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On-site screening and GC–MS analysis of cocaine and heroin metabolites in body-packers urine

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Abstract

The illicit transportation of cocaine and heroin either swallowed or inserted into the rectum and/or vagina of individuals, defined as "body-packers", is becoming increasingly common. Assessment of smuggling by urinalysis from body-packers has been sparsely reported and on-site rapid screening methods are essentially lacking.

We screened the presence of cocaine and heroin metabolites in urine from suspected body-packers by an on-site immunochromatographic test and confirmed the obtained results by gas chromatography–mass spectrometry and X-ray examination.

Samples were collected from 64 individuals (45 men, 19 women) stopped at Fiumicino and Ciampino airports of Rome (Italy) for suspicion of internal concealment of cocaine and heroin between October 2006 and July 2007. Urine was immediately screened on-site by Cozart® rapid urine test. Irrespective of test results, individuals underwent X-ray examination and urine samples were analyzed by gas chromatography–mass spectrometry (GC–MS). In 48 out of 64 cases (24 positives and 24 negatives) screening results were confirmed by GC–MS assay and X-ray examination. In 5 cases, positive to the on-site test and GC–MS analysis, abdominal radiography was negative and individuals resulted to be drug users. In 11 cases, negative to the on-site test and radiological investigation, GC–MS analysis found benzoylecgonine in 10 cases and morphine in one case. Concentration of both substances was in all cases lower than 50 ng/ml and compatible with personal drug use.

From obtained results, on-site detection of cocaine and heroin metabolites in the urine of suspected body-packers appears to be a reliable screening test to disclose internally concealed drugs and justify subsequent radiological investigations.

Keywords: On-site test; GC–MS analysis; Cocaine; Heroin; Body-packers

1. Introduction

Body-packers or "mules" are people who ingest packets filled with illicit drugs (typically cocaine or heroin) in the attempt to pass undetected through airport customs. From the first report of 1973 about an individual who swallowed a condom filled with hashish in Toronto [1], the smuggling of illicit drugs either swallowed or inserted into the rectum and/or vagina is becoming increasingly common [2,3]. The detection of this practice is of major importance, not only in the apprehension and prosecution of these smugglers, but also eventual severe health consequences [4]. The "body-packer syndrome" can show the features of acute drug intoxication, intestinal occlusion and delirium leading in some cases to sudden death [5–7].

Suspicious circumstances (e.g. travel route, person's behaviour, refusal of meals during flight, signs of intoxication, information by foreign or local police) can induce customs agents to stop suspected "body-packers" at airport after disembarking. The diagnosis of body packing can be started based on physical examination (e.g. abdominal and rectal examinations), but subsequent abdomen radiography is necessary to confirm internal concealment of drugs and induce packets passing after a purge [2,4]. Nonetheless, some types of packaging may not be always visible on abdominal radiographs [8]. Moreover, to avoid detection pregnant women are used as body-packers, since

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exploration by X-rays is prohibited in these individuals, unless strong evidences of smuggling are available [9].

Detection of illicit drugs in urine from body-packers by immunological screening method, confirmed by gas chromatography–mass spectrometry (GC–MS), has been sparsely reported during the nineties and the application of on-site rapid screening methods was essentially lacking. Contradictory results led some authors to conclude for beneficial role of urinalysis and some others for its limited outcome [10–12].

We aimed to assess the reliability of an on-site immunochromatographic test for screening the presence of cocaine and heroin metabolites in urine from suspected body-packers, confirming the obtained results by gas chromatography–mass spectrometry and X-ray examination.

2. Experimental section

2.1. Chemicals and materials

Cozart® rapid urine multi-panel test was gently donated by Cozart Italia (Pomezia, Roma, Italy).

Cocaine-HCl, benzoylecgonine tetrahydrate (BZE), morphine-HCl, nalorphine-HCl, used as internal standard, I.S., were purchased from Salars (Como, Italy). *N*,*O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was obtained from Sigma–Aldrich (Milano, Italy). Bond Elut Certify solid-phase extraction (SPE) columns were from Varian (Palo Alto, CA, USA). Ultrapure water and all other reagents of analytical grade were obtained from Carlo Erba (Milano, Italy).

2.2. Urine samples collection and on-site rapid test for drugs of abuse

From October 2006 to July 2007, 64 individuals (45 men, 19 women) suspected of body packing for the above-reported suspicious circumstances were stopped by the customs officers at the Fiumicino and Ciampino airports of Rome, Italy. Urine samples were collected from the individuals at the airport and an aliquot was immediately tested for the presence of cocaine and heroin metabolites by Cozart® rapid urine test. This is an on-site immunochromatographic test strip which in few minutes turns positive to the presence of any drug of abuse when no line is present in the line region corresponding to the considered drug. Since this screening test is a multi-panel test, able to screen also for cannabinoids, amphetamines, benzodiazepines and barbiturates, information on the presence of these latter substances in urine from suspected individuals was available.

Irrespective of test results, suspects were referred to the emergency room of a hospital to undergo abdominal X-ray examination. Contemporarily, urine samples were brought to Department of Therapeutic Research and Medicines Evaluation at the Istituto Superiore di Sanità to be analyzed using GC–MS by laboratory personnel, unaware of results from on-site screening.

2.3. Sample preparation for GC–MS confirmation

Samples preparation involved a solid-phase extraction procedure of both non-hydrolyzed and chemically hydrolyzed urine samples in order to measure both free and conjugated morphine (e.g. 3 and 6 morphine glucuronides) as heroin metabolites.

For determination total morphine, 1 ml urine spiked with 10 μl I.S. working solution (10 μg/ml), was incubated in 1 ml 0.1 M HCl at 80 °C for 30 min. Then, urine pH was adjusted to 6.0 using 50 μl 1N NaOH.

Hydrolyzed and non-hydrolyzed urine samples (these latter added with 10 μl I.S. working solution) with 1 ml 0.1 M phosphate buffer pH 6.0, underwent solid-phase extraction procedure using Bond Elut Certify columns. Briefly, columns were preconditioned with 2 ml methanol and 2 ml 0.1 M phosphate buffer (pH 6.0), washed with 1ml 1.0 M acetic acid and 4 ml methanol. Then, samples were loaded and analytes eluted with 2 ml ethyl acetate–2% ammonium hydroxide.

The eluate was evaporated to dryness at 40 °C under a nitrogen stream. The dried sample was derivatized in capped test tubes with 25 μl BSTFA-1%TMS and 25 μl acetonitrile at 70 °C for 30 min. For GC/MS analysis, a 1 μl volume was injected.

2.4. GC–MS confirmation method for cocaine and heroin metabolites in urine

GC–MS analyses were carried out on a 6890 Series Plus gas chromatograph equipped with an Agilent 7683 autosampler and coupled to a 5973 N mass selective detector (Agilent Technologies, Palo Alto, CA, USA). Data acquisition and analysis were performed using standard software supplied by the manufacturer (Agilent Chemstation, Palo Alto, CA, USA). Analytes separation was achieved on a fused silica capillary column (DB-5MS, 30 m × 0.25 mm i.d., film thickness 0.25 μm) (Agilent Technologies). The oven temperature was programmed at 80 °C for 1 min, increased to 230 °C at 35 °C/min, and then raised to 290 °C at 10 °C/min and held for 10 min. Split injection mode (15:1) and helium (purity 99%), with a flow rate of 1 ml/min as carrier gas were used. The injection port, ion source, quadrupole, and interface temperatures were: 260 °C, 230 °C, 150 °C and 280 °C, respectively.

Ions monitored, in the selected-ion-monitoring (SIM) mode, were: *m/z* 82, 182, and 303 for cocaine; *m/z* 82, 240 and 361 for BZE-*O*-TMS; *m/z* 236, 401 and 429 for morphine bis-*O*-TMS and 455 for nalorphine (I.S.). The underlined ions were used for quantification. The methodology was completely validated, applying the internal protocol and in accordance to the internationally established criteria [13–15].

3. Results and discussion

Of the 64 analyzed urine samples, on-site screening gave 29 positive results (26 for cocaine, 2 for cocaine and heroin and 1 for heroin) and 35 negative results (Table 1). Of the 29 positive screening results, all confirmed by GC–MS for the presence of cocaine and/or heroin metabolites, 24 were from body-packers as demonstrated by X-ray examination (Table 1). Specifically,

Table 1
Drug disclosed by on-site urine screening, GC–MS urinalysis and X-ray examination of 24 body-packers

Sample ID	Gender	Drugs	COZART urinalysis screening	GC–MS confirmation (ng/ml)	
17,292 (A)	Female	Cocaine (100 pieces)	COC (+)	Cocaine BZE	12659.59 129.77
17,390 (B)	Male	Cocaine (25 pieces)	COC (+)	Cocaine BZE	ND 370.97
17,461 (D)	Female	Cocaine (81 pieces)	COC (+)	Cocaine BZE	ND 460.39
17,477 (E)	Male	Cocaine (57 pieces)	COC (+)	Cocaine BZE	312.98 14602.29
17,745 (L)	Female	Cocaine (73 pieces)	COC (+)	Cocaine BZE	6.71 320.02
17,825 (M)	Female	Cocaine (92 pieces)	COC (+)	Cocaine BZE	ND 387.39
17,857 (N)	Female	Cocaine (1 piece)	COC (+)	Cocaine BZE	42.99 446.60
17,858 (S)	Female	Cocaine (1 piece)	COC (+)	Cocaine BZE	ND 570.24
17,917 (O)	Male	Cocaine (97 pieces)	COC (+)	Cocaine BZE	ND 335.15
18,139 (T)	Male	Cocaine (50 pieces)	COC (+)	Cocaine BZE	ND 444.55
18,233 (P)	Male	Cocaine (65 pieces)	COC (+)	Cocaine BZE	ND 400.02
A/7 (R)	Male	Cocaine (77 pieces) Heroin (1 piece)	COC (+), OPI (+)	Cocaine BZE Morphine	ND 390.64 Free, 50.46; total, 340.02
18,425 (V)	Female	Cocaine (50 pieces)	COC (+)	Cocaine BZE	10.19 282.18
18,426 (Z)	Female	Cocaine (1 piece)	COC (+)	Cocaine BZE	ND 380.10
18,439 (W)	Female	Cocaine (96 pieces)	COC (+)	Cocaine BZE	554.94 12400.14
18,646 (AG)	Male	Cocaine (60 pieces)	COC (+)	Cocaine BZE	ND 469.64
18,781 (AL)	Female	Cocaine (16 pieces)	COC (+)	Cocaine BZE	76.53 735.23
18,916 (AS)	Male	Cocaine (84 pieces)	COC (+)	Cocaine BZE	ND 570.45
18,920 (AT)	Male	Cocaine (82 pieces)	COC (+)	Cocaine BZE	34.46 7414.93
19,096 (AW)	Female	Cocaine (26 pieces)	COC (+)	Cocaine BZE	ND 325.44
19,111 (AY)	Male	Cocaine (81 pieces)	COC (+)	Cocaine BZE	ND 352.14
19,141 (BB)	Male	Cocaine (79 pieces)	COC (+)	Cocaine BZE	ND 324.12
A/30 (BH)	Male	Cocaine (91 pieces)	COC (+)	Cocaine BZE	19514.09 271.41
A/31 (BI)	Male	Heroin (77 pieces)	OPI (+)	Morphine	Free, 67.22; total, 356.28

COC, cocaine; OPI, opiates; 6-MAM, 6-acetylmorphine.

Table 2
Drug disclosed by on-site urine screening, GC–MS urinalysis in individuals negative to X-ray examination

Sample ID	Gender	Confessed drugs	COZART urinalysis screening	GC/MS confirmation (ng/ml)	
17,720 (I)	Male	None	OPI (+)	Morphine	Free, 5.60; total, 370.44
				Codeine	Free and total, ND
A/5 (U)	Male	None	COC (+)	Cocaine	ND
				BZE	303.68
18,578 (AE)	Male	None	COC (+)	Cocaine	ND
				BZE	415.95
A/16 (AN)	Male	None	COC (+)	Cocaine	ND
				BZE	396.54
19,555 (BO)	Female	None	COC (+)	Cocaine	ND
				BZE	323.98

abdominal radiographs disclosed 19 cases of body packing by ingestion, 1 by ingestion and rectal insertion, 1 only by rectal insertion and finally 3 by vagina insertion. The number of found packages ranged from 1 to 100. In all the cases but one, the concealed drug was cocaine, as revealed by gas chromatographic mass spectrometric analysis of the powder (method applied was the above reported for urine samples, opportunely modified and validated for powder analysis). In one case, one heroin packet was found together with the 77 ones containing cocaine; and in another case only 77 packets of heroin were found. This evidence was in agreement with the results of urinalysis of the two body-packers: indeed urine sample of the first one contained both cocaine and heroin metabolites, while the second only contained heroin metabolites (Table 1). The smugglers were 13 men (age: 32.8 ± 7.32 years) and 11 women (age 33.7 ± 7.76 years), nobody presented signs of drug intoxication and no surgical extraction of packets was needed since they were eliminated by the use of laxatives.

The five remaining samples, positive to on-site screening test and GC–MS analysis were from individuals, which resulted negative to X-ray examination. These individuals admitted personal drug use and absence of drug packages was further confirmed by evacuation of normal faeces without packets.

With respect to the 35 negative results by on-site screening test, 24 were confirmed by both X-ray examination and GC–MS analysis. In the remaining 11 cases, negative to the on-site test and radiological investigation, GC–MS analysis found BZE in 10 cases and morphine in one case. Concentration of both substances was in all cases lower than 50 ng/ml (from 6.89 to 40.45 ng/ml BZE in 10 cases and 43.69 ng/ml total morphine)

Table 3
Drug disclosed in urine samples negative to on-site urine screening and X-ray examination, but positive to GC–MS

Sample ID	Gender	COZART urinalysis screening	GC–MS confirmation (ng/ml)	
18,536 (X)	Male	(-)	Morphine	Free, ND; total, 43.69
A/14 (AH)	Male	(-)	Cocaine	ND
			BZE	10.80
A/15 (AM)	Female	(-)	Cocaine	ND
			BZE	6.89
	Male	(-)	Cocaine	ND
			BZE	15.64
A/18 (AQ)	Male	(-)	Cocaine	ND
			BZE	8.68
18,914 (AR)	Male	(-)	Cocaine	ND
			BZE	8.26
A/19 (AU)	Male	(-)	Cocaine	ND
			BZE	40.45
A/20 (AV)	Male	(-)	Cocaine	ND
			BZE	22.37
A/21 (AZ)	Female	(-)	Cocaine	ND
			BZE	10.11
A/22 (BE)	Female	(-)	Cocaine	ND
			BZE	19.48
A/24 (AX)	Female	(-)	Cocaine	ND
			BZE	7.34

in one case and compatible with personal drug use, which was indeed admitted by the individuals (Tables 2 and 3).

From the obtained results, it can be concluded that the Cozart® rapid urine multi-panel test showed a great sensitivity and specificity in detecting internal concealment of cocaine and heroin in suspected individuals. Indeed, the test gave five results that can be defined as "false positive for internal concealment of drugs", since individuals were true positive for cocaine or heroin, but due to personal use of drugs. Most importantly, the test did not give any "false negative for internal concealment of drugs" since all the negative samples were not body-packers, even if they were consumers in some cases, as revealed by GC–MS confirmation.

For the first time, our proposed method implies the use of an on-site test, which can be easily used in a non-medical setting (e.g. directly at the airport site). Differently from the sparse methods reported during the nineties which typically coupled a laboratory-machine screening immunological method (e.g. EMIT or TDx) with chromatographic confirmation [10–12], this applied on-site screening test result was not only rapid, but also selective and sensitive enough, when compared to the confirmatory GC–MS analyses.

It has to be said that in this study the on-site test has not been applied to a random population, but only to individuals already suspected of "body packing". Never-the-less, when in presence of suspicious circumstances, this rapid and simple screening test can help in singling out suspected individuals and in justifying subsequent radiological investigations, especially in the particular case of pregnant and paediatric body-packers [9,16].

4. Conclusion

Detection of body packing can be a difficult task because most of the smugglers behave normally, and due to the increasing of sophistication of packaging it is not always possible to disclose internal concealment of drugs by abdominal radiographs.

In this study, we showed that there is a striking relation between the presence of drugs in urine and body packing of cocaine and heroin. The on-site Cozart® rapid urine multi-panel test used to screen the presence of cocaine and heroin metabolites in urine from suspected body-packers proved to be a rapid, simple method that seems to be a reliable test useful to justify subsequent radiological investigations in suspected individuals.

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