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SHORT COMMUNICATION

Comparison of urine results concerning co-consumption of illicit heroin and other drugs in heroin and methadone maintenance programs

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Abstract Urine samples of patients from a heroin maintenance program (HMP) and a methadone maintenance program (MMP) were chromatographically analyzed 1 month before and 6 and 12 months into treatment for the presence of classical markers of heroin use as well as for the presence of markers for illicit heroin abuse. Furthermore, the samples were immunochemically tested for cannabinoids, cocaine metabolites, amphetamine, methylendioxyamphetamines and benzodiazepines. A co-consumption of illicit heroin (HER) in the HMP was determined to be 50% but was significantly lower compared to the MMP with a co-use of 71%. The incidence was high because not only acetylcodeine (AC) as a very specific marker was considered but also other marker substances for illicit HER use. Amphetamines played only a minor part in both collectives, and the proportion of HER and methadone patients using cocaine was similar and decreased during treatment. Also, the benzodiazepine use decreased, and cannabis use was high in both collectives during treatment. Considering only the AC in the present study, a co-use of illicit HER in the HMP was similar to previous reports concerning HER-assisted treatment programs. If additional marker substances were examined, the suspicion of a co-use of illicit HER is markedly enhanced.

Keywords Morphine · Morphine-glucuronides · Codeine · Acetylcodeine · Noscapine · Papaverine · Heroin · Urine · Liquid chromatography-mass spectrometry · Substitution · Markers · Heroin maintenance

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Introduction

In the year 2002, a heroin maintenance program (HMP) for opiate addicts was started in Germany [http://www.heroinstudie.de]. A basic requirement in such opioid substitution programs is the patients' abstinence from any other illicit drugs. Recently, we presented results from hair analyses, which were compared with patients from a methadone maintenance program (MMP) [1]. Part of the German HMP was also the examination of a co-consumption of illicit heroin and other drugs on the basis of urine analysis and a comparison of the results with those of participants of a MMP.

In several studies, the use of illicit heroin could be demonstrated by the detection of further opium alkaloids additionally to the heroin metabolites 6-monoacetylmorphine (6AM), morphine (MOR), and MOR glucuronides. Codeine (COD) was most frequently detected as an additional marker substance in urine of illicit heroin users, but only its acetylated form, acetylcodeine (AC), was considered as a definite biomarker [2]. Otherwise, AC was found in fewer specimens and in lower concentrations than 6AM [3, 4], and due to a short half-life of approximately 237 min [5], it was not detectable in urine for as long as morphine following heroin abuse [5, 6]. Therefore, it was concluded that the detection of urinary metabolites of the opiate alkaloids noscapine (NOS)

and papaverine (PAP) is useful for an analytical differentiation between the use of illicit heroin and pharmaceutical diacetylmorphine, which is free from further substances [7, 8]. Especially desmethyl papaverine was considered as a marker for illicit heroin use, which was also demonstrated to be more sensitive than 6AM in urine samples. However, interpretation of positive results can be difficult because of the presence of opiate alkaloids in medicines and foods (e.g., poppy seeds) [9-13]. Recently, we demonstrated that after ingestion of poppy seeds besides positive urinary opiate immunoassay results in a 48-h monitoring period, peak concentrations of MOR, COD, and their glucuronides appeared in 4–8 h, and concentrations of total MOR higher than 10 µg/ml could be observed [14]. Furthermore, it was demonstrated that neither NOS nor PAP was detectable in urine samples after the consumption of poppy seeds containing doses up to 94 µg NOS and up to 3.3 µg PAP. NOS and PAP were rapidly metabolized. On the other hand, desmethyl papaverine and especially its glucuronide were determined in urine samples of poppy seed consumers even 48 h after consumption [15]. According to these results, PAP metabolites should not be regarded as definite markers of illicit heroin abuse. To exclude external sources influencing further results, PAP metabolites were not analyzed in the present study where a fully validated procedure was used for the simultaneous determination of general markers of heroin use (6AM, MOR, M3G, M6G) together with markers of illicit heroin (COD, C6G, AC, NOS, PAP). Additionally, urine samples were checked for the presence of cannabinoids, cocaine-like substances, amphetamine and methylendioxyamphetamines, methadone, and benzodiazepines.

Material and methods

Specimen collection

The anonymous collective consisted of 46 Caucasian subjects who took part in the German heroine-maintenance program in Bonn under controlled conditions and self-administered HER hydrochloride intravenously on a regular basis. HER was administered twice or three times daily, with the daily doses varying between 10 and 1000 mg. Furthermore, urine samples were obtained from an anonymous collective of 35 Caucasian subjects enrolled in a parallel 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 far in advance out of a collective of heroin-addicted persons. The subdivision of the patients into these two substitution groups was random. A urine specimen was obtained from enrolled patients in both substitution programs 1 month prior to commencement of the treatment (T−1) and four to five samples weekly after 6 months (T6) and 1 year of participation in the program (T12).

Immunoassays

Urine samples were analyzed for the presence of cocaine metabolites, cannabinoids, amphetamines, methylendioxyamphetamines, and benzodiazepines using standard immunoassay screening tests and cutoff levels proposed by the manufacturer (CEDIA DAU, Microgenics, Passau, Germany) on a Hitachi 912 automatic analyzer.

Liquid chromatography-tandem mass spectrometry

For a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) determination, a fully validated procedure was used which was previously published in detail [15, 16].

Results

The chromatographic routine procedure for the determination of heroin marker substances in urine samples was useful in the present study. In Table 1, the positive urine results for opium alkaloids and metabolites at T−1, T6, and T12 are demonstrated for both groups. It has to be considered that these are the summarized results from up to five urine samples per patient at T6 and T12. A co-consumption of illicit HER could be determined in 24 out of 48 subjects from the HMP (50%) and in 17 patients (35%) above once. However, the co-consumption of illicit HER in the MMP was significantly higher ($p<0.001$) with 32 out of 45 patients (71%) and in 23 subjects (51%) twice or more.

In Table 2, the positive urine results for street heroin markers and for other drugs of interest are summarized. A significant difference between both groups was only found for the street heroin markers with a lower consumption rate in the HMP ($p<0.001$). Amphetamines played only a minor part in both collectives. The proportion of HER and MET patients using cocaine is similar and decreased compared to T−1. Also, the benzodiazepine use decreased, but diazepam was prescribed at least once during the study treatment to 15.5% of the HER patients and to 14.4% of the MET patients. Cannabis use was high in both collectives also during treatment.

Discussion

According to the immunochemical urine results, amphetamine use plays only a minor part in opiate addicts, and the proportion of HER and MET patients using cocaine was similar and decreased in both groups compared to T−1. Also, the

Table 1 Positive results for opium alkaloids and their metabolites at T−1, T6, and T12 in urine samples

	Collection	HMP (%)	MMP (%)
n	T−1	44	46
	T6	221	161
	T12	226	161
MOR	T−1	97.7	84.8
	T6	97.3	35.4
	T12	94.2	26.7
6AM	T−1	81.8	71.2
	T6	81.9	25.5
	T12	81.9	19.9
M3G	T−1	97.7	87.0
	T6	98.6	41.0
	T12	97.3	30.4
M6G	T−1	97.7	87.0
	T6	98.6	41.0
	T12	97.3	29.8
COD	T−1	86.4	84.8
	T6	14.5	27.3
	T12	17.7	23.0
C6G	T−1	93.2	84.8
	T6	18.1	29.8
	T12	19.5	26.1
AC	T−1	77.3	73.9
	T6	10.0	16.2
	T12	13.7	18.0
NOS	T−1	88.6	84.8
	T6	16.3	28.6
	T12	18.6	23.0
PAP	T−1	84.1	76.1
	T6	13.1	24.2
	T12	16.8	21.1

benzodiazepine use decreased but remained at a high frequency in both programs. It has to be considered that approximately 15% of the patients received diazepam via prescription at least once during the study treatment. Additionally, it was confirmed that many opioid addicts use cannabis more or less regularly, even if the window of detection for cannabinoids in urine samples is higher than for other drugs [17].

The LC-MS/MS procedure used in the present study permitted the simultaneous determination of nine substances which are considered as general markers of heroin use or rather as specific markers of illicit heroin abuse [2, 4-6]. The procedure is useful for the analytical differentiation between prescribed pharmaceutical heroin use and illicit heroin abuse on the basis of urine analysis. A significant decrease of illicit HER use in the HER maintenance program was confirmed compared to the MET program. In contrast to the present study with relatively high rates of co-consumption of illicit heroin as well as of other drugs, in the official clinical study report, a treatment response was defined in another way [http://www.heroinstudie.de]. A decrease of street heroin abuse was considered if not more than two of the five urine tests were positive in the 12th month of treatment. If only four tests were available, only one analysis may have been positive for street heroin, and if only three tests were available, none may have been positive. This is the reason for lower rates of co-use of all tested drugs in the official study report.

Recently, Rook et al. [18] analyzed plasma samples of patients in a HMP for the presence of COD and AC and found 16% AC positives and 17% positives for COD or AC. In other HER-assisted treatment settings, AC was tested positive in 14–32% in urine samples of patients [5, 7] which is also comparable to our results. However, in the present study, a co-consumption of illicit HER in the HMP was determined with 50% but was significantly lower compared to the MMP with a co-use of 71%. The reasons for these findings are:

- Urine was analyzed instead of plasma samples [18], and the window of detection for substances is markedly enhanced in this matrix;

Table 2 Positive results for markers of illicit HER and other drugs at T−1, T6 and T12 in urine samples

	Collection	HMP (%)	MMP (%)
Parameter (*n*)	T−1	44	46
	T6	221	161
	T12	226	161
Street heroin	T−1	97.7	95.5
	T6	18.1	41.0
	T12	19.5	30.4
Cannabinoids	T−1	54.5	63.0
	T6	62.9	57.1
	T12	69.5	48.5
Cocaine	T−1	29.5	37.0
	T6	17.6	22.4
	T12	16.4	16.8
Amphetamines	T−1	0.0	2.2
	T6	2.7	1.2
	T12	1.8	0.6
Benzodiazepines	T−1	63.6	54.4
	T6	48.9	32.9
	T12	47.8	32.9

- the spectrum of marker substances was markedly enhanced with respect to the short window of detection for AC.

As described by others, also in the present study, COD and especially the metabolite C6G were most frequently detected as marker substances in urine. AC, considered as a definite biomarker, was found in fewer specimens and in lower concentrations than all other substances determined with the present method. MOR and especially the MOR glucuronides were most frequently detectable. This fact—for all intents and purposes a disadvantage for the MET patients—has to be considered if the co-consumption of illicit HER is compared between both maintenance programs. NOS and PAP were also useful for an analytical differentiation between the use of illicit heroin and pharmaceutical diacetylmorphine and were more frequently detected in urine specimens than 6AM or AC. However, it must be kept in mind that only AC may be regarded as absolute specific marker of nonprescription heroin. All other compounds may appear in urine after ingestion of morphine- or codeine-containing medicines or other sources of opium alkaloids (e.g., poppy seeds).

A new strategy was recently tested in the Dutch heroin-assisted treatment program [19]. For monitoring co-use of illicit HER, a deuterated analog of HER was added (1:20) to the pharmaceutical preparation. In urine samples, a concentration ratio of 6AM/6AM-d_3 above 32.8 was considered indicative of co-use of illicit HER, and this value was associated with a false positive rate of only 1%. However, the ratio was detectable in urine only 4–9.5 h after smoking pharmaceutical HER.

Recently, two cases of patients with opioid dependence were presented who maintained their dependence with poppy tea [20]. In general, patients in a heroin substitution program should be advised not to ingest any medications containing MOR/COD and not to consume foods containing poppy seeds. Otherwise, after two cases of intoxication—one in a 6-week-old infant who ingested a mixture of honey and poppy seeds in milk [21] and the other in a young female who ingested 80 g of powdered poppy seeds over spaghetti [22]—in Germany, the Federal Institute for Risk Assessment derived a provisional guidance MOR value of 4 mg/kg together with low values for other alkaloids [23]. Maybe in the future, a so-called poppy-seed defense after a positive opiate test will no longer be expected if the guidelines concerning alkaloid values in foods get a legal hearing.

At least the present urine results have to be discussed with regard to the results of simultaneously performed hair analyses for opiates and cocaine in the same collectives, which have already been published [1]. On the basis of hair analysis, the incidence of additional cocaine use (64.6% at T−1 and 45.8% at T12 in the HMP as well as 71.4% at T−1 and 60.0% at T12 in the MMP) was significantly higher compared to the results revealed from urine analyses. Considering 6AM as specific marker substance in hair analysis, the incidence of opiate abuse at T−1 was 100% in both collectives, which was not demonstrated by means of urine analysis. Additionally, in the MMP at T12, the incidence of a co-consumption of HER determined by positive results for 6AM in 54.3% was much higher than revealed by urine analysis (30.4% positives). Otherwise, the detection rate of COD and AC as markers for illicit HER abuse in hair analysis was lower compared to the present results of urine analysis due to the not sufficient limits of detection for these congeners of HER preparations in hair analysis. Therefore, a sophisticated analysis of urine considering the typical markers of illicit HER seems to be of advantage to prove a co-consumption in a HMP. But, in general hair testing for 6AM and MOR show also an advantage to determine opiate abuse compared to urine analysis. These findings correspond to the previous assumption that opiate and especially cocaine hair tests appear to be highly sensitive in identifying past drug use even in settings of negative urine tests [24].

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