Relationship between hypoglycaemic response and plasma concentrations of BTS 67 582 in healthy volunteers

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- 1 The relationships between blood glucose, plasma insulin and plasma BTS 67 582 concentrations were studied in a randomised, placebo-controlled, four-way crossover study involving 16 healthy male volunteers aged between 19 and 43 years. Single oral doses of 125, 250 and 500 mg BTS 67 582 were studied.
- 2 Fasting blood samples were taken pre-dose and half-hourly for 8 h post-dose. Mean peak plasma concentrations of BTS 67 582 were 518, 1076 and 2435 ng ml⁻¹ for doses of 125, 250 and 500 mg, respectively. Mean maximum reductions in blood glucose were 1.13, 1.59 and 1.78 mmol l⁻¹, and mean maximum increases in plasma insulin were 26, 14 and 21 µu ml⁻¹ for the three doses, respectively. Changes in incremental area under the curve (AUC) of blood glucose were correlated with changes in plasma BTS 67 582 AUC. The maximum reduction in blood glucose was correlated with the peak plasma BTS 67 582 concentration. No correlations between plasma insulin and plasma BTS 67 582 concentrations were observed.
- 3 Anticlockwise hysteresis was evident in concentration-effect curves, but less evident following subtraction of placebo data, and was mainly due to an underlying downward trend in fasted blood glucose levels with time evident under placebo treatment. This suggests that hypoglycaemic effects were related to systemic BTS 67 582 concentrations, suggesting that active metabolites of the drug do not make a major contribution to acute hypoglycaemic effects.
- 4 A log-linear model described the relationship between blood glucose and plasma BTS 67 582 concentrations for 14 of the 16 volunteers. Log-linear and E_{max} models described this relationship when the data were pooled.
- 5 A single compartment pharmacokinetic model with no lag time was fitted to the plasma concentration data of BTS 67 582. The pharmacokinetics of the drug were generally linear over the three doses studied.
- 6 Concentration-effect and pharmacokinetic models were combined to produce a predictive model of the reduction in blood glucose with time following doses of BTS 67 582. A potential application of this model is for the prediction of reductions in glycosylated haemoglobin under longterm administration of BTS 67 582, using reported correlations with blood glucose.

Keywords BTS 67 582 oral hypoglycaemic pharmacokinetics pharmacodynamics
E_{max} model hysteresis

Introduction

Current drug treatment of non-insulin dependent diabetes mellitus (NIDDM) is mainly with sulphonylureas and biguanides. Sulphonylureas are prescribed for non-obese NIDDM patients who do not respond

adequately to dietary control. However, those who initially respond to sulphonylureas may only respond for a limited time, and the risk of failure of sulphonylurea treatment increases with the duration of disease

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[1]. In addition, it has been proposed that chronic treatment with sulphonylureas could induce 'sulphonylurea resistance' leading to drug failure [2]. Often a biguanide will be administered as an add-on therapy when sulphonylurea treatment fails. The secondary failure rate for metformin, currently the only prescribed biguanide, is reported to be between 5 and 10% per year [3], and is similar to that for sulphonylureas [4]. In the UK metformin accounts for around 25% of prescriptions for oral hypoglycaemic drugs, about half of these being given in combination with a sulphonylurea [5].

Guanidine derivatives, including the biguanides, have been investigated for antihyperglycaemic effect since Watanabe demonstrated the activity of guanidine in 1918 [6]. Structurally diverse guanidines have demonstrated direct insulin secretagogue action [7], improvements in insulin sensitivity [8] and inhibition of monosaccharide uptake from the intestinal tract [9, 10]. The principal side effect of the antidiabetic guanidines, lactic acidosis, which led to the withdrawal of phenformin, is thought to be related to the lipophilicity of the side chain moiety [11]. Gastrointestinal side effects are a common feature of biguanides such as metformin and guanidine insulin secretagogues such as linoglitide. Developmental work on linoglitide was halted owing to CNS toxicity and work on its analogue, piroglitide, was ceased owing to elevations in hepatic transaminase in some patients [7].

BTS 67 582 (1,1-dimethyl-2-[2-morpholinophenyl] guanidine monofumarate) is an orally active hypoglycaemic agent in animals and man. It is structurally distinct from the sulphonylureas and biguanides, and may offer a novel treatment for NIDDM. In several animal species BTS 67 582 has been shown to increase plasma insulin concentrations and to exert hypoglycaemic effects in streptozotocin-induced diabetic rats, suggesting both pancreatic and extra-pancreatic actions (Marita & Kaul, unpublished). In animals it does not cause lactic acidosis and, based on limited human exposure, it appears to be well tolerated.

Few formal studies of relationships between plasma concentration and effect have been reported for oral hypoglycaemic agents. This study examines these relationships in 16 fasted healthy volunteers after receiving placebo and 125, 250 and 500 mg doses of BTS 67 582 in a randomised four-way crossover trial.

Methods

Study design

Sixteen healthy male Caucasian volunteers entered and completed this double-blind, randomised, placebo-controlled four-way crossover study. Volunteers were aged from 19 to 43 years, had body weights between 61.7 and 87.2 kg, heights ranging from 169 to 193 cm and body mass indexes between 18.4 and 24.9 kg m⁻². No volunteer received any other

prescribed medication for the duration of the study and for 14 days prior to the first study day. All volunteers provided written informed consent to participation, and the study was approved by a local ethics committee. The treatments studied were single doses of 125 mg, 250 mg, 500 mg BTS 67 582 (Boots Pharmaceuticals, Nottingham) and placebo. Each treatment consisted of two identical capsules. Volunteers received each treatment once, in a pre-defined order according to a William's square design [12]. Treatment periods were separated by a drug-free washout of at least 7 days.

Volunteers were admitted to the clinic at approximately 17.00 h on the evening before each study day and remained fasted from midnight. Medication was administered at 07.00 h. Blood samples (10 ml) were taken from an indwelling venous cannula pre-dose and at half-hourly intervals until 8 h, followed by samples at 10 h and 12 h. Blood glucose samples were stored in fluoride treated tubes at 4°C for subsequent assay. Drug and insulin samples were centrifuged immediately and plasma stored at -20°C until analysed. Volunteers remained fasted until the 8 h blood sample, after which they were provided with a standard meal. Data collected beyond the 8 h blood sample were not included in the investigation of a concentration-effect relationship.

Analytical methods

Blood glucose was measured by a u.v. enzymatic method using glucose hexokinase as the primary enzyme, with a test kit (BCL Lewes, East Sussex, UK). The assay coefficient of variation was 2%. Plasma concentrations of BTS 67 582 were determined by h.p.l.c following purification of the samples on C18 solid-phase extraction cartridges. The h.p.l.c analysis was carried out on a 25 cm nitrile column using a mobile phase of 0.00125 M pentane sulphonic acid and 0.022 M acetic acid in water/acetonitrile (1/3) flowing at 1.7 ml min⁻¹, with u.v. detection of the eluent at 225 nm. The precision of the assay of BTS 67 582 in plasma was below 15% over the range 50 to 5000 ng ml⁻¹, with 50 ng ml⁻¹ being considered the limit of quantification. The BTS 67 582 concentrations were all measured as the fumarate salt (Underwood, unpublished). Plasma insulin was measured using a radioimmunoassay kit (Amersham International Plc, UK). The assay coefficient of variation was 5%.

Data analysis

Plasma concentrations of BTS 67 582 in each individual were fitted by a one compartment model with first-order input using ordinary non-linear least squares regression [13]. A population model was derived from the average of the pharmacokinetic parameter estimates in individuals.

Relationships between incremental AUC(0,8h) values for blood glucose and plasma insulin and the plasma AUC(0,t) of BTS 67 582; maximum reduction in blood glucose, maximum increase in plasma insulin and peak plasma concentration of BTS 67 582

were explored by linear regression (Pearson correlation coefficients). AUC values were calculated using the linear trapezoidal rule, and incremental AUCs were defined as the differences in area above and below baseline (pre-dose level). Dose-related differences in the above measures of glucose and insulin were assessed using four-way analysis of variance with factors for treatment, subject, period and first-order carryover. Where appropriate, William's test was used to compare active and placebo treatments. Placebo effects were evaluated by a linear regression of blood glucose against time. Three models of the relationship between blood glucose and plasma BTS 67 582 were examined: a linear model $E = a + bC$; a log-linear model: $E = a + b \ln(C)$; and an $E_{\max}$ model: $E = E_{\max} C^n / (EC_{50}^n + C^n)$; where E is the percent reduction in blood glucose after adjustment for placebo effect, C is the plasma drug concentration, $E_{\max}$ is the maximum percent reduction in blood glucose, EC_{50} is the plasma drug concentration at half maximum effect, and n is the Hill coefficient describing the steepness of the logistic concentration-response curve. Models were fitted separately to individual subject data and to pooled data. Goodness of fit was evaluated by inspection of residual plots, coefficients of determination (R^2) and Akaike Information Criteria (AIC) [14]. Any hysteresis affecting the concentration-response relationship was assessed graphically. Profiles indicating a specific direction of hysteresis were defined subjectively as those spanning an area in that direction exceeding 2/3 of the total area covered. The direction of hysteresis observed was compared for a dose effect using Friedman's test, and between doses using pairwise Wilcoxon signed-ranks tests. Similar analyses were applied to assess the relationship between plasma insulin and BTS 67 582 data. The kinetic and dynamic models were combined to predict the time-course of drug-effect after different drug doses. Assuming additivity in the pharmacokinetics, predicted reductions in 24 h mean blood glucose measurements during chronic administration of 500 mg BTS 67 582 twice daily were used to predict longterm reductions in glycosylated haemoglobin (HbA1) from regression equations relating fasting blood glucose to HbA1 reported in patient studies [15].

Results

Pharmacokinetic model

Mean plasma concentrations of BTS 67 582 at each dose level are shown in Figure 1a.

Figure 1 Mean plasma concentrations of BTS 67 582 (a), blood glucose concentrations (b) and plasma insulin concentrations (c) after single oral doses of 125 (▲), 250 (●) and 500 (■) mg BTS 67 582 and placebo (○) in fasted subjects.

A one compartment model with no lag time provided an adequate description of the plasma drug data for each individual at each dose level (Figure 2a). There was no evidence for changes in kinetics as a function of dose (Table 1).

Kinetic-dynamic relationship

Inspection of the time-courses of plasma BTS 67 582, blood glucose and plasma insulin (Figures 1a, 1b and 1c) suggested that the glucose lowering effect of BTS 67 582, but not the change in insulin, mirrored the plasma drug concentration profile. Reductions in incremental glucose AUC with increasing AUC of BTS 67 582 were observed (Table 2, $r = 0.67$, $P < 0.001$). No similar relationship could be found between incremental insulin AUC and the AUC of BTS 67 582 (Table 2, $r = 0.01$, $P = 0.93$). The maximum reduction in blood glucose was related to the

Table 1 One compartment model parameters describing individual time courses of plasma BTS 67 582 in fasted volunteers

Dose (mg)	Mean parameter estimates (95% confidence interval)			Mean R^2 (range)
	k_a (h^{-1})	k (h^{-1})	V (l)	
125	0.97 (0.76,1.19)	0.36 (0.28,0.44)	228 (182,275)	0.88 (0.67,0.98)
250	0.73 (0.60,0.88)	0.32 (0.27,0.37)	266 (218,313)	0.89 (0.64,0.98)
500	0.62 (0.58,0.66)	0.32 (0.25,0.39)	267 (228,306)	0.88 (0.69,0.97)

Table 2 Pharmacokinetic measurements and blood glucose and plasma insulin responses to single oral doses of 125, 250 and 500 mg BTS 67 582 in 16 fasted healthy volunteers

Variable	Placebo	Treatment			Pearson correlation with plasma BTS 67 582	
		BTS 67 582 125 mg	BTS 67 582 250 mg	BTS 67 582 500 mg	r	P
AUC(0,t) plasma BTS 67 582 (ng ml ⁻¹ h)	—	1849.9 ± 644.4	4352.1 ± 1202.4	9538.8 ± 2547.2	—	—
iAUC ¹ (0,8h) blood glucose (mmol ml ⁻¹ h)	-1.6 ± 2.0	-3.1 ± 2.0	-4.6 ± 2.0	-6.6 ± 1.6	0.67 ²	p < 0.001 ²
iAUC ¹ (0,8h) plasma insulin (µu ml ⁻¹ h)	16.9 ± 65.2	12.9 ± 69.6	-38.6 ± 106.4	-12.6 ± 138.8	0.01 ²	p = 0.93 ²
Peak plasma BTS 67 582 (ng ml ⁻¹)	—	517.9 ± 178.0	1076.0 ± 263.6	2434.9 ± 712.8	—	—
Maximum reduction in blood glucose (mmol l ⁻¹)	1.03 ± 0.40	1.13 ± 0.48	1.59 ± 0.64	1.78 ± 0.56	0.49 ³	p < 0.001 ³
Maximum increase in plasma insulin (µu ml ⁻¹)	24.2 ± 14.4	25.5 ± 24.8	14.4 ± 11.2	20.9 ± 14.4	0.07 ³	p = 0.66 ³

Results are quoted as mean ± sd.

¹iAUC = incremental area under the curve.

²Correlation against plasma AUC(0,t) BTS 67 582.

³Correlation against peak plasma BTS 67 582 concentration.

Figure 2 Observed (—, closed symbols) and predicted (---, open symbols) plasma concentrations of BTS 67 582 (a) and blood glucose (b) following single oral doses of 125 (▲, △), 250 (●, ○) and 500 (■, □) mg BTS 67 582 based on pharmacokinetic and combined pharmacokinetic and log-linear concentration-effect models, for a representative subject (volunteer 8).

peak plasma concentration of BTS 67 582 (Table 2, $r = 0.49$, $P < 0.001$), but a relationship between maximum increase in plasma insulin and peak plasma drug concentrations was not observed (Table 2, $r = 0.07$, $P = 0.66$). The minimum effective doses in reducing the AUC of the glucose response and increasing the maximum reduction in blood glucose

compared with placebo were 125 mg and 250 mg, respectively ($P < 0.05$). Significant dose-related differences in AUC and peak plasma insulin concentration were not observed.

Subjective assessment of plots of the reduction in blood glucose against plasma BTS 67 582 concentrations revealed no evidence of anticlockwise hysteresis at the higher two doses when the data were adjusted for placebo (Figures 3a, 3b, 3c). For the 125 mg dose there was some evidence of anticlockwise hysteresis from around 1 h to 5 h post-dose (Figure 3a). Conversely, the 500 mg data suggested clockwise hysteresis from around 1 h to 6 h post-dose (Figure 3c). Thus, there was some evidence of dose-related differences in the direction of hysteresis observed (Friedman test, $P = 0.06$). Of the 16 volunteers receiving 125 mg BTS 67 582, the data from 10 indicated anticlockwise hysteresis and two clockwise hysteresis. The numbers suggesting anticlockwise and clockwise hysteresis were five and two, and two and eight for the 250 mg and 500 mg doses, respectively. Pairwise comparisons revealed a significant difference in the nature of hysteresis observed between the 125 mg and 500 mg doses ($P = 0.02$).

A significant linear-relationship ($r = 0.33$ to 0.79 , $P < 0.05$) was observed between the placebo corrected percentage reduction in blood glucose concentration and the logarithm of the plasma BTS 67 582 concentration in 14 of the 16 volunteers studied. The population model was described by the equation $E = -43.26 + 8.52 \ln(C)$. The log-linear model produced superior fits to the linear and $E_{\max}$ models.

When the pharmacokinetic and pharmacodynamic models were combined, the predicted time course of blood glucose concentrations followed the trends observed for each single dose of BTS 67 582 (Figure 2b).

Figure 3 Relationships between mean reduction in fasted blood glucose (adjusted for placebo) and mean plasma BTS 67 582 concentration after single doses of 125 mg (a), 250 mg (b) and 500 mg (c) BTS 67 582.

A naive pooled approach to modelling the concentration-hypoglycaemic effect relationship suggested significant fits of both $E_{\max}$ (Figure 4) and log-linear models. ($E_{\max}$ model: $n = 1$, $E_{\max} = 35\%$, $EC_{50} = 499$ ng ml⁻¹ ($R^2 = 0.71$); log-linear model: $E = -36.84 + 7.71 \ln(C)$ ($r = 0.51$, $P < 0.01$)). When extended to investigate multiple doses of BTS 67 582, the $E_{\max}$ model predicted a reduction in 24 h mean glucose concentration of 14.9 mg dl⁻¹ compared with placebo. Using reported regressions between mean blood glucose measurements and glycosylated haemoglobin (HbA1c) levels in patients [15], this predicted improvement in glycaemic control forecasted a 0.85% reduction in HbA1c.

No concentration-effect relationship was observed between the plasma insulin and BTS 67 582 concentrations.

Discussion

This study has demonstrated a relationship between the glucose lowering effect of BTS 67 582 and plasma drug concentrations. Incremental AUCs and maximum reductions in blood glucose were correlated with plasma BTS 67 582 AUCs and peak concentrations, respectively. This is in keeping with the effect of sulphonylureas [16] where, for example, correlations between the fall in glucose levels and drug concentrations were observed during the first 4 h after dosage of gliclazide in healthy volunteers [17]. Although BTS 67 582 has been seen to exert a hyperinsulinaemic effect in animal studies (Marita & Kaul, unpublished), no correlation between plasma insulin and plasma BTS 67 582 was observed in this study. This may be a feature of the use of an oral formulation, instead of an intravenous formulation, in

Figure 4 $E_{\max}$ model: Observed and predicted percent reduction in blood glucose (adjusted for placebo) vs plasma concentration of BTS 67 582. $E_{\max} = 35\%$, $EC_{50} = 499$ ng ml⁻¹.

fasted healthy volunteers [18]. It has been suggested that early treatment of NIDDM with a sulphonylurea (glipizide) may produce a reduction in blood glucose without chronic hyperinsulinaemia [19]. However, measurement of insulin levels in the portal vein may be more revealing than systemic levels.

The glucose lowering effect was adequately described by a log-linear model and, when the data were pooled, by an $E_{\max}$ model where the maximum effect was a 35% reduction in fasted blood glucose and the EC_{50} was 500 ng ml⁻¹. Similarities between blood glucose and plasma drug concentration profiles have also been reported for sulphonylureas in healthy subjects [20, 21]. These models are, however, likely to over simplify an extremely complex feedback system. Physiologically based models may enhance predictions [22].

After adjustment for the placebo response, little evidence of anticlockwise hysteresis in the relationship between blood glucose and plasma drug concentrations was evident at the higher doses, suggesting that BTS 67 582 does not produce an active metabolite. Some evidence of clockwise hysteresis from 1 h to 6 h was observed at the highest dose of BTS 67 582. The samples at 1 h and 6 h post- 500 mg dose corresponded to mean plasma BTS 67 582 concentrations close to the maximum plasma drug concentration observed for the 250 mg dose. It may be that around this concentration (approximately 800 ng ml⁻¹, which correlates with a 20% reduction in blood glucose) other mechanisms, such as the secretion of glucagon and adrenaline operate due to increased hypoglycaemic stimulus. However, these hypotheses require further support and are unlikely to be the case in NIDDM patients, as blood glucose is likely to remain at a higher concentration. Such problems are, nevertheless, likely to be an inherent feature of complex feedback systems, such as the control of blood glucose or blood pressure.

Combining pharmacokinetic and concentration-effect models permitted pharmacokinetic and pharmacodynamic time courses to be predicted for different doses of BTS 67 582. Such models can be used to perform simulation experiments to provide information useful in optimising future dosing regimens, although greater benefit from this approach may be gained from a non-fasted study of pharmacokinetics and dynamics in NIDDM patients. Reported correlations between mean blood glucose measurements and glycosylated haemoglobin (HbA1) levels in patients [15] were useful in predicting drops in HbA1 following chronic administration of BTS 67 582. The predicted drop in 24 h mean glucose concentration of 14.9 mg dl⁻¹ compared with placebo corresponded to a 0.85% reduction in HbA1. A 0.8% reduction in HbA1c has been reported under long term sulphonylurea therapy [23]. A 1.0–1.5% reduction in HbA1c is likely to be the desired primary efficacy endpoint for new oral hypoglycaemic agents [24]. The estimated reduction provides some indication of longterm efficacy, although it is based on fasted levels in healthy volunteers and is dependent on the estimation of $E_{\max}$ and EC_{50} values with few data at very high plasma BTS 67 582 concentrations. The above methods should be applied to further studies on BTS 67 582 in NIDDM patients to produce more valuable and realistic inferences to aid future study design, although fasted volunteers may be a good model of the effects in patients whose post-prandial glucose levels remain difficult to control.

The study was performed at GH Besselaar Associates Clinical Research Unit, Leeds. Mr L. M. Underwood and Mr I. D. Hind, Boots Pharmaceuticals, were responsible for the BTS 67 582 assay. Dr M. Cooling, Boots Pharmaceuticals, was responsible for the insulin assay. Glucose assay was performed at GH Besselaar Associates.

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