Lack of relationship between glibenclamide metabolism and debrisoquine or mephenytoin hydroxylation phenotypes

M.-L. DAHL-PUUSTINEN¹, C. ALM¹, L. BERTILSSON¹, I. CHRISTENSON², J. ÖSTMAN³, E. THUNBERG² & I. WIKSTRÖM²

Departments of ¹Clinical Pharmacology, ²Pharmacy, and ³Internal Medicine, Huddinge University Hospital, S-141 86 Huddinge, Sweden

The pharmacokinetics of a single oral dose of 1.75 mg glibenclamide were studied in 15 healthy Caucasians including five poor metabolisers of debrisoquine and five poor metabolisers of S-mephenytoin. Plasma glibenclamide concentrations and the urinary concentrations of trans-4- and cis-3-hydroxyglibenclamide were analyzed by h.p.l.c. Thirty-six $\pm$ 6% (mean $\pm$ s.d., $n = 15$) of the given dose of glibenclamide was excreted in 48 h urine as hydroxylated metabolites, 27 $\pm$ 4% as trans-4-hydroxyglibenclamide and 8 $\pm$ 2% as cis-3-hydroxyglibenclamide. There were no differences in the plasma pharmacokinetics of glibenclamide or in the urinary excretion of the metabolites between poor and extensive metabolisers of debrisoquine, neither between the two mephenytoin hydroxylator phenotypes. The study thus indicates that the disposition of glibenclamide is not influenced by these two independent polymorphisms of drug oxidation.

Keywords glibenclamide pharmacokinetics debrisoquine polymorphism mephenytoin polymorphism

Introduction

Glibenclamide (glyburide), one of the so-called second-generation sulphonylurea hypoglycaemic drugs, is extensively metabolized in the liver (Rupp *et al.*, 1969). Two hydroxylated metabolites, trans-4- and cis-3-hydroxyglibenclamide (4-OH- and 3-OH-glibenclamide), have been identified in urine, 4-OH-glibenclamide being the major metabolite in man (Rupp *et al.*, 1969). Very large variations in the steady-state plasma concentrations of glibenclamide have been reported in out-patients treated with the same dose of the drug (Sartor *et al.*, 1980). Fifty-one cases of glibenclamide-associated hypoglycaemia, reported to the Swedish Adverse Drug Reactions Advisory Committee, have been reviewed by Asplund and co-workers (1983). The median daily dose of glibenclamide was the same in the hypoglycaemia cases as in a random control group of patients prescribed glibenclamide.

Interindividual, genetically determined differences in the metabolism of glibenclamide were suggested as one possible factor which might contribute to the development of hypoglycaemia.

The metabolism of a number of drugs including β -adrenoceptor antagonists, tricyclic antidepressants, some antiarrhythmic drugs, and certain neuroleptics has been shown to covary with the polymorphic hydroxylation of debrisoquine (Brøsen & Gram, 1989). 4-Hydroxylation of S-mephenytoin also exhibits a bimodal distribution in the population, 3–5% of Caucasians being poor metabolisers of mephenytoin (Küpfer & Preisig, 1984; Nakamura *et al.*, 1985). So far, only the metabolism of mephobarbitone (Küpfer & Branch, 1985), hexobarbitone (Knodell *et al.*, 1988), diazepam (Bertilsson *et al.*, 1989), propranolol (Ward *et al.*, 1989) and omeprazole (Andersson *et al.*, 1990) have been

Correspondence: Dr Marja-Liisa Dahl-Puustinen, Department of Clinical Pharmacology, Huddinge University Hospital, S-141 86 Huddinge, Sweden

reported to be associated with the mephenytoin hydroxylator phenotype. In the present study we have investigated whether the disposition of glibenclamide is related to either of these two polymorphisms of drug hydroxylation. While this study was being performed, Peart and co-workers (1989) reported a lack of relationship between glibenclamide disposition and debrisoquine hydroxylation phenotype. The relationship of glibenclamide metabolism and mephenytoin hydroxylation has however not been investigated previously.

Methods

Fifteen Caucasians (six men, nine women) 21 to 58 years of age and weighing 53 to 85 kg took part in the study. All were healthy as assessed by clinical status and routine laboratory analyses of haematological, kidney and liver function, and none was taking any medication. All subjects gave their informed consent. The study was approved by the Ethics Committee at Huddinge Hospital, Huddinge, Sweden.

The volunteers had all been previously phenotyped for their ability to hydroxylate debrisoquine and S-mephenytoin according to methods described earlier (Sanz *et al.*, 1989; Steiner *et al.*, 1988). Five of the volunteers were poor metabolisers (PM) of debrisoquine, i.e. they had a debrisoquine/4-OH-debrisoquine ratio higher than 12.6 in a 6 h urine collected after oral administration of 10 mg debrisoquine (Declinax®, Roche). The remaining subjects were extensive metabolisers (EM) of debrisoquine with a metabolic ratio of less than 1.0. Five of the extensive debrisoquine metabolisers were poor metabolisers of S-mephenytoin as defined by an S/R enantiomeric ratio of mephenytoin of about 1 in both the 0–8 and 24–32 h urines after intake of 100 mg racemic mephenytoin (Mesantoin®, Sandoz); extensive metabolisers all had a ratio < 0.65 (Sanz *et al.*, 1989).

The subjects took a single oral dose of 1.75 mg glibenclamide (Daonil®, Hoechst) in tablet form at 08:00 h after an overnight fast just before breakfast. Venous blood samples were drawn from the antecubital vein 10 min before drug intake and at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 and 32 h after the dose. Plasma was collected after centrifugation and stored frozen at -20°C until analyzed for glibenclamide. Glucose was analyzed in fresh whole-blood samples. During the first 4 h, capillary blood samples were also taken at every 15 min for an immediate reflectometric analysis of blood glucose level to ensure the safety of the experiment. Urine was

collected in 2 h fractions for the first 10 h, in one fraction between 10 and 14 h and between 14 and 24 h and finally in 8 h fractions up to 48 h after the dose. The volume of each urine fraction was measured and 20 ml aliquots were stored frozen at -20°C until analysis.

Plasma glibenclamide concentration was measured by h.p.l.c. according to the method of Emilsson *et al.* (1986) with some minor modifications. The method of Emilsson *et al.* (1986) is a modification of the original h.p.l.c. method of Wählin-Boll & Melander (1979). In brief, plasma was mixed with glibornuride (internal standard) and hydrochloric acid and extracted with ethyl acetate. The organic phase was evaporated to dryness and the residue was dissolved in the mobile phase. A Spherisorb ODS column (4.5×250 mm, $5\text{ }\mu\text{m}$ particles, Jones Chromatography), equilibrated with acetonitrile–0.01 M sodium phosphate buffer pH 3.5 (50:50) as mobile phase, at a flow rate of 1.15 ml min^{-1} , was used. U.v.-absorption was recorded at 230 nm. The detection limit varied between 4 and 10 nmol l^{-1} of glibenclamide. The within-assay variation was less than $\pm 5\%$.

Trans-4-OH-glibenclamide and cis-3-OH-glibenclamide were analyzed in urine in a similar way using glipizide as internal standard. Acetonitrile–0.01 M sodium phosphate buffer pH 3.5 (34:66) was used as mobile phase at a flow rate of 1.5 ml min^{-1} . The within-assay variation was $\pm 5\%$.

The area under the plasma drug concentration-time curve (AUC) was estimated by the trapezoidal rule and extrapolation of the area to infinite time. A terminal half-life was estimated from the last three to four concentrations. The extrapolated area varied between 3 and 17% of the total area. Oral plasma clearance (CL_0) was calculated as dose/AUC. Urinary recovery of the metabolites of glibenclamide was calculated from the sum of the amounts excreted in urine during 48 h. Partial metabolic clearance by the formation of 3-OH-glibenclamide ($\text{CL}(3\text{OHG})$), and by that of 4-OH-glibenclamide ($\text{CL}(4\text{OHG})$), was calculated as the oral plasma clearance multiplied by the fraction of the dose recovered in urine as the respective metabolite.

Student's *t*-test was used for the statistical analysis.

Results

There were no differences in any of the calculated pharmacokinetic parameters between PM and EM of debrisoquine, or between the two mephenytoin hydroxylator phenotypes after a

Figure 1 Plasma concentrations of glibenclamide (◆) and the urinary excretion rates of 4-OH- (○) and 3-OH-glibenclamide (●) in one subject after a single oral dose of 1.75 mg glibenclamide.

single oral dose of 1.75 mg glibenclamide (Table 1). Peak plasma concentrations of glibenclamide were reached at 0.5 to 2 h after the intake of glibenclamide and ranged between 131 and 297 nmol l⁻¹. Glibenclamide could be measured in plasma for 6 to 10 h after the dose. The dis-

appearance of glibenclamide from plasma was not monoexponential, but showed a tendency for increasing apparent half-life with time. There was no deviation from an apparent monoexponential decline in urinary excretion rate of the two hydroxylated metabolites.

Of the given dose of glibenclamide, $27 \pm 4\%$ ($n = 15$) was recovered in 48 h urine as 4-OH-glibenclamide, and $8 \pm 1\%$ as 3-OH-glibenclamide (Table 1). There was little variation between individuals with respect to the urinary recovery of the metabolites, and neither the PM of debrisoquine nor those of mephenytoin differed from the EM in this respect. The partial metabolic clearances to 3-OH- and 4-OH-glibenclamide did not differ between the phenotypes. Representative urinary excretion rates of the hydroxylated metabolites in one volunteer are shown in Figure 1 together with the plasma drug concentration-time curve.

All subjects developed mild symptoms of hypoglycaemia and had blood glucose levels around 2 mmol l⁻¹ at 1.5–2 h after the dose. At this point, all subjects were given enough fruit juice to return the blood glucose level to normal and to relieve the symptoms of hypoglycaemia.

Discussion

The plasma pharmacokinetics of a single oral dose of glibenclamide did not differ significantly between PM and EM of debrisoquine or between PM and EM of mephenytoin. Moreover, no differences in the urinary excretion of the two hydroxylated metabolites, 4-OH-glibenclamide

Table 1 Pharmacokinetic parameters of glibenclamide (1.75 mg p.o.) in poor (PM) and extensive metabolisers (EM) of debrisoquine and of mephenytoin. The values are tabulated as mean $\pm$ s.d.

	All (n = 15)	Debrisoquine hydroxylator phenotype		P	Mephenytoin hydroxylator phenotype		P
		EM (n = 10)	PM (n = 5)		EM (n = 10)	PM (n = 5)	
$C_{\max}$ (nmol l ⁻¹)	213 $\pm$ 48	210 $\pm$ 53	218 $\pm$ 42	NS	221 $\pm$ 41	198 $\pm$ 63	NS
CL_O (ml h ⁻¹ kg ⁻¹)	79.0 $\pm$ 23.8	86.5 $\pm$ 25.5	64.1 $\pm$ 10.1	NS	72.4 $\pm$ 15.9	92.3 $\pm$ 33.1	NS
$CL(4OHG)$ (ml h ⁻¹ kg ⁻¹)	21.6 $\pm$ 7.4	23.3 $\pm$ 8.5	18.0 $\pm$ 3.0	NS	19.9 $\pm$ 4.8	24.9 $\pm$ 11.1	NS
$CL(3OHG)$ (ml h ⁻¹ kg ⁻¹)	6.5 $\pm$ 2.1	7.0 $\pm$ 2.4	5.4 $\pm$ 1.1	NS	6.1 $\pm$ 1.8	7.4 $\pm$ 2.7	NS
AUC (nmol l ⁻¹ h)	703 $\pm$ 234	649 $\pm$ 246	811 $\pm$ 182	NS	719 $\pm$ 175	669 $\pm$ 347	NS
Recovery in 0–48 h urine, % of dose							
4-OH- glibenclamide	27 $\pm$ 5	27 $\pm$ 5	28 $\pm$ 2	NS	28 $\pm$ 4	27 $\pm$ 5	NS
3-OH- glibenclamide	8 $\pm$ 2	8 $\pm$ 2	8 $\pm$ 1	NS	8 $\pm$ 2	8 $\pm$ 1	NS
Total	36 $\pm$ 6	35 $\pm$ 7	37 $\pm$ 3	NS	36 $\pm$ 6	35 $\pm$ 6	NS

P statistical significance between EM and PM using Student's *t*-test for unpaired data

and 3-OH-glibenclamide could be observed between the different hydroxylator phenotypes (Table 1). Thus, the data indicate that the disposition of glibenclamide is not related to either of these genetic polymorphisms of drug metabolism. A similar lack of relationship between glibenclamide metabolism and debrisoquine hydroxylation was reported recently by Peart & coworkers (1989).

There appeared to be a second slower elimination phase of glibenclamide. However, we do not think that a more complex pharmacokinetic analysis is possible with the few data obtained or necessary for the aim of this study. Using the same h.p.l.c. method for glibenclamide, we have been able to follow its plasma concentration-time curve during the 24 h dosing interval at steady-state in eight patients treated with glibenclamide. All of these patients showed a multi-exponential plasma drug concentration-time curve (unpublished results). These data are in accordance with those of Balant & coworkers (1975) who found similar results after an oral dose of radioactively labelled glibenclamide.

Thirty-six $\pm$ 6% (mean $\pm$ s.d., $n = 15$) of the dose of glibenclamide was recovered in 48 h urine as the two previously identified metabolites, trans-4- and cis-3-OH-glibenclamide (Table 1). 4-OH-glibenclamide was the major metabolite. The ratio between the urinary excretion rates of

these two metabolites remained constant throughout the collection period (Figure 1), and varied little between individuals. Balant *et al.* (1975) also found that 35% of the oral dose of the [14 C]-glibenclamide was recovered in urine, mainly as metabolites. No conjugated metabolites of glibenclamide have been identified.

The blood glucose level was followed initially in all subjects in order to evaluate whether the possible pharmacokinetic differences would be reflected in the pharmacodynamic, clinical effects of the drug. All subjects developed mild hypoglycaemic symptoms and had a blood glucose level around 2 mmol l $^{-1}$ at 1.5–2 h after the dose. Neither the PM of debrisoquine nor those of mephenytoin differed from the other subjects with respect to development of hypoglycaemia.

In conclusion, the present data show a lack of relationship between the disposition of glibenclamide and the debrisoquine and mephenytoin hydroxylator phenotypes. Thus, the metabolism of glibenclamide by 4- and 3-hydroxylation appears not to be catalyzed by the cytochrome P-450 isoenzymes responsible for debrisoquine and mephenytoin hydroxylation polymorphisms.

The study was supported by the Swedish Medical Research Council (3902) and carried out in coordination with COST B1.

References

- Andersson, T., Regårdh, C.-G., Dahl-Puustinen, M.-L. & Bertilsson, L. (1990). Slow omeprazole metabolisers are also poor S-mephenytoin hydroxylators. *Ther. Drug Monit.* (in press).
- Asplund, K., Wiholm, B.-E. & Lithner, F. (1983). Glibenclamide-associated hypoglycaemia: A report on 57 cases. *Diabetologia*, **24**, 412–417.
- Balant, L., Fabre, J. & Zahnd, G. R. (1975). Comparison of the pharmacokinetics of glipizide and glibenclamide in man. *Eur. J. clin. Pharmacol.*, **8**, 63–69.
- Bertilsson, L., Henthorn, T. K., Sanz, E., Tybring, G., Säwe, J. & Villén, T. (1989). Importance of genetic factors in the regulation of diazepam metabolism: Relationship to S-mephenytoin, but not debrisoquin hydroxylation phenotype. *Clin. Pharmacol. Ther.*, **45**, 348–355.
- Brøsen, K. & Gram, L. F. (1989). Clinical significance of the sparteine/debrisoquine oxidation polymorphism. *Eur. J. clin. Pharmacol.*, **36**, 537–547.
- Emilsson, H., Sjöberg, S., Svedner, M. & Christenson, I. (1986). High-performance liquid chromatographic determination of glibenclamide in human plasma and urine. *J. Chromatogr.*, **383**, 93–102.
- Knodell, R. G., Dubey, R. K., Wilkinson, G. R. & Guengerich, F. P. (1988). Oxidative metabolism of hexobarbital in human liver: relationship to polymorphic S-mephenytoin 4-hydroxylation. *J. Pharmacol. exp. Ther.*, **245**, 845–849.
- Küpfer, A. & Preisig, R. (1984). Pharmacogenetics of mephenytoin: A new drug hydroxylation polymorphism in man. *Eur. J. clin. Pharmacol.*, **26**, 753–759.
- Küpfer, A. & Branch, R. A. (1985). Stereoselective mephobarbital hydroxylation cosegregates with mephenytoin hydroxylation. *Clin. Pharmacol. Ther.*, **38**, 414–418.
- Nakamura, K., Goto, F., Ray, W. A., McAllister, C. B., Jacqz, E., Wilkinson, G. R. & Branch, R. A. (1985). Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin. Pharmacol. Ther.*, **38**, 402–408.
- Peart, G. F., Boutagy, J. & Shenfield, G. M. (1989). The metabolism of glyburide in subjects of known debrisoquin phenotype. *Clin. Pharmacol. Ther.*, **45**, 277–284.
- Rupp, W., Christ, O. & Heptner, W. (1969). Resorption, Ausscheidung und Metabolismus nach intravenöser und oraler Gabe von HB 419– 14 C an Menschen. *Arzneimittel-Forsch.*, **19**, 1428–1434.
- Sanz, E. J., Villén, T., Alm, C. & Bertilsson, L.

- (1989). S-mephenytoin hydroxylation phenotypes in a Swedish population determined after co-administration with debrisoquin. *Clin. Pharmac. Ther.*, **45**, 495–499.
- Sartor, G., Melander, A., Scherstén, B. & Wählin-Boll, E. (1980). Serum glibenclamide in diabetic patients, and influence of food on the kinetics and effects of glibenclamide. *Diabetologia*, **18**, 17–22.
- Steiner, E., Bertilsson, L., Säwe, J., Bertling, I. & Sjöqvist, F. (1988). Polymorphic debrisoquine hydroxylation in 757 Swedish subjects. *Clin. Pharmac. Ther.*, **44**, 431–435.
- Ward, S. A., Walle, T., Walle, K., Wilkinson, G. R. & Branch, R. A. (1989). Propranolol's metabolism is determined by both mephenytoin and debrisoquin hydroxylase activities. *Clin. Pharmac. Ther.*, **45**, 72–79.
- Wählin-Boll, E. & Melander, A. (1979). High-performance liquid chromatographic determination of glipizide and some other sulfonylurea drugs in serum. *J. Chromatogr.*, **164**, 541–546.

(Received 17 January 1990,
accepted 10 May 1990)