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Profiles of urine samples from participants at rave party in Taiwan: prevalence of ketamine and MDMA abuse

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Abstract

Drug abuse patterns are different due to cultural, social and geographical differences. Methamphetamine (MA) is the most important drug of abuse in Taiwan followed by opiates. Recently, there has been an increase of ketamine and MDMA abuse in disco dancing clubs. Here, we report the patterns of drug abuse by the participants in a metropolitan city disco-dancing club and the general public in Taiwan. The positive rates of common drugs of abuse detected in samples collected from participants in a dancing club were as follows: MDMA, 75.7%; ketamine, 47.0%; MA, 41.6%; opiates, 0%. Marijuana and cocaine were detected at much lower rates (3.4 and 4.7%, respectively). Ketamine and one of the amphetamines were detected together in 42.9% of the samples. The positive rates in samples collected from police detainees suspected of drug abuse in the general public were as follows: MA, 76.0%; OPA, 37.0%; MDMA, 6.0%; ketamine, 2.0%.

Keywords: Ketamine; MDMA; Rave party

1. Introduction

Ketamine (K) was synthesized by a scientist at Parke-Davis Laboratories and marketed as an anesthetic drug for human and animal use in the 1970s [1,2]. It is a ''dissociative'' anesthetic [2]. At sub-anesthetic doses, ketamine possess psychedelic properties similar to phencyclidine (PCP) [3]. Ketamine induces hallucination like PCP is less toxic and with a shorter duration of action. Psychedelic effects of ketamine include the following: mind revealing; increased empathy and insights; revealing old memories; out-of-body experience and near-death experience [1,4]. Reports of ketamine abuse appeared soon after its introduction into clinical use [5]. Recently, there were reports of increased abuse of ketamine and ketamine-related deaths [6–8].

Amphetamines (AMPs) are sympathomimetic drugs, and are used to treat narcolepsy, attention-deficit disorder and obesity [9]. They are also powerful central nervous system stimulants and have potent effects on the reward (pleasure) center of the brain that leads to euphoria and so are widely abused. The most important AMPs are amphetamine (A) and methamphetamine (MA) [9]. The designer drugs of amphetamine, such as: 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) are psychotropic drugs [10]. MDMA was widely abused in the US [11] in the 1980s. In England, MDMA was commonly abused at ''rave'' parties where electronic music was mixed with video and laser shows [12]. This counter culture with the combination of a rave party and MDMA has been exported worldwide [13].

MDMA causes a drop in defense mechanism or fear response, a reduced inhibition, an increased empathy for others, an intensification of feeling, a desire for communication and euphoria [11,12,14,15]. The adverse effects of MDMA include tachycardia, jaw clenching, nystagmus, anorexia, nausea, vomiting and ataxia [16,17]. In higher doses, MDMA can cause paranoid psychosis, panic attack and seizure [17]. Death due to MDMA abuse usually is associated with hyperthermia and dehydration [12,16].

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In Taiwan, MA is the most important drug of abuse, followed by opiates [18,19]. There were also sporadic reports of MDMA and ketamine abuse. However, the populations that abuse MDMA and ketamine, and the prevalence rates of their abuse have not been determined. In this report, we collected urine samples from two different sources and compared the patterns of drug abuse.

2. Materials and methods

2.1. Drug standard and chemicals

Amphetamine, MA, MDA, MDMA, K, NK and the deuterated analogs, A-d_5, MA-d_8, K-d_4 and NK-d_4 were all obtained from Cerilliant Corp. (Austin, TX, USA).

Trichloroacetic anhydride (Fluka, Switzerland), *N*,*O*-bis-(trimethylsilyl) acetamide (Tri sil BSA) (Pierce, USA) and other chemicals were purchased through local distributors.

Immunoassay reagents for AMPs, OPA, BZD, THC, COC were purchased through local distributors of DRI (Diagnostic Reagents Inc., Sunnyvale, CA, USA). DRI reagents are enzyme immunoassays based on the Emit® principle [20].

2.2. Urine samples

Urine samples (149) were collected from participants of a disco-dancing club in Taipei City (referred to as Club samples) in 2001. Another 100 urine samples were collected from police detainees suspected of drug abuse in the general public (referred to as Detainee's samples) in the same period. Samples were kept in the refrigerator at 4 °C until analyses.

2.3. Immunoassays

Urine samples were screened with IA reagents adapted to a Cobas Mira *Plus* (Roche Diagnostic Systems Inc., Branchburg, NJ, USA) automatic clinical chemistry analyzer according to the manufacturer's recommendations. Concentrations of the cutoff calibrators for the immunoassays are as follows: AMPs, d-amphetamine 500 ng/ml; OPA, morphine 300 ng/ml; BZD, oxazepam 200 ng/ml; COC, benzoylecgonine 300 ng/ml; THC, 11-nor-9-carboxy-delta^9-tetrahydrocannabinol 50 ng/ml.

2.4. Determination of the amphetamines with GC/MS

Samples were extracted with organic solvent, followed by chemical derivatization before GC/MS analysis. Briefly, 2 ml of urine samples were adjusted to pH 9.5 with bicarbonate buffer and extracted with 3 ml of ethylacetate (EA). The organic layer was evaporated to dryness under a stream of nitrogen gas. The dried extracts were derivatized with trichloroacetic anhydride and analyzed with GC/MS. Deuterated analogs of A (A-d_5) and MA (MA-d_8) were employed as internal standards for the quantification of A/MDA and MA/MDMA, respectively. The retention times, quantitation and qualification ions are summarized in Table 1. A Hewlett-Packard GC (6890) coupled to MSD (5973) (Agilent, Palo Alto, CA, USA) equipped with a capillary column HP-5MS (12 m × 0.2 mm i.d., 0.33 μm film thickness) was used for all the GC/MS analysis.

Table 1
Retention times and ions monitored for the GC/MS analysis of amphetamines and ketamine

Analytes	Retention times (min)	Ions monitored (*m*/*z*) (underlines: quantitation ions)		
Amphetamine	4.64	118	188	190
Amphetamine-d_5	4.63	123	194	196
Methamphetamine	5.11	202	204	118
Methamphetamine-d_8	5.10	209	211	213
MDA	5.85	162	135	188
MDMA	6.31	162	135	202
Ketamine	14.33	209	180	182
Ketamine-d_4	14.27	184	213	186
Norketamine	13.60	166	195	168
Norketamine-d_4	13.55	170	199	172

The temperature program for GC was as follows: oven temperature began at 100 °C and held for 0.3 min, then increased to 270 °C at 30 °C/min and held for 2 min. Mass data were collected with scan mode (scan range: *m*/*z* from 45 to 350) [21].

2.5. Determination of ketamine and norketamine with GC/MS

To 2 ml of urine samples were added 200 ng of internal standard (K-d_4 and NK-d_4 at 10 μg/ml in methanol) and hydrolyzed with 0.5 ml of concentrated hydrochloric acid at 120 °C for 20 min. After cooling down to room temperature and neutralizing with concentrated KOH solution, the samples were made alkaline with 2 ml of 1N NaOH and extracted with 3 ml of EA. The organic layer was evaporated to dryness under a stream of nitrogen gas. The dried extracts were dissolved in 100 μl of EA and 3 μl was injected to GC/MS. The retention times and ions monitored are presented in Table 1. The temperature program of the GC was as follows: oven temperature began at 110 °C and held for 0.5 min, then increased to 180 °C at 5 °C/min and held for 0.2 min, finally increased at 45 °C/min to 270 °C and held for 0.5 min. Splitless, selected ion monitoring mode was used to collect data [22].

3. Results

3.1. Pattern of drug abuse

Results of initial immunoassay screening and GC/MS confirmation are shown in Table 2. Ketamine was not

Table 2
Positives rates of common drugs of abuse in urine samples collected from two different sources

Analytes	Positive rate (%)			
	Club samples		Detainees's samples	
	IA	GC/MS[a]	IA	GC/MS[a]
AMPs	77.2	83.2	80.0	77.0
OPA	0.7	0	38.0	37.0
THC	3.4	3.4	5.0	2.0
COC	4.7	4.7	0	0
K	ND[b]	47.0	ND[b]	2.0
BZD	11.4	ND[b]	10.0	ND[b]

[a] GC/MS positive sample are defined as follows: AMPs: A, MA, MDA or MDMA $\geq$ 500 ng/ml; OPA: morphine or codeine $\geq$ 300 ng/ml; THC: THC $\geq$ 15 ng/ml; COC: benzoylecgonine $\geq$ 150 ng/ml; K: ketamine or norketamine $\geq$ 50 ng/ml.
[b] ND: not done.

screened because there was no commercially available immunoassay. The presumptive positive samples of OPA, THC and COC by IA were confirmed with GC/MS. In the Club samples, the OPA positive sample confirmed negative while all the THC and COC positive samples confirmed positive. The BZD positive samples were not analyzed with GC/MS. Every sample was also analyzed with GC/MS for AMPs, K and NK. The cutoff concentration is 500 ng/ml for A, MA, MDA and MDMA; and 50 ng/ml for K and NK. The positive rates for AMPs and K/NK were 83.2 and 47.0%, respectively. There were 9 samples (6%) that tested positive for AMPs by GC/MS, but tested negative by immunoassay (false negatives).

For comparison, the results of urine samples collected from police detainees suspected of drug abuse are also shown here. The presumptive positive samples for OPA and THC by IA were further analyzed with GC/MS. The positive rates obtained with GC/MS for OPA and THC were 37 and 2.0%, respectively.

Every Detainee's urine sample was analyzed for AMPs, K and NK with GC/MS. With the same cutoff concentration as described above, the positive rates for AMPs and N/NK were 77.0 and 2.0%, respectively. There were three AMPs false positive samples by the immunoassay and no false negatives.

3.2. Profile of drugs of abuse

The profile of drugs of abuse in the Club samples is shown in Table 3. Since A and MDA are metabolites of MA and MDMA, respectively, individual urine sample was designated as A or MDA positive only, when the concentration of A or MDA was equal to or greater than 500 ng/ml and MA or MDMA was less than 500 ng/ml. When the concentration of MA or MDMA was equal to or greater than 500 ng/ml, the sample was designated as MA or MDMA positive irrespective of the concentration of A or MA. A complicated poly-drug use patterns were observed. In the Club samples, up to five drugs were detected in some samples. The most prominent combination was MDA, MA and K (18.1%). The positive rates for individual drug were as follows: MDMA, 75.7%; K, 47.0%; MA, 41.6%; BZD, 11.4%; COC, 4.7%; THC, 3.4%; MDA, 2.7%; A, 0.7%.

Table 3
Profile of drugs of abuse in the Club samples

Drugs analyzed								Samples (%)
MDMA	MDA	MA	A	K	BZD	COC	THC	
+	−	−	−	−	−	−	−	24.2
+	−	+	−	+	−	−	−	18.1
+	−	−	−	+	−	−	−	10.7
+	−	+	−	−	−	−	−	9.4
+	−	+	−	+	+	−	−	4.0
−	−	−	−	+	−	−	−	4.0
+	−	+	−	+	+	+	−	2.0
−	−	+	−	+	−	−	−	2.0
+	−	−	−	−	+	−	−	1.3
+	−	−	−	+	+	−	−	1.3
+	−	+	−	−	+	−	−	1.3
+	−	+	−	+	−	−	+	1.3
−	+	−	−	−	−	−	−	1.3
+	−	−	−	−	−	−	+	0.7
+	−	+	−	+	−	+	+	0.7
+	−	−	+	+	−	−	−	0.7
−	+	+	−	+	−	−	−	0.7
−	+	+	−	−	−	−	−	0.7
−	−	+	−	+	+	+	−	0.7
−	−	+	−	+	+	−	+	0.7
−	−	−	−	−	−	−	−	15.5

The profile of drugs of abuse in the Detainee's samples is shown in Table 4. Designation of A, MA, MDA or MDMA positives was described as above. In the Detainee's samples, there was no sample that tested positive for A or MDA alone.

Table 4
Profile of drugs of abuse in Detainee's samples

Drugs analyzed						Samples (%)
MDMA	MA	OPA	K	BZD	THC	
−	+	−	−	−	−	41.0
−	+	+	−	−	−	23.0
−	−	+	−	−	−	7.0
−	−	+	−	+	−	4.0
+	+	−	−	−	−	3.0
−	+	+	−	+	−	3.0
−	+	−	−	+	−	3.0
+	+	−	+	−	−	2.0
+	−	−	−	−	+	1.0
−	+	−	−	−	+	1.0
−	−	−	−	−	−	12.0

Table 5
Distribution pattern of amphetamines and ketamine in urine samples from two different sources

Drugs analyzed			Samples (%)	
MDA/MDMA	A/MA	K	Club	Detainee's
+	+	+	27.5	2.0
+	+	−	11.4	3.0
+	−	+	12.0	0
+	−	−	27.5	1.0
−	+	+	3.4	0
−	+	−	0	70.0
−	−	+	4.0	0
−	−	−	15.5	24.0

MA and OPA was the most important combination detected (23.0%). The positive rates for individual drug were as follows: MA, 76.0%; OPA, 37.0%;, BZD, 10.0%; MDMA, 6.0%; K, 2.0%; and THC, 2.0%.

3.3. Combined use of ketamine and amphetamines

The samples were reorganized into MDA/MDMA, A/MA and K positives. Results are shown in Table 5. For the Club samples, the positive rate for ketamine with one of the amphetamines was 42.9%. For the Detainee's samples, the positive rate for K and MA was 2.0%.

4. Discussion

The present study shows a high prevalence of ketamine and MDMA detection in urine samples from participants in a disco-dancing club in Taiwan. Detailed analysis of the drugs of abuse profiles in Club urine samples and Detainee's samples revealed a very different pattern. Ketamine and MDMA positive rates were extremely high (47.0 and 75.7%, respectively) in Club urine samples. A substantial number of Club samples also tested positive for MA (41.6%). MDA and A were detected only occasionally (2.7 and 0.7%, respectively). There were only a few samples tested positive for marijuana and cocaine (3.4 and 4.7%, respectively) and none was positive for opiates. The relatively high positive rate for BZD is a cause for concern. Because BZD are legitimate clinical therapeutic drugs, how many of the positive samples were from the legitimate use and how many were the illegal abuse is not known. There are many drugs in the benzodiazepine family. We did not attempt to identify the individual drug in the BZD positive samples.

In the Detainee's samples, there was no sample that tested positive for A or MDA alone. MA was the most important drug (76.0% positive), followed by opiates (37.0% positive). This agrees with the drug abuse pattern we observed previously [19]. Only a few MDMA (6.0%), K (2.0%) and THC (2.0%) positive samples were detected. The positive rate for BZD was similar to the Club samples (10.0%).

There were nine Club samples that were tested negative by immunoassay and positive by GC/MS (false negative by IA). All the samples contained low levels of either MDA or MDMA except two samples with relatively high MDA (5626 ng/ml) or MDMA (1927 ng/ml). This discrepancy was due to the low cross reactivity of the reagent employed for this study toward MDA and MDMA (40 and 20%, respectively) [20]. The immunoassay was designed with maximum specificity toward d-amphetamine. There were three Detainee's samples that tested positive by IA and negative by GC/MS (false positives). Two of the samples contained low level of amphetamine. The DRI amphetamine reagents employed in this study were capable of detecting AMPs in most of the samples.

Ketamine and MDMA were detected together in 42.9% of the Club samples. However, in the Detainee's samples, only 2.0% of the sample was positive for both K and MDMA.

5. Conclusion

The patterns of drug abuse were very different between the participants in disco-dancing club and the general public in Taiwan. In the Club samples, MDMA was the most important drug detected (75.7%), followed by ketamine (47.0%) and MA (41.6%). The positive rate for K and MDMA poly-drug use was 42.9%. In the Detainee's samples, MA (76.0%) was the most important drug detected, followed by opiates (37.0%), MDMA (6.0%) and ketamine (2.0%). The positive rates for THC and COC were low in the two groups of samples tested.

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