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Determination of Cocaine in Human Hair by Gas Chromatography/Mass Spectrometry

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Summary. A qualitative method for the determination of cocaine alone without its metabolites in human hair by gas chromatography/mass spectrometry (GC/MS) was developed. The assay used helium as carrier gas, a 30-m bonded phase fused silica OV-1 capillary column, and solid injection at 290°C evaporator temperature.

The cocaine concentrations in hair were determined also by radioimmunoassay (RIA). The values obtained are the sum of cocaine and its metabolites.

Both GC/MS and RIA meet the requirements for the determination of drug abuse by two different methods in forensic science.

Key words: Cocaine, gas chromatography/mass spectrometry – Human hair, determination of cocaine

Zusammenfassung. Diese Studie beschreibt die Bestimmung von Kokain in menschlichen Haaren mittels Gaschromatographie/Massenspektrometrie (GC/MS). Als Trägergas wurde Helium benützt; die Säule war eine Kapillarsäule OV-1 und die Injektion eine Feststoffinjektion, bei einer Verdampfungstemperatur von 290°C.

Die Kokainkonzentrationen in Haaren, die von einem Kokainabhängigen gewonnen wurden, wurden auch mittels Radioimmunoassay (RIA) bestimmt. Die Konzentrationen stellen die Summe von Kokain und seinen Metaboliten dar.

Beide Methoden GC/MS entsprechen den Anforderungen der Forensischen Medizin, einen Drogenkonsum mit zwei verschiedenen Methoden zu belegen.

Schlüsselwörter: Kokain, Gaschromatographie/Massenspektrometrie – Haare, Kokainnachweis

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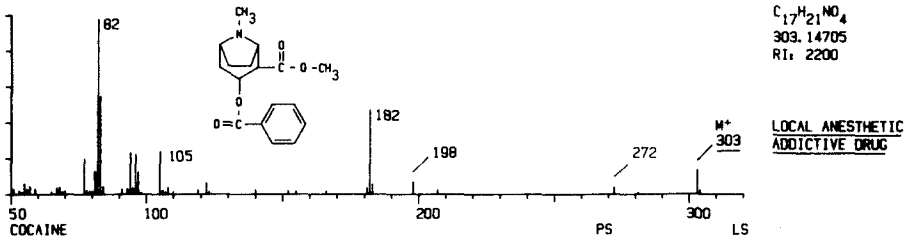

Fig. 1. Cocaine-selected ions

Cocaine is an alkaloid isolated from coca leaves. The drug has been used as stimulant and local anesthetic for hundreds of years. However, recently cocaine abuse has become epidemic. The drug has a potent psychotropic effect comparable with that of amphetamines, increases the physical capacity of the body and produces both euphoria and aphrodisia. Overdosage of cocaine results in nausea, vomiting, convulsions, paralysis of muscles, loss of vital function, and death.

In the body cocaine is rapidly metabolized largely to benzoylecgonine. However, also unchanged cocaine is present in the body fluid and tissues.

Determination of cocaine concentrations in hair by radioimmunoassay has been reported (Valente et al. 1981; Baumgartner et al. 1982; Smith and Liu 1986; Balabanova et al. 1987). The antibody used in the assay is benzoylecgonine antiserum which reacts with benzoylecgonine, but also with cocaine, ecgonine, and ecgonine methyl ester. Consequently, the concentrations measured are the sum of cocaine and its metabolites.

Determination of cocaine by GC/MS has been performed (Fig. 1). Cocaine concentrations measured by GC/MS in plasma, urine, and tissue samples, but not in hair, were reported (Javaid et al. 1975; Jindal et al. 1978; Chinn et al. 1980; Lewin et al. 1980; Griesemer et al. 1983). However, in forensic toxicology the determination of drugs is obliged to be performed by two different methods. In this study we describe a second specific method for the cocaine determination in hair.

Materials and Methods

Hair samples were obtained from (a) cocaine user 24 h after death and (b) sheep after repeated administration of cocaine. Cocaine hydrochloride (2 mg/kg b. wt.) was given daily i.v. as 80 mg into 1 ml aqueous solution for 40 days. Hair was collected immediately before and daily after the administration.

After washing of the hair the extraction was proved according to the method of Valente et al. (1981). Briefly, 50 mg hair was crushed with 1 ml 0.1 *M* HCl and incubated overnight at 45°C. The acid extracts were neutralized with 100 µl 1 *M* NaOH and diluted with phosphate buffer (pH 7.4) up to 2 ml. The determination of the cocaine was proved by (a) GS/MS and (b) radioimmunoassay.

GC/MS

GC/MS-analysis was performed on a DANI 6500 gas chromatograph connected to a Hewlett-Packard 5970 B mass selective detector using direct coupling device. Helium was used as carrier gas at a flow rate of 0.9 ml/min. A 30-m bonded phase fused-silica OV-1 capillary column, 0.2 mm i.d., was employed. Solid injection at 290°C evaporator temperature and 50°C oven temperature. Following injection, the oven temperature was rapidly increased to 210°C and kept for 2 min, the programmed at a rate of 2°C/min to a final temperature of 220°C, and kept for 8 min at this temperature. The ionization mode was electron impact, ionizing voltage; 70 eV.

Selected ions; m/z 303 (M^+) and mass fragments: m/z, 272, 198, 182, 105, 82 were monitored repeatedly and stored for subsequent processing in a Hewlett-Packard 9000/300 personal computer.

The lowest detection limit of cocaine-HCl under this conditions was 100 fg/injection.

RIA

Of the hair extract diluted with phosphate buffer 100 µl was used directly for RIA. The anti-benzoylecgonine serum was prepared in goat, the tracer was ^{125}I-labeled benzoylecgonine. The standard was prepared as concentrated solution of cocaine hydrochloride diluted in phosphate buffer (pH 7.4). The lower limit of detection was 0.3 ng/mg hair. The intra-assay coefficient of variation was 7.7% ($n = 10$). The cpm for the unknown samples were converted to nanogram equivalent per milliliter by use of the calibration curve, and then converted to ng/mg hair.

Cross-reactivity with morphine, codeine, methamphetamine, and methadone was not observed. All chemicals used were reagent grad. The standard used for both methods was cocaine hydrochloride. In addition, the deposition of cocaine in tissue after lethal dose (1.6 g) administration to sheep was investigated. Kidney was removed immediately after killing the animal, and 1 g was homogenized with 5 ml 0.9% NaCl. The homogenate was centrifuged (10 min × 1000 g), and the supernatant was used for the GC/MS and RIA-determination of cocaine.

For GC/MS the supernatant was extracted with chloroform after alkalinization. The chloroform extract was injected into the GC.

For RIA, 100 µl of the supernatant obtained by centrifugation was assayed directly.

Results

The mass spectrum of cocaine alone with molecular ion at m/e 303; and mass fragments at m/e 182, 82, 105 and 272; and the ion chromatogram are depicted in Fig. 2.

The mass spectrum obtained from hair and tissue extracts showed the same ions and clean and symmetrical peaks of the ion chromatogram as the standard (Fig. 2).

In the hair samples obtained from the addict, the cocaine concentration measured by RIA were 7.3 ng/mg hair, respectively. Measurable cocaine concentrations in sheep hair were found after 12 days of repeated administration. The concentrations reached 2.7 ng/mg hair and remained approximately unchanged during 60 days. The results are given in Fig. 3. The concentrations found in the kidney measured by RIA were 9 µg/g.

Comparison of peak areas from known quantities of standard with those from hair sample extracted in an identical manner make it possible to estimate approximately the cocaine concentrations in the sample.

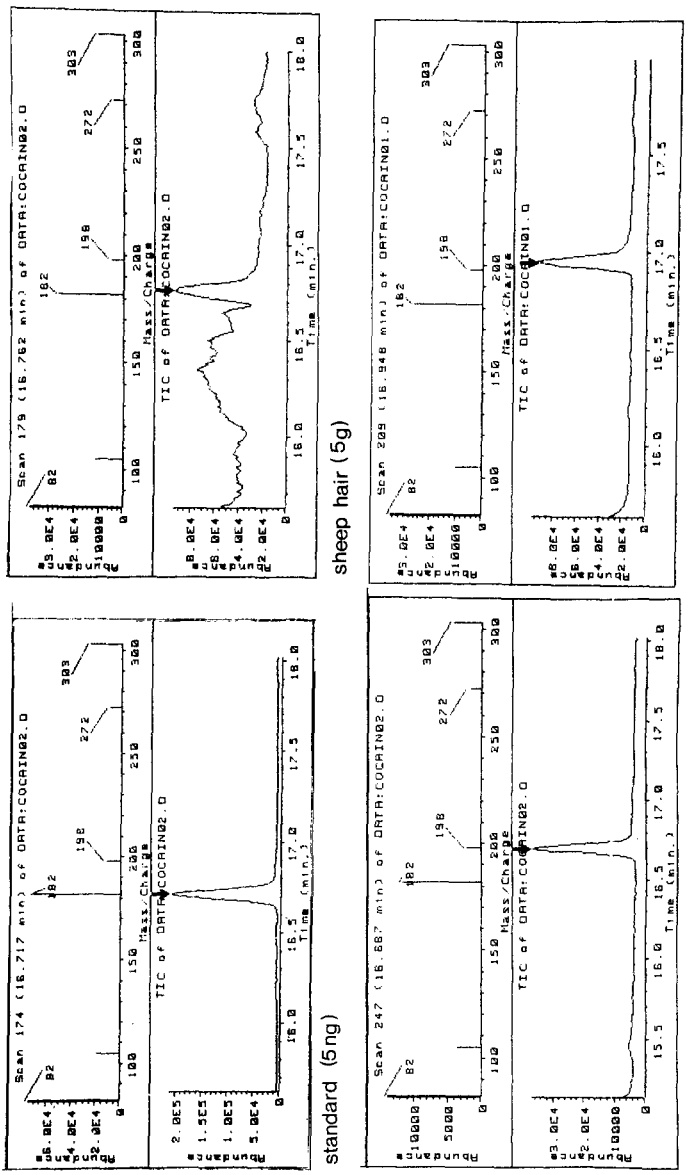

Fig. 2. Cocaine-selected ions and chromatograms: standard, human hair, sheep hair, and kidney tissue

Discussion

This study showed the presence of cocaine in hair (human and sheep) and in kidney of sheep, determined by two different methods.

Cocaine is very rapidly excreted and degraded in the body. However, cocaine remained partly also unchanged. The cocaine concentrations found in kidney are similar to those found in dogs (Woods et al. 1951) or human (DiMaio and Garriott 1978; Poklis et al. 1985).

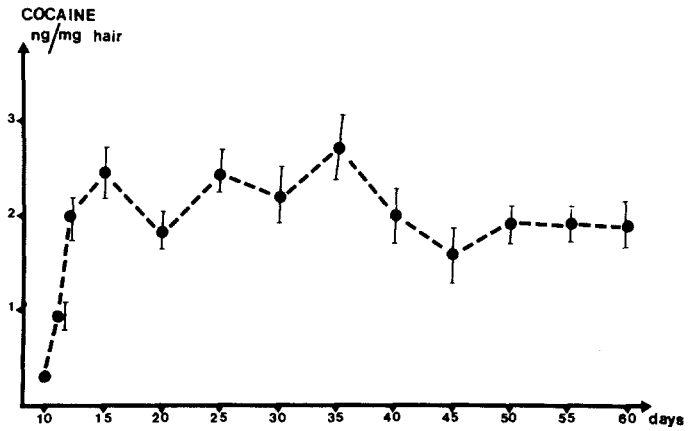

Fig. 3. Cocaine concentrations in hair after repeated sublethal doses administration to sheep

The determination of cocaine by RIA lacks specificity. As described above, the values measured are the sum of cocaine and its metabolites. The GC/MS determination is specific and showed the presence of cocaine alone.

However, the RIA method is sensitive, simple, rapid, and practicable for routine determination. In the case of positive results, the hair samples have to be investigated by GC/MS. Thus, analysis performed by both RIA and GC/MS fulfil the requirements in forensic science.

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