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Short Communication

Chiral analysis of methadone in plasma by high-performance liquid chromatography

OLOF BECK*, LARS O. BOREUS, PIERRE LAFOLIE and GUN JACOBSSON

Department of Clinical Pharmacology, Karolinska Hospital, P.O. Box 60 500, S-10401 Stockholm (Sweden)

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ABSTRACT

A method for the chiral high-performance liquid chromatographic analysis of methadone in plasma has been developed. The method employed organic solvent extraction, enantiomeric separation on a Chiral AGP column, and ultraviolet absorption detection at 212 nm. The intra-day variation in the quantification of methadone enantiomers was less than 9% at the 100 ng/ml level, and the values obtained correlated well with those from a gas chromatographic–mass spectrometric method. Results from patients indicate inter- and intra-individual differences in the ratio between *l*- and *d*-methadone in plasma during therapy with racemic methadone. In one patient, a higher level of *d*-methadone in plasma was caused by both faster elimination and lower bioavailability of *l*-methadone.

INTRODUCTION

Methadone (Mtd) therapy is important for the effective treatment of opiate dependence in heroin addiction. Because of inter-individual differences in the pharmacokinetics of Mtd, it is necessary for optimal clinical results that doses are individualized in order to achieve recommended target plasma mean or trough levels [1–3]. In addition, adjustments of dose may be required because of intra-individual changes in pharmacokinetic parameters over time [4].

Mtd contains a chiral carbon atom (Fig. 1) and therefore exists in two ste-

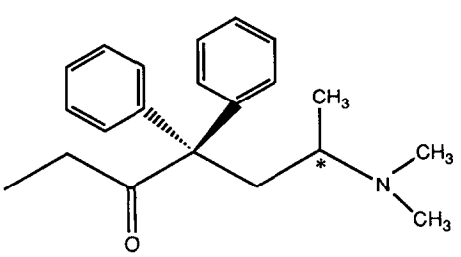

Fig. 1. Structure of methadone showing the position of the chiral carbon atom (asterisk).

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reoisomeric forms. Although the enantiomers show different pharmacodynamic and pharmacokinetic parameters, it is usually the racemate that is administered. The *l*-form is associated with the pharmacological effect [5,6], and seems to be the obvious species to monitor when analysis is performed. Unfortunately, methods for this are lacking. Recently, however, the Chiral AGP column, which has α_1-acid glycoprotein bound to silica as the separating principle, was introduced. Since the enantiomers of Mtd are known to separate on this type of chromatographic column [7] we undertook to develop a method for the quantitation of Mtd enantiomers in human plasma using the Chiral-AGP column.

EXPERIMENTAL

Chemicals

Racemic Mtd (*d,l*-6-dimethylamino-4,4-diphenyl-3-heptanone) hydrochloride was obtained from Apoteksbolaget (Stockholm, Sweden) and *l*-Mtd hydrochloride from Hoechst (Frankfurt am Main, Germany). Other chemicals used were of analytical quality. Glassware was silanized by treatment with Aqua-Sil (Pierce, Rockford, IL, USA).

Plasma samples

Plasma samples were obtained from patients participating in the Mtd maintenance programme of Stockholm County. All subjects were outpatients (both sexes) and had a history of heroin abuse. The dose range of racemic Mtd hydrochloride in the patients was 60–240 mg per day. Blood was collected by venous puncture in heparinized Vacutainer tubes. The plasma was transferred to plastic tubes and delivered to the laboratory within 24 h. The plasma samples were stored at −180°C prior to analysis. Mtd in plasma was stable for at least six months under these conditions.

Preparation of samples

Aliquots of plasma (0.5 ml) were pipetted into 15-ml ethanol-washed and newly silanized glass test-tubes, followed by the addition of 0.5 ml of 1 *M* K_2CO_3 and 3.5 ml of 2% butanol in *n*-hexane. The layers were thoroughly mixed for 1 min and subsequently separated by centrifugation at 1000 *g* for 10 min at ambient temperature (20–22°C). The organic layers were transferred to a new set of ethanol-washed and newly silanized test-tubes, and evaporated to dryness under a stream of nitrogen. Finally, the residues were redissolved in 250 μl of the mobile phase and stored at −180°C.

Chromatography

The chromatographic system consisted of a Constametric 3000 pump (LDC, Riviera Beach, FL, USA), a Rheodyne 7125 loop injector (Rheodyne, Cotati, CA, USA) with a loop volume of 100 μl, a 100 mm × 4.6 mm I.D., a 5 μm Chiral

AGP column (ChromTech, Norsborg, Sweden), a Spectromonitor 3100 UV detector (LDC) set at 212 nm, and a Hitachi D-2500 chromato-integrator (Hitachi, Tokyo, Japan) for peak-area integration.

The mobile phase was a solution of 16% (v/v) acetonitrile in 10 m*M* NaH_2PO_4 (pH 6.6) and was degassed in an ultrasonic bath before use. The flow-rate was 0.6–0.8 ml/min.

Calibration samples were prepared by spiking human blood plasma with known amounts (0–1000 ng/ml) of racemic Mtd. Quantitation was achieved by comparing the peak-area counts of unknown samples with those obtained for the calibration samples.

RESULTS AND DISCUSSION

This method was based on organic solvent extraction and had a recovery of Mtd from plasma of *ca.* 95%. The *l*- and *d*-isomers were baseline-separated (α = 1.2, R_s = 1.6) under the conditions used, with an analysis time of *ca.* 15 min (Fig. 2). No interfering peaks were observed during the analysis of over a hundred human plasma samples. The intra-assay variation was determined by spiking seven 0.5-ml aliquots of human blood plasma with 100 ng/ml of each of *l*- and *d*-Mtd. The mean level (n = 7) of *l*-Mtd was found to be 96.6 ng/ml with a coefficient of variation (C.V.) of 8.3%, and the mean level of *d*-Mtd was 97.4 ng/ml with a C.V. of 6.1%. The lower limit of determination was *ca.* 10 ng/ml. The peak areas obtained were linearly correlated with the amount of Mtd enantiomers added.

The quantitation of Mtd was further validated by comparing the sum of *l*- and *d*-Mtd with the results obtained with a gas chromatographic–mass spectrometric (GC–MS) method [8]. The results from the two methods were found to correlate

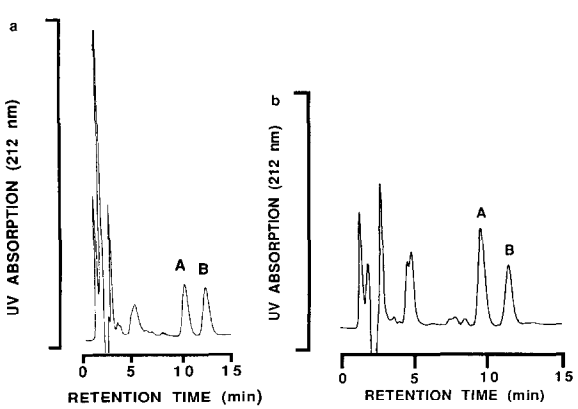

Fig. 2. Chromatograms obtained from the analysis of (a) blank plasma spiked with 100 ng/ml racemic methadone and (b) a sample of plasma from a patient undergoing methadone maintenance treatment. The resolution (R_s) between enantiomers was 1.6 and the separation factor (α) was 1.2. Peaks: A = *l*-Mtd; B = *d*-Mtd.

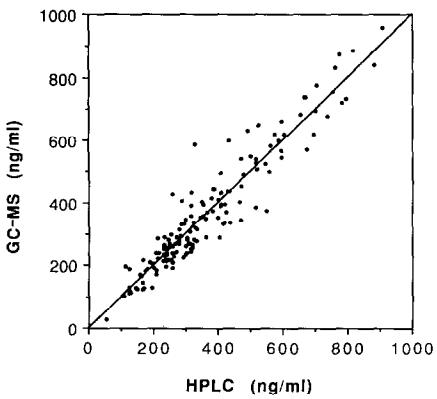

Fig. 3. Quantitation of methadone with the chiral HPLC method correlated with a gas chromatographic–mass spectrometric method. Linear regression analysis showed a relation according to the equation GC–MS = HPLC × 1.0 + 0.2, and a correlation coefficient of 0.95 (n = 152).

well (Fig. 3), according to the equation GC–MS = HPLC × 1.0 + 0.2, and with a correlation coefficient of 0.95. The ratio in quantitative results between the methods (HPLC to GC–MS) was 1.03 ± 0.18 (S.D., n = 152).

The method has been successfully used for the routine measurement of *l*- and *d*-Mtd levels during the dose interval in patients on Mtd therapy. Fig. 4 shows results from a male patient receiving 110 mg of Mtd orally per day. The measurement was made in the steady-state condition. There is a quantitative predom-

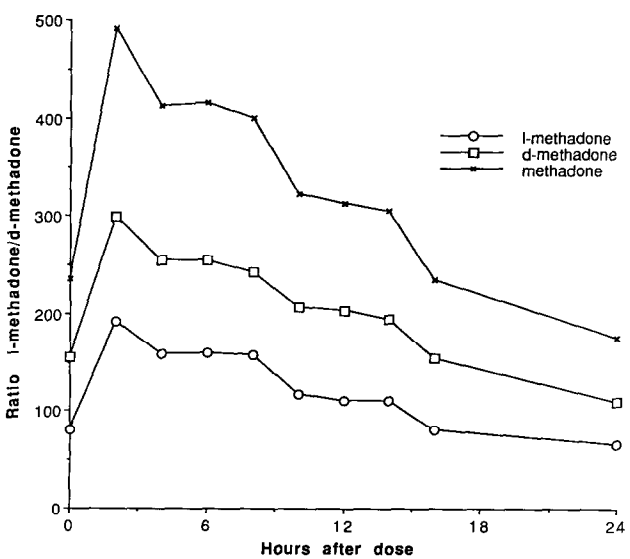

Fig. 4. Plasma concentrations profile of total, *l*- and *d*-methadone in a male patient with a daily oral dose of 110 mg of methadone.

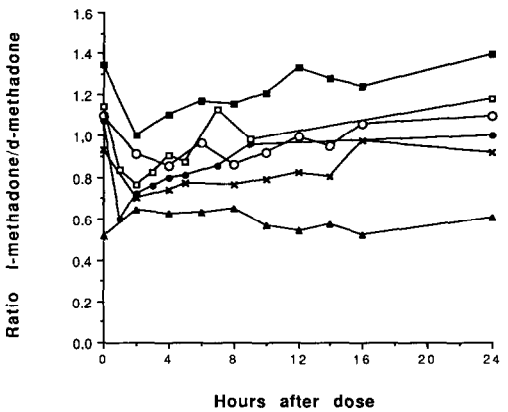

Fig. 5. Plasma ratio between *l*- and *d*-methadone in six patients on methadone therapy.

inance of *d*-Mtd over *l*-Mtd in this patient, which is explained by the shorter elimination half-life ($t_{1/2\beta}$ = 13.8 h *versus* 15.6 h) and the lower bioavailability ($AUC_{0-24\ h}$ = 2754 *versus* 4667 ng/ml h) of *l*-Mtd. It was further found that the ratio between the enantiomers varies not only between individuals but also within an individual during the dose interval (Fig. 5).

Previous studies have indicated that differences between the pharmacokinetics of *l*- and *d*-Mtd [9–11] exist. Our results are in accordance with earlier studies but show, for the first time, the existence of inter- and intra-individual differences in the ratio and pharmacokinetics of Mtd enantiomers in plasma during maintenance therapy. This should probably be taken into account when Mtd levels are being monitored, and this will be evaluated in future studies.

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