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Tolbutamide as a model drug for the study of enzyme induction and enzyme inhibition in the rat

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1 The effects of various drugs on the pharmacokinetics of tolbutamide have been examined in the rat.

2 Phenobarbitone pretreatment caused a significant decrease in half life and area under the curve (AUC) and a significant increase in clearance and volume of distribution (Vd).

3 Acute administration of primaquine significantly increased half life and AUC and decreased clearance. In contrast, the related animoquinolone chloroquine, was without effect.

4 Acute administration of cimetidine produced similar changes to primaquine but of lesser magnitude.

5 Formation of the major metabolite hydroxytolbutamide, was markedly enhanced by phenobarbitone and reduced by primaquine and cimetidine.

6 We conclude that due to its single pathway of metabolism, tolbutamide is a good substrate to use when examining pharmacokinetic interactions involving hepatic enzyme induction and inhibition.

Introduction

The metabolism of tolbutamide (1-butyl-3-p-tolylsulphonylurea, I, Figure 1) has been extensively studied. In the rat, 80% of an orally administered dose of tolbutamide is excreted in urine predominantly as hydroxytolbutamide (1-butyl-3-p-hydroxymethylphenylsulphonylurea, II). In man, however, carboxytolbutamide (1-butyl-3-p-carboxyphenylsulphonylurea, III) is present in urine to a greater extent than hydroxytolbutamide (Thomas & Ikeda, 1966). Tagg *et al.* (1967), have shown that tolbutamide is converted in the liver to hydroxytolbutamide by NADPH-linked microsomal enzymes. The oxidation pathway appears to be almost quantitative in the rat and man (Thomas & Ikeda, 1966; Rowland & Matin, 1973). Hydroxytolbutamide is further metabolised to carboxytolbutamide, presumably via tolbutamide aldehyde, by enzymes in the cytoplasmic soluble fraction (Hansen & Christensen, 1977). It can therefore be seen that oxidation of tolbutamide to hydroxytolbutamide is the rate limiting step in elimination of the drug and its metabolites (Rowland & Matin, 1973; Hansen & Christensen, 1977).

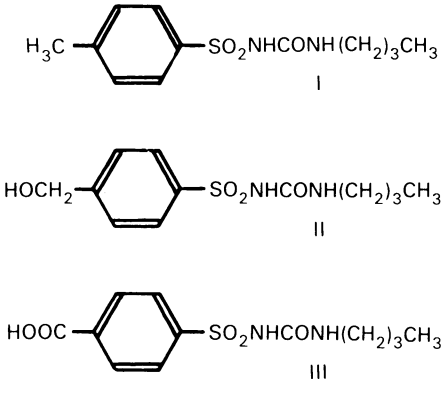

Figure 1 Tolbutamide (I) and metabolites (II and III).

As a result of hypoglycaemic crises in patients stabilized on tolbutamide who have then received additional drug therapy e.g. sulphaphenazole, dicoumarol, phenylbutazone and chloramphenicol (Hansen & Christensen, 1977), drug interactions with tolbutamide have received extensive study. The best studied interaction is that between the sulphonamide, sulphaphenazole and tolbutamide. Sulphaphenazole is reported to increase the half life of tolbutamide in man by up to 8 fold (Christensen *et al.*, 1963; Schulz & Schmidt, 1970; Pond *et al.*, 1977) and in rats by 3 fold (Sugita *et al.*, 1981).

In the present study we have examined the effect of

various drugs on the pharmacokinetics of tolbutamide in the rat with the aim of demonstrating that this compound could be a good marker for assessing drug interactions *in vivo* both qualitatively and quantitatively. The main advantage of tolbutamide compared to antipyrine is that the latter, which is often used in drug interaction studies *in vivo* (Danhof *et al.*, 1979; Back *et al.*, 1983) has at least three major routes of metabolism (Brodie & Axelrod, 1959; Yoshimura *et al.*, 1968; Baty & Price-Evans, 1973; Danhof *et al.*, 1979) which are catalysed by different forms of cytochrome P450 (Huffman *et al.*, 1973; Boobis *et al.*, 1981; Dunhof *et al.*, 1982) and hence perturbation of one pathway may not necessarily give rise to an overall pharmacokinetic change. Such a problem does not arise with tolbutamide since if a drug either induces or inhibits tolbutamide hydroxylation there will be a change in overall clearance.

Methods

Animals

Adult male rats of the Wistar strain, weighing 250–400g 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). The carotid artery and femoral or jugular vein were cannulated with polyethylene tubing (PE50). Heparanised saline (200 units) was injected to prevent coagulation of the blood samples collected from the carotid artery.

Phenobarbitone was administered twice daily for 4 days at a dose of 40 mg kg^{-1} and the study performed on the sixth day.

Cimetidine, chloroquine and primaquine (50 mg kg^{-1}; 25 mg ml^{-1} in physiological saline) were injected intraperitoneally (i.p.) 30 min before the administration of tolbutamide (50 mg kg^{-1}; 50 mg ml^{-1}) into the peripheral vein. Blood samples were collected at 0, 15, 30, 60, 90, 120, 180, 240, and 300 min. After each sample was taken, 0.3 ml saline was returned to the blood system. Blood samples were centrifuged for 3 min and the plasma stored deep frozen for analysis by high performance liquid chromatography (h.p.l.c.).

Urinary excretion

Eighteen rats were individually housed in metabolism cages. The rats were placed in the metabowls 24 h before the start of the study to allow them to adapt to their new surroundings. Six rats were controls and groups of 4 rats were either pretreated with phenobarbitone (40 mg kg^{-1} twice daily for 4 days) or given cimetidine or primaquine (50 mg kg^{-1}). Tolbutamide (50 mg kg^{-1}) was injected i.p. and urine collected at intervals for 24 h. After determination of the volume, aliquots were taken and stored deep frozen for analysis by h.p.l.c.

Assay of tolbutamide and hydroxytolbutamide

Plasma tolbutamide and hydroxytolbutamide and urinary hydroxytolbutamide were measured by h.p.l.c. substantially according to the method of Nation *et al.*, (1978). A model 110A pump (Altex) linked to a model 110–10 spectrophotometer (Hitachi) monitoring at 230 nm was used. Separations were performed at room temperature on a Partisil 10/25 ODS–2 (0.46 cm i.d. × 25 cm; Whatman) protected by an in-line guard column (Whatman column survival kit) packed with Co : Pell ODS. The mobile phase used was methanol : 0.05% phosphoric acid in proportions 50 : 50 by volume. The flow rate was 1.6 ml min^{-1}.

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 2000 g; 20 μl of supernatant was injected onto the column. The ratio of the peak height of tolbutamide or hydroxytolbutamide to I.S was plotted against the concentration of each compound to provide standard curves. A similar procedure was adopted for urine.

Samples of plasma or urine (100 μl) from rats injected with tolbutamide were pipetted into pyrex tubes (75 × 12 mm), followed by the addition of I.S and methanol. Samples were then analysed as described above.

The coefficient of variation for tolbutamide was 3.5% at a concentration of 247 μg ml^{-1} and for hydroxytolbutamide 3.0% at a concentration of 54 μg ml^{-1}.

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 (Cp_0).

Tolbutamide clearance was calculated from

$$Clp = \frac{0.693 \times Vd}{t_{\frac{1}{2}}}$$

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

Drugs and chemicals

Drugs and chemicals used were: tolbutamide and hydroxytolbutamide (Hoechst), chlorpropamide, primaquine diphosphate and chloroquine diphosphate (all Sigma). Cimetidine was obtained from Smith Kline & French and phenobarbitone from BDH.

Results

Pretreatment of rats with phenobarbitone caused a significant decrease in tolbutamide half life from 128.6 ± 25.1 to 86.3 ± 9.4 min (mean ± s.d.), a significant decrease in AUC from 513 ± 34 to 307 ± 47 $\mu g\,ml^{-1}\,h^{-1}$, a significant increase in clearance from 1.21 ± 0.18 to 2.09 ± 0.22 $ml\,min^{-1}$ and a significant increase in volume of distribution from 219.7 ± 11.7 to 258.7 ± 21.3 $ml\,kg^{-1}$ (Table 1; Figure 2).

Primaquine administration significantly increased half life to 246.9 ± 59.8 min (92% increase) and AUC to 678 ± 56 $\mu g\,ml^{-1}\,h^{-1}$ (32% increase) and there was a concomitant decrease in clearance to 0.70 ± 0.15 $ml\,min^{-1}\,kg^{-1}$ (42% decrease). There was no change in the volume of distribution (Table 1; Figure 2).

Cimetidine produced similar qualitative changes to primaquine but the magnitude of the changes in half life (187.7 ± 18.8 min.; 46% increase), AUC (631 ± 59 $\mu g\,ml^{-1}\,h^{-1}$; 21% increase) and clearance (0.83 ± 0.07 $ml\,min^{-1}$; 31% decrease) were less than those produced by primaquine (Table 1).

Administration of chloroquine had no significant

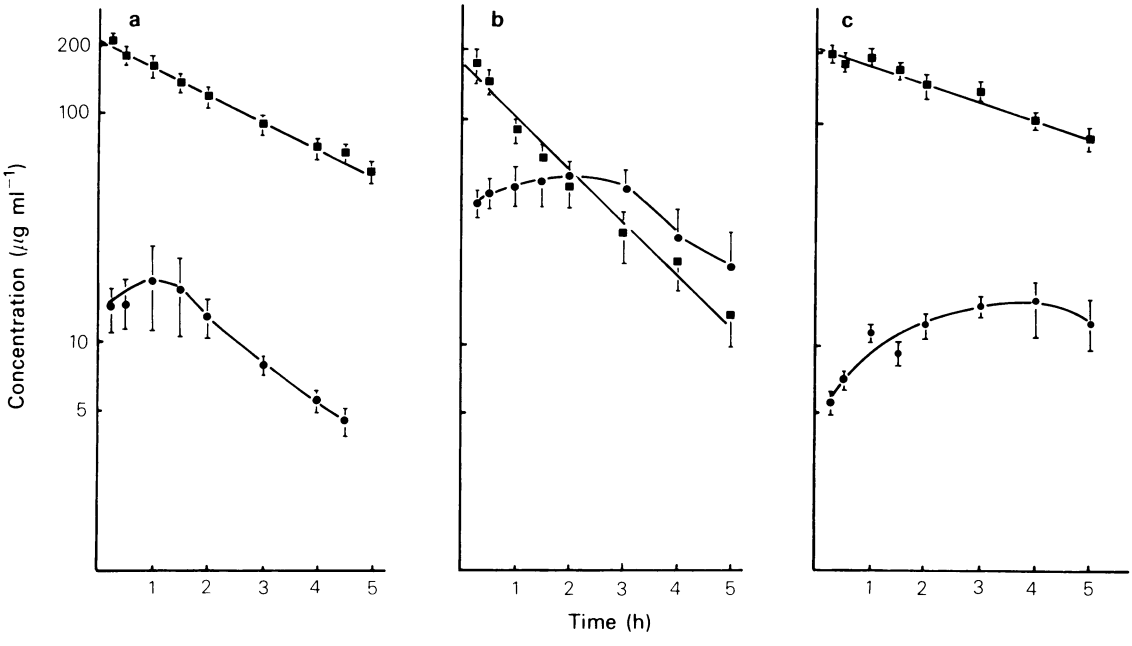

Figure 2 Concentration of tolbutamide (■) and hydroxytolbutamide (●) in the plasma of control rats (a), rats pretreated with phenobarbitone (b) and rats given primaquine (c). For details see text. Values are mean of 4 experiments; vertical lines show s.d.

Table 1 Effect of phenobarbitone pretreatment (40 mg kg^{-1} twice daily for 4 days) and of acute cimetidine (50 mg kg^{-1}), chloroquine (50 mg kg^{-1}) and primaquine (50 mg kg^{-1}) administration on plasma tolbutamide pharmacokinetic parameters

	Saline	*Phenobarbitone*	*Cimetidine*	*Chloroquine*	*Primaquine*
$t_{\frac{1}{2}}$ (min)	128.6 ± 25.1	86.3* ± 9.4	187.7* ± 18.8	150.7 ± 21.8	246.9** ± 59.8
AUC (μg ml^{-1} h^{-1})	513 ± 34	307*** ± 47	631* ± 59	510 ± 30	678** ± 56
Clp (ml min^{-1} kg^{-1})	1.21 ± 0.18	2.09*** ± 0.22	0.83* ± 0.7	1.14 ± 0.24	0.70** ± 0.15
Vd (ml kg^{-1})	219.7 ± 11.7	258.7* ± 21.3	236.6 ± 72.9	243.7 ± 23.0	239.5 ± 19.6

Each value is the mean ± s.d. of 4 experiments.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, significantly different from controls.

effect on any of the pharmacokinetic parameters although there was a 17% increase in half life.

Plasma concentrations of hydroxytolbutamide are shown in Figure 2. At peak, there was approximately three times the plasma concentration of the metabolite following phenobarbitone pretreatment compared to control. After primaquine administration, hydroxytolbutamide levels were initially very low and slowly increased to peak at approximately 3-4 h.

The urinary recovery data (Figure 3) are consistent with the effects seen on plasma concentrations. Tolbutamide was not detectable in urine. Excretion of hydroxytolbutamide was significantly increased by phenobarbitone up to 8 h but at 24 h there was no significant difference. Both primaquine and cimetidine significantly reduced excretion of the metabolite.

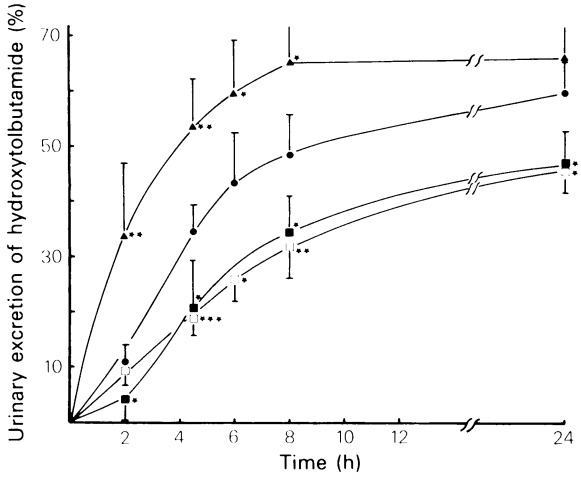

Figure 3 The cumulative urinary excretion of hydroxytolbutamide in control rats (●), rats pretreated with phenobarbitone (▲), and rats given either primaquine (□) or cimetidine (■). For details see text. Values are mean of at least 4 rats vertical lines show s.d.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$: significantly different from control.

Discussion

Sugita *et al.* (1981) have previously studied the interaction of tolbutamide and sulphonamides in the rat and shown that both sulphaphenazole and sulphadimethoxine decrease the total body clearance of the hypoglycaemic drug. Their study was performed with radiolabelled tolbutamide and plasma hydroxytolbutamide was not measured. The present study has shown that utilizing a simple h.p.l.c. method for measurement of both tolbutamide and hydroxytolbutamide, tolbutamide is a particularly sensitive probe drug for the study of pharmacokinetic drug interactions.

Phenobarbitone caused a marked increase in plasma tolbutamide clearance mainly due to an increase in tolbutamide hydroxylation. Urinary recovery of hydroxytolbutamide was consequently increased. There was also an increase in the volume of distribution which probably is a reflection of an increase in liver size (Back *et al.*, 1980).

Cimetidine has in a short period of time attained the status of something akin to a classical inhibitor of microsomal oxidation. Giving rise to a Type II spectral change (Pelkonen & Puurunen, 1980), cimetidine has been shown in the rat 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). In the present study, cimetidine decreased tolbutamide clearance, reduced plasma hydroxytolbutamide concentrations and reduced urinary excretion of the metabolite.

We have previously shown (Back *et al.*, 1983) that the antimalarial drug primaquine is a potent inhibitor of hepatic drug metabolism. Although chloroquine also gave evidence of inhibition with some substrates there was clearly a marked difference in the metabolic effects of the two aminoquinolones. It was suggested that the most probable explanation is a structure-activity phenomenon similar to that high-

lighted for the imidazole group of compounds (Wilkinson *et al.*, 1972; 1974 a,b; Rogerson *et al.*, 1977). In the present study, primaquine caused significant inhibition of tolbutamide metabolism (evident from both plasma and urinary data), whereas chloroquine was without effect, thus reinforcing the previous work.

In conclusion, we suggest that in pharmacokinetic interaction studies when assessing the possibility of enzyme induction or inhibition, tolbutamide can be a useful test drug and may be used in conjunction with other test compounds such as antipyrine (Park 1982). The advantages of tolbutamide are that it has a single pathway of metabolism and there is a rapid h.p.l.c. method for both parent drug and metabolite. Figure 2 clearly indicates the effect of an inducer and an inhibitor of tolbutamide metabolism. The disadvantage of tolbutamide is that it is highly protein bound and hence if the interacting drug displaces tolbutamide from binding (Sugita *et al.*, 1981) there will be more complicated pharmacokinetics.

We have used the approach outlined to considerable benefit in the teaching of principles of drug metabolism and disposition.

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