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Moclobemide treatment causes a substantial rise in the sparteine metabolic ratio

L. F. GRAM, K. BRØSEN and the DANISH UNIVERSITY ANTIDEPRESSANT GROUP*
Department of Clinical Pharmacology, Institute of Medical Biology, Odense University, Denmark

A sparteine test was carried out immediately before (n = 37) and during (n = 33) moclobemide treatment (200 mg twice daily) in 37 patients participating in a controlled clinical trial. The sparteine metabolic ratio (MR) did not correlate with the plasma concentration of moclobemide and/or its oxidized metabolite Ro 12-8095, and four sparteine poor metabolisers (PM, MR > 20) had plasma moclobemide concentrations similar to those in extensive metabolisers (EM, MR < 20). The Ro 12-8095/moclobemide ratio tended to correlate negatively with the sparteine MR before and during treatment (r_s = −0.32, −0.37). During moclobemide treatment the sparteine MR rose substantially by a factor of 1–103 (median 4.7), and two EM became phenotypically PM. In the PM subjects as well as in one EM patient on cimetidine during both tests, no change in sparteine MR occurred.

Keywords moclobemide sparteine metabolic ratio

Introduction

Moclobemide is a reversible, selective monoaminoxidase A inhibitor recently introduced as an antidepressant (Fitton *et al.*, 1992). In man it is eliminated mainly by metabolism with an oral clearance of 0.6–1.5 l min^{-1}. The volume of distribution is about 60–100 l, and the half-life is about 1–2 h after a single dose, but becoming more prolonged after multiple dosing (Fitton *et al.*, 1992; Jauch *et al.*, 1990; Raaflaub *et al.*, 1984). Accumulation is greater than predicted from single dose data (Guentert *et al.*, 1990; Wiesel *et al.*, 1985). In healthy volunteers many metabolites of moclobemide have been detected in the urine (Jauch *et al.*, 1990). In blood two metabolites have been detected in measurable concentrations, formed by morpholine C-oxidation (lactam metabolite, Ro 12-8095) and morpholine *N*-oxidation (Ro 12-5637). The former is present in blood at concentrations comparable with those of moclobemide, whereas blood concentrations of the latter are generally low and often undetectable 6 h after moclobemide administration (Fitton *et al.*, 1992).

An increasing number of drugs, including several classes of psychotropic drugs (tricyclic and newer antidepressants, neuroleptics) have been shown to be metabolised by the polymorphic sparteine/debrisoquine oxygenase (CYP2D6), and some of these drugs are also potent inhibitors of CYP2D6 (Brøsen & Gram, 1989). The role of the sparteine/debrisoquine oxidation polymorphism in the elimination of moclobemide is unclear. Retrospective analyses with relatively few poor metabolisers (PM) have not been conclusive (Schoerlin *et al.*, 1987; 1990; Wiesel *et al.*, 1985). The present study was carried out as part of a controlled clinical trial to elucidate further the role of CYP2D6 in the pharmacokinetics of moclobemide.

Methods

The patients studied participated in a randomised clinical trial comparing moclobemide 400 mg day^{-1} with clomipramine 150 mg day^{-1}. The study was carried out in depressed in-patients at six clinical centres in Denmark within the Danish University Antidepressant Group (DUAG, 1993). Patients were started on a single-blind placebo treatment for 1 week and then given active treatment in a fixed dose for 4–6 weeks. Moclobemide (or clomipramine) was given in two equal doses at 08.00 and 20.00 h. Compliance was monitored by observing ingestion of drug and by weekly plasma drug concentration measurement. A sparteine test was scheduled for the placebo-week and for the 3rd week of active treatment. The study was approved by the regional Ethics Committees and the Danish National Health Service (Sundheds-styrelsen).

In the clinical study (DUAG, 1993) 57 patients were

Correspondence: Dr L. F. Gram, Department of Clinical Pharmacology, IMB, Winsløwparken 19,3, DK-5000 Odense C, Denmark
*Steering Committee: P. Bech, T. G. Bolwig, L. F. Gram (Chairman), P. Kragh-Sørensen, N. Reisby, P. Vestergaard.

included in the moclobemide group, but 20 were subsequently excluded owing to early drop out or failure to perform the sparteine test. Thus, 37 patients were included, 26 women and 11 men aged 23 to 68 (median 50) years. In 33 patients the sparteine test was carried out both before and during moclobemide treatment. In 29 of these cases and in the 4 cases having only a premedication sparteine test, average plasma concentrations of moclobemide could be estimated.

Concurrent medication was taken in unchanged doses during the placebo and the active treatment period. Nearly all patients received oxazepam. Two were taking oral contraceptives, two were receiving disulfiram and one cimetidine.

The sparteine test was started in the evening (20.00 h) by ingestion of 100 mg sparteine sulphate (Depasan®, Giulini) followed by urine collection for 12 h. Sparteine and 2,3- and 5,6-dehydrosparteine in urine were assayed by gas chromatography (Vinks *et al.*, 1982). The sparteine metabolic ratio (MR) was calculated as the ratio between sparteine and 2,3- plus 5,6-dehydrosparteine excreted in the 12 h urine. Control experiments with urine from moclobemide-treated patients (without sparteine test) showed no interference with the sparteine assay.

Blood for the assay of moclobemide and metabolites was drawn in heparinised tubes in the morning at 12 h after the last moclobemide dose.

Moclobemide and its primary oxidized metabolite were assayed in plasma by quantitative thin layer chromatography using techniques adapted from earlier studies with imipramine (Gram *et al.*, 1983) and newer antidepressants (DUAG, 1990). Briefly 1000 μl plasma was extracted with 500 μl dichlorethane and after washing (0.1 N NaOH, 0.5 ml), 300 μl of the organic phase was transferred directly to high performance t.l.c. plates (Merck) using a Desaga Autospotter. The t.l.c. plates were developed in chloroform:butanol:ammonia (25% v/v) 50:5:0.25. This allowed separation of moclobemide ($R_f = 0.38$) and its metabolites (Ro 12-8095: $R_f = 0.62$; Ro 12-5637: $R_f = 0.24$). However, measurment of the minor metabolite Ro 12-5637 had to be abandoned because of interference by oxazepam. Densitometric *in situ* scanning of the t.l.c. plates was carried out at 245 nm with a Zeiss KM 3 Chromatogram Spectrophotometer. Blanks and standard curves were run on each t.l.c. plate. Standard curves were linear in the concentration range 0–800 nM and samples with higher concentrations were reanalysed after dilution. The lower level of quantitation was about 50 nM for moclobemide and about 100 nM for Ro 12-8095. All samples were assayed in duplicate and the between-day reproducibility was 6–8% (coefficient of variation). Standards were provided by Roche Ltd, Basel, Switzerland.

Statistical testing was carried out by Spearman Rank Correlation and the Wilcoxon-Pratt paired rank sum test using the MEDSTAT program package version 2.12 (Astra, Albertslund, Denmark).

Results

Plasma concentrations of moclobemide and Ro 12-8095 fluctuated markedly in each patient. In six patients single measurements were clearly deviant (> factor 2.5 from mean) probably due to sampling after the morning dose. Omitting these measurements, average concentrations could be calculated with an accuracy of ± 10–30% (coefficient of variation, 3–6 measurements). These fluctuations probably reflect the short half-life of moclobemide and to a smaller degree the reproducibility of the assay. Interpatient variability in plasma moclobemide concentration (×65, range 70–4580 nM) and in plasma Ro 12-8095 (×18, range 110–2020 nM) clearly exceeded intrapatient variability. There was a significant correlation between the average plasma concentrations of moclobemide and Ro 12-8095 ($r_s = 0.91$, $n = 33$; $P < 0.001$), but the metabolite/parent compound ratio decreased with increasing plasma drug concentrations.

The premedication sparteine test identified four patients as PM (MR > 20). There was no correlation ($P > 0.05$) between the premedication MR and the average plasma concentration of moclobemide, Ro 12-8095 or moclobemide + Ro 12-8095. Correlation coefficients for the MR during moclobemide treatment with the average plasma concentration of moclobemide, Ro 12-8095 and the sum were slightly higher but not significant. The ratio Ro 12-8095/moclobemide showed weak correlations with the premediction MR ($r_s = -0.32$, $n = 33$, $P = 0.068$) and the MR during moclobemide treatment ($r_s = -0.37$, $n = 29$, $P = 0.048$).

As shown in Figure 1 there was pronounced increase in the sparteine MR ($P < 0.001$) during moclobemide treatment. In extensive metabolisers (EM, MR < 20) it increased by a factor of 1 to 103 (median 4.7). Two EM became phenotypically PM during moclobemide treatment. Urinary recoveries of sparteines and dehydrosparteines are shown in Table 1.

The patient taking cimetidine had one of the highest MR (2.4) within the EM-group and this did not change during moclobemide. The increase in MR in the other subjects did not correlate with plasma moclobemide or Ro 12-8095 concentrations. The two values of MR (before and during moclobemide) correlated significantly ($r_s = 0.78$, $n = 33$, $P < 0.001$). In the three PM tested twice, no change in MR occurred (Figure 1).

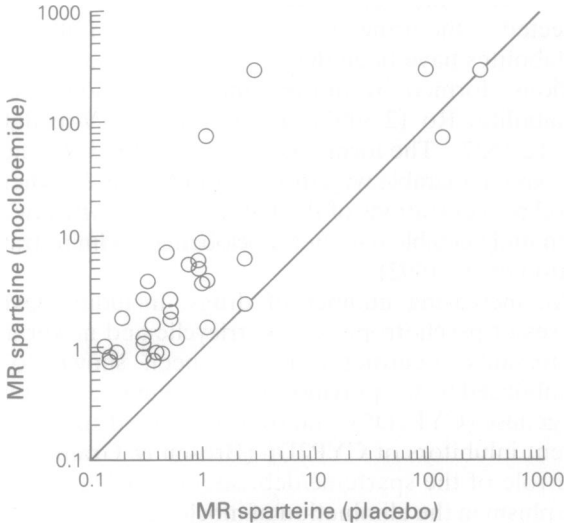

Figure 1 Sparteine MR before (placebo) and during moclobemide treatment ($n = 33$). The diagonal line is the line of identity.

Table 1 Excretion of sparteine and 2,3- plus 5,6-dehydrosparteine in % of dose (*median* (range)) before (placebo) and during the second week of moclobemide treatment:

	EM[a] (n = 29)		*PM* (n = 3)	
	Placebo	*Moclobemide*	*Placebo*	*Moclobemide*
Sparteine	*16.8* (6.6–41.5)	*38.4*[b] (13.7–75.7)	*53.5* (37.9–69.0)	*72.9* (50.1–87.2)
Dehydrosparteines	*28.2* (10.3–65.1)	*14.8*[b] (0.1–34.8)	*0.4* (0.1–0.6)	*0.4* (0.2–0.6)
Total	*55.0* (30.3–74.6)	*54.4* (24.8–88.1)	*50.5* (38.2–69.0)	*77.9* (54.3–87.3)

a: Patient taking cimetidine omitted
b: $P < 0.001$ compared with placebo.

Discussion

This study showed that plasma moclobemide concentrations do not correlate with the sparteine MR. Thus, CYP2D6 appears to play no major role in the elimination of moclobemide. The relatively low precision of the determination of individual steady-state plasma moclobemide concentrations might have precluded the detection of a partial cosegregation, as suggested by the weak correlations between the metabolite ratio (Ro 12-8095/moclobemide) and the sparteine MR.

Our results indicate that moclobemide and/or its metabolites are potent inhibitors of CYP2D6 *in vivo*. This contrasts with the findings of *in vitro* studies with human liver microsomes where moclobemide was found to be a weak inhibitor of CYP2D6 (K_i = 140 μM, Skjelbo & Brøsen, 1992) and two of its metabolites were non-inhibitory (Brøsen, unpublished data). The observed effect of moclobemide on the sparteine MR corresponds to that seen with haloperidol (10–20 mg day^{-1}) in patients (Gram *et al.*, 1989) and quinidine (5–10 mg single doses) in volunteers (Nielsen *et al.*, 1990). *In vitro* these drugs are potent (haloperidol K_i = 1 μM, Inaba *et al.*, 1985) or very potent (quinidine K_i = 0.06 μM, Otton *et al.*, 1984) inhibitors of CYP2D6. The explanation for the discrepancy between the inhibitory potency of moclobemide *in vitro* and *in vivo* is unknown.

Cimetidine is known to inhibit the 4-hydroxylation of debrisoquine (Philip *et al.*, 1989) and also the metabolism of moclobemide (Fitton *et al.*, 1992). Apparently the administration of cimetidine in one of our subjects prevented further inhibition by moclobemide.

The inhibitory effect of moclobemide on CYP2D6 could influence the elimination of other drugs which are substrates of this isozyme such as tricyclic antidepressants.

This study represents a contribution to the aims of COST B1.

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