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Pharmacokinetic and pharmacodynamic studies of glibenclamide in non-insulin dependent diabetes mellitus

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1 The pharmacokinetic and pharmacodynamic properties of oral glibenclamide have been studied in 31 hospitalised in-patients and 79 ambulant out-patients with diabetes mellitus.
2 Breakfast was found to have no significant influence on the kinetic behaviour of glibenclamide or on the effect of this drug on blood glucose utilisation.
3 The time course of glibenclamide kinetics after 20 mg dosing was adequately described by a two-compartment open model, yielding mean half-lives of 3.3 ± 1.5 h ($t_{\frac{1}{2},\lambda_1}$) and 9.7 ± 1.2 ($t_{\frac{1}{2},z}$) for the initial and terminal elimination phases respectively.
4 No significant accumulation or change in kinetic profile occurred in patients who had normal renal and hepatic function, were treated continuously with glibenclamide, and then rechallenged after 8–12 weeks.
5 Despite inter-individual variations in drug absorption, peak plasma concentrations (C_{max}) and the area under the plasma concentration-time curve (AUC(0–24)) were dose-dependent over the dose range 5–20 mg. No significant dose-response behaviour was observed in respect of glucose utilisation, suggesting that there is little clinical benefit in using doses of glibenclamide above 5 mg day^{-1}.
6 Comparison of plasma glibenclamide concentrations at different time-bands following doses of 5 and 10 mg showed a wider range in ambulant out-patients than in age-, sex-matched in-patients treated with the same dosages of drug. Mean plasma drug concentrations attained at all time bands up to 8 h after dosing were higher in out-patients than in in-patients, suggesting a tendency to 'over-compliance' by patients in anticipation of attendance at clinic.

Keywords glibenclamide non-insulin dependent diabetes pharmacodynamics pharmacokinetics

Introduction

Glibenclamide is a second-generation sulphonylurea which has been widely used in the management of non-insulin dependent diabetes mellitus in Europe since 1969 and in the United States since 1984, where it is known as glyburide (Feldman, 1985). Many aspects of its clinical pharmacology remain incompletely characterised. Recent reports have emphasised the danger of hypoglycaemia with glibenclamide, even at low dose, especially in the elderly (Asplund *et al.*, 1983; Ferner & Neil, 1988). Drug interactions and impaired renal function are suspected to

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contribute to hypoglycaemic episodes, but little is known about their effect on glibenclamide pharmacokinetics (Asplund *et al.*, 1983; Ferner & Chaplin, 1987; Pearson *et al.*, 1986). These problems and the lack of understanding of the dose-response relationship for the drug (Nattrass, 1986; Wahlin-Boll *et al.*, 1982), complicate the clinical use of the drug.

A number of reports have drawn attention to inter-individual variations in absorption (Balant *et al.*, 1977; Ikegami *et al.*, 1986), steady-state circulating concentrations (Sartor *et al.*, 1980a) and elimination rates (Pearson *et al.*, 1986), features that have been noted also with other sulphonylureas (Kobayashi *et al.*, 1984; Melander *et al.*, 1978). The effect of food on the bioavailability of glibenclamide is unclear, some reports showing no effect (Sartor *et al.*, 1980a), whilst others demonstrate distinct alterations in drug absorption (Balant *et al.*, 1977). The result is that standard advice on when the drug should best be given, in relation to breakfast, varies (British National Formulary, 1989; George, 1984).

The apparent lack of consistency in the pharmacokinetics of glibenclamide, coupled with the incomplete information on the characteristics of its dose-response relationship (Ferner & Chaplin, 1987; Marchetti & Navalesi, 1989; Nattrass, 1986), often makes it difficult to determine optimum drug dosage in the individual diabetic patient. Some of the reported inconsistencies reflect the fact that kinetic profiles for the drug have been determined in animals or healthy volunteers rather than in diabetic patients (Adams *et al.*, 1982; Ings *et al.*, 1981). Further, with the considerably increased potency on milligram basis of second-generation sulphonylureas, as compared with older agents, there has been need for highly specific and sensitive methods of drug analysis in order to follow the fate and actions of glibenclamide *in vivo*. Even where sophisticated technology has become available, as for example with radioimmunoassay, published reports have often been misleading because of failure to take into account cross-reactivity between native glibenclamide and its hydroxylated metabolites (Kawashima *et al.*, 1979; Pearson, 1985; Royer *et al.*, 1976).

In the present study, we have employed a specific and sensitive h.p.l.c. assay to investigate a number of aspects of the pharmacokinetic and pharmacodynamic responses to glibenclamide in a group of 31 hospitalised patients with non-insulin dependent diabetes mellitus. The effect of food on the drug kinetics and the hypoglycaemic effect dose-response relationship have been studied over a glibenclamide dose range of 5–20 mg day^{-1}. A comparison has also been made between the plasma concentrations achieved at 5 and 10 mg dose levels in hospitalised in-patients with those obtaining at these same dosages in a matched group of 79 out-patients on long-term treatment with glibenclamide.

Methods

In-patient studies

A total of 22 hospitalised in-patients with non-insulin dependent diabetes mellitus participated in these studies (thirteen females and nine males). Their ages ranged from 54–80 years and body weights from 44–95 kg. All gave informed consent to participation and the study protocol was approved by the relevant Hospital Ethics Committees. Patients were studied only after a minimum stay of 48 h in hospital, most were in a convalescent phase of their illness. All received their individualised hospital diet and the only sulphonylurea therapy given was glibenclamide BP (H. N. Norton & Co, London). All these hospitalised patients were receiving a range of ancillary medical treatments which were continued and remained unaltered during repeated study days. All patients were stabilised on the challenge dose of glibenclamide at least 48 h before the study day. Routine haematology, as well as assessment of plasma proteins, renal and hepatic function yielded normal findings, compatible with the age of the patients, in all cases.

Group I Seven patients (six females and one male) received oral glibenclamide, 20 mg, either 30 min before, or at the end of, a standard breakfast consisting of wholemeal bread (60 g), cornflakes (25 g), butter (30 g), marmalade (30 g), milk (200 ml) and coffee (200 ml). The order of administration in relation to breakfast was randomised and a minimum of 1 week elapsed between individual kinetic studies.

Blood (8–10 ml) was taken from an indwelling venous cannula prior to dosing, and subsequently at half-hourly intervals until 8 h, followed by hourly samples to 12 h, and a final sample at 24 h. Samples were collected into fluoride/oxalate and lithium heparin tubes, immersed in ice. Drug assay samples were centrifuged immediately at 0° C and stored at −20° C until analysed. Blood glucose samples were stored at 4° C until assayed within 1 day.

In four patients (numbers 2, 3, 4 and 6), separate 20 mg oral challenges with glibenclamide

were repeated in identical fashion after intervals of 8–12 weeks in order to check for evidence of drug accumulation and/or alteration in the kinetic profile following repeated administration of the drug. Between challenges, the patients were maintained on glibenclamide 10–20 mg day^{-1}.

Group II Five patients (four female and one male) received an oral dose of 10 mg glibenclamide either 30 min before, or at the end of, a standard breakfast. The order of drug administration in relation to breakfast was again randomised and a minimum of 1 week elapsed between each study. Blood samples were taken as in group I above, but at the following times: hourly until 6 h, then 2-hourly to 12 h, with a final sample at 24 h.

Group III Thirteen patients (seven male and six female) received in each case 5, 10, 20 mg doses of glibenclamide in random order, drug doses being taken with a standard breakfast. A minimum of 5 days elapsed between each dose of glibenclamide. Blood samples were withdrawn through an indwelling venous cannula, as described for group II above.

Blood samples from one patient could not be satisfactorily analysed for glibenclamide because of interference by coincidental drug therapy with the assay; this patient was withdrawn from the study.

Out-patient studies

A comparison was undertaken between the steady-state plasma concentration of glibenclamide achieved on 5 mg or 10 mg day^{-1} in chronic therapy between ambulant out-patients and hospitalised patients. Seventy-nine out-patients who were routine attenders at one of our morning or afternoon diabetic clinics participated, and were matched for sex and age with a group of 31 in-patients, some of whom had also taken part in the studies in groups II and III above. For the 5 mg dosage studies, the age range of out-patients was 41–81 years (mean ± s.e. mean = 65 ± 1 years; n = 43) as compared with 54–80 years (70 ± 2 years; n = 14) for in-patients. For the 10 mg dosage studies, the age ranges were 50–86 years (out-patients, mean ± s.e. mean = 69 ± 1 years; n = 36) and 58–80 years (in-patients, 69 ± 2 years; n = 17).

Out-patients were only included for study if their antidiabetic management consisted of controlled diet and glibenclamide therapy. None of the out-patients had significant renal or hepatic dysfunction. During routine attendances at clinic, and without prior warning, patients were asked at what time they had taken their morning dose of glibenclamide, the dose taken and the relationship to breakfast. Single blood samples were taken for glibenclamide assay so as to fall within the time bands: 0–2, 2–4, 4–6 and 6–8 h after dosing. As some of the out-patients were taking Daonil (Hoechst) or Eugluclon (Roussel) rather than the standard formulation of glibenclamide used for in-patient studies, a note was also made of the particular brand of glibenclamide each patient was taking at the time of study.

Analytical methods

1: Chemical Plasma glucose was analysed by a glucose oxidase method using a Beckman glucose-analyser (Beckman, High Wycombe, UK). Plasma urea, creatinine, electrolytes and liver function tests were measured by routine laboratory methods.

Glibenclamide was assayed by modifications of the h.p.l.c. techniques of Wahlin-Boll *et al.* (1979) and Rogers *et al.* (1982). Aliquots of plasma (1 ml) were mixed with 80 μl 1.3 M HCl to which were added 50 μl of gliburnuride (10 μg ml^{-1}) as internal standard and 1 ml of distilled water. Dichloromethane (7 ml) was added and glibenclamide extracted for 30 min. The aqueous layer was discarded and the organic layer evaporated to dryness on a rotary evaporator. The residue was redissolved in 100 μl of methanol and 30 μl injected into the h.p.l.c. system, which consisted of a modular dual piston solvent pump (model 600A, Waters Associates, Mass, USA) linked to a U6K injector. H.p.l.c. was performed on a Hypersil 3 ODS column (Technicol, Cheshire, UK) using 62% methanol in 0.08 M phosphate buffer (pH 4.1) as the mobile phase. The effluent was monitored by a variable wavelength u.v. detector (SpectroMonitor III, Laboratory Data Control, Fla, USA) set at 228 nm, coupled to a CR1b integrator (Shimadzu, Kyoto, Japan). All chemicals used were Pronalys AR grade (May & Baker, Dagenham, UK). Glibenclamide and gliburnuride, pure substances, were kindly provided by Hoechst UK and Roche UK respectively. The detectability limit for glibenclamide was 10 ng ml^{-1}. Recovery was 98 ± 6%, with intra- and inter-assay coefficients of variation of 6.1% and 6.2% respectively.

2: Kinetic analysis The pharmacokinetic profiles of glibenclamide were evaluated by a least-squares-fitting computer programme (Johnston & Woollard, 1983). The data were most appropriately fitted by a two-compartment open

model. Using such a model, the following measurements were derived: t_{max} = the time after drug administration that the maximum plasma glibenclamide concentration was observed (h); C_{max} = the maximal plasma glibenclamide concentration attained (ng ml^{-1}); $t_{½,\lambda_1}$ and $t_{½,z}$ = computer-estimated half-lives of elimination on the basis of first-order kinetics and a biphasic pattern of decay curve. The elimination rate constants were calculated by linear regression analysis of data from the slopes of the log plasma concentration-time curves; AUC = integrated area (determined by the trapezoidal rule) under the plasma concentration/time curve over the time interval, 0–t (h) expressed as ng ml^{-1} h. Mean 24 h plasma glucose concentration and AUC(0–24) for plasma glucose were calculated in a similar manner.

3: Statistical methods Results have been expressed as either mean values ± s.e. mean or as means with their 95% confidence intervals.

Statistical significance was calculated using Student's t-test for paired or unpaired data, and two-way analyses of variance (SPSS Inc, 1988).

Results

In-patient studies

Group I – a) Kinetic dynamic profiles and the effect of food Pharmacokinetic profiles were undertaken in seven patients following treatment with single oral doses of glibenclamide, 20 mg, given before and after breakfast on separate occasions. The times taken to reach peak plasma concentration (t_{max}) were (mean ± s.e. mean) 3.2 ± 0.9 h and 3.5 ± 0.4 h respectively (Table 1). Two of the seven patients in this group (numbers 6 and 7) had delayed absorption, as shown by t_{max} values considerably longer than 4 h (7.1 and 5.5 h respectively), whilst in three patients (numbers 2, 4 and 5) t_{max} values of less than 2 h were achieved. Peak plasma drug concentrations (C_{max}) attained were (mean ± s.e. mean) 354 ± 33 ng ml^{-1} with drug taken before, and 360 ± 49 ng ml^{-1} with drug taken after breakfast. The areas under the time *vs* plasma concentration curve, covering the time of drug administration to 24 h later, (AUC (0–24)) were: 2968 ± 283 ng ml$^{1-1}$ h and 2810 ± 405 ng ml^{-1} h respectively. Elimination half-lives reflecting the initial rapid and terminal slow phases ($t_{½,\lambda_1}$ and $t_{½,z}$) were (mean ± s.e. mean) 3.1 ± 1.0 h and 10.4 ± 1.8 h respectively, when glibenclamide was taken before breakfast, compared with 3.5 ± 1.2 h and 9.0 ± 1.3 h, when

Table 1 Detailed pharmacokinetic measurements and plasma glucose responses to single oral doses of 20 mg glibenclamide, taken 30 min before the start of or at the end of a standard breakfast by seven non-insulin dependent diabetic patients (Group I). The values obtained from doses before breakfast were not significantly different from values obtained with doses postprandially.

	Pre-breakfast						*Post-breakfast*					
	Glibenclamide pharmacokinetics				*Plasma glucose*		*Glibenclamide pharmacokinetics*				*Plasma glucose*	
Patient	t_{max} *(h)*	C_{max} *(ng ml^{-1})*	$t_{½,\lambda_1}$ *(h)*	$t_{½,z}$ *(h)*	*AUC(0–24) (ng ml^{-1} h)*	*24 h mean (mmol l^{-1})*	*AUC(0–24) (mmol l^{-1} h)*	t_{max} *(h)*	C_{max} *(ng ml^{-1})*	$t_{½,\lambda_1}$ *(h)*	$t_{½,z}$ *(h)*	*AUC(0–24) (ng ml^{-1} h)*	*24 h mean (mmol l^{-1})*	*AUC(0–24) (mmol l^{-1} h)*
1	3.0	231	2.7	11.0	2193	6.3	121	4.0	294	4.0	16.0	3078	5.9	105
2	1.5	452	3.0	5.7	3562	8.4	151	2.5	284	2.8	7.8	4452	9.2	167
3	2.5	456	4.3	7.1	3614	7.5	118	3.5	437	4.2	9.2	3659	8.5	109
4	1.1	333	3.6	18.1	4007	7.5	107	4.4	263	2.5	6.3	1424	6.8	135
5	1.5	290	4.0	14.5	2142	8.7	132	1.5	245	5.7	9.0	2188	7.8	136
6	7.1	299	2.1	11.0	2707	12.9	177	5.1	241	2.9	5.6	1793	12.6	203
7	5.5	415	1.7	5.7	2549	13.3	207	3.5	555	2.7	9.4	3078	14.3	216
Mean	3.2	354	3.1	10.4	2968	9.2	145	3.5	360	3.5	9.0	2810	9.3	153
s.e. mean	0.86	33	1.0	1.8	283	1.0	13.6	0.45	49	1.2	1.3	405	1.2	16.6

the drug was taken after breakfast. None of these pharmacokinetic variables, pre- and post-breakfast, differed significantly.

In the same group of patients, the mean of the plasma glucose concentrations over 24 h (mean plasma glucose) and the mean area under the plasma glucose concentration *vs* time curve over 24 h (glucose AUC(0–24)) were (mean ± s.e. mean) 9.2 ± 1.0 mmol l^{-1} and 145 ± 13.6 mmol ml^{-1} h respectively, with drug dosing before breakfast, and 9.3 ± 1.2 mmol l^{-1} and 153 ± 16.6 mmol ml^{-1} h respectively, with drug dosing after breakfast. These glucose values were not significantly different from one another, and analysis of variance of the individual plasma glucose concentrations failed to show any significant effect of taking the drug before or after breakfast.

Group I – b) Effect of repeated drug challenge In four patients (numbers 2, 3, 4 and 6) a kinetic and dynamic profile was repeated after dosing with 20 mg glibenclamide before breakfast on at least three occasions over a period of up to 12 weeks. The patients received 10–20 mg glibenclamide once daily in the interim. There were no significant differences in the pharmacokinetic or glucose utilisation profiles of the drug on repeated challenge (Figure 1). There was no indication of drug accumulation as evidenced by the lack of significant change in basal or zero-time plasma glibenclamide concentrations with passage of time. The coefficient of variation (from 14 challenges on four individuals) of glibenclamide AUC(0–24) was 19%.

Group II In a second group of five patients, kinetic and dynamic drug profiles were studied following treatment with single oral doses of glibenclamide, 10 mg given before or after breakfast on separate occasions. The t_{max} achieved at this dose was (mean ± s.e. mean) 2.1 ± 0.3 h for pre-breakfast dosing and 2.7 ± 0.4 h for post-breakfast dosing (Table 2). The respective pre- and post-breakfast mean values for C_{max} were 241 ± 69 ng ml^{-1} and 262 ± 68 ng ml^{-1} respectively; the mean values for AUC(0–24) were 1558 ± 547 ng ml^{-1} h and 1450 ± 371 ng ml^{-1} h respectively. None of these differences was statistically significant. Neither were the differences between the mean plasma glucose

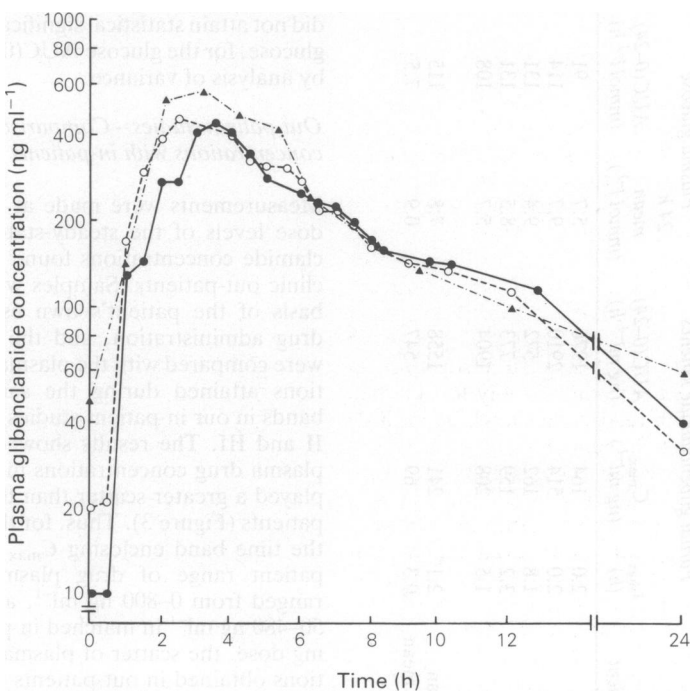

Figure 1 Plasma concentration-time curves after single oral doses of glibenclamide 20 mg, repeated on three separate occasions, over a period of 12 weeks continuous therapy with daily doses of 15 mg glibenclamide in individual patient (number 3, Group I). Individual study days are shown: ▲ = first challenge, AUC(0–24) 5136, ○ = second challenge, AUC(0–24) 3614, ● = third challenge, AUC(0–24) 3659 ng ml^{-1} h.

Table 2 Partial pharmacokinetic profiles and plasma glucose responses to single oral doses of 10 mg glibenclamide, taken 30 min before the start of or at the end of a standard breakfast by five non-insulin dependent diabetic patients (Group II). The values obtained from doses before breakfast were not significantly different from values obtained with doses postprandially.

	Pre-breakfast					*Post-breakfast*				
	Partial glibenclamide kinetics			*Plasma glucose*		*Partial glibenclamide kinetics*			*Plasma glucose*	
Patient	t_{max} (*h*)	C_{max} (*ng ml*$^{-1}$)	*AUC(0–24)* (*ng ml*$^{-1}$ *h*)	*24 h mean* (*mmol l*$^{-1}$)	*AUC(0–24)* (*mmol l*$^{-1}$ *h*)	t_{max} (*h*)	C_{max} (*ng ml*$^{-1}$)	*AUC(0–24)* (*ng ml*$^{-1}$ *h*)	*24 h mean* (*mmol l*$^{-1}$)	*AUC(0–24)* (*mmol l*$^{-1}$ *h*)
1	2.0	164	1976	5.7	91	2.5	471	2547	5.1	118
2	2.0	514	2910	9.7	114	2.2	374	2117	10.5	120
3	1.8	162	572	9.4	131	4.1	190	832	6.5	108
4	3.2	159	773	8.5	131	2.0	155	697	8.9	145
5	1.5	208	1904	5.7	108	2.0	119	1056	6.5	123
Mean	2.1	241	1558	7.8	115	2.7	262	1450	7.5	123
s.e. mean	0.3	69	547	0.9	7.5	0.4	68	371	1.0	6.1

concentrations over 0–24 h or between the associated glucose AUC(0–24) values for pre- and post-breakfast challenges of glibenclamide.

Group III–Dose-response studies When graded increases in glibenclamide dosage of 5, 10 and 20 mg were studied in a third group of 12 patients, the results showed a trend toward prolongation of t_{max} after 10 and 20 mg dosage as compared with 5 mg. Two out of the twelve patients had a t_{max} value exceeding 4 h after 5 mg dosing, whilst three out of the twelve patients given 10 mg displayed a $t_{max} > 4$ h. Overall, however, the prolongations of t_{max} at the different doses of glibenclamide did not attain statistical significance (Table 3). A clear dose-response relationship emerged however in respect of C_{max} and AUC(0–24). C_{max} increased approximately three-fold from (mean ± s.e. mean) 152 ± 26 ng ml^{-1} to 436 ± 48 ng ml^{-1} (5 mg *vs* 20 mg; $P < 0.001$) whilst the AUC(0–24) increased from (mean ± s.e. mean) 1154 ± 367 to 3490 ± 673 ng ml^{-1} h (5 mg *vs* 20 mg; $P < 0.001$). Although there was a consistent trend downwards in both mean plasma glucose concentrations and plasma glucose AUC(0–24), with increasing doses of glibenclamide (Figure 2), the observed changes did not attain statistical significance for the mean glucose, for the glucose AUC(0–24) (Table 3) or by analysis of variance.

Out-patient studies – Comparison of plasma drug concentrations with in-patients

Measurements were made at 5 mg and 10 mg dose levels of the steady-state plasma glibenclamide concentrations found in a group of 79 clinic out-patients. Samples were taken on the basis of the patient's own estimated time of drug administration, and the values obtained were compared with the plasma drug concentrations attained during the same time-interval bands in our in-patient studies involving groups II and III. The results showed that individual plasma drug concentrations in out-patients displayed a greater scatter than those found in in-patients (Figure 3). Thus, for the 10 mg dose, in the time band enclosing C_{max} (2–4 h) the out-patient range of drug plasma concentration ranged from 0–800 ng ml^{-1}, as compared with 50–480 ng ml^{-1} in matched in-patients. At the 5 mg dose, the scatter of plasma drug concentrations obtained in out-patients during each time band was similarly greater than the equivalent concentrations determined in hospitalised in-patients. The plasma glibenclamide levels were not different in out-patients taking different formulations of drug. Overall the geometric

Table 3 Partial pharmacokinetic profiles and plasma glucose responses (mean ± s.e. mean) to single oral doses of 5, 10 and 20 mg glibenclamide, taken by 12 non-insulin dependent diabetic patients. In six patients the dosage was taken before breakfast, in the other six at the end of breakfast. As the pre- and post prandial responses did not differ significantly, only pooled results ($n = 12$) are presented. Results are as expressed as mean ± s.e. mean. Results significantly different, between pairs of results marked * and † ($P < 0.05$); and between pairs of results marked ** and †† ($P < 0.01$).

	Glibenclamide partial pharmacokinetics			*Plasma glucose*	
Dose (mg)	t_{max} (h)	C_{max} (ng ml^{-1})	*AUC(0–24)* (ng ml^{-1} h)	*24 h mean* (mmol l^{-1})	*AUC(0–24)* (mmol l^{-1} h)
5	2.71 ± 0.44	152* ± 26	1154† ± 351	11.9 ± 1.0	296 ± 25
10	3.55 ± 0.70	245*†† ± 32	1999†** ± 326	11.2 ± 1.2	274 ± 28
20	3.34 ± 0.45	436†† ± 48	3490** ± 673	10.7 ± 1.0	261 ± 20

* $P < 0.025$, ** $P < 0.020$, † $P < 0.01$, †† $P < 0.001$

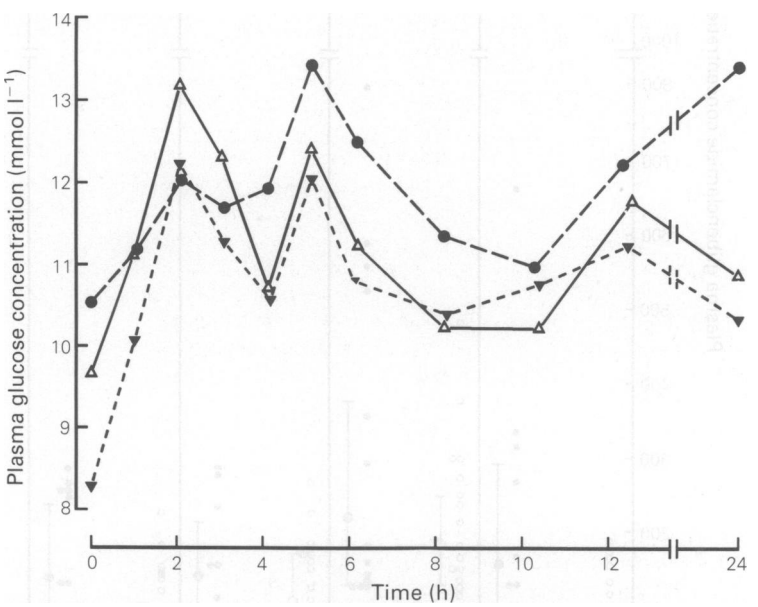

Figure 2 Plasma glucose concentration-time curves after single oral doses of glibenclamide, taken by 12 non-insulin dependent diabetic patients (see Table 3). Glibenclamide taken at time 0. ● is response to 5 mg dose, △ 10 mg dose, ▼ 20 mg dose. Error bars not shown for reasons of clarity.

mean plasma drug concentrations achieved in each time band after both 5 and 10 mg doses were significantly higher in out-patients as compared with in-patients ($P < 0.002$, by analysis of variance). The individual out-patients with the very highest plasma concentrations of glibenclamide were found to have normal renal and hepatic function.

Discussion

The effect of food on the pharmacokinetics and pharmacodynamics of drugs has not been widely studied (George, 1984; Welling, 1984; Winstanley & Orme, 1989). For glibenclamide, the sulphonylurea most commonly used in the U.K., there is uncertainty about the relationship between dosing, food and drug efficacy. There is a discrepancy between the recommendations of George (1984), who suggested that glibenclamide, along with other sulphonylureas, should be administered up to 30 min before food, and the British National Formulary (1989) as well as the official data sheets for glibenclamide which recommend that the drug is taken at breakfast or with the first main meal of the day. Sartor *et al.* (1982) found that giving gliben-

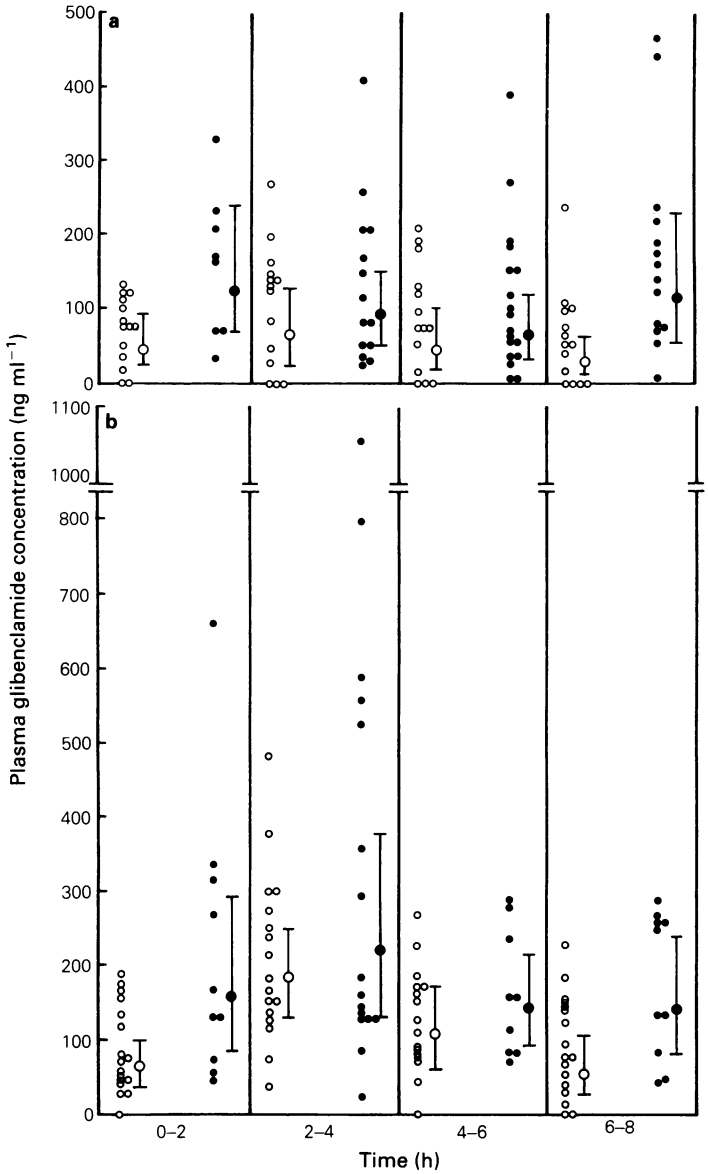

Figure 3 Comparisons of plasma glibenclamide concentrations, at various times post drug administration, between in-patients (○), and out-patients (●). In the upper panel (a), dosage was 5 mg glibenclamide; in lower panel (b), dosage was 10 mg glibenclamide. In-patient data pooled from studies in patients from groups II and III (n = 31). Out-patient data from age-, sex-matched patients (n = 79). Bars are geometric means with 95% confidence limits.

clamide on an empty stomach to non-insulin dependent diabetic patients improved the pharmacodynamic effect by evoking lower plasma glucose concentrations, although the pharmacokinetic profile remained unaltered. Similar improved glucose utilisation has been found with tolbutamide when the drug is given 30 min before food (Samanta *et al.*, 1984). Our results have shown that changing the relationship between dose and breakfast had no effect on either glibenclamide plasma concentrations or on glucose utilisation, using 10 and 20 mg doses in hospitalised diabetic patients. Reasons for the differences between our findings and

those of Sartor *et al.* (1982) with glibenclamide may relate to the different dosing schedules used by the latter group; they gave 7.5 mg either as a single dose or in divided doses. Their finding, of significantly lower blood glucose concentrations with drug dosing before breakfast, was only noted with the effects of the first-ever dose of glibenclamide.

A number of difficulties arise when comparing the results of glibenclamide kinetics reported by different research groups. First, differing brands of glibenclamide have often been used and there is evidence that formulation may significantly affect bioavailability of the drug (Arnquist *et al.*, 1983; Chalk *et al.*, 1986; McEwen, 1984; Rupp *et al.*, 1972). Some have studied glibenclamide entering the circulation so quickly that a rapid distribution phase could be confused with a first elimination half-life (Rogers *et al.*, 1982). Second, some have studied normal subjects (Ings *et al.*, 1981; Neugebauer *et al.*, 1985; Sartor *et al.*, 1980b), whilst others have, like ourselves, studied diabetic patients (Matsuda *et al.*, 1983; Sartor *et al.*, 1982) and the question may be raised as to whether the observed variations in drug handling reflect alterations in gastrointestinal motility secondary to autonomic neuropathy complicating the diabetic state (Ikegami *et al.*, 1986). Third, different analytical methods have been used to measure plasma glibenclamide concentrations and the sensitivities as well as the specificities of the techniques have varied considerably.

Some studies have measured total radioactivity after giving radiolabelled drug (Balant *et al.*, 1975; Rupp *et al.*, 1972); others have determined immunoreactive glibenclamide (Balant *et al.*, 1977) or employed high performance liquid chromatography (Rogers *et al.*, 1982; Wahlin-Boll & Melander, 1979) or gas liquid chromatography (Castoldi & Tofanetti, 1979). Use of radioimmunoassay over-estimates glibenclamide-derived plasma activity because of cross-reactions with hydroxylated metabolites (Pearson, 1985). This has led to unjustified assumptions regarding possible 'third compartments' of drug distribution (Balant *et al.*, 1977). We have found that linear regression of semi-log data is adequately described by a two-compartment open model, and that there is no evidence of a further slowly-equilibrating compartment of drug distribution.

Since the sensitivities of the g.l.c. and h.p.l.c. methods of analysis allow satisfactory detection of glibenclamide signals at concentrations below 10 ng ml^{-1}, there is reasonable agreement between the published values of $t_{\frac{1}{2},\lambda_1}$, of different groups, to within a fairly narrow coefficient of variation (Peart *et al.*, 1989). In respect of the terminal phase of distribution, the position is less uniform, mainly because most studies, including our present one, have only been able to include a smaller number of sample points beyond the major kinetic segment of peak plasma drug concentrations. Some workers have unfortunately only studied the kinetic profile *in toto* for 6–10 h (Arnquist *et al.*, 1983; Castoldi & Tofanelli, 1979; Ikegami *et al.*, 1986) and so have generated data compatible with a one-compartment open model (Uihlein & Sistovaris, 1982). Ethical considerations allowed us only to include a maximum of four sample points beyond 8 h in the group I patients in whom we sought to model accurately the time-course of glibenclamide action both in kinetic and dynamic terms. An added problem in trying to compare kinetic parameters derived by different laboratories is the relative lack of information in many reports as to the precise way in which the data handling has been undertaken. Such differences in technique account for the reports of glibenclamide $t_{\frac{1}{2}}$ ranging from 1.5 h for an early distribution phase (Rogers *et al.*, 1982) to a terminal half-life of 9–10 h (Marchetti & Navalesi, 1989; Pearson, 1985). Our $t_{\frac{1}{2},\lambda_1}$ of approximately 3 h agrees with that of other workers using h.p.l.c. (Peart *et al.*, 1989; Prendergast, 1984).

The majority of the kinetic studies with oral glibenclamide have employed doses of up to 7.5 mg day^{-1} in either healthy humans or diabetic patients (Adams *et al.*, 1982; Arnquist *et al.*, 1983; Ayanoglu *et al.*, 1983; McEwen, 1984; Matsuda *et al.*, 1983; Prendergast, 1984; Sartor *et al.*, 1980b; Uihlein & Sistovaris, 1982). Our present study using dosages up to 20 mg has thus yielded similar kinetic characteristics for glibenclamide to those reported by other investigators who have used drug specific analytical methods (Adams *et al.*, 1982; Marchetti & Navalesi, 1989; Pearson *et al.*, 1986). A feature of our results has been the inter-individual variations that emerged in response to identical drug dosage, a finding highlighted also by Ikegami *et al.* (1986) and attributed by these workers to changes in drug absorption caused by diabetic autonomic neuropathy. 'Slow' and 'fast' absorbers of gliclazide have also been identified (Campbell *et al.*, 1980). We were unable to link our observed variations to the extent of neuropathy, or to differences in renal or hepatic function (Huupponen *et al.*, 1982). A possible contribution to small, delayed disturbances in the kinetic profile of glibenclamide may come from the effect of meals, as has been noted by Pearson (1985). However such effects are of small magnitude, fall within the limits of accuracy of the

h.p.l.c. drug assay, and cannot therefore account for the more striking differences in response noted between patients. The possibility of drug accumulation during chronic administration was raised by the findings of Balant *et al.* (1977), but may represent the significant contribution after multiple dosing of less-active glibenclamide metabolites to the drug assay. Our results showed no evidence of drug accumulation after repeated challenge with the same dose of glibenclamide for up to 12 weeks.

Inter-individual variations may also be the reason why it is often stated that there is no consistent dose-response relationship for glibenclamide either in respect of plasma drug concentrations or with regard to the response of blood glucose concentrations (Huupponen *et al.*, 1982; Marchetti & Navalesi, 1989; Pearson, 1985). This is particularly relevant when, in most instances, the design of reported studies has involved giving different groups of patients differing drug dosages. In the present study, the same patients were challenged individually with variable dosage, and in these circumstances we found that C_{max} and AUC(0–24) were indeed dose-dependent, and there was a similar tendency also for t_{max}, though the latter did not attain statistical significance. However, increasing drug dosage only caused marginal changes in the extent of lowering of corresponding blood glucose concentrations. This suggests that there is little to be gained in most patients by increasing doses of glibenclamide above 5 mg day^{-1} and may imply a plateau of biological response (Arnquist *et al.*, 1983; Nattrass, 1986). Such a possibility is currently being investigated further in our laboratory.

Circulating concentrations of sulphonylureas in diabetic out-patients are often far from optimal and it has been claimed that effectiveness of diabetic control might be increased further if plasma drug concentrations were monitored (Melander *et al.*, 1978; Sartor *et al.*, 1980a). Such a view presupposes effective patient compliance with prescribed regimes and, interestingly, Swift *et al.* (1979) only observed a strong positive correlation between dose and steady-state plasma concentrations of chlorpropramide in diabetic clinic out-patients after 'non-compliers' had been excluded. Our results show a wide scatter of plasma glibenclamide concentrations at similar post-dosing intervals when out-patients were compared with in-patients with a consistent tendency for mean plasma drug concentrations to be higher in out-patients than in-patients, both after 5 mg and 10 mg daily dosing.

This might, in part, reflect variations in the formulations of glibenclamide used by out-patients as well in accuracy of dosing times when patients take therapy at home as opposed to hospital. However there was no systematic difference in the formulations taken by those with the highest plasma concentrations, and timing errors alone cannot account for the highest plasma concentrations of glibenclamide seen. Another possible explanation of the high glibenclamide concentrations is sulphonamide and other drug interactions (Ryan & Oyston, 1988; Semple *et al.*, 1986) although their importance is disputed (Sjöberg *et al.*, 1987), and in any case, in-patients were taking more other medications than out-patients. Perhaps a more likely explanation of the higher plasma drug concentrations is 'over-compliance', that is, self-administration of an excessive dose of drug, by a proportion of out-patients, shortly before their clinic attendances. Such a possibility may make interpretation of plasma drug concentrations, as monitored in clinic out-patients, difficult.

In conclusion, this study has shown that food has insignificant effects on the kinetic profile of oral glibenclamide or on the effect of the drug on glucose utilisation in diabetic patients. Whereas, over the dose range 5–20 mg day^{-1}, kinetic parameters show dose-dependence, glucose utilisation is little improved by higher doses of drug. Despite a mean terminal half-life of approximately 10 h, no evidence for drug accumulation was noted after repeated administration. Comparison of steady-state plasma concentrations of glibenclamide between out-patients and in-patients revealed greater scatter and, in general, higher mean plasma drug concentrations in out-patients, implying a tendency for many patients to take excessive drug dosage in anticipation of their attendances at clinic.

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