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Inhibition of tolbutamide metabolism by substituted imidazole drugs *in vivo*: evidence for a structure-activity relationship

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1 Tolbutamide has been used as a model drug for an examination of the effects of eleven substituted imidazole compounds on hepatic metabolism *in vivo*.

2 The 1-substituted compounds 1-methylimidazole, miconazole, clotrimazole and ketoconazole produced marked alterations in tolbutamide kinetics (increased half-life, decreased clearance). However, if there was substitution in the 2- position, irrespective of a substituent on N-1, then the compound did not appear to inhibit metabolism (e.g. 2-methylimidazole, 1,2-dimethylimidazole, methimazole, metronidazole). The 4- substituted compounds, 4-methylimidazole and cimetidine were inhibitors.

3 A structure-activity relationship for the inhibitory actions of the substituted imidazoles is thus evident *in vivo*.

Introduction

Certain substituted imidazoles have been shown to be potent inhibitors of microsomal mono-oxygenase activity, producing Type II spectral changes with cytochorome P-450 (Wilkinson *et al.*, 1972; Mailman *et al.*, 1974). Wilkinson *et al.* (1974a,b) have demonstrated that some 4(5)- and 1-substituted aryl imidazoles bind to cytochrome P-450 with a very high affinity but substitution in the 2-position of the imidazole decreases the binding affinity by approximately 2 orders of magnitude. The 1- and 4(5)-substituted compounds are therefore among the most potent *in vitro* inhibitors of cytochrome P-450 mediated drug oxidations (Wilkinson *et al.*, 1983); *in vivo* they prolong barbiturate sleeping time (Wilkinson *et al.*, 1972; Leibman & Oritz, 1973; Palmer & Cawthorne, 1974). Structure-activity studies have clearly shown that both binding and inhibition are dependent on the presence of a sterically unhindered nitrogen atom at the 3-position of the imidazole ring (Rogerson *et al.*, 1977). These findings support the proposal (Wilkinson *et al.*, 1972) that inhibition results primarily from coordination of the non-bonded electrons at N-3 with the fifth or sixth ligand of the heme iron of cytochrome P-450.

Although to date many studies have involved series of non-therapeutic compounds, there are numerous widely used drugs which are imidazole derivatives. Cimetidine, a 4(5)-substituted imidazole, is a well proven inhibitor of microsomal oxidation. Thus it has been shown to inhibit the metabolism of aminopyrine and benzpyrene (Pelkonen & Puurunen, 1980), ethoxycoumarin (Rendic *et al.*, 1979), hexobarbitone (Puurunen & Pelkonen, 1979), warfarin, phenobarbitone and zoxazolamine (Serlin *et al.*, 1980) and acetaminophen (Galinsky & Levy, 1982). The antimycotic drugs, clotrimazole, econazole, miconazole and ketoconazole (all 1- substituted imidazoles) have also been found to inhibit certain mono-oxygenase activities *in vitro* (Kahl *et al.*, 1980; Drew & Scott, 1983) and *in vivo* (Niemegeers *et al.*, 1981; Drew & Scott, 1983).

In the present study we have examined the effect of various imidazoles (Figure 1) on the pharmacokinetics of tolbutamide in the rat with the aim of demonstrating that alterations in the *in vivo* metabolism and kinetics of this drug are related to particular structural features of the imidazole. To date, few studies with imidazoles have related inhibition to plasma concentrations of a substrate drug. Tolbutamide is a particularly useful model drug since it has a single pathway of metabolism (to hydroxytolbutamide) and there is a rapid high performance liquid chromatography (h.p.l.c.) method for the measurement of both parent drug and metabolite (Back *et al.*, 1984).

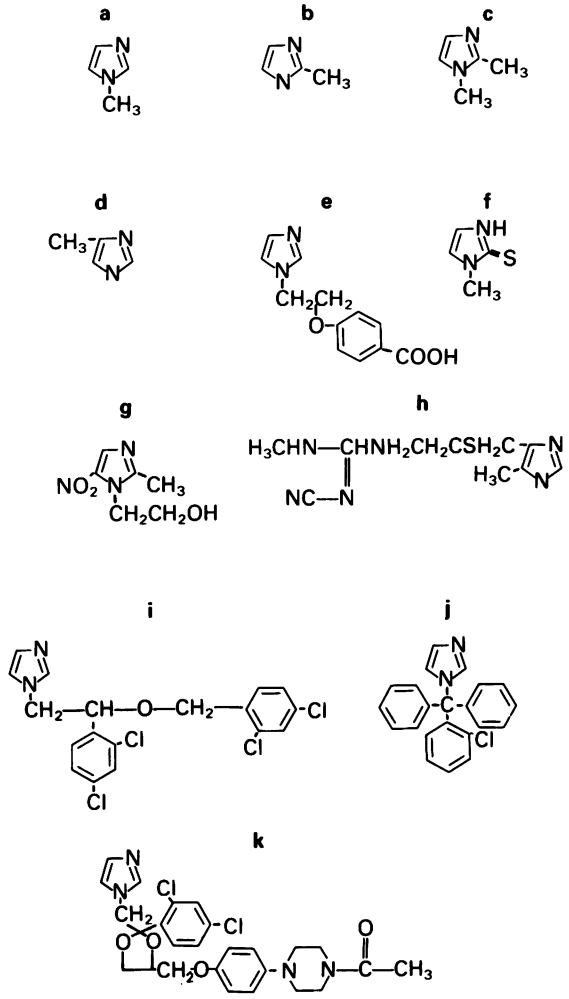

Figure 1 The structures of the substituted imidozoles used in this study. (a) 1-Methylimidazole, (b) 2-methylimidazole, (c) 1,2-dimethylimidazole, (d) 4-methylimidazole (e) dazoxiben, (f) methimazole, (g) metronidazole, (h) cimetidine, (i) econazole, (j) clotrimazole, (k) ketoconazole.

Methods

Animals

Adult male rats of the Wistar strain, weighing 250–350 g were housed in well ventilated cages and kept at a temperature of approximately 24°C. They were allowed to feed *ad libitum* on pelleted food (Oxoid breeding diet, Oxoid Ltd, London) and tap water.

Tolbutamide elimination

Rats were anaesthetized with pentobarbitone sodium (Sagatal, M & B Ltd; 60 mg kg^{-1}, 0.1 ml 100 g^{-1} body weight) and remained anaesthetized for the duration of the study. The femoral artery and femoral vein were cannulated with polyethylene tubing (PE50). Heparinized saline (200 units) was injected to prevent coagulation of the blood samples collected from the artery.

Rats were divided into groups of five rats per group. There were three control groups injected with the different vehicles (0.9% w/v NaCl solution (saline), dimethylsulphoxide (DMSO) and 0.07 N HCl) used to obtain solutions of the compounds studied as follows: 1-methylimidazole (1-MI), 2-methylimidazole (2-MI), 4-methylimidazole (4-MI), 1,2-dimethylimidazole (1,2-DiMI), dazoxiben and methimazole (50 mg kg^{-1}; 25 mg ml^{-1} in saline); metronidazole, miconazole and clotrimazole (50 mg kg^{-1}; 25 mg ml^{-1} in DMSO); cimetidine and ketoconazole (50 mg kg^{-1}; 25 mg ml^{-1} in 0.07 N HCl). All the above compounds were injected intraperitoneally (i.p.) 30 min before the administration of tolbutamide (50 mg kg^{-1}) into the peripheral vein. Blood samples (0.3 ml) were collected at 0 (blank), 15, 30, 60, 90, 120, 180, 240, 300 and 360 min and after each collection the blood taken was replaced by 0.3 ml saline. These blood samples were centrifuged for 2 min at 12,000 *g* and the plasma stored at − 20°C until analyzed by h.p.l.c.

Assay of tolbutamide and hydroxytolbutamide

Plasma tolbutamide and hydroxytolbutamide were measured by h.p.l.c. as described previously (Back *et al.*, 1984). In brief, blank rat plasma (100 µl) was spiked with known amounts of tolbutamide (5–60 µg) and hydroxytolbutamide (5–52.5 µg). The internal standard (I.S., chlorpropamide, 25 µl of 1 mg ml^{-1} solution) was added followed by methanol (125 µl). The contents were vortexed for a few seconds and then centrifuged for 10 min at 2,000 *g*; 20 µl of supernatant was injected onto the column (Partisil 10/25 ODS-2; 0.46 cm i.d. × 25 cm; Whatman). The mobile phase was methanol:0.05% phosphoric acid, 50:50, v/v. The flow rate was 1.6 ml min^{-1}. The ratio of the peak height of tolbutamide or hydroxytolbutamide to I.S. was plotted against the concentration of each compound to give standard curves.

Samples of plasma (100 µl) from rats injected with tolbutamide were pipetted into pyrex tubes (75 × 12 mm), I.S. and methanolol added and analyzed as described above.

The coefficients of variation for measurement of tolbutamide and metabolite have been found previously (Back *et al.*, 1984).

Pharmacokinetic analysis

All data were tested for statistical significance using Student's non-paired t test. Values are expressed as mean ± s.d.

Tolbutamide half-life ($t_{\frac{1}{2}}$) was calculated from the elimination rate constant (k) obtained by least squares regression analysis of plasma drug concentrations. The apparent volume of distribution (Vd) was calculated by dividing the dose by the plasma concentration at zero time (C_{p0}).

Tolbutamide clearance (Cl_{tol}) was calculated from:

$$(Cl_{tol}) = \frac{0.693 \times Vd}{t_{\frac{1}{2}}}$$

The area under the curve (AUC_{0-t}) was calculated by the trapezoidal rule using a Hewlett-Packard programmable calculator. The area under the curve to infinity was calculated from:

$$AUC_{0-\infty} = \frac{C_{p0}}{k}$$

Drugs and chemicals

Drugs and chemicals were obtained as follows: tolbutamide and hydroxytolbutamide (Hoechst), chlorpropamide, 1-methylimidazole, 2-methylimidazole, 1,2-deimethylimidazole, methimazole, metronidazole, miconazole and clotrimazole (Sigma), 4-methylimidazole (Aldrich), dazoxiben (Pfizer), cimetidine (Smith, Kline & French) and ketoconazole (Janssen). Solvents were of h.p.l.c. grade and obtained from Fisons.

Results

The pharmacokinetic data are summarized in Table 1. Only compounds substituted in the 1- position produced significant prolongation of tolbutamide half-life, with the exception of dazoxiben. Thus, 1-methylimidazole miconazole, clotrimazole and ketoconazole increased the half-life of tolbutamide by 70, 54, 68 and 100% respectively when compared to controls. The area under the curve (AUC) was increased and plasma clearance decreased by each of these drugs. With 1-methylimidazole and clotrimazole there was a small change in apparent volume of distribution although the reason for this remains unclear. The kinetics of tolbutamide following dazoxiben were not significantly different from control.

When a compound was substituted in the 2- position (i.e. 2-methylimidazole, 1,2-dimethylimidazole, methimazole and metronidazole) there was no evidence of altered kinetics irrespective of additional substitution at N-1 (i.e. 1,2-dimethylimidazole and metronidazole). Compounds substituted at the 4- position, 4-methylimidazole and cimetidine (also substituted at 5- position) gave rise to significant increases in the half-life of tolbutamide (by 124 and 42% respectively) and the AUC (136 and 44%). However, they caused no change in the volume of distribution.

Table 1 Effect of acute administration of various imidazole compounds (50 mg kg^{-1}) on plasma tolbutamide pharmacokinetic parameters

Treatment	*Position of substituents*	$t_{\frac{1}{2}}$ (h)	*Vd* (ml kg^{-1})	$AUC_{0-\infty}$ (mg ml^{-1} min)	Cl_{tol} (ml min^{-1} kg^{-1})
Control (saline)		3.10 ± 0.48	184.7 ± 15.8	72.4 ± 6.2	0.70 ± 0.06
1-Methylimidazole	1	5.26 ± 0.93**	161.5 ± 8.4	141.4 ± 25.6**	0.36 ± 0.06***
2-Methylimidazole	2	3.67 ± 0.37	198.2 ± 11.5	80.2 ± 8.0	0.63 ± 0.06
4-Methylimidazole	4	6.93 ± 1.50**	176.1 ± 3.1	170.3 ± 34.7	0.30 ± 0.06***
1,2-Dimethylimidazole	1,2	3.58 ± 0.24	196.8 ± 8.3	79.0 ± 4.3	0.64 ± 0.04
Dazoxiben	1	3.34 ± 0.24	203.6 ± 23.8	72.6 ± 6.2	0.69 ± 0.06
Methimazole	1,2	3.35 ± 0.17	181.5 ± 9.6	80.1 ± 7.8	0.63 ± 0.06
Control (DMSO)		3.83 ± 0.58	209.2 ± 18.0	75.2 ± 10.9	0.68 ± 0.10
Metronidazole	1,2,5	3.92 ± 0.33	190.9 ± 10.1	89.1 ± 10.4	0.57 ± 0.07
Miconazole	1	5.90 ± 0.79**	194.5 ± 8.9	131.8 ± 21.3***	0.39 ± 0.02***
Clotrimazole	1	6.44 ± 0.45	182.4 ± 13.2*	153.7 ± 11.8***	0.33 ± 0.02***
Control (0.07 N HCl)		3.18 ± 0.31	189.9 ± 11.2	72.6 ± 6.4	0.69 ± 0.03
Cimetidine	4,5	4.53 ± 0.45***	188.2 ± 17.5	104.3 ± 8.3***	0.48 ± 0.04***
Ketoconazole	1	6.37 ± 0.49***	170.6 ± 9.4	161.6 ± 9.1***	0.31 ± 0.01***

Results are means ± s.d. ($n = 5$). Abbreviations: $t_{\frac{1}{2}}$, half-life of tolbutamide; Vd, apparent volume of distribution; $AUC_{0-\infty}$, area under the curve to infinity; Cl_{tol}, plasma clearance of tolbutamide.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$, significantly different from controls.

Table 2 Effect of acute administration of various imidazole compounds (50 mg kg^{-1}) on the ratio of the areas under the plasma tolbutamide (tol) and hydroxytolbutamide (OHtol) concentration time curves (AUC_{tol}/AUC_{OHtol}) for each drug treatment for the time period 0–3 h

Treatment	AUC_{tol}/AUC_{OHtol}
Control	25.3 ± 8.1
1-Methylimidazole	63.0 ± 10.5***
1,2-Dimethylimidazole	23.7 ± 2.7
2-Methylimidazole	35.3 ± 3.3*
4-Methylimidazole	> 100†
Dazoxiben	33.3 ± 5.7
Methimazole	31.4 ± 2.3
Metronidazole	26.7 ± 3.3
Miconazole	45.3 ± 10.7**
Clotrimazole	75.0 ± 32.0***
Cimetidine	37.7 ± 6.8*
Ketoconazole	96.6 ± 23.4***

Results are means ± s.d. ($n = 4$ or 5). There were no significant differences between the ratios obtained in the 3 control groups. Saline controls are shown in the Table.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, significantly different from controls.
† In 2 rats in this group hydroxytolbutamide was not detected.

Table 2 gives the ratios of the area under the plasma tolbutamide (tol) and hydroxytolbutamide (OHtol) concentration time curves (AUC_{tol}/AUC_{OHtol}; 0–3 h) for each drug administered. Significant increases in the ratio were seen with 1-MI, 2-MI, 4-MI, miconazole, clotrimazole, cimetidine and ketoconazole. It is difficult to explain the increase with 2-MI since there were no significant changes in any of the other kinetic parameters measured (Table 1).

Discussion

All the 1-substituted compounds with the exception of dazoxiben significantly inhibited the metabolism of the co-administered drug tolbutamide. The three antimycotic drugs were comparable in the degree of inhibition they produced (as judged by the increase in half-life, decrease in clearance and appearance of metabolite). It should be noted that the appearance of metabolite and the validity of using an increase in the ratio AUC_{tol}/AUC_{OHtol} to imply inhibition of metabolism will be dependent upon the renal clearances of drug and metabolite being unchaged by the pretreatments.

Dazoxiben is a thromboxane synthetase inhibitor (Tyler, 1983), there being good evidence (Ullrich & Graf, 1984) that thromboxane A_2 (TXA_2) synthetase is a P-450 protein. The reason why dazoxiben did not apparently inhibit tolbutamide hydroxylation is open to speculation. It is possible that absorption is erratic after an i.p. injection. However, it is more likely that dazoxiben only inhibits certain forms of cytochrome P-450 which are distinct from the protein responsible for tolbutamide oxidation. There is evidence that the ability of a particular concentration of an imidazole derivative to inhibit cytochrome P-450-dependent mono-oxygenase activites depends on the reaction catalyzed (Little & Ryan, 1982). Alternatively, access to the enzyme may be limited because of the physicochemical properties of the drug.

In agreement with previous *in vitro* studies (Wilkinson *et al.*, 1974), all the compounds possessing a substituent at the 2- position failed to cause significant inhibition. Thus irrespective of substitution at 1- (i.e. 1,2-diMI, methimazole and metronidazole), the presence of an additional group at 2- prevents the compound acting as an inhibitor. Therefore, this study failed to show metronidazole as an inhibitor of drug oxidation despite the finding that it inhibits warfarin metabolism in man (O'Reilly, 1976). More recently, Staiger *et al.* (1984) found that metronidazole had no effect on antipyrine metabolism in man. It is difficult to see, given our understanding of the necessity for an unhindered N-3 (Rogerson *et al.*, 1977), how metronidazole could produce any appreciable degree of inhibition.

Drew & Scott (1983) have indicated that the antimycotic imidazoles are approximately 3,000–10,000 times more potent *in vitro* inhibitors of mixed function oxidases than cimetidine. Such a finding cannot quantitatively be extrapolated to the *in vivo* situation. However, it is interesting that in the present study the degree of inhibition of tolbutamide metabolism produced by cimetidine was considerably less than that produced by the 1-substituted compounds.

There are obvious pitfalls in extrapolating findings *in vitro* to the *in vivo* situation and from rat to man and therefore we need to be cautious in making statements which may be too general. Of particular relevance is that considerable metabolism of the imidazoles may occur during the experimental period so that in effect it is not only the parent drug which comes under the spotlight but metabolites as well. Also it could be argued that the present data can only be applied to tolbutamide metabolism in rats, especially since the identity of other substrates for the form(s) of cytochrome P-450 catalyzing the hydroxylation is unknown. Hence, we reiterate a comment made previously (Back *et al.*, 1984), that tolbutamide could be a useful test drug for investigation of pharmacokinetic interactions when used in conjunction with other substrates such as antipyrine.

It is unlikely that displacement of tolbutamide from protein binding was of major significance in the interactions studied. The volume of distribution did not increase (which would be indicative of an increase in free fraction) in the presence of any of the co-administered drugs. Sugita *et al.* (1984) have demonstrated that the effects of sulphaphenazole on the kinetics of tolbutamide are very different in the rat and rabbit. In the rat, sulphaphenazole gives rise to a prolongation of tolbutamide half life due to a marked inhibitory effect on metabolism and minimal effects on protein binding. In contrast, in the rabbit, displacement of tolbutamide from binding is important and leads to an increase in free fraction (with a concomitant increase in apparent volume of distribution) and consequently an increase in metabolism (since tolbutamide is a capacity limited drug); this counteracts the inhibitory effects of sulphaphenazole which are evident *in vitro*.

In conclusion, the present results provide some further evidence of the structure-activity relationship which exists for the inhibition produced by the substituted imidazoles.

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