (QUARTZ).

2.3. Standards

Drug identification was confirmed and controlled using certified analytical standards from accredited suppliers. Mixed standard solutions were prepared and stored at − 20 °C prior to analysis. Deuterated internal standards (IS) were amphetamin-D11, buprenorphin-D4, cocain-D3, diazepam-D5, LSD-D3, MDMA-D5, methadon-D9, morphin-D6 and pentobarbital-D5.

2.4. Sample preparation

Whole-blood was sampled by medical staff into Vacuette sodium fluoride and potassium oxalate (FX) tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) and delivered to Section of Forensic Toxicology. For analysis, 0.25 g of whole-blood was aliquoted. Ten µl IS mix was added followed by protein precipitation carried out by adding 500 µl acetonitrile to each sample tube. Samples were then mixed using a whirl-mixer for 1 min and cold-centrifugated for 10 min at 15,000 rpm. Supernatant was extracted and diluted 1:1 by mixing 200 µl sample with 200 µl type 1 ultrapure water (Milli-Q® IQ 7000 Ultrapure Water System, Merck KGaA, Darmstadt, Germany).

2.5. Data processing and graphical representation

Statistical tests were performed using GraphPad Prism 9 for Windows (LLC Inc.). Groups did not pass normality tests; thus, nonparametric tests were used for groups comparisons with a 0.05 significance level. Nonparametric tests were Kruskal-Wallis test with Dunns' test (uncorrected) for multiple comparison. Box and whisker plots were used for graphical representation of data. Boxes are 25th to 75th percentiles and the line in the middle represents the median. Whiskers are 10th to 90th percentiles but for groups where n < = 3 range was plotted. Obtained concentrations were compared to the widely recognized comatose-fatal (from) reference concentrations in plasma reported in [14]. In case of methadone, the comatose-fatal plasma concentration was converted to whole blood using a blood/plasma (b/p) ratio of 0.7 [15]. In case of MDMA a b/p of 1.2 was used [15]. In Danish forensics, quantitative results for psychoactive substances are reported in mg/kg instead of mg/L (or ng/mL) as they are based on weighed samples. Reported concentrations in mg/kg can be considered equivalent to mg/L under the assumption that the density of the sampled blood is ≈ 1 g/mL.

3. Results

Total eligible cases are listed in Table 1.

Morphine levels in the case and control groups were illustrated in Fig. 1. Median morphine levels were significantly higher in both

Table 1
Eligible cases in the groups.

	Cases (n)		Controls (n)		
Drug	Group A	Group B	Group C	Group D	Total
Morphine	12	48	8	192	260
Methadone	8	60	9	189	266
Cocaine	2	18	4	682	706
Amphetamine	2	8	6	1529	1545
MDMA	1	2	0	180	183

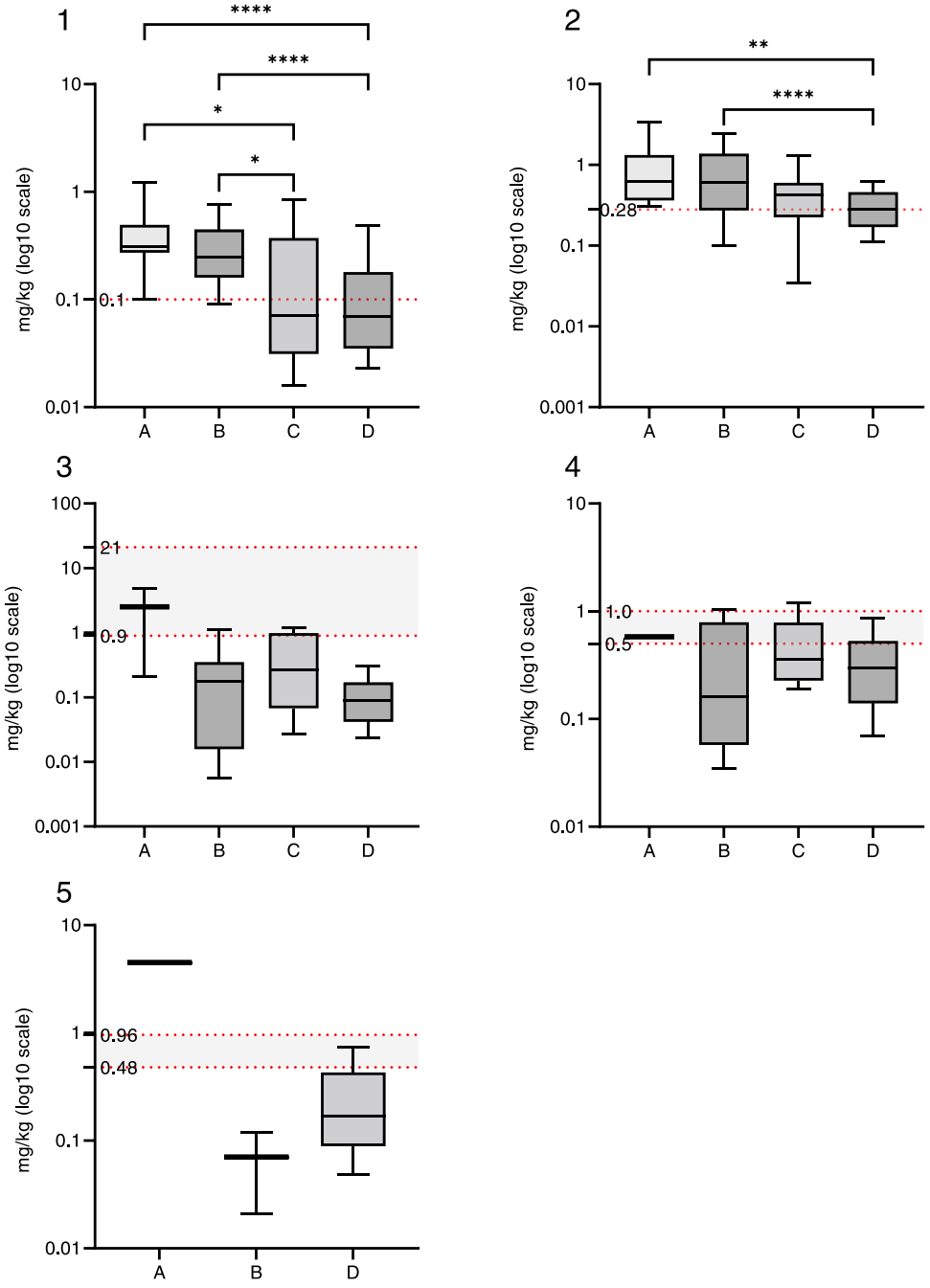

Fig. 1. 1; Morphine, 2; Methadone, 3; Cocaine, 4; Amphetamine, 5; MDMA (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Red dotted lines indicate recognized comatose-fatal (from) reference concentrations in plasma [14]. Methadone and MDMA plasma concentrations were adjusted to whole blood concentrations using blood/plasma ratios from [15]. Gray area used when reference works indicated ranges.

the fatal mono-intoxications and the fatal combination-intoxications cases compared to control groups. The 10th percentile in group A and B corresponded to the recognized comatose-fatal lower limit listed in [14]. Notably, 39% of individuals in the DUID control group exhibited whole blood concentrations above the comatose-fatal lower limit with 5 individuals having extreme concentrations above 2.0 mg/kg.

In Fig. 1 methadone levels were significantly higher in the intoxication groups A and B compared to DUID cases (group D). The 10th percentile in group A corresponded with the comatose-fatal lower limit as do median levels in DUID cases.

Cocaine in Fig. 1 showed no statistical difference between groups and overlaps between cases and controls were smaller than for morphine and methadone. Very few fatal mono-intoxications with cocaine existed and the large majority of DUID cases had concentrations below the comatose-fatal lower limit.

In the case of amphetamine (Fig. 1) few fatal intoxications existed but 101 (7%) of the DUID cases had concentrations in the comatose-fatal lower area.

MDMA in Fig. 1 few or none eligible cases existed in groups A, B and C, and comparison between groups should not be made. However, more than 20% of the DUID cases had concentrations in the comatose-fatal area.

4. Discussion

4.1. DUIDs are often above the lower limit of fatal intoxication

The present study focused on heroin/morphine, methadone, cocaine, amphetamine and MDMA concentrations in drugs addicts and DUID cases. These drugs are commonly encountered in both fatal intoxications and DUID cases and the potential for abuse is well known. DUID cases did in many instances exhibit whole blood concentrations well above the established lower limit of fatal intoxication. A considerable overlap was to be expected due to tolerance of especially the opioids. However, in case of heroin/morphine, we did not expect to see 39% of cases above the reported comatose-fatal lower limit. Furthermore, the examples above 2.0 mg/kg are considerable above concentrations seen in most of our fatal mono-intoxications. It should be noted that we do not know the exact circumstances of these cases, but individuals in the DUID group were involved in driving a vehicle at time of arrest without immediate subsequent autopsy. The 5 individuals in the DUID group having extreme heroin/morphine concentrations well above 2.0 mg/kg are all suspected to be tolerant users due to concomitant methadone and benzodiazepines in the blood samples. Methadone is an indicator of active treatment for opioid dependence and the combination with benzodiazepines is commonly seen in drug addicts. Also, a single individual is represented in two of the extreme cases, thus displaying repeat offenses. Even though median levels are still significantly different between cases and controls these findings underline the importance of case history when evaluating fatal intoxications among drug addicts and tolerant individuals. A case compilation has listed large morphine concentrations in surgical patients from 0.8 to 2.6 mg/L; all patients required assisted ventilation when subjected to these concentrations [15]. The same reference work listed concentrations from 0.2 to 2.3 mg/L in fatalities. Here we report that these ranges are also possible in living individuals driving under the influence, underlining that it is practically impossible to evaluate cases on the heroin/morphine concentrations alone. Similar observations were made for methadone and amphetamine. For methadone, DUID controls grouped evenly around the lower comatose-fatal limit from [14], meaning that about half the individuals were driving with comatose-fatal levels. Amphetamine is often compared to cocaine in terms of effects, user groups and side effects. In our data, amphetamine in DUID controls had a markedly bigger cross-over into comatose-fatal levels than cocaine. This was also true for MDMA.

The DUID group would in all probability consist of a mixture of both naïve and tolerant users, pain patients, drug addicts and represent various routes of administration (nasal, inhalation, intravenous etc.) making it useful for distribution analysis – especially towards the higher end of the scale, due to the high number of cases. It should be noted that only cases above legal limits are represented which is expected to shift median levels slightly upward compared to all apprehended drivers. Morphine and methadone 10th percentiles of fatal mono-intoxications corresponds to the lower comatose-fatal limit. This is expected when stringently applying reference concentrations for assessing intoxication. Thus, these data should not be used for corroborating existing limits, as they themselves are based hereupon.

In case of morphine, the lower comatose-fatal limit reported in [14] seem to be based on a single observation in [16]. The case is an intravenous multiple drug overdose of a 45-year-old woman. Others have put ranges for morphine fatalities from 0.2 – 2.3 mg/L and 0.3–0.43 mg/L for heroin fatalities [15]. Thus, the 0.1 mg/L lower comatose fatal limit is probably an extreme low, where intoxication potentially was aided by combining other drugs.

To summarize, in the DUID group, the opioids heroin/morphine and methadone and the stimulants amphetamine and MDMA were often seen in concentrations above the reported lower comatose-fatal levels whereas cocaine was almost always below. It is not the study's objective to question the limits, merely to supplement current knowledge about the possible span and potential overlaps of measured concentrations together with a data-based evaluation of which drugs are most prone to false assessment regarding fatal intoxications. Based on our data, the potential for false assessment of intoxication cases when comparing to reported comatose-fatal limits appear greatest on lower end concentrations of heroin/morphine, methadone, amphetamine and MDMA, whereas false assessment of cocaine appears less likely because almost all control cases are below reported comatose-fatal levels.

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CRediT authorship contribution statement

Simon Kjær Hermansen: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Dorte Jensen Christoffersen:** Conceptualization, Data curation, Writing – review & editing, Supervision.

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Declarations of interest

None.

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