Determination of Cocaine Metabolites in Hair Samples – Comparison with street cocaine samples

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Aims: Hair testing is a common technique for the determination of drug abuse. As drugs which are commonly smoked or sniffed (e.g. cocaine), can contaminate the hair through smoke or dust, testing for metabolites, especially hydroxy metabolites, is highly recommended. To check if the detection of hydroxy metabolites gives a definite proof of ingestion, the presence of these metabolites in street cocaine samples has to be checked. **Methods:** For this study 451 cocaine (COC) (COC >0.1 ng/mg) positive hair samples were analysed by LC-MS/MS for the metabolites benzoylecgonine (BE), norcocaine (NC), cocaethylene (CE), ortho-, meta- and para-hydroxy-cocaine (o-, m-, p-OH-COC), meta- and para-hydroxy-benzoylecgonine (m-, p-OH-BE), and meta- and para-hydroxy-norcocaine (m-, p-OH-NC). The results were compared with the cocaine metabolite concentrations in 146 street cocaine samples, confiscated by the Bavarian police, by comparison of the concentration ratios for BE/COC, NC/COC and CE/COC and the area ratios for hydroxy-metabolites/COC. **Results and Discussion:** The following concentration/area ratios were found in the street cocaine samples: BE/COC 0.03 - 1.2%, NC/COC 0 - 4.1%, p-OH-COC up to 0.04 %, m-OH-COC up to 0.09% and o-OH-COC 0.18%. CE, OH-BE or OH-NC were not detected in any of the seized samples. Similar area ratios for the hydroxy-metabolites were found for p-OH-COC in 5.1 % of the hair samples, in 6.8 % of the samples for m-OH-COC and in 88.7 % for o-OH-COC. **Conclusion:** m- and p-OH-COC area ratios which exceed the ratios of street cocaine more than twice plus additional identification of OH-BE and OH-NC will be implemented as new in-house criteria to distinguish between contamination and ingestion. It is expected that the more hydrophilic hydroxy-metabolites of cocaine are incorporated into the hair-matrix in a lesser extent than the cocaine itself. Detection of the meta- and para-hydroxy-metabolites using the above mentioned criteria is a reliable tool to distinguish between ingestion and external contamination.

1. Introduction

Hair testing for drugs of abuse has become a valuable tool e.g. in forensic toxicology, work place testing and family care situations. As drugs which are commonly smoked or sniffed such as cocaine (COC) can contaminate the hair through smoke or dust, testing for metabolites is highly recommended. However, norcocaine (NC), cocaethylene (CE) and benzoylecgonine (BE) have also been detected in illicit samples probably formed during the isolation procedure of COC from leafs of Erythroxylon coca. Therefore, detection of these metabolites may not unequivocally prove COC ingestion. A small proportion of COC is also metabolized by hydroxylation. These hydroxy-metabolites were described to be present in hair samples [2,3]. To check if detection of hydroxy metabolites may enable a definite proof of ingestion, the presence of these metabolites has been investigated in addition to BE, NC and CE in street cocaine samples as well as in hair samples. Subsequently, metabolite to COC ratios were

estimated to further decide whether they provide a more accurate measure to differentiate active use from passive exposure [1].

2. Methods

For method development extracts (methanol/ultrasonication) prepared from COC-positive hair samples in the high concentration range were investigated for presence of hydroxy metabolites of cocaine using a LC-QTOF 5600 system (Sciex, Darmstadt, Germany). Accurate mass determination revealed three peaks for OH-COC, and two peaks for OH-BE and OH-NC, respectively. Identification of the fragmentation pattern of the seven compounds enabled identification of the hydroxy-metabolites. The most sensitive transitions for the targeted quantification of hydroxy-metabolites were implemented into an in-house assay for various drugs of abuse on a QTrap 6500 system (Sciex, Darmstadt, Germany). Synthesis of hydroxy-metabolites allowed to qualitatively assign the elution order of particular isomers.

For the present study, 576 extracts (methanol/ultrasonication) prepared from COC-positive hair samples (> 0.1 ng COC/mg hair) were investigated with this method for meta- and para-hydroxy-norcocaine (m-, p-OH-NC), ortho-, meta- and para-hydroxy-cocaine (o-, m-, p-OH-COC) as well as for meta- and para-hydroxy-benzoylecgonine (m-, p-OH-BE).

The same analytical method had been applied to determine COC metabolite concentrations from 146 street COC samples which had been confiscated by the Bavarian police. Subsequently, the concentration ratios for BE/COC, NC/COC and CE/COC and the area ratios for hydroxy-metabolites/COC have been derived from hair and clandestine samples to be used in a comparison.

3. Results

In all hair samples under investigation, p-OH-COC, m-OH-COC and o-OH-COC were present in 90.5, 84.7 and 54.2%, respectively. p-OH-BE and m-OH-BE were detectable in 64.8 and 63.2% hair specimens, respectively, whereas 63.2% were positive for p-OH-NC and 61.1% for m-OH-NC. Regarding street COC samples, only hydroxylated COC could be identified; 68.7, 72.7 and 91.8% of confiscated samples contained p-, m- and o-OH-COC, respectively.

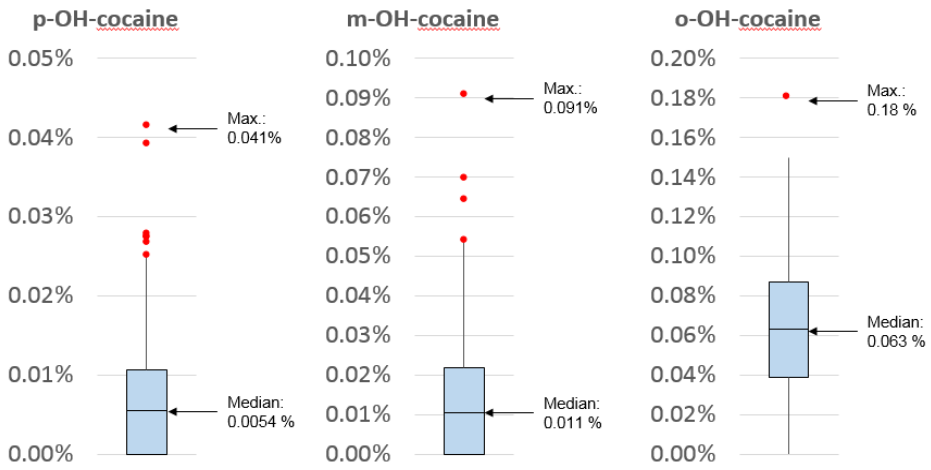

Fig. 1. Box-plots of peak area ratios of p-, m- and o-hydroxy-cocaine to cocaine estimated from illicit cocaine samples.

As a certified reference material for hydroxy-metabolites of COC was not available, the area ratios from the target ions of p-, m- and o-OH-COC and COC, respectively, were used for an estimation of the ratios for both, hair and illicit samples. Concentration ratios derived from clandestine preparations are depicted in Fig. 1.

Referring to hair samples, the following median area ratios could be calculated from the analytical data: 4.2, 4.3 and 0.2% for p-, m- and o-OH-COC, respectively. The upper 75% of all estimated ratios exceeded 1.4% for the p-, 1.2% for the m- and 0.1% for the o-isomer.

4. Discussion

Estimating ratios from peak area ratios of hydroxy-metabolites to COC revealed a noticeable distinction between hair and illicit samples. p-, m-, o-OH-COC/COC ratios in hair exceeding the respective ratios in street samples by more than twice may serve as a new criterion to differentiate an arbitrary use from exposure or contamination. Detection of p- or m-OH-BE and of p- or m-OH-NC can be regarded as a second distinctive feature.
Re-evaluation of the COC-positive hair specimens from this study applying these two novel criteria showed that use of COC could be substantiated in 92% of all cases.

5. Conclusion

Detection of hydroxy-metabolites of COC, BE and NC is suitable to differentiate active use of COC from exposure or contamination. Quantification of hydroxy-metabolites using reference material is in progress.

6. References

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