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Monitoring of drugs of abuse and medicaments in hair and nails – applications and pitfalls in forensic toxicology

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1. Introduction

The analysis of keratinized matrices, such as hair and nails, has achieved considerable importance in forensic toxicology as drug intake or drug exposure can be detected for a much broader time frame than in classical specimens, such as blood and urine. Head hair can serve as a chronological record of consumption with a growth rate of 0.6 to 1.4 cm per month [1]. Hair analysis is applied on a regular basis for the retrospective long-term monitoring of drugs, drugs of abuse and alcohol consumption behaviour or for abstinence control in the process of regranting driver’s license [1]. Pitfalls of hair analysis are contamination of the specimen by dust/smoke from the environment as it was reported for drugs of abuse such as cocaine, cannabis, and heroin [2-4]. Therefore, it is important to distinguish contamination of a hair sample from drug intake or from exposure to the drug. Bias in analysis results can also occur because of hair colour; it was demonstrated that incorporation of basic drugs into hair is increasing with higher melanin content in hair [1, 5]. The lack of a suitable hair sample because of cosmetic treatment or the removal of all head and body hair constitutes another pitfall. In such cases, nails may be an alternative matrix to hair but the application of nail analysis still has to be evaluated. Several drugs of abuse (e.g. cocaine, amphetamines, opiates, and tetrahydrocannabinol), drugs (e.g. benzodiazepines, antidepressants) and the alcohol marker ethyl glucuronide have already been detected in fingernails or toenails, respectively [6-11]. Unlike hair, nails grow continuously throughout one’s life [12]. Growth rates of fingernails range from 1.9 to 4.4 mm per month with an average of 3 mm per month [13]. Toenails grow slower with an average of 1.6 mm per month [14]. The nail is formed at the germinal nail matrix and nail bed which are well supplied by blood from digital arteries [15]. Hence, these compartments constitute regions of drug incorporation into the nail plate. Although melanocytes are located in the germinal nail matrix and nail bed, the nail plate lacks melanin granules under healthy conditions [16].

2. Aims and scopes

Within the scope of this work, applications and pitfalls of hair and nail analysis should be discussed. The aim of the first study was to investigate the contamination pitfall of hair analysis when monitoring consumption of drugs. The use of metabolite to parent drug concentration ratios in hair to distinguish intake of tramadol from external contamination of hair was investigated. In the second study, incorporation pathways of drugs into fingernails were identified after a single intake of zolpidem by continuous collection of nail clippings. Moreover, the window of detection in fingernails should be determined. The collection of fingernail clippings was evaluated as an alternative to hair by comparing analysis results. In the third study, toenails were assessed as an alternative matrix to hair for the retrospective long-term moni-
toring of cocaine use and changes of cocaine consumption. Analysis of scraped horizontal layers of a post-mortem nail was performed to provide further insights into drug incorporation pathways. For all studies, liquid chromatography-tandem mass spectrometry methods were developed and validated according to international guidelines.

3. Results and Discussion

First study: A person’s hair sample was tested positive for tramadol and its two major metabolites, N-desmethytramadol (NDMT) and O-desmethytramadol (ODMT), at an abstinence control [17]. The concentration for tramadol, NDMT and ODMT was 1710 pg/mg, 180 pg/mg and 6 pg/mg hair, respectively. The person denied intake and claimed that he was working at a tramadol production company. In order to differentiate contamination of the hair sample from active tramadol intake a study was designed. Eight employees from a tramadol production company were willing to provide hair samples which should represent contamination and possible passive exposure to drugs. To investigate concentrations for active drug intake, 75 hair samples from tramadol patients were analysed. All hair samples were tested for tramadol and its metabolites and metabolite to parent drug concentration ratios were compared. Tramadol concentrations in hair samples from employees were ranging from 130 to 21,460 pg/mg hair. NDMT was present in all employees’ hair samples ranging from 13 to 2,950 pg/mg hair. ODMT was only detected in four samples from employees working at the drug production site at concentrations ranging from 15 to 50 pg/mg hair. The median concentration for tramadol, NDMT and ODMT in patients’ hair samples was 650 pg/mg, 130 pg/mg and 71 pg/mg hair, respectively. Metabolite to parent drug concentration ratios for NDMT were rather lower in employees compared with patients and significantly lower for ODMT (p=0.001). Differentiation of contamination from intake was not possible based on the NDMT/tramadol concentration ratio. The case could be assigned to the employees group based on the ODMT/tramadol concentration ratio but assignment was not unambiguous because of overlapping with both cohorts, patients and employees. To further investigate contamination, wash water of hair samples from all employees and eight patients was analysed. Wash water to hair concentration ratios for tramadol in samples from employees were ranging from 0.1 to 0.6 being significantly higher compared to patients (p=0.0009). With a concentration ratio of 0.1 the suspect was in the range of employees’ ratios suggesting external contamination of hair with tramadol probably caused by powder from the working environment. Nevertheless, the person must have also ingested tramadol, either in an active or passive way, because metabolites were detected. To conclude, metabolite to parent drug concentration ratios of hair samples can be considered for the assessment of tramadol intake versus external contamination and passive exposure. However, it has to be kept in mind that tramadol metabolism can be altered by induction or inhibition of CYP2D6, 2B6 and 3A4 enzymes involved in tramadol metabolism. Moreover, CYP2D6 and 2B6 are polymorphically expressed enzymes which can explain interindividual differences in metabolic concentration ratios. Wash water to hair concentration ratios may provide additional information in ambiguous cases as they can be indicative of recent external contamination.

Second study: Incorporation pathways were systematically investigated in fingernails after a single dose of the sedative drug zolpidem. Zolpidem was also reported to be involved in drug facilitated sexual assaults (DFSA) [18]. Fingernail clippings from nine volunteers were collected from the same finger one week before intake, 24 hours and then weekly after the intake. A hair sample was additionally collected six weeks after the intake. It could be demonstrated that a single dose of zolpidem can be detected in a single fingernail clipping displaying three peaks in the zolpidem concentration-time profile. Drug could be detected at highest con-
Concentrations already 24 hours after ingestion when outgrowth of the nail proportion in which the drug was incorporated via the bloodstream is not possible [19]. This suggests that sweat and/or sebum contributes to a high extent to drug incorporation into fingernails which was also stated by another group [20]. Another concentration peak was found after 2 to 3 weeks after the intake in five of nine participants which indicated the start of outgrowth of drug incorporated by the nail bed. This was also observed by other authors for antimycotic drugs [21-23]. Decreasing concentrations after peak 2 could be explained by washout of the drug by daily hygiene. Ten to 18 weeks (median: 12 weeks) after the intake a third peak at concentrations ranging from 0.15 pg/mg to 0.9 pg/mg nail (median: 0.52 pg/mg nail) was detected in all participants. Most probably, this nail proportion was formed by the nail matrix while the drug circulated in the blood. Therefore, weekly collection of nail clippings provides sufficient time resolution for monitoring a single intake compared to the collection every second week [20]. The determined window of detection of one fingernail clipping in our study was on average 3.5 months after a single intake of zolpidem. Furthermore, our hair analysis results have shown that highest zolpidem concentrations were found in the 0.7-cm-hair segment corresponding to the calculated time of intake. Concentrations were ranging from 1.1 to 10.8 pg/mg hair which is comparable to those found by Villain et al. [24]. Concentrations in nail clippings corresponding to the time of intake were lower than in hair segments indicating that incorporation is favoured by melanin. This was confirmed by the analysis of seven grizzled hair samples from patients taking zolpidem. Hair samples were divided in a non-pigmented and pigmented proportion. Pigmented hair proportions exhibited significantly higher zolpidem concentrations than non-pigmented. Consequently, in contrast to hair, this bias may not exist for drug incorporation into nails. The study provided important insights into pharmacokinetics of zolpidem into fingernails and suggests that nails may be an alternative matrix and complement to hair analysis, e.g., in DFSA cases, when collection of clippings is performed weekly.

Third study: Retrospective long-term monitoring of cocaine consumption behaviour was performed by paired hair and toenail analysis for cocaine users participating in the large Zurich Cocaine Cognition Study at the Psychiatry Hospital of the University of Zurich, Switzerland [25-27]. Data on drug consumption and classification into dependent and chronic users were assessed in standardized interviews by trained psychologists. Participants declared constant cocaine consumption behaviour throughout the study periods. Hair samples, toenail clippings and scrapings were collected from 21 participants after a study period of 12 months. Three participants provided samples at the follow-up assessment 12 months later. Hair segments of one to 6 cm length and toenail samples were analysed for cocaine and its metabolites. Dependent users displayed significantly higher cocaine concentrations in hair and toenail samples compared to recreational users (1,700 to 41,500 pg/mg hair versus 130 to 5,000 pg/mg hair) [28]. Concentrations of cocaine and its metabolites were higher in hair than in nails which was in line with previous studies [29, 30]. Toenail scrapings displayed higher concentrations compared to clippings which may suggest that scrapings are susceptible to contamination. Moreover, recently incorporated drug via sweat could lead to higher concentrations in scrapings. The analysis of samples collected at the follow-up assessment revealed that increased cocaine consumption resulted in increased cocaine and metabolite findings in hair and toenails whereas steady cocaine consumption within the two periods resulted in concentrations in the same range for the sum of cocaine and metabolites. Abstinence of cocaine for 6 and 8 months, respectively, could be monitored in two cases. Further, the distribution profile of cocaine through the nail was investigated [28]. A post-mortem fingernail sample was collected and analysed in nine horizontal layers by scraping the nail from the dorsal to the ventral side. Highest cocaine and metabolite concentrations were observed in the dorsal layer which may be explained by contamination or recent drug intake and sweat-
mediated incorporation. They were followed by sharply decreasing concentrations of all analytes towards the inner layers which may be explained by drug penetration through the nail. A slight increase in concentrations in the two ventral layers could arise from incorporation via nail bed after recent ingestion and, hence, confirm our previous study outcomes. The study suggests that nails could be used as an alternative to hair, as negative results can confirm abstinence, and cocaine exposure could be successfully monitored. However, positive results should be interpreted with caution until further studies are performed. The collection of hair samples should remain the first choice for retrospective long-term monitoring because, unlike nails, hair can serve as a chronological record when segmental analysis is performed.

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5. References


