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Dose-Proportionality of a Final Market Image Sitagliptin Formulation, an Oral Dipeptidyl Peptidase-4 Inhibitor, in Healthy Volunteers

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ABSTRACT: Sitagliptin is a highly selective orally active dipeptidyl peptidase-4 inhibitor recently approved in the United States for the treatment of type 2 diabetes. Ten healthy subjects received single oral doses of 25, 50, 100, 200 and 400 mg final market image tablets in five separate treatment periods in randomized fashion to assess dose proportionality. Blood (up to 72 h post-dose) and urine (up to 24 h post-dose) samples for sitagliptin pharmacokinetic analysis were collected at pre-specified times following administration of sitagliptin. Dose-proportionality of $AUC_{0-\infty}$, C_{max} and $C_{24\,h}$ was assessed using a power-law model. The results of this study indicate that plasma $AUC_{0-\infty}$ increased in a dose-proportional manner over the 25–400 mg dose range. Over the same dose range, plasma C_{max} increased in a greater than dose-proportional manner and $C_{24\,h}$ increased in a modestly less than dose proportional manner. No clinically meaningful differences in T_{max} or apparent $t_{1/2}$ were noted across the dose range. Differences in the percentage of the sitagliptin dose excreted unchanged in urine (72.5% pooled across doses) and renal clearance (344 ml/min pooled across doses) were not statistically significant. Sitagliptin was generally well tolerated at all the doses evaluated. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: sitagliptin; dose proportionality; final market image

Introduction

Sitagliptin ((2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8 H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine) is an orally active, potent and selective inhibitor of dipeptidyl peptidase-4 (DPP-4) recently approved in the United States for the treatment of type 2 diabetes [1]. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes which functions to stimulate glucose-dependent insulin release and reduce glucagon levels, by inhibiting the inactivation of incretins, particularly glucagon-like peptide-1 [GLP-1] and GIP, thereby improving glycemic control [2,3].

The current study was performed to evaluate the dose proportionality of final market image tablets of sitagliptin. The doses selected, 25, 50, 100, 200 and 400 mg, bracketed the proposed clinical dose of 100 mg. The selection of these doses was based on prior preliminary information from rising single dose studies which suggested that sitagliptin $AUC_{0-\infty}$ increased approximately dose proportionally with increasing dose, whereas C_{max} increased modestly greater than dose proportionally across the dose

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range of 1.5–600 mg following administration of a capsule dosage form [4]. Sitagliptin $AUC_{0-\infty}$ was selected as the primary estimation endpoint of interest *a priori* as it is considered to be the most relevant pharmacokinetic parameter dictating safety and efficacy for a chronically administered DPP-4 inhibitor with no peak-related adverse experiences identified to date.

Methods

Subjects

Ten healthy male ($n = 5$) and non-pregnant female ($n = 5$) volunteers were enrolled in this study. All subjects were non-smokers with a mean age of 38.4 years (range 23–45 years), mean body weight of 78.4 kg (range 60.9–96.4 kg) and had normal plasma glucose levels. Subjects were in good general health according to routine medical history, physical examination, 12-lead electrocardiogram (ECG), vital signs and routine laboratory data. Subjects were excluded if they had any history of renal, hepatic, cardiovascular, gastrointestinal, neurologic disease or diabetes or impaired glucose tolerance. Every subject gave written informed consent. The protocol was approved by the Southern Institutional Review Board, Miami, Florida and the study was conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the Declaration of Helsinki.

Study design

This was an open-label, randomized, 5-period, balanced, crossover study to assess the dose proportionality of sitagliptin final market image tablets within the 25–400 mg dose range in 10 healthy adult subjects. In each period, the subjects received a single 25, 50, 100, 200 or 400 mg dose of sitagliptin. Each subject received the doses of sitagliptin at the same time in each period. The order in which the subjects received each dose was randomized according to a computer generated allocation schedule. In each period, after an overnight 8 h fast, each subject received a single dose of sitagliptin administered with 240 ml of water. Water was restricted 1 h prior to and after drug administration. There was at least a 7 day washout interval separating administration of sitagliptin in each period. After drug administration in each period, blood and urine samples for sitagliptin assay were collected at specified time points up to 24 h (urine) and 72 h (blood) postdose. Since $AUC_{0-24\,h}$ represents approximately 80% of the plasma $AUC_{0-\infty}$, 24 h of urine collection was deemed sufficient to compute the renal clearance and urinary excretion of sitagliptin. Subjects were sequestered at the clinical research unit (CRU) until collection of the 48 h samples, discharged, and then returned for the collection of subsequent blood samples for plasma drug assay.

Blood (4 ml) was drawn via an indwelling intravenous catheter in a forearm vein and processed by centrifugation for determination of plasma sitagliptin concentration at predose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, 32, 48 and 72 h postdose. Urine collections occurred predose and at the following intervals; 0 to 6, 6 to 12, and 12 to 24 h postdose. Urine was kept on ice during collection, the volume recorded to the nearest 5 ml and a 4 ml aliquot was saved for analysis. Plasma and urine samples were stored at –70°C until assayed.

Safety

Physical examinations, vital signs, 12-lead ECG and safety laboratory measurements were performed prestudy, at various time points post dosing and at poststudy. Adverse experiences were monitored throughout the study. An investigator evaluated all clinical adverse experiences in terms of intensity (mild, moderate or severe), duration, severity, outcome and relationship to the study drug.

Bioanalytical methods

Plasma and urine samples were analysed for sitagliptin concentrations as described previously [5]. Briefly, samples were directly injected onto a Cohesive Technologies high turbulent liquid chromatography system (HTLC). Analyte and internal standard were detected by tandem mass spectrometry (MS/MS) using selected reaction monitoring (SRM) with turbo-ionspray interface

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in the positive ion mode. The lower limit of quantitation (LLOQ) for the plasma assay was 0.5 ng/ml and the linear calibration range was 0.5–1000 ng/ml. The LLOQ for the urine assay was 0.1 μg/ml and the linear calibration range was 0.1–50 μg/ml.

Pharmacokinetic methods

The apparent terminal rate constant (λ) was estimated by regression of the terminal log-linear portion (determined by inspection) of the plasma concentration-time profile; $t_{1/2}$ was calculated as the quotient of ln(2) and λ. Area under the plasma concentration-time curve (AUC) to the last time point was calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations [6]. The $AUC_{0-\infty}$ value was estimated as the sum of AUC to the last measured concentration and the extrapolated area given by the quotient of the last measured concentration and λ. The C_{max}, T_{max} and $C_{24\,h}$ values were obtained by inspection of the plasma concentration data.

The amount of sitagliptin excreted unchanged in urine in each collection interval was determined by the product of the urine concentration and the urine volume. The fraction of the sitagliptin dose that was excreted unchanged in urine over 24 h ($f_{e,0-24\,h}$) was determined by the quotient of the sum of sitagliptin collected over all collection intervals and the dose administered. The fraction of the sitagliptin dose that was excreted unchanged in urine ($f_{e,0-\infty}$) was determined as the product of $f_{e,0-24\,h}$ and the $AUC_{0-\infty}/AUC_{0-24\,h}$ ratio. Renal clearance (Cl_R) was determined as the quotient of $f_{e,0-24\,h}$● dose and $AUC_{0-24\,h}$.

Statistical methods

The dose proportionality of sitagliptin over the dose range 25–400 mg was assessed by fitting a power-law model where log[$AUC_{0-\infty}$] of sitagliptin was modeled as a function of subject, period and log[dose], with subject being considered as a random factor. The slope of log[$AUC_{0-\infty}$] versus log[dose] and its 90% confidence interval (CI) were estimated based upon the power-law model. The power-law model assumes a linear relationship between log[$AUC_{0-\infty}$] and log[dose]. A plot of the log[$AUC_{0-\infty}$] of sitagliptin versus log[dose] with a supportive power-law regression line and the corresponding 95% CI was used to provide an additional assessment for the lack of fit of the power-law model.

In the case of perfect dose proportionality, the slope equals 1.00. Thus the model (not counting the terms for subject and period) was written as:

$$\log[AUC_{0-\infty}] = \log(\alpha) + \beta * \log[dose] + \varepsilon \quad (1)$$

On the back transformed scale, this model is

$$AUC_{0-\infty} = \alpha * dose^{\beta} \quad (2)$$

where log(α) and β are the intercept and slope, respectively, in the power-law model. ε is the error term.

The deviation of the slope from 1.00 reflects the degree of departure in the dose-exposure relationship from strict dose proportionality. A slope of less than 1.00 indicates that $AUC_{0-\infty}$ increases in a less than dose proportional manner and a slope greater than 1.00 indicates that $AUC_{0-\infty}$ increases in a greater than dose proportional manner. To assess more explicitly the degree of non-proportionality, the true increase in $AUC_{0-\infty}$ resulting from a 16-fold increase in dose (i.e. over the whole dose range 25–400 mg) was estimated using the relationship $AUC_{400-mg}/AUC_{25-mg} = 16^{slope}$, which was derived from the power-law model. The CI for this ratio was obtained by substituting the lower and upper bound of the 90% CI for the slope in the above expression.

The 90% CI of the geometric least-squares mean ratio (GMR) of dose-adjusted $AUC_{0-\infty}$ between the highest dose (400 mg) and the reference dose (100 mg) was estimated as an additional check that the power-law model was not missing a clinically relevant increase or decrease in $AUC_{0-\infty}$ at the top of the dose range.

A similar procedure was conducted to assess dose proportionality for the plasma C_{max} and $C_{24\,h}$ over the range 25–400 mg. Note that the carryover effect was assumed to be negligible from the above analysis since there was a sufficient washout period (at least 7 days) between administrations of sitagliptin. Statistical trend tests were carried out for T_{max}, apparent terminal $t_{1/2}$, renal clearance (Cl_R) and $f_{e,0-\infty}$ to assess the changes with doses via an analysis of

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variance (ANOVA) model containing factors for subject, period and doses (in continuous scale).

Results

Safety

Administration of single oral sitagliptin doses of 25, 50, 100, 200 and 400 mg was well tolerated. No serious clinical adverse experiences were reported and there were no discontinuations due to an adverse experience. There were no laboratory adverse experiences reported during the study. Three of the 10 subjects reported a total of four non-serious clinical adverse experiences, all of which were considered by the investigator to be mild and transient, the most frequently reported adverse experience being headache. There were no consistent treatment-related changes in laboratory, vital signs or ECG parameters during any of the study treatments.

Pharmacokinetics

Figure 1 illustrates the plasma sitagliptin concentration vs time profile following single dose administration of 25, 50, 100, 200 and 400 mg doses. Sitagliptin exhibited peak concentrations at approximately 1–4 h post-dose. The average apparent terminal half-life ($t_{1/2}$) ranged from 11 to 13 h across the various doses. The pharmacokinetic parameters of sitagliptin following single dose administration of 25, 50, 100, 200 and 400 mg sitagliptin are summarized in Table 1. Since the amount of *AUC* extrapolated in the determination of $AUC_{0-\infty}$ was less than 10%, $AUC_{0-\infty}$ was used as the exposure parameter for evaluation. For $AUC_{0-\infty}$, the slope and its 90% CI estimated by the power-law model was 1.00 and (0.98, 1.01) (see Figure 2). The sitagliptin dose-adjusted (to 100 mg) $AUC_{0-\infty}$ geometric least-squares mean ratio (GMR, 400 mg/100 mg) was 1.02 with a corresponding 90% CI of (0.99, 1.06) and geometric least-squares means of dose-

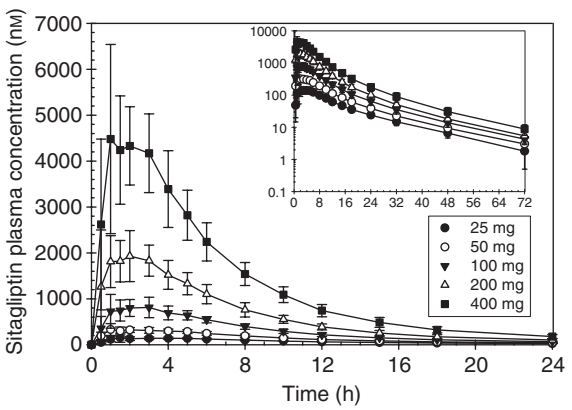

Figure 1. Mean sitagliptin plasma concentrations following administration of single oral doses of 25, 50, 100, 200 and 400 mg of sitagliptin to healthy male and female subjects

Table 1. Mean sitagliptin pharmacokinetic parameters following single oral doses of final market image sitagliptin tablets to healthy male and female subjects ($n = 10$)

Sitagliptin Parameter	LS mean (SD)[a]					Slope (90% CI)[b]	GMR (90% CI)[c] 400 mg/100 mg
	25 mg	50 mg	100 mg	200 mg	400 mg		
$AUC_{0-\infty}$ (μM.h)	2.20 (0.126)	4.17 (0.394)	8.52 (0.733)	16.7 (1.79)	34.9 (3.34)	1.00 (0.98, 1.01)	1.02 (0.99, 1.06)
C_{max} (nM)	177 (65.5)	378 (78.8)	950 (239)	2184 (707)	4949 (1348)	1.21 (1.17, 1.26)	1.30 (1.13, 1.50)
$C_{24\,h}$ (nM)	23.8 (4.83)	38.3 (7.43)	63.5 (16.7)	95.7 (30.4)	170 (44.4)	0.70 (0.67, 0.72)	0.67 (0.62, 0.72)
T_{max} (h)[d]	3.5 (1.8)	2.5 (1.6)	1.3 (1.4)	2.0 (1.1)	2.5 (1.4)		
$t_{1/2}$ (h)[e]	13.1 (2.14)	13.0 (1.82)	12.4 (1.68)	11.7 (1.48)	11.3 (1.40)		
Cl_R (ml/min)	325 (71.0)	357 (53.4)	350 (64.2)	342 (77.7)	347 (64.4)		
$f_{e,\,0-\infty}$[f]	0.707 (0.119)	0.733 (0.098)	0.738 (0.118)	0.703 (0.089)	0.744 (0.096)		

[a] Geometric least-squares mean and between-subject standard deviation, back-transformed from log scale.
[b] Slope of log[PK parameter] versus log[dose] from power-law model.
[c] Ratio of dose adjusted (to 100 mg) geometric least-squares means (400 mg/100 mg).
[d] Median and between-subject standard deviation.
[e] Harmonic least-squares mean and Jackknife standard deviation.
[f] Arithmetic least-squares mean and between-subject standard deviation.

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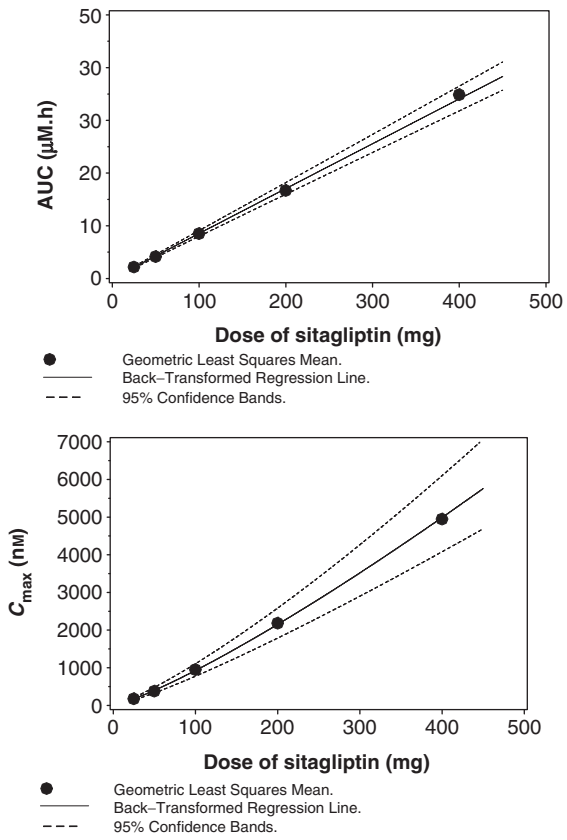

Figure 2. Sitagliptin $AUC_{0-\infty}$ and C_{max} versus dose following administration of single oral doses of 25, 50, 100, 200 and 400 mg of sitagliptin to healthy male and female subjects

adjusted $AUC_{0-\infty}$ values were generally similar among all treatments ($p = 0.056$). The estimated increase in $AUC_{0-\infty}$ from a 16-fold increase in dose (i.e. over the dose range 25–400 mg) was approximately 16.0-fold with a corresponding 90% confidence interval of (15.1, 16.4) (Figure 2).

For C_{max}, the slope and its 90% confidence interval estimated by the power-law model was 1.21 and (1.17, 1.26) (see Figure 2). The estimated increase in C_{max} across this 16-fold dose range (25–400 mg) is approximately 28.6-fold with a corresponding 90% CI of (25.6, 32.9). The sitagliptin dose-adjusted (to 100 mg) C_{max} GMR (400 mg/100 mg) was 1.30 with a corresponding 90% CI of (1.13, 1.50) and geometric least-squares means of dose-adjusted C_{max} values were statistically significantly different among all treatments ($p < 0.001$).

For $C_{24\,h}$, the slope and its 90% confidence interval estimated by the power-law model was 0.70 and (0.67, 0.72). The estimated increase in $C_{24\,h}$ across this 16-fold dose range (25–400 mg) is approximately 7.00-fold with a corresponding 90% CI of (6.4, 7.4). The sitagliptin dose-adjusted (to 100 mg) $C_{24\,h}$ GMR (400 mg/100 mg) was 0.67 with a corresponding 90% CI of (0.62, 0.72) and geometric least-squares means of dose-adjusted $C_{24\,h}$ values were statistically significantly different among all treatments ($p < 0.001$).

Apparent terminal $t_{1/2}$ tended to decrease slightly as dose increased ($p < 0.001$). There were no meaningful differences in T_{max}, renal clearance (Cl_R) and fraction of dose excreted unchanged in the urine ($f_{e,0-\infty}$) across the entire dose range ($p > 0.200$).

Discussion

This study assessed the dose proportionality of sitagliptin final market image tablets within the proposed therapeutic range in healthy adult subjects. The crossover design used in this study, wherein each subject was administered each of the sitagliptin final market image doses (25, 50, 100, 200 and 400 mg as 2×200 mg), enabled a more definitive characterization of the relationship between dose and sitagliptin pharmacokinetics. The study bracketed the once-daily sitagliptin doses that were evaluated in pivotal Phase III clinical studies [7]. The study was not powered to assess the impact of gender on pharmacokinetics, however, no meaningful changes were discerned in the pharmacokinetics of sitagliptin.

Following administration of single oral doses, plasma sitagliptin $AUC_{0-\infty}$ was found to increase dose proportionally as dose was increased from 25 to 400 mg. The slope of log[$AUC_{0-\infty}$] versus log[dose], evaluated with the power-law model, was approximately 1 and no statistically significant differences in dose-adjusted (to a reference dose of 100 mg) $AUC_{0-\infty}$ values were noted. This result is consistent with previous results following administration of a capsule formulation of sitagliptin indicating that AUC increases approximately proportionally with increasing dose

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following rising single or multiple oral doses [4,8]. Additionally, following single intravenous doses of sitagliptin ranging from 25 to 100 mg, it was previously shown that there were no statistically significant differences in total plasma sitagliptin clearance with dose. Results of this study also indicate that neither renal clearance of sitagliptin (which accounts for approximately 70% of total plasma clearance in healthy subjects), nor the fraction of the sitagliptin dose excreted unchanged in urine, change with dose. Together, the results of this study and earlier studies examining oral bioavailability as well as the pharmacokinetics of intravenous doses of sitagliptin [data on file] suggest that neither total plasma clearance nor bioavailability of sitagliptin change with dose over the 25–400 mg dose range.

Sitagliptin C_{max} was found to increase in a greater than dose proportional manner and $C_{24\,h}$ was found to increase in a modestly less than dose proportional manner with dose. These results are consistent with previous results following single or multiple oral doses [4,8]. Additionally, the apparent terminal $t_{1/2}$ of sitagliptin tended to decrease slightly with increasing dose. Since the clearance and bioavailability of sitagliptin do not appear to change with dose, these differences in C_{max}, $C_{24\,h}$ and apparent $t_{1/2}$ with dose are likely due to changes in the distributional or absorption pharmacokinetics of sitagliptin with increasing dose that are not considered to be clinically meaningful.

The pharmacokinetics of sitagliptin exhibited low variability, with approximate within-subject coefficient of variation in $AUC_{0-\infty}$, C_{max}, and $C_{24\,h}$ being 4.7%, 18.7% and 10.5%, respectively, over the dose range 25–400 mg. Based on a theoretical rationale and preclinical data, *AUC* is anticipated to be the most relevant pharmacokinetic parameter for a DPP-4 inhibitor [9; data on file]. Since no peak-related adverse experiences have been identified for sitagliptin to date, the modest deviations from dose-proportionality observed for C_{max} are not considered likely to be clinically meaningful.

Conclusions

In conclusion, sitagliptin $AUC_{0-\infty}$ increases in a dose proportional manner within the 25–400 mg dose range. Sitagliptin C_{max} increases in a modestly greater than dose proportional manner and $C_{24\,h}$ increases in a modestly less than dose proportional manner within the 25–400 mg dose range. The renal clearance of sitagliptin does not appear to change with dose within the 25–400 mg dose range. Sitagliptin was found to be generally well tolerated following administration of single oral 25–400 mg doses to healthy male and female subjects.

Acknowledgements

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