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HAIR ANALYSIS FOR DRUGS OF ABUSE. PLAUSIBILITY OF INTERPRETATION

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Over more than 20 years hair analysis for drugs has been gaining increasing attention and recognition in various toxicological fields as preemployment and employment screening, forensic sciences, doping control of banned substances, clinical diagnostics in health problems. Hair analysis for drugs can expand the toxicological examination of conventional materials and thus contribute with additional important information to the complex evaluation of a certain case. Hair is a unique material for the retrospective investigation of chronic drug consumption, intentional or unintentional chronic poisoning in criminal cases, gestational drug exposure or environmental exposure to pollutants and adulterants and with specific ultrasensitive procedures allow to demonstrate even a previous single dose administration in a very low amount. Assuming the ideal hair steady and uniform growth, segmental hair analysis can provide the information about the time course of the substance use or exposure. However, the physiological background of hair growth, mechanisms of drug incorporation are not simple, not yet understood in full details and need not be evaluated exactly in all cases. The hair sampling, storage, sample preparation, analytical performance themselves are also very important for final results. Different laboratory attitudes can produce different results. The full information on circumstances of the case examined must be taken into account during interpretation. The pitfalls in hair analysis should be known and avoided to assure the responsible and correct interpretation of laboratory results adequate to an individual case.

INTRODUCTION

Using hair as a medium to analyze drug use has been receiving increased attention during years because of less embarrassing circumstances of collection and because hair does not decompose like other body fluids or tissues. Hair testing offers also a wider detection window after drug exposure than urine testing.

The heavy metals were the first toxic substances which could be analyzed in hair matrix by means of atomic absorption spectroscopy to document the exposure in the past time. Later on, gradual development of analytical technologies, offering methods with sufficient sensitivity, enabled hair analysis also for organic substances[1]. In 1979, Baumgartner et al.[2] extracted opiates from hair of heroin user and analyzed the final buffer extract with Abuscreen RIA. The concentration along the hair shaft differed and corresponded with the time of drug intake. After tenths years of scientific research the analytical part itself has reached a general standard[3–4]. Preliminary immunochemical tests for selected drug groups may be accepted as the first step[5–6]. GC-MS is a method of choice in general practice and various tandem mass spectrometry methods (GC-MS-MS or LC-MS-MS) are used for targeted analyses of low dosed compounds (e. g. fentanyl, buprenorphine, flunitrazepam), or for detection of some important specific metabolites present in trace concentrations in hair (e. g. carboxy metabolite of delta-9-tetrahydrocannabinol), or detection single doses in previous time[7].

However, hair analysis is much more complex problem. Several comprehensive reviews were published on this topic e. g. by Pragst[8], Kintz[9], Nakahara[10], and scientific conferences were held and focused on hair analysis for drugs and the presentations were published in special issues of some journals[11]. Hair is a solid tissue which biology and physiology have not been understood in all details yet. The basics of hair anatomy and physiology have been explained in papers of Harkey[12], Cone and Huestis[13] and others. Hair is an annex of skin, originated from the hair follicle in which the germination centre is formed by matrix cells. The matrix cells give rise to the different layers of the hair shaft, including cuticle, cortex and medulla. In the root, cells are in active proliferation, whereas within the hair shaft above the skin the metabolism is negligible. The most important components of hair are fibrous proteins (keratins), melanins and lipids. Hair follicles are located 3-4 mm below the surface of the skin and are surrounded by rich blood capillary system. Three glands are associated with the hair follicle – the apocrine, sebaceous and sweat ones. The secretions of the first two glands bathe the hair shaft in the follicle and the one of the sweat gland bathe it above the surface of the skin.

Hair grows in cycles: the anagen (active growing stage), the catagen (transition stage) and telogen (resting stage).

The individual length of hair depends on the mutual duration of these stages and on the growth rate. Average values for the anagen stage in human are 4–8 years, the catagen a few weeks, and the telogen stage 4–6 months. The scalp hair growth rate is reported to be in the range 0.6–1.4 cm per month in general[7,8]. There are significant differences both in the proportions anagen/telogen hair and both in the growth rate between hair from various anatomical part of the body[8,14]. The both parameters are dependent on race, sex, age, health stage. On the scalp of an adult, the approximately 85 % of the hair is in the growing phase (anagen) and the remaining 15 % is in a resting phase (telogen). The consequence of the cyclic hair growth is the nonhomogenity of the hair bunch at the horizontal level, at a certain distance from the skin.

Table 1. Expected concentration ranges in hair of drug users[28]

COMPOUND	ng/mg hair
Cocaine	0.1 – 28.9
Cocaethylene	0 – 2.6
Benzoylecgonine	0 – 4.4
Ecgonine methylester	0 – 4.4
Heroin	0 – 16
6-Monoacetylmorphine	0.1 – 67
Morphine	0.1 – 10
Codeine	0 – 4.2
Methamphetamine	3.1 – 126
Amphetamine	0.8 – 12

INCORPORATION OF DRUGS INTO HAIR

The precise mechanisms involved in the incorporation of drugs into hair have not been clarified completely and more research is still necessary. The ideal model assumes that drugs or chemicals enter hair by passive diffussion from blood capillaries into the growing cells at the base of hair follicle. However, experimental data indicate that drugs may enter hair in different locations and in different times from different sources by various mechanisms. The drugs can be transported from blood and also from deep skin compartments not only into hair growing cells but with some time delay also into keratogenous zone during hair shaft formation. The other mechanisms are diffusion from sweat or sebum secretions. A contamination from external environment need not be excluded on the hair surface exiting the skin. This multicompartment model has been demonstrated in details by Henderson[15] and it has been accepted in general[7,8,16].

The three key factors which influence the drug incorporation into hair are melanin content in a hair, lipophilicity and basicity of a drug substance. The physicochemical properties of drugs, lipophilicity and basicity related to molecular structure clearly affect the drug incorporation into hair and on the other side, hair structure and its colour plays a very important role too. The pH of melanocytes is between 3 and 5 and significant melanin afinity for basic drugs has been demonstrated in several experimental studies both with animals[17,18] and humans[19–22] or *in vitro*[23–25]. It was confirmed that drug concentration in pigmented hair was much higher than in blond or grey hair after the same dosage[21,22].

The second important factor is the polarity of a drug or its metabolite. It has been many times documented that more polar metabolites benzoylecgonine, morphine or amphetamine enter the hair in a lesser extent than their more lipophilic precursors cocaine or 6-monoacetylmorphine or methamphetamine[8,26,27]. Some examples according to[28] are shown in Table 1.

The acidity of basicity of a drug substance is the third important factor. The matrix of hair is more acidic than blood pH 7.4, therefore the resulting pH gradient is more convenient for transfer of bases than for neutral molecules or acids. For example the acidic carboxy metabolite of delta-9-tetrahydrocannabinol enters the hair only in tiny traces[27,29].

All aspects of bioavailability and disposition of drugs in hair must be taken into account in evaluation of a real case. The genetic context, the age, sex and health conditions have been discussed with the capability of drug deposition in the inner space of hair[7,8]. The retention and stability of drugs in hair is considered to be good, nevertheless it can be affected by cosmetic treatments as bleaching or dyeing and permanent wave application. Cocaine was less affected than morphine or 6-monoacetylmorphine. The extent of concentration decline of a drug is dependent on the value prior to cosmetic treatment and also on individual hair matrix[30–32]. The long term effects of wheather (sunshine, rain, wind) may cause the damage of hair shaft with impacts to changes of concentration in hair[33]. The findings indicate that not only the stability of a particular drug is important but also the influence of UV light and water on the hair pigment. In case of long hair, above all the structure of distal part could be damaged and its analysis should be avoided.

PERFORMANCE OF HAIR ANALYSIS

Sample collection should be performed by a responsible authority not necessarily a physician, respecting ethical and legal principles. If possible hair should be collected from the posterior vertex of the scalp. Hairs should be tight together and cut as close to the skin as possible. A sufficient amount of the sample should be collected to allow further analyses if needed. At least 200 mg sample is recommended. During collection, hair specimen orientation must be marked so that the root or tip end could be clearly identified. Analysis of hair involves a series of steps generally, starting with documenting weight, length, colour and potential chemical treatment of hair and the anatomical part of the body where the sample was collected. Prior to analysis, the hair sample must be decontaminated by washing with a variety of solvents

to remove oily or potential surface drug contamination. Potential contaminants from the hair surface should be evaluated and compared with the positive results from the hair matrix interior. After washing and drying, the hair strand is weighted again and it can be divided to several segments along its length. Hair segments are cut into small snipetts or mechanically ground to a powder to open the inner space of the hair. Usually 100–20 mg of fine hair pieces or powder is taken for hair incubation or digestion in various media and these procedures must be selected with respect to stability of target analytes. After this step, additional purification by extraction of analytes are necessary for following specific analysis. In general, final analyses of prepared hair extracts are accomplished by a variety of GC-MS or LC-MS methods.

PRACTICAL APPLICATIONS

Human performance toxicology

Over past decades it has been noticed the rise in the number of employers requiring their staff to undergo testing to ascertain whether they have been taking illicit drugs or consume alcohol to excess. The wish to ensure public safety and corporate security, as well as achieving a "drug free workplace", should override individual civil liberties. There is no specific legislation to date in Europe or other part of world and there are no generaly accepted guidelines for applying to hair test for drugs in the workplace or in traffics even if some scientific recommendations have been issued. Many companies have established their own policy for drug testing which is based usually on urine testing. The knowledge and experience with drug analyses in hair have been advancing rapidly. However, there are many unanswered questions that influence the acceptance of data from hair that can have serious impacts on an individual. Recently, USA court decisions indicate that hair test results provide information that the court should consider.

In his review Cone[34] summarized the current state on use alternate biological matrices including hair on the global scale. In the USA the use of hair testing is not addressed by federal law. Some states permit testing, tests are permitted in criminal investigation, child custody, divorce cases. In the private sector in the USA over 75 % the States either allow use or have no limiting regulation that prohibit use of hair matrice for drug testing (Cone[34]). In majority of European countries the hair tests for drugs are commonly limited to criminal investigations. In Germany, hair testing is allowed in monitoring chronic drug use in healthcare sector, in regranting driver's licence. Analogous attitude is practised in Italy.

Tagliaro et al.[35] reported on integrated diagnostic strategy to check the physical and mental fitness of individuals, formerly users of illicit drugs, to obtain a driving licence after a period of abstinence (after some months). According to the Italian law, this problematic individual must provide evidence to have quit his drug abuse habits and to show no risk of relapse in the future. These subjects undergo medical examination involving hair and urine analysis on eight series collected over about 40 days. The hair samples (4–5 cm in lenghth from vertex posterior) were washed, cut into small pieces (no segments), incubated and screened by RIA for opiates, cocaine and ecstasy adopting cut off level 0.1 ng/mg and positives were confirmed by HPLC, CE or GC/MS. In 1998 the prevalence of positives for morphine , cocaine and ecstasy was 4.8, 11.3 and 2.6 %, respectively. Testing for cannabinoids was not involved into the programme due to their slow clearance and other problems. Montagna et al.[36] reported on similar program with substantial modification. The sampling protocol consisted of collection of one hair sample (A) of 5 cm length, and one urine sample, which were analyzed for opiates and cocaine. When both samples were positive or both were negative the protocol was concluded.. In case of disagreement in both results, the second hair sample (B) was collected 6 weeks later and 1 cm proximal segment analysed. In the Italian province Brescia[37], a control programme was adopted including analysis of opiates and cocaine in two hair segments (0–3 and 3–6 cm) and in urine for sake of regranting driving licence to drug addicts or occassional abusers. In cases of regranting driving licence to previous drug addicts in Germany, hair from vertex posterior 6 cm in length are analyzed.

Alcoholism is one of the most frequent addiction and it is of particular interest in traffic medicine. Yegles et al.[38] presented a study on investigation hair for ethylglucuronide (EtG) and fatty acid ethyl esters (FAEE). The both metabolites proved to be suitable qualitative hair markers of chronic excessive alcohol consumption. Using cut-off value of the sum of FAEE. > 1 ng/mg and/or positive EtG result in hair, excessive alcohol consumption can be confirmed using hair analysis. No significant correlation between the EtG and FAEE concentrations in the positive cases was found. The possible differences between EtG and FAEE in mechanisms of formation and incorporation into hair and elimination from hair were suggested in discussion.

The abuse of amphetamines and particularly methylendioxymethamphetamine (ecstasy, MDMA) or other derivatives of these "new synthetic drugs" has become popular among young people at techno and rave parties. The abuse of these drugs with health risk potential and many adverse effects can cause impairment and it can be the reason for suspending driving licence and hair analysis for these drugs may be of interest. It is important to know which region of hair corresponds to certain time of consumption and how long the drug can be detected after the abuse was stopped. Rothe et al.[39] studied these relationships in correlation to self-reported abuse history of twenty amphetamine and ecstasy users. The authors stressed the significant role of sweat in incorporation mechanism and suggested that sweatting can be the reason for the high concentration of ecstasy also in hair segments which were formed before the abuse. A transport of drugs from more distal to more proximal segments by sweat was not observed. The authors recommend to compare the results between segments to avoid incorrect interpretation.

Results of drug testing in hair in the workplace of US police performed between 1985 and 1999 on large scale of subjects (several thousands annualy) were presented by T. Mieczkowski[40]. Drugs tested both in hair and urine were amphetamines, cannabis, cocaine, opiates, phencyclidine. Hair analysis with wider detection window than urine analysis, displayed a higher prevalence rate. The mean rate for hair analysis of drug positive specimens was found to be 1.36 times higher than for urine analysis.

Generally, hair analysis if performed may complete and clarify some results in urine. However, in doping control in various sport disciplines, the official rules state that a positive case is established by unequivocal chemical detection of banned substance in urine. Recently, some discussions have appeared on possibility using scalp hair in doping testing to show the retrospectivity of drug intake. Anabolic agents when taken to improve the physical performance, could be detectable in hair after chronic consumption[41, 42]. However, Rivier[43] raised the question of individual or ethnic equality against the controls. The drug incorporation into hair and its retention are strongly influenced by pigmentation, cosmetic treatment, washing processes. It is obvious that more knowledge on these phenomena is necessary and to date hair analysis in doping control has doubted or limited application.

Chronic intoxications. Gestational drug exposure

Hair samples are more useful to prove chronic intoxications than urine samples.

There are not many references in literature focused on cases related with long term exposure to unknown drug other than drugs of abuse. When some observable pathological symptoms (hepatotoxicity, neurotoxicity) are evident and can not be explained, the idea of poisoning with unknown substance can be taken into consideration. The long-term environmental polution, food adulteration with some ingredients or hidden criminal offences can gradually induce various harmful effects. Kadoumi et al.[44] published a paper concerned adulteration of chinese herbal medicine with N-nitrosofenfluramine (appetite suppressant) and determination of fenfluramine and norfenfluramine as metabolites in hair of hospitalized patients with hepatal problems. The authors detected fenfluramine in the ranges 43–1389 pg/mg and norfenfluramine 18–680 pg/mg in hair and concluded that the patients might ingest N-nitrofenfluramine at least 5 months.

The majority of published papers concerns the drugs of abuse. From the perspective of long term exposure the neonatal hair analysis may be of interest of many authors. Callahan et al[45]. compared the analytical findings in maternal and infant's hair and of meconium and urine with the drug history of cocaine use obtained by interview for 59 women. According to the 3 trimesters of pregnancy, the mothers'hair samples were divided into three equal segments. Specified to to the respective trimesters, the agreement of the hair test results with the interview data was 94–100% for admitted abuse and 61–66% for denied abuse. The infants' hair samples were investigated as a whole and gave positive results in 25 of 32 exposed cases. Nakahara reported in his review[10] some papers of his group and in one of them methamphetamine was detected from all 12 hair segments (every 1 cm from roots) of a mother at concentrations ranging from 4 to 84 ng/mg. In baby's hair metamphetamine was identified in three sections in the range 3–1 ng/mg. Even if the drug concentrations in baby's hair were much lower than that in mother's , the trends in corresponding sections were quite paralel. They reported also on findings methylephedrine, dihydrocodeine, chlorpheniramine in neonate hair. The mothers were abusing these substances during pregnancy. Vinner et al.[46] assesed opiates in hair of 17 mother/neonates couples. A newborn exposed to drugs in utero can suffer from varying degree of withdrawal syndrome a few days after the birth. Withdrawal syndrom can be treated but it is not easy to recognize its origin because of atypical symptoms presented by neonates and when maternal addiction has not been revealed. Gestational opiate exposure profiles were drawn up and linked with the observed withdrawal syndromes. The results obtained in the neonatal hair were in accordance, for the majority, with the appearance of a neonatal withdrawal syndrome. Neonatal hair analysis could contribute to assess in utero drug exposure to opiates, particularly when results in urine and meconium are negative or when these matrices are not available.

Maternal smoking is another health hazard for the fetus. Kintz et al.[47] investigated 40 mother/infants pairs and found 0.15–11.8 ng/mg nicotine in neonatal hair and 0.37–63.5 ng/mg in mothers'hair. A significant correlation (83%) was confirmed between infants' and mothers' nicotine hair concentrations. Klein et al. [48] found that maternal hair concentration of nicotine decreased during pregnancy without any reported reduction in smoking, while the cotinine concentration remained constant. It may indicate increased nicotine metabolism during pregnancy. The amount of nicotine and cotinine in hair provides a cumulative index of tobacco exposure.

Postmortem toxicology

Hair analysis is useful in any situation in which the history of the past rather than recent drug use is considered and numerous applications have been published in forensic literature. The time window of drug detection in hair is much wider (weeks-months-years) in comparison to traditional media as blood or urine (hours-days) and therefore hair can be complementary specimen for investigation in postmortem toxicology when the history of drug use may be difficult to obtain. Hair is not a material useful to prove an acute lethal intoxication generally. Nevertheless, Nakahara et al.[49] demonstrated that using hair roots in some special situation, acute poisoning can be proved. They analyzed hair roots of four men who died mainly due to the acute poisoning of methamphetamine and found extremely high concentrations (250.8–41.7 ng/mg).

Long term drug abuse or chronic poisoning can gradually induce certain harmfull effects on the human organism and can exacerbate some preexisting diseases. For example chronic abuse of methamphetamine is known

to be associated with cardiovascular diseases. During autopsy certain types of morphological alterations are found in the hearts of stimulant addicts. The rapid increase in blood pressure after an intravenous methamphetamine dose can be risky for addicts with arteriosclerosis which can develop after long term abuse. However, the information on the life style of a deceased person need not be available to explain the pathological cardiovascular alterations observed during the autopsy and to classify the cause of death correctly. The findings in hair segments may be useful in this respect. The results in four 2 cm segments and in the fith distal 7 cm segment were used to explain pathomorphological alterations found during autopsy of an methamphetamine addict with bleeding into cerebellum. The results provided a clear evidence that the man was methamphetamine abuser for more than 8 months[50].

Cirimele et al.[51] described a case where a female nurse known as abuser of anaesthetics for years was found dead at home. Segmental analysis of 6 cm hair strand revealed the presence of midazolam and propofol in each 2 cm segment. Self administration of midazolam and propofol without respiratory assistance and medical control contributed to the death.

A significant factor in interpretation postmortem blood methadone concentration (or other opioids) is chronic or single consumption If the subject is a methadone long term addict his peripheric blood concentration need not be toxic due to the tolerance. Only hair can resolve the question whether the application of methadone was chronic or not[7]. In our laboratory we have examined the fatal case of a young woman shortly after delivery who committed suicide by heroin application. Her hair strand collected from the scalp, in the length corresponding the period of pregnancy was divided into several segments and analyzed for opiates. The results (Fig. 1) demonstrated the drop in heroine consumption during pregnancy and consequently the changes in her tolerance to opiates before the death.

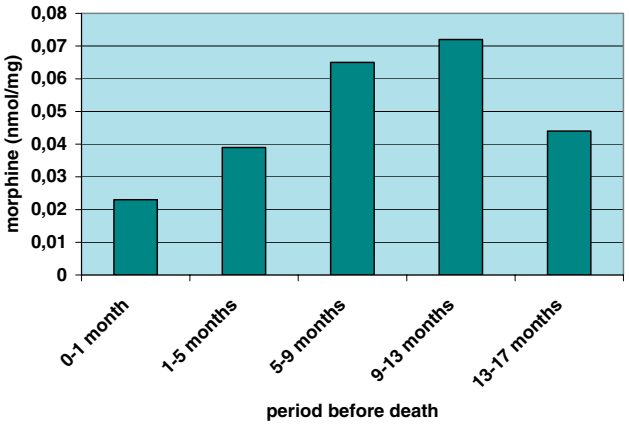

Fig. 1. Example of distribution of opiates in equivalents of morphine along the growing hair from the scalp of a woman who died 14 days after delivery of fatal overdose by heroin. The assumed hair growth rate is taken 1cm/month.

Segmental hair analysis can provide a retrospective calendar of individual's drug use, period of abstinence, or the evidence of switching from one drug to another or of mixing various drugs e. g. heroin, dihydrocodeine, hydrocodone[52].

Criminal assaults to modify human behaviour

It is not a new phenomenon to misuse drugs with the intention to govern other person's perception and behaviour. Except the known drug misuse in the political context in the past, the drugs are used for personal criminal profit, as in robberry, sexual assaults, children's abuse. Drugs involved in such crimes are various pharmaceuticals (sedatives, hypnotics, aneasthetics), ethanol or drugs of abuse (cannabis, LSD, GHB, ecstasy, etc). To obtain a successful toxicological evidence of the crime committed, or to verify some story, some prerequisites should to be fulfilled:

1) The time elapsed between the drug application and specimens (blood, urine) collection should be as short as possible until a drug and its metabolites are eliminated from the body. However, in reality hours or days can pass until the victim becomes conscious or some indications of the comitted assault can appear. In these cases with some time lag hair analysis can be useful.
2) The drugs which are misused can be very potent pharmacologically, therefore the effective dosage can be very low (LSD, buprenorphine, some benzodiazepines, fentanyl). They are often applied as single dose and the resulting concentration in hair can be only in pg/mg range. If they are searched in hair later, they must be correctly localized in the hair strand. Very sensitive analytical technology for targeted assay is necessary (GC/MS, GC/MS/NCI, GC/MS/MS or LC/MS or LC/MS/MS).
3) If hair analysis for drugs is of concern, the understanding the principles of drug incorporation into hair is assumed for correct sample preparation and results interpretation.

Benzodiazepines are the most common so-called "rape drugs" with flunitrazepam (Rohypnol) mentioned most frequently. Negrush and coworkers[53] designed a study in which 10 volunteers, 8 women and 2 men, with various hair pigmentation and treatment participated. The aim of the study was to ascertain whether flunitrazepam and its major metabolite 7-aminoflunitrazepam could be detected in hair collected from volunteers after receiving a single 2 mg dose of Rohypnol using GC/MS/NCI. The hair samples were collected from volunteers just before administration and then 1, 3, 5, 14, 21, 28 days after. For analysis of each sample were used 1.5 cm lenghts from the root side, pulverized and 50 mg aliquots digested. The metabolite was detected after 24 hours in five volunteers and remained in hair throughout the entire study period (0.6–8.0 pg/mg). In two volunteers the metabolite appeared in hair 14 days after the drug intake (0.5–5.4 pg/mg) and in other 2 volunteers 21 days after the intake (0.5–2.7 pg/mg). In one subject the metabolite appeared

in slight concentration below quantitation limit (approx. 0.4 pg/mg) on day 14 and 21.

Kintz[7] reported on recent and repetitive exposure of a 14-year boy to buprenorphine with nordazepam as sedatives. The boy was found dead at the home of well known sex offender. At the autopsy only pulmonary and visceral congestion were noted. The concentrations of buprenorphine achieved by LC/MS were 1 ng/ml in blood and 23 pg/mg in hair. The death of the boy was attributed to accidental asphyxia in a facilitated repetitive sexual abuse situation. The hair concentrations of buprenorphine was comparable with values after chronic administration. In the controled long term (maximum 180 days) administration of buprenorphine 8 mg sublingual to 12 subjects, buprenorphine concentrations in hair segments ranged from 3.1–123.8 pg/mg[54].

Lorazepam possesses amnesic properties and can impair individual rapidly. In the study of Kintz et al.[7], lorazepam 2.5 mg was administered orally to 3 volunteers and after 4 weeks hair samples were collected. After decontamination 2 cm segments, 20 mg aliquots were used for incubation in phosphate buffer. Despite the limit of quantitation 1 pg/mg (using LC/MS/MS) lorazepam could not be detected. Another study with other benzodiazepines was performed by Chéze et al[55]. They studied the disposition of bromazepam into hair of a volunteer after single peroral dose 6 mg (Lexomil)) and clonazepam in another subject's hair after single peroral dose 2mg (Rivotril). Head hair was collected 1 month after the exposure. Bromazepam was detected in hair at 28 pg/mg and 7-aminoclonazepam at 22 pg/mg. No clonazepam was detectable. This method was applied to two forensic cases. It allowed to determine bromazepam in cut head hair at 6.7 pg/mg only in the first 2cm proximal segment. Hair sample was collected 3 weeks after an offence. The other case showed the presence of 7-aminoclonazepam at about 3.2 pg/mg in axilliary hair collected 4 months after an offence.

New hypno-sedatives have been preferred over conventional benzodiazepines lately. Among them zolpidem (Stilnox) has been indicated to treat short-term insomnia. The recomended single dose per night is 10 mg. Zolpidem is misused also in drug-facilitated crimes and it can impair an individual rapidly. Due to its amnesic properties, the victim is less able to accurately recall the circumstances under which the offence occurred. In the study of Villain et al.[56] three volunteers received 10 mg peroral dose. 3–5 weeks after the single dose of zolpidem hair samples were collected, decontaminated, prepared three 2 cm segments of finely cut hair, from which 20 mg were taken for incubation. Zolpidem was detected in root hair segment of each volunteer in the range 1.8–9.8 pg/mg. In case of two chronic zolpidem consumers, the concentrations in hair were much higher, 1123 and 2211 pg/mg. The developed method was applied to two possible criminal cases. In the first case, zolpidem was positive in corresponding hair segment at 4.4 pg/mg. In the second case zolpidem was detected in all segments analyzed demonstrating chronic drug use additionaly to possibly recent exposure.

Zopiclone (Imovane) is another drug which can be compound of choice to sedate a victim. Zopiclone was administered orally to two volunteers who ingested a single Imovane 7.5 mg tablet. Hair samples were collected one month after the dose in vertex posterior and 20 mg cut hair was taken for incubation. Zopiclone was detectable in the proximal 2 cm hair segment of both volunteers at a concentration 5.4 and 9.0 pg/mg[57].

Thiopental and pentobarbital were found in head and pubic hair sample of a woman who had been sexually assaulted during hospitalization[58]. In the three 1.5 cm proximal head hair segments the concentrations (ng/mg) of thiopental/pentobarbital were 0.30/0.40, 0.20/0.20, 0.15/0.20. In the distal hair segments no barbiturates were detected. The results indicated that drug administration could have been occurred in the time period about 1–2 months before hair sampling which corresponds to the time of hospitalization.

Therapy compliance control

The idea, that hair analysis for drugs can provide the information on drug administration over a longer time period, requires the existence of some relationship between the amount of drug taken, plasma and hair concentration. It has been demonstrated that essential prerequisites of TDM like an intraindividually valid correlations between therapeutic region of hair concentration and reproducible serum level are not fulfilled. The detailed compliance monitoring to ascertain whether the patient has taken his therapy in exact accord with medical prescription appears to be inapplicable due to enormous intraindividual and interindividual variation of quantitative data[59,8].

Goullé at al.[60] compared the values of phenobarbital in serum of epileptic patients with the ones in hair and found greater variability of phenobarbital in hair. Williams et al.[61] monitored 23 epileptic patients on chronic therapy with carbamazepine for 6 months. The relationships between dose and hair and plasma concentrations were assessed as well as the monthly variability in the concentration of carbamazepine in both matrices. Pragst et. al.[62] investigated the long-term therapeutic compliance of 56 patients who were under permanent treatment of tricyclic antidepressants. They found that in comparison to therapeutic plasma levels, the nor-metabolites always accumulate less in hair than parent drugs. No correlation was found between hair concentration and the daily dose. Cirimele et al.[63] studied clozapine dose-concentration relationships in plasma, hair and sweat specimens of schizophrenic patients. They found wide variations for patients at the same posology. The idea of using quantitative results in hair for exact therapy compliance monitoring was not supported. Takiguchi et al.[64] analyzed flecainide in hair of 15 patients for assessing the individual drug-taking behavior and evaluated the results as useful for getting retrospective qualitative information on the individual history of drug intake.

Even if intraindividual significant correlations were found the enormous interindividual variations in relationship drug dose taken and hair concentration existed[59].

The qualitative or semiquantitative monitoring whether the patient takes the drug or not or whether the long-term regularity of intake exists, it can be seen from the concentrations in hair segments series The examination of shorter hair segments (0.5 cm better than 1 cm) is convenient for evaluation the time of drug intake[8].

Therapy compliance control can be considered also in the relation to the medical treatment of drug addiction. For example the treatment of opiate addiction by substitution by buprenorphine or methadone requires gradual abstinence from street heroin and other drugs. Whether this requirement has been fulfilled or not and substitute is taken as prescribed, this can be monitored in the long term perspective according to the presence of individual drugs in hair segments[7, 65, 66] . Kintz[7] demonstrated by segmental hair analysis the gradual substitution of 6-acetylmorphine (decreasing concentration from distal to proximal hair segments) by buprenorphine (increasing concentration from distal to proximal hair segments).

INTERPRETATION TO DATE AND FUTURE PROSPECTS

Continuous advances in analytical technologies have been resulting in lowering detection limits of analytical methods, improving their accuracy and thus allowing better scientific understanding and interpretation of test data. This will influence their acceptance as useful and objective tool of evidence or important information for subsequent measures with impacts to an individual. So far the target drugs in the analysis are mostly typical drugs of abuse. With modern laboratory facilities, lower and lower quantities will be detectable in hair and thus some other harmful substances will be of analytical interest.The weak points of nowadays hair analysis are well known and should be considered:

- It is difficult to prepare reference hair standards containing accurate concentration of drugs which are necessary for calibration.
- The question of efficiency of drug extraction from solid matrix is very important and this parameter need to be evaluated for each type of drug in every laboratory. The standardization of decontamination and extraction procedures is also desirable.
- Minimal performance standards should be kept in different laboratories to assure interlaboratory comparability of test results. A sufficient LOD values, comparable cut-off values will support correct identification of drugs and metabolites in hair.

To facilitate consistent interpretation and consensus approach, the Society of Hair Testing (established in Europe in 1995, www.soht.org) has made some reccommendations, about specimen collection, decontamination and specimen handling procedures, criteria for obtaining positive results, metabolites to be assayed and metabolite-to parent drug ratios[67–69].

At present state of knowledge, the data of hair analysis are rather of semiquantitative value and in segmental analysis the data have mutual relative character. The interpretation on time and dose from results of segmental analysis may not be possible in full exact details and must be careful considering many aspects of the individual case. Frequency of drug consumption need not to be quite known and lag time between consumption time and drug appearance in hair above the skin may be variable from a person to a person. Variability in hair rate growth, multiple mechanisms of drug incorporation, role of hair pigmentation, stability and retention of drugs in hair under cosmetic treatment – all these factors and related phenomena are to be taken into account during interpretation. There are still remaining unresolved questions in hair analysis and more scientific understanding and further research are necessary in this developing technology. Hair analysis may be useful in any situation in which the history of past rather than recent drug intake is taken into account.

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