Concentration-effect relations of glibenclamide and its active metabolites in man: modelling of Pharmacokinetics and Pharmacodynamics

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Aims The main purpose of this paper is to describe the relationship between serum concentrations of glibenclamide and its main metabolites and the effects on blood glucose levels, the clinically most relevant parameter to assess in diabetes.

Methods Serum concentrations and blood glucose lowering effects (expressed as percent blood glucose reduction vs placebo) of glibenclamide (Gb) and its active metabolites, 4-trans-hydroxy-(M1) and 3-cis-hydroxy-glibenclamide (M2), were analysed in eight healthy subjects participating in a placebo-controlled, randomized, single-blind crossover study, using intravenous administration of each compound as well as oral administration of Gb.

Results Plots of % blood glucose reduction vs log serum concentration demonstrated counter-clockwise hysteresis for parent drug and its metabolites. An effect compartment was linked to appropriate pharmacokinetic models and pharmacokinetic and pharmacodynamic modelling was used to fit the pharmacokinetics of Gb by both routes and the metabolites for each individual. Based on the individual concentration-time profiles a PK/PD—model was applied to all effect data simultaneously. An increase in the steady-state serum concentration when the effect is 50% of maximal, C

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Ess50,, was found in the sequence M1 (23 ng ml⁻¹), M2 (37 ng ml⁻¹) and Gb (108 ng ml⁻¹). Corresponding interindividual variabilities expressed as CV% were 25%, 47% and 26%. The elimination rate constants from the effect site (kE0) were estimated and increased in the order M1 (0.178 h⁻¹, CV 13%), M2 (0.479 h⁻¹, CV 8.5%) and Gb (1.59 h⁻¹, CV 36%). Corresponding equilibration half-lives for the effect site (kE0-HL) were 3.9 h, 1.4 h and 0.44 h. Estimated E

max

-values obtained for M1, M2 and Gb were 40% (CV 30%), 27% (CV 56%) and 56% (CV 14%), respectively.

Conclusions It is concluded that the two major metabolites of Gb are hypoglycaemic in man, that they may have higher activity at low concentrations and that they may have a longer effect duration than the parent drug.

Keywords: glibenclamide, metabolites, sulphonylurea, diabetes mellitus, non-insulin dependent, pharmacokinetics, pharmacodynamics, pharmacodynamic modelling, glucose

Introduction

Glibenclamide (Gb) is a commonly used second generation sulphonylurea drug. Gb is metabolized by the liver and is eliminated as hydroxylated derivatives, 4-trans-hydroxy-(M1) and 3-cis-hydroxy-glibenclamide (M2) [1–7]. The basal pharmacokinetics and urinary excretion pattern of these metabolites in man have been studied both after intravenous administration of each metabolite per se, and after intravenous and oral administration of Gb [8]. Independent of the route of administration of Gb one third of the administered dose was excreted as metabolites (proportion, M1/M2=4) in urine within 10 h postdose [8]. Recently, we showed that both M1 and M2 possess pronounced hypoglycaemic activity in man [9]. Subsequent studies showed that M1 and M2 have similar pharmacokinetic profiles but differ from Gb in clearance and volume of distribution [8].

It is uncertain how the hypoglycaemic effect of sulphonylurea varies with drug concentration [10]. The current study compared the concentration-effect relations of Gb, M1 and M2 by modelling of pharmacokinetics and pharmacodynamics. The nonlinear mixed-effects model population program NONMEM [11] was used for the analysis. Thorough reviews of PK/PD-modelling and population pharmacokinetics and pharmacodynamics are given in [12–14] and [15–17], respectively.

Methods

Protocol

Table 1 shows characteristics of subjects and individual fasting blood glucose and insulin levels for each test. The details of the subjects, treatment, sampling and analysis have

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levels were drawn at regular intervals between 0 and 10 h state, the problem with counterclockwise hysteresis can be reduced, but not eliminated, by collecting the effect concentrations at the time of each effect measurement. Therefore, effect raw data were transformed to percent change in blood glucose (relative reductions of blood glucose) using the general equation:

\[ E = \text{percent effect (i)} = 100 \times \frac{\text{placebo}(i) - \text{treatment}(i)}{\text{placebo}(i)} \]

where \( E \) percent effect (i) is the percent effect at time (i), determined from the glucose levels at corresponding times during placebo (placebo(t)) and treatment (treatment (t) periods). The equation defines the effect at every time during placebo treatment as 0 percent, and for blood glucose the maximum possible effect (reduction) as 100 percent (20–21). For each test, each subject was evaluated by plotting the corresponding effect-concentration data pairs between 0 and 5 h, and different basic pharmacodynamic models, log-linear, \( E_{\text{max}} \) and sigmoid \( E_{\text{max}} \) models were tried and fitted to the data (22–24).

Data pairs were collected after distribution equilibrium (1–5 h for i.v. data and 2–5 h for oral data) was obtained between the central and peripheral compartments; i.e. monoexponential portion of the descending serum concentration-time curve. For experiments not performed at steady-state, the problem with counterclockwise hysteresis can be reduced, but not eliminated, by collecting the effect-concentration data pairs in this manner (20–25).

**Linked pharmacokinetic (PK) and pharmacodynamic (PD) modelling**

The theory behind this modelling technique was described in 1979 [26] and has been tested and extended [27, 28]. The approach has been used in several studies [29–32]. This technique allows fitting of the serum drug concentration and the effect data from non-equilibrium phases, i.e. initial distribution phases after single-dose administration. The model has an effect site connected to a PK model. The drug is assumed to enter and leave the effect compartment according to first-order kinetics, described by the rate constants, \( k_{\text{in}} \) and \( k_{\text{out}} \).

**Pharmacokinetic and pharmacodynamic analysis**

Nonlinear mixed-effects modelling were used to characterize the pharmacokinetics and pharmacodynamics in the actual population. This modelling approach is appropriate for analyses of all subjects simultaneously, taking the interindividual variability into account [15]. The analysis was performed with NONMEM [11]. Graphical analyses were performed using the Xpose package [33] running under Splus, version 3.3 [34], on a Hewlett Packard 9000 computer, at the Department of Pharmacy, Division of Biopharmaceutics and Inorganic Chemistry, Lund University, and the study protocol was approved by the Medical Ethics Committee at Lund University, Lund, Sweden. Eight healthy Caucasian subjects (four of each sex) participated in the placebo-controlled, randomized, single-blind crossover study with five single-dose tests, 3 months apart; placebo intravenously, 3.5 mg doses of M1, M2, Gb intravenously and, in addition, a 3.5 mg tablet of Gb (Daonil®, Hoechst GmbH, Frankfurt, Germany) were administered in the fasting state. Standardized breakfast and lunch were eaten 0.5 and 5.5 h postdose. The meals had energy contents of 1,800 kJ (430 kcal) and 3,150 kJ (750 kcal), respectively. Apart from the meals, no food or liquid intake was allowed. Venous blood samples for analyses of serum drug concentrations and blood glucose levels were drawn at regular intervals between 0 and 10 h (0.083, 0.17, 0.33, 0.50, 0.67, 0.83, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0 and 10.0 h) after drug dosing. Quantitative urine collections were made at 1, 2, 4, 6, 8 and 10 h. Serum concentrations of Gb were measured by h.p.l.c. with a detection limit of 1 ng ml\(^{-1}\) [18], and serum and urine concentrations of M1 and M2 by essentially the same method, with a detection limit of 5 ng ml\(^{-1}\). Metabolite concentrations in serum from the tests giving Gb were not analysed. The coefficients of variation (between-day) for the assays of the three different sulphoxyflur compounds varied between 2.5–9.8% [8]. Blood glucose was assayed by a glucose oxidase method. Serum insulin concentrations were analysed by a specific enzyme-linked immunosorbent method [19].

**Data analysis**

To examine the time course of effects after placebo and drug administration, plots of effect (raw data) vs time were made, and, in order to fit data to a pharmacodynamic model, the effects were also plotted vs drug serum concentrations at the time of each effect measurement. Beforehand, effect raw data were transformed to percent change in blood glucose (relative reductions of blood glucose) using the general equation:

\[ E = \text{percent effect (i)} = 100 \times \frac{\text{placebo}(i) - \text{treatment}(i)}{\text{placebo}(i)} \]

Table 1 Characteristics of subjects and individual fasting blood glucose and serum insulin levels for each test.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>M1</th>
<th>M2</th>
<th>Gb</th>
<th>Gb</th>
<th>M1</th>
<th>M2</th>
<th>Gb</th>
<th>Gb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>M</td>
<td>3.6</td>
<td>3.8</td>
<td>4.1</td>
<td>3.9</td>
<td>16</td>
<td>24</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>M</td>
<td>4.2</td>
<td>4.3</td>
<td>4.2</td>
<td>4.2</td>
<td>38</td>
<td>35</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>M</td>
<td>4.3</td>
<td>4.4</td>
<td>4.3</td>
<td>4.8</td>
<td>23</td>
<td>66</td>
<td>35</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>M</td>
<td>4.7</td>
<td>4.4</td>
<td>3.6</td>
<td>4.4</td>
<td>34</td>
<td>48</td>
<td>13</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>F</td>
<td>4.1</td>
<td>4.6</td>
<td>4.4</td>
<td>3.7</td>
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<td>23</td>
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<tr>
<td>6</td>
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<td>F</td>
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<td>7</td>
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<td>F</td>
<td>3.6</td>
<td>3.8</td>
<td>4.1</td>
<td>4.1</td>
<td>9.0</td>
<td>12</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>F</td>
<td>4.0</td>
<td>3.5</td>
<td>4.0</td>
<td>4.5</td>
<td>17</td>
<td>18</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

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Pharmacokinetics, Biomedical Centre, Uppsala, Sweden. Initially, NONMEM was used to separately fit the pharmacokinetics of Gb by both routes (intravenous and oral together) and each metabolite, and then to fit a linked PK/PD-model to all data simultaneously, but keeping the kinetic parameters constant. The data relating to the pharmacokinetics of Gb (serum data) and the metabolites (serum and urine data from intravenous administration of M1 and M2, respectively and urine data from intravenous and oral administration of Gb) were then placed together in one large PK/PD-model.

The PK model best fitted for both M1 and M2 was the tri-exponential intravenous model with bolus input and first order output. Intravenous and oral Gb were evaluated simultaneously with a bi-exponential intravenous model and a two-compartment oral model with first-order input, first-order output and a lag time. Those models described the data best, and were used in later model development. Estimates of $k_{01}, k_{12}, V_{1}$, initial dilution volume ($V_{1}/V_{2}$), $k_{13}$, $k_{12}$, $f$, absorption rate constant ($k_{a}$) and lag time ($t_{lag}$) were sought. Following the administration of Gb, no serum measurements of M1 or M2 were made, yet knowledge of those profiles are necessary to properly account for the contribution of these metabolites to the effect. Prediction of these profiles were based on the following assumptions: (i) the fraction of Gb forming M1 (or M2) is equal to the fraction of the dose excreted into the urine in the form of M1 (or M2), (ii) the CL of Gb is constant over time, (iii) the disposition characteristics of M1 (M2) as determined from the intravenous administration of M1 (M2) apply also when the metabolite is formed from Gb.

As a PD model for each metabolite, a separated end version of the ordinary sigmoid $E_{max}$ model was used to relate the intensity of effect, $E$, to the amount or concentration of metabolite (M1 or M2) in the hypothetical effect compartment.

$$E = \frac{E_{max} \cdot C}{1 + \frac{C}{C_{e_{50}}}}$$

where $E_{max}$ is the maximum percentage decrease in blood glucose levels, $C_{e_{50}}$ is the steady-state serum concentration when the observed effect is 50% of maximal, $C$ is the drug concentration in the hypothetical effect compartment, and $\gamma$ is the sigmoidicity factor, which affects the sigmoidal shape of the curve [28, 31]. For combined drug action of Gb, M1 and M2, we used a standard model for the competitive interaction of three agonists [35]. It was written as:

$$E_{Gb + M1 + M2} = E_{max} \times \left\{ \frac{C_{Gb}}{C_{e_{50Gb}}} + \frac{C_{M1}}{C_{e_{50M1}}} + \frac{C_{M2}}{C_{e_{50M2}}} \right\}$$

where

$$X_{Gb} = \frac{C_{Gb}}{C_{e_{50Gb}}}$$

and

$$X_{M1} = \frac{C_{M1}}{C_{e_{50M1}}}$$

and

$$X_{M2} = \frac{C_{M2}}{C_{e_{50M2}}}$$

and

$$X_{Gb} = \frac{C_{Gb}}{C_{e_{50Gb}}}$$

and

$$C = \frac{C_{M1}}{C_{e_{50M1}}} + \frac{C_{M2}}{C_{e_{50M2}}}$$

$$+ \frac{C_{Gb}}{C_{e_{50Gb}}}$$

After giving initial estimates for each test, the curve-fitting was performed and the parameters were adjusted by the NONMEM iterations to provide the best fit to the observed concentration-time and effect-time data. Weighting with both an additional and a proportional weighting term was used. Comparison between models were based mainly on visual examination of the residual scatterplots, and comparison of the minimum objective function values. The objective function computed by NONMEM is equal to minus twice the log likelihood [15, 16]. A stepwise approach was used to determine which parameters should be included in the final optimal model that has the best fit (lowest-2 log likelihood). Parameter estimates of $k_{01}, E_{max}, C_{e_{50}}$, and $\gamma$ for M1, M2 and Gb were sought. The half-life determining time for drug loss from the effect site, $k_{01}$, was calculated as (ln 2)/$k_{01}$. Effect data after 5 h were not used since little effect on blood glucose levels vs placebo could be discerned after lunch.

**Statistical analysis**

All data are shown as means± s.d. or as means and CV%. The obtained parameters were compared by paired, two-tailed Student tests (two sample test) or at multiple comparisons by repeated measures analysis of variance (ANOVA). A $P$ value <0.05 was considered significant.

**Results**

Plots of individual percent effect data vs serum concentration data exposed a time dependent concentration--effect relationship (counterclockwise hysteresis), which is shown for a representative subject (number 1) (Figure 1a and b). Oral administration of Gb delayed the effect more than intravenous Gb. From urine data it was concluded that the metabolites appeared in serum after Gb administration between 0.5–1 h and that $t_{lag}$ of both metabolites were around 3–4 h.

A plot of pooled percent effect data from all subjects vs corresponding serum concentrations indicated a sigmoidal relationship. The mean percent effect data from the monoexponential portion of the descending serum concentration-time curve in each test plotted vs mean logarithmic serum concentration at corresponding times of each compound were almost linear (Figure 2). All slopes in the figure differed significantly ($P<0.05$), except that between M1
and M2 \((P=0.06)\). The resulting parameters after linear regression analysis were summarised together with the concentrations estimated to produce 20% effect \((EC_{20})\) and for Gb also \(EC_{50}\) (Table 2).

Individual plots showing observed and predicted values of serum concentrations of Gb after intravenous and oral administration of Gb are shown in Figure 3. The resulting population pharmacokinetic parameters are shown in Table 3. The "final" linked PK/PD-model that had the best fit assumed no baseline and also the same value of \(\gamma\) in all subjects. Figure 4 shows individual plots of observed and predicted percent effects in two representative subjects (no. 2 and 7) for each test. The resulting population pharmacodynamic parameters of the eight subjects are shown in Table 4.

\(EC_{50}\) values increased in the sequence M1, M2 and Gb and the concentration producing 50% effect was significantly lower for both metabolites than for Gb \((P<0.05)\). Corresponding estimated \(E_{\text{max}}\) values were 40%, 27% and 56% (Table 4). The elimination rate constant for Gb also \(EC_{50}\) (Table 2).

Discussion
This study re-affirms that both major metabolites of glibenclamide possess hypoglycaemic activities in man after intravenous administration [9], and despite single-dose test and lack of steady-state data assessed the effect response
Concentration-effect relationship of glibenclamide

Figure 2 Percent reduction in β-glucose vs log serum concentration after intravenous bolus doses of M1 (solid triangles), M2 (open squares) and Gb (solid circles) as well as a single-dose of oral Gb (solid squares).

Table 2 Values of doses and resulting concentrations corresponding to 20% (EC20) and 50% (EC50) reduction in β-glucose levels after linear regression of the descending part of the log serum concentration -% effect curves (1–5 h for i.v. data and 2–5 h for oral data).

<table>
<thead>
<tr>
<th></th>
<th>Intravenous M1</th>
<th>Intravenous M2</th>
<th>Intravenous Gb</th>
<th>Oral Gb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>17.7</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>95% confidence interval of slope</td>
<td>7.9–27.4</td>
<td>23.0–23.7</td>
<td>35.2–23.8</td>
<td>60.0–79.5</td>
</tr>
<tr>
<td>EC20 (ng ml⁻¹)</td>
<td>—</td>
<td>—</td>
<td>50¹</td>
<td>87⁴</td>
</tr>
<tr>
<td>EC50 (ng ml⁻¹)</td>
<td>—</td>
<td>—</td>
<td>243⁴</td>
<td>233⁴</td>
</tr>
</tbody>
</table>

¹Not true, but apparent values, since the response after Gb is administered will be a summation of that due to the parent drug and due to the two active metabolites.

Table 3 Population pharmacokinetic parameters of eight healthy volunteers estimated by NONMEM, mean and interindividual variability (CV%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M1¹</th>
<th>M2²</th>
<th>Gb²</th>
</tr>
</thead>
<tbody>
<tr>
<td>k₁₀ (h⁻¹)</td>
<td>4.44 (81%)</td>
<td>2.79 (4.2%)</td>
<td>1.30 (10%)</td>
</tr>
<tr>
<td>k₂₀ (h⁻¹)</td>
<td>2.71 (63%)</td>
<td>2.50 (10%)</td>
<td>0.447 (15%)</td>
</tr>
<tr>
<td>k₁₂ (h⁻¹)</td>
<td>2.76 (82%)</td>
<td>2.31 (8.2%)</td>
<td>0.916 (21%)</td>
</tr>
<tr>
<td>V₁ (l)</td>
<td>4.17 (39%)</td>
<td>3.56 (8.4%)</td>
<td>3.63 (17%)</td>
</tr>
<tr>
<td>k₁₃ (h⁻¹)</td>
<td>1.89 (13%)</td>
<td>0.863 (57%)</td>
<td></td>
</tr>
<tr>
<td>k₃₁ (h⁻¹)</td>
<td>0.526 (1%)</td>
<td>0.399 (5%)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.82 (29%)</td>
<td>0.756 (60%)</td>
<td></td>
</tr>
<tr>
<td>kₐ (h⁻¹)</td>
<td>0.40 (13%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Tri-exponential intravenous model with bolus input and first order output was used for M1 and M2.
²Intravenous and oral Gb were evaluated simultaneously with a bi-exponential intravenous model and a two-compartment oral model with first-order input, first-order output and a log time.

than the parent drug at lower concentrations. These results are in contrast to a previous claim that M1 is about 6.5 times less potent than Gb, following intraperitoneal administration of different doses of Gb and M1 in six rats [6]. A possible explanation apart from species difference, could be that the animal study concerned dose-response while the current one investigated concentration-effect relationships. Another possible explanation could be that the animal study used 20 times higher dose per kg bodyweight.

In a non-crossover, single-dose placebo-controlled study at four dosage levels of oral glibenclamide (1.25–5.0 mg), in five groups of eight subjects each, serum concentrations were measured by a radioimmunoassay which did not separate metabolites from glibenclamide [3]. The correlation with fasting serum glucose was weak. In fact, similar drug levels could be associated with different degrees of glucose lowering, depending upon dose and time after dosing [3]. The findings would fit with a counterclockwise hysteresis effect after oral administration. The authors also defined a minimum effective glibenclamide/metabolite level at 30–50 ng ml$^{-1}$. In the 1.25 dosage group they had a significant decrease in glucose levels down to the 6 ng ml$^{-1}$ level [3]. These data agree with the assumption that Gb and especially its metabolites are active at low concentrations, as suggested by the $EC_{20}$ and $C_E_{50}$-values seen in the current study. A higher free fraction of the metabolites compared with the parent drug may be an explanation to the higher activity of metabolites. There are published data indicating that Gb is more protein-bound in blood than M1 and M2 [1, 36].

Administration of Gb appeared to generate at least three bioactive compounds; Gb itself with a rapid effect onset and a short duration ($k_{e0-HL}$, 0.44 h), M2 with an intermediate effect duration ($k_{e0-HL}$, 1.4 h) and M1 with a slower onset of effect and longer duration ($k_{e0-HL}$, 3.9 h). The prolonged effect duration of the metabolites can be explained in terms of the linked PK/PD model. As the response-time curve is dependent on the slowest rate constant describing the concentration in the effect site, as the $k_{e0}$ values for M1 (0.178 h$^{-1}$) and M2 (0.479 h$^{-1}$) were smaller than their metabolites from glibenclamide [3]. The correlation with fasting serum glucose was weak. In fact, similar drug levels could be associated with different degrees of glucose lowering, depending upon dose and time after dosing [3]. The findings would fit with a counterclockwise hysteresis effect after oral administration. The authors also defined a minimum effective glibenclamide/metabolite level at 30–50 ng ml$^{-1}$. In the 1.25 dosage group they had a significant decrease in glucose levels down to the 6 ng ml$^{-1}$ level [3]. These data agree with the assumption that Gb and especially its metabolites are active at low concentrations, as suggested by the $EC_{20}$ and $C_E_{50}$-values seen in the current study. A higher free fraction of the metabolites compared with the parent drug may be an explanation to the higher activity of metabolites. There are published data indicating that Gb is more protein-bound in blood than M1 and M2 [1, 36].

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Concentration-effect relationship of glibenclamide

Table 4 Population pharmacodynamic parameters of eight healthy volunteers estimated by NONMEM, mean and interindividual variability (CV%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M1</th>
<th>M2</th>
<th>Gb</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{e0}$ (h$^{-1}$)</td>
<td>0.178 (3.3%)</td>
<td>0.479 (8.5%)</td>
<td>1.59 (36%)</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (%)</td>
<td>40 (30%)</td>
<td>27 (56%)</td>
<td>56 (14%)</td>
</tr>
<tr>
<td>$C_{\text{Ess50}}$ (mg ml$^{-1}$)</td>
<td>23 (20%)</td>
<td>33 (47%)</td>
<td>108 (26%)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>4.6a</td>
<td>4.6a</td>
<td>2.4a</td>
</tr>
</tbody>
</table>

"The 'final' model assumed the same value of $\gamma$ in all subjects, therefore no interindividual variability is shown.

In conclusion, while there is no simple, direct relationship between sulphonylurea concentrations and the hypoglycaemic effect, consideration of pharmacokinetic and pharmacodynamic time dependencies by means of population PK/PD modelling with NONMEM demonstrates a relationship, involving both Gb and its active metabolites. Indeed, the metabolites may have higher activity at low concentrations and may have a longer effect duration than the parent drug per se. This should be clinically relevant.

Dosage is sufficient in most patients. Significant prolongation in the elimination half-life [38–40] and an increased volume of distribution of Gb has also been observed during chronic dosing [40]. In addition, a recent study indicates that the maximum effect of sulphonylurea is reached at lower doses and concentrations than previously thought. The maximum effect of Gb in healthy volunteers would be obtained by 5 mg or less [41]. The $C_{\text{Ess50}}$ values in the current study supports this view.
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References

Concentration-effect relationship of glibenclamide


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