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ORIGINAL ARTICLE

Cocaine and opiate concentrations in hair from subjects in a heroin maintenance program in comparison to a methadone substituted group

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Abstract One month before (T-1) and 12 months after (T12) controlled i.v. administration of pharmaceutical heroin–HCl (10–100 mg/day) in the context of a heroin maintenance program (HMP), concentrations of opiates and cocaine as well as its metabolites were determined in head hair (n=46) using a validated gas chromatographic–mass spectrometric method. In addition, a patient collective of a methadone maintenance program (MMP, daily doses 15–260 mg) was examined (n=35). The incidence of additional cocaine consumption decreased in both groups during the study period (T-1 to T12): in HMP from 64.6% to 45.8% and in MMP from 71.4% to 60.0%. A significant reduction of cocaine consumption was defined as an at least 30% reduction of analyte concentrations in hair (Δc>30%). Accordingly, in HMP, a decrease in 45.8% of initially (T-1) cocaine-positive patients was determined; in MMP, the reduction was 48.6%. In 22.9% of HMP and 37.1% of MMP, an increase of cocaine concentrations was detected. Codeine and acetylcodeine were found in 50.0% and 43.5% (T-1) and 13.0% and 10.9% (T12) of the samples of the HMP, as well as in 45.7% and 25.7% (T-1) and 17.1% and 5.7% (T12) in MMP, respectively. The missing of acetylcodeine, in particular at T-1, questions its applicability as a characteristic marker of a preceding consumption of illicit heroin in hair analysis.

Keywords Heroin maintenance program · Hair analysis · Opiates · Acetylcodeine · GC/MS

Introduction

For drugs of abuse, hair analysis complements blood and urine analyses by providing long-term information on an individual's drug use and is routinely applied in clinical and forensic toxicology [1–5]. Several groups have analysed opiates and cocaine in hair to prove illicit consumption of heroin and cocaine using immunoassay methodologies [6–10] and especially using gas chromatographic–mass spectrometric (GC/MS) procedures [11–17]. In the present study, a validated GC/MS method was used for determination of cocaine (COC), the cocaine metabolites benzoylecgonine (BE) and cocaethylene (CE) and of heroin (HER), 6-monoacetylmorphine (MAM), morphine (MOR), codeine (COD), acetylcodeine (AC) in hair samples of patients taking part in a heroin maintenance program (HMP). A basic requirement in such a substitution program is that participants do not use any other illicit drugs, particularly non-prescription heroin. HER intake can be checked by detection of the parent drug itself together with MAM, the major metabolite found in hair, and MOR.

Various preparation techniques have been developed to analyse hair specimens for opiates, such as organic solvent incubation [12, 13, 17–24], enzymatic hydrolysis [11, 16] and acid [14, 25–27] or alkaline hydrolysis [28]. For the current study, alkaline hydrolysis has to be avoided because of the possible hydrolysis of HER and MAM to MOR. Methanol extraction reveals best results and is the only procedure that has been proposed to identify HER in hair

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[29, 30]. For this reason, a procedure with methanolic ultrasonication and further cleanup for the simultaneous determination of HER, MAM, MOR, COD and AC was used.

However, hair analysis does not necessarily deliver conclusive data on the relationship between intake doses and determined drug concentrations. Obstacles to the determination of such relationships are individually different hair growth rates, cosmetic hair treatment and, most importantly, the incorrect and often understated consumption quoted by drug abusers in self-reports [31]. Furthermore, the unknown purity of the illicit compounds, a considerable variation in uptake of drug from blood to hair as well as the rate of sweating, the amount of apocrine and sebaceous gland secretions between individuals, have to be considered. These factors can result in low correlations between administered dose and measured concentrations.

Acetylcodeine (AC), a manufacturing impurity of illicit HER, is proposed to be used as a specific marker of illicit HER abuse because pharmaceutical heroin does not contain this compound [32, 33]. Therefore, the determination of AC in addition to the other opiates was included in the analytical method. Furthermore, especially the additional consumption of COC before and during the substitution program should be checked by means of hair analysis. The collected data should be used to cross-check the hypothesis that a reduction of additional consumption of cocaine in HMP is more significant than in a methadone maintenance program (MMP) [34].

Materials and methods

Reagents and materials

The substances heroin (HER, HER-d_9), 6-monoacetylmorphine (MAM, MAM-d_3), morphine (MOR, MOR-d_3), codeine (COD, COD-d_3), cocaine (COC, COC-d_3), benzoylecgonine (BE, BE-d_3) and cocaethylene (CE, CE-d_8) were purchased as stock solutions (1 mg/ml) from Promochem (Wesel, Germany), while acetylcodeine (AC, AC-d_3) were obtained as stock solutions (1 mg/ml) from Lipomed (Bad Saeckingen, Germany). All reagents were stored at 8°C and used after dilution to the required concentrations. Further chemicals were purchased from Merck (Darmstadt, Germany). Chromabond drug solid-phase extraction columns (3 ml, 200 mg) and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) were supplied by Macherey-Nagel (Dueren, Germany).

Specimen collection

The anonymous collective consisted of 46 white subjects who took part in the German heroin maintenance program in Bonn under controlled conditions and self-administered HER hydrochloride intravenously on a regular basis [35]. HER was administered two or three times daily, with the daily doses varying between 10 and 1000 mg. Furthermore, hair samples were obtained from an anonymous collective of 35 white subjects enrolled in a methadone maintenance program under similar supervised conditions. All subjects received oral doses of racemic methadone ranging from 15 to 260 mg/day. Participants of both studies were selected in advance out of a collective of heroin-addicted persons, allowing a prospective collection of the hair samples 1 month before the programs started (T-1) and after 1 year of participation (T12).

The hair samples were obtained using scissors to cut strands of hair as closely to the scalp as possible. The strands were secured in their original position using a piece of string, marked at the scalp end and stored wrapped in aluminium foil. Special hair treatment procedures, such as bleaching or cosmetic treatment with dye or other chemicals, were documented. The samples were stored at room temperature in a dry place, and analysis took place until analysis.

The analysed proximal hair segments were 1 cm in length in both subject groups.

Sample preparation

The hair specimens were washed successively for 5 min using 5 ml each of deionised water, petroleum benzine and dichloromethane. The washing solutions were analysed by GC–MS to exclude contamination. Subsequently, the specimens were dried and prepared for analysis by cutting into smaller segments of approximately 1-mm length.

After addition of 8 ml of methanol and 50 µl of each deuterated internal standard (5 µg/ml) to 50 mg of hair, extraction was performed by ultrasonication for 5 h at 50°C; the methanol phases were evaporated to dryness under a stream of nitrogen (at 50°C) and reconstituted in 2 ml of phosphate buffer (pH 6).

Solid-phase extraction columns were consecutively conditioned with 2 ml each of methanol, deionised water and phosphate buffer. Following the sample loading step, the columns were rinsed with 1 ml water, 1 ml 0.1 M phosphoric acid (twice) and 1 ml of methanol. Elution was performed with 2 ml of dichloromethane/propanol-2/ammonia (80:20:2).

The elution solvent was evaporated and the extract derivatised by addition of 70 µl of MSTFA with 30 µl of pyridine and 100 µl of isooctane for 15 min at 90°C, and a 1-µl aliquot was injected into the GC–MS system.

Gas chromatography–mass spectrometry

Hair analyses were performed on a model 6890 Series Plus gas chromatograph in combination with an Agilent 5973 N MSD mass spectrometer (Chromtech, Idstein, Germany). Substances were separated on a fused silica capillary column (HP-5MS, 30 m×0.25 mm I.D., film thickness 0.25 µm). The

Table 1 GC–MS procedure: linear regression (m = gradient, b = axis intercept, r = correlation coefficient) and limits of detection (LOD) and quantitation (LOQ)

	Linearity (ng/mg)	m	b	r	LOD (g/mg)	LOQ (ng/mg)
COD	0.5–7.5; 5–25	0.052; 0.051	−0.010; −0.016	0.999; 0.999	0.02	0.04
MOR	0.5–25	0.014	0.023	0.992	0.03	0.11
AC	0.5–25	0.035	0.043	0.992	0.02	0.13
HER	0.5–25	0.049	0.002	0.999	0.04	0.21
MAM	0.5–10; 5–25	0.073; 0.081	−0.001; −0.016	0.993; 0.993	0.02	0.15
COC	0.5–5; 2.5–25	0.034; 0.030	−0.007; 0.008	0.998; 0.998	0.01	0.11
BE	0.5–7.5; 5–25	0.035; 0.039	0.011; −0.009	0.997; 0.997	0.03	0.26
CE	0.5–10; 5–25	0.034; 0.031	−0.007; 0.008	0.995; 0.996	0.05	0.21

temperature programs were applied as follows: 180°C maintained for 1 min, 15°C/min ramping to 190°C, 190°C maintained for 10 min, 5°C/min ramping to 250°C with following 30°C/min ramping to 290°C (hold for 2 min). The temperatures for the injection port, ion source, quadrupole and interface were set at 280°C, 230°C, 150°C and 290°C, respectively. Injection was carried out in splitless injection mode, and helium was used as carrier gas at a flow rate of 1.0 ml/min.

Electron impact mass spectra for each of the analytes were recorded in full scan mode in order to detect the characteristic mass fragments. For each analyte, three ions were chosen for selected ion monitoring (SIM) mode: HER (*m/z 369*, 327, 268), HER-d_9 (*m/z 378*, 334, 272), MAM-TMS (*399*, 340, 287), MAM-d_3-TMS (*402*, 343, 290), MOR-TMS (*429*, 414, 236), MOR-d_3-TMS (*432*, 417, 239), COD-TMS (*371*, 234, 196), COD-d_3-TMS (*374*, 237, 199), AC (*229*, 341, 282), AC-d_3 (*232*, 344, 285), COC (*182*, 303, 272), COC-d_3 (*185*, 306, 275), BE (*240*, 361, 346), BE-d_3 (*243*, 364, 349), CE (*196*, 317, 212) and CE-d_8 (*204*, 325, 220).

For quantification purposes, area calculation was performed using the mass traces of the SIM ions indicated in italic print. Evaluation was carried out by relating the peak area ratio of each analyte and its respective deuterated internal standard to the given substance concentration.

Validation protocol

The GC–MS method was fully validated according to international guidelines [36, 37] using the program VALISTAT® for statistics [38]. In order to evaluate method selectivity, blank hair samples from different sources were prepared as described, but without adding any analyte or internal standard mix (n=6). Furthermore, blank samples were analysed to check for the absence of analyte ions in the presence of internal standards (n=2), and no interferences were found. For calibration, blank hair samples were spiked with analytes in various concentrations. Results showed that the analytes acetylcodeine, heroin and morphine demonstrat-

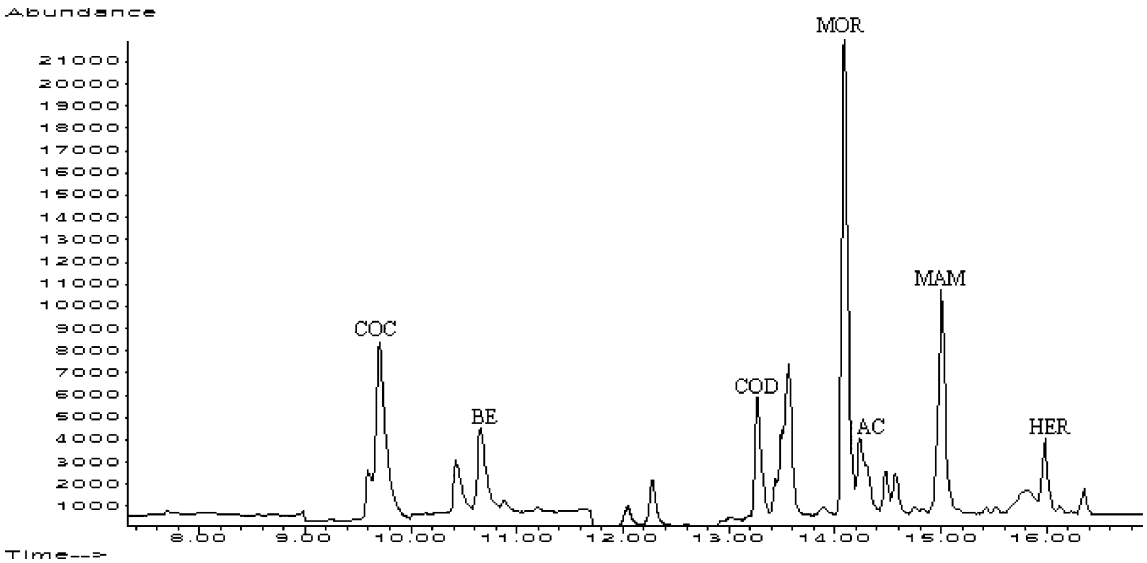

Fig. 1 Chromatogram of an authentic hair sample containing cocaine (*COC*), benzoylecgonine (*BE*), codeine (*COD*), morphine (*MOR*), acetylcodeine (*AC*), 6-monoacetylmorphine (*MAM*) and heroin (*HER*)

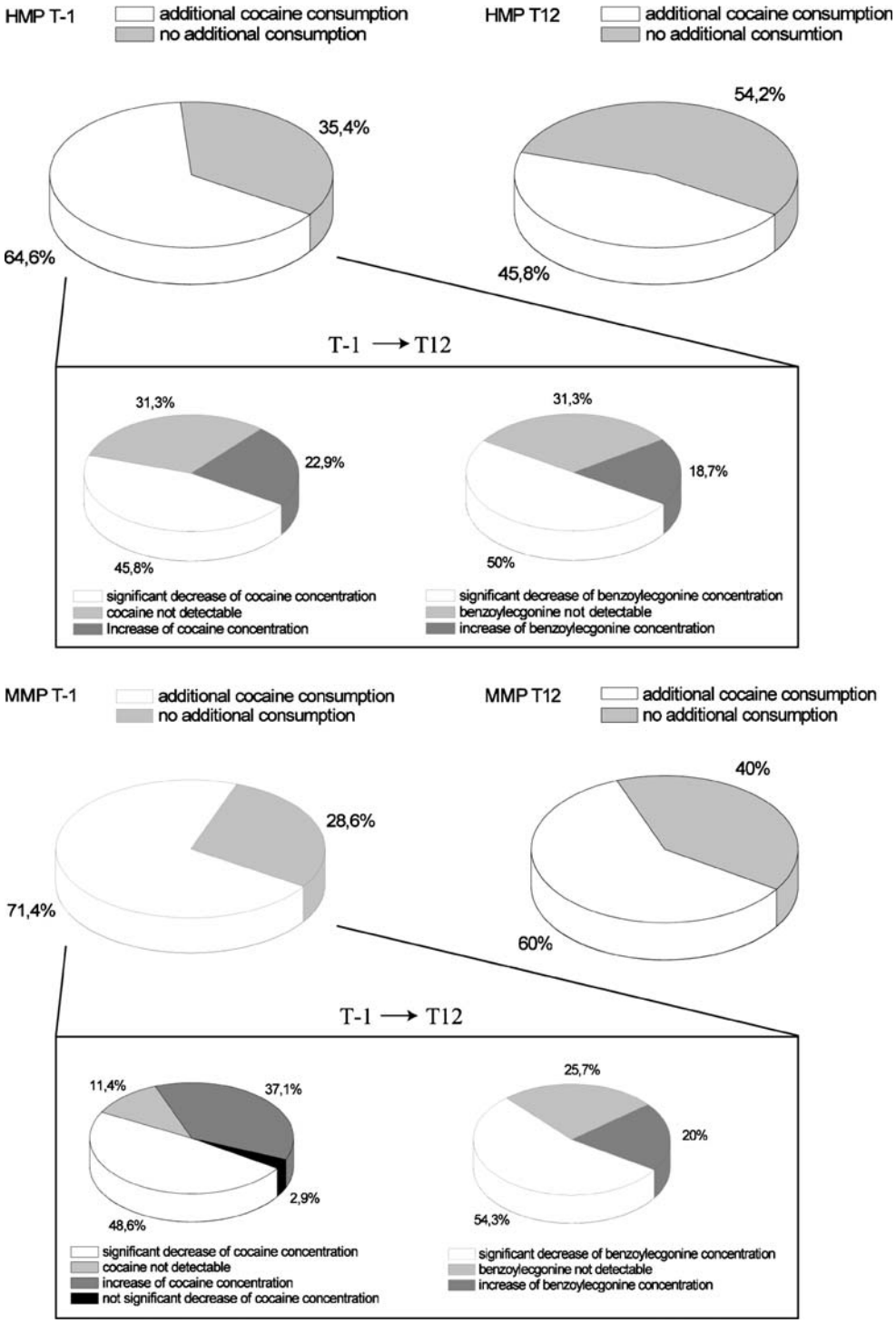

Fig. 2 Incidences of an additional cocaine consumption for HMP (n=48) in comparison to MMP (n=35) in the course of a year

ed a linear behaviour over the whole measured range (0.5–25 ng/mg). For the analytes 6-monoacetylmorphine, codeine, cocaine, benzoylecgonine and cocaethylene, two linear calibration curves, one each for high and low concentration ranges, were established. Table 1 contains both linear calibration curves as well as the corresponding correlation coefficients. Analytical limits were recorded according DIN 32645 using VALISTAT® and refer to an amount of 50 mg of hair. Additionally, three quality control samples (high, medium and low concentration with 25, 5 and 0.5 ng/mg, respectively) were prepared and analysed in duplicate over 8 days. The values obtained were used to determine within and between precision (P) as well as accuracy (A; ESM Table S1). The applicability of the procedure was proven by the successful participation in inter-laboratory tests and by routine analysis of standard reference materials. A typical

chromatogram of an authentic hair sample is demonstrated in Fig. 1.

Results and discussion

The concentrations in the proximal hair segments (1 cm) of patients in the HMP were between 0.03 and 8.26 ng/mg for cocaine and metabolites at T-1. For the same analytes at T12, concentrations ranged between 0.01 and 10.50 ng/mg, respectively. The obtained concentrations for opiates ranged between 0.02 and 32.41 at T-1. At T12, the concentrations ranged between 0.02 and 8.37 ng/mg (ESM Table S2).

The obtained concentrations in the proximal hair segment in the MMP were between 0.01 and 22.80 ng/mg for cocaine and metabolites at T-1 and between 0.02 and 8.37 ng/mg for cocaine and metabolites at T12. The obtained concentrations for opiates ranged between 0.02 and 28.88 ng/mg at T-1 and between 0.03 and 4.66 ng/mg at T12 (ESM Table S3).

There were no statistically significant differences in the mean cocaine and opiate concentrations determined for T-1 and T12 between the HMP and MMP. After 1 year of participation in the program, no significant differences in cocaine and benzoylecgonine concentrations could be determined comparing the HMP with the MMP, indicating that both groups had consumed similar doses of cocaine.

The comparison of the opiate concentrations in hair from both groups after 1 year showed no statistically significant differences in the mean values, the only exception being morphine, which was lower in MMP. This result is not surprising, since the additional consumption of illicit heroin during methadone substitution can be expected to be much lower then the MOR level resulting from daily administration of pharmaceutically pure HER in the HMP.

At T-1 in 64.6% of the cases of the HMP and in 71.4% of the MMP, an additional consumption of cocaine was determined (Fig. 2). An additional consumption of cocaine was considered when the hair samples were tested positive for cocaine and/or benzoylecgonine. The incidence of an additional consumption of cocaine decreased during the duration of the treatment to 45.8% (HMP) and 60.0% (MMP), respectively. A decrease of the additional consumption was regarded as significant when at least 30% reduction of analyte concentration was determined in the hair samples. In the HMP, the cocaine concentrations in hair decreased in 45.8% of the former (T-1) cocaine-positive patients during the treatment period. The mean decrease was 90.8%. The analogue reduction for benzoylecgonine was 89.8% in 50.0% of the patients.

In the MMP, the cocaine concentrations in hair decreased in 48.6% of the patients during the treatment period, and the mean decrease was 89.2%. The analogue reduction for benzoylecgonine was 80.3% in 54.3% of the patients (Fig. 2).

In 22.9% of the patients of the HMP, an increase of cocaine concentrations was detected; the concentrations of benzoylecgonine increased in 18.7% of the cases. For the MMP, higher cocaine concentrations were determined in 37.1% of analysed hair samples, and the concentration of benzoylecgonine increased in 20.0% of the cases.

In 31.3% of analysed hair samples from the HMP, cocaine and benzoylecgonine could no longer be detected after 1 year. In the MMP, cocaine was not detectable in 11.4% of analysed hair samples, and benzoylecgonine was not detectable in 25.7% of the cases. Patients in whose hair samples neither cocaine nor benzoylecgonine could be determined were considered free of additional cocaine consumption.

It became apparent that in both groups, a comparable number of participants consumed additional cocaine. In the HMP, the reduction of an additional consume was more distinctive than in the MMP after 1 year. The hypothesis of Backmund [34] describing this larger reduction in the HMP was confirmed. The more frequent use of illicit drugs during the MMP in Switzerland was explained by a presumably higher activity in the drug scene in this group [39].

Furthermore, results showed that participants of both groups consumed comparable amounts of cocaine because the detected hair concentration of cocaine and benzoylecgonine were similar. The significant decrease of cocaine and benzoylecgonine concentrations in both groups during the treatment period was likewise comparable.

The increase of cocaine concentrations in hair of participants of the MMP can be explained by the fact that in contrast to heroin, methadone does not lead to the desired “kick”, which can be compensated with higher doses of cocaine. However, this fact does not seem to be a categorical problem in methadone substitution because an increase of cocaine concentrations occurred only in a few cases and was not statistically significant. Furthermore, the additional cocaine consumed was very distinctive in both groups after 1 year of substitution, which disproves the hypothesis that participants of a MMP are more prone to cocaine abuse than those of HMP: The missing “kick” after methadone administration does not seem to be the leading factor of supplementary cocaine consumption.

Table 2 Incidences for codeine and acetylcodeine in hair samples in various studies

	COD (T-1, %)	COD (T12, %)	AC (T-1, %)	AC (T12, %)
HMP	50.0	13.0	43.5	10.9
MMP	45.7	17.1 (n=35) 31.6 (n=19)	25.7	5.7 (n=35) 10.5 (n=19)
Fatalities [32]	66		44	
HMP [33]		32.6		11.6

No significant advantage of one of the therapies can be determined solely on the basis of additional cocaine consumed during the treatment period. However, addicted persons accept heroin more than methadone, which leads to a higher motivation in maintenance programs [39].

Codeine and especially acetylcodeine have been suggested as markers of illicit heroin abuse. Codeine is a natural constituent of illicit heroin preparations isolated from *Papaver somniferum* L., which is subjected to acetylation and purification. In contrast to codeine, only acetylcodeine is suggested as an absolutely specific marker of illicit heroin. The acetylcodeine content in illicit heroin preparations ranges from 0.25% to 10.2% [40], whereas pharmaceutical medication heroin is pure. Therefore, the presence of codeine and especially of acetylcodeine in samples of patients indicates that they may be supplementing their prescribed heroin doses with non-prescription heroin. With respect to medicines containing morphine or codeine or other legal sources of opium alkaloids (e.g. poppy seeds), patients of a HMP are advised not to ingest these during treatment.

In a former study, concentrations of codeine and acetylcodeine were detected in 50.0% and 43.5% of samples at T-1 (HMP) and in 45.7% and 25.7% of samples at T-1 (MMP) [41], respectively (Table 2). These findings correspond to an observation analysing hair from opiate-associated fatalities where codeine was detected in 66% and acetylcodeine was detected in 44% of the cases. Neither codeine nor acetylcodeine was identified in hair collected from addicts taking part in the Swiss HMP in Bern [32].

As previously described, in our studies after 1 year of participating in the HMP, codeine and acetylcodeine were found in only 13.0% and 10.9% of the samples, respectively [41]. In a similar study surveying opiate concentrations in hair of patients who participated in a HMP in Geneva (Switzerland), codeine was detected in 32.6% and acetylcodeine in 11.6% of the samples [33], which corresponds to our results. In hair of illicit heroin users, codeine and acetylcodeine detection rates were extraordinarily high with 94.5% and 91.8%, respectively, using a method with a limit of quantitation (LOQ) of 0.09 ng/mg.

After 1 year of participating in the MMP, codeine and acetylcodeine were found in 17.1% and 5.7% of samples of all patients (n=35). Considering that participants of the MMP obtained the additional heroin illegally, the consumed heroin can be expected to be an impure "street heroin" preparation containing considerable amounts of codeine and acetylcodeine. An additional consumption of illicit heroin was considered when the hair samples were tested positive for at least one of the opiates. 6-Monoacetylmorphine with the highest detection rate was determined in 54.3% (n=19) of samples, and of these, 31.6% and 10.5% were tested positive for codeine and acetylcodeine, respectively, which corresponds to the results of the HMP in Geneva (Switzerland) [33].

Because of its low concentration or absence in about 50–60% of specimens tested positive for 6-monoacetylmorphine at T-1, it is arguable whether acetylcodeine should be considered a suitable biomarker in hair analysis following heroin abuse. However, acetylcodeine is an interesting biomarker of illicit heroin consumption during the course of maintenance programs. Regarding the incidences for acetylcodeine in both groups (10.9% for HMP, 10.5% for MMP, Table 2), it can be assumed that the additional illicit heroin consumed was similar in the HMP as well as the MMP.

Thus, on the basis of the additional consumption of illicit heroin during the treatment period, no significant advantage of one of both therapies can be determined.

Conclusion

Neither the HMP nor the MMP showed major advantages or disadvantages regarding an additional consumption of cocaine or illicit heroin. In both groups, concomitant consumption is prevalent; nevertheless, controlled maintenance programs improve the participants' situation, i.e. improve their state of health and reduce their need to commit drug-related crimes [35].

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