Pharmacokinetics of Intravenous Glibenclamide Investigated by a High Performance Liquid Chromatographic Assay

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Summary. A simple high performance liquid chromatographic assay for the determination of plasma glibenclamide concentrations is described. This resolved glibenclamide from normal plasma constituents. The calibration curve of the assay was linear over the range 10–500 µg/l and the minimum level of detection was 2 µg/l. Within-assay coefficients of variation were 11.6% (20 µg/l); 5.3% (50 µg/l); 6.8% (100 µg/l); between-assay coefficients of variation were 8.4% (20 µg/l); 4.7% (50 µg/l) and 7.4% (100 µg/l). The assay was used to study the pharmacokinetics of a 1 mg intravenous dose of glibenclamide in eight normal subjects. The mean half-life was found to be 1.47 ± 0.42 h (SD) and no evidence for a non-linear β -phase or slowly equilibrating 'deep' compartment

was found although this could not be rigorously excluded. The mean systemic drug clearance was 78 ± 29 ml·h⁻¹·kg⁻¹ and the apparent volume of distribution in the β -phase was 155 ± 44 ml/kg. The median time of maximum response of plasma immunoreactive insulin was 25 min and the median time of maximum blood glucose response was 53 min. No correlation could be found between the pharmacokinetics of glibenclamide and these responses in fasted normal individuals.

Key words: Glibenclamide, pharmacokinetics, high performance liquid chromatography, plasma insulin, blood glucose.

Glibenclamide (N-4-(2-(5-chloro-2-methoxybenzamido)-ethyl)-benzenesulphonyl-N'-cyclohexylurea) is one of the most widely used of the oral hypoglycaemic sulphonylureas. Because of its high potency and low plasma concentrations its estimation has presented technical problems and even now, some 15 years after its introduction into clinical practice, there remain some unresolved questions regarding the details of its pharmacokinetics. The majority of the studies on the pharmacokinetics and metabolism of glibenclamide have been performed using radiolabelled drug [1–3] but this assay is not suitable for routine purposes. More recently radioimmunoassay techniques have been developed [4–6] although these may lack specificity because of cross-reaction with glibenclamide metabolites.

This study describes a simple high performance liquid chromatographic assay for glibenclamide and details the results of a pharmacokinetic study of the drug after administration of a low intravenous dose to normal subjects.

Subjects and Methods

Normal Volunteer Study

Eight subjects (four males; four females, aged 21–32 years; body weights 57–90 kg) gave informed consent to take part in a pharmacokinetic study, the protocol of which was approved by the Ethical

Committee of Guy's Hospital Medical School. Subjects were confirmed as normal by routine clinical chemistry and haematology tests (females also had negative urinary pregnancy tests) and were known to have normal oral glucose tolerance tests.

After 10 h of fasting, subjects were put to bed and an indwelling venous cannula for blood sampling was inserted into a forearm vein. A slow saline (0.9%) IV infusion was given into the other arm and through this 1 mg glibenclamide (Boehringer, Mannheim, FRG) was administered over 3 min. Venous blood samples for glibenclamide determination were taken before and at 1, 15, 20, 30 and 45 min and 1, 2, 4, 6, 8, 12 and 24 h after the injection. Blood was also taken at 0, 3, 6, 9, 12, 15, 20, 30, 45, 60, 90 and 120 min for estimation of blood glucose and immunoreactive insulin concentrations. Four hours after the glibenclamide injection the volunteers received a standard meal (60 g carbohydrate; 30 g protein; 30 g fat). Caffeine-containing beverages were not allowed until the evening meal but water was taken as required. Alcohol and smoking were not permitted during the study.

Glibenclamide Assay

Determination of glibenclamide in plasma was by high pressure liquid chromatography on a Magnusphere C₁₈ 5 µm reverse phase column (75 cm × 4.6 mm) supplied by Magnus Scientific, Sandbach, Cheshire, UK. Samples were applied to the column using a Rheodyne 7101 injection valve fitted with a 50 µl injection loop. The mobile phase comprised 0.05 mol/l ammonium phosphate – methanol (38:62, v/v) delivered at 0.8 ml/min by an Altex 110A pump. Detection was by absorbance at 225 nm with a Pye LC3 variable wavelength detector (Pye, Cambridge, UK). All materials used were purchased from British Drug Houses, Poole, Dorset, UK and were used as supplied except for N-desmethylglibenclamide which was a gift from Hoechst UK, Hounslow, Middlesex, UK.

Table 1. Pharmacokinetic parameters of glibenclamide in eight normal subjects following a 1 mg intravenous dose

Subject	Sex	Age (years)	Body weight (kg)	Distribution rate constant α (per h)	Disposition rate constant β (per h)	Half-life $t_{1/2}$ (h)	Systemic clearance (ml · h ⁻¹ · kg ⁻¹)	Apparent volume of distribution (ml/kg)
1	F	32	60	7.84	0.946	0.73	136	144
2	F	30	62	3.20	0.383	1.81	70	183
3	F	24	57	1.86	0.371	1.86	81	218
4	F	33	83	4.32	0.474	1.46	57	120
5	M	21	90	1.97	0.583	1.19	80	137
6	M	21	84	2.40	0.573	1.21	69	120
7	M	21	88	2.73	0.354	1.96	37	105
8	M	21	73	1.96	0.448	1.55	95	212
Mean $\pm$ SD				3.29 $\pm$ 2.02	0.517 $\pm$ 0.194	1.47 $\pm$ 0.42	78 $\pm$ 29	155 $\pm$ 44

Fig. 1. Representative plasma concentration-time profile of glibenclamide (subject 7). The solid line is a computer-fitted model to observed plasma concentrations (●) **Fig. 2.** Response of plasma insulin and blood glucose concentrations shown as medians and median absolute deviations (bars) for eight fasting subjects following 1 mg glibenclamide intravenously

Glibenclamide was extracted from plasma as follows: To 2 ml of plasma in a 15 ml glass-stoppered centrifuge tube were added 20 μ l of the internal standard solution (10 μ g/l N-desmethyl-glibenclamide in methanol) and 8 ml of dichloromethane. Extraction was performed for 20 min using a Luckhams Rolamix (Luckhams, Burgess Hill, Sussex, UK). After centrifugation the top aqueous phase was carefully discarded and the organic phase transferred to a conical glass tube and evaporated to dryness at 45 °C under nitrogen. The residue was dissolved in 80 μ l of the mobile phase and 50 μ l injected on to the column.

A calibration curve was prepared with each batch of test samples by adding known amounts of glibenclamide to blank serum and treating in the manner described above. Peak heights of glibenclamide and the internal standard were measured and a calibration curve, of the ratio of the peak height ratio of glibenclamide/peak height of internal standard versus the concentration of glibenclamide, was constructed.

Pharmacokinetic Analysis

Plasma concentration-time data were found to be adequately fitted by a model represented by the biexponential function $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ where C_t is concentration at time t , α and β are the apparent first order distribution and disposition rate constants and A and B are coefficients with the units of concentration. These parameters were estimated by fitting the model to the data by computer using a non-linear regression simplex algorithm [7]. Initial parameter estimates were provided by the exponential curve stripping program ESTRIP [8]. The half-life was found from $t_{1/2}\beta = \frac{0.693}{\beta}$.

Areas under the plasma concentration-time curve were calculated by the linear trapezoidal rule up to the last measured concentration (C_{t^*}). The curve was extrapolated to infinity ('corrected area under curve') by addition of the approximation $\frac{C_{t^*}}{\beta}$. The total body clearance (Cl) was calculated from $\frac{\text{Dose}}{(\text{corrected area under curve})}$ and the apparent volume of distribution in the β phase from $Vd\beta = \frac{Cl}{\beta}$.

Blood glucose was estimated by a glucose oxidase method [9] implemented on a Technicon Mk II analyser.

Plasma insulin was estimated using a commercial radioimmunoassay kit (Liso-phase, Lepetit, Milan, Italy). The minimum level of detection of this method is 2 mU/l with a within-assay coefficient of variation of 1.6% at 31.9 mU/l and 3.2% at 65.8 mU/l.

Results

Glibenclamide was found to be accurately resolved as a single, sharp peak. N-desmethylglibenclamide is not a

metabolite of glibenclamide and no interference was noted from normal endogenous plasma constituents or with glipizide, tolbutamide, chlorpropamide, phenformin or metformin. The elution volumes were 6.4 ml and 9.6 ml for internal standard and glibenclamide respectively. The calibration curve was linear over, at least, the range 10–500 µg/l and the minimum level of detection (giving a detector response of at least twice baseline noise) was 2 µg/l. Within-assay replicate studies give coefficients of variation of 11.6%, 5.3% and 6.8% at 20, 50 and 100 µg/l respectively ($n = 5$ for each). Between-assay coefficients of variation were 8.4% at 20 µg/l ($n = 11$); 4.7% at 50 µg/l ($n = 11$); 7.4% at 100 µg/l ($n = 11$) and 9.7% at 400 µg/l ($n = 5$).

Pharmacokinetic parameters derived for glibenclamide are shown in Table 1. Figure 1 shows a representative plasma concentration time profile and the computer-fitted model data. In only one subject (no.4) was glibenclamide detected in the plasma 12 h after this IV dose: this is consistent with the relatively short half-life proposed for this drug.

The responses of glucose and insulin to an IV dose of 1 mg glibenclamide are summarised in Figure 2. The insulin responses fell into two groups with pronounced responses in two subjects who showed peak insulin concentrations of four to five times those of the other individuals. Because of this non-normal distribution of the variates, these responses are shown as median and median absolute deviations. The median time of maximal response of plasma insulin to glibenclamide was 25 min and the median maximum blood glucose response occurred at 53 min. In no subject was it necessary to administer glucose to counter severe hypoglycaemia although four of the subjects noted tachycardia, sweating, headache and mild abdominal discomfort at about 30 min which passed off by 75–90 min after drug administration.

Discussion

The present study reports a simple high pressure liquid chromatographic assay for the estimation of plasma glibenclamide concentrations. It has comparable sensitivity to radioimmunoassay [5] but requires a larger volume of plasma. On the other hand, it requires no special reagents and is easily performed using readily available laboratory equipment. The specificity of high pressure liquid chromatography is greater than that of radioimmunoassay since cross-reactivity of antibodies raised against glibenclamide with metabolites of the drug has been a problem with radioimmunoassay [4, 5].

The sensitivity of the method presented here was sufficient to allow a pharmacokinetic investigation of the disposition of glibenclamide following a 1 mg intravenous dose. The pharmacokinetic parameters found in this study were broadly comparable with those published by others but differ in detail. It was found satis-

factory to fit the data to a two-compartment open model with linear pharmacokinetic behaviour and in this our conclusions are similar to others [1, 2]. Some studies, however, have found that the apparent half-life of total radioactivity [3] or immunoreactive glibenclamide [4] increased with time and that the plasma concentration-time profile was curved, concave upwards. On the basis of this, it was concluded that a slowly equilibrating 'deep' (in a pharmacokinetic sense) compartment was present and that on repeated administration accumulation of large amounts of drug could occur in this compartment. On multiple dosing the slow release from this compartment could account for an increase in apparent half-life of the drug after each dose [4]. It has been suggested that this prolongation of half-life with time could explain the occurrence of late hypoglycaemia many hours after taking glibenclamide [10]. Our study revealed no evidence of a slowly equilibrating deep compartment and the mean half-life was found to be only 1.47 h. This value is similar to the recent estimates of 1.7 h in fasted and 1.6 h in post-prandial normal subjects determined by radioimmunoassay [6]. Such a short half-life is consistent with the lack of accumulation of drug noted on multiple dosing [4]. However, the existence of a 'deep' compartment producing sustained plasma glibenclamide concentrations below the lower limit of detection of our assay cannot be entirely excluded. Alternatively, the presence of pharmacologically active metabolites of glibenclamide could explain the occurrence of late hypoglycaemia.

Our estimated half-life is shorter than that of 6.6 h reported for the decay of total radioactivity in the only other comparable study of glibenclamide given intravenously [1]. Studies after oral administration of the drug in larger doses (the oral bioavailability is 100% [1, 2]) give values of 5 h [1] and 10 ± 2 h [11] for the half-life of decay of total radioactivity in the plasma. These estimates are, however, relatively inaccurate being based upon graphical analysis, the slope often being determined by only two observations. In a radio-label study in which correction was made for unchanged drug by thin-layer chromatography, the half-life of glibenclamide was found to be 3.72 and 7.7 h in two normal subjects [2]. Clearly the influence of errors of analysis on pharmacokinetic interpretation must be taken into account. In a two-compartment model, if the initial concentrations are high, the later values low and the analytical method has an intrinsic fixed error, then the terminal segment of the blood concentration-time plot may curve upward [12]. This may result in erroneously long estimates of half-life.

Only one estimate for the apparent volume of distribution of unchanged glibenclamide has been published previously [2]. The estimates obtained were 9.96 and 10.71 litres for two normal subjects, which are very similar to those found in the present study and are consonant with the characteristics of a drug which is more than 97% bound to serum proteins [1].

The responses of plasma immunoreactive insulin and blood glucose were similar in magnitude and time-course to those found by others [13]. No correlation was found between the area under the plasma concentration-time curve for glibenclamide and the area under the plasma insulin response curve or the maximum increment in plasma insulin concentration. Clearly the dose response relationship between insulin and glibenclamide concentration is neither simply expressed nor direct. It has been suggested that there is a threshold concentration which must be exceeded before activity occurs and, above which, an all-or-none response occurs [14]. A complicating factor is that the stimulatory effect of glibenclamide and other sulphonylureas on insulin release increases with rising blood glucose [15]. Further studies will therefore be required to delineate these dose-response relationships: the availability of this simple assay technique should facilitate these studies.

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