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Determination of methadone in human hair by radioimmunoassay

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Summary. The concentrations of methadone in human hair were measured. The washed hair was cut in 1-mm pieces approximately, then incubated overnight at 45°C with 0.1 m HCl. The extracts were alcalized by 1 m NaOH and diluted by phosphate buffer, pH 7.4. The methadone concentrations were determined by radioimmunoassay. The method is simple, rapid, and practicable for routine determination.

Key word: Methadone, radioimmunologic determination in hair

Zusammenfassung. Die Konzentration von Methadon im menschlichen Haar wurde bestimmt. Die gewaschenen Haarproben wurden in ca. 1 mm lange Stücke geschnitten und mit 0.1 m HCl über Nacht bei 45°C inkubiert. Die Extrakte wurden mit 1 m NaOH alkalisiert und mit Phosphat-Puffer pH 7.4 verdünnt. Die Methadonkonzentrationen wurden mittels Radioimmunoassay gemessen. Diese Methode stellt eine einfache und schnelle Routinebestimmung dar.

Schlüsselwort: Methadon, radioimmunologische Bestimmung im Haar

Introduction

Methadone is a synthetic long-acting analgesic promoted as a treatment in detoxification and maintenance programs of heroin addiction (Isbell and Vogell 1949; Dole and Nyswandes 1965). However, methadone use results also in habit formation (Isbell and Vogell 1949; Symonds 1977; Ettinger et al. 1979). Consequently, the use of methadone represents a serious problem and indicates the necessity of control. In this study, we describe the determination of methadone in human hair by radioimmunoassay.

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Material and methods

Hair samples were obtained from nonaddicted subjects ($n = 10$) and from two patients treated with L-methadone. The hair samples were washed with 10 ml distilled water and 10 ml ethanol 3 times each. Fifty milligram hair samples were cut in 1-mm pieces approximately, then incubated overnight at 45°C with 1 ml 0.1 m HCl. The acid extracts were neutralized with 100 µl 1 m NaOH. To the extracts 0.9 ml phosphate bufer (pH 7.4) was added.

The concentrations of methadone were determined by a modification of the radioimmunoassay for methadone in urine (Biermann, FRG). The standard was methadone hydrochloride diluted in phosphate buffer (pH 7.4). In addition, the standard was also diluted in hair extract obtained from nonaddicted subjects. The standard curve covers the range 2.5 ng/ml–500 ng/ml. The antibody was coated to the tubes. The tracer was 125.I-labeled methadone hydrochloride. The intraassay coefficient of variation was 5.1% ($n = 6$). The cpm for the unknown samples were converted to nanogramm equivalents per milliliter by use of the calibration curve, and then converted to nanogramm per milligram hair.

Results

The standard curves obtained by methadone hydrochloride dilution in phosphate puffer or hair extract are identical (Fig. 1). This indicates that the hair extracts have no effects on the methadone determination. Dilution of methadone present in the patient hair gave a displacement curve parallel to that of standard methadone (Fig. 2). The nonspecific binding of the assay, the hair samples of the patients or the nonaddicted subjects were: 971 cpm, 1,009 cpm, and 1,002 cpm, respectively (mean values of three determinations each). The cpm values measured in hair samples of the control group were within the range of 27,200–28,554 cpm (mean ± SD: 27,996 ± 405).

The methadone concentrations found in hair of the patients treated with methadone were: 5.2 ng/mg hair and 4.9 ng/mg hair, respectively.

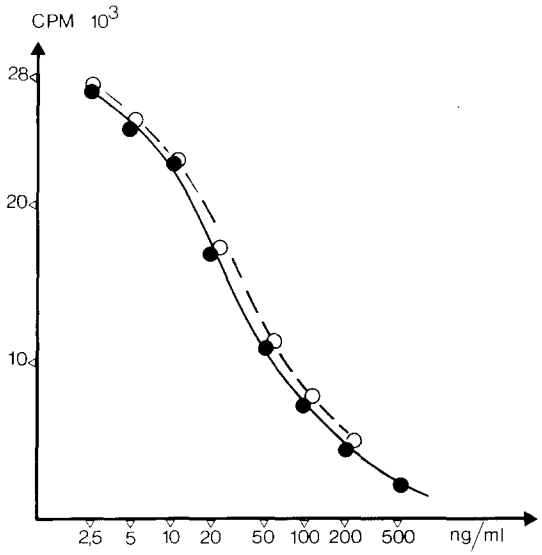

Fig. 1. Standard curves of methadone hydrochloride diluted in extract of human hair (○) and in phosphate buffer (pH 7.4) (●). Mean values of two determinations each

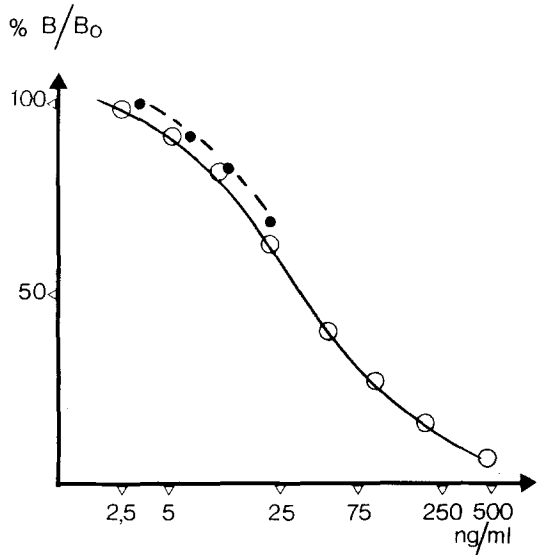

Fig. 2. Parallel displacement curves of standard methadone hydrochloride (○) and methadone present in human hair (●). Mean values of two determinations each

The recovery of methadone hydrochloride in the preparation by extraction was examined as follow: 100 ng and 50 ng standard were added to 50 mg washed hair obtained from the control group and then extracted as above. The recovery (% ± SD) was 74 ± 6 and 62 ± 7.2, respectively (mean values of three determinations each).

Cross-reactivity between the methadone antiserum and morphine, codeine, phenobarbitone, methaqualone, propoxyphene, methamphetamine (10.000 µg/l each) was not observed.

Discussion

Methadone is metabolized largely to 2-ethylidene-1.5-dimethyl-3.3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3.3 diphenylpyrroline (EMDP) (Pohland et al. 1971). However, no pharmacologic activity of both methabolites EDDP or EMDP was observed. No extent in plasma or tissues EDDP or EMDP during usage of methadone was also described (Baselt 1982; Liu et al. 1983). The metabolism is pH-dependent. After acidification of the urine, the amount of methadone excreted is elevated (Baselt 1982). The antibody used in the present study reacts with methadone but also with its metabolites. However, as described previously, in plasma the metabolites are not present to a significant extent. The lack of cross-reactivity with morphine, codeine, barbiturate, etc., as described above, as well as the like displacement curves of standard methadone and methadone in hair (Fig. 2), indicate the specifity of the antibody used in the assay.

Determination of methadone in urine, blood and tissue by radioimmunoassay is described (Coumbis and Kaul 1974; Manning et al. 1976). Studies for the methadone determination in hair are not present. The results of our present

investigation indicate that the radioimmunologic method used is practicable for the routine determination of methadone in hair. As obligatory in forensic toxicology, the concentrations measured by radioimmunoassay may be verified by a second method, e.g., by gas chromatography/mass spectrometry.

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