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Evaluation of poly-drug use in methadone-related fatalities using segmental hair analysis

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ABSTRACT

In Denmark, fatal poisoning among drug addicts is often related to methadone. The primary mechanism contributing to fatal methadone overdose is respiratory depression. Concurrent use of other central nervous system (CNS) depressants is suggested to heighten the potential for fatal methadone toxicity. Reduced tolerance due to a short-time abstinence period is also proposed to determine a risk for fatal overdose. The primary aims of this study were to investigate if concurrent use of CNS depressants or reduced tolerance were significant risk factors in methadone-related fatalities using segmental hair analysis. The study included 99 methadone-related fatalities collected in Denmark from 2008 to 2011, where both blood and hair were available. The cases were divided into three subgroups based on the cause of death; methadone poisoning ($N = 64$), poly-drug poisoning ($N = 28$) or methadone poisoning combined with fatal diseases ($N = 7$). No significant differences between methadone concentrations in the subgroups were obtained in both blood and hair. The methadone blood concentrations were highly variable (0.015–5.3, median: 0.52 mg/kg) and mainly within the concentration range detected in living methadone users. In hair, methadone was detected in 97 fatalities with concentrations ranging from 0.061 to 211 ng/mg (median: 11 ng/mg). In the remaining two cases, methadone was detected in blood but absent in hair specimens, suggesting that these two subjects were methadone-naive users. Extensive poly-drug use was observed in all three subgroups, both recently and within the last months prior to death. Especially, concurrent use of multiple benzodiazepines was prevalent among the deceased followed by the abuse of morphine, codeine, amphetamine, cannabis, cocaine and ethanol. By including quantitative segmental hair analysis, additional information on poly-drug use was obtained. Especially, 6-acetylmorphine was detected more frequently in hair specimens, indicating that regular abuse of heroin was common among the deceased. In conclusion, continuous exposure of methadone provide by segmental hair analysis suggested that reduced tolerance of methadone was not a critical factor among methadone-related fatalities. In contrast, a high abundance of co-ingested CNS depressants suggested that adverse effects from drug-drug interactions were more important risk factors for fatal outcome in these deaths.

1. Introduction

Methadone is a long-acting opioid primarily used in the treatment of opioid-dependence. However, it is also widely sold on the illicit market. In Denmark, fatal poisoning among drug addicts is commonly related to methadone, and an increase in methadone-related fatalities has occurred through the years [1].

The primary mechanism contributing to fatal opioid overdose is respiratory depression. Drug–drug interactions during concurrent use of central nervous system (CNS) depressants will heighten the risk of acute fatal respiratory depression [2]. Several studies have shown that an abundance of co-ingested drugs is common among methadone-related deaths [3–5]. Especially, sedative drugs such as benzodiazepines and alcohol are frequent in these cases followed by the major illicit drugs: cannabis, amphetamine and morphine/heroin. When alcohol and benzodiazepines are ingested alone the respiratory-depressant effect is mild, however concurrent use with opioids will increase or pro-long the respiratory-effect of opioids [2].

Reduced tolerance due to a drug-free period prior to death also determines a risk for opioid poisoning. Loss of tolerance is a gradual process which occurs within days to weeks. The degree of tolerance is difficult to determine and in the absence of a suitable

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biomarker different approaches have been used to investigate the phenomena of tolerance [5–11]. Metabolite to parent drug ratios in blood and urine have been used as a tool to evaluate short-time abstinence in opioid-related deaths. A low ratio would indicate a recent single intake while a high ratio would indicate a continuous intake [5,8–10,12]. Another approach is to use segmental hair analysis, which can provide a historical calendar of drug intake [7,11]. The usefulness of segmented hair analysis has been demonstrated for the investigation of abstinence in 28 heroin overdose deaths [7]. In 18 cases, morphine was absent in the proximal segment indicating reduced tolerance. Although, the survey showed that careful segmental hair analysis could reveal a recent opioid abstinence, the authors concluded that concurrent intake of other drugs was more important for these deaths.

The main purpose of this study was to evaluate poly-drug use in 99 methadone-related fatalities using segmental hair analysis. To study the abuse pattern in the last 2.5 month prior to death, the hair samples were carefully sectioned into three short segments; S1: 5 mm, S2: 10 mm and S3: 10 mm. Additionally, we compared the blood specimen with the proximal hair segment to reveal a possible abstinence of methadone. Chiral analysis of methadone was performed to examine the variation between concentrations of the active *R*-enantiomer and total methadone.

2. Materials and methods

2.1. Subjects and biological specimens

Our study included 99 methadone-related fatalities collected in Denmark in the period from January 2008 to December 2011. A criterion for the selection of cases was that both femoral blood and hair were available. The cause of death was determined by two physicians based on the case circumstances, forensic autopsy and toxicological analysis, and the cause of death was classified as: (a) methadone poisoning ($N = 64$), (b) poly-drug poisoning including methadone ($N = 28$), or (c) methadone poisoning in combination with fatal diseases such as untreated diabetes, severe pneumonia or cancer ($N = 7$).

Femoral blood and hair samples were routinely collected during forensic autopsies by trained forensic technicians. Blood samples were stabilised with 10 mg sodium fluoride and 22.5 mg oxalate in a 10-mL container and stored at −20 °C until analysis. Hair samples of approximately 1 cm in diameter were collected from the posterior vertex region as close to the scalp as possible and stored in tinfoil at room temperature until analysis.

2.2. Analytical procedures

Initially, a toxicological screening was performed in whole blood using HPLC–TOF–MS, and all positive findings were quantified by HPLC–MS/MS or UHPLC–MS/MS as a standard procedure in our laboratory [13–15]. Chiral analysis of methadone and the main metabolite 2-ethylidine-1,5-dimethyl-3,3-diphenyl-pyrrolinium (EDDP) in blood was performed with a LC–MS/MS method described by Holm and Linnet [16].

Hair samples were carefully aligned and sectioned into three segments; 5 mm long for the proximal segment (S1) and 10 mm long for the two outer segments (S2 and S3). Assuming a hair growth of approximately 0.35 mm/day [17], a positive detection in S1 corresponds to drug exposure within 14 days prior to overdose. Absence of methadone in S1 combined with a positive detection in blood would then provide an indication of a possible withdrawal period.

To achieve enough hair material, two bundles of hair collected from the same case were analysed as genuine duplicate measurements with an UPLC–MS/MS method described by Nielsen et al. [18]. Briefly, the hair segments were washed with 1 mL 2-propanol for 5 min, two times with 1 mL purified water for 5 min and at last with 1 mL 2-propanol for 1 min. The last washing water was analysed to check for external contamination. The hair segments were dried overnight, and about 10 mg of each segment was weighted, pulverised and incubated in a mixture of methanol, acetonitrile and 2 mM ammonium formate (25:29:24, v/v) for 18 h. The method included 31 commonly abused drugs as follows: alprazolam, amphetamine, benzoylecgonine (BZE), bromazepam, chlordiazepoxide, clonazepam, 7-aminoclonazepam, cocaine, codeine, diazepam, nordiazepam, EDDP, flunitrazepam, 7-aminoflunitrazepam, ketamine, ketobemidone, methadone, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxy-methamphetamine (MDMA), morphine, 6-acetylmorphine (6-AM), nitrazepam, 7-aminonitrazepam, oxazepam, oxycodone, temazepam, tramadol, *O*-desmethyltramadol, zolpidem and zopiclone. Antidepressants and antipsychotics were not included in the hair method, since regular treatment with these substances in most cases was apparent from the medical records. In 13 cases, only two segments were analysed due to the length of the hair. Chiral analysis of methadone and EDDP in hair extracts was performed with the same LC–MS/MS method used for blood analysis.

The chiral LC–MS/MS method was fully validated for hair analysis prior to the conduct of this study. The limit of quantification was 0.0125 ng/mg ($N = 12$) for all four enantiomers with precision (CV%) less than 14% and accuracy within 97–112%. A linear range was established from 0.0125 to 25 ng/mg for all four enantiomers. For levels above the limit of quantification, the precision was $\leq$16% and the accuracies were within 91 and 104% for all four enantiomers. CVs of three control samples analysed in 20 series were below 6% for *R*- and *S*-methadone and 10% for *R*- and *S*-EDDP, respectively. The accuracies were 93–113% for *R*- and *S*-methadone and 79–121% for *R*- and *S*-EDDP, respectively. The mean absolute recoveries were between 105 and 109% for all four enantiomers.

2.3. Statistical analysis

Analysis of differences between two groups was performed with the Mann–Whitney test and analysis of differences between multiple groups was performed with the Kruskal–Wallis test. Comparison between concentrations of *R*-enantiomer and *S*-enantiomer was performed with Wilcoxon's rank test. Correlation between the concentrations of *R*-methadone and total methadone were evaluated by the Spearman rank correlation coefficient.

3. Results

In total, 99 methadone-related fatalities were included in the study, where methadone contributed to the cause of death either as single drug poisoning (a), poly-drug poisoning (b) or single drug poisoning combined with fatal diseases (c). The deceased were most commonly males (76%) with a mean age of 42 years (range: 20–68 years). Poor health conditions were prevalent among the deceased, and the most common pathological findings were hepatitis, cirrhosis and pneumonia.

The methadone concentrations in blood and hair are summarised in Table 1. The methadone blood concentrations were highly variable and ranged between 0.015 and 5.3 (median: 0.52 mg/kg) regardless of the cause of death. There was no significant difference between methadone blood concentrations in the subgroups ($P > 0.05$).

In hair, methadone was detected in 97 fatalities. In 96 of these cases, the methadone hair concentrations ranged from 0.040 to 76 ng/mg. In the last case, high methadone concentrations were detected in both hair (150–211 ng/mg) and blood (5.3 mg/kg). The