Drugs in prehistory: chemical analysis of ancient human hair

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Abstract

Concern about drug abuse in modern populations has led to the development of specific methods for identification of cocaine, opiates and cannabis in human hair. Drug use in prehistory can provide indirect evidence of interpopulational contact and social stratification. This paper reports drug evaluation in nineteen ancient hair samples from archaeological sites in northern Chile. Each sample was tested for the presence of traces of cocaine, opiates and cannabis, in order to establish a standard methodology for studies of drug use among prehistoric groups. Although results are negative, this absence of evidence could be due to two main causes: (1) the individuals evaluated did not use any drugs, which does not mean that other members of their cultural group did, or (2) the wide range of known drugs studied did not consider some group specific drugs, derived from local or imported plants, thus meaning that a greater drug range must be tested. In any case, our study confirms that drug testing in prehistoric samples is viable. However, in order to determine what kind of substances were used in prehistoric times new patterns that incorporate all drugs which are not part of the western pharmacopeia must be created. Finally, a methodology for the study of drug use among prehistoric groups using ancient hair samples is described.

Keywords: Ancient hair; Drug use; Chemical analysis; GC–MS; Mummy

1. Introduction

In the last decade, forensic scientists have paid increasing attention to hair as a drug exposure marker. Moreover, new investigations of 'trace evidence' involving the chemistry of ancient hair samples have demonstrated the presence of the cocaine metabolite benzoylecgonine (BZE) on Chilean mummies dated 2000 B.C. to 1500 A.D., suggesting that coca-leaf-chewing originated in Chile around 2000 years ago and became a common practice among subsequent populations [1,2]. Also, traces of nicotine have been found in hair samples from Nubian burial sites, including both children and adults, indicating that plants containing nicotine may have been used as medical stimulants [3].

The analytical requirements for the chemical study of ancient hair samples are different from those of fresh samples. Thus, when studying prehistoric samples, consideration should be given to the high porosity status of the strands and the degradation of the inaccessible domain. Both facts can be demonstrated by the methylene blue staining procedure and microscopic examination.

This paper explores the use of GC–MS analysis to examine drug use in prehistoric populations from northern Chile. We analyzed adult hair samples to track traces of cocaine, opiates and cannabis, in order to establish a standard methodology for studies of drug use among prehistoric groups.

2. Materials and methodology

The hair samples studied in this investigation were obtained from mummies excavated at the Formative cemeteries of Topater (100 B.C.) and Chiu-Chiu 273 (10 B.C. to 140 A.D.), northern Chile. Dating was determined by ^{14}C and thermoluminescence analysis.

Nineteen ancient and two recent (control) adult hair samples were analyzed; the latter two gathered from individuals living today in the same geographic area. When the scalp was available, hair was sectioned as close as possible to the skin and its orientation recorded. Sex and age were estimated, where possible, using current standard anthropological procedures (Table 1) [4,5].

Table 1
Distribution of prehistoric hair samples from northern Chile by structure, archaeological context, age and sex

	M1–M7	M8	M9	M10	M11	M12	M13	M14–M19
Structure		5A	2A	2A	5A	5A	4B	–
Excavation date	29/11/96	26/11/96	13/08/95	10/08/95	26/11/96	27/11/96	10/08/95	20/08/97
Material	Human hair	Human hair	Human hair	Human hair	Human hair	Human hair	Human hair	Human hair
Context	–	Burial 1	Burial C	Burial C	Burial 1	Burial 5	–	
Age (years)	20–30	20–30	15–20	20–30	20–30	20–30	15–20	35–40
Sex	Undetermined	Female	Female	Male	Female	Male	Undetermined	Male

2.1. Hair preparation and microscopic examination

After selecting the hair strands and prior to washing, gross debris was removed and the porosity status evaluated using 0.5% methylene blue staining, followed by copious rinsing and microscopic examination [6]. All samples showed a high degree of porosity, possibly due to the fact that hair was maintained in an unfavourable environment for centuries. The two recent samples showed no porosity degradation. Extreme care was taken in the selection of the strands in order to cut and pulverise only hair [7].

Hair strands were cut into 1-mm segments (50–100 mg), placed in a pre-weighed reservoir and weighed. Due to their high porosity, a non-polar solvent was applied initially, then hexane washing for 15 min and finally dry ethanol. The hexane fraction was evaporated to dryness, reconstituted with a buffer (200 μl) and checked with ELISA immunoassay for the possible presence of THC metabolites.

The ethanolic eluate was collected on a 12-port vacuum manifold glass tube, evaporated to dryness and dissolved in a buffer (200 μl), applying qualitative checking with ELISA immunoassay (cocaine: cocaine detection limit of 0.4 ng/ml; opiates: codeine detection limit of 0.1 ng/ml and morphine 1.08 ng/ml).

2.2. Acid extraction (cocaine and opiates), derivatization procedures and alkaline extraction (cannabis) [8]

After the washing and drying, the remaining hair in the reservoir was sealed, 2 ml of 25 mM HCl was added and it was then vortexed. The sealed reservoir was incubated overnight in a water bath at 37°C, the acid extract collected and saved. The acid solution was neutralised with a 0.1 M NaOH solution and adjusted to pH 6 with a phosphate buffer. Columns for SPE were conditioned with methanol (1 ml: twice), distilled water (twice), followed by a phosphate pH 6 buffer (1 ml); the sample being applied 1–2 ml/min. After washing the column with water (1 ml), 0.25 mM HCl (1 ml) and methanol (2 ml), it was aspirated to dryness. Analytes were eluted from the column with 2 ml of dichloromethane–2-propanol (8:2, v/v) with 2% ammonium hydroxide — 1 to 2 ml/min. Evaporated eluates were reconstituted with 20 μl of acetonitrile and derivatizated with 100 μl of MSTFA containing 1% TMCS, transferred to autosampler vials, sealed, heated to 60°C (20 min) and analysed by GC–MS.

Similarly to the acid extraction procedure, after the washing and drying, the remaining hair in the reservoir was sealed, 2 ml of 1 M NaOH added and it was then vortexed. The sealed reservoir was incubated in a water bath at 90°C for 45 min, the alkaline extract collected and saved. The alkaline solution was extracted with a 3 ml of hexane–ethylacetate (6:1, v/v), evaporated, reconstituted with 50 μl of ethylacetate and transferred to autosampler vials. The vials were sealed, heated to 70°C (20 min) and analysed by GC–MS.

2.3. GC–MS determination

Fresh extracts were analyzed using the following sequence: blank, negative hair samples and spiked hair. For the GC–MS analysis a 5890/5972 mass selective detector

(MSD) equipped with a 7673A autosampler, HP ultra 1 column and 25 m×0.2 mm I.D.×0.11 μm film thickness was used. The inlet was operated in the splitless mode. A transfer line of 300°C and injector maintained temperature at 250°C. Temperature program: initial temperature: 150°C (0 min); rate: 20°C/min; final temperature: 300 °C. Final time: 10 min. Run time. 17.5 min. The instrument was operated in the SIM mode.

A minimum of five concentrations per analyte were used for the construction of calibration curves. Control spiked hair with known concentrations were analyzed using the same procedure (Fig. 1). Analytes in spiked hair were identified by retention times and relative abundance of confirming ions in relation to known standards. Two control samples containing 0.2 and 0.5 ng/mg of cocaine were included (Table 2). The monitored ions (in parenthesis for quantitation) were:

Cocaine	
Cocaine	*m/z* 82, (182), 303
BZE	*m/z* 82, (240), 361
EME (Ecgonine methylester)	*m/z* 82, (96), 271
Anhydrous ecgonine	*m/z* 42, (152), 181
Coca ethylene	*m/z* 82, (196), 317
Opiates	
Morphine	*m/z* 236, 414, (429)
Codeine	*m/z* 178, 234, (371)
6-Monoacetylmorphine	*m/z* 287, 340, (399)
Heroin	*m/z* 310, (327), 369
Cannabis	

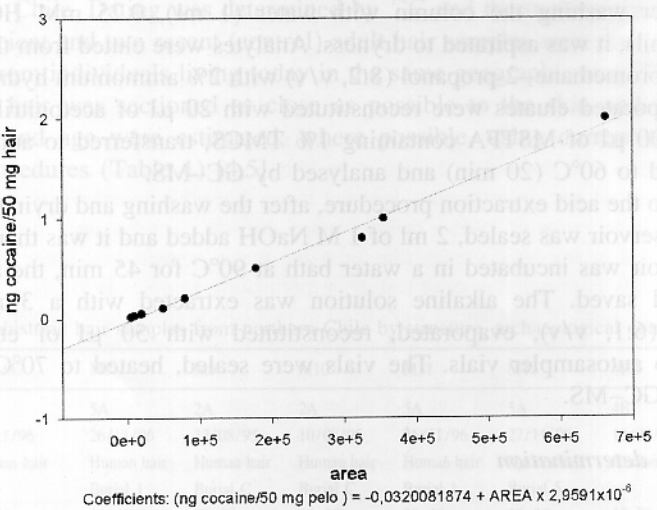

Fig. 1. Cocaine calibration curve for human hair.

Table 2
GC–MS analysis of cocaine, opiates, thc and their metabolites in prehistoric hair samples from northern Chile

Code	Sex	Age (years)	Cocaine[a] (ng/mg)	Opiates[b] (ng/mg)	Cannabis[c] (ng/mg)
M1	Indet.	20–30	–[d]	–	–
M2	Indet.	20–30	–	–	–
M3	Indet.	20–30	–	–	–
M4	Indet.	20–30	–	–	–
M5	Indet.	20–30	–	–	–
M6	Indet.	20–30	–	–	–
M7	Indet.	20–30	–	–	–
M8	Female	20–30	–	–	–
M9	Female	15–20	–	–	–
M10	Male	20–30	–	–	–
M11	Female	20–30	–	–	–
M12	Male	20–30	–	–	–
M13	Indet.	15–20	–	–	–
M14	Male	35–40	–	–	–
M15	Male	35–40	–	–	–
M16	Male	35–40	–	–	–
M17	Male	35–40	–	–	–
M18	Male	35–40	–	–	–
M19	Male	20–30	–	–	–
LOD (S/N>3)			0.0125		
LOQ (S/N>5)			0.025		
Control 1			0.2		
Control 2			0.5		

[a] Cocaine, BZE, EME, anhydrous ecgonine and coca ethylene.
[b] Morphine, codeine, 6-monoacetylmorphine and heroin.
[c] Cannabidiol, Δ9-THC and cannabinol.
[d] –, negative.

Cannabidiol	*m/z* 231, 299, (314)
Δ9-THC	*m/z* 231, 299, (314)
Cannabinol	*m/z* 238, (295), 310

The ion dwell time was 20 ms.

Replicate analysis for the three drug groups was performed at the Institute de Médicine Légale et Médicine Sociale, Strasbourg, France.

3. Results

Nineteen ancient adult hair samples were tested for traces of cocaine, opiates and cannabis. Tables 2 shows the GC–MS results for each sample. Results are negative for all groups analyzed. Table 3 summarizes the GC–MS analysis of the hair washings while Table 4 summarizes the GC–MS analysis of the hexane washings. Both tables show negative results for all the groups studied. These results, obtained at the Doping

Table 3
GC–MS analysis of hair washings of prehistoric hair samples from northern Chile

Code	Cocaine[a] Extract wash ng/mg hair	Opiates[b] Extract wash ng/mg hair	Cannabis[c] Extract wash ng/mg hair
M1–M19	–[d]	–	–

[a] Cocaine, BZE, EME, anhydrous ecgonine and coca ethylene.
[b] Morphine, codeine, 6-monoacetylmorphine and heroin.
[c] Cannabidiol, Δ9-THC and cannabinol.
[d] –, negative.

Laboratory of the Faculty of Chemistry of the University of Chile, were confirmed by the replicate analysis carried out at the Institute de Médicine Légale et Médicine Sociale, Strasbourg, France. It must be indicated that analytical techniques for drug detection are standardized, thus differences in sensitivity to chemical compounds are the result of more powerful equipment.

4. Discussion

Chemical analysis of ancient hair samples to trace drug use in prehistoric populations is a rather recent avenue of research in bioanthropology. Verification of possible 'importation' of substances from other regions (through trade or migration) or an association between use and social stratification are potential contributions of these studies to the understanding of the biology and culture of ancient peoples.

This research originates in the archaeological confirmation of the use of hallucinogenic substances either through the nose, mouth or anus, by the groups studied. Although the results of the chemical tests are negative, the absence of evidence could be due to two main causes: (1) the individuals evaluated did not use any drugs, which does not mean that other members of their cultural group did not, or (2) the wide range of known drugs studied did not consider some group specific drugs, derived from local or imported plants, thus meaning that a greater drug range must be tested.

At present the use of hallucinogenic substances for ritual purposes by rain forest tribes from South America is well documented [9]. Moreover, consumption of a hallucinogenic substance derived from the roasted seeds of the leguminous plant *Anadenanthera peregrina* has been suggested by the archaeological evidence in other groups from the region [9].

In spite of the negative results we can conclude that (1) detection of drug use in

Table 4
GC–MS analysis of hexane washings of prehistoric hair samples from northern Chile[a]

Code	THC (ng/mg hair)	CBD (ng/mg hair)	CBN (ng/mg hair)
M1–M19	–	–	–

[a] CBD, cannabidiol; THC, Δ9-THC; CBN, cannabinol; –: negative.

ancient human hair is possible, (2) in order to determine what kind of substances were used in prehistoric times we must create new patterns that incorporate all drugs which are not part of the western pharmacopeia, and (3) the identification of drug use in ancient populations can be used as a powerful tool for understanding interpopulational contacts and social stratification. Finally, a comprehensive one-step GC–MS method has been developed to allow simultaneous detection of cocaine and opiates and their metabolites in ancient hair samples. THC and its metabolites have also been incorporated into this analysis.

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