Br J clin Pharmac 1995; **39**: 77–80

Evidence for a role of cytochrome P450 2D6 and 3A4 in ethylmorphine metabolism

ZHURONG LIU[1], ÖRJAN MORTIMER[1], C. A. DALE SMITH[2], C. ROLAND WOLF[3] & ANDERS RANE[1]
[1]Department of Clinical Pharmacology, Akademiska Hospital, Uppsala University, S-751 85 Uppsala, Sweden,
[2]Department of Pathology, Medical School, University of Edinburgh, Edinburgh EH8 9NG and
[3]Biomedical Research Centre, University of Dundee, Dundee DD1 9SY, UK

Ethylmorphine is metabolised by *N*-demethylation (to norethylmorphine) and by *O*-deethylation (to morphine). The *O*-deethylation reaction was previously shown *in vivo* to co-segregate with the *O*-demethylation of dextromethorphan indicating that ethylmorphine is a substrate of polymorphic cytochrome P450(CYP)2D6. To study further the features of ethylmorphine metabolism we investigated its *N*-demethylation and *O*-deethylation in human liver microsomes from eight extensive (EM) and one poor metaboliser (PM) of dextromethorphan. Whereas *N*-demethylation varied only two-fold there was a 4.3-fold variation in the *O*-deethylation of ethylmorphine, the lowest rate being observed in the PM. Quinidine, at a concentration of 1 μM, inhibited *O*-deethylation in microsomes from an EM, but was unable to do so in microsomes from the PM. The immunoidentified CYP2D6 and CYP3A4 correlated with the rates of *O*-deethylation ($r = 0.972$) and *N*-demethylation ($r = 0.969$), respectively. We conclude that the *O*-deethylation of ethylmorphine is catalysed by the CYP2D6 in human liver microsomes consistent with previous findings in healthy volunteers.

Keywords cytochrome P4502D6 polymorphism human liver microsomes ethylmorphine

Introduction

Ethylmorphine has been employed widely as an *in vitro* substrate in studies of the *N*-demethylation capacity of the liver. Previous studies with human liver indicated that this reaction is catalysed by a member of the cytochrome P4503A family [1]. In rats, it was found to be catalysed by CYP3A [2] and CYP2C11 [3].

Ethylmorphine *O*-deethylation was recently described in isolated rat hepatocytes [4]. In humans, we have shown that the *O*-deethylation of ethylmorphine to morphine co-segregates with the debrisoquine hydroxylation (CYP2D6) polymorphism *in vivo* [5], poor metabolisers showing higher ethylmorphine AUC values than extensive metabolisers. Chemically, ethylmorphine is similar to codeine, the *O*-demethylation of which is also polymorphic, both *in vivo* [6, 7] and *in vitro* [8]. For both of these drugs, the product of the *O*-dealkylation is morphine.

To establish further the role of CYP2D6 in ethylmorphine *O*-deethylation, we have investigated this reaction in liver microsomes from patients phenotyped and genotyped for the polymorphic CYP2D6 isoform.

Methods

Chemicals

Ethylmorphine, morphine, norethylmorphine and quinidine (Nordic Pharmacopeia) were obtained from the hospital pharmacy. Biochemicals, chemical agents and solvents were of analytical grade from Sigma Chemical Company (St Louis, MO, USA), Sigma or Bio-RAD (Stockholm, Sweden).

Immunoblotting

Immunoblotting of liver microsomes was performed as described previously [8] with an anti-CYP2D6 peptide antibody and a polyclonal antibody against rat CYP3A1 [9].

Microsomes

Human liver samples from one PM and eight EMs with normal liver function tests were obtained as wedge biopsies at abdominal surgery (Table 1). The patients were *genotyped* with respect to CYP2D6 [10]. Phenotyping was performed with dextromethor-

Correspondence: Professor Anders Rane, Department of Clinical Pharmacology, Akademiska Hospital, S-751 85 Uppsala, Sweden

phan [8]. The study was approved by the Ethics Committee of the University and performed after informed consent from the patients.

Incubations

Isolation of microsomes, protein analysis, and incubation with ethylmorphine were performed as described previously [1]. The enzyme kinetics experiments were carried out with ethylmorphine at concentrations ranging from 0.1 to 4.0 mM. Linearity with time and protein concentration was ascertained.

Inhibition studies

Antibodies against CYP3A1 (or preimmune IgG) were preincubated with microsomes at concentrations of 1, 3, and 5 mg ml^{-1} for 15 min at 23° C. As the antibody to CYP2D6 was not inhibitory, a potent competitive inhibitor of CYP2D6, quinidine, was used at a concentration of 1 μM [11]. Quinidine was dissolved in 0.1 M sodium phosphate buffer (pH = 7.4) and preincubated as described above.

Analytical methods

Morphine and norethylmorphine were assayed by h.p.l.c. according to Ladona *et al.* [1].

Results are presented as the mean of duplicate assays.

Results

The formation of morphine by *O*-deethylation varied 4.3-fold betwen individuals (5.7 to 24.4 pmol min^{-1} per mg protein). The specific *O*-deethylation activity (at a substrate concentration of 1 mM) was 5.7 pmol mg^{-1} min^{-1} in the PM *vs* 13.5 ± 6.3 in the EMs. The *N*-demethylation rate varied only two-fold (39.9 to 63.1 pmol mg^{-1} protein min^{-1}). There was no correlation between the rates of formation of these metabolites.

Western blot analysis revealed a high correlation between the rates of *O*-deethylation and the level of CYP2D6 protein (Figure 1a; $r = 0.972$). As expected, the PM had no visible CYP2D6 protein band, yet there was a significant although low *O*-deethylation rate. The level of CYP3A4 protein in the PM was close to the average value of the group. The CYP3A4 levels were closely related to the *N*-demethylation rates (Figure 1b; $r = 0.969$) but did not correlate with the *O*-deethylation rates.

The CYP3A1 antibody (3 mg ml^{-1} incubate) inhibited the *N*-demethylation reaction by 46 and 20% in two preparations of liver microsomes. No inhibitory effect of this antibody on the *O*-deethylation reaction was observed. Indeed, there seemed to be an apparent stimulation of the reaction in some incubations.

Quinidine inhibited *O*-deethylation by 74 and 14% in the EM and PM sample, respectively; it did not affect the *N*-demethylation of ethylmorphine.

Table 1 Clinical features of the patients

Patient	*Sex*	*Age (years)*	*Smoker (Yes/No)*	*Disease*	*Drug treatment*	*CYP2D6 genotype*	*CYP2D6 phenotype*
1	F	60	No	Cholecystolithiasis	Ranitidine Sex hormone	wt/wt	EM
2	F	61	No	Cholecystolithiasis	Enalapril	wt/CYP2D6B	EM
3	F	49	No	Cholecystolithiasis	0	wt/wt	EM
4	F		?	Cholecystolithiasis		wt/wt	EM
5	F	44	No	Adipositas VBG	Glibenclamide Enalapril Medroxyprogesterone Terfenadine	wt/wt	EM
6	F	48	No	Cholecystolithiasis	Codeine Cephalosporin	wt/wt	EM
7	F	63	No	Cholecystolithiasis	0	wt/wt	EM
8	F	32	No	Cholecystolithiasis	0	wt/CYP2D6B	EM
9	F	39	No	Cholangiocarcinoma	0	CYP2D6B/ CYP2D6B‡	PM

*Abbreviations: EM = extensive metaboliser, PM = poor metaboliser, VGB = ventricular banded gastropexia.
‡This patient could be a heterozygous CYP2D6B/CYP2D6D, but this possibility could not be determined by our assay. No CYP2D6A alleles were identified.

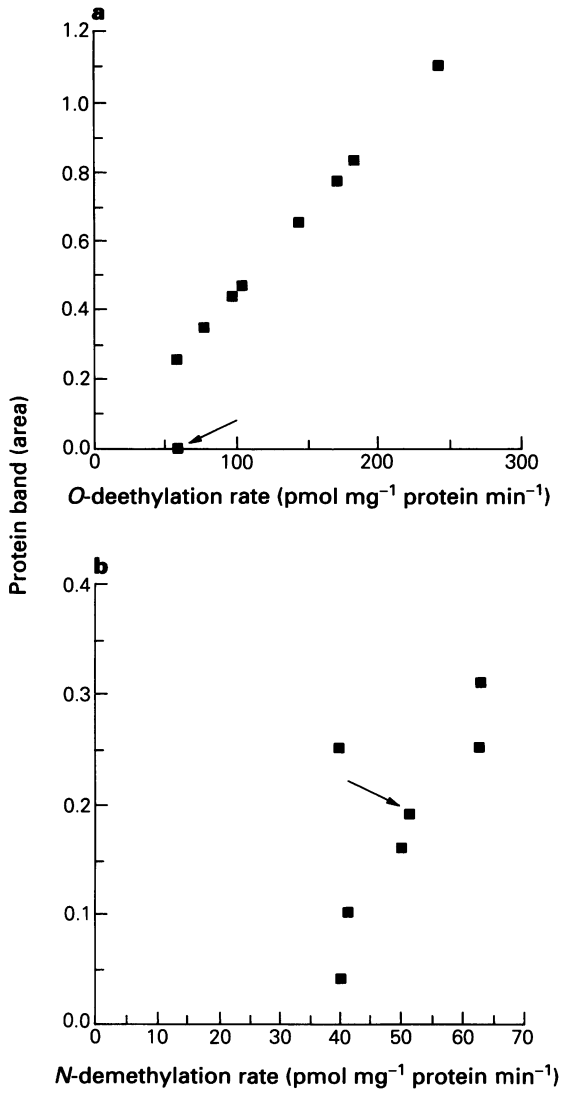

Figure 1 Relation between ethylmorphine metabolism activities and the areas of protein bands in Western blots. a) shows the *O*-deethylation rate *vs* immunoblotting band density of CYP2D6, and b) shows the *N*-demethylation rate *vs* the CYP3A4 band density. The data represent results from studies of liver microsomes from nine patients (one poor—PM and eight extensive—EM metabolisers of dextromethorphan). The PM is indicated in the figure by an arrow. The incubates contained 100 μg microsomal protein, an NADPH-regenerating system and ethylmorphine at a concentration of 1 mM.

Discussion

Ethylmorphine is a common ingredient in many cough remedies and has been widely used as a standard substrate in studies of drug metabolism in microsomal preparations. Our results demonstrate that *O*-deethylation of ethylmorphine is at least in part catalyzed by the polymorphic CYP2D6 enzyme which is also active in the metabolism of several clinically important groups of drugs such as β-adrenoceptor blocking agents, tricyclic antidepressants, many neuroleptics etc. The inter-individual variation of polymorphic *O*-deethylation was greater in the group of EMs than that of *N*-demethylation in the total panel. Our results indicate that another isoform is responsible for the low *O*-deethylation rate observed in the PM (indicated by arrow in Figure 1a).

The role of CYP2D6 in the *O*-deethylation of ethylmorphine was substantiated by demonstrating that the CYP2D6 inhibitor quinidine was a potent inhibitor in the EM sample but not in that from the PM. Similarly, Dayer *et al.* [11] were unable to find any inhibitory effect of quinidine on the *N*-demethylation of codeine.

The results also demonstrate the involvement of CYP3A4 in the *N*-demethylation of ethylmorphine. Figure 1b shows that another isoform must be involved in the reaction since a substantial metabolic rate was observed when no protein band was visible (regression line intersected the positive x-axis at zero density of the protein band, as read on the y-axis). These findings are consistent with our previous demonstration of a 66% inhibition of this reaction in rat microsomes [3] using an inhibitory monoclonal antibody against the rat cytochrome P4502c/RLM 5/ CYP2C11.

The *in vitro* results reported here support the conclusion of our previous studies in human healthy volunteers [5] that the *O*-deethylation and the *N*-demethylation of ethylmorphine are catalysed by different isoforms of cytochrome P450.

The expert technical assistance of Mrs Charlotte Thyr and Mrs Birgitta Ask is gratefully acknowledged. This work was supported by grants from the Swedish Medical Research Council (04X-04496) and the Swedish Cancer Society.

References

1 Ladona MG, Spalding DJM, Ekman L, Lindström B, Rane A. Human fetal and adult liver metabolism of ethylmorphine: relation to immunodetected cytochrome P-450 PCN and interactions with important fetal corticosteroids. *Biochem Pharmac* 1989; **38**: 3147–3155.

2 Elshourbagy NA, Guzelian PS. Separation, purification, and characterization of a novel form of hepatic cytochrome P-450 from rats treated with pregnenolone-16α-carbonitrile. *J biol Chem* 1980; **255**: 1279–1285.

3 Rane A, Ask B. A conspicuous down-regulating effect of morphine on essential steroid hydroxylation reactions and on certain drug *N*-demethylations. *J Steroid Biochem mol Biol* 1992; **41**: 91–98.

4 Xu BQ, Bjørnboe A, Ripel Å, Aasmundstad TA, Christophersen AS, Mørland J. Ethylmorphine metabolism in isolated rat hepatocytes. *Pharmac Tox* 1993; **73**: 35–40.

5 Rane A, Modiri AR, Gerdin E. Ethylmorphine *O*-deethylation co-segregates with the debrisoquine genetic metabolic polymorphism. *Clin Pharmac Ther* 1992; **52**: 257–264.

6 Chen ZR, Somogyi AA, Bochner F. Polymorphic O-demethylation of codeine. *Lancet* 1988; **ii**: 914–915.

7 Yue OY, Svensson JO, Alm C, Sjöqvist F, Säwe J. Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. *Br J clin Pharmac* 1989; **28**: 629–637.

8 Mortimer Ö, Persson K, Ladona MG, Spalding D, Zanger UM, Meyer UA and Rane A. Polymorphic formation of morphine from codeine in poor and extensive metabolisers of dextromethorphan. Relationship to the presence of immunoidentified cytochrome P-450IID1. *Clin Pharmac Ther* 1990; **47**: 27–35.

9 Forrester LM, Henderson CJ, Glancey MJ, Back DJ, Park BK, Ball SE, *et al.* Relative expression of cytochrome P450 isoenzymes in human liver and association with the metabolism of drugs and xenobiotics. *Biochem J* 1992; **281**: 359–368.

10 Smith CAD, Gough AC, Leigh PN, *et al.* Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. *Lancet* 1992; **339**: 1375–1377.

11 Dayer P, Desmeules J, Leemann T, Striberni R. Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation (cytochrome P-450 dbl/bufI). *Biochem Biophys Res Comm* 1988; **152**: 411–416.

(Received 7 March 1994, accepted 23 September 1995)