THE DETECTION OF MORPHINE AND CODEINE IN HUMAN TEETH: AN AID IN THE IDENTIFICATION AND STUDY OF HUMAN SKELETAL REMAINS

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ABSTRACT

When studying unidentified putrefied or skeletonised human remains it may be difficult to obtain information on drug habits which may prove important for the construction of a biological profile or lead to hypotheses on the manner of death. The detection of morphine and codeine in teeth from human remains may prove crucial in obtaining such information and thus give forensic odontology and anthropology a further tool for identification. Because teeth can be an important deposit of exogenous substances accumulated both in the pulp and in the calcified tissues, they are an invaluable source of data from a toxicological point of view. The authors therefore tested 3 groups of teeth for morphine and codeine: the first group consisted of artificially aged teeth from individuals known to have died of heroin overdose; the second, of teeth from individuals with no history of drug abuse; the third, of teeth from cases of burnt, putrefied and skeletonised remains found in conditions strongly suggestive of a drug-related death. Results showed that in groups 1 and 3 morphine and codeine could still be identified in the teeth, proving that these tissues may be a reliable source for toxicological information concerning the history of the individual. Further studies are needed to verify whether the substances detected reflect drugs in circulation in an acute phase (and therefore present in blood vessels in the pulp) or whether they represent drugs which have percolated and been stored in dentine and enamel and thus denote a history of drug abuse. Nonetheless this study shows that teeth may be an important source of toxicological information in the forensic scenario.
(J Forensic Odontostomatol 2003;21:1-5)

Keywords: Forensic odontology, identification, forensic toxicology, morphine, codeine, drug abuse

INTRODUCTION

When human skeletal and dental remains are found the two main problems which arise are identification of the person and determination of the cause and manner of death. Lack of preservation of soft tissues not only impairs identification procedures but often makes it very difficult to determine the cause of death in the absence of obvious skeletal trauma.

An average of 20 cases of badly putrefied, burnt or skeletonised human remains are found every year in Milan.[1] In most cases a biological profile (i.e. gender, age, race, pathology, occupational traits, habits, etc.) of the person has to be constructed as there are no indications of the person's identity and, furthermore, in most cases, the cause of death remains undetermined.

Forensic toxicology may be a valuable aid if applied to potentially one of the best preserved tissues, the teeth. In fact the sensitivity and specificity of modern forensic toxicological analyses have given investigators very powerful means for detecting even small quantities of xenobiotic substances.

Although the detection of morphine or other drug-related substances from teeth will certainly not *per se* solve the problem of identification nor provide a certain cause of death, it can give important indications as to particular "habits" or indicate a history of drug abuse. This is a piece of information which is obviously important in reconstructing a person's biological and – to a certain extent – social profile, and is equally important, when no other cause

of death is found, in suggesting – but not proving – a drug-related death. In the past ten years the number of heroin addicts dying of overdose has passed 100 cases in the city of Milan alone and many of these individuals die in suspicious circumstances, with virtually no identity.

It is known that teeth are a remarkable "diary" of a person's life history. Not only can lifestyle, occupational and dietary habits (eg. tobacco, alcohol, etc.) leave behind visible traces but many types of drugs taken in life by the person may leave chemical foot prints in the teeth, such as tetracycline,[2] certain alcohols and monocarbonic acids.[3] There is therefore reason to believe that teeth may provide excellent biochemical markers concerning the "drug" history of the person to whom the remains belong, in particular concerning the consumption of opiates.

It is not known how difficult the retrieval of such substances may be from old dental material. Many studies have concentrated on verifying the survival and diagenesis of biomolecules of "non-toxicological" interest from dental tissues and already much is known about survival of DNA,[4] albumin and other important biomolecules [5,6] in the teeth. Diagenetic research however has never been performed on teeth to study the survival of toxic substances of forensic interest (in particular those concerning drug abuse), whereas such studies, aimed at the detection of opiates in particular and other substances of abuse, have been performed on non-calcified tissues such as hair and nails.[7,8,9] It is apparent now that many substances will survive in time within the dental tissues and the chemistry of the inorganic component of teeth may in fact be well capable of retaining parts of molecules which could be informative of that specific person's history. Collins and Westbroek[10] have proved that biomineral will provide some degree of protection of biomolecules from biological or chemical degradation and further, that specific targeting of certain drugs onto hydroxyapatite crystals by small peptide conjugation has been demonstrated.

We therefore performed a pilot study on artificially aged teeth from individuals known to have died of drug overdose and on teeth from real forensic cases in order to verify the detectability of two commonly found substances, morphine (a metabolite of heroin) and codeine, in fresh and aged teeth with a post-mortem interval (PMI) of up to two years.

MATERIALS AND METHODS

The study was performed on three groups of teeth. A first sample of teeth (group 1) consisted of artificially aged positive-control ten lower first molars (each tooth taken from a separate cadaver), ranging in weight from 1.5g to 3.5 g, extracted from well preserved cadavers of individuals who had died of heroin overdose. This was done in order to verify the methods of morphine extraction and detection from teeth on a "known" sample which initially presented optimal preservation of blood and soft tissues. These individuals had all been found with circumstantial evidence of heroin injection (a syringe near the cadaver) and had shown blood levels of morphine greater than 1 μg/ml, and were also positive for codeine. After extraction the teeth were cleaned and washed in distilled water and artificially aged at room temperature, under a hood, in a dry environment. One tooth was then crushed for testing at postmortem intervals (PMIs) of 5 days, 1 month, 6 months, 8 months, 4 teeth at 12 months and 2 teeth at 18 months.

Group 2 consisted of 10 molars, ranging in weight from 1.6 g to 3.5 g, (each tooth taken from a separate cadaver), from individuals with no history of heroin addiction and with no circumstantial evidence for antemortem heroin, morphine or codeine use; these teeth were aged in the same manner and tested at the same PMIs as group 1, as negative controls. For this group blood tests from the cadaver for morphine and codeine resulted negative.

Finally, group 3 was composed of teeth from 4 cases of unidentified human remains. Case 1 was a saponified body of a young adult male who had been in water (river) for approximately one month. Cases 2 and 3 were those of badly burnt and decomposed bodies with an estimated PMI of 15 days. Case 4 was the skeletal remains of a young male with an estimated PMI of 2 years. Cases 2, 3 and 4 had syringes containing heroine found next to them. Analyses on the viscera and remaining body fluids of all individuals except Case 4 (the skeletonised remains) had previously tested positive for both

CASE	TOOTH (wt in g)	MORPHINE (ng/g)	CODEINE (ng/g)
No. 1 saponified cadaver	molar (1.7)	38.83	28.00
PMI = 1 month	premolar (1.1)	35.09	15.81
	incisor (1.1)	76.75	8.88
No. 2 burnt cadaver (1)	molar (3.6)	22.59	12.46
PMI = 15 days	premolar (1.8)	28.60	8.74
No. 3 burnt cadaver (2)	molar (2.0)	8.24	5.47
PMI = 15 days	premolar (1.4)	6.94	2.99
No. 4 skeleton	molar (3g)	83.03	16.97
PMI = 2 yrs			

Table 1: *Results of morphine and codeine tests on single teeth from group 3*

Positive controls: all teeth from drug addicts with drug related cause of death , artificially aged up to 18 months tested positive for morphine and codeine.
Negative controls: all teeth from individuals with no history of drug abuse and natual cause of death tested negative for morphine and codeine.

morphine and codeine. For case 1, one molar (1.7g), one incisor (1.1g) and one premolar (1.1g) were examined. In cases 2 and 3 one premolar and one molar for each burnt body were tested (1.8g and 1.4g respectively for the premolars, and 3.6 and 2.0g respectively for the molars). In case 4 one molar (3g) was tested (Table 1). (For group 3 the study was extended to all teeth according to availability).

Teeth were washed and cleaned of all blood and soil residues in distilled water and were gently polished (both crown and root) in order to eliminate any external contaminant. They were then incubated at room temperature in vials containing distilled water, rotated for 24 hrs and pulverised with an IKA ceramic mill* (the mill was cleaned overnight between teeth with "Ausilab" detergent),† after which 1.8g of tooth powder was incubated in 0.25M HCL overnight in an oven at 60°C and hydrolysed for 18 hrs in 2 ml of 0.25 M HCl at 50°C.

Reagents
Morphine hydrochloride, codeine hydrochloride and derivatising agents were obtained from Sigma‡ and the solvents and HCl were obtained from Merck.§

Extraction
Fifty µl of morphine-d_3 (1µg/ml) were added to the acid as an internal standard and purification was performed with n-heptane-isoamylalcohol (98.5:1.5). After mixing and centrifugation the solvent was discarded, the acid converted to alkaline (pH 9) and extracted with 4 ml of chloroform:isopropanol:n-heptane (50:33:17). Test tubes were then shaken for 15 min and centrifuged at 3500 rpm for 10 min, the solvent was separated and evaporated in a rotating evaporator and the residue was derivatised with 100 µl of pentafluoranhydride and 70 µl of pentafluoropropanol at 90°C for 15 min. The derivatising agent was dried with nitrogen and the residue reconstituted in 50 µl of ethylacetate. Codeine and morphine standards were now extracted at concentrations of 5, 10, 25, 50, 100 ng with the same quantity of internal standards (morphine-d_3)

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‡ *Sigma, 3050 Spruce St., St. Louis, MO 63103, USA.*
§ *Merck, P.O. Box 100, Whitehouse Station, NJ, 08889, USA.*

GC/MS conditions (Gas Chroma-tography/Mass Spectrometry)
GC/MS analyses (on 2 _l) were performed using a Hewlett Packard gas chromatograph[¶] mod. 6890 equipped with a 7673 HP autosample and MSD 5973 HP.[†] The gas chromatography was carried out with a MS5 (HP),[¶] length 12 m, internal diameter 0.2mm and 0.33 micron film thickness. The injection port temperature was 260°C (pulsed splitless mode) and the carrier gas was helium (8.4 psi head pressure, working at constant flux conditions) 1 ml/min constant flux. The oven temperature was held at 70°C for 2 min following injection and then programmed to progressively increase to 200°C at a rate of 20°C/ min and successive increments of 8 min up to 250°C and a final increase of 40°C per minute up to 280°C. The MSD detector was selected for single ion acquisition and precisely for ions 414, 430 and 577 m/z for morphine, ions 266, 282 and 445 m/z for codeine and ions 417, 433 and 580 m/z for deutered morphine. Temperature of the quadrupole was set at 150°C; source 230°C and 2 µl of each sample, followed by the standards, were then injected into the GC/MS. The identification of morphine and codeine was performed by comparing retention times, by the presence of ions selected and their ratios.

RESULTS

For groups 1 and 2 which had been tested in an initial part of the study aimed only at giving a qualitative assessment, the teeth were simply scored as positive or negative for morphine and codeine. All teeth from group 1 (artificially aged for up to 18 months) tested positive for morphine and codeine while all teeth from group 2 tested negative for these two substances, which confirmed that teeth from cadavers with no history of drug abuse test negative. Teeth from individuals known to have a history of drug abuse and to have died of drug overdose always gave positive results for morphine and codeine.

Results obtained for group 3 are illustrated in Table 1 which shows that morphine and codeine were found in all specimens, even in the case of skeletal remains with a PMI of 2 years.

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DISCUSSION

The scope of this pilot study was to test the detectability of morphine and codeine in human teeth from individuals with different post-mortem intervals and in different conditions of preservation and to prove that in known drug abusers morphine can be detected in their teeth up to 2 years after death.

Until now the detection of substances of forensic toxicological interest had been performed on many types of difficult substrates, for example hair and nails,[7,8,9] but never on teeth in different states of preservation and from individuals with different PMIs. The potential of a method for detecting even small quantities of morphine in old dental tissues is enormous. When nothing but bones and teeth remain, detecting drugs such as morphine or codeine may be a crucial element in building a biological profile. In fact, other signs of drug abuse such as syringes may not be present at the crime scene and the individual may not be suspected of being a drug abuser. The presence of morphine and codeine in teeth may therefore be the only clue to drug abuse, placing the person in the drug abuser category and helping investigating authorities to search for missing persons among specific social groups. Furthermore, it may lead to hypotheses on the cause of death (i.e drug overdose) although a correlation between the presence of morphine in teeth and in the circulation still needs to be investigated.

This study leads to further questions: are the morphine and codeine residues telling us what was in circulation at the time of death or do they represent old deposits accumulated during life? If what we have extracted from the teeth actually derives from the pulp, then is it likely that we have been extracting substances present in the pulp vessels at the time of death? Or might we be detecting substances accumulated during life which have penetrated and been stored in dentine or enamel? Future studies ought therefore to concentrate on separating these two dental sources (pulp and other tissues) in order to verify the route of contamination. This may also help explain why apparently "smaller" teeth sometimes yield larger quantities of morphine. For identification purposes a positive or negative qualitative result may be sufficient to provide information on a person's drug history. If the

presence of morphine however is to be used to verify a possible cause of death then information on the amount of morphine present in the vessels in the pulp is necessary and this requires isolation of morphine from the pulp and its quantification.

CONCLUSIONS

This study provides a novel perspective in the analysis of human remains for reconstructing the history of drug abuse of a person. The detection of morphine and codeine in artificially aged human teeth (up to 18 months from death) from individuals known to have a history of drug abuse and known to have died of drug overdose, and with a PMI of up to 2 years, proves that once again teeth are a remarkable source of data on the toxicological history of a person. Although further studies are needed to verify that the substances detected reflect drugs present in the circulation in an acute phase (and therefore in the blood vessels in the pulp) or whether they represent drugs which have percolated and been stored in dentine and enamel and thus denote a history of drug abuse, this study shows that teeth are an important source of information. The detection of morphine in teeth from human remains may give clues concerning a potential drug-related death but it can especially provide a "personal descriptor" crucial to constructing a biological profile which may lead to identification.

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