

the charge balance equation, Eq. 7a). On solving the quadratic equation, Eq. 9 is obtained.

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Pharmacokinetics of Gliclazide in Healthy and Diabetic Subjects

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Abstract □ The pharmacokinetics of total and free gliclazide, 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(*p*-tolylsulfonyl)urea, a potential hypoglycemic drug, was studied in healthy ($n = 12$) and diabetic ($n = 12$) subjects. The serum level of gliclazide was determined by a high-performance liquid chromatographic method (HPLC). The free fraction of gliclazide was obtained from serum by an ultrafiltration technique using a collodion membrane. The mean adsorption of gliclazide to the membrane was ~50% when the membrane was used more than twice. Therefore, the gliclazide level in the filtrate was corrected by doubling the apparent value. The ratio of gliclazide-protein binding remained constant at ~92% in serum after administration to healthy and diabetic subjects. The mean pharmacokinetic parameters of elimination rate (k_e), time to reach the peak level (t_{max}), elimination half-life ($t_{1/2}$), and volume of distribution (V_d) were 0.07 h^{-1} , 2.8 h, 12.3 h, and 17.4 L, respectively. The parameters did not differ significantly between healthy and diabetic subjects or between single and successive administrations; moreover, they did not differ between the free and total drug level. Although there were intersubject variations, the therapeutic effects of oral administration of gliclazide on serum glucose and insulin levels were found in four diabetic patients. The results of this study show that the pharmacokinetics of the total gliclazide level reflect those of the free gliclazide in serum.

Keyphrases □ Gliclazide—protein binding in healthy and diabetic human serum □ Pharmacokinetics—serum gliclazide level after administration in healthy and diabetic subjects □ Ultrafiltration—protein binding of gliclazide in healthy and diabetic human serum

The pharmacokinetic study of serum drug levels is important in the assessment of intrinsic properties of a drug (e.g., absorption, distribution, metabolism, and excretion) to plan effective drug administration. Sulfonyleureas, such as tolbutamide and chlorpropamide, bind to several circulating serum proteins (1). In particular, serum albumin strongly interacts with many sulfonyleureas (2–4) and other drugs (5). Moreover, free sulfonyleureas are the forms that exert the pharmacological effect of hypoglycemic activity (6–8). Therefore, the pharmacokinetics of the free sulfonyleurea level in blood may be useful in programming drug administration.

The drug level in a protein-free solution must be measured to determine the free drug level in blood. Ultrafiltration (9), equilibrium dialysis (2, 3, 10), and gel filtration (11) techniques have been used to measure free drug level. Equilibrium dialysis has been commonly used to study the binding of drugs and proteins, but the time required to reach equilibration (8–24 h) is a major disadvantage. Due to simplicity, convenience, and speed, an ultrafiltration technique was used to separate the protein-free phase from serum in the present study.

In this study, a sensitive high-performance liquid chromatographic (HPLC) method (12) was used to determine the total and free levels of gliclazide (13), during a pharmacokinetic study.

EXPERIMENTAL SECTION

Subjects—Twelve male volunteers (age, 32–42 years; weight, 54–72 kg) served as test subjects. All were healthy according to clinical examinations and routine tests. Twelve patients (seven males, five females; age, 35–76 years; weight, 45–80 kg) were patients with maturity-onset diabetes mellitus (FBS: 121–302 mg/100 mL). They did not have impaired renal function, nor hepatic or endocrine disease.

Methods of Drug Administration—One tablet containing 40 mg of gliclazide¹ was administered orally before breakfast to the healthy subjects who had fasted overnight. Blood samples were obtained without an anticoagulant before and at 1, 2, 3, 4, 6, 10, and 24 h after drug administration. After the blood had clotted, the tube was centrifuged at 2500 rpm for 10 min and the supernatant serum was separated. All serum samples were stored at -20°C until use. The volunteers had regular mealtimes throughout the experiment. Twelve diabetic patients were orally administered two tablets containing 40 mg of gliclazide (therapeutic dose) in the morning; eight of them continued to take the drug (40 mg \times 2) daily for 7 d. Blood samples were obtained as

¹ Dainippon Pharmaceutical Industries Co., Osaka, Japan.

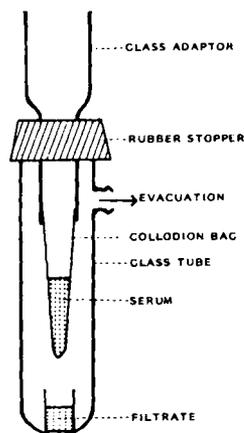


Figure 1—Ultrafiltration assembly.

described for the healthy subjects before drug administration and at 1, 2, 4, 6, 8, 12, and 24 h or 2, 6, and 24 h after administration.

Ultrafiltration—Ultrafiltration was performed using the apparatus shown in Fig. 1. A collodion bag² with an M_r cutoff of 13,200 was soaked in distilled water overnight before use. The serum samples (3 mL) were ultrafiltered under reduced pressure (40 mm Hg) at room temperature (25–27°C). The extent of drug-membrane binding was assessed with the same apparatus by substituting 0.066 M phosphate buffer (pH 7.4) containing various concentrations of gliclazide (3–20 $\mu\text{g}/\text{mL}$) for serum. The concentration of free gliclazide in the filtrate (1–2 mL) was determined by HPLC (12). The same collodion bag was used repeatedly by washing with distilled water. Gliclazide level in the filtrate was corrected by doubling the apparent concentration based on the results of a drug-absorption experiment.

Measurements of Serum Gliclazide and Blood Glucose and Insulin Levels—The serum gliclazide level was determined by HPLC³ (12). The free gliclazide level in the serum was found by determining the level in the filtrate that had passed through a collodion membrane filter at room temperature (25–27°C). Blood glucose and insulin (immunoreactive insulin) levels were measured with an autoanalyzer⁴ and a single-antibody RIA (polyethylene glycol method) (14), respectively.

Calculations—Several pharmacokinetic parameters including the elimination rate (k_e , h^{-1}), the elimination half-life ($t_{1/2}$, h), and the area under the serum drug concentration curve (AUC_{0-24} , $\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$) from 0 to 24 h were calculated by conventional methods, and the peak level (C_{max} , $\mu\text{g}/\text{mL}$) and the time to reach the peak level (t_{max} , h) were graphically estimated from the decay curve. The volume of distribution (V_d) was calculated from the following equation proposed by Gibaldi *et al.*: $V_d = \text{dose} / \int_0^\infty C_p dt \cdot k_e^{-1}$, where dose, C_p , $\int_0^\infty C_p dt$, and k_e are the dose of administered drug, the serum level of drug, the area under the serum concentration curve ($\text{AUC}_{0-\infty}$), and the elimination rate, respectively. The Student's t test was used for statistical comparisons.

RESULTS AND DISCUSSION

The ultrafiltration techniques using a membrane filter⁵ and a collodion membrane were tested for the measurement of free drug level. We chose a collodion membrane for the ultrafiltration because it was more convenient for multisample measurements and was more mechanically stable than other membranes. Many drugs, including sulfonylureas, are adsorbed on the membrane filter during ultrafiltration. We found that the degree of drug adsorption varies with use. We examined the recovery of gliclazide that had filtered through the collodion membrane (Table I). When a fresh membrane was used for the ultrafiltration, the mean recovery of gliclazide in the filtrate was ~27% of the original concentration (3–20 $\mu\text{g}/\text{mL}$). The recovery of gliclazide remained at ~50% when the membrane was used more than twice. If a constant amount of gliclazide was adsorbed in the membrane, the recovery would decrease with low concentrations, but this was not observed; thus, even when the gliclazide concentration was low, the binding constant between the drug and membrane filter remained the same. Based on the result from the

Table I—Effect of Reuse of Collodion Membrane on Recovery of Gliclazide

n^a	Recovery of Drug in Filtrate, % ^b				Mean \pm SD
	Original Conc. of Gliclazide, $\mu\text{g}/\text{mL}$				
	3	5	10	20	
1	31.1	24.5	24.3	28.3	27.1 \pm 3.3
2	51.1	45.6	51.4	49.8	49.5 \pm 2.7
3	53.7	47.9	50.3	48.5	50.1 \pm 2.6
4	56.7	40.6	—	51.5	49.6 \pm 8.2
5	50.0	30.7	—	55.8	45.5 \pm 13.1

^a Number of times membrane was used for ultrafiltration. ^b Each value represents the mean of 2–4 experiments. The ultrafiltration was carried out at room temperature (25–27°C).

drug-absorption experiment, the free gliclazide level in serum was measured by ultrafiltering the serum sample (using the collodion membrane one time). The gliclazide level in the filtrate was corrected by doubling the apparent concentration.

Although it is known that the pharmacological activity of a sulfonylurea is exerted by free drug in the blood (6), to our knowledge no information on the pharmacokinetics of free sulfonylurea level in the serum has been reported. The decay curve and the pharmacokinetic data for the free gliclazide levels in healthy and diabetic subjects are demonstrated in the present paper. The decay curves of total and free gliclazide levels in the healthy volunteers orally administered the drug (40 mg of gliclazide) are shown in Fig. 2. Individual variation in serum gliclazide level and the time to reach the peak level (t_{max}) was large. The mean values of t_{max} for the total and free gliclazide levels in the healthy subjects were 2.8 and 2.7 h, respectively (Table II). The C_{max} and AUC_{0-24} for the total gliclazide level in the healthy subjects were 2.8 $\mu\text{g}/\text{mL}$ and 37.2 $\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$ (40 mg of gliclazide). The mean ratio of free drug level (D_f) to total drug level (D_t) was ~8% during the 24-h period after gliclazide administration. We have reported that the value of C_{max} for the free gliclazide level was 0.11 $\mu\text{g}/\text{mL}$ (4) which was half the amount in the present study (0.22 $\mu\text{g}/\text{mL}$). The apparent gliclazide concentration in the filtrate was not corrected, in the former study, for the amount adsorbed to the collodion membrane during ultrafiltration.

Decay curves of blood gliclazide level in diabetic patients who took single and successive administrations of the drug (80 mg/d) are shown in Fig. 3. The decay curve in the single administration had a profile similar to that of the healthy subjects, except for the C_{max} and the drug level 24 h after drug administration. In the case of successive oral administrations of the drug for 7

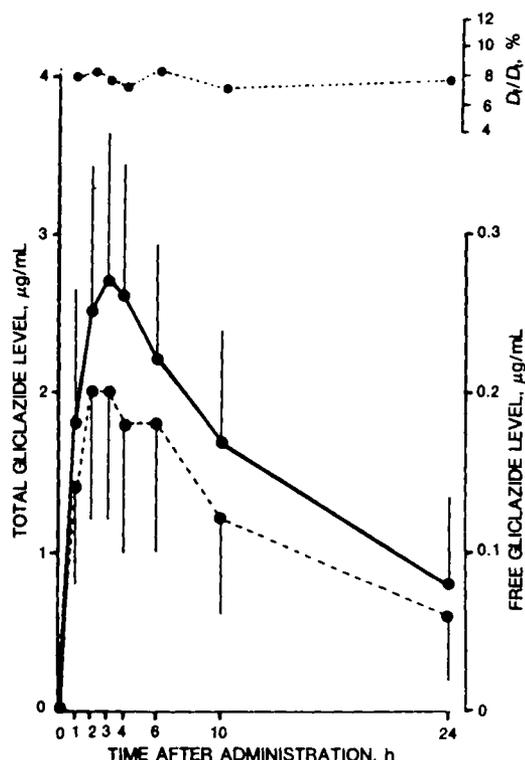


Figure 2—Decay curves of total (—) and free gliclazide levels (---) in healthy subjects ($n = 6$). Each point represents the mean; the bar, SD.

² Collodion bags, SM-13200; Sartorius GmbH, 3400 Göttingen, FRG.

³ High-pressure liquid chromatograph, TRIROTAR-II; Japan Spectroscopic Co., Ltd., Tokyo, Japan.

⁴ Autoanalyzer-II; Technicon Instruments Co., Tarrytown, N.Y.

⁵ YMB membrane MPS-1; Micropartition system; Amicon, Ltd., Mass.

Table II—Pharmacokinetic Parameters Obtained after Oral Administration of Gliclazide ^a

Method of Administration	Parameters						n
	k_e, h^{-1}	$C_{max}, \mu g/mL$	t_{max}, h	$t_{1/2}, h$	$AUC_{0-24}, \mu g \cdot h \cdot mL^{-1}$	Vd_{β}, L	
Healthy Subject							
Single Dose							
D_t	0.07 ± 0.03	2.8 ± 0.8	2.8 ± 0.9	12.3 ± 6.0	37.2 ± 13.1	17.4 ± 6.7	12
D_f	0.07 ± 0.04	0.22 ± 0.06	2.7 ± 0.8	12.0 ± 0.8	2.8 ± 1.16		6
Diabetic Subject							
Single Dose							
D_t	0.07 ± 0.02	4.5 ± 2.2	3.0 ± 1.9	11.6 ± 5.2	71.8 ± 6.8	17.9 ± 8.3	12
Successive Dose							
D_t	0.07 ± 0.01^b	7.7 ± 2.0^c	3.3 ± 1.5^b	11.4 ± 2.7^b	92.5 ± 19.5^d	14.2 ± 7.9	8

^a Values are mean \pm SD. ^b Not significantly different from single dose. ^c Significantly different from single dose ($p < 0.1$). ^d Significantly different from single dose ($p < 0.01$).

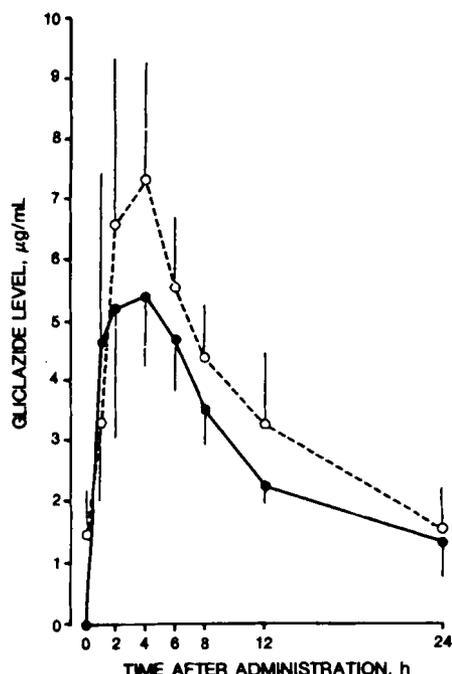


Figure 3—Decay curves of total gliclazide level in diabetic patients. Each point represents the mean \pm SD in seven patients who were administered a single dose of gliclazide (80 mg) (●), and four patients who took successive administration of gliclazide (80 mg/d) for 7 d (○).

d, $t_{max} = 3.3$ h, was longer compared with 3.0 h for the single administration. The subjects also had higher levels of C_{max} ($7.7 \mu g/mL$) and AUC_{0-24} ($92.5 \mu g \cdot h \cdot mL^{-1}$) (Table II).

The decay curves of total and free serum gliclazide levels were examined in diabetic patients (Figs. 4A and B). A perfect decay curve for the free gliclazide level in the diabetic patients was not obtained because the amount of serum sample required for the ultrafiltration could not be repeatedly taken. Thus, the pharmacokinetic parameters could not be obtained by computer calculation (Table II). From the available data, the value of t_{max} for total drug level in the diabetic patients was almost equal to that in the healthy volunteers.

The total gliclazide levels reached a maximum ($\sim 7.7 \mu g/mL$) 2 h after successive administration, which was higher than that obtained by single administration ($4.5 \mu g/mL$ at 6 h). The values of C_{max} for free gliclazide levels were nearly equal for the different administration methods ($0.47-0.55 \mu g/mL$). The ratio of free drug level (D_f) to total drug level (D_t) in the diabetic subjects administered a single dose was $\sim 8-9\%$, but the ratio in the diabetic subjects administered successive doses was lower ($\sim 7\%$).

The apparent volume of distribution (Vd) is important in the pharmacokinetic characterization of drugs. However, the calculation of volume of distribution is based on many assumptions and cannot, therefore, be estimated with a high degree of accuracy. Gibaldi *et al.* demonstrated that the volume of distribution was identical to the apparent volume of distribution obtained from the area under the serum concentration curve (AUC) determined by the equation for a two-compartment (α and β) open system (15). The volume of distribution (Vd_{β}) of gliclazide in healthy and diabetic subjects was $14.2-17.9$ L (Table II). There was large intersubject variation in Vd but this could be due to variations in total adsorption that is known to occur with sulfonlureas. The distribution of gliclazide with high protein binding was expected to be confined more to the serum or extracellular fluid volume, as is the case of tolbutamide and chlorpropamide (16).

Effects of oral administration of gliclazide on blood glucose and insulin levels in four diabetic patients (A, B, C, and D) are summarized in Table III. The range of fasting blood glucose levels for the patient was $100-302$ mg/100 mL. Although there are intersubject variations, the values of 24-h cumulative blood glucose levels were lowered by the administration of gliclazide ($\Delta, -177$ to -1081 mg/100 mL, determined eight times in 24 h). On the other hand,

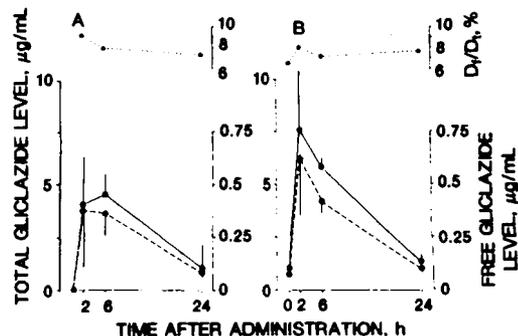


Figure 4—Decay curves of total (—) and free gliclazide levels (---) in diabetic patients, who took single (A) and successive administrations (B) of gliclazide. Each point represents the mean \pm SD in 3-4 diabetic patients.

Table III—Effect of Oral Administration of Gliclazide on Blood Glucose and Insulin Levels in Diabetic Patients

Subject	FBS, mg/100 mL ^a	Σ BGS, mg/100 mL ^a			Σ IRI, $\mu U/mL^a$			Σ Drug, $\mu g/mL^a$
		Predose	Postdose	Δ	Predose	Postdose	Δ	
A	302	3511	2430	-1081	128	221	+93	35.8
B	100	885	708	-177	—	—	—	26.1
C	224	2153	1406	-747	97	127	+30	24.7
C* ^b	224	2153	1176	-977	97	133	+36	32.2
D	121	1633	1075	-558	—	—	—	31.9

^a FBS shows the fasting blood glucose level. Σ BGS, Σ IRI, and Σ Drug, respectively, shows the sum level of blood glucose, immunoreactive insulin, and serum gliclazide determined eight times through 24 h. ^b C* is a diabetic patient administered gliclazide successively for 7 d.

the values of 24-h cumulative blood insulin levels were increased by the administration of the drug (Δ , 30–93 μ U/mL), resulting in the stimulation of insulin release from pancreatic B cells in all cases. The difference between single and successive administration on cumulative blood glucose levels and cumulative blood insulin levels was not significant in patient C (Table III, footnote b).

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Secondary Antithyroid Action of Drugs in Relation to Structure

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Received July 13, 1982, from the *Department of Chimica Therapeutica, Faculty of Medicine and Pharmacy, 87032 Limoges Cedex, France.* Accepted for publication November 29, 1983.

Abstract □ Molecular interactions between iodine and disulfiram, clomethiazole, and tolinaftate were investigated by electron spectroscopy. Iodine forms charge transfer complexes with these molecules, with 1:1 stoichiometry and of the $n-\sigma$ type. The formation constants were compared with those obtained with antithyroid molecules. Only disulfiram appears to have any effect on the intrathyroid cycle of iodine.

Keyphrases □ Charge transfer complexes—iodine and disulfiram, clomethiazole, tolinaftate □ Disulfiram—charge transfer complexes with iodine □ Clomethiazole—charge transfer complexes, iodine □ Tolinaftate—charge transfer complexes, iodine

It has been shown that molecules possessing an NCS moiety can form charge transfer complexes with iodine (1, 2). Both qualitative and quantitative studies have shown that certain antithyroid drugs (those possessing the NCS function) form charge transfer complexes involving the transfer of charge from the pair of free electrons on the nitrogen and/or the sulfur atoms to the antibonding orbital of the iodine (3, 4). The intensity of this action can be determined from the complex formation constant and the thermodynamic parameters. A correlation has been demonstrated between the constant (K_c) and antithyroid activity (5). A structure–activity relationship has been developed to classify all known antithyroid molecules (6). Iodine fixation by complex formation is one action mechanism of synthetic antithyroid agents.

Synthetic antithyroid drugs can also inhibit peroxidase (7). This enzyme is necessary for the oxidation of circulating iodine, for its integration into thyroglobulin, and for coupling monoiodotyrosines and diiodotyrosines to form triiodotyrosines (T_3) and tetraiodothyronines (T_4). While antithyroid agents

display variable activity towards peroxidase, they can all complex iodine, so that the latter is unavailable for thyroid hormone synthesis.

This NCS function is found in many drugs belonging to other therapeutic classes. Hence, if we hope to understand biological activity it is important to investigate the possible formation of complexes between these molecules (tolinaftate, disulfiram, and clomethiazole) and iodine. This can help determine whether these molecules possess secondary antithyroid activity.

EXPERIMENTAL SECTION

Materials—An ultrapure iodine was prepared by sublimation and stored in a desiccator containing P_2O_5 . Disulfiram¹ [tetraethylthioperoxydicarbonic diamide (I)], tolinaftate² [*O*-2-naphthyl-*m*,*N*-dimethylthiocarbonyl (II)], and clomethiazole³ [5-(2-chloroethyl)-4-methylthiazole (III)] were pharmaceutical grade; purity was determined by HPLC⁴. UV-grade carbon tetrachloride⁵ was used.

UV and visible spectra were recorded on a double-beam spectrophotometer⁶ equipped with a Peltier effect thermoelectric cell holder. Hellma quartz cells with a path length of 1 cm were employed.

Methods—The glassware was thoroughly dried with dry nitrogen to eliminate any effects due to hydration of the complex solutions. Volumetric solutions were prepared from initial solutions obtained by weighing. The spectra were recorded immediately after solution preparation.

¹ Millot; Solac Laboratories, Paris, France.

² Unicet; Cetranc Laboratories, Levallois-Perret, France.

³ Debat Laboratories, Paris, France.

⁴ Model 244 U/45; Waters, S.A. Paris, France.

⁵ Merck uvasol Art. 2209; E. Merck, Darmstadt.

⁶ Model 554 UV-Vis; Perkin-Elmer.