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Pharmacological studies on meprobamate incorporation in human beard hair

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Summary. The time course of appearance of meprobamate in beard hair after single oral administration (400, 800, or 1200 mg) was monitored in 3 groups of 4 subjects by GC/MS. Meprobamate appeared in beard hair approximately 4–5 days after administration and peaked during the 7–9th day. Drug levels in beard hair appeared to be dose-related.

Key words: Hair – Beard – Meprobamate – Pharmacology

Zusammenfassung. Der zeitliche Verlauf der Ablagerung von Meprobamat in Barthaaren wurde nach einmaliger Einnahme von 400, 800, bzw. 1200 mg in 3 Gruppen von je 4 Personen mittels GC/MS untersucht. Meprobamat wurde in Haar ca. 4–5 Tagen nach Einnahme nachweisbar; höchste Haarkonzentrationen wurden nach ca. 7–9 Tagen erreicht. Es wurden Korrelationen zwischen Haarkonzentration an Meprobamat und eingenommener Dosis festgestellt.

Schlüsselwörter: Haare – Bart – Meprobamat – Pharmakologie

Introduction

The analysis of drugs of abuse in human hair, first reported in 1979 [1], is increasingly being recognized as a useful test to complement urine and blood analysis [2–12]. This detection was subsequently extended to therapeutic agents [13–17] and non-abused drugs [17, 18]. Hair complements urine by providing long-term information (from weeks to months) of an individual's drug use, particularly concerning the severity and pattern of drug use.

Several applications have been described using hair analysis as a diagnostic tool: forensic science, justice, workplace, compliance monitoring, addiction recovery programs, toxic psychosis and prenatal drug exposure.

Different opinions on the application of hair analysis have emerged and were evaluated in the USA by the Society of Forensic Toxicologists (SOFT), the National Institute of Drug Abuse (NIDA) and the National Institute of Justice (NIJ). Their consensus opinion was published in 1990 [20] and according to this, too many critical questions remain to be answered before the results can be accurately interpreted, particularly in the case of employee and pre-employment drug testing. Moreover, standardization of analytical methods, definition of appropriate cut-off levels and reference materials are missing. In conclusion, they suggested some research areas, including pharmacological studies on the rate of absorption (time for appearance of a positive detection), dose-concentration relationships or individual differences. To answer some of these questions, we evaluated some parameters on meprobamate incorporation in beard hair which is considered to be a suitable alternative to head since growth rates are similar [3] and collection could be on a daily basis.

Materials and methods

Subjects. 16 healthy male volunteer subjects (laboratory personnel) participated in the study and informed oral consent was obtained. Subjects were aged 27–32 years, and had never been treated with meprobamate.

Study protocol. The protocol was closely related to the protocol developed by Cone [3]. Prior to induction into the study, the urine samples were tested for the presence of meprobamate. Groups of 4 subjects received either a single oral placebo, 400, 800 or 1200 mg of meprobamate at 10.00 p.m. on the first day. The doses were administered under blind conditions. The subjects were instructed to shave their face and neck areas daily (prior to 9.00 a.m) with a clean electric razor. No liquid or powder preshave products were used. Collection of beard hair continued for 21 days.

Daily beard collection was accomplished by careful removal of chopped hair particles from inside the razor head, which were stored until analysis in plastic tubes. After collection, the razor

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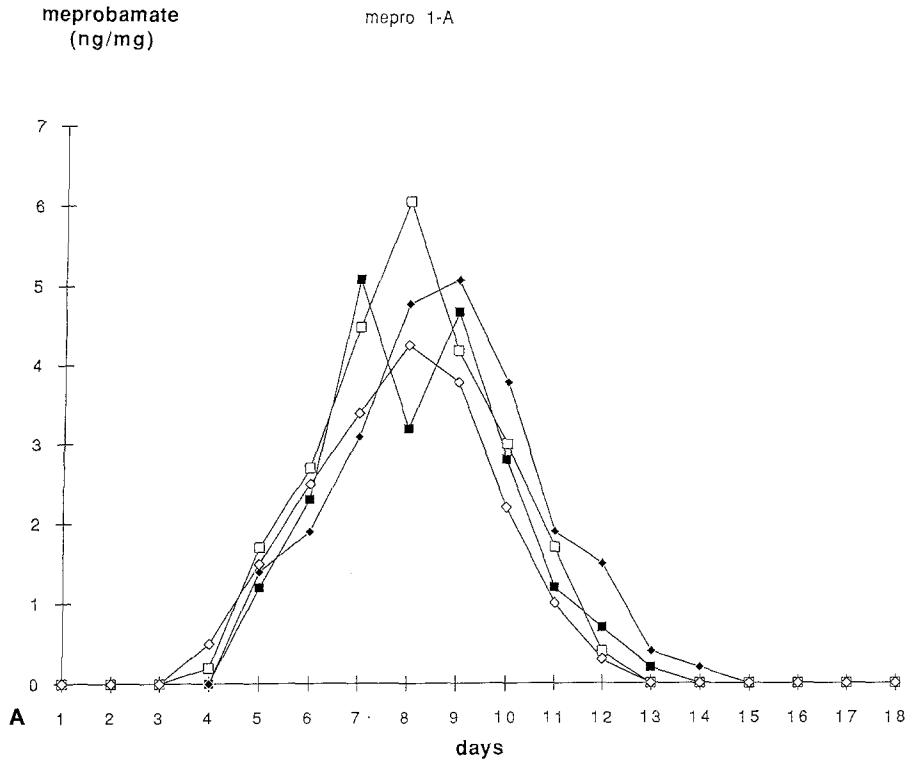

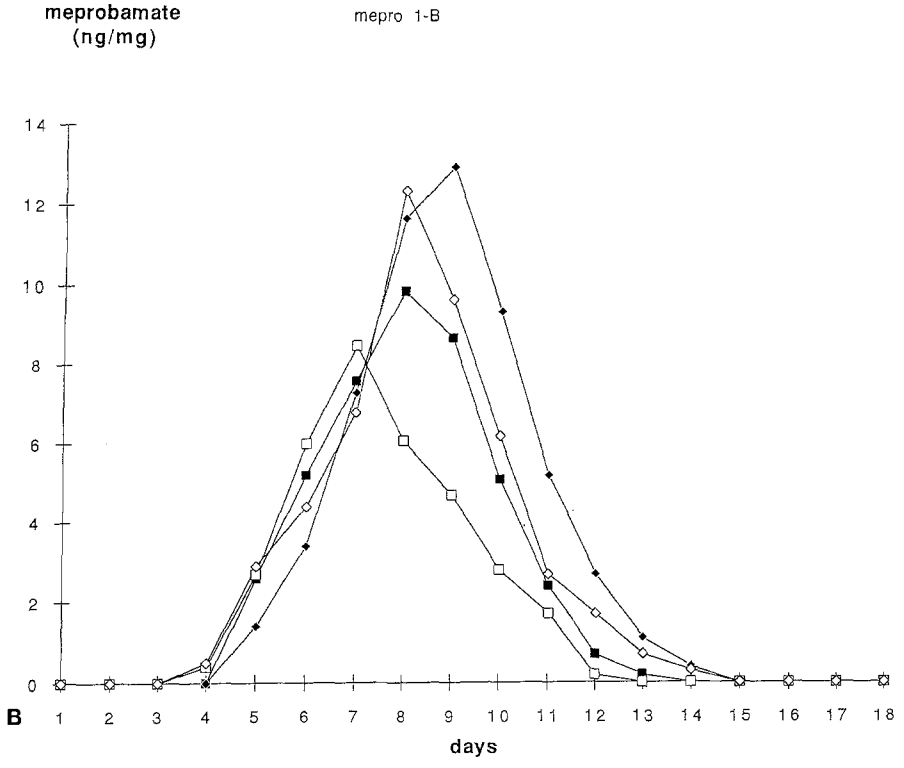

Fig. 1A–C. Pattern of meprobamate beard concentrations after single oral administration from 4 individuals. Each curve is the result of one subject. **A** after 400 mg; **B** after 800 mg; **C** after 1200 mg

head and interior were cleansed with methanol and dried with an air jet for reuse. Daily hair collection weights for individual subjects ranged from 7–38 mg.

Chromatographic analysis. The analytical procedure was performed using a previously described technique [21]. Briefly, the hair was decontaminated by washing in 5 ml dichloromethane for 15 min at 37°C. The protein matrix of the hair was homogenised by incubation in 1 ml 1 N HCl overnight at 60°C. Phosphate buffer (50%, pH 5.5) was added and meprobamate was extracted in 5 ml chloroform in the presence of 20 µl vinylbarbital (10 mg/l). After agitation and centrifugation the organic phase was evaporated to dryness. The residue was derivatized by adding 20 µl of Meth-Prep II (Alltech) at room temperature for 20–30 min and a 2 µl aliquot

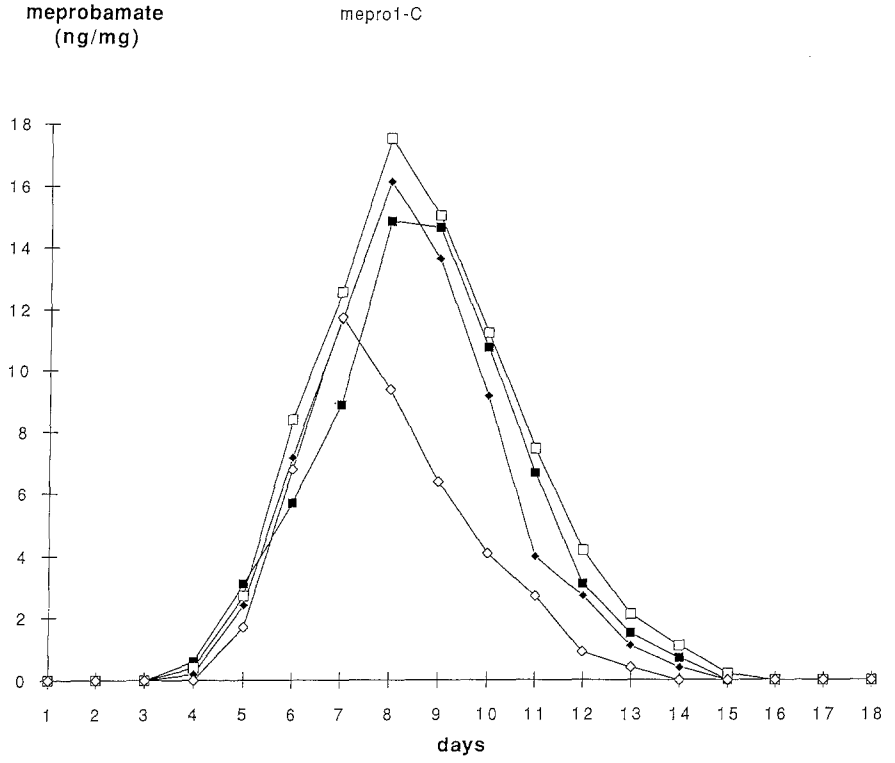

Fig. 1C

was injected into a 12 m × 0.22 mm fused-silica capillary column HP-1. The GC (Hewlett Packard 5890 serie II) oven temperature was initially 100°C for 3 min, programmed at 30°C/min to 280°C and held for 3 min. The flow of carrier gas (helium, purity N55) through the column was 1.8 ml/min. Splitless injection (split valve off-time of 0.75 min) was employed. A model 5971 Hewlett Packard mass spectrometer was used in the electron impact mode at 70 eV.

The ions monitored for meprobamate and vinylbarbital and their respective retention times were as follows: meprobamate, m/z 162, 6.31 min; vinylbarbital, m/z 182, 6.49 min.

The assay had a 80.1% extraction efficiency; the limit of quantification was approximately 0.2 ng/mg using a 30 mg sample.

Results and discussion

Meprobamate is a sedative antianxiety and muscle relaxant agent that is available for oral administration in doses of 400 mg. Therapeutic doses are in the range 400–1600 mg daily without side-effects. For this reason meprobamate was chosen for this first study. It could be expected that after oral intake of such a drug concentration, the incorporation in beard hair would be consistent and therefore more easy to evaluate by GC/MS.

During the period of the study, meprobamate levels in beard hairs remained negative in the placebo control samples. In the other samples, meprobamate levels in beard hairs were negative for the first 4–5 days and after 12–14 days. Meprobamate levels peaked during the 7–9th day irrespective of the administered dose (Fig. 1). Figure 2 illustrates the response obtained from one subject on day 7 after administration of 800 mg.

These findings suggest a time lag of approximately 4–5 days between the administration and appearance in hairs, probably as a result of the growth time necessary for the hair shaft to emerge from the bulb area in the follicle to a height above the skin surface sufficient for razor collection. Other authors have also found similar time lags, of various durations: 1 day for codeine [23], 5 days for pholcodine [13] and 7–8 days for morphine or codeine [3]. The two latter observations are in accordance with our findings. The pattern of meprobamate presence in beard hair was similar at 400, 800 and 1200 mg, for a period of 8–10 days after administration, which is consistent with previous data.

After oral administration of 400, 800 and 1200 mg of meprobamate, peak concentrations were in the range 4.27–6.08, 8.54–13.01, and 11.89–17.64 ng/mg, respectively.

One critical question about hair analysis which remains controversial is the relationship between dose and concentration. Although the limited subject data (4 subjects for 3 doses) in the present study preclude generalization, the results are strongly suggestive that a dose-response relationship exists between drug levels in beard hairs and the administered dose. This seems particularly true in such controlled studies, where a drug is taken for the first time or under close supervision. In case of chronic abuse, daily doses vary significantly from day to day, and the establishment of a dose-response relationship requires a large amount of data to take individual differences into consideration. No relationships could be established between opiate levels in hairs and morphine administration in carcinoma patients [23]. On the other hand, some papers reported significant relationships for digoxine, cocaine, phencyclidine, cannabis, morphine [5], haloperidol [16], nicotine [19], or amitriptyline [15].

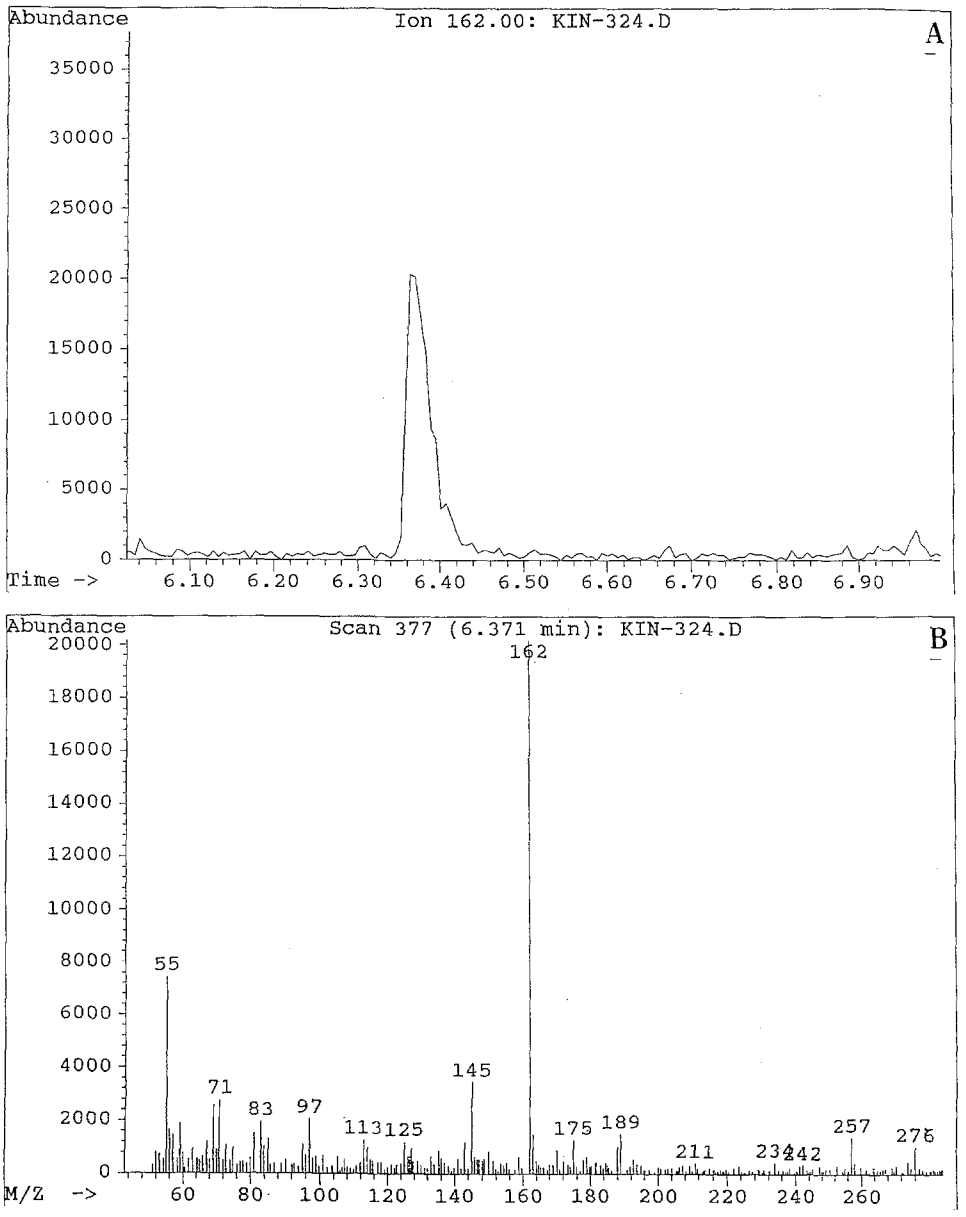

Fig. 2. A Selected ion monitoring at m/z 162 for meprobamate derivative in extract of a subject's beard obtained on day 7 after administration of 800 mg; concentration: 6.42 ng/mg. **B** Electron impact of meprobamate derivative (tetramethyl)

As proposed by Cone [3], these observations would also hold for drugs in head hair as both head and beard hair grow at a similar rate.

Numerous factors may influence the incorporation of drugs into hair, such as the nature of the compound (pKa, lipid solubility, metabolization pattern), and variations in hair growth cycles. Until these mechanisms are elucidated, the quantitative results and extrapolation to the amount of drug intake of such hair analyses should be considered with extreme caution.

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