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Short communication

Challenges in interpretation of forensic toxicological findings for opiates: case report and a literature review

Marija Bujaroska*, Nataša Bitoljanu, Ljupčo Čakar, Renata Jankova-Ajanovska, Zlatko Jakjovski, Verica Poposka, Aleksej Duma

Institute of Forensic Medicine, Criminology and Medical Deontology, Medical Faculty, Ss. Cyril and Methodius University, Skopje, Macedonia

Introduction

The routine screening in the Laboratory of Forensic Toxicology encompasses assays for determination of psychoactive substances, commonly used drugs and presence of ethanol. The positive finding on opiates does not always imply the presence of heroin (HER) and/or its metabolites because various drug formulations contain an opiate as an active substance. The detection of HER and its main metabolite 6-monoacetylmorphine (6-MAM) as a general marker of HER use, poses some analytical challenges, mainly due to their short half-lives (Goldberger et al.,1994; Paterson and Cordero, 2006; Rook et al., 2006). The scope of this study includes 3 forensic cases with positive findings on opiates, where the presence of HER and 6-MAM could not be detected, but some additional markers of HER use were identified.

Materials and methods

The analyses which are subject of this study were conducted by routine screening for the presence of psychoactive substances, using fluorescence polarization immunoassay and/or biochip array technology for urine and blood samples, respectively. The positive results were confirmed by gas chromatography-mass spectrometry (GC-MS), after previous sample preparation using ion exchange solid phase extraction (SPE) columns. In the case of urine samples, acid hydrolysis was performed prior to SPE, in order to determine free opioid alkaloids.

Results

Case 1: The routine screening of the postmortem urine samples did not show positive results for the presence of psychoactive substances. However, further analyses using GC-MS were conducted due to the information on the presence of a syringe and an unknown powdered substance near the body of the deceased. The GC-MS analysis of the serum revealed the presence of the following substances: caffeine, morphine (MOR), acetaminophen and traces of noscapine, papaverine and hydrocodone. In the unknown powder sample and the syringe, acetaminophen, caffeine, codeine (COD), 6-MAM, papaverine, MOR and noscapine were detected.

Case 2: Positive results on the presence of opiates and methadone in postmortem blood and urine samples were obtained during the routine screening. Further GC-MS analyses of the biological samples were conducted and the following substances were identified in the serum: nicotine, cotinine, caffeine, theophylline, theobromine, paracetamol, methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), metamizol, MOR, meconine and traces of COD. The same substances were identified in the urine sample, along with noscapine, hydrocotarnine and desmethylpapaverine.

Case 3: Screening of postmortem urine samples showed positive finding on opiates, whereas postmortem blood screening showed presence of opiates, methadone, benzodiazepines and cannabinoids. The GC-MS analysis confirmed the presence of caffeine, nicotine, tramadol, methadone and meconin in serum. The same substances were detected in the urine sample, along with COD, MOR, papaverine, hydrocotarnine and paracetamol.

* bujaroska_m@yahoo.com

Discussion

HER is quite unstable in aqueous medium and is rapidly converted to 6-MAM due to spontaneous hydrolysis (Knight et al., 2014; Rook et al., 2006). This can be noticed in the results of the first case presented, where only 6-MAM was detected in the extracts from the syringe and the unknown powdered substance. The same hydrolysis occurs in the serum, resulting in the deacetylation of HER to 6-MAM, which is further metabolized to MOR (Rook et al., 2006). Different data on the half-lives of HER and 6-MAM have been reported in the literature. It is thought that HER can be detected in blood 10-40 min after intravenous administration, while 6-MAM 1-3 h, reaching maximum plasma concentration immediately after injection (Rook et al., 2006). In our cases, larger number of opioid alkaloids and their metabolites were identified in urine rather than in blood. Several studies conducted using postmortem biological samples suggest that the most suitable medium for detection of 6-MAM is the cerebrospinal fluid (CSF), while others consider the vitreous humor to be the sample with the greatest number of positive findings on 6-MAM compared to other fluids and tissue samples from the same forensic cases, with CSF having the second greatest number of positive results (Goldberger et al., 1994; Pragst et al., 1999; Wyman and Bultman, 2004). 6-MAM was not detected in any available biological sample from the three forensic cases, thus further analysis is needed to determine the origin of the opiates. Even though MOR is an end product of HER metabolism, it can also be a metabolite of COD, which is an active substance in some formulations. Some studies have compared MOR/COD concentration ratio, suggesting that MOR/COD concentration ratio >1 indicates HER use (Bogusz et al., 2001; Ceder and Jones, 2001; Konstantinova et al., 2012). Other studies suggest the use of acetylcodeine (AC), a by-product of HER synthesis, as a marker of illicit HER use. AC is considered as the only definite marker of illicit HER use, along with 6-MAM, but its short half-life aggravates its usefulness in forensic applications (Bogusz et al., 2001; Goldberger et al., 1994; Musshoff et al., 2010). This is also confirmed by the results of our study, where AC was not detected in any death case. Though some authors propose the presence of papaverine, especially its metabolites as a reliable marker of HER use, several studies have shown that papaverine metabolites can be detected in urine after the consumption of poppy seeds (Musshoff et al., 2010; Paterson and Cordero, 2006). COD and noscapine (and its metabolites meconin and cotarnine) are also considered as markers of HER use (Bogusz et al., 2001; Paterson and Cordero, 2006). However, they can also be detected in biological samples after food consumption as papaverine (Bogusz et al., 2001; Musshoff et al., 2010; Paterson and Cordero, 2006). In our cases, almost all opium alkaloids were identified, but 6-MAM was not detected. Pharmaceutically prepared HER is not available as a treatment option in Republic of Macedonia, therefore it can be concluded that the deceased had used illicit HER.

Conclusion

The absence of 6-MAM in postmortem biological samples is not an indicator of HER non-use. When interpreting the results for the opiate presence due to the use of HER or different opioids, the results of numerous researches can be utilized, such as MOR/COD ratio as the most relevant marker or the presence of AC as a definite marker of HER use. The determination of other opioid alkaloids such as papaverine, noscapine and its metabolites, together with additional forensic evidences can be useful in autopsy cases.

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