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Radioimmunological Determination of Cocaine in Human Hair

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Summary. A simple procedure for the determination of cocaine in human hair was described. After washing hair samples were crushed in 0.1 *m* HCl and incubated overnight at 45°C. The acid extracts were neutralized with 1 *m* NaOH. Phosphate buffer (pH 7.4) was added to the extracts. The cocaine concentrations were measured by radioimmunoassay. Detection in hair was achieved in all hair samples obtained from cocaine users. This method appears to be suitable for the routine determination of cocaine.

Key word: Cocaine, radioimmunological determination in hair

Zusammenfassung. Diese Arbeit beschreibt eine Methode für die Bestimmung von Kokain im menschlichen Haar. Das Haar wurde gewaschen, zermalt mit 0,1 *m* HCl und über Nacht bei 45°C inkubiert. Nach Neutralisierung mit 1 *m* NaOH wurde Phosphat-Puffer (pH 7,4) zugesetzt. Die Kokain-Konzentrationen wurden mittels Radioimmunoassay bestimmt. In allen Haarproben von Kokainabhängigen wurden meßbare Konzentrationen nachgewiesen. Dieses Verfahren stellt eine schnelle und einfache Routinemethode dar.

Schlüsselwort: Kokain, radioimmunologische Bestimmung im Haar

Introduction

The increasing use of cocaine is a subject of intensive research with respect to its medical and social problems. The analysis of cocaine in blood, urine and tissue is valuable in proving the drug abuse in forensic toxicology. One of the useful indicators for monitoring drug use is also the hair. The major advantage of hair analysis is the valuable long-term information on prior drug use. The hair of cocaine users seems to contain amounts of the cocaine and its primary metabolite benzoylecgonine. The Abuscreen Radioimmunoassay for cocaine meta-

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bolite is a specific test to detect the presence of benzoylecgonine in urine. However, the benzoylecgonine antiserum react also with the cocaine contained in hair. Consequently, the measured concentrations are the sum of cocaine and benzoylecgonine.

In this paper we present the determination of cocaine and the metabolite in hair by a simple modification of the radioimmunoassay for cocaine metabolite in urine. The term cocaine is used for the sum of cocaine and benzoylecgonine.

Materials and Methods

Cocaine in hair was measured by radioimmunoassay (Abuscreen, Hoffmann La Roche). Standard was prepared as concentrated solution of cocaine hydrochloride and then diluted in (a) phosphate buffer (pH 7.4) and (b) extracts of hair. Hair samples were obtained from non-addicted subjects. To remove external contaminations, 100 mg hair was washed with 10 ml distilled water and 10 ml ethanol 3 times each. The extraction was proved according to the method of Valente et al. (1981). Briefly, 50-mg hair samples were 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. To the extracts 0.9 ml phosphate buffer (pH 7.4) was added.

The lower limit of detection was 0.3 ng/mg hair. The anti-benzoylecgonine serum reagent was prepared in goat. The tracer was ^{125}I-labelled benzoylecgonine. The intra-assay coefficient of variation was 7.5% ($n = 6$). Extracts of hair samples obtained from 18 non-addicted subjects were assayed as control.

In addition, hair samples were obtained from ten cocaine users. The duration of drug use was 2–12 months. After washing, 50 mg of each hair sample were extracted as described previously. For the determination of cocaine in hair 100 µl of each extract was used. All determinations were performed in duplicate. The cocaine concentrations were measured also in hair segments at different distances from the root (0–2, 4–8, 8–12, 13–16, and 17–19 cm).

The cpm for the unknown samples were converted to nanogram equivalents per millilitre by use of the calibration curve, and then converted to nanogram per milligram hair.

Results

The standard curves obtained with standards diluted in hair extract or phosphate buffer were identical (Fig. 1). The nonspecific binding of the assay, the hair samples of nonaddict subjects, and the hair of cocaine users were 1470, 1361 and 1412 cpm, respectively (mean value of two determinations each).

Control tests on hair samples from the controls were consistently negative. The cpm values were within the range of 69,300–71,845 cpm (mean 70,804 ± 833).

The recovery of cocaine in the preparation by extraction was examined as follows: 150 ng and 75 ng cocaine were added to 50 mg of washed hair obtained from the control group and then extracted as described above. The recovery was (% ± SD): 61 ± 4.2 and 76 ± 7.8, respectively (mean values of three determinations each).

The cocaine concentrations found in the hair from seven addicts ranged from *0.6* to 6.4 ng/mg hair. The cocaine concentrations found after three months of abstinence were 0.3 and 0.5 ng/mg hair (Fig. 2). The concentrations found in hair segments at different distances from the root are depicted in Fig. 3.

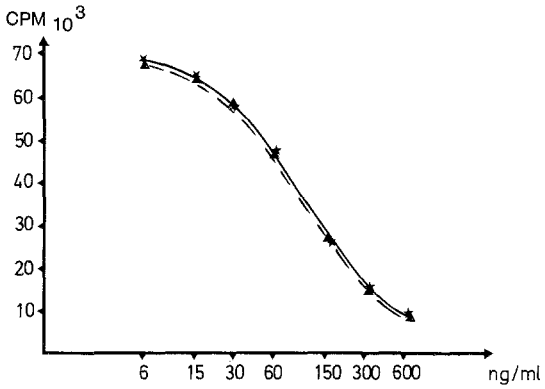

Fig. 1. Standard curves of cocaine hydrochloride diluted in extract of human hair (★) and in phosphate buffer (pH 7.4) (▲) (mean values of two determinations each)

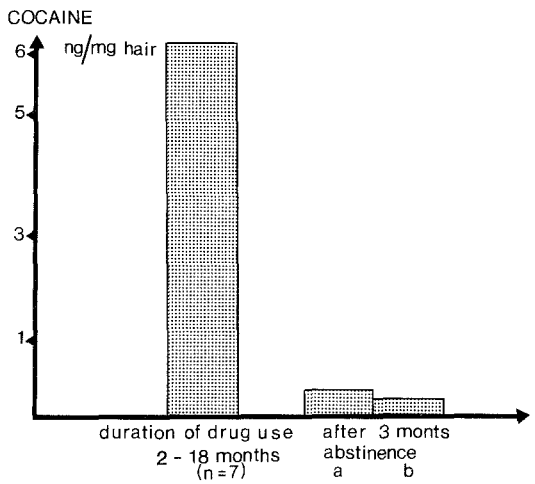

Fig. 2. Cocaine concentrations found in nine cocaine users

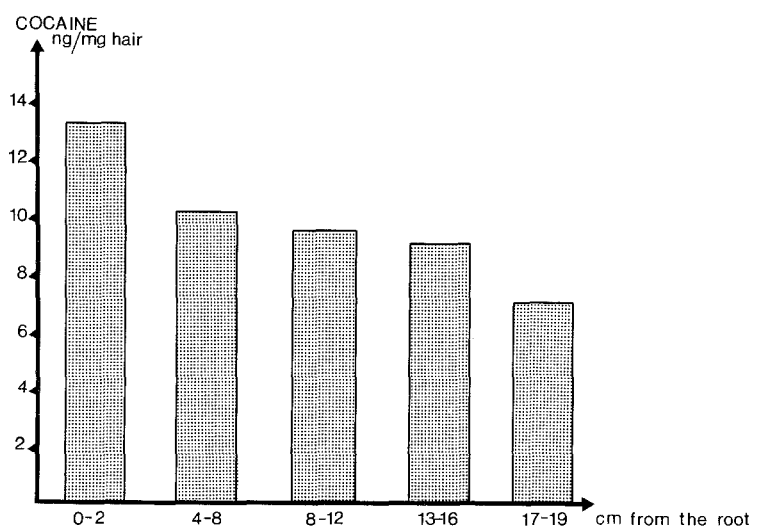

Fig. 3. Cocaine concentrations found in hair segments at different distances from the root

The following compounds were tested as above 10,000 µg/l for cross-reactivity in cocaine radioimmunoassay and found to not cross-react: codeine, methamphetamine, methadone, and morphine.

Discussion

It is apparent that the method described in this study is sensitive and specific for cocaine determination. Because of its ability to detect the drug in a concentration of 0.3 ng/mg hair within 2 days, the method appears to be suitable for routine determination of cocaine in human hair. The measurement of drugs in the hair makes it possible to provide past and present drug use as shown in Fig. 2. The detection of the drugs after successive hair washing indicates that the substances were contained within the hair and not on the surface (Goldblum et al. 1954; Harrison et al. 1974; Klug 1980; Baumgartner et al. 1981; Valente et al. 1981; Baumgartner et al. 1982; Niwaguchi et al. 1983; Puschel et al. 1983; Suzuki et al. 1984).

Cross-reactivity of benzoylecgonine antiserum with cocaine has been demonstrated before (Cleeland et al. 1976). The recovery of cocaine described in the results showed also that the antiserum reacts with cocaine up to 70% approximately. In 1975 Valente et al. reported that cocaine in hair can be detected qualitatively by benzoylecgonine antiserum. In 1982 Baumgartner et al. and recently Smith and Liu (1986) demonstrated quantitative radioimmunological determination of cocaine in the hair, expressed in benzoylecgonine equivalents. However, the measured concentrations are the sum of cocaine and benzoylecgonine because both are present in the body or samples, respectively. As mentioned above, cocaine is rapidly metabolized largely to benzoylecgonine, inside and outside the body. The degradation of cocaine to benzoylecgonine is pH-dependent. Alkaline pH stimulates the degradation (Liu et al. 1982). In our method the samples were analysed immediately after their removal from the body. The extraction was performed under acid conditions. The final samples were diluted with phosphate buffer (pH 7.4). Under these conditions the degradation to benzoylecgonine outside the body may be stopped. However, the concentrations measured by RIA are the sum of cocaine and its metabolites.

Determination of drugs in body fluids, tissues, stains, and hair by radioimmunoassays has been documented (Spector and Parker 1970; Spector and Flynn 1971; Catlin et al. 1973; Cheng et al. 1973; Berman et al. 1975; Cleeland et al. 1975; Mulé et al. 1975; Shaler et al. 1978; Baumgartner et al. 1979, 1981; Smith 1981; Smith and Pomposini 1981; Valente et al. 1981). The sensitivity of this method makes it possible to detect the agent a long time after drug abuse or administration without pretreatment of the samples. The simultaneous determination of several samples and drugs, as well as the rapid detection and quantitation of the drugs, increase the significance of this method in forensic toxicology. However, in forensic science the determination of drugs is obliged to be performed by two different methods. Determination of cocaine by gas liquid chromatography/mass spectrometry/high performance liquid chromatography

has been described (Javaid et al. 1975; Jindal et al. 1978; Chinn et al. 1980; Lewin et al. 1980; Clark 1981; Griesemer et al. 1983).

The radioimmunological determination of cocaine described in this study presents a second sensitive, specific and rapid method for the cocaine determination in hair.

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