DOSE LINEARITY ASSESSMENT OF GLIMEPIRIDE (AMARYL®) TABLETS IN HEALTHY VOLUNTEERS

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SUMMARY

Twelve healthy fasting male volunteers received glimepiride in 1, 2, 4 or 8 mg single oral doses. On the days when glimepiride was taken, the subjects were given a standardised carbohydrate diet (18 bread exchange units) and drank 125 ml of water hourly. Blood and urine samples were taken before drug administration and afterwards for up to 36 hours (blood) and 48 hours (urine) to determine serum and urinary concentrations of glimepiride and its hydroxy- and carboxy-metabolites (M1 and M2). The areas under the curve for glimepiride after oral doses of 1 to 8 mg and the urinary recovery of its metabolites M1 and M2 were dose linear. All confidence intervals were well contained within the bioequivalence range of 80-125%. There was a statistically significant difference for C_{max} values of glimepiride between doses after dose normalisation. A dose-dependent increase for C_{max} was nevertheless clearly observed with a correlation coefficient of $r=0.90$. The pharmacokinetics of glimepiride are dose linear in the dose range 1 to 8 mg, and glimepiride was safe and well tolerated in healthy volunteers.

KEY WORDS

dose linearity, glimepiride, healthy volunteers, pharmacokinetics

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INTRODUCTION

Glimepiride (Amaryl®) is a new oral hypoglycaemic agent of the sulphonylurea class. As with other sulphonylureas, glimepiride appears to lower plasma glucose levels by stimulating the release of insulin from the pancreas /1,2/. Glimepiride is completely bioavailable /3/, and highly plasma protein bound (99.4%) /4/.

When administered orally to healthy volunteers in increasing doses of up to 8.0 mg, the minimum dose for hypoglycaemic effect was observed at 0.5 mg /5/. Single doses of up to 2.0 mg, given intravenously to healthy volunteers, and multiple oral doses of between 1 and 32 mg daily in patients with non-insulin dependent diabetes mellitus were safe and well tolerated /5,6/.

Elimination of glimepiride is by formation of two main metabolites, the hydroxy-metabolite M1 and the carboxy-metabolite M2, which are excreted renally /4,7,8/. After oral and intravenous administration, approximately 50% of the drug is recovered in the urine as the sum of M1 and M2 /3/. Animal pharmacology studies have shown M1 to exhibit some hypoglycaemic effect in rats, although this was three times less than that of the parent drug. M2 showed no pharmacological activity /9/.

The aim of this study in healthy volunteers was to determine whether ascending single oral doses of glimepiride over the therapeutic range (1 to 8 mg) exhibit dose linearity.

METHODS

This double blind, four period cross-over study was conducted at a single centre in Austria. Healthy male volunteers, aged between 18 and 40 years, with normal physical examinations and normal ECGs, chest X-rays and laboratory values, were considered for entry into the study. Inclusion criteria required subjects to have a normal oral glucose tolerance test in the last six months and a body weight within + 10% and – 15% of normal weight according to Broca (Broca's formula for normal weight = height in cm – 100).

Subjects who had a serious medical disease in the four weeks prior to the study or who had the presence or history of gastrointestinal, hepatic or renal disease which could interfere with drug pharmacology were excluded from the study, as were subjects known to be abusing

alcohol or who had a hypersensitivity to sulphonylureas or a related compound. Subjects were also excluded from the study if they were receiving, or had received prior to the start of the study, any treatment which could interact with glimepiride or the assessment of its bio-availability.

The trial protocol was approved by the local ethics committee. All volunteers gave their written consent to participate in the trial.

Treatment

On entry into the study, subjects were randomised, according to a Latin square design, to receive single oral doses of glimepiride 1, 2, 4 or 8 mg with 150 ml of water. A washout period of 7 to 14 days occurred between doses. Each subject received each dose of glimepiride using the following double blind design: 1 mg dose = one 1 mg glimepiride tablet + one placebo tablet; 2 mg dose = two 1 mg glimepiride tablets; 4 mg dose = one 4 mg glimepiride tablet + one placebo tablet; 8 mg dose = two 4 mg glimepiride tablets.

On the day before dosing, food and fluid intake were standardised in order to obtain a standard baseline situation across all volunteers. On the morning of the four main trial days, the subjects reported to the Clinical Pharmacology Unit after an overnight fasting period of 12 hours. Thirty minutes prior to drug administration, an indwelling cannula was fixed into a suitable vein and was left in place for up to 24 hours after medication. The total amount of blood withdrawn for the trial including the safety pharmacology was about 500 ml over the study period.

To avoid hypoglycaemia, the subjects received a standardised carbohydrate diet on the main trial days. Only carbohydrates which were not rapidly absorbed (e.g. rye bread, fruit and vegetables) were used in the subjects' diet. Carbohydrate intake was measured in bread exchange units (BEU) (1 bread exchange unit = 12 g carbohydrate) and subjects received a total of 18 bread exchange units as follows:

2 hours after medication: 3 BEU with 250 ml herbal tea (breakfast)
4 hours after medication: 4 BEU (lunch)
7 hours after medication: 3 BEU with 250 ml herbal tea (tea-time)
10 hours after medication: 4 BEU (dinner)
12 hours after medication: 2 BEU (snack)
14 hours after medication: 2 BEU (snack)

The subjects drank 125 ml of water hourly up to 12 hours after dosing; fluid intake was then unrestricted. At breakfast and tea time, the 125 ml of water was replaced by 250 ml of herbal tea. The subjects remained seated for the first four hours after dosing. Strenuous activity was not permitted at any time.

Assessments

Blood samples (5 ml) were taken five minutes before administration of glimepiride and 15, 30, 45 minutes, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32 and 36 hours afterwards.

Total urine output was collected two hours before administration of glimepiride and afterwards at the following time intervals: 0-2, 2-4, 4-8, 8-12, 12-24 and 24-48 hours.

Serum and urinary concentrations of glimepiride and the two main metabolites, M1 and M2, were determined by high-performance liquid chromatography (HPLC) /10/.

Clinical chemistry, haematology and urinalysis tests were performed at screening, before the first dose and 48 hours after the last dose of glimepiride. Monitoring of vital signs (blood pressure and heart rate) was carried out, after at least 10 minutes in the recumbent position, before medication and 1, 2, 3, 4, 6, 8, 10 and 24 hours after medication. Twelve-lead ECGs were performed at screening and 24 hours after each administration of glimepiride. Any adverse events which occurred during the trial were noted. For safety purposes, blood glucose concentrations were measured by the hexokinase method in all blood samples during the trial and, if considered necessary by the investigator, blood glucose was also determined by means of the 'Reflolux II' rapid analysis technique (Haemo-Glukotest® 20-800R, Boehringer Mannheim, Germany).

Data analysis

The following model-independent pharmacokinetic parameters for glimepiride and its metabolite M1 in serum were calculated: maximum serum concentration (C_{max}), time to peak concentration (t_{max}), area under the concentration time data completed by extrapolation (AUDC), dominant half-life ($t_{1/2}$), terminal half-life ($t_{1/2.z}$) and relative total clearance (CL).

The terminal half-life ($t_{1/2,z}$) of a compound may often be observed as apparently increasing with increasing doses. This is either because of an insufficient sampling scheme or because the limit of detection is too high to follow the time course of the compound for the time needed to evaluate a real terminal phase. Since this was a study of ascending single oral doses of glimepiride, the evaluation of a terminal phase was questionable and likely to show a high degree of variation due to the effects described above. The dominant half-life ($t_{1/2}$) was therefore also calculated as this was liable to give more consistent and reliable results.

Urinary recovery of the metabolites M1 and M2 was expressed in mg and as a percentage of the dose of glimepiride. The latter was calculated as the sum of the excreted metabolites M1 and M2 after molecular weight correction.

The determination of dose linearity was based on C_{max}, the AUDC for glimepiride and on the urinary recovery of metabolites M1 and M2. C_{max} and AUDC were normalised to the highest dose by multiplication of the original values with the factors 8, 4, 2 and 1 for the 1, 2, 4 and 8 mg doses, respectively. Urinary recovery was based on absolute amounts. The data were tested by an analysis of variance (ANOVA); the level of significance was set at p=0.05%. Point estimates and the corresponding conventional 90% confidence intervals were calculated /11/. The point estimates of the ratios would be 100% for perfect dose linearity. Linear least-squares regression analysis was performed on the original (non-normalised) dose related parameters.

RESULTS

Twelve healthy white male volunteers participated in the study. Mean age of the subjects was 28 years (range 23-32), mean weight 78 kg (64-98) and mean height 178 cm (173-189). All subjects received the appropriate dose of glimepiride on each of the four main study days and completed all the examinations.

Pharmacokinetics in serum and urine

Profiles of the mean serum concentrations for glimepiride are plotted in Figure 1. The mean pharmacokinetic parameters (± SD) for glimepiride and M1 are given in Table 1. No pharmacokinetic parameters were calculated for M2 since most of the serum

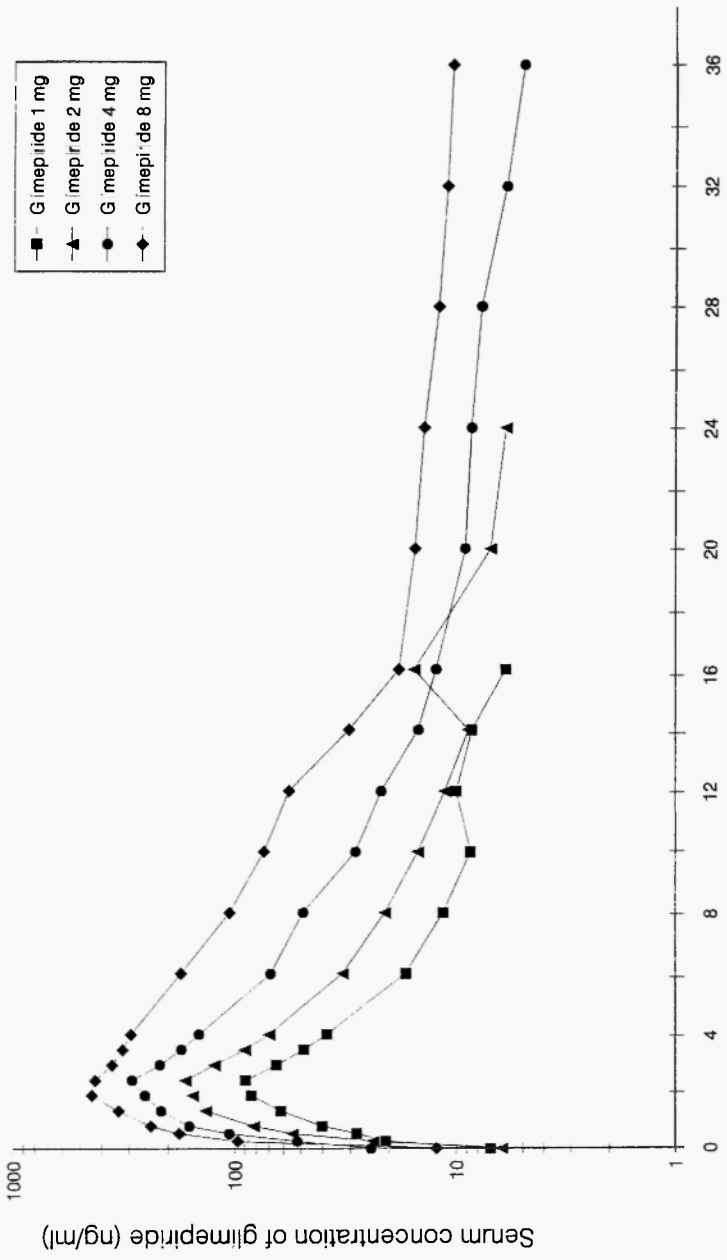

Fig. 1: Mean serum concentrations of glimepiride following oral 1, 2, 4 and 8 mg doses.

TABLE 1
Pharmacokinetic parameters of glimepiride and M1 [mean (± SD)]

	Glimepiride				M1			
Parameter	**1 mg**	**2 mg**	**4 mg**	**8 mg**	**1 mg**	**2 mg**	**4 mg**	**8 mg**
C_{max} (ng/ml)	103.2 (± 34.3)	176.8 (± 44.1)	307.8 (± 69.4)	550.8 (± 151.9)	24.0 (± 5.7)	42.1 (± 9.6)	76.7 (± 19.9,	135.9 (± 40.4)
t_{max} (h)	2.3 (± 0.5)	2.4 (± 0.5)	2.1 (± 0.6)	2.8 (± 1.2)	2.8 (± 0.8)	2.8 (± 0.5)	3.3 (± 0.7)	3.4 (± 1.1)
$t_{1/2}$ (h)	1.2 (± 0.5)	1.3 (± 0.4)	1.5 (± 0.5)	1.5 (± 0.4)	3.1 (± 0.9)	2.5 (± 0.7)	3.0 (± 0.8)	3.2 (± 1.1)
$t_{1/2,z}$ (h)	3.2 (± 1.4)	3.6 (± 1.4)	5.6 (± 4.1)	8.8 (± 4.1)	-	-	-	-
AUDC (ng.h/ml)	326 (± 97)	668 (± 178)	1297 (± 303)	2634 (± 890)	133 (± 48)	210 (± 56)	479 (± 172)	853 (± 250)
CL (ml/min)	55.3 (± 16.3)	53.5 (± 15.5)	53.6 (± 10.6)	56.5 (± 21.1)	146 (± 72)	170 (± 48)	166 (± 90)	169 (± 47)

concentration values were below or close to the detection limit for the 1 mg and 2 mg doses.

Unchanged glimepiride was detectable in the urine of one subject after 2 mg, five subjects after 4 mg and nine subjects after 8 mg. Concentrations above the detection limit were mainly measured in the urinary fraction 2-4 hours after dosing, with values between 23 ng/ml and 124 ng/ml. The recovery of unchanged glimepiride ranged from 0.03% to 0.27% of the dose administered. The mean urinary recovery of the sum of M1 and M2, after molecular weight correction, expressed as a percentage of the dose is shown in Figure 2.

Dose linearity of glimepiride

The C_{max} values of glimepiride normalised to the highest glimepiride dose (8 mg) were 825 ng/ml for the 1 mg dose and 707, 616 and 551 ng/ml for the 2, 4 and 8 mg doses, respectively. The point estimates and 90% confidence intervals for the ratios of the respective doses with respect to the true mean C_{max} of glimepiride normalised for the 8 mg dose are shown in Table 2. ANOVA showed a statistically significant difference between doses after dose normalisation. Linear regression analysis of C_{max} versus dose produced a coefficient of correlation of r=0.90. The estimated intercept was calculated at 58 ng/ml and differed significantly from zero.

The AUDC values of glimepiride normalised to the highest glimepiride dose of 8 mg were 2612 ng.h/ml for the 1 mg dose and 2673, 2593 and 2634 ng.h/ml for the 2, 4 and 8 mg doses, respectively. The point estimates and 90% confidence intervals for the ratios of the respective doses with respect to the true mean AUDC of glimepiride normalised for the 8 mg dose are shown in Table 3 and Figure 3. ANOVA did not show statistically significant differences between doses. The power of the test (i.e. the probability of detecting a difference of 20% between two normalised doses) was 83%. Linear regression analysis of AUDC versus dose produced a coefficient of correlation of r=0.88 (Figure 4). The estimated intercept was calculated at 68 ng.h/l and did not differ significantly from zero.

Mean urinary recovery of the sum of M1 and M2 (after molecular weight correction) expressed as a percentage of the dose was 45.9% for the 1 mg dose and 50.7%, 48.6% and 46.0% for the 2, 4 and 8 mg doses, respectively. The point estimates and 90% confidence intervals for the ratios of the respective doses with respect to the true mean

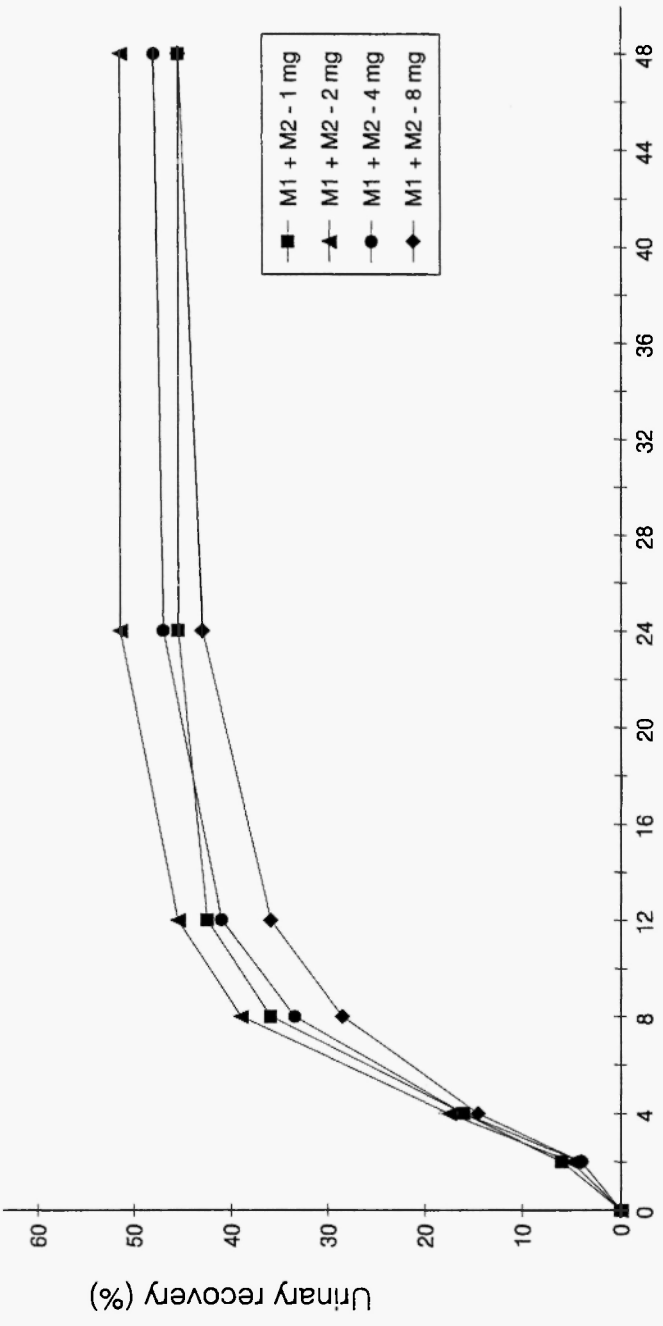

Fig. 2: Mean urinary recovery of the sum of M1 and M2 as a percentage of glimepiride dose (after molecular weight correction).

TABLE 2
Point estimates and 90% confidence intervals for C_{max} ratios of glimepiride normalised to the 8 mg dose

Normalising factor	x	Dose (mg)	:	Normalising factor	x	Dose (mg)	Point estimate (%)	90% confidence interval (%)
8	x	1		1	x	8	150	130-170
4	x	2		1	x	8	130	109-148
2	x	4		1	x	8	114	92-131
8	x	1		2	x	4	131	116-152
4	x	2		2	x	4	114	97-132
8	x	1		4	x	2	115	101-132

TABLE 3
Point estimates and 90% confidence intervals for AUDC ratios of glimepiride normalised to the 8 mg dose

Normalising factor	x	Dose (mg)	:	Normalising factor	x	Dose (mg)	Point estimate (%)	90% confidence interval (%)
8	x	1		1	x	8	103	91-113
4	x	2		1	x	8	106	93-115
2	x	4		1	x	8	103	89-111
8	x	1		2	x	4	100	91-114
4	x	2		2	x	4	103	93-116
8	x	1		4	x	2	97	84-112

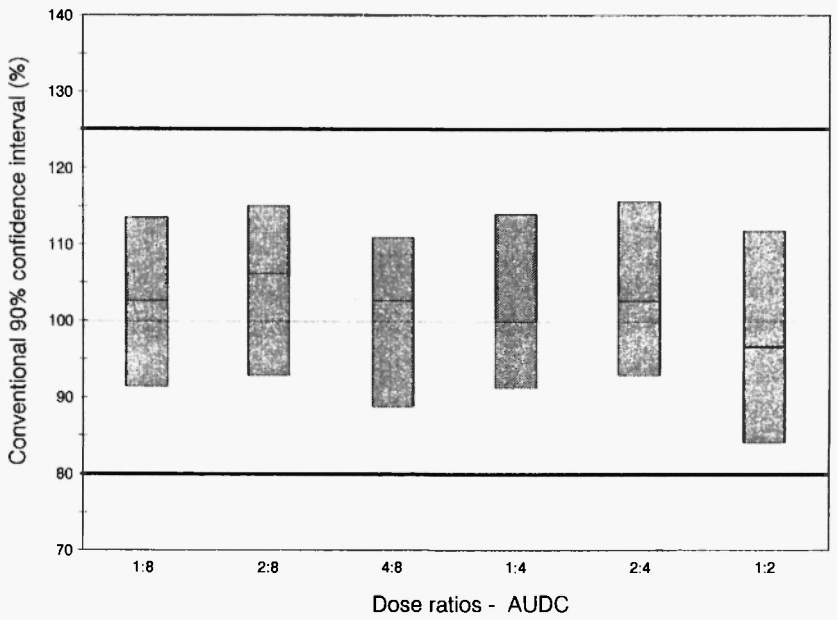

Fig. 3: Confidence intervals for the different dose ratios of glimepiride AUDC.

urinary recovery, expressed as a percentage of the dose, are given in Table 4 and Figure 5. ANOVA did not show statistically significant differences between doses. The power of the test to detect a difference of 20% was 91%. Linear regression analysis of the urinary recovery of metabolites M1 and M2 expressed in absolute amounts (mg) produced a coefficient of correlation of r=0.97 (Figure 6). The estimated intercept was calculated at 0.07 mg and did not differ significantly from zero.

Safety

One subject experienced headache and vomiting 7.5 hours after receiving 1 mg glimepiride in the second period of the trial. The symptoms were moderate and lasted for 3.5 hours. His blood glucose was 4.44 mmol/l (normal range: 3.61-5.55 mmol/l) at the time of the event. The patient had a history of frequent headaches.

There were no clinically important or drug-related changes in vital signs, laboratory tests or urinalysis for any of the subjects.

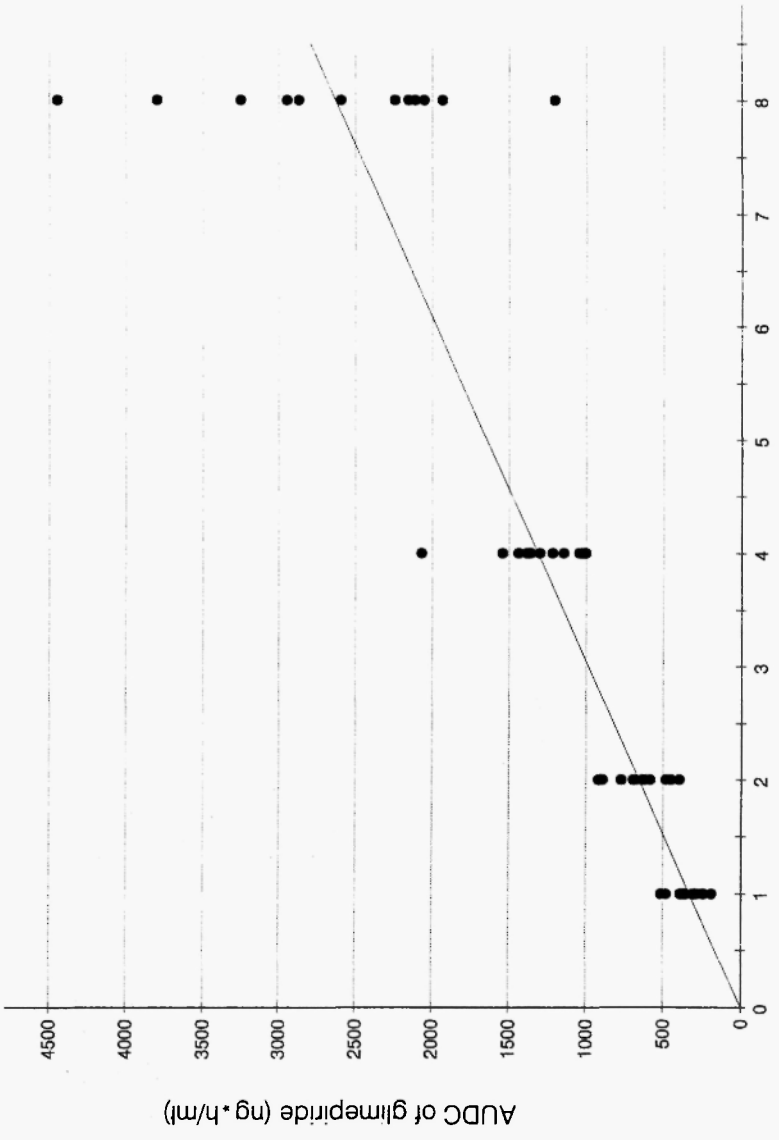

Fig. 4: Linear regression analysis of glimepiride AUDC versus dose.

TABLE 4
Point estimates and 90% confidence intervals for urinary recovery of M1 and M2 expressed as a percentage of the glimepiride dose

Dose (mg)	:	Dose (mg)	Point estimate (%)	90% confidence interval (%)
1		8	100	90-110
2		8	91	82-100
4		8	95	85-104
1		4	106	96-116
2		4	96	87-105
1		2	111	100-120

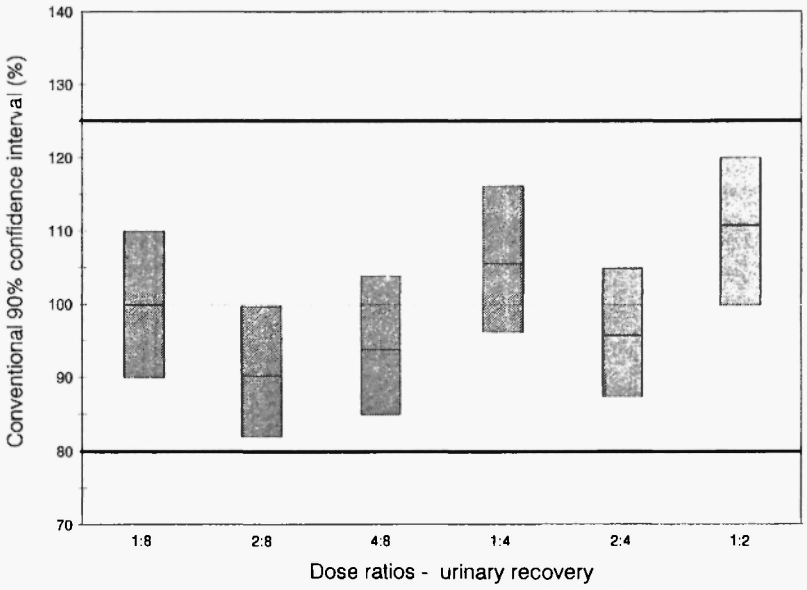

Fig. 5: Confidence intervals for the different dose ratios of the urinary recovery of the sum of M1 and M2.

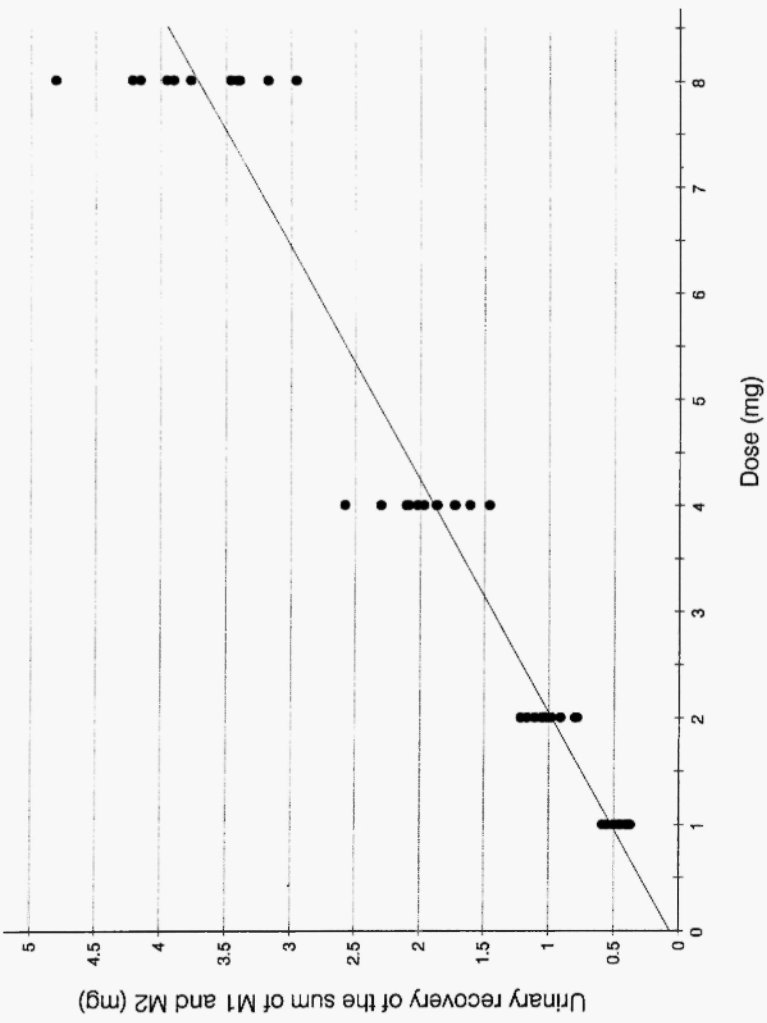

Fig. 6: Linear regression analysis of urinary recovery of metabolites M1 and M2 expressed in absolute amounts (mg).

Measurements of the mean blood glucose levels for safety purposes showed a dose related decrease in blood glucose. Although one subject recorded an extremely low blood glucose value of 0.89 mmol/l 90 minutes after administration of the 8 mg glimepiride dose, there were no hypoglycaemic episodes which required countermeasures in any of the subjects.

DISCUSSION

In this double blind cross-over study of healthy male volunteers, the AUDC for glimepiride after oral doses of 1 to 8 mg, normalised for dose, exhibited dose linearity. A close relationship between dose, C_{max} and AUDC was also seen for M1. Pharmacokinetic parameters were not calculated for M2 since most of the serum concentration values were below or close to the detection limit for 1 mg and 2 mg doses. However, as M2 is inactive, no contribution would have been made to the study by such calculations.

Although unchanged glimepiride was recovered in the urine of nine subjects in the collection period 2-4 hours after dosing, the levels were negligible, representing 0.03% to 0.27% of the dose administered. Mean urinary recovery of the sum of M1 and M2 expressed as a percentage of the dose ranged from 45.9% to 50.7% for glimepiride doses of 1 to 8 mg. The dose linearity observed with AUDC measurements was supported by urinary recovery of metabolites M1 and M2. All the confidence intervals calculated for glimepiride AUDC and urinary recovery of the sum of M1 and M2 were well contained within the defined bioequivalence range of 80-125% representing dose linearity.

A dose-dependent increase for the C_{max} of glimepiride was clearly observed with a linear regression coefficient of r=0.90. However, there was a statistically significant difference in glimepiride peak serum concentrations between doses (1, 2, 4 and 8 mg) after dose normalisation. This was not unexpected in the light of previous studies of glimepiride tablets in healthy volunteers which have shown certain intra-individual and inter-individual variability in the rate of absorption of glimepiride /5/. These differences in C_{max} between doses, whether statistically significant or not, should not be relevant from a therapeutic viewpoint.

As previously observed in other studies /3,12/, glimepiride was well tolerated by the healthy volunteers. There were no clinically important or drug-related changes in vital signs, laboratory tests or urinalysis for any of the subjects. One subject with a frequent history of headache experienced moderate headache and vomiting approximately 8 hours after 1 mg of glimepiride; however, no headache or vomiting was reported for this subject after the 2, 4 or 8 mg doses of glimepiride.

In a previous pilot study in which volunteers were given 3, 5 and 8 mg doses of glimepiride, a special diet based on bread exchange units (to enable calculation of carbohydrate intake) was found to be important in avoiding hypoglycaemia for volunteers on higher doses /5/. A similar technique was used in the present study. Although subjects had dose-related decreases in blood glucose, none of the subjects experienced hypoglycaemic episodes which required counter-measures, showing that the diet was well chosen.

In conclusion, the pharmacokinetics of glimepiride is dose linear in the dose range 1 to 8 mg, and glimepiride was safe and well tolerated in healthy volunteers.

REFERENCES

1. Geisen K. Special pharmacology of the new sulfonylurea glimepiride. Arzneim Forsch Drug Res 1988; 38: 1120-1129.
2. Leclercq-Meyer V, Akkan AG, Marchand J, Malaisse WJ. Effects of glimepiride and glibenclamide on insulin and glucagon secretion by the perfused rat pancreas. Biochem Pharmacol 1991; 42: 1634-1637.
3. Badian M, Korn A, Lehr K-H, Malerczyk V, Waldhäusl W. Determination of the absolute bioavailability of glimepiride (HOE490), a new sulfonylurea. Clin Pharmacol Ther Toxicol 1992; 30: 481-482.
4. Hoechst AG. Glimepiride. Drugs Future 1992; 17: 774-778.
5. Data on file, Hoechst AG.
6. Ratheiser K, Korn A, Waldhäusl W, et al. Dose relationship of stimulated insulin production following intravenous application of glimepiride in healthy man. Arzneim Forsch Drug Res 1993; 43: 856-858.
7. Weyer R, Hitzel V. Acylureidoalkylphenylsulfonylureas with blood glucose lowering efficacy. Arzneim Forsch Drug Res 1988; 38: 1079-1080.
8. Badian M, Korn A, Lehr K-H, Malerczyk V, Waldhäusl W. Pharmacokinetic interaction between propranolol and glimepiride in healthy volunteers. Klin Pharmakol akt 1993; 4: 25 (Abstract P1.17).
9. Donaubauer HH, Mayer D. Acute, subchronic and chronic toxicity of the new sulfonylurea glimepiride in rats. Arzneim Forsch Drug Res 1993; 43: 547-549.

10. Lehr K-H, Damm P. Simultaneous determination of the sulphonylurea glimepiride and its metabolites in human serum and urine by high-performance liquid chromatography after pre-column derivatization. J Chromatogr 1990; 526: 497-505.
11. Locke CS. An exact confidence interval from untransformed data for the ratio of the two formulation means. J Pharmacokinet Biopharm 1984; 12: 549-655.
12. Badian M, Korn A, Lehr K-H, Malerczyk V, Waldhäusl W. Pharmacokinetics and pharmacodynamics after intravenous administration of the hydroxymetabolite (M1) of glimepiride (HOE490). 34th Spring Meet Dtsch Ges Pharmakol Toxikol, Mainz (March 1993): in Abstracts 1993; R27: abstract 105.