K70 Urinary Metabolites in Fatal Intoxications With Methoxyacetylfentanyl Could Indicate Time Until Death

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Learning Overview: The goal of this presentation is to increase attendees' knowledge of methoxyacetylfentanyl toxicology by providing urinary data in postmortem cases and discussing the potential value of such data in case interpretation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by increasing knowledge of methoxyacetylfentanyl toxicology and providing data to facilitate interpretation of urinary findings in fentanyl analog intoxications.

Methoxyacetylfentanyl is a fentanyl analog in which the propionamide group is replaced by a 2-methoxyacetamide group. It contributed to 11 intoxications in Sweden between 2016 and 2018. In this study, urinary metabolites in eight fatal cases involving methoxyacetylfentanyl (all male, age 27–41) were analyzed. Femoral blood concentrations ranged from 17-140ng/g and the Cause Of Death (COD) was intoxication, except for one case listing COD as acute complications of an underlying heart disease but with possible contribution from methoxyacetylfentanyl. The hypothesis was that urinary metabolite profiles might correlate to the time between intake and death, potentially indicating a short period of abstinence before the last dose.

Urinary metabolites were analyzed by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry LC/qTOF/MS (Agilent® 1290/6550) with and without hydrolysis with β-glucuronidase/arylsulfatase. Samples were diluted 1:4 in 1M sodium acetate buffer pH 5 and a negative urine sample was included as a control. The metabolites were separated on an Acquity® HSS T3 column (150 × 2.1mm, 1.8µm, Waters®) using a 13min gradient from 1%–40% acetonitrile with 0.1% formic acid in both solvents (60°C, 0.5mL/min). The method was not quantitative; but for individual analytes, peak areas generally correlate well with concentration. For confirmation of identified metabolites, methoxyacetylfentanyl was incubated with hepatocytes (5µM, 1 million cells/mL). Duplicate incubations for zero, one, three, and five hours were analyzed with the same method as the urine samples.

In total, 25 urinary metabolites were found, including eight glucuronides. Major biotransformations were O-demethylation, dealkylation to form the nor-metabolite, mono- and dihydroxylations of the phenethyl moiety (the latter with subsequent methylation), as well as combinations thereof. Methoxyacetylfentanyl was detected as the parent compound in all cases, and the most abundant metabolites in hydrolyzed urine samples included O-desmethyl-,; O-desmethyl, phenethyl-hydroxy-; phenethyl-hydroxymethoxy-; and normethoxyacetylfentanyl.

When looking at the abundances of the parent compound methoxyacetylfentanyl, M21 (O-desmethyl) and M18 (glucuronide of M21) in urine, three distinct groups were observed. In three cases, the abundances of methoxyacetylfentanyl, M18 and M21 were all low (<380k, <21k, and <270k counts, respectively), which in combination with a substantial concentration in femoral blood (21–140ng/g) may indicate an acute intake while abstinent and a "rapid" death. In two of those cases, a syringe was found at the scene, suggesting intravenous administration and a rapid onset.

In two other cases, the abundances of methoxyacetylfentanyl (>2,400k) and M21 (>1,200k) were high while the abundance of M18 was low (<280k). This could indicate an acute intake while abstinent but a more delayed death where phase I metabolite M21 was formed and at least some phase II metabolite M18 was produced. Femoral blood concentrations were 17 and 31ng/g. In hepatocytes, M21 reached a plateau after 1h while M18 was still increasing at 5h of incubation.

In the remaining three cases, the abundances of methoxyacetylfentanyl (>3,000k) as well as both M18 (>2,300k) and M21 (>9,300k) were high, which could be indicative of an acute intoxication in a chronic user. (This group includes the case in which methoxyacetylfentanyl was a possible contribution to complications of heart disease.) Femoral blood concentrations were 18–51ng/g.

Based on eight cases, the major urinary metabolites of methoxyacetylfentanyl were identified. Differences in the abundance of methoxyacetylfentanyl and its major metabolites could be interpreted to indicate fatal intoxications in abstinent or chronic users. It is postulated that urinary concentrations of methoxyacetylfentanyl and two metabolites, in combination with the methoxyacetylfentanyl concentration in femoral blood, might be good indicators of the time between administration and death as well as prior use. However, to verify this, further (preferably quantitative) measurements of urinary methoxyacetylfentanyl, M18, and M21 in cases with well-established case histories are needed.

Methoxyacetylfentanyl, Fatal Intoxication, Metabolites

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