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Quantitative analysis of methamphetamine in hair of children removed from clandestine laboratories – Evidence of passive exposure?

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ABSTRACT

In New Zealand many children have been removed from clandestine laboratories following Police intervention. In the last few years it has become standard procedure that these children have hair samples taken and these samples are submitted to the laboratory for analysis.

There are various mechanisms for the incorporation of drugs into hair. The hair follicle has a rich blood supply, so any drug that may be circulating in the blood can be incorporated into the growing hair. Another mechanism is via external contamination, such as spilling a drug on the hair or through exposure to fumes or vapours.

Hair samples were analysed for methamphetamine and amphetamine. From the 52 cases analysed 38 (73%) were positive for methamphetamine (>0.1 ng/mg) and amphetamine was detected in 34 of these cases. In no case was amphetamine detected without methamphetamine. The hair washes (prior to extraction) were also analysed (quantified in 30 of the positive cases) and only 3 had a wash to hair ratio of >0.1 (all were <0.5), which may be indicative of a low level of external contamination. This low level of evidence of external contamination suggests that the children are exposed to methamphetamine and are incorporating it into the hair through the blood stream.

1. Introduction

Production of methamphetamine (MA), a Class A drug, in clandestine laboratories is a big issue in New Zealand. The number of such laboratories identified by Police has increased from 9 in 2000 to 135 in 2009 [1]. In a third of these laboratories children are associated with the property in some way and the children are present in around a third of these (therefore around 1/6th of total cases) at the time of a Police raid. In the United States of America the Drug Enforcement Agency reported more than 15 000 children present in clandestine laboratory related incidents in the years 2000–2005 [2]. The manufacture of MA is associated with high levels of fumes and vapours (with an associated high risk of explosion) and properties where manufacture has been carried out require extensive decontamination after the laboratory is dismantled.

In a clinical review of children removed from MA laboratories there were serious health concerns raised over exposure to both the chemicals used in production of MA and to the drug itself [2]. The most significant health effects are due to the inhalation of chemicals causing respiratory irritation and distress, contact with skin causing burns and irritation of the eyes. The most common effect seen in children presenting to emergency departments following exposure to amphetamines is an altered mental state – agitation, hallucination and confusion. Other effects reported to be associated with MA exposure include: tachycardia, vomiting, uncontrolled crying, seizure, rapid eye movements and hypertension [2].

MA is present in high amounts on surfaces, furniture and appliances in the clandestine laboratory [2]. It is likely that children living in these properties are exposed in this way, and through breathing any airborne drug present. It has been reported that urine samples from children are more likely to be positive for MA if collected within 6 h of the most recent exposure [3], with no child testing positive greater than 6.5 h after removal from exposure. Occupational exposure of laboratory workers handling large quantities of MA leads to low level positive urine samples, with levels in the urine that would not be deemed positive by most screening tests [4]. These subclinical doses are eliminated from the body rapidly but repeated subclinical exposure may accumulate in the hair.

Hair testing has been examined as a tool for detecting exposure to MA in children removed from clandestine laboratories. In a previous study (Farst et al. [5]) 103 hair samples were analysed and 46 (45%) tested positive for MA at a level above 0.1 ng per milligram of hair (ng/mg) [5] (no levels were reported). Hair testing has also been used to detect the presence of cocaine in hair samples from children in cases of exposure [6,7].

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Passive exposure and subsequent incorporation into hair is not well studied with drugs of abuse. There are three main modes of incorporation of drugs described: from circulating blood in the hair follicle; from sweat or sebum resting on the hair and external passive contamination from the environment (particularly for a smoked or powdered drug). An important first step in analysis is therefore the washing of the sample to remove any drug resting on the external surface of the hair before extraction. The hair washes are kept and analysed alongside the extracted samples to determine the extent of environmental exposure. The likelihood of a positive result being from external exposure can be determined by the ratio of the analysed wash to the extracted hair sample [8]. In cases of child exposure it is not so important to determine whether the drug had been absorbed through the blood stream or through contact with the hair, most law enforcement agencies describe either as unacceptable. However, by washing the samples and analysing the washes it is possible to propose whether it is likely the child has incorporated the drug into the hair through their blood or if the drugs presence in the hair is more likely to be through external contamination.

This study shows the results of hair analysed from children, aged from 2 months to 15 years, removed from clandestine laboratories during 2008–2010. The levels are compared with those detected in adult users from the same period. To the best of our knowledge there are no other studies showing the levels of MA achieved in children's hair samples following exposure to MA manufacture or determining the likelihood of external contamination by analysis of the washes.

2. Methods

2.1. Reagents and materials

The substances amphetamine (AMP), AMP-D_5, MA and MA-D_5 were purchased as liquid standards from Cerilliant (through BDG Synthesis, NZ). Solid phase extraction cartridges (Bond Elute Certify, 130 mg sorbent mass, 120 μm particle size) were from DKSH, New Zealand. All solvents were Mallinckrodt reagent grade from Biolab, NZ.

2.2. Sample collection

The samples were collected from children aged from 2 months to 15 years during a medical examination following their removal from a clandestine laboratory. Other than age (reported in the majority of cases), no demographic information was made available to the laboratory. The samples (where possible) were collected from the posterior vertex [9]. The samples were tied at the root end and transported to the laboratory for analysis. The protocol was to analyse three times two centimetre segments starting from the root (so a 6 month coverage), the results are presented here as a mean of that 6 month period.

2.3. Extraction from the hair

The extraction was undertaken as previously published [10,11]. Briefly, each sample (20 mg) was washed with methanol (1 mL) three times, the final wash was dried down for analysis. The samples were extracted overnight at 37 °C by the addition of HCl (1 mL, 0.1 M). The samples were then centrifuged (2700 rpm, 5 min) and adjusted to approximately pH 7 using NaOH (100 μL, 1 M). Phosphate buffer was added (0.1 M, pH 7), the tubes vortexed briefly and centrifuged (2700 rpm, 5 min). The liquid sample was then transferred to a clean tube for solid phase extraction (SPE).

2.4. Calibration curve preparation

A standard mix, which included MA and AMP, was prepared in methanol and diluted down as appropriate to produce a six point calibration curve. The curve had values of 0, 0.1, 0.25, 0.5, 2.5, 10 ng/mg (spiked to a 20 mg blank hair sample). A blank hair sample without internal standard was prepared with each batch. Internal standards (D_5-MA and D_5-AMP) were added at 50 ng per sample (2.5 ng/mg) to all samples and washes.

2.5. Solid phase extraction

The SPE column (Bond Elut Certify) was conditioned by addition of methanol (2 mL) and phosphate buffer (2 mL, 0.1 M, pH 7). The extracted sample was transferred to the SPE column and washed with water (2 mL), acetic acid (0.1 M, 1 mL) and methanol (2 mL). The column was dried for 5 min and the analytes eluted with dichloromethane: propan-2-ol and ammonium hydroxide (2 mL × 2 mL, 80:20:3, v/v/v). Acidic methanol (50 μL of 15 μL HCl in 25 mL methanol) was added to the collected fraction before evaporation under a gentle stream of nitrogen.

Table 1
Transitions monitored in MRM mode for the detection of methamphetamine and amphetamine and deuterated standards.

Compound	Transition 1	Transition 2	Transition 3
AMP	136.1/91.1	136.1/119.1	136.1/65.07
AMP-D_5	141.1/93.1	N/A	N/A
MA	150.0/91.1	150/119.3	150.01/65.2
MA-D_5	155.2/92.1	N/A	N/A

2.6. Instrumental analysis

The samples were reconstituted in 100 μL of the mobile phase for analysis by LC–MS/MS. Analysis was performed using an Ionics EP10+ HSID API 3/5 mass spectrometer with a Dionex UltiMate 3000 HPLC system. A 10 μL aliquot of the extract was applied to a Phenomenex Luna® SCX column (150 mm × 2.0 mm) with a SCX 4 mm × 2.0 mm guard column in place. The analysis was performed using isocratic elution, with acetonitrile (75%) and ammonium formate buffer (100 mM) + 0.5% formic acid (25%) as mobile phase at a flow rate of 200 μL/min.

The mass spectrometric detection was carried out by a tandem mass spectrometer equipped with a Turbo Ion Spray atmospheric pressure interface operating in positive ionisation mode using multiple reaction monitoring mode (MRM) acquisition for analyte confirmation. The transitions monitored were determined by infusion of each compound directly into the mass spectrometer and are presented in Table 1. Three transitions are monitored for each drug of interest and one for each of the deuterated internal standards.

Some method performance characteristics of this assay are showed in Table 2. All points on the calibration curve had to be within 20% of their expected value. The calibration curves all showed linear regression coefficient of $R^2 > 0.99$ over the range analysed. The lowest calibrant point had a signal to noise of much greater than 5:1, and at 0.1 ng/mg it was half the value of the maximum recommended limit of quantitation (LOQ) from the Society of Hair Testing for amphetamine-type stimulants (0.2 ng/mg) [9], as such it was deemed to be sufficient to be used as the LOQ for this assay. This was also the cut off value for methamphetamine in the hair used by Farst et al. [5].

3. Results and discussion

From the analysis of 52 case samples, MA was detected in 38 (73%) above the LOQ (0.1 ng/mg), with a mean of 7.03 ng/mg of hair. Within these 38 samples, there were also 34 where AMP was also detected (65% of total, or 88% of the MA positive cases). No samples were positive for AMP without MA being detected. A sample was deemed to have MA detected if a peak was present above the lowest calibrant level of 0.1 ng/mg. Of those cases where MA was detected, 5 had levels above the highest calibration point (10 ng/mg) and these numbers are extrapolated using the linear regression analysis. There was not enough sample remaining to repeat these analyses and dilute to within the calibration range. The quantitative results are summarised in Table 3. For amphetamine the levels reported above and below the calibrants are also extrapolated from the calibration line. Where a result is reported as detected for amphetamine below the calibration line, the peak had a signal to noise of greater than 5 and methamphetamine was above 0.1 ng/mg.

Table 2
Intra and interday validation parameters for methamphetamine (MA) and amphetamine (AMP) extracted from spiked blank hair samples.

	Intraday ($n = 6$)			Interday ($n = 18$, 3×6)		
	Mean (SD) (ng/mg)	CV	Accuracy (%)	Mean (SD) (%)	CV (%)	Accuracy (ng/mg) (%)
MA	0.23 (0.01)	5.0	92	0.23 (0.04)	16.6	90
	2.28 (0.11)	4.9	91	2.34 (0.14)	6.0	94
AMP	0.27 (0.01)	2.7	108	0.26 (0.02)	8.9	106
	2.50 (0.12)	4.7	100	2.43 (0.14)	5.8	97