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Note

Gas—liquid chromatographic determination of plasma and urinary levels of amantadine in man

PIERRE M. BÉLANGER* and ODETTE GRECH-BÉLANGER

École de Pharmacie, Université Laval, Sainte-Foy, Québec, G1K 7P4 (Canada)

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Amantadine hydrochloride (Symmetrel) is an antiviral drug which has been increasingly used as antiparkinsonism agent in recent years. Gas—liquid chromatographic (GLC) procedures employing flame ionization [1, 2] and electron-capture [3, 4] detection have been reported for the quantitative determination of amantadine in biological fluids and tissues. According to Sioufi and Pommier [4] the former procedures reportedly lacked the sensitivity for the reliable determination of plasma levels of amantadine; besides in one method reported [2] 2-phenylethylamine, an endogenous compound which is excreted in urine in significant amounts [5—11] was used as an internal reference standard. GLC methods based on electron-capture detection can determine levels as low as 10 ng/ml in plasma but necessitate the formation of a suitable derivative which is time consuming.

We now report a simple GLC method for the direct determination of plasma and urinary levels of amantadine using flame ionization detection which can be used to determine amantadine plasma levels either routinely or for pharmacokinetic studies in man.

EXPERIMENTAL

Compounds and materials

Amantadine hydrochloride and chlorphentermine hydrochloride were kindly provided by Endo Lab. (Garden City, NY, U.S.A.) and Warner-Lambert Research Institute (Morris Plains, NJ, U.S.A.). The materials for GLC were all purchased from Chromatographic Specialities (Brockville, Canada). All other chemicals used were purchased from the usual commercial sources.

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Instrumentation

A Hewlett-Packard Chromatograph Model 5711A equipped with a flame ionization detector and a Hewlett-Packard chart recorder Model 7123A were used. A glass column of 1.2 m × 4 mm I.D. which was packed with Gas-Chrom Q (100—120 mesh) and coated with Apiezon L 5% was used. The column was preconditioned at 250°C for 16 h and treated with hexamethyldisilazane before use. The injection port and detector temperatures were set at 250°C and 300°C, respectively. The analyses were carried out using the following conditions: oven temperature, 150°C, air, 233.8 ml/min, hydrogen 58.6 ml/min, nitrogen flow-rate, 60 ml/min. The retention times of amantadine and the reference standard chlorphentermine were 5 and 8 min, respectively.

Assays procedures

Extraction from urine. A 1-ml aliquot of urine sample was rendered alkaline by the addition of sodium hydroxide (2 *N*, 0.5 ml); chlorphentermine HCl (90 μl, 1 ml) was added as internal reference standard and the mixture was extracted with freshly distilled diethyl ether (three times). Isopropanol (0.1 ml) was added to the combined ethereal extracts from each sample and the contents were then analyzed after concentration to 20—40 μl at 44°C.

Extraction from plasma. Aliquots (1—3 ml) of plasma were extracted under alkaline conditions as described for the urine samples except that a solution of 10 μg/ml of chlorphentermine HCl was used as internal standard. Hydrochloric acid (2 *N*, 3 ml) was then added to the combined ethereal extracts from each sample and the solutions were mixed on a Vortex mixer for 1 min. The ethereal phase was then discarded, sodium hydroxide (10%, 3 ml) was added and the resulting mixture was extracted with diethyl ether (three times). Isopropanol (0.1 ml) was added to the extracts which were then concentrated and analyzed by GLC.

Calibration curves

Freshly prepared solutions of amantadine HCl and chlorphentermine HCl were added to either urine or plasma. The range of concentrations of amantadine HCl used varied from 4—160 μg in urine and from 0.1—5.0 μg in plasma and the concentrations of chlorphentermine HCl used were 90 and 10 μg/ml in the urine and plasma samples, respectively. All samples were extracted and analyzed using the procedures described above. Calibration curves based on the peak height ratios of amantadine to the internal reference standard were constructed using six different concentrations of amantadine HCl analyzed in duplicate for each biological sample. The data were subjected to linear regression analysis to give the appropriate calibration factors.

Drug administration to man

Four healthy male subjects, two smokers and two non-smokers, who had not taken any drug for several days were given a 100-mg single oral dose of amantadine HCl (Symmetrel) in capsule form with 200 ml of tap water after an overnight fast. They were not allowed to ingest any solid food till 3 h had elapsed after administration of the drug.

Blood samples were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after drug

administration. The blood samples (11—13 ml) were collected in heparinized Vacutainer tubes, 10-ml aliquots were transferred to clean tubes and centrifuged to separate the plasma. Complete urine collections were taken at the same intervals as for the blood and continued up to eight days after administration of the drug. The pH of each urine was determined shortly after collection. Control urine and blood samples were collected just before the drug was taken. All samples were stored at 4°C and analyzed within a few days of initiation of the trials.

RESULTS AND DISCUSSION

The chromatographic conditions described were suitable for the analysis of amantadine in plasma (Fig. 1) and urine (Fig. 2) samples after administration of a 100-mg dose of amantadine HCl using chlorphentermine as internal reference standard. Ethereal extracts of the control samples of plasma and urine did not give any peak that interfered with the GLC analysis of amantadine and chlorphentermine and both compounds gave symmetrical peaks suitable for quantitative analysis. In the case of plasma, acid washing of the ethereal extracts was necssary in order to obtain clean extracts which could be analyzed at high sensitivities with minimum noise from the detector. These analytical conditions were specific to amantadine and there was no interference from its metabolic products N-hydroxyamantadine [12], 1-nitrosoadamantane [12] and 1-acetamidoadamantane [1] identified in animal studies. Also, there was no interference from other drugs used in the treatment of parkinsonism like benztropine mesylate (Cogentin), biperiden HCl (Akineton), *l*-dopa (Larodopa), procyclidine HCl (Kemadrin), trihexylphenidyl HCl (Artane), bromocriptine

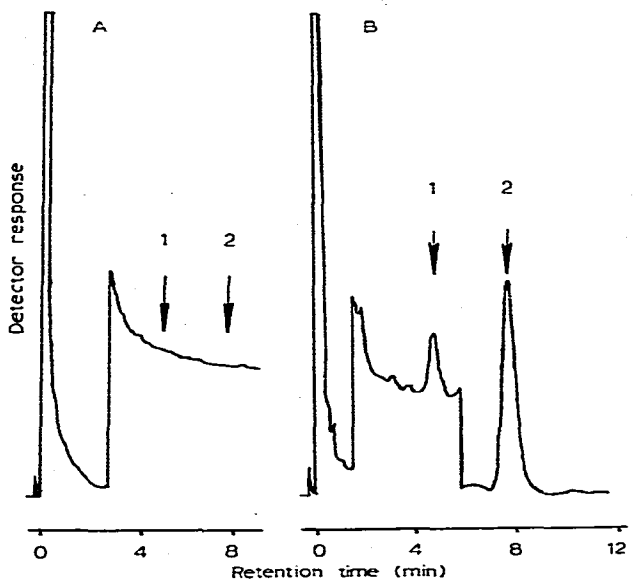

Fig. 1. Representative chromatograms of (A) untreated predose human plasma extract and (B) extract containing 456.5 ng/ml of amantadine HCl and the internal standard. Peaks: 1 = amantadine; 2 = internal standard, chlorphentermine.

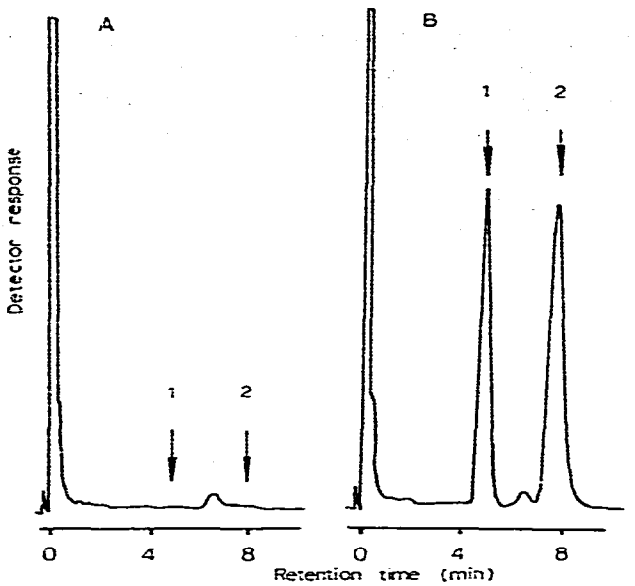

Fig. 2. Representative chromatograms of (A) untreated human urine extract before the dose and (B) urine extract containing 62.4 μg/ml of amantadine HCl and the internal standard. Peaks: 1 = amantadine; 2 = internal standard, chlorphentermine.

mesylate (Parlodel), diphenhydramine (Benadryl), meprobamate (Equanil), ethopropazine HCl (Parsitan), orphenadrine HCl (Disipal) and promethazine HCl (Phenergan).

During the first attempts to construct calibration curves for amantadine using the methods described herein, except that isopropanol was omitted, a high coefficient of variance between duplicate analyses of the same solutions was observed. Separate studies conducted to determine the cause of this variance showed that amantadine was volatile under the experimental conditions used but that this problem could be corrected by the addition of isopropanol (0.1 ml) to the ethereal extracts prior to evaporation.

Tables I and II give the recovery of amantadine added to control plasma and urine respectively at the concentrations of 0.1—5 μg for the plasma and 4—160 μg in the urine. In both systems, the coefficient of variation of duplicate

TABLE I

RECOVERY OF AMANTADINE HYDROCHLORIDE ADDED TO HUMAN PLASMA SAMPLES

Amount added (ng/ml)	Amount found (ng/ml) (mean ± S.D., $n = 2$)	Coefficient of variation (%)	Recovery (%)
100.0	99.7 ± 3.2	3.2	99.7
200.0	207.1 ± 8.6	4.2	103.6
500.0	492.4 ± 1.4	0.3	98.5
1000	1100 ± 70	6.1	110.0
2000	2100 ± 70	3.3	105.0
5000	4700 ± 40	0.7	94.0
		Mean ± S.D. = 101.8 ± 5.6	

TABLE II

RECOVERY OF AMANTADINE HYDROCHLORIDE ADDED TO HUMAN URINE

Amount added (μg/ml)	Amount found (μg/ml) (mean ± S.D., $n = 2$)	Coefficient of variation (%)	Recovery (%)
4	4.2 ± 0.3	7.1	105.0
10	99.0 ± 0.1	0.7	99.0
40	40.3 ± 3.2	7.9	100.8
80	76.2 ± 1.8	2.4	95.3
100	100.1 ± 0.0	0	100.1
160	161.2 ± 2.8	0.6	100.8
		Mean ± S.D. =	100.2 ± 3.1

analyses was satisfactory and the average recovery was essentially quantitative.

In order to show the application of the above analytical procedures we studied the pharmacokinetics of an oral dose of amantadine HCl (equivalent to 80.56 mg of the free base) in four healthy male volunteers. Fig. 3 shows the mean plasma levels of amantadine versus time following the oral administration of a capsule of 100 mg of amantadine HCl (Symmetrel). The plasma levels of amantadine varied between 0.20 and 1.05 μg/ml. These values are within the range of 0.1—1.3 μg/ml reported by various authors [3, 4, 13—15] and show that the proposed analytical method is satisfactory for determining plasma levels of amantadine either routinely or during pharmacokinetic studies in man. Amantadine was mainly excreted unchanged in the urine and Fig. 4 shows a typical cumulative excretion curve of amantadine under varying conditions of urinary pH. In this case, 95% of the dose ingested was recovered unchanged in urine over a period of eight days. An average urinary recovery value of 92% of the dose of amantadine has been reported [1].

In conclusion, the proposed GLC assay is simpler and faster than other methods that have been published. It consists of a simple extraction procedure using diethyl ether, a solvent which is easily concentrated in a water bath at 42—45°C and straightforward analysis of amantadine in the concentrate on a 5% Apiezon L column isothermally without prior derivatization. It has been

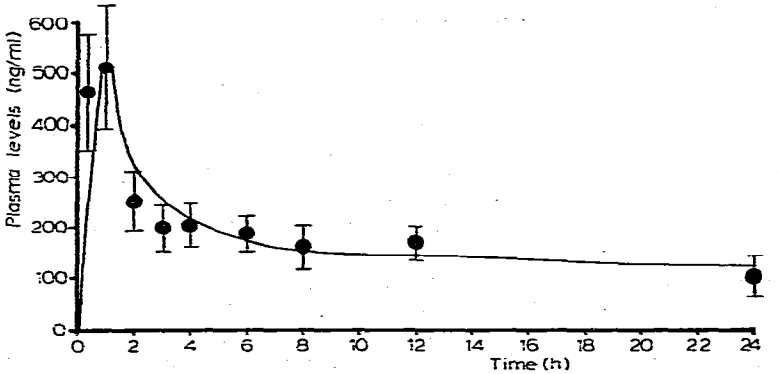

Fig. 3. Average (± S.E.) plasma concentrations of amantadine versus time obtained in four healthy male subjects after the oral administration of 100 mg of amantadine HCl (Symmetrel).

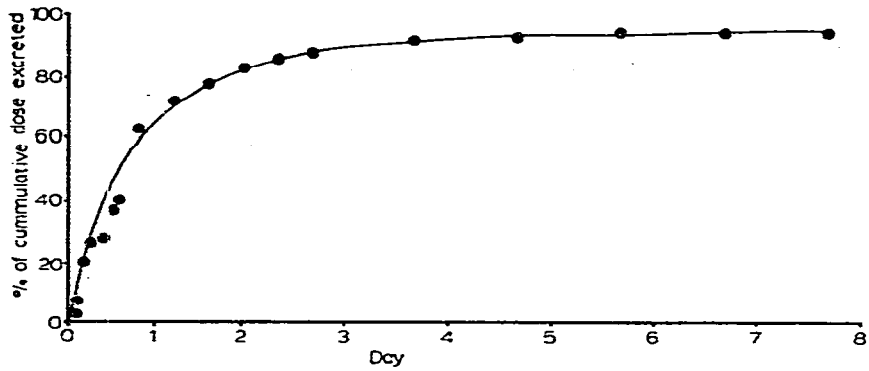

Fig. 4. A typical cumulative urinary excretion curve of amantadine (subject M.L.) under varying conditions of urinary pH after oral administration of 100 mg of amantadine HCl.

shown that the method is sufficiently sensitive to accurately determine plasma and urine levels of amantadine after administration of a therapeutic dose in man. Neither the known metabolic products of amantadine that have been identified in laboratory animals nor the presence of several antiparkinsonism agents that are often prescribed with amantadine interfere with the proposed assay. To date this is the first assay that demonstrates specificity for amantadine.

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