Metabolism of verapamil in a family pedigree with deficient *N*-oxidation of trimethylamine

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The oxidative *N*-dealkylation of verapamil has been studied in a family of five members with two probands with an inherited deficiency of trimethylamine *N*-oxidation (fish-odour syndrome). The results were assessed for possible co-segregation of the trimethylamine *N*-oxidation phenotype and any observed deficiency in oxidative *N*-dealkylation. The general pattern of metabolism of verapamil in the five subjects studied was similar to that reported in earlier investigations. Moreover, there were no differences between the two affected subjects and other family members with respect to the metabolic pattern. It is concluded that there is no functional segregation with respect to the mechanisms controlling trimethylamine *N*-oxidation and verapamil *N*-dealkylation.

Keywords verapamil trimethylamine *N*-oxidation Ziegler's enzyme polymorphism

Introduction

Racemic verapamil is a calcium channel antagonist widely used in the management of a variety of cardiovascular disorders (McTavish & Sorkin, 1989). In man oral doses of verapamil are readily absorbed from the gastrointestinal tract after which it undergoes extensive first-pass metabolism prior to renal excretion (Eichelbaum *et al.*, 1979; McIlbenny, 1971). Less than 5% of an oral dose is excreted unchanged. To date about 50% of an oral dose of the drug can be accounted for as identifiable metabolites (Eichelbaum *et al.*, 1979; Nelson & Olsen, 1988). The metabolic pattern is complex since the drug is metabolised along several pathways, namely, *N*-dealkylation, *O*-dealkylation and conjugation to generate an array of urinary metabolic products (Mikus *et al.*, 1990). The *N*-dealkylation reaction of verapamil appears to be the dominant pathway of metabolism for this drug. It has been suggested that unlike the *O*- and *N*-demethylation pathways which are probably mediated by cytochrome P-450 isozymes, the major *N*-dealkylation pathway may be catalyzed by a flavin-containing monooxygenase enzyme (Cashman 1989). This is based on the observation that flavin-containing monooxygenase activity can give rise to *N*-dealkylation products via Cope-type elimination reactions of initially formed tertiary amine *N*-oxides formed by this monooxygenase. Indeed, Cashman (1989) has adduced evidence that rat and hog liver microsomes metabolise verapamil by a FAD-containing monooxygenase and that furthermore the oxidation process is markedly stereoselective.

These observations on the possible role of a flavin-containing monooxygenase in the *N*-dealkylation of verapamil prompted us to study this phenomenon in a family pedigree with two probands with the fish-odour syndrome. The latter is congenital and inherited and arises from a transmitted defective ability to *N*-oxidise the simple aliphatic amine, trimethylamine (Al-Waiz *et al.*, 1987, 1988). The object of the study was to compare the *N*-dealkylation of verapamil in these two family members with that in other family members and, in particular, to assess any co-segregation of the trimethylamine *N*-oxidation deficiency with impaired *N*-dealkylation of verapamil. The defective *N*-oxidation syndrome appears to arise at the molecular level of the gene regulating one of the various forms of the flavin-containing monooxygenase.

Methods

Subjects

A single entire family of five members (Figure 1) in which two probands for the inherited deficiency of trimethylamine *N*-oxidation had previously been characterised was studied (Al-Waiz *et al.*, 1988). Both parents were defined as genotypically heterozygotes (carriers) for the metabolic deficiency while the third sibling was homozygous for normal *N*-oxidation status as revealed

Figure 1 Pedigree of subjects with a genetically determined deficiency of the *N*-oxidation of trimethylamine. It shows the percentage of the normal dietary load of trimethylamine (TMA) excreted as the *N*-oxide while values in parenthesis show the percentage of a single oral dose of verapamil (80 mg) excreted in the urine within 24 h as *N*-dealkylation products.

by trimethylamine challenge tests (Al-Waiz *et al.*, 1989). The protocol was approved by the St Mary's Hospital Ethics Committee and each volunteer gave their informed consent for the study. All participants had normal kidney and liver functions as assessed by appropriate laboratory tests. Electrocardiograms revealed no physiological abnormalities.

Following an overnight fast, each volunteer received a single oral dose of racemic verapamil hydrochloride (80 mg) and no food was allowed for 2 h. None of the participants was receiving other medications during the study. The five volunteers were monitored clinically for up to 8 h after dosing. No effects attributable to the drug treatment were recorded. Urine was collected serially from each subject at 0–3, 3–6, 6–9, 9–12, 12–15 and 15–24 h after dosing. The urine volumes were measured and aliquots (40 ml) from each time period was stored at -20° until analysis which was completed within 2 months of the drug administration.

Analysis

Trimethylamine and trimethylamine *N*-oxide were measured in the 0–8 h control urine samples for the five subjects as described before (Al-Waiz *et al.*, 1988). Urine samples were also analyzed by high-performance liquid chromatography for unchanged verapamil and the following metabolic products (Mikus *et al.*, 1990): norverapamil; the major metabolite produced by *N*-dealkylation, 2,8-bis-(3,4-dimethoxyphenyl)-2-isopropyl-6-azaocitanitrile (D-617); 5-amino-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (D-620): formed by *N*-dealkylation and *N*-demethylation; 2-(4-hydroxy-3-methoxyphenyl)-5-methylamino-2-isopropylvaleronitrile (D-717): formed by *N*-dealkylation and *O*-demethylation; 2-(4-hydroxy-3-methoxyphenyl)-8-(3,4-dimethoxyphenyl)-2-isopropyl-6-azaocitanitrile (D-715) which is formed from norverapamil by *O*-demethylation and finally 2-(3,4-dimethoxyphenyl)-8-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-isopropyl-6-azaocitanitrile formed by *O*-demethylation of verapamil (D-703).

Results

Table 1 shows the urinary recoveries of racemic verapamil and its metabolites following the oral administration of a single dose (80 mg) to each member of a family, two of which are propositi for the inherited deficiency of *N*-oxidation (the fish-odour syndrome). Little unchanged drug was excreted (mean 0.53% dose). The major urinary metabolite (mean 12.4%) was D-617 which arises from the *N*-dealkylation of verapamil. Metabolites D-620 and D-717 are further biotransformation products of D-617 and accounted for mean values of 4.1 and 2.4% dose, respectively. The product of the *N*-demethylation of verapamil, namely, norverapamil, was excreted in small

Table 1 Urinary recoveries of metabolites of verapamil following its oral administration (80 mg) to a family with the fish-odour syndrome

Family subject	Urinary metabolites (% dose)								Total excretion
	Verapamil	Norverapamil	D-617	D-620	D-717	D-617 D-620 D-717	D-703	D-715	
1 Mother* (58 years)	0.25	1.10	11.0	2.8	2.3	16.1	1.46	1.3	20.40
2 Father+ (66 years)	1.50	2.04	7.3	2.7	2.6	12.6	2.70	1.7	20.50
3 Unaffected daughter (28 years)	0.30	1.80	14.4	5.2	2.6	22.2	2.00	1.2	27.5
4 Affected daughter I (25 years)	0.40	1.0	15.4	3.1	1.4	19.9	1.6	0.8	23.80
5 Affected daughter II (22 years)	0.20	1.46	14.0	6.5	1.3	21.9	2.2	1.5	27.25

* Smoked 20 cigarettes day⁻¹; + Occasional recourse to a Ventolin inhaler and aminophylline (single slow release, daily dose 300 mg).

amounts with a mean value of 1.5% of dose. Two other metabolites which were detected and measured were D-703 which arises from *O*-demethylation and D-715 which is formed from the *O*-demethylation of nor-verapamil. The mean values for the excretion of these two metabolites were 0.09 and 1.3% dose, respectively.

Discussion

The pattern of metabolism of verapamil in the five subjects included in this study was similar to that reported in previous studies except that both total urinary recoveries and that for the metabolites was somewhat lower (Eichelbaum *et al.*, 1979; Nelson & Olsen, 1988). There is no obvious reason to explain this discrepancy. Scrutiny of the results for each metabolite for each member of the family reveal no consistent differences. With particular respect to metabolite D-617, which is produced by *N*-dealkylation, there are no obvious inter-subject differences. Of particular significance are daughters I and II, both of whom have a well-characterized deficiency for the *N*-oxidation of trimethylamine and in whom the *N*-dealkylation of verapamil was unaffected. If the recoveries of the three metabolites produced by *N*-dealkylation (D-617 + D-620 + D-717) are summed there are still no obvious differences between the two affected daughters and the other three family members.

It would appear from these findings that the *N*-dealkylation of verapamil and the *N*-oxidation of trimethylamine are under separate genetic regulation. This is in contrast to data obtained for nicotine *N*-oxidation where a co-segregation with the TMA oxidation pathway is suggested (Ayesh *et al.*, 1988). However, there is no co-segregation between the *N*-oxidation of the antihypertensive drug pinacidil and TMA *N*-oxidation (Ayesh *et al.*, 1989).

The apparent lack of co-segregation of the *N*-dealkyla-

tion of verapamil with that of trimethylamine *N*-oxidation bears comment as it could have several explanations. Firstly, the *N*-dealkylation of verapamil in man may be mediated by a form of the flavin-containing monooxygenase which is different to that responsible for TMA oxidation. There is evidence of the occurrence of multiple forms of the FAD monooxygenases with different tissue distributions, substrate specificities and genetic regulation (Hlavica *et al.*, 1990; Ziegler, 1990). Secondly, Ziegler (1990) has pointed out that a genetic defect of the FMO enzyme would primarily affect the *N*-oxidation of substrates of low affinity and relatively high K_m values such as trimethylamine. Thus, Ziegler (1988) has suggested that a 5–10 fold change in activity of FMO would produce only minor changes in the metabolic profiles of high affinity, low K_m substrates. Verapamil is a relatively good substrate for the FMO enzyme with a K_m value similar to that of other tertiary amines with high affinity for this enzyme. If Ziegler's arguments are correct it follows that the *N*-dealkylation of verapamil may not necessarily be defective in trimethylaminuric subjects if adequate functional enzymic activity is present. Yet a third explanation is that, unlike in rat and pig where there is strong evidence of the *N*-dealkylation of verapamil being mediated by an FMO enzyme, in man this may be catalyzed by a cytochrome P-450 isoenzyme. That this may be at least partly the case, is suggested by the observation that the *N*-dealkylation of verapamil *in vitro* is inhibited by antibodies against the P-450 IIIA family (Eichelbaum & Kroemer, 1990, unpublished observations).

We conclude that in the single trimethylaminuric family studied there is no functional segregation in the affected subjects with the deficiency of trimethylamine *N*-oxidation. While a role for the FMO enzyme system cannot be ruled out on the basis of this study the *N*-dealkylation of verapamil in man may be mediated, at least in part, by isoenzymes of the P-450 system.

References

- Al-Waiz, M., Ayesh, R., Mitchell, S. C., Idle, J. R. & Smith, R. L. (1987). A genetic polymorphism of the *N*-oxidation of trimethylamine in humans. *Clin. Pharmacol. Ther.*, **42**, 588–594.
- Al-Waiz, M., Ayesh, R., Mitchell, S. C., Idle, J. R. & Smith, R. L. (1988). Trimethylaminuria (fish-odour syndrome) a study of an affected family. *Clin. Sci.*, **74**, 231–236.
- Al-Waiz, M., Ayesh, R., Mitchell, S. C., Idle, J. R. & Smith, R. L. (1989). Trimethylaminuria: the detection of carriers using a trimethylamine load test. *J. Inher. Metab. Dis.*, **12**, 80–85.
- Ayesh, R., Al-Waiz, M., Crother, J., Cholerton, S., Mitchell, S. C., Idle, J. R. & Smith, R. L. (1988). Deficient nicotine *N*-oxidation in two sisters with trimethylaminuria. *Br. J. clin. Pharmacol.*, **25**, 664.
- Ayesh, R., Al-Waiz, M., McBurney, A., Mitchell, S. C., Idle, J. R., Ward, J. W. & Smith, R. L. (1989). Variable metabolism of pinacidil: lack of correlation with the debrisoquine and trimethylamine C and *N*-oxidative polymorphisms. *Br. J. clin. Pharmacol.*, **27**, 423–428.
- Cashman, J. R. (1989). Enantioselective *N*-oxygenation of verapamil by hepatic flavin-containing monooxygenase. *Mol. Pharmacol.*, **36**, 497–503.
- Eichelbaum, M., Ende, M., Remberg, G., Schomerus, M. & Dengler, H. (1979). The metabolism of DL-[¹⁴C] verapamil in man. *Drug Metab. Dispos.*, **7**, 145–148.
- Hlavica, P., Kellermann, J., Henschen, A., Mann, K. H. & Künzel-Mulas, U. (1990). Evidence of the existence of structurally distinct hepatic and pulmonary forms of microsomal flavin-containing monooxygenase in the rabbit. *Biol. Chem.*, **371**, 521–526.
- McIlbenny, H. M. (1971). Metabolism of [¹⁴C] verapamil. *J. med. Chem.*, **14**, 1178–1184.
- McTavish, D. & Sorkin, E. M. (1989). Verapamil: an updated review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in hypertension. *Drugs*, **38**, 19–76.
- Mikus, G., Eichelbaum, M., Fischer, C., Gumulka, S., Klotz, U. & Kroemer, H. K. (1990). Interaction of verapamil and cimetidine, stereochemical aspects of drug metabolism, drug disposition and drug action. *J. Pharmacol. exp. Ther.*, **253**, 1042–1048.

- Nelson, W. L. & Olsen, L. D. (1988). Regiochemistry and enantioselectivity in the oxidative *N*-dealkylation of verapamil. *Drug Metab. Dispos.*, **16**, 834–841.
- Ziegler, D. M. (1988). Flavin-containing monooxygenases: catalytic mechanism and substrate specificities. *Drug Metab. Rev.*, **19**, 1–32.

- Ziegler, D. M. (1990). Flavin-containing monooxygenase enzymes adapted for multisubstrate specificity. *Trends pharmac. Sci.*, **11**, 321–324.

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