XENOBIOTICA, 2001, VOL. 31, NO. 7, 387–397

Characterization of moclobemide *N*-oxidation in human liver microsomes

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Received 11 January 2001

1. Moclobemide undergoes morpholine ring *N*-oxidation to form a major metabolite in plasma, Ro12-5637.

2. The kinetics of moclobemide *N*-oxidation in human liver microsomes (HLM) ($n = 6$) have been investigated and the mixed-function oxidase enzymes catalysing this reaction have been identified using inhibition, enzyme correlation, altered pH and heat pretreatment experiments.

3. *N*-oxidation followed single enzyme Michealis-Menten kinetics (0.02–4.0 mM). $K_{m\ app}$ and V_{max} ranged from 0.48 to 1.35 mM (mean ± SD 0.77 ± 0.34 mM) and 0.22 to 2.15 nmol mg^{-1} min^{-1} (1.39 ± 0.80 nmol mg^{-1} min^{-1}), respectively.

4. The *N*-oxidation of moclobemide strongly correlated with benzydamine N-oxidation, a probe reaction for flavin-containing monooxygenase (FMO) activity, (0.1 mM moclobemide, $r_S = 0.81, p < 0.005$; 4 mM moclobemide, $r_S = 0.94, p = 0.0001$). Correlations were observed between moclobemide *N*-oxidation and specific cytochrome P450 (CYP) activities at both moclobemide concentrations (0.1 mM moclobemide, CYP2C19 $r_S = 0.66, p < 0.05$; 4 mM moclobemide, CYP2E1 $r_S = 0.56, p < 0.05$).

5. The general P450 inhibitor, *N*-benzylimidazole, did not affect the rate of Ro12-5637 formation (0% inhibition versus control) at 1.3 mM moclobemide. Furthermore, the rate of Ro12-5637 formation in HLM was unaffected by inhibitors or substrates of specific P450s (< 10% inhibition versus control).

6. Heat pretreatment of HLM in the absence of NADPH (inactivating FMOs) resulted in 97% inhibition of Ro12-5637 formation. *N*-oxidation activity was greatest when incubated at pH 8.5. These results are consistent with the reaction being FMO mediated.

7. In conclusion, moclobemide *N*-oxidation activity has been observed in HLM *in vitro* and the reaction is predominantly catalysed by FMOs with a potentially small contribution from cytochrome P450 isoforms.

Introduction

Moclobemide is a reversible selective inhibitor of monoamine oxidase-A (MAO-A) that is widely prescribed for the treatment of depression. It undergoes extensive first-pass metabolism and following oral administration < 0.5% of the parent drug is excreted unchanged in the urine (Jauch *et al.* 1990). Moclobemide undergoes *N*- and *C*-oxidation to form its two major metabolites in plasma, Ro12-5637 and Ro12-8095, respectively (Jauch *et al.* 1990) (figure 1). Ro12-5637 is a

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Xenobiotica ISSN 0049–8254 print/ISSN 1366–5928 online © 2001 Taylor &
http://www.tandf.co.uk/journals
DOI: 10.1080/00498250110055488

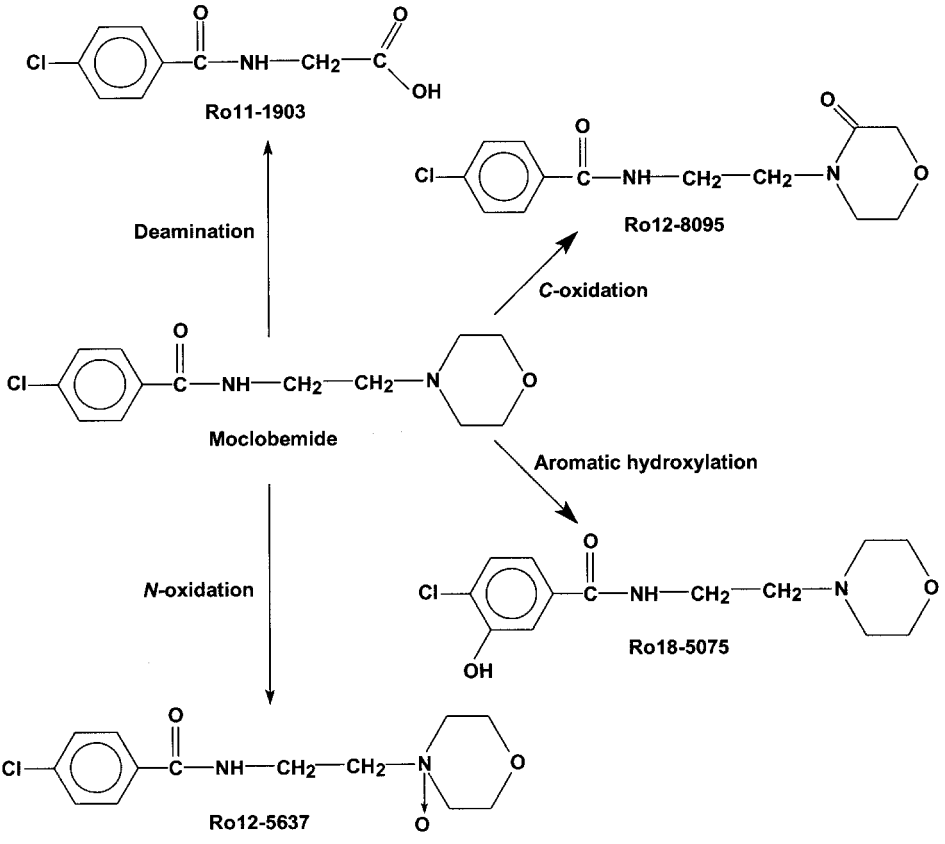

Figure 1. Primary metabolic pathways of moclobemide.

weak inhibitor of MAO-A, whereas Ro12-8095 has no MAO-A inhibitory activity (Da Prada *et al.* 1989). About 35% of an orally administered dose of moclobemide is recovered in urine as Ro12-5637, whereas Ro12-8095 accounts for $< 1\%$ of the dose (Mayersohn and Guentert 1995).

The enzymes catalysing moclobemide oxidation have not been characterized. *In vivo* evidence suggests that the C-oxidation pathway is partially mediated by cytochrome P450 2C19 (Gram *et al.* 1995). *N*-oxidation of the tertiary amines of many psychoactive drugs, including imipramine, clozapine and olanzapine, is predominantly catalysed by FMOs rather than by cytochrome P450s (Lemoine *et al.* 1990, Ring *et al.* 1996, Tugnait *et al.* 1997). *N*-oxidation of moclobemide occurs at the tertiary amine of the morpholine ring suggesting that this reaction may be FMO rather than P450 mediated.

Cytochrome P450s and FMOs are present in the microsomal fraction prepared by differential centrifugation of homogenized hepatic cells. The hepatic oxidation of a drug can be characterized *in vitro* using microsomes prepared from human liver samples. Straightforward experiments can be employed to determine whether a reaction is FMO- or P450-mediated (Grothusen *et al.* 1996) and enzyme correlation and chemical inhibition experiments can be performed to identify the specific P450 isoforms involved (Chang *et al.* 1994, Newton *et al.* 1995, Bourrie

et al. 1996, Ono *et al*. 1996). The objective of the current study was to characterize moclobemide *N*-oxidation in human liver microsomes (HLM) *in vitro* and to identify the mixed-function oxidase(s) that catalyse(s) this reaction.

Materials and methods

Chemicals

Dextromethorphan hydrobromide, coumarin, α-naphthoflavone, phenacetin, quinidine, salicylic acid, tolbutamide, troleandomycin (TAO) and β-$NADP^+$ (β-nicotinamide adenine dinucleotide phosphate, potassium salt) were purchased from Sigma Chemical Co. (St Louis, MO, USA). *S*-mephenytoin and (±)-4′-hydroxymephenytoin were purchased from Salford Ultrafine Chemicals and Research Ltd (Manchester, UK) and didiethylcarbamate (DDC) and *N*-benzylimidazole from Aldrich Chemical Co. (Milwaukee, WI, USA). Other drugs and drug metabolites were generously provided as follows: moclobemide, Ro12-8095, Ro12-5637 and dextrorphan tartrate from Roche Pty Ltd (Sydney, NSW, Australia), ketoconazole from Janssen-Cilag Pty Ltd (North Ryde, NSW, Australia), sulphaphenazole from Ciba-Geigy Australia Ltd (Pendle Hill, NSW, Australia), and benzydamine and benzydamine *N*-oxide from 3M Pharmaceuticals Pty Ltd (Sydney, NSW, Australia). Glucose 6-phosphate and glucose 6-phosphate dehydrogenase were purchased from Boehringer Mannheim (Sydney, NSW, Australia). Methanol and triethylamine were HPLC grade and purchased from BDH (Kilsyth, VIC, Australia). Potassium phosphate salts, 70% perchloric acid, 85% orthophosphoric acid and magnesium chloride were analytical grade and purchased from Ajax Chemicals (Auburn, NSW, Australia). Methanolic solutions were prepared of moclobemide and inhibitors and stored at −20°C. Ro12-5637 was prepared in double-distilled water.

Ro12-5637 high-performance liquid chromatography (HPLC) assay

The reversed-phase HPLC method of Geschke *et al*. (1987) was modified to measure moclobemide and Ro12-5637 in microsomal incubates. A mobile phase consisting of methanol:triethylamine:33.5 mM potassium dihydrogen orthophosphate (25 : 0.1 : 74.9 v/v/v), adjusted to pH 3 using orthophosphoric acid, was pumped at a flow rate of 1.3 ml min^{-1} using an LC-10AT pump (Shimadzu Oceania Pty Ltd, Rydalmere, NSW, Australia) with a back pressure of 150–180 kg cm^{-2}. An SIL-10AXL Autoinjector (Shimadzu Oceania) was used to inject 0.13 ml of the incubates and calibration standards onto a Spherisorb 5 μm C6 column 150 × 4.6 mm i.d. (Phenomenex, Torrance, CA, USA). An SPD-10A UV-Vis detector (Shimadzu Oceania) set at 240 nm detected moclobemide and Ro12-5637. The system was controlled and chromatograms integrated using CLASS LC10 (v.1.60) software (Shimadzu Oceania). Calibration standards of 0.04-12.3 μM Ro12-5637 in 0.2 ml 0.1 M potassium phosphate buffer (pH 7.4) and 10 μl 17.5% perchloric acid were prepared in 1.7 ml centrifuge tubes (Edwards Instrument Co., Narellan, NSW, Australia). Standards were vortexed and centrifuged for 8 min at 6800*g*. Standard curves were estimated by least-squares linear regression of Ro12-5637 concentration versus peak height. Concentrations of Ro12-5637 in the microsomal incubates were calculated from the regression lines.

Microsomal preparation

Microsomes from HL2-32 ($n = 11$) were prepared as described by Sutton *et al*. (Sutton *et al*. 1997). HLM1-6 were prepared from healthy hepatic tissue obtained at biopsy that would otherwise have been discarded as surgical waste. After tissue removal it was immediately placed into liquid nitrogen and stored at −70°C. The cytochrome P450 content and protein concentration were determined as previously described (Rieutord *et al*. 1995).

Moclobemide N-*oxidation microsomal assay*

Methanolic solutions of moclobemide and inhibitor solutions (prepared in methanol and stored at −20°C) were added to 10 ml polypropylene tubes (Sarstedt Australia Pty Ltd, Technology Park, SA, Australia) and the methanol was evaporated using a Speedivac Rotary Evaporator (Savant Instruments, Inc., Farmingdale, NY, USA) prior to incubation. A typical incubation (final volume of 0.2 ml) contained moclobemide, potassium phosphate buffer (0.1 M, pH 7.4) and an NADPH-generating system (final concentrations: 10 mM glucose 6-phosphate, 10 mM $MgCl_2$, 1 mM $NADP^+$, 0.4 U glucose 6-phosphate dehydrogenase). Samples were pre-incubated for 5 min at 37°C in a constant-temperature SW-20°C agitated water bath (Julabo Labortechnik, Seelbach, Germany) and reactions were initiated by adding HLM. The reaction proceeded without a lid so that the incubate was in equilibrium with

oxygen in the air. The reaction was stopped by adding 10 µl 17.5% perchloric acid and samples were then vortexed and centrifuged for 8 min at 6800*g*.

The relationship between rate of *N*-oxide formation and HLM protein concentration was assessed from 0.025 to 0.3 mg ml^{-1} at 37°C for 60 min. The relationship between rate of formation and incubation time was assessed from 5 to 120 min at 37°C at a protein concentration of 0.1 mg ml^{-1}. To determine the reproducibility of the microsomal assay, moclobemide was incubated with HLM2 in duplicate on four separate occasions to assess between-day reproducibility and in quadruplicate on a single occasion to assess within-day reproducibility. The conditions selected for investigation were a protein concentration of 0.1 mg ml^{-1} and an incubation time of 60 min. For kinetic studies, samples from HLM1, 2–6 and HL27 were incubated with increasing concentrations of moclobemide (0.02–4.0 mM). For activity correlations the rate of Ro12-5637 formation was measured at 0.1 and 4 mM moclobemide in 11 HLM samples. All other experiments were performed at 1.3 mM moclobemide (average apparent K_m). To investigate the contribution of FMO to the *N*-oxidation of moclobemide, microsomes (HLM2) were pre-incubated with and without the NADPH-regenerating system at 50°C for 1 min and stored on ice for < 10 min before being incubated with moclobemide. To assess the effect of pH on moclobemide *N*-oxidation, the rate of Ro12-5637 formation was measured at pHs 7.0, 7.4, 8.0, 8.5, 9.0 and 9.5 in HLM2. For these incubations, 0.2 M Tris-HCl was prepared and adjusted to the appropriate pH by the addition of 0.1 M HCl. All incubations were performed in duplicate and the average value is reported.

Activity of specific P450 isoforms and FMOs

Eleven HLM samples were assessed for the following activities: testosterone 6β-hydroxylation (CYP3A4; 50 µM), 7-ethoxyresorufin-*O*-deethylation (CYP1A2; 2.5 µM), *N*-dimethylnitrosoamine *N*-demethylation (CYP2E1; 4 mM), aniline 4-hydroxylation (CYP2E1; 5 mM) and tolbutamide 4-hydroxylation (CYP2C9; 0.3 mM) as described (Sutton *et al.* 1997). Dextromethorphan *O*-demethylation (CYP2D6; 40 µM) (Kerry *et al.* 1993), *S*-mephenytoin 4′-hydroxylation (CYP2C19; 0.2 mM) (Chiba *et al.* 1993) and benzydamine *N*-oxidation (FMO; 0.5 mM) (Baldock *et al.* 1990, Lang *et al.* 1998) were also assessed.

Chemical inhibition experiments

Potential chemical inhibitors were pre-incubated with moclobemide as described above and the reactions were initiated by adding HLM2. The mechanistic inhibitors, TAO and DDC, were pre-incubated with HLM and an NADPH-regenerating system for 30 min at 37°C and the moclobemide *N*-oxidation reaction initiated by adding moclobemide in 0.1 M phosphate buffer (pH 7.4). The final concentrations of putative P450 inhibitors and substrates were 1 mM *N*-benzylimidazole (general P450 inhibitor), 10 and 25 µM coumarin (CYP2A6), 10 and 50 µM DDC (CYP2E1), 2 and 5 µM ketoconazole (CYP3A4/5), 50 and 250 µM S-mephenytoin (CYP2C19), 0.1 and 1.0 µM α-naphthoflavone (CYP1A1/2), 50 and 250 µM phenacetin (CYP1A1/2), 0.5 and 5 µM quinidine (CYP2D6), 10 and 20 µM sulphaphenazole (CYP2C9), 0.2 and 0.5 mM tolbutamide (CYP2C9), and 10 and 50 µM TAO (CYP3A4). Salicylic acid (0.25 mM) was used as a negative control. The final concentrations were selected on the basis of literature reports of each compound's maximum inhibition selectivity (Grothusen *et al.* 1996) and the K_m of the substrates (Chiba *et al.* 1993, Chang *et al.* 1994, Newton *et al.* 1995, Bourrie *et al.* 1996, Ono *et al.* 1996).

Data analysis

The rate of metabolite formation (v) was calculated as nanomoles of Ro12-5637 formed per milligram of protein per min (nmol mg^{-1} min^{-1}). Apparent K_m and V_{max} derived from Eadie-Hofstee plots were used as initial estimates for fitting the kinetic data to the single-enzyme Michaelis-Menten equation using extended least-squares non-linear regression. The relationships between moclobemide *N*-oxidation and the activities of FMOs and specific P450s were assessed by calculating the magnitude and significance of the Spearman rank correlation coefficients.

Results

Using the HPLC technique described, the retention times of moclobemide and Ro12-5637 were 5.0 and 6.9 min, respectively, with a final run time of 20 min. A representative chromatogram of moclobemide incubated with HLM and an NADPH-regenerating system is shown in figure 2A. The calibration curve was linear for the measurement of Ro12-5637 over the concentration range studied with regression coefficients > 0.99. A typical equation for the Ro12-5637 calibra-

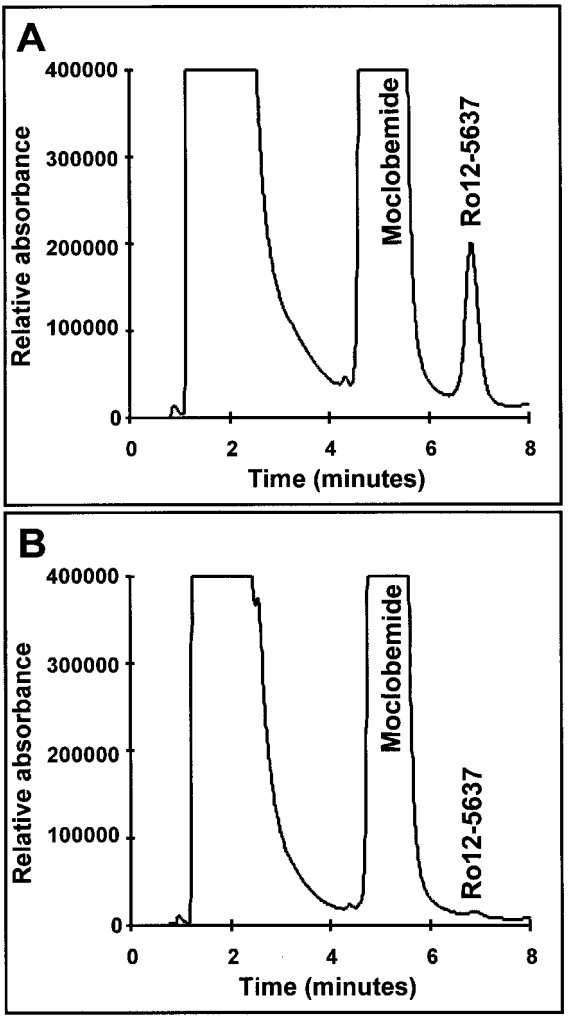

Figure 2. Representative chromatograms of moclobemide incubated with human liver microsomes (0.1 mg protein ml^{-1}) at 37°C for 60 min. Chromatogram (A) is an incubation of moclobemide (1.3 mM) with HLM2 and (B) is an incubation of moclobemide (1.3 mM) with HLM2 pre-incubated at 50°C for 1 min in the absence of an NADPH-regenerating system. FMOs are unstable at 50°C in the absence of NADPH.

tion curve was $y = 5555x - 540$. The limit of quantification for the Ro12-5637 assay was 0.04 μM.

Formation of Ro12-5637 was observed in the presence of an NADPH-regenerating system but not in the absence of NADPH under the same conditions (table 1). Over the range of moclobemide concentrations studied, <10% of the parent drug was metabolized. At a moclobemide concentration of 1.3 mM the within-day coefficient of variation of Ro12-5637 formation ($n = 4$) was <4.0%. The between-day coefficient of variation for the incubation assay determined at 0.1 mM moclobemide on four separate occasions was <4.0%.

Ro12-5637 formation was linear with time from 5 to 120 min and was also linear with protein concentration from 0.025 to 0.3 mg ml^{-1} (data not shown). The

Table 1. Velocity of moclobemide *N*-oxidation to Rol2-5637 under different experimental conditions. Moclobemide (1.3 mM) was incubated with human liver microsomes (HLM2) for 60 min at 37°C.

HLM pre-incubation conditions	Incubation co-factor[+]	Velocity (nmol mg^{-1} min^{-1})[‡]
No preincubation	+NADPH	1.26
No preincubation	−NADPH	0.00
Heat[§]	+NADPH	0.03
Heat, NADPH[§]	+NADPH	1.12

[+]Cofactor was an NADPH regenerating system consisting of glucose-6-phosphate, $MgCl_2$, $NADP^+$ and glucose-6-phosphate dehydrogenase.

[‡]Each value represents the average of duplicate measurements.

[§]Microsomal protein was preincubated with moclobemide for 1 min at 50°C in the presence or absence of NADPH regenerating system and stored on ice < 10 min before being incubated with moclobemide as per usual.

rate of Ro12-5637 formation was subsequently determined over the concentration range of 0.02-4 mM moclobemide. Eadie-Hofstee plots for metabolite formation were monophasic for all six livers tested so kinetic data was fitted to the Michaelis-Menten equation for a single enzyme system (figure 3). The apparent K_m varied 2.8-fold from 0.48 to 1.35 mM (mean ± SD = 0.77 ± 0.34 mM) and V_{max} varied 9.8-fold from 0.22 to 2.15 nmol mg^{-1} min^{-1} (mean ± SD = 1.39 ± 0.80 nmol mg^{-1} min^{-1}).

In a bank of 11 HLM samples, large interindividual variability in moclobemide *N*-oxidation was observed at both 0.1 mM (26-fold) and 4 mM (65-fold). One

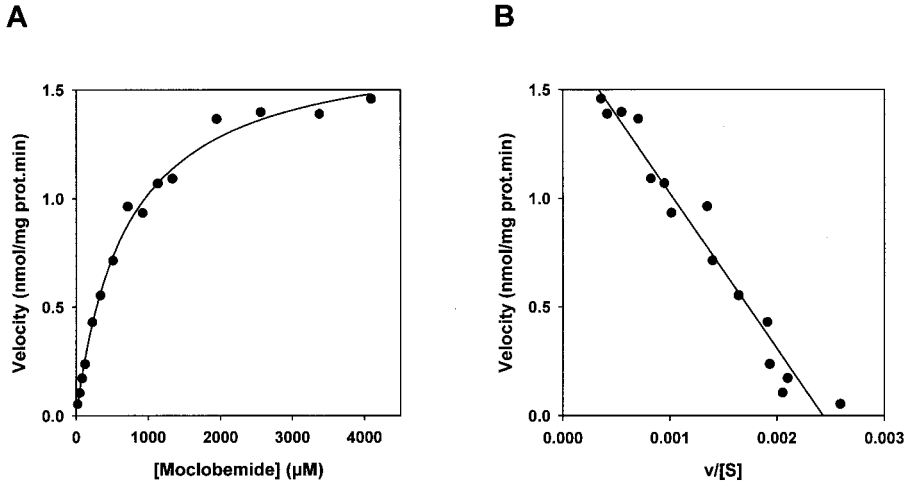

Figure 3. Moclobemide (0.02-4.0 mM) incubated with human liver microsomes (HLM6; 0.1 mg protein ml^{-1}) at 37°C for 60 min. Data are presented as Michaelis-Menten (A) and Eadie-Hofstee (B) plots. Each point represents the average of duplicate measurements.

sample had very low *N*-oxidation activity (moclobemide 0.1 mM, $v = 0.007$ nmol mg^{-1} min^{-1}; 4 mM, 0.023 nmol mg^{-1} min^{-1}) relative to other HLM samples and so was considered an outlier and excluded from further analysis. The activities of the 10 human liver preparations varied 4-fold at 0.1 mM moclobemide and 6-fold at 4 mM. The Spearman rank correlations between moclobemide *N*-oxidation and isoform specific P450 and FMO activities are presented in table 2. At both moclobemide concentrations high correlations were observed between the rate of Ro12-5637 formation and the N-oxidation of benzydamine, a probe reaction for FMO activity. At 0.1 mM moclobemide, but not 4.0 mM, a weaker correlation was observed between moclobemide *N*-oxidation and *S*-mephenytoin 4′-hydroxylation. At the high moclobemide concentration a weak correlation was observed between moclobemide *N*-oxidation and one of the CYP2E1 probe reactions, aniline 4-hydroxylation, but not for another CYP2E1 mediated reaction, *N*-dimethylnitrosoamine *N*-demethylation. No significant relationships between moclobemide *N*-oxidation and 7-ethoxyresorufin *O*-deethylation, dextromethorphan *O*-demethylation, tolbutamide 4-hydroxylation and testosterone 6β-hydroxylation were observed suggesting that CYP1A2, CYP2D6, CYP2C9 and CYP3A4 respectively do not mediate this reaction at the moclobemide concentrations tested.

At 1.3 mM moclobemide, the general P450 inhibitor, *N*-benzylimidazole, had no effect on Ro12-5637 formation by HLM. In addition, all of the isoform specific inhibitors and substrates tested produced $< 10\%$ inhibition of *N*-oxidation (data not shown). Heat treatment of HLM in the absence of an NADPH-regenerating system (Grothusen *et al.* 1996) decreased Ro12-5637 formation to 2% of control in HLM (table 1). The NADPH-generating system protected the reaction from thermal inactivation (88% of control activity) (table 1). At pH 7.4 the rate of moclobemide *N*-oxidation was greater for moclobemide incubated with HLM in Tris-HCl buffer rather than phosphate buffer (1.14 versus 1.00 nmol mg^{-1} min^{-1}, respectively). Ro12-5637 formation was greater at pH > 7.4 and maximal at pH 8.5 (2.10 nmol mg^{-1} min^{-1}) (figure 4).

Table 2. Correlations (r_S) between moclobemide *N*-oxidation and flavin-containing monooxygenase (FMO) activity and cytochrome P450 (CYP) isoform-specific activities in a panel of human liver microsomal preparations ($n = 10$).

	Correlation coefficient (r_S) with moclobemide *N*-oxidation	
Isoform-selective activity	0.1 mM	4 mM
Benzydamine *N*-oxidation (FMO)	0.806**	0.939***
7-Ethoxyresorufin *O*-deethylation (CYP1A2)	0.527	0.394
Tolbutamide 4-hydroxylation (CYP2C9)	0.358	0.503
S-mephenytoin 4′-hydroxylation (CYP2C19)	0.661*	0.249
Dextromethorphan *O*-demethylation (CYP2D6)	0.376	−0.394
N-dimethylnitrosomaine *N*-demethylation (CYP2E1)	−0.134	0.146
Analine 4-hydroxylation (CYP2E1)	0.430	0.564*
Testosterone 6β-hydroxylation (CYP3A4)	0.394	0.297

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$.

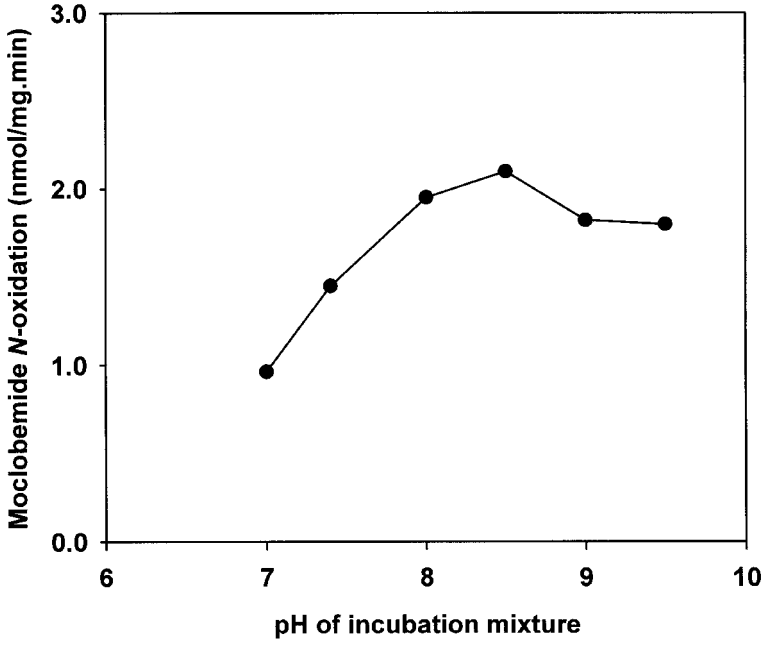

Figure 4. Effect of pH on *N*-oxidation of moclobemide. Moclobemide (1.3 mM) was incubated for 60 min at 37°C with HLM2, an NADPH-generating system and 0.2 M Tris-HCl buffer at increasing pH. Each point represents the average of duplicate measurements.

Discussion

The formation of the *N*-oxide metabolite of moclobemide, Ro12-5637, was NADPH dependent indicating that the reaction is FMO and/or cytochrome P450-mediated. FMOs are inactivated selectively by pre-incubating HLM at 50°C for 1 min (Grothusen *et al.* 1996) in the absence of an NADPH-regenerating system. These heat conditions greatly diminished the formation of Ro12-5637 whereas HLM heat-treatment in the presence of an NADPH-regenerating system protects the enzyme from inactivation. Furthermore, the reaction velocity is maximal at pH 8.5, a characteristic of FMO-mediated reactions (Cashman *et al.* 1993, Brunelle *et al.* 1997), and the rate of Ro12-5637 formation is strongly correlated with the *N*-oxidation of benzydamine, an FMO substrate (Lang *et al.* 1998). Together these observations are consistent with FMOs catalysing the *N*-oxidation of moclobemide in human liver, *in vitro*. Five isoforms of FMO (1–5) have been identified in humans. FMO3 and FMO5 are expressed in the adult human liver and FMO3 is the major isoform (Overby *et al.* 1997). A recent study using recombinant human FMOs showed that FMO3 catalyses benzydamine *N*-oxidation with greater efficiency than FMO5 (FMO3: $K_m = 60$ μM; $V_{max} = 46$ min^{-1}, FMO5: $K_m > 2$ mM; $V_{max} < 1$ min^{-1}) (Lang and Rettie 2000), suggesting benzydamine *N*-oxidation is a specific probe reaction for FMO3 activity in HLM. Given the strong relationship between benzydamine and moclobemide *N*-oxidation it is likely that the formation of Ro12-5637 is also mediated by FMO3 in HLM.

Large variability in moclobemide *N*-oxidation activity was observed (26- and 65-fold), however, when the HLM sample with the lowest activity was excluded, the variability (6-fold) was similar to that reported for other FMO mediated

reactions (Overby *et al*. 1997, Tugnait *et al*. 1997, Lang *et al*. 1998). Individuals homozygous for a missense Pro153 to Leu153 mutation in exon 4 of *FMO3* have impaired *N*-oxidation of trimethylamine (Dolphin *et al*. 1997, Zschocke *et al*. 1999). These individuals suffer from 'fish-odour' syndrome because they excrete the fishy smelling trimethylamine in their sweat, urine and breath. A number of other mutations of *FMO3* have been identified, some of which result in amino acid changes that may contribute to interindividual variation in FMO catalytic activity (Cashman *et al*. 2000, Kang *et al*. 2000). The variability observed in the group of human liver microsomal samples tested in this study may be due to interindividual variability in *FMO3* genotypes, a hypothesis which remains to be confirmed.

To the authors' knowledge there is one report of benzydamine *N*-oxidation activities in HLM (Lang *et al*. 1998). Using the same incubation conditions, the benzydamine *N*-oxidation activities observed in the present study are lower than those previously reported (0.15–2.3 versus 2.8–9.5 nmol mg^{-1} min^{-1}) (Lang *et al*. 1998). These lower activities may reflect different microsomal preparation techniques (Sadeque *et al*. 1993). FMOs are very sensitive to thermal inactivation in the absence of NADPH and labile to mechanical disruption (Cashman 1999). Although our HLM have lower FMO catalytic activities possibly reflecting lower levels of viable FMOs, a high correlation between benzydamine and moclobemide *N*-oxidation was still observed supporting a major role for FMOs in moclobemide *N*-oxidation.

The general P450 inhibitor, *N*-benzylimidazole, did not affect the formation of Ro12-5637 suggesting a lack of P450 involvement in moclobemide *N*-oxidation. Enzyme correlation experiments, however, indicate minor roles for CYP2C19 and CYP2E1 in catalysis of *N*-oxidation at low and high moclobemide concentrations, respectively. However, these observations are not supported by the chemical inhibition results. Experiments using heterologously expressed P450 enzymes would assist in elucidating the specific P450 isoforms involved in catalysing the reaction and to what extent they contribute to the formation of Ro12-5637.

In conclusion, the *N*-oxidation of moclobemide of human liver microsomes highly correlates with the flavin-containing monooxygenase mediated *N*-oxidation of *N*-benzydamine, indicating that moclobemide *N*-oxidation is predominantly catalysed by FMOs. The role of this group of enzymes was confirmed by the observation that Ro12-5637 formation is maximal at pH 8.5 and that heat pretreatment of the human liver microsomes at 50°C for 1 min in the absence of NADPH abolishes the formation of Ro12-5637. Enzyme correlation and chemical inhibition experiments suggest only minor roles for cytochrome P450s in catalysing the reaction.

Acknowledgements

J. H. was funded by a Dora Lush (Biomedical) Scholarship awarded by the National Health and Medical Research Council of Australia.

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