Forensic Science International 188 (2009) e1–e5

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case report

# Sudden death associated with intravenous injection of toad extract

Chris Kostakis [a], Roger W. Byard [a,b,*]

[a] Forensic Science SA, Adelaide, Australia
[b] Discipline of Pathology, The University of Adelaide, Adelaide, Australia

ARTICLE INFO

*Article history:*
Received 23 October 2008
Received in revised form 27 January 2009
Accepted 7 February 2009
Available online 19 March 2009

*Keywords:*
Toad
Ecstasy
Substitution
Sudden death
Chinese herbal medicine
*Chan Su*

ABSTRACT

A 24-year-old male died suddenly following the intravenous injection of what was believed to be the ring-derivate amphetamine 'ecstasy' (MDMA). Toxicological analyses of the victim's blood and the injected material, however, failed to reveal MDMA, but showed instead low levels of bufotenine, a tryptamine derivative alkaloid found in the secretions of various toads. In addition, resibufogenin, cinobufagin and bufalin, bufadienolides that are also found in toad venom, were identified in the injected material. While these substances also occur in certain South American plants, the finding of paracetamol, promethazine and diclofenac would be in keeping with ingredients found in the traditional Chinese herbal product *Chan Su* that derives from the skin glands and secretions of toads and that is often adulterated with standard pharmaceutical drugs. This case demonstrates the problems that users and sellers may encounter from the unknown composition of street drugs and herbal medicines, and the danger that may be incurred from the injection of such materials. It also shows the difficulties that may be associated with attempting to identify low levels of organic toxins in postmortem specimens necessitating a targeted screening approach guided by information obtained at the death scene.


## 1. Introduction

In our experience illicit drug administration by intravenous injection may cause death due to a variety of mechanisms including central respiratory depression and cardiotoxicity. Sedation and respiratory depression are most often due to opiate drugs such as heroin and morphine resulting in lethal cerebral hypoxia. The history from witnesses is often of prolonged and deep sleep with snoring after drug administration, followed by failure to rouse and eventually death [1]. Alternatively, intravenous administration of an opioid drug may result in rapid death; the drug may be self-administered or used in a homicide. Amphetamines and other psychostimulants, such as cocaine, may also be injected and may cause hyperpyrexia or have a direct cardiotoxic action [2]. These drugs may also impair reflexes or lead to increased risk taking, both of which may predispose to traumatic deaths. Injected drugs may have an additive or synergistic effect with other drugs or alcohol that may have been previously or concurrently taken. The combined effects of a variety of drugs may also result in death from aspiration of gastric contents if emesis is stimulated and swallowing mechanisms are impaired.

Occasionally a case of witnessed intravenous drug administration occurs that is followed by collapse and rapid death where routine toxicological evaluation does not reveal common drugs of abuse. The details of such a case are reported involving the self-administration of what was thought by the user to be a ring derivative amphetamine.

## 2. Case report

A 24-year-old male was observed to collapse and die soon after an intravenous injection of 35–40 ml of what was thought to be 'ecstasy' (methylenedioxy-methamphetamine or MDMA). A friend had injected a smaller volume (20 ml) and had vomited but survived. The decedent was a known intravenous amphetamine user but was not known to abuse any other illicit drugs. The body was transferred to FSSA for autopsy along with a plastic bag containing the injected material and the empty syringe.

At autopsy the body was that of a young adult male with no injuries or significant underlying organic illnesses that could have caused or contributed to death. Recent and remote injection sites were present in the left cubital fossa characterized by interstitial hemorrhage and fragments of non-polarizable foreign-body type material with surrounding chronic inflammation and hemosiderin-containing macrophages. The lungs showed congestion and edema with no evidence of aspiration of gastric contents.

Routine postmortem toxicological screening of femoral venous blood, urine and gastric contents, utilizing immunoassay,

* Corresponding author at: Discipline of Pathology, Level 3 Medical School North Building, The University of Adelaide, Frome Road, Adelaide 5005, Australia. Tel.: +61 8 8303 5441; fax: +61 8 8303 4408.
*E-mail address:* byard.roger@saugov.sa.gov.au (R.W. Byard).

0379-0738/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2009.02.006

chromatography and mass spectrometry was undertaken without detecting common illicit or prescribed drugs or pesticides.

Given the circumstances of death the empty syringe was rinsed with ethanol and the resultant solution concentrated and analysed by gas chromatography/mass spectrometry (GC–MS). The GC–MS analyses were performed using an Agilent Technologies 6890N GC coupled to a 5973N mass selective detector and equipped with a 15 m × 0.25 mm internal diameter fused-silica capillary column with a bonded 0.25 μm DB-5ms phase. Injections (2 μl) were made in the pulsed splitless mode with an inlet temperature of 250 °C and a pulsed pressure of 10 psi for 2 min. The purge flow was set to 50 ml/min. The carrier gas was helium set to a constant pressure of 1.5 psi. Oven temperature program was 60 °C (2 min) to 280 °C (10 min) at a rate of 15 °C/min. The transfer line was set at 280 °C, and the MS source and quadrupole set points were 230 and 150 °C, respectively. The analyses were performed in full scan EI (electron impact) mode with a scan range of 40–550 amu. All data was processed using Agilent Chemstation software. Drug confirmations were achieved by retention time and mass spectral matches to reference samples analysed on the same system.

While MDMA was not detected, examination of chromatograms revealed low levels of paracetamol and promethazine. A small amount of the injected material was transferred to a second tube and ethanol was added. Following mixing and centrifugation, the ethanol was removed and concentrated. Again the presence of paracetamol and promethazine was confirmed along with diclofenac. These drugs were not detected in the blood of the deceased.

Further examination of the injected material was undertaken with extraction of material under alkaline (pH ∼ 10) and acidic (pH ∼ 2) conditions. The extracts were prepared by the addition of distilled water (1 ml) and 0.1 ml of a 0.5-M sulphuric acid solution (acidic conditions) or 0.3 ml of concentrated ammonia solution (alkaline conditions) to separate tubes containing a portion of the injected material. The mixtures were sonicated, vortexed and then had ethyl acetate (2 ml) added. The capped tubes were shaken, centrifuged and the ethyl acetate layers transferred to separate tubes, concentrated and analysed using the same GC–MS conditions described earlier. The acidic extract was again found to contain paracetamol, promethazine and diclofenac, as well as several bufadienolides, including resibufogenin, cinobufagin and bufalin (Figs. 1 and 2). Fig. 2 shows the mass spectrum highlighting the characteristic ions and intensities of each bufadienolide identified in the injected material. Tentative identification of the bufadienolides was achieved by mass spectral library matches to those listed in the NIST mass spectral database [3]. Identification of these compounds was further confirmed by mass spectral comparisons with those reported by Barry et al. [4]. The presence of bufadienolides and additional lipids in the acid extract correlated with the findings of Barry et al. who identified these compounds in their analysis of the traditional Chinese herbal medicine *Chan su* [4].

The basic extract was also found to contain paracetamol, promethazine and diclofenac, in addition to bufotenine. Re-analysis of the decedent's blood, using a targeted procedure, revealed a trace of bufotenine that had not been detected through the general screening process. The procedure first involved an enzyme hydrolysis step using β-glucuronidase type IX-A isolated from *Eshcerichia coli* and based on the method outlined by Kamata et al. [5]. Psilocin, a structural isomer of bufotenine, is metabolised by conjugation with glucuronic acid known as glucuronidation [5,6]. It is anticipated that bufotenine will also exist in its conjugated form and cleavage of the glucuronide bond by enzymatic hydrolysis was undertaken. Following hydrolysis, bufotenine and its internal standard, psilocin, were extracted from postmortem blood using mixed-mode solid-phase extraction (SPE). Hydrolysed blood samples (3 ml) were loaded onto X-trackt® SPE cartridges (XRDAH-203; UCT, Inc.) pre-conditioned with 3 ml of methanol followed by 3 ml of acetate buffer (0.1 M; pH 5.7). The extraction cartridges were washed with 3 ml of acetate buffer (0.1 M; pH 5.7) followed by 1 ml of 0.1 M acetic acid and finally with 3 ml of methanol. Bufotenine and psilocin were then eluted with 3 ml of 2% concentrated ammonia solution in a dichloromethane/isopropanol mix (80:20). The eluant was evaporated to dryness, reconstituted into 50 μl ammonium formate (10 mM; pH 3.5) and analysed by liquid chromatography tandem mass spectrometry (LC–MS–MS).

Chromatographic separation was carried out on an Agilent 1100 HPLC with an Agilent ZORBAX SB-C18 column (150 mm × 2.1 mm, 5.0 μm). A mobile phase consisting of acetonitrile and ammonium formate (10 mM; pH 3.5) was delivered with a constant flow rate of 0.2 ml/min. The following step-wise gradient elution program was used: the acetonitrile concentration was maintained at 5% (v/v) for 5 min, then increased to 90% over 12 min, remaining constant for the last 2 min. The sample injection volume was 20.0 μl. Mass spectra were obtained using an Agilent LC/MSD Trap "Classic" (Model G2445A) equipped with an electrospray ionisation source in positive ion mode. Data acquisition was performed using multiple reaction monitoring (MRM) of the fragmentation products of protonated pseudo-molecular ions ($m/z$ 205 > 160 for both bufotenine and psilocin). System control and data analysis was provided by the Agilent LC–MS Chemstation software.

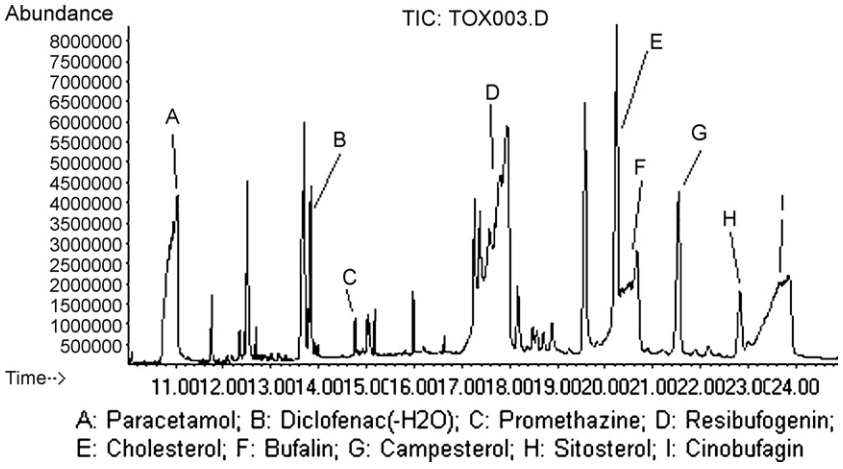


**Fig. 1.** GC–MS analysis of the injected material following acid extraction.