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Note

Measurement of moclobamide, a new monoamine oxidase inhibitor, by gas chromatography with nitrogen-selective detection

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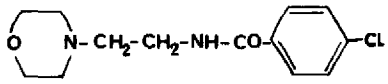
Moclobamide [*p*-chloro-N-(2-morpholinoethyl)benzamide, Fig. 1] is a new monoamine oxidase (MAO) inhibitor. Its inhibitory effect on MAO in the brain is reversible and specific for the A form of the enzyme. An open evaluation suggested the drug had a rapid onset of antidepressant effect and low toxicity [1]. Its antidepressant efficacy has since been confirmed in a double-blind comparison with amitriptyline [2].



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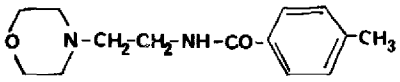

B

Fig. 1. The structures of moclobamide (A) and the internal standard (B).

To evaluate both the pharmacokinetics of moclobamide and the relationship between plasma concentrations and antidepressant efficacy, we have developed an analytical method based on gas chromatography (GC) with nitrogen-selective detection.

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EXPERIMENTAL

Materials

Moclobamide (Ro 11-1163) and Ro 11-9506 (Fig. 1) were obtained from Hoffmann-La Roche (Basle, Switzerland). The latter was used as the internal standard. Ethyl acetate, HPLC grade was obtained from Waters Assoc. (Milford, MA, U.S.A.).

All glassware was soaked overnight in a 5% solution of phosphate-free detergent, Lipsol (Lip. Ltd. Shipley, West Yorkshire, U.K.), thoroughly rinsed in tap water then finally rinsed with glass-distilled water.

Extraction procedure

Samples of 0.5 or 1 ml of plasma were diluted to 5 ml with glass-distilled water in 20-ml glass tubes. Unknown samples, four plasma standards and a quality control were included in each extraction. The internal standard solution (Ro 11-9506 in ethanol) was added to give a concentration of 250 ng per ml of sample. After mixing, the samples were alkalinized with 0.5 ml of 5 *M* sodium hydroxide, and 5 ml of ethyl acetate were added. The samples were shaken for 10—15 min on a horizontal shaker and centrifuged for 5 min at 1400 *g*. A maximum aliquot of the solvent phase was transferred to clean tubes containing 1 ml of 0.1 *M* hydrochloric acid. The plasma phase was re-extracted with a further 5 ml of ethyl acetate and a maximum aliquot of the solvent was added to the first extract. The combined solvent phases were extracted with the acid by shaking for 10—15 min followed by centrifugation as before. The acid phase was removed to clean 5-ml glass tubes, alkalinized with 0.3 ml of 5 *M* sodium hydroxide, and extracted by gentle rotation (10—15 min) with 2 ml of ethyl acetate. After centrifugation the solvent phase was transferred to 5-ml V-shape tubes. The solvent was evaporated under air at 37°C and the

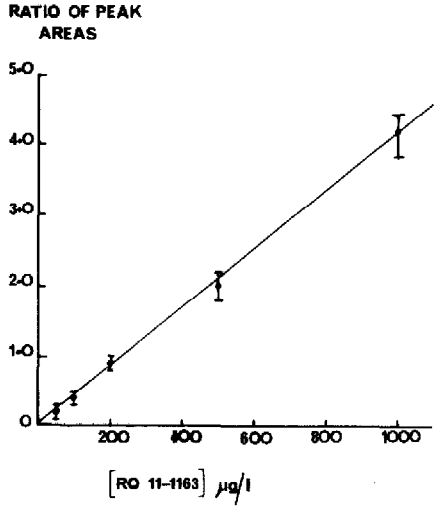

Fig. 2. Plasma standard curve for moclobamide (Ro 11-1163). Calculated linear regression line was $y = 0.0042x - 0.0053$, $r = 0.9994$. The error bars represent ± 1 S.D. and the number of determinations at each concentration was 5.

samples were stored at −4°C. Prior to analysis, samples were reconstituted in 10 μl of ethanol. Aliquots of 1—5 μl were injected into the gas chromatograph depending on the expected concentration.

Gas chromatography

The analysis was carried out on a Hewlett-Packard Model 5710A gas chromatograph. Gas chromatographic separation was carried out on a silanised glass column (2 m × 2 mm I.D.) packed with a 3% OV-101 on Gas-Chrom W HP (80–100 mesh).

Nitrogen was used as carrier gas at a flow-rate of 20 ml/min. The injection port was maintained at 260°C and the oven was held initially at 220°C for 2 min and then programmed to 240°C at 4°C/min. Moclobamide and the internal standard eluted after 5.2 and 4.5 min, respectively. Peak areas were integrated using a Hewlett-Packard 3380A integrator. The peak area ratio of moclobamide/internal standard was calculated for each sample and standard curves were constructed using linear regression analysis (Fig. 2).

Precision studies

Drug-free plasma (Blood Bank) was used to prepare standards to which known amounts of moclobamide were added. These were used each run to prepare standard curves and to evaluate the day-to-day precision of the assay.

Single-dose experiments

Five depressed patients were given 50 mg of moclobamide. Samples were taken over 8 h via an indwelling heparinized cannula, the plasma separated and stored frozen until analysed. The half-life of elimination for each patient was calculated by linear regression analysis of the terminal plasma concentrations.

Multiple-dose experiments

Blood samples were obtained from ten patients participating in a double-blind comparative trial of moclobamide versus amitriptyline [2]. Samples were obtained weekly (prior to the 0800 or 1200 dose) over a four-week period, the plasma separated and stored frozen until analysed.

RESULTS AND DISCUSSION

There has not been many published studies concerning the measurement of monoamine oxidase inhibitors in plasma since platelet MAO activity is easily measured and serves as an indication of whether the dosage is sufficient to elicit a clinical response. Platelet MAO is the B form of the enzyme and moclobamide is specific for the A form. To look at either pharmacokinetics or clinical efficacy, the actual drug concentrations in plasma must be determined for moclobamide.

Gas chromatography has been used previously to measure concentrations of MAO inhibitors in biological media [3, 4], and combined gas chromatography—mass spectrometry has also been used [5]. In the latter case, the plasma concentrations of phenelzine were extremely low (< 10 ng/ml), hence the better sensitivity achieved using the combined technique was required.

TABLE I

REPRODUCIBILITY OF THE ASSAY FOR MOCLOBAMIDE CONCENTRATIONS IN PLASMA

n = 10 at all concentrations.

Expected concentration (μg/l)	Found concentration (μg/l)	± S.D.	± C.V. (%)
50	54.8	8.6	15.7
100	103.1	9.8	9.5
200	207.6	13.9	6.7
500	508.8	34.5	6.8
1000	999.2	62.1	6.2

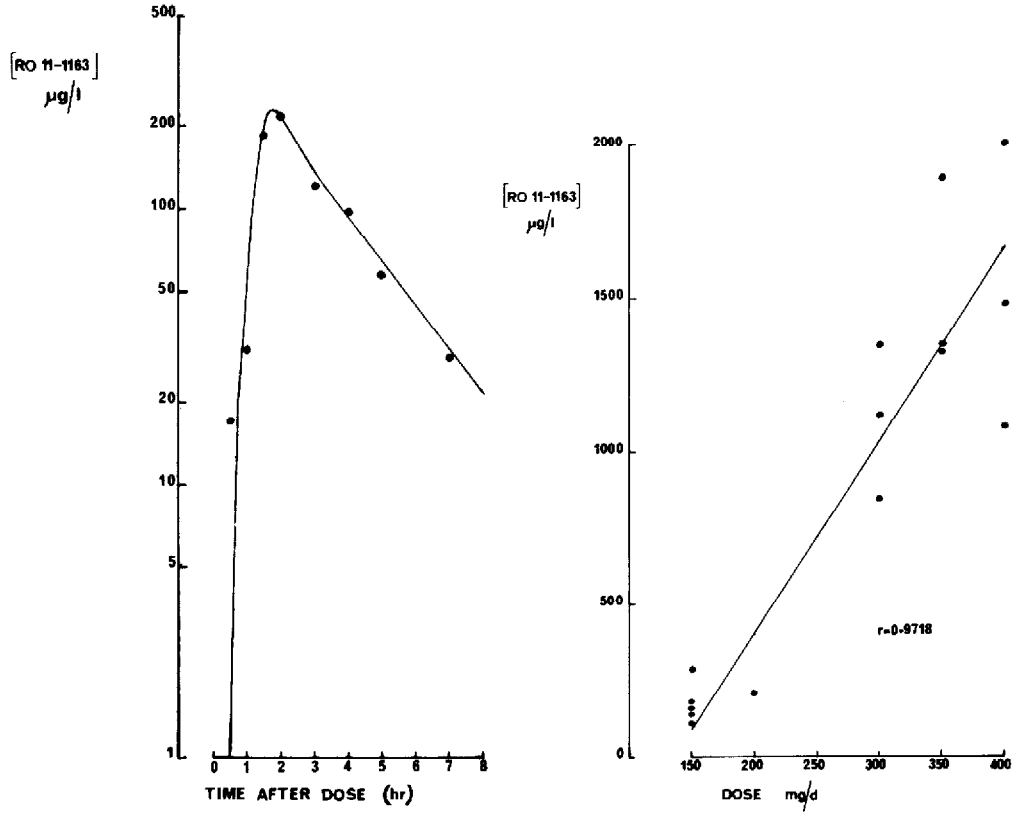

Fig. 3. Mean plasma concentrations of moclobamide (Ro 11-1163) following a single oral dose of 50 mg in five depressed patients.

Fig. 4. Steady-state plasma concentrations versus daily dose of moclobamide (Ro 11-1163) in ten depressed patients.

Adequate sensitivity for moclobamide was achieved using GC with nitrogen-selective detection.

Ethyl acetate was found to be a satisfactory solvent for extracting moclobamide but a three-step extraction procedure was found necessary to provide clean extracts for analysis. The average recovery for moclobamide through the procedure was 70% and the sensitivity was 10 μg/l. The second ethyl acetate extraction could be omitted when analysing steady-state concentrations as the sensitivity would still be adequate. The reproducibility over the concentration range 50—1000 μg/l is shown in Table I.

The assay is suitable for the measurement of plasma concentrations following either a single oral dose or after multiple doses. This is demonstrated in Figs. 3 and 4. Following a 50-mg single dose in five depressed patients, a mean peak concentration of 365 μg/l was reached at 1.7 h post-dose. The mean elimination half-life was 1.5 h. Pharmacokinetic data obtained for another MAO inhibitor, phenelzine, was similar to that for moclobamide [6]. A fuller treatment of the pharmacokinetics will be published separately.

Little published data is available on the metabolism of moclobamide. Urinary excretion studies show that only 0.4% of the dose is excreted as unchanged drug, that the metabolite pattern is complex and the major metabolites are as yet unidentified [7].

The steady-state concentrations achieved by patients treated over four weeks with varying dosages of moclobamide were measured. A linear relationship between the dosage administered and the steady-state plasma concentrations achieved was obtained (Fig. 4). High concentrations (1000 μg/l) were achieved with the highest doses, and considerable interindividual variability in the plasma concentrations was observed at each dose.

The method as described is simple to perform, has the required sensitivity for single-dose kinetic analysis and is reproducible over the range required for steady-state plasma concentration analysis.

ACKNOWLEDGEMENTS

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