PHARMACOKINETICS AND PHARMACODYNAMICS OF THE HYDROXY-METABOLITE OF GLIMEPIRIDE (AMARYL®) AFTER INTRAVENOUS ADMINISTRATION

M. Badian[1], A. Korn[2], K.-H. Lehr[1], V. Malerczyk[1] and W. Waldhäusl[2]

[1]*Hoechst AG, Frankfurt/M, Germany*
[2]*Medizinische Universitätsklinik, Vienna, Austria*

SUMMARY

Glimepiride is a new sulphonylurea which is eliminated by the formation of a hydroxy-metabolite (hydroxy-gli) and a carboxy-metabolite (carboxy-gli). Animal studies have shown hydroxy-gli to exhibit some hypoglycaemic effects while carboxy-gli does not appear to have any pharmacological activity. Pharmacokinetic and pharmacodynamic effects of hydroxy-gli were assessed in humans. 12 healthy male volunteers received an intravenous injection of hydroxy-gli (1.5 mg) or placebo in a single blind, randomised, cross-over study. Samples were collected for up to 24 hours (blood) or 48 hours (urine) following administration of hydroxy-gli or placebo. Hydroxy-gli significantly decreased the minimum serum concentration (C_{min}) of glucose by 12% and the average serum glucose concentration over the first four hours of treatment (C_{avg0-4}) by 9% compared with placebo ($p \leq 0.05$). In addition, maximum serum C-peptide concentration (C_{max}) and C_{avg0-4} were both increased by 7% after hydroxy-gli ($p < 0.05$). Serum insulin concentrations (C_{max} and C_{avg0-4}) increased by 4% but the differences from placebo were not statistically significant. No adverse events were reported during the study. In conclusion, the hydroxy-metabolite of glimepiride shows pharmacological activity in human subjects.

Author for correspondence:
M. Badian
Hoechst Aktiengesellschaft
Clinical Research, H 840
D-65926 Frankfurt am Main
Germany

KEY WORDS

pharmacokinetics, pharmacodynamics, glimepiride, hydroxy-glimepiride, healthy volunteers

INTRODUCTION

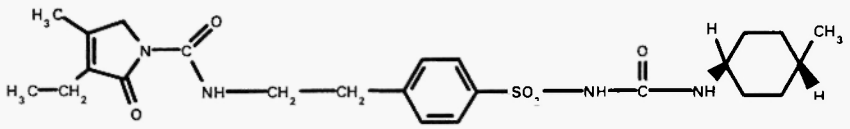
Glimepiride (Amaryl®) is a new oral hypoglycaemic agent of the sulphonylurea class /1/ which is under clinical investigation for the treatment of patients with type II non-insulin dependent diabetes mellitus. The drug appears to lower plasma glucose levels by stimulating the release of insulin from the pancreas, its effect dependent upon functioning beta cells in the pancreatic islets /2/.

Glimepiride is characterised by complete bioavailability /3/, a high degree of protein binding /4/, and dose linear pharmacokinetics /5/. Elimination of glimepiride is by the formation of two metabolites, a hydroxy-metabolite (hydroxy-gli) and a carboxy-metabolite (carboxy-gli), both of which are excreted renally /4,5/. The structures of glimepiride and its two main metabolites are shown in Figure 1. The hydroxy derivative is formed by oxidation of the methyl group in the cyclohexyl ring of the molecule whereas the carboxy derivative is derived from the hydroxy metabolite by further oxidation. Following oral or intravenous administration, approximately 50% of the drug is recovered in the urine as the sum of these two main metabolites /3,6/.

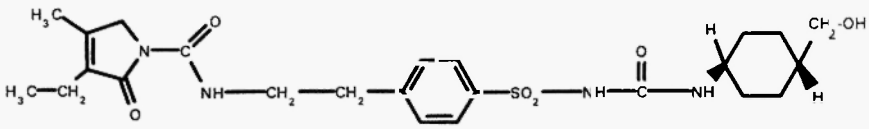
Of these two metabolites, hydroxy-glimepiride has been shown in animal pharmacology studies to exhibit some hypoglycaemic effects with the blood glucose lowering effects of the metabolite approximately three times less than those caused by the parent compound (Hoechst AG, data on file). In the same studies, carboxy-glimepiride exhibited no pharmacological activity. In acute and chronic animal toxicology studies, both metabolites were well tolerated /6,7/.

The finding of a major metabolite which contributes to the pharmacodynamic effects of the parent compound is unusual for the class of sulphonylurea drugs. The aim of the present study was to investigate the potential pharmacodynamic activity of hydroxy-glimepiride in human subjects. A single intravenous dose of hydroxy-glimepiride (1.5 mg) was administered to healthy volunteers and its effects on serum glucose, insulin and C-peptide were compared with those of placebo.

Glimepiride (Amaryl®)



Hydroxy-glimepiride



Carboxy-glimepiride

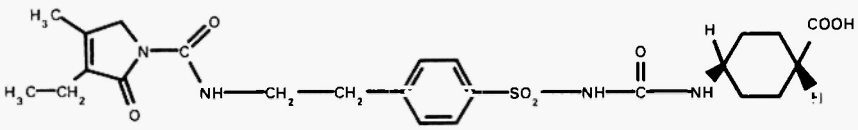


Fig. 1: Structure of glimepiride and its metabolites hydroxy-glimepiride and carboxy-glimepiride.

METHODS

This single blind, randomised, cross-over study with a washout period between treatments of 7 to 14 days, was conducted at a single centre in Austria.

Healthy male volunteers were considered for entry into the study if they were aged between 18 and 40 years with normal physical examinations and with normal ECGs and laboratory values. 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 with symptoms of major medical disease in the four weeks prior to the study or who had the presence or history of gastro-intestinal, 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.

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

Treatment

Subjects were randomised to receive a single intravenous 6 ml dose of either hydroxy-gli 1.5 mg (dry powder reconstituted in water for injection) or 0.9% NaCl (placebo). 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 dosing, the subjects reported to the Clinical Pharmacology Unit after an overnight fasting period of 12 hours. Thirty minutes prior to drug administration, an indwelling catheter was fixed into a suitable vein and was left in place for up to 12 hours after medication. The total amount of blood withdrawn for the trial was about 400 ml over the study period. Intravenous medication was injected according to the randomisation plan over one minute into a vein in the arm contralateral to the one used for blood sampling. No food was permitted until 10 hours after medication, at which time a standardised meal was served. The subjects drank 125 ml of water hourly up to 10 hours after dosing; fluid intake was then unrestricted. The subjects remained seated for the first four hours after dosing and strenuous activity was not permitted at any time.

Assessments

Blood samples were taken five minutes before administration of hydroxy-gli and 5, 10, 15, 20, 30, 40, 50 minutes, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 hours afterwards.

Total urine output was collected two hours before administration of hydroxy-gli 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 hydroxy-gli and carboxy-gli were determined in all samples by high-performance liquid chromatography (HPLC) /8/. Glucose, insulin and C-peptide were determined in all serum samples. Serum glucose was determined enzymatically by the hexokinase method. Serum insulin and serum C-peptide were determined by radioimmunoassay.

Clinical chemistry, haematology and urinalysis tests were performed at screening, before the first dose and 24 hours after the last dose of medication. 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 the last dose of study medication. Any adverse events which occurred during the trial were recorded.

Data analysis

Although a two-compartment model was chosen to approximate the concentration profiles of hydroxy-gli and a one-compartment model for fitting the concentration profiles of carboxy-gli, the following model-independent parameters are reported here: maximum serum concentration (C_{max}), time to peak concentration (t_{max}), area under the concentration time data completed by extrapolation (AUDC), terminal half-life ($t_{½,z}$) and total clearance (CL).

Urinary recovery of the metabolites hydroxy-gli and carboxy-gli was expressed in absolute amounts and as a percentage of the dose of hydroxy-gli. The latter was calculated as the sum of the excreted metabolites hydroxy-gli and carboxy-gli after molecular weight correction.

Profile parameters for serum glucose, serum insulin and serum C-peptide were calculated. The following pharmacodynamic parameters were calculated. maximum serum concentration (C_{max}), time to peak concentration (t_{max}), minimum serum concentration (C_{min}), time to minimum concentration (t_{min}) and mean concentration over the first four hours (C_{avg0-4}). The pharmacodynamic parameters were tested by an analysis of variance (ANOVA) with the level of significance preset at $p=0.05$. Point estimates and 90% confidence intervals were calculated for the respective ratios after hydroxy-gli or placebo administration /9/.

RESULTS

Thirteen volunteers were enrolled in this study because one dropout had to be replaced for not complying with the urinary sampling scheme on the first day of the study. Twelve healthy white male subjects completed the study. Mean age of the subjects was 26 years (range:

22-33), mean weight was 75 kg (69-85) and mean height 180 cm (173-186). All 12 subjects included in the study received the appropriate doses of medication on the dosing days and completed all the examinations.

Pharmacokinetics

Profiles of the mean serum concentrations for hydroxy-gli and carboxy-gli are plotted in Figure 2. The mean pharmacokinetic parameters (± SD) for hydroxy-gli and carboxy-gli are given in Table 1.

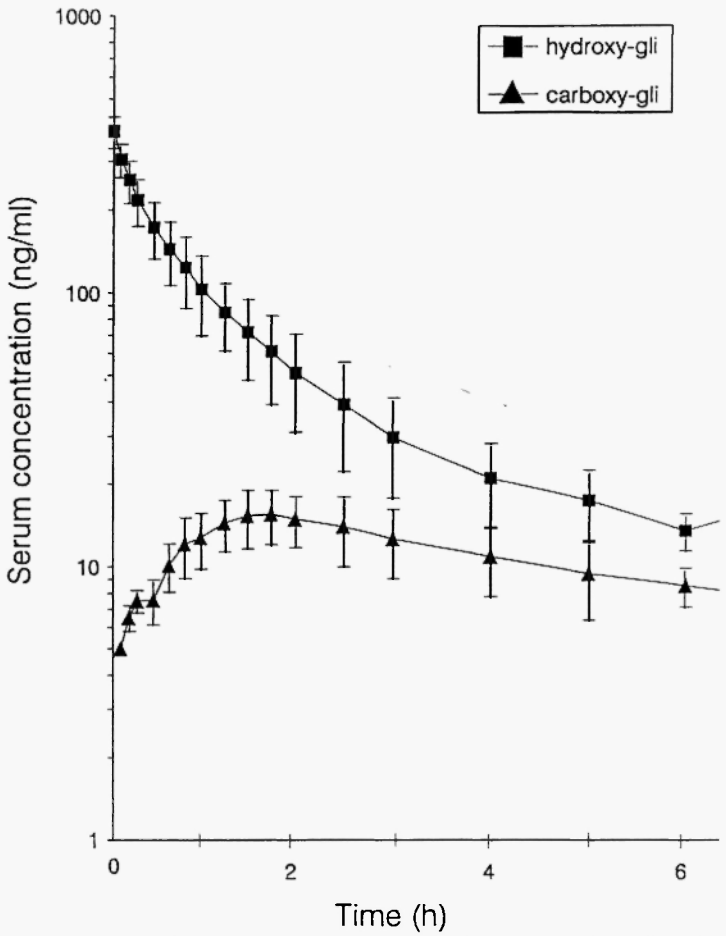


Fig. 2: Mean (± SD) serum concentrations of hydroxy-gli and carboxy-gli following intravenous administration of 1.5 mg hydroxy-gli.

TABLE 1

Pharmacokinetic parameters of hydroxy-gli and carboxy-gli

Parameter	Hydroxy-gli	Carboxy-gli
C_{max} (ng/ml)	383.3 ± 49.2*†	16.2 ± 3.4
t_{max} (h)	†	1.5 ± 0.3
AUDC (ng.h/ml)	349 ± 110	90 ± 40
$t_{1/2.z}$ (h)	1.2 ± 0.4	2.8 ± 1.1
CL (ml/min)	78.3 ± 24.3	331.7 ± 139.2#

* Results represent mean ± SD
† First sample taken 5 minutes after start of injection
# Relative total clearance

The mean (± SD) cumulative urinary recovery of hydroxy-gli and carboxy-gli in absolute amounts was 0.853 mg (± 0.117) and 0.200 mg (± 0.033), respectively (Fig. 3). Mean urinary recovery of the sum of hydroxy-gli and carboxy-gli (after molecular weight correction) expressed as a percentage of the dose was 69.9% (Fig. 4).

Pharmacodynamics

The main profile parameters for serum glucose, serum insulin and serum C-peptide after intravenous injection of hydroxy-gli and placebo are shown in Tables 2, 3 and 4, respectively, together with the point estimates and confidence intervals for the ratio hydroxy-gli to placebo. There were statistically significant differences between treatments for the profile parameters C_{min} and $C_{avg0\text{-}4}$ for serum glucose and C_{max} and $C_{avg0\text{-}4}$ for serum C-peptide (ANOVA, preset level of significance p=0.05). There was no statistically significant difference between treatments for any of the profile parameters for serum insulin. Mean serum glucose, serum insulin and serum C-peptide concentrations after

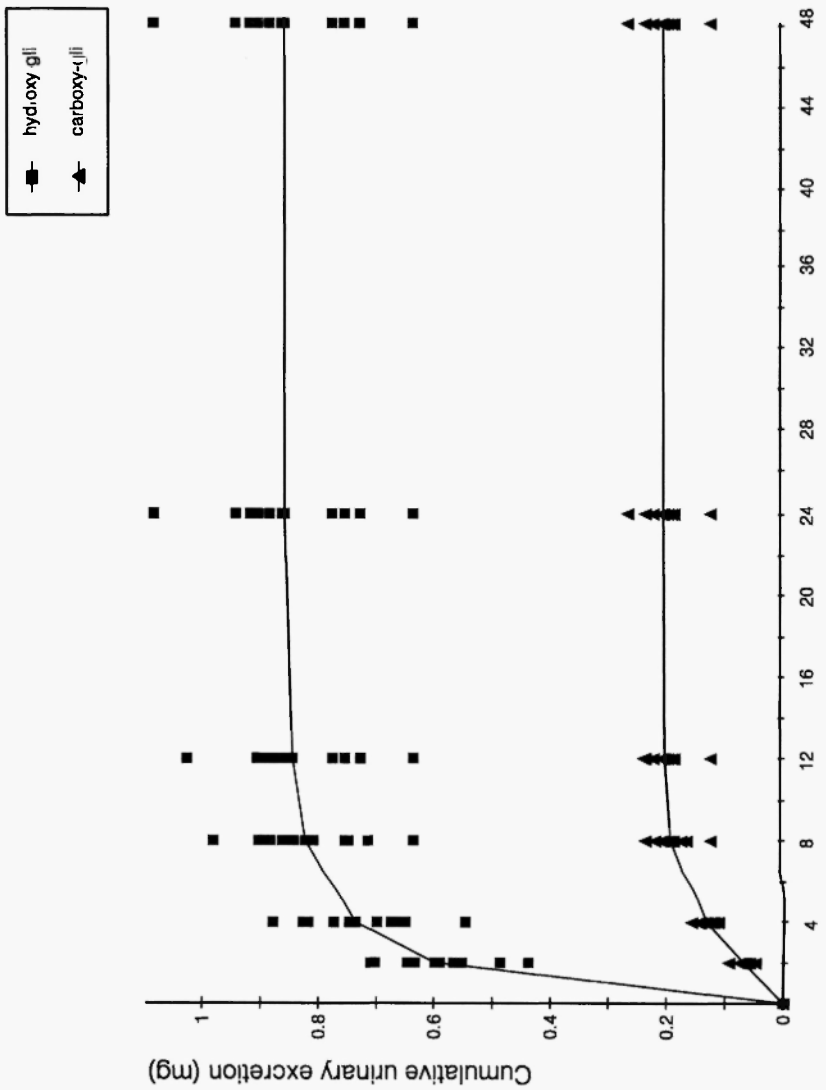


Fig. 3: Cumulative urinary recovery (mean plus original values) of hydroxy-gli and carboxy-gli in absolute amounts (mg) following intravenous administration of 1.5 mg hydroxy-gli.

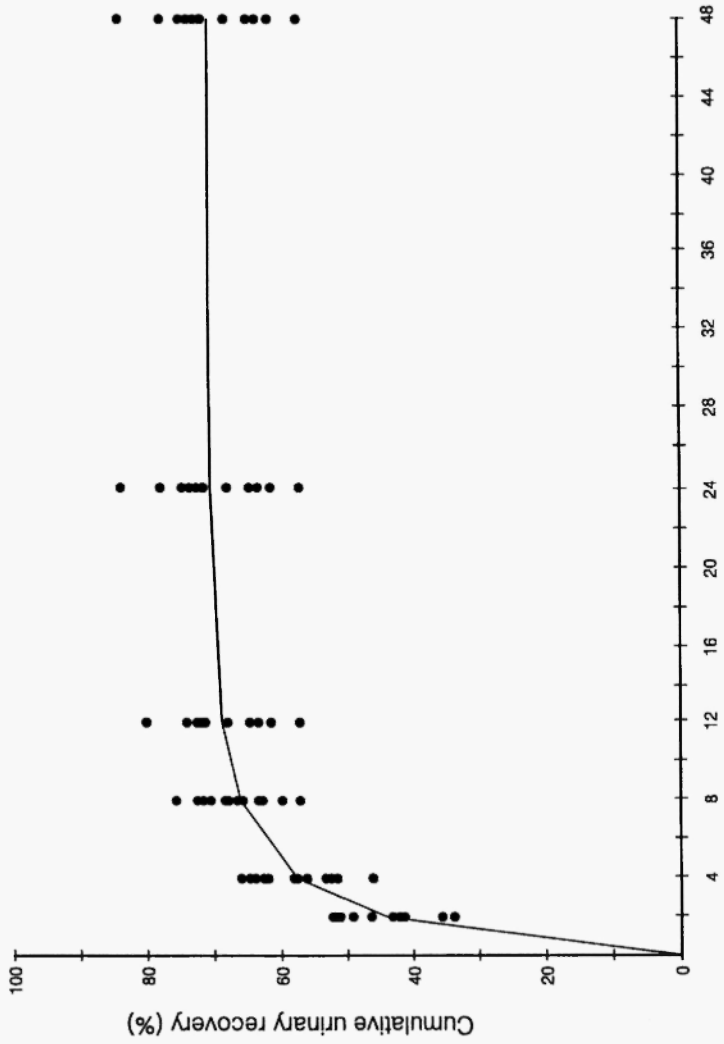


Fig. 4: Cumulative urinary recovery (mean plus original values) of the sum of hydroxy-gli and carboxy-gli as a percentage of the dose of hydroxy-gli (after molecular weight correction).

TABLE 2

Pharmacodynamic parameters of serum glucose

Parameter	Placebo	Hydroxy-gli	Point estimate [%]	90% confidence interval [%]
C_{min} (mmol/l)	4.6 ± 0.4*	4.1 ± 0.3	88	86-91
t_{min} (h)	1.9 + 0.9	2.0 ± 0.8	107	65-140
C_{avg0-4} (mmol/l)	4.9 ± 0.3	4.5 ± 0.3	91	89-93

*Results represent mean ± SD

TABLE 3

Pharmacodynamic parameters of serum insulin

Parameter	Placebo	Hydroxy-gli	Point estimate [%]	90% confidence interval [%]
C_{max} (mIU/l)	18.3 + 2.1*	19.1 ± 2.8	104	97-112
t_{max} (h)	0.3 ± 0.3	0.5 ± 0.4	163	82-253
C_{avg0-4} (mIU/l)	14.9 ± 2.2	15.4 ± 2.2	104	97-110

* Results represent mean ± SD

hydroxy-gli and placebo are plotted in Figures 5, 6 and 7. For reference, the left hand inset in Figure 5 superimposes the glucose lowering activity of 1.5 mg of the parent drug glimepiride administered i.v. under similar experimental conditions (Hoechst AG, data on file).

The power of the pharmacodynamic tests (i.e. the probability of detecting a difference of 20% between hydroxy-gli and placebo), based on a level of significance of 0.05, was greater than 98% in all tests.

TABLE 4

Pharmacodynamic parameters of serum C-peptide

Parameter	Placebo	Hydroxy-gli	Point estimate [%]	90% confidence interval [%]
C_{max} (µg/l)	2.0 ± 0.2*	2.2 ± 0.3	107	101-113
t_{max} (h)	0.4 ± 0.5	0.8 ± 0.6	185	100-271
C_{avg0-4} (µg/l)	1.7 ± 0.2	1.8 ± 0.2	107	102-112

*Results represent mean ± SD

Safety

No adverse events were reported during the study. There were no clinically important or drug-related changes in vital signs, laboratory tests or urinalysis for any of the subjects.

DISCUSSION

Results from this placebo-controlled, cross-over study in human volunteers demonstrate that intravenous administration of hydroxy-glimepiride, the main metabolite of the new sulphonylurea glimepiride, has a significant hypoglycaemic effect. In terms of minimum serum glucose concentrations, hydroxy-glimepiride at a dose of 1.5 mg produced a 12% decrease compared with placebo. Similarly, over the first four hours after dosing, hydroxy-glimepiride lowered serum glucose concentrations by 9% compared with placebo. Both of these differences were statistically significant. Hydroxy-glimepiride also significantly increased serum C-peptide concentrations; although serum insulin concentrations were elevated following dosing with the metabolite, the difference from placebo was not statistically significant.

In a previous study conducted under similar experimental conditions, the parent drug glimepiride was administered intravenously to six healthy human volunteers at a dose of 1.5 mg (Hoechst AG, data

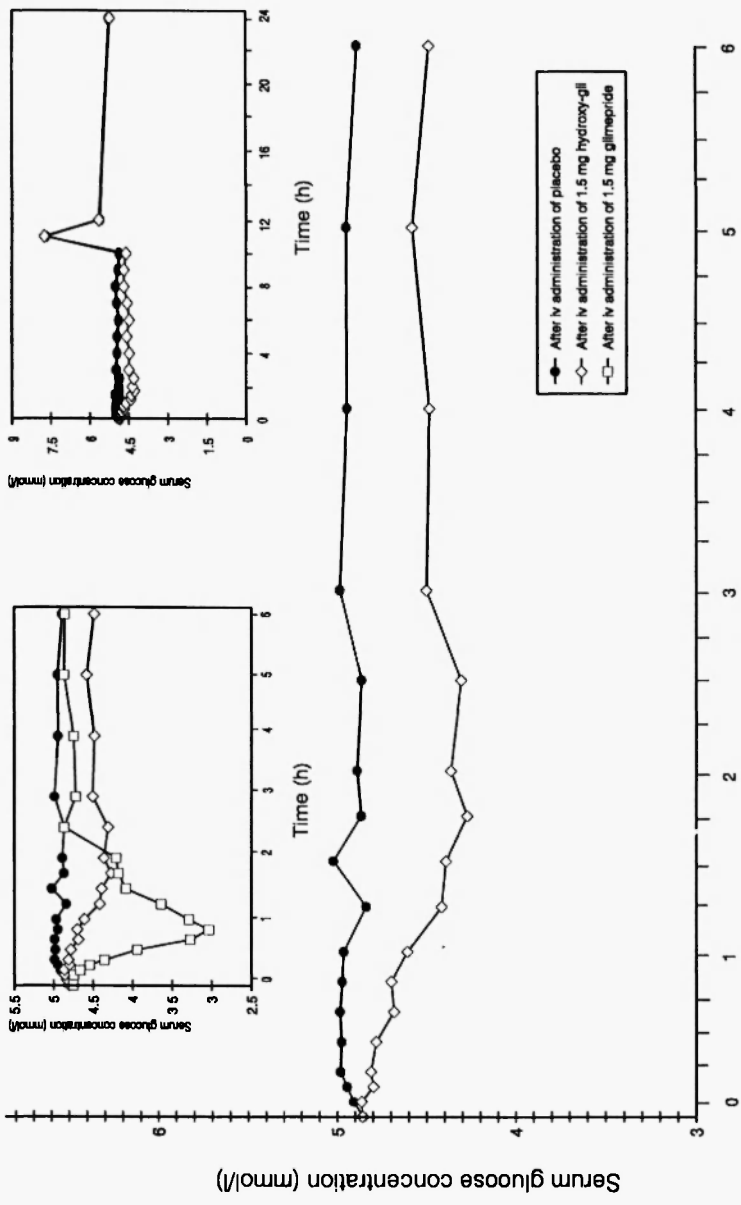


Fig. 5: Mean serum glucose concentration following intravenous administration of placebo and 1.5 mg hydroxy-gli. The left-hand inset superimposes the glucose lowering activity of 1.5 mg of the parent drug glimepiride administered i.v. under similar experimental conditions.

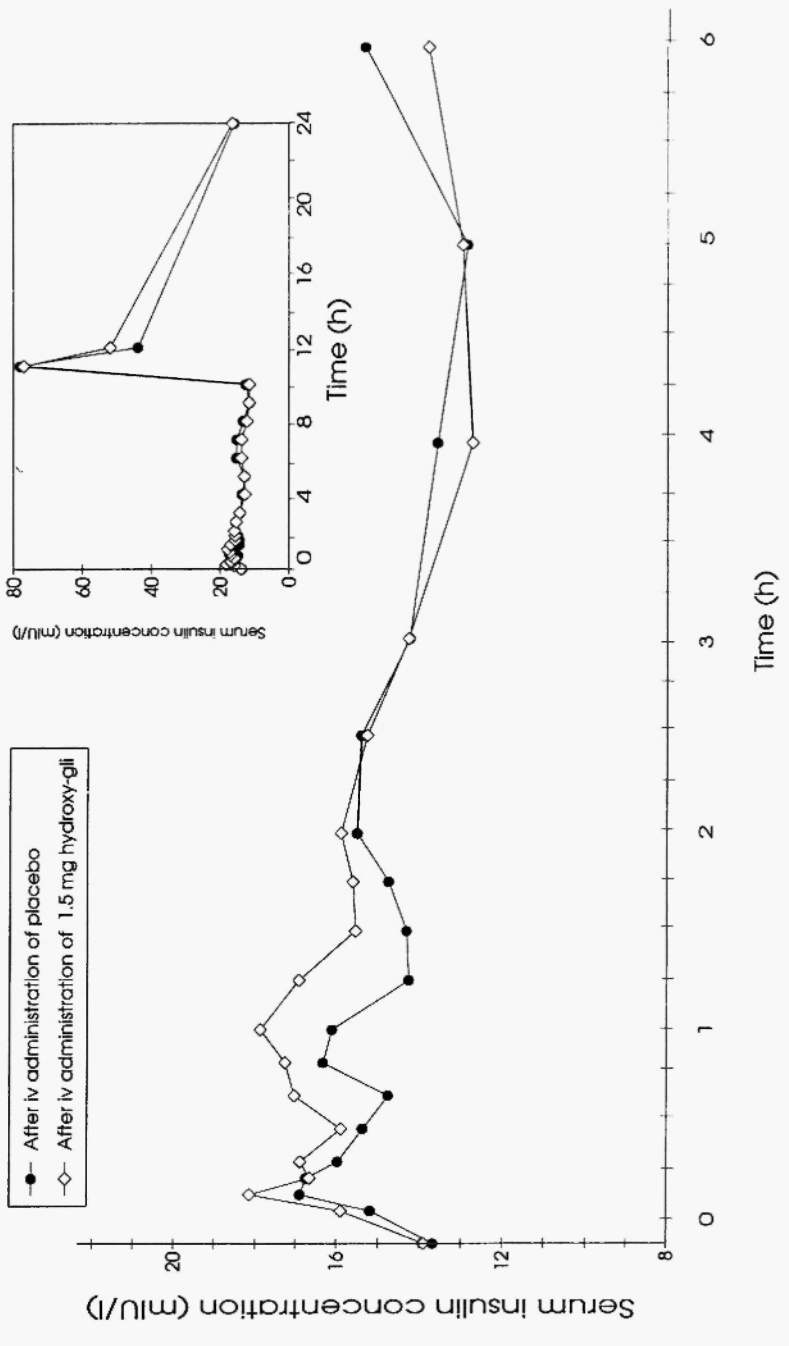


Fig. 6: Mean serum insulin concentration following intravenous administration of placebo and 1.5 mg hydroxy-gli.

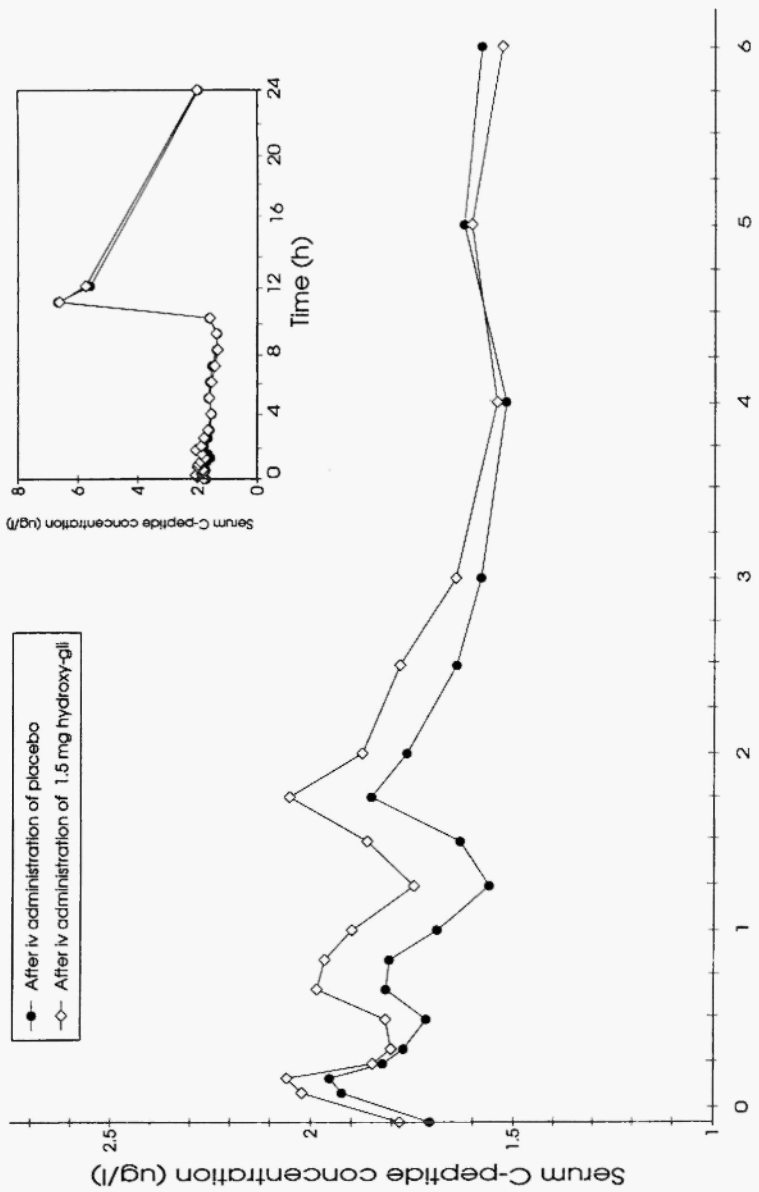


Fig. 7: Mean serum C-peptide concentration following intravenous administration of placebo and 1.5 mg hydroxy-gli.

on file). Mean serum glucose concentrations decreased from 4.76 mmol/l to minimum of 3.04 mmol/l after 50 minutes, a mean decrease from baseline of approximately 36%. In the present study, minimum serum glucose concentrations following hydroxy-glimepiride were achieved after nearly 2 hours, decreasing from 4.83 to 4.27 mmol/l (a mean decrease from baseline of approximately 12%). Taken together, the results of these two studies indicate that glimepiride is a potent hypoglycaemic agent with a major metabolite that provides a significant contribution to the overall pharmacodynamic effect. It is important to note that no clear differentiation can be made between the total activity and the contribution made to the total activity by hydroxy-glimepiride because the active metabolite is formed continuously from the parent drug. Nevertheless, the fact that glimepiride has a slowly formed metabolite which also possesses hypoglycaemic activity is an important factor in the rationale for administration of glimepiride in a once daily dosing regimen.

This phenomenon of an active metabolite is unusual among the sulphonylureas. Apart from the first generation compound, acetohexamide, for which most of its *in vivo* activity is due to its hydroxy-metabolite /10/, the majority of sulphonylureas have metabolites which show little or negligible hypoglycaemic activity. For example, tolbutamide (which is metabolised in a similar fashion to glimepiride) has a hydroxy-metabolite which may show some activity; however, plasma concentrations are 100 times lower than those of the parent tolbutamide, and therefore the metabolite expresses minimal hypoglycaemic effects /10,11/. Glibenclamide, probably the most commonly prescribed second generation sulphonylurea /12/, has somewhat different metabolism from glimepiride, being transformed by the liver into 4-*trans*-hydroxy-glibenclamide and 3-*cis*-hydroxy-glibenclamide /13/. Both of these metabolites are virtually inactive showing weak to absent hypoglycaemic activity in animal models /14,15/. Furthermore, the metabolites of glipizide, which is biotransformed in a similar fashion to glibenclamide, are devoid of any pharmacological activity /13/.

In the present study, urinary recovery of hydroxy-glimepiride and carboxy-glimepiride was 70% when expressed as a percentage of the dose of hydroxy-glimepiride administered. This figure is greater than that previously reported from human studies /3,5,16/ in which the combined urinary recovery of hydroxy-glimepiride and carboxy-glimepiride was approximately 50% of the total glimepiride dose, independent of the route of administration. This observed difference in

urinary excretion of the metabolites is attributed to the direct intravenous administration of hydroxy-glimepiride rather than the parent compound.

Another difference noted in this study was that the terminal half-life observed for hydroxy-glimepiride was shorter (1.2 hours) than that reported from earlier studies (2.7 to 6.1 hours) /3,16/. This difference may be explained by the "flip-flop" effect which describes the inability to decide whether absorption/biotransformation or elimination is the rate-limiting step in determining the half-life.

In conclusion, the hydroxy-metabolite of glimepiride possesses a significant degree of hypoglycaemic activity in comparison with placebo. This finding is unusual for the class of sulphonylureas, many of which have weak or inactive metabolites, and may have clinical implications in terms of being able to effectively administer glimepiride in a once-daily dosing regimen.

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