

Deuterodiacetylmorphine as a Marker for Use of Illicit Heroin by Addicts in a Heroin-Assisted Treatment Program

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Abstract

In preparation for a treatment program concerning the medical coprescription of heroin and methadone to treatment-resistant addicts in the Netherlands, we studied a novel strategy for monitoring co-use of illicit (nonprescribed) heroin. A deuterated analogue of heroin was added (1:20) to pharmaceutical, smokable heroin (a powder mixture of 75% w/w diacetylmorphine base and 25% w/w caffeine anhydrate), to be used by inhalation after volatilization ("chasing the dragon"). Plasma and urine samples were collected from nine male patients who had used pharmaceutical, smokable heroin during a four-day stay in a closed clinical research unit, and these samples were analyzed by liquid chromatography coupled with tandem mass spectrometry. Ratios of deuterated and undeuterated diacetylmorphine and 6-acetylmorphine (MAM/MAM-d₃) in plasma and urine were calculated from peak areas of these substances in the respective chromatograms. The MAM/MAM-d₃ ratios in plasma and urine were normally distributed (with small standard deviations) and independent from concentrations of 6-acetylmorphine and from time after use of pharmaceutical heroin. A MAM/MAM-d₃ ratio in urine above 32.8 was considered indicative of co-use of illicit heroin, and this value was associated with a false-positive rate of only 1% (95% confidence interval: -1 to 3%). The MAM/MAM-d₃ ratio was detectable in urine for 4–9.5 h after use of pharmaceutical, smokable heroin. Addition of stable, isotopically labelled heroin to pharmaceutical, smokable heroin is considered to be a feasible strategy for the detection of co-use of illicit heroin by patients in heroin-assisted treatment.

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Introduction

In the Netherlands, a trial on the medical coprescription of heroin to treatment-resistant heroin-dependent patients in a methadone maintenance program was performed to evaluate its effect on mental and physical health and social functioning of the participants (1). Because the results were positive, this is expected to lead to the introduction of a heroin prescription program intended to treat a specific subgroup of treatment-resistant addicts undergoing methadone maintenance treatment. Optimal results of this treatment can only be expected when prescribed heroin completely substitutes the addict's use of illicit (nonprescribed) heroin; therefore, monitoring of any illicit heroin consumption is required.

To date, the search for suitable markers for (concomitant) use of illicit heroin has been limited to common alkaloid constituents of illicit heroin, like 6-acetylcodeine, noscapine, papaverine, and their metabolites. Several studies on the detection of these substances in hair (2) and urine (3–7) have been conducted. The main problems of this approach, however, are false-negative results due to the variable composition of illicit heroin and the small amounts of these alkaloids present and false-positive results due to consumption of medicines or food containing these or related alkaloids. 6-Acetylcodeine is the alkaloid most commonly used. It is found in most samples of illicit heroin, but usually only in small amounts: 1–5% (7), < 5% (8), 3–11% (9), or 4.3–7.4% (10). A specific problem of this marker for routine monitoring in a heroin-assisted treatment program is the possibility of transacetylation from high doses of diacetylmorphine to co-used oral codeine to form 6-acetylcodeine (7). Papaverine and noscapine are less common in street heroin and quantities vary greatly: up to 3 and 19% were found, respectively, in one study (8), while another found

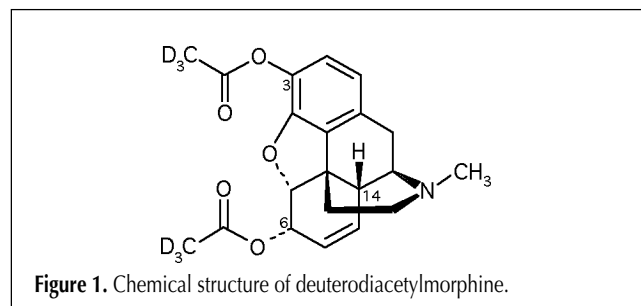
1.2–2.5% papaverine and 0.7–8.5% noscapine (10). Another general limitation of these markers is that they cannot be used to determine how much illicit heroin has been co-used, while this is potentially possible (with some knowledge on the time of co-use) if a deuterated marker is used. A stable isotope of heroin could be added to pharmaceutical heroin, which would be expected to show similar effects and side-effects as heroin, as well as negligible additional safety risks for the patient (11,12). The ratio of the labelled and unlabelled compound (or metabolite) in plasma and urine samples can be expected to be constant, so that a shift in this ratio would be indicative of co-use of illicit heroin.

This approach was proposed by Gyr et al. in a study on pharmaceutical heroin for prescription to addicts (13), but to our knowledge, no paper on this subject has been published to date. We studied the suitability of deuterated diacetylmorphine as a marker by adding it to pharmaceutical, smokable heroin, a product intended for inhalation after volatilization. Five percent of the diacetylmorphine base in the 3:1 w/w mixture of diacetylmorphine base and caffeine anhydrate was replaced by diacetylmorphine- d_6 (Figure 1). The stable, isotopically labelled diacetylmorphine contains three deuterium atoms in each acetyl group, resulting in a deuterated metabolite (6-acetylmorphine- d_3) and nondeuterated morphine after hydrolysis. Diacetylmorphine, 6-acetylmorphine, their deuterated analogues, and morphine were measured in plasma and urine samples of patients using pharmaceutical heroin by “chasing the dragon” in a closed clinical research unit. These data were used to study the feasibility of using a deuterium ratio in plasma or urine samples as potential proof for co-use of illicit heroin by addicts in heroin-assisted treatment.

Experimental

Materials

The pharmaceutical heroin to be used by “chasing the dragon” was prepared by mixing diacetylmorphine- d_6 (Figure 1) with diacetylmorphine base and caffeine anhydrate, resulting in a 3:1 w/w powder mixture of diacetylmorphine/caffeine, with a diacetylmorphine/diacetylmorphine- d_6 w/w (DAM/DAM- d_6) ratio of 20. Diacetylmorphine and diacetylmorphine- d_6 were provided through the Central Committee on the Treatment of Heroin Addicts (Utrecht, The Netherlands) and caffeine anhydrate was purchased from Bufa (Uitgeest, The Netherlands). Diacetylmorphine base



and diacetylmorphine- d_6 were equally pure, containing < 2% related substances.

Methods

Liquid chromatography–mass spectrometry–mass spectrometry (LC–MS–MS) analysis

A previously described LC–MS–MS method was used for quantification of diacetylmorphine, 6-acetylmorphine, morphine, and morphine-3- and -6-glucuronide in plasma samples (14). It was modified slightly, using morphine- d_3 instead of diacetylmorphine- d_6 as an internal standard for quantification of diacetylmorphine and 6-acetylmorphine, to avoid interference in the calculation of DAM/DAM- d_6 and 6-acetylmorphine/6-acetylmorphine- d_3 (MAM/MAM- d_3) ratios. Plasma samples were simultaneously screened for the presence of 6-acetylcodeine, codeine, cocaine, and its metabolites benzoylecgonine and norcocaine (14). Urine samples were analyzed using essentially the same method with some modifications in the sample pretreatment procedure. For quantification of morphine and the morphine glucuronides, samples were pretreated separately: the solid-phase extraction procedure was substituted for dilution with blank urine and mobile phase, followed by direct injection into the LC–MS–MS system. Sample pretreatment for analysis of 6-acetylmorphine and its deuterated analogue in urine involved a solid-phase extraction procedure: 1 mL of urine (acidified to pH 4 with 1M HCl) was subjected to the same procedure as the plasma samples. DAM/DAM- d_6 and MAM/MAM- d_3 ratios were calculated from the peak areas of the respective components. The lower limit of detection of the deuterated compounds was set at a signal-to-noise ratio of 4 and a minimum peak area of 1000 cps (mass spectrometer signal in counts per second).

Sampling

Patients were recruited from the Dutch Heroin Trial (1) and were considered eligible when they had used prescribed heroin for inhalation for at least 12 months and were considered responders in the trial. For this study, nine male patients were admitted to a closed research facility for a period of 4 days. Prescribed medication consisted of a daily dose of methadone and pharmaceutical, smokable heroin twice daily, to be used via “chasing the dragon” in a maximum of 30 min. The morning dose of heroin was varied; each patient used 66, 100, or 150% of his regular dose (overall dose range 133–450 mg heroin). The use of alcohol, cannabis, cocaine, and nonprescribed opiates was not allowed. Blood samples were collected after the morning dose via an intravenous cannula in the underarm, 10 min before the start of the smoking session, 2 min after using 40% of the heroin dose, and 2, 5, 10, 15, 30, 45, 60, 115, 180, 240, and 480 min after completing the total dose. Urine samples were collected on patients’ demand and analyzed as separate fractions instead of 24-h accumulation samples to avoid dilution to concentrations below the lower limit of quantification. Detailed pharmacokinetic and pharmacodynamic results of this study are described elsewhere (15).

Results

All nine patients completed the pharmacokinetic study, resulting in 36 plasma concentration-time curves for diacetylmorphine, 6-acetylmorphine, morphine, and morphine-3- and -6-glucuronide and 213 urine samples (a mean of 6 samples per patient per day). All plasma samples were screened for the presence of 6-acetylcodeine, codeine, cocaine, and its metabolites benzoylecgonine and norcocaine; all were found negative, except for the samples from the first days of admission to the clinical research unit. Samples from eight out of nine patients contained traces of benzoylecgonine on the first two days of ad-

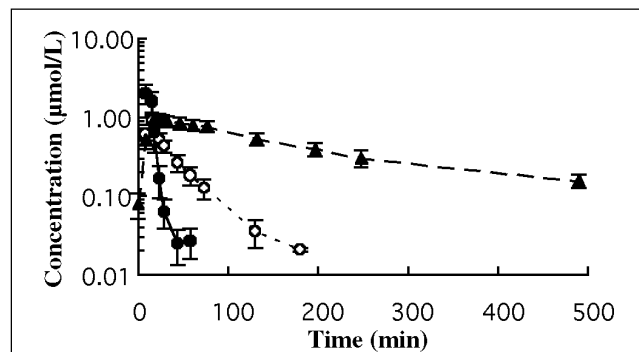


Figure 2. Mean concentration-time curves for diacetylmorphine (closed bullets), 6-acetylmorphine (open bullets), and morphine (closed triangles) in plasma after inhalation of 300 mg pharmaceutical heroin ($n = 15$ curves). Error bars indicate the 95% confidence intervals.

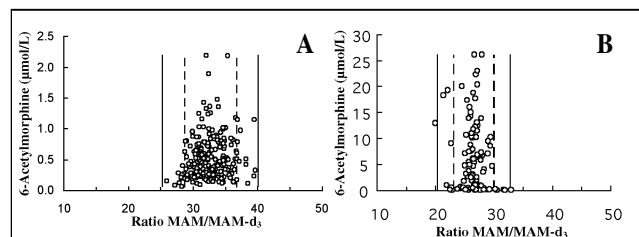


Figure 3. MAM/MAM- d_3 ratio in plasma (A) and urine (B) as a function of 6-acetylmorphine concentration. The dashed lines represent the 90% (mean $\pm 1.645 \times$ standard deviation) range around the mean; the solid lines represent the 99.74% range ($\pm 3 \times$ standard deviation).

Table I. MAM/MAM- d_3 Ratios in Plasma and Urine Samples of Patients A–I

Patient	A	B	C	D	E	F	G	H	I	Overall
Plasma										
Mean*	33.4	31.8	33.9	32.6	34.3	33.2	33.1	30.3 [‡]	31.7	32.6
SD	2.4	2.2	2.1	1.9	1.8	3.1	2.4	1.8	1.8	2.5
N [†]	20	24	32	32	26	22	22	32	29	239
Urine										
Mean*	27.1	25.8	27.3	23.3 [‡]	27.5	27.0	27.3	24.9 [‡]	26.7	26.5
SD	1.6	0.8	1.5	2.5	2.3	1.4	1.9	1.6	1.8	2.1
N [†]	12	9	12	7	15	9	13	12	12	101

* Mean values are given with standard deviations (SD) per patient, as well as overall statistics.

[†] N = number of calculated ratios.

[‡] Indicates the value differs significantly ($p < 0.05$) from other values.

mission to the clinical research unit. Peak areas decreased over time, indicating that cocaine use had occurred prior to entering the facility and had not been repeated. The absence of traces of 6-acetylcodeine and codeine indicated that no co-use of illicit heroin had occurred.

Plasma

Eight patients used a 300 mg diacetylmorphine dose once or twice in the study; the resulting mean plasma concentration-time curves ($n = 15$ curves) for diacetylmorphine, 6-acetylmorphine, and morphine are given in Figure 2. These curves illustrate the rapid decrease in the plasma concentrations of diacetylmorphine and 6-acetylmorphine, resulting in half-lives of 3 and 26 min for these compounds, respectively. As a result, the DAM/DAM- d_6 ratio could be calculated in only 84 of the 467 plasma samples (18%), because of the rapid decrease of the amount of diacetylmorphine- d_6 in plasma to below the lower limit of detection. Diacetylmorphine- d_6 was detectable until 5 min after ending the smoking session, resulting in a maximum of 3 deuteration ratios per smoking session. The DAM/DAM- d_6 ratio was found to vary from 35.1 to 193.0, with a mean of 80.4 ± 35.1 (standard deviation). The DAM/DAM- d_6 ratios showed large differences between patients and doses, but as expected, no significant linear relation between ratio and dose was found.

Because of the longer half-life of 6-acetylmorphine, ratios of MAM/MAM- d_3 could be calculated in 239 (51%) of the plasma samples; they were normally distributed (Kolmogorov-Smirnov test, $p = 0.2$) around a mean of 32.6 ± 2.5 (range 25.9–39.6) (Figure 3A). 6-Acetylmorphine- d_3 was detectable until 60 (range 45–180) min after ending the smoking session. Differences in mean plasma MAM/MAM- d_3 ratios between individual patients were small, but statistically significant in some cases (ANOVA, Bonferroni post hoc multiple comparisons, $p < 0.001$) (Table I). A significant difference in the MAM/MAM- d_3 ratio in plasma was found between the 200-mg and 250-mg doses (ANOVA, Bonferroni post hoc multiple comparisons $p = 0.034$, Table II), but no significant correlation between ratio and dose was found (Pearson correlation 0.09, $p = 0.174$). Time since the last dose was weakly, but significantly correlated with the MAM/MAM- d_3 ratio in plasma (Pearson correlation -0.14 , $p = 0.03$).

Urine

Concentration-time curves for morphine, morphine-6-glucuronide, and morphine-3-glucuronide in urine are given in Figure 4. Diacetylmorphine and diacetylmorphine- d_6 were not detected in urine samples. All 213 urine samples were 6-acetylmorphine-positive, and in 101 (47%) samples, 6-acetylmorphine- d_3 was detectable during a period of 4–9.5 h after the end of the smoking session. The MAM/MAM- d_3 ratio in urine samples was normally distributed around a mean of 26.5 ± 2.1 (range 19.9–32.9, Kolmogorov-Smirnov test: $p = 0.35$, Figure 3A), differing significantly from the mean value found in plasma (t -test, $p < 0.001$) (Figure 3B). The ratio

MAM/MAM-d₃ was not significantly correlated with the 6-acetylmorphine concentration (Pearson correlation -0.137 , $p = 0.173$) nor was a significant correlation between the ratio and the time after the last dose observed (Pearson correlations 0.138 , $p = 0.170$) (Figure 5). No differences in MAM/MAM-d₃ ratios were found between doses (ANOVA, $p = 0.165$, Table II), but statistically significant differences between some of the patients were found (ANOVA, Bonferroni post hoc multiple comparisons, $p < 0.001$) (Table I).

Discussion

Until now, the search for a marker for use of illicit heroin has focused on common alkaloid constituents of illicit heroin, like 6-acetylcodeine, noscapine, papaverine, and their metabolites. Addition of a marker substance to the prescribed pharmaceutical heroin could be considered a novel approach in the search for an appropriate indicator for co-use of illicit heroin. Our study was performed in a specially selected patient group, that consisted of "stable" patients from a heroin-assisted treatment program using a heroin dose that was titrated to their needs (1). Moreover, they were kept closed off from the outside world during the study and were monitored carefully for use of illicit drugs. These circumstances allowed us to determine the feasibility of deuterodiacylmorphine as a marker for co-use of illicit heroin by studying its pharmacokinetics and the variability of the deuteration ratio in the intended dose range and population. The study design was validated by the fact that there was no evidence for use of illicit heroin by patients during this study: neither 6-acetylcodeine nor codeine was detected in plasma samples. Small amounts of cocaine and benzoylecgonine were present in samples from the first day(s) of the study, indicating that some of the participants used cocaine before they entered the research facility.

The DAM/DAM-d₆ ratio in plasma was found to be higher (80.4) than the dose ratio of 20 in the pharmaceutical heroin and quite variable (standard deviation 35.1). This might be explained by small differences between diacetylmorphine-d₆ and diacetylmorphine in pharmacokinetic profile (volume of dis-

tribution and affinity for metabolic enzymes) and/or in volatilization properties or bioavailability. Small absolute differences between the compounds could be magnified by the high rates of absorption and hydrolysis. In a later phase of heroin metabolism, the deuteration ratio in vivo was closer to the dose ratio: MAM/MAM-d₃ in plasma was 32.6 ± 2.5 and MAM/MAM-d₃ in urine was 26.5 ± 2.1 . The MAM/MAM-d₃ ratios in plasma and urine were found to be normally distributed and independent of the heroin dose, 6-acetylmorphine concentration, and time after administration, which would make them suitable parameters for routine monitoring of co-use of illicit heroin by patients in a heroin-assisted treatment program. The observed differences between patients were not considered relevant, the differences were small and the "deviant" ratios were lower than the mean, indicating that they were unlikely to cause false-positive results.

The most important advantage of urine in routine monitoring is the noninvasive sample collection that does not require supervision, but no medically trained personnel (16). Moreover, in this study, 6-acetylmorphine-d₃ was detectable in plasma for 60 min (45–180 min), while it was detected in urine for 4–9.5 h after the end of the smoking session. In general, plasma is preferred for quantitative accuracy (16), but the determination of the deuteration ratio could be considered a pseudo-quantitative analysis, which was performed as accurate in urine as in plasma. Moreover, knowledge of the exact 6-acetylmorphine concentrations in urine often will not be necessary to prove co-use of illicit heroin, even though the described method of analysis can easily provide this information. These considerations, combined with the larger detection window and easy sample collection, make urine the preferred matrix for routine monitoring.

When the mean MAM/MAM-d₃ ratio in urine (as found in this study) is considered to be the reference value for a patient who does not co-use illicit heroin, a urine MAM/MAM-d₃ ratio larger than 32.8 (mean + 3 × standard deviation) (Figure 3) could be considered the lower limit ratio proving co-use of illicit heroin with 99.87% certainty (one-tailed application of the normal distribution). When this limit value was applied to our study population (Figure 3), one false-positive sample was found (1% and 95% confidence interval: -1 to 3%) that had a ratio of

32.9, although it was extremely unlikely that the patient had co-used (illicit) heroin. Ratios of 27.0 and 27.1 were observed in two urine samples from the same patient, collected sooner after use of the same dose; moreover, the false-positive result was associated with a low concentration of 6-acetylmorphine in urine ($0.15 \mu\text{mol/L}$), which might have resulted in a less reliable ratio calculation (Figure 3B). Similarly, the lower limit for the MAM/MAM-d₃ ratio in plasma samples would be 40.1 and no false-positive results were observed in our population.

In summary, a MAM/MAM-d₃ ratio above 32.8 in urine (and above 40.1 in plasma) could be considered indicative of co-use of illicit heroin in a heroin-assisted treatment program

Table II. MAM/MAM-d₃ Ratios in Plasma and Urine Samples After Inhalation of Different Doses

Dose (mg)	133	167	200	250	300	375	450	Overall
Plasma								
Mean*	31.3	34.1	32.0	34.3	32.5	34.7	32.8	32.6
SD	2.8	2.0	2.2	2.0	2.7	1.2	2.1	2.5
N†	5	6	56	14	103	6	49	239
Urine								
Mean*	25.1	28.5	25.7	27.0	26.5	29.3	26.6	26.5
SD		2.3	1.8	2.3	2.2	0.5	1.4	2.1
N†	1	2	18	11	58	2	9	101

* Mean values are given with standard deviations (SD) per dose of heroin, as well as overall statistics.

† N = number of calculated ratios.

in which the previously mentioned pharmaceutical product was used, provided that it was not associated with very low concentrations of 6-acetylmorphine ($< 0.25 \mu\text{mol/L}$). Another important aspect of monitoring co-use of illicit heroin, the percentage of false-negative results could not be calculated as no urine samples were available from addicts that had co-used labelled pharmaceutical and illicit heroin. However, using the previously mentioned upper limit for the MAM/MAM- d_3 ratio in urine means that the presence of a minimum of 23% "extra" 6-acetylmorphine ([upper limit = 32.8]/[mean ratio = 26.5]) in urine due to use of illicit heroin could be detected. This value is important in estimating the chance of false-negative results, together with the detection window. The MAM/MAM- d_3 ratio was detectable in urine for 4–9.5 h, which was considered a workable detection window, but some planning would be required in a heroin-assisted treatment program where heroin is administered 2–3 times daily, to ensure that useful urine samples (with detectable amounts of 6-acetylmorphine- d_3) are collected. The number of false-negative results will depend on the time of co-use of illicit heroin compared to the time of use of pharmaceutical heroin and the sampling time.

A larger detection window would greatly increase the flexi-

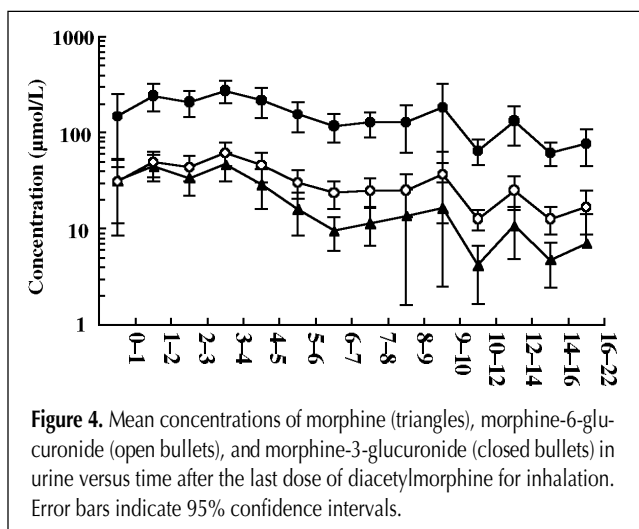


Figure 4. Mean concentrations of morphine (triangles), morphine-6-glucuronide (open bullets), and morphine-3-glucuronide (closed bullets) in urine versus time after the last dose of diacetylmorphine for inhalation. Error bars indicate 95% confidence intervals.

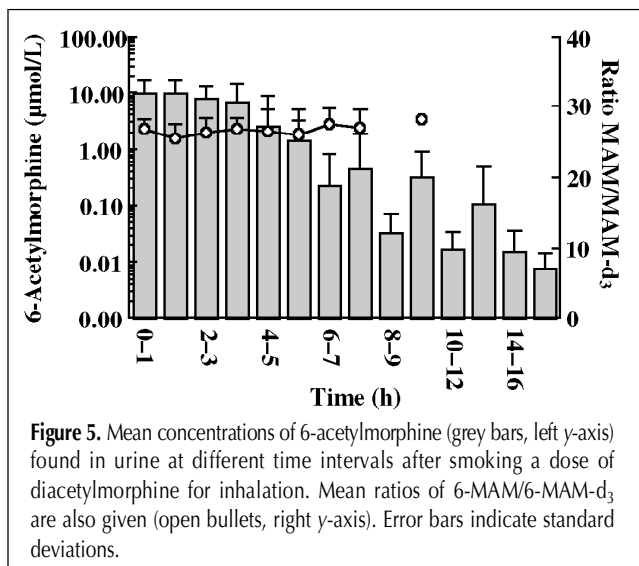


Figure 5. Mean concentrations of 6-acetylmorphine (grey bars, left y-axis) found in urine at different time intervals after smoking a dose of diacetylmorphine for inhalation. Mean ratios of 6-MAM/6-MAM- d_3 are also given (open bullets, right y-axis). Error bars indicate standard deviations.

bility and decrease the chance of false-negative results of the monitoring program for detection of co-use of illicit heroin. We expect that the detection window in urine samples could be improved if a diacetylmorphine isotope was available that contained deuterium atoms in the morphinan structure, as was also proposed by Gyr et al. (13). Use of this isotope would result in deuterated morphine and morphine glucuronides in urine that would be detectable for much longer than 6-acetylmorphine (Figures 4 and 5). However, while our diacetylmorphine- d_6 could be manufactured by simply acetylating morphine with deuterated acetic anhydride, the morphine structure is still synthesized most effectively by the opium poppy and is therefore much less easy to manipulate. Even though synthesis of diacetylmorphine- d_9 (intended for use as an internal standard in a mass spectrometric analysis) has been described (17), it is likely that this would be a very costly process, especially considering the large quantities that would be required to routinely add to the smokable, pharmaceutical heroin used in a heroin-assisted treatment program. Authorities might consider dispensing pharmaceutical heroin containing diacetylmorphine- d_6 (or diacetylmorphine- d_9) as part of coordinated spot checks, for example in individuals who were suspected of co-use by the nursing staff. This could greatly reduce the cost of such a program, as smaller amounts of deuterated marker are required. This strategy could be effective in detecting co-use of illicit heroin and have a preventive effect as well.

Conclusions

A novel strategy for monitoring co-use of illicit (nonprescribed) heroin by participants in a heroin-assisted treatment program was tested. Addition of a deuterated analogue of heroin to the pharmaceutical smokable heroin yielded constant ratios of MAM/MAM- d_3 in plasma and urine. The ratios were normally distributed (with small standard deviations) and they were independent from plasma/urine concentrations of 6-acetylmorphine or time after use of pharmaceutical heroin. In a program using pharmaceutical heroin with a deuteration ratio of 20, a deuteration ratio of 6-acetylmorphine in urine above 32.8 was considered indicative of co-use of illicit heroin. A percentage of false-positive results of 1% (95% confidence interval: -1 to 3%) was observed and the ratio was detectable in urine for 4–9.5 h after use of pharmaceutical, smokable heroin. The detection window could possibly be improved by using a heroin analogue that contained stable, isotopically labelled atoms in the morphinan structure.

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