BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2011.03937.x

Effect of rifampicin on the pharmacokinetics and pharmacodynamics of saxagliptin, a dipeptidyl peptidase-4 inhibitor, in healthy subjects

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Keywords
dipeptidyl peptidase-4 (DPP-4) inhibitor, rifampicin, rifampin, saxagliptin

Received
17 August 2010

Accepted
8 February 2011

Accepted Article
18 February 2011

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Rifampicin, an anti-tubercular antibiotic, is a potent inducer of cytochrome P450 (CYP) 3A4 enzymes. Rifampicin is also a potent inducer of some drug efflux transporters and an inhibitor of certain uptake transporter proteins.
- Saxagliptin is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme in the treatment of type 2 diabetes.
- Saxagliptin is metabolized by CYP3A4/3A5 to its major metabolite, 5-hydroxy saxagliptin, which is also a potent inhibitor of DPP-4.
- A drug–drug interaction study of saxagliptin with an archetypal CYP3A4 inducer, rifampicin, provides essential information for clinical use of saxagliptin with respect to the need for dose adjustment when co-administered with CYP3A4 inducers.

WHAT THIS STUDY ADDS

- Rifampicin significantly reduced exposure to saxagliptin. There was no decrease in the area under the plasma concentration–time curve and slight increase in the peak plasma concentration for the active metabolite 5-hydroxy saxagliptin. The saxagliptin total active moieties exposure was slightly lower when co-administered with rifampicin.
- There was no change in the maximum DPP-4 inhibition and area under the effect–time curve for DPP-4 inhibition when saxagliptin was co-administered with rifampicin.
- Saxagliptin was generally safe and well tolerated when co-administered with rifampicin in this study.
- The lack of clinically meaningful change of pharmacodynamic effect (plasma DPP-4 activity) of saxagliptin when co-administered with rifampicin is consistent with the observed slight reduction in systemic exposure to the total active moieties. Based on these findings, it is not necessary to adjust the saxagliptin dose when co-administered with rifampicin.

AIM

To investigate the effect of co-administration of rifampicin, a potent inducer of cytochrome P450 (CYP) 3A4 enzymes, on the pharmacokinetics (PK) and pharmacodynamics (PD) of saxagliptin and 5-hydroxy saxagliptin in healthy subjects. Saxagliptin is metabolized by CYP3A4/3A5 to 5-hydroxy saxagliptin, its major pharmacologically active metabolite.

METHODS

In a non-randomized, open label, single sequence design, 14 healthy subjects received single oral doses of saxagliptin 5 mg with and without steady-state rifampicin (600 mg once daily for 6 days). PK (saxagliptin and 5-hydroxy saxagliptin) and PD (plasma DPP-4 activity) were measured for up to 24 h on days 1 and 7.

RESULTS

Concomitant administration with rifampicin resulted in 53% (point estimate 0.47, 90% CI 0.38, 0.57) and 76% (point estimate 0.24, 90% CI 0.21, 0.27) decreases in the geometric mean C_{max} and AUC values of saxagliptin, respectively, with a 39% (point estimate 1.39, 90% CI 1.23, 1.56) increase in the geometric mean C_{max} and no change (point estimate 1.03, 90% CI 0.97, 1.09) in the AUC of 5-hydroxy saxagliptin. Similar maximum % inhibition and area under the % inhibition–time effect curve over 24 h for DPP-4 activity were observed when saxagliptin was administered alone or with rifampicin. The saxagliptin total active moieties exposure (AUC) decreased by 27% (point estimate 0.73, 90% CI 0.66, 0.81). Saxagliptin with or without rifampicin in this study was generally well tolerated.

CONCLUSIONS

Lack of change of PD effect of saxagliptin is consistent with the observed 27% reduction in systemic exposure to the total active moieties, which is not considered clinically meaningful. Based on these findings, it is not necessary to adjust the saxagliptin dose when co-administered with rifampicin.

Introduction

Saxagliptin is a highly potent, selective, reversible, dipeptidyl peptidase-4 (DPP-4) inhibitor [1]. The DPP-4 inhibitors are an emerging new therapeutic class designed to treat type 2 diabetes [2]. The DPP-4 enzyme actively converts the key insulinotropic hormone glucagon-like peptide-1 (GLP-1) from intact GLP-1 to an inactive form and is responsible for the short half-life of intact GLP-1 *in vivo* [3]. Inhibitors of DPP-4 increase concentrations of endogenous intact GLP-1 and thus potentiate its physiological actions, augmenting postprandial insulin secretion and improving the overall glycaemic profile in patients with type 2 diabetes [4]. Because DPP-4 inhibitors stimulate insulin secretion in a glucose-dependent manner, this mechanism of action is expected to present low risk of hypoglycaemia and may not lead to weight gain [1].

The clinical pharmacodynamic (PD) properties of saxagliptin 5 mg are consistent with once daily dosing. The usual clinical dose in adults is 5 mg administered orally once daily [5]. Saxagliptin has predictable and dose-proportional pharmacokinetics (PK), with minimal accumulation with once daily dosing [6]. Upon oral administration, saxagliptin is rapidly and extensively absorbed, and is cleared via metabolic and renal routes [5]. The major pharmacologically active metabolite of saxagliptin is 5-hydroxy saxagliptin, with an *in vitro* DPP-4 inhibitory potency at 37°C of approximately one-half that of the parent compound saxagliptin [5].

In vitro metabolism studies (individual recombinant cytochrome P450 [CYP] enzymes and human liver microsomes) indicate that saxagliptin is metabolized primarily by CYP3A4 (major) and CYP3A5 (minor) enzymes to 5-hydroxy saxagliptin [5]. *In vitro* incubation of 5-hydroxy saxagliptin with CYP450 isozymes showed a low turnover and no specific CYP isozyme has been identified that catalyzes metabolic elimination of 5-hydroxy saxagliptin. The elimination of 5-hydroxy saxagliptin is almost exclusively by non-metabolic routes (urinary excretion [major] and biliary excretion [minor]). Ketoconazole, a potent inhibitor of CYP3A4/5 enzymes, increased the C_{max} and $AUC_{0-\infty}$ of saxagliptin in healthy subjects by approximately 62% and 145%, respectively, and decreased the C_{max} and $AUC_{0-\infty}$ of 5-hydroxy saxagliptin by approximately 95% and 88%, respectively [7]. Similarly, diltiazem, a moderate inhibitor of CYP3A4/5, increased the C_{max} and $AUC_{0-\infty}$ of saxagliptin by 63% and 109%, respectively, and decreased the C_{max} and $AUC_{0-\infty}$ of 5-hydroxy saxagliptin by 43% and 34%, respectively [8]. These *in vitro* studies and clinical findings clearly establish a major role of CYP3A4/5 in the disposition of saxagliptin and further indicate that concomitant administration of saxagliptin with CYP3A4/5 inducers in humans would be expected to decrease systemic exposure to saxagliptin.

Rifampicin is a potent inducer of CYP3A4 [9, 10], and has been shown to induce various drug efflux transporters such as P-glycoprotein (P-gp) [9, 11] and multi-drug resistance protein 2 (MRP2) [12, 13] via the pregnane X receptor-dependent mechanism [14]. Recent experimental evidence also suggests that rifampicin inhibits certain uptake transporters such as organic anion transporter polypeptide (OATP-1) and MRP2 [15–19]. The European Agency for the Evaluation of Medicinal Products and the US Food and Drug Administration (FDA) recommend rifampicin as a CYP3A4 inducer for use in clinical drug–drug interaction studies in which CYP induction may be of clinical importance [20–22].

The present study was designed to examine the effect of the co-administration of saxagliptin with an archetypal CYP inducer, rifampin (rifampicin), on the PK and PD of saxagliptin, to determine if the combination is safe and tolerable and to guide any potential dose adjustment for saxagliptin when rifampicin is co-administered. Once daily dosing for 5 days with rifampicin 600 mg, which is the highest recommended once daily dose, has been shown to achieve marked CYP induction [10, 23]. Accordingly, in this study, a daily dose of rifampicin 600 mg was administered orally for 6 days to ensure maximum induction of CYP3A4 prior to saxagliptin administration. A 5 mg dose of saxagliptin was used in this study, because it represents the usual once daily clinical dose of saxagliptin.

Methods

This study was conducted at the Bristol-Myers Squibb Clinical Research Center, Hamilton, New Jersey, USA.

Ethics

All study participants gave written informed consent before entering the study. The study protocol and informed consent form were approved by an institutional review board (New England Institutional Review Board, Wellesley, Massachusetts, USA). The study was conducted according to federal regulations [24, 25] and the Declaration of Helsinki. Per statistical considerations, 14 subjects who met inclusion/exclusion criteria were needed.

Subjects

Male and female subjects 18 to 45 years of age, determined as healthy by medical history, physical examination, 12-lead electrocardiogram (ECG), routine haematology and serum chemistry analysis and urinalysis participated in the study. Female subjects were not breast-feeding, pregnant or of childbearing potential and had negative pregnancy tests within 24 h prior to dosing. Subjects with known allergies to DPP-4 inhibitors or rifampicin were excluded. Potential confounding effects of the use of any other medicinal agents were mitigated by excluding subjects who were on: (i) any prescription drugs or over the counter gastric acid controllers or St John's wort (CYP3A and P-gp inducer) within 4 weeks prior, (ii) any other drugs

including over the counter medications and herbal preparations within 1 week and (iii) any injectable or implantable hormonal contraceptive agent within 3 months prior to study drug administration.

Design and procedure

This was a non-randomized, open label, single sequence study (Figure 1), in which subjects who met study requirements within 21 days prior to study enrolment were admitted to the clinical facility on day −1. Each subject received a single oral dose of saxagliptin 5 mg on day 1, 600 mg once daily oral doses of rifampicin on days 2–6, and a single dose of saxagliptin 5 mg concomitantly with a single dose of rifampicin 600 mg on day 7. At the time of dosing, 240 ml of water was taken by the subject orally along with study medication. Except during the rifampicin alone treatment period (days 2–6), subjects had nothing to eat or drink except water for 10 h prior to and until 4 h after study drug administration. Also, subjects did not drink water in the period 1 h before or after study drug administration except when dosing. Subjects remained confined to the research unit until day 8 (after the PK/PD collection and all safety assessments were completed) and were not allowed strenuous exercise, smoking or consumption of alcoholic beverages, grapefruit containing products, Seville oranges or caffeine containing food or beverages during the study.

Safety assessments

Physical examinations, vital sign measurements, 12-lead ECGs and clinical laboratory evaluations (haematology studies, serum chemistry analysis and urinalysis) were performed at selected times throughout the study. Subjects were closely monitored for any adverse events (AEs) throughout the study.

PK and PD sampling

Blood samples were collected for PK (plasma saxagliptin and 5-hydroxy saxagliptin concentrations) and PD (plasma DPP-4 activity) prior to saxagliptin dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16 and 24 h after saxagliptin dosing on days 1 and 7. Urine samples were collected prior to dosing and during the following intervals: 0–12 h and 12–24 h after saxagliptin dosing on days 1 and 7. The first collection interval (0–12 h) began with a complete void at pre-dose and each interval concluded with a complete void. The volume of urine obtained was recorded at the end of each collection interval. The plasma (PK and PD) and urine samples were stored at −20°C until analysis within the known period of stability.

Quantitative determination of saxagliptin and 5-hydroxy saxagliptin in plasma and urine

Saxagliptin and its major active metabolite, 5-hydroxy saxagliptin, were analyzed in the plasma and urine samples using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Sample preparation for plasma and urine samples involved solid phase extraction, and saxagliptin-$^{13}CD_2$ and 5-hydroxy saxagliptin-$^{13}CD_2$ were used as internal standards. The lower limits of quantitation (LLQ) of the assays were 1 ng ml^{-1} for saxagliptin and 2 ng ml^{-1} for 5-hydroxy saxagliptin in plasma, and 5 ng ml^{-1} for saxagliptin and 10 ng ml^{-1} for 5-hydroxy saxagliptin in urine. The accuracy and precision values for the assay were well within the widely accepted criteria of ±15% deviation and coefficient of variation (CV) percentage set forth for quantitative determination of an analyte in biological matrices [26]. All samples for a subject from both treatments (with and without rifampicin) were analyzed together. Stability of both saxagliptin and 5-hydroxy saxagliptin in human plasma was established for 12 months at −20°C and for 736 days in human urine at −20°C, and all samples were analyzed within this period of analyte stability.

Plasma DPP-4 (PD) activity determination

Plasma samples were analyzed to determine DPP-4 activity using a validated endpoint enzyme activity assay. Briefly,

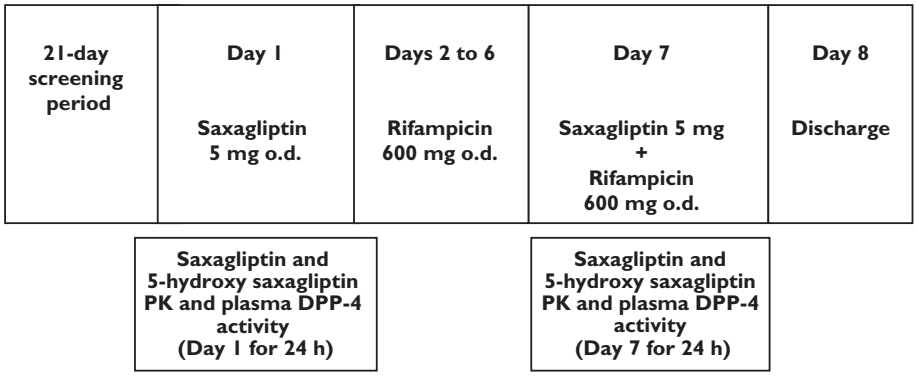

Figure 1
A schematic of the study design

10 µl of plasma was incubated with 100 µl of 2000 µmol l^{-1} glycyl-prolyl-p-nitro-aniline for 90 min at pH 7.4 and 25°C and the rate of p-nitro-aniline product formation was determined as a measure of DPP-4 activity. The measured change in absorbance at 405 nm (p-nitro-aniline formation) was directly proportional to the plasma DPP-4 activity. The reportable assay range was 0.31–40 U l^{-1}. Deviations of the mean measured quality control samples were within ±1.7% and the between-run and within-run variability was ≤6.55% CV.

PK analysis

PK parameters were generated by non-compartmental methods and other specific methods, as described following using the program Kinetica (version 4.4.1, Thermo Electron Corporation, Philadelphia, Pennsylvania, USA). The metabolite : parent $AUC_{0-\infty}$ ratio was calculated by dividing the molar $AUC_{0-\infty}$ value for the metabolite, 5-hydroxy saxagliptin, by the molar $AUC_{0-\infty}$ value for the parent drug, saxagliptin. The saxagliptin total active moiety exposure values were calculated by adding the molar $AUC_{0-\infty}$ value for saxagliptin to one-half of the molar $AUC_{0-\infty}$ value for 5-hydroxy saxagliptin. One-half of the 5-hydroxy saxagliptin $AUC_{0-\infty}$ value was used to account for the 2-fold lower *in vitro* potency for DPP-4 inhibition of 5-hydroxy saxagliptin relative to that of saxagliptin [5].

PD analysis

PD parameters were generated by non-compartmental methods using the program Kinetica. Prior to non-compartmental analysis of the plasma DPP-4 activity–time data, DPP-4 activity–time data were converted to percentage inhibition of plasma DPP-4 activity (DPP-4 % inhibition) using each individual subject's baseline DPP-4 activity, i.e. DPP-4 activity prior to saxagliptin dosing for each treatment period (pre-dose, time 0 h). The following formula was used:

$$\text{DPP-4 \% inhibition} = \frac{[(\text{DPP-4 activity at time 0 h} - \text{DPP-4 activity at time } t) \times 100]}{\text{DPP-4 activity at time 0 h}}$$

Statistical analysis

The log-transformed PK parameters (C_{max}, $AUC_{0-\infty}$) for saxagliptin and 5-hydroxy saxagliptin were analyzed using analysis of variance (ANOVA). Point estimates and 90% confidence intervals for treatment differences on the log scale were exponentiated to obtain point estimates and 90% confidence intervals for the day 7 (saxagliptin with rifampicin) and day 1 (saxagliptin alone) ratio of geometric means for C_{max} and $AUC_{0-\infty}$ of saxagliptin and 5-hydroxy saxagliptin, on the original scale of measurement. Secondary PK and PD parameters and safety variables were analyzed in a descriptive manner by summary statistics. All statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, North Carolina, USA).

Analysis of pooled urine samples to evaluate relative changes in metabolites

Two pooled urine samples from the two treatments representing day 7 (saxagliptin with rifampicin) and day 1 (saxagliptin alone) were prepared by combining equal volumes (100 µl each) from both the 0–12 h and 12–24 h post-dose cumulative urine samples of all the subjects for the two treatments separately. The pooled human urine samples were centrifuged at 13 000 rpm for 5 min in a microcentrifuge and 50 µl portions were injected and analyzed by positive ion electrospray LC-MS/MS. Metabolites of saxagliptin that had been previously identified in human studies were monitored using selected multiple reaction monitoring transitions. The peak areas of these metabolites in the two samples were determined and relative changes in metabolite amounts were reported as the peak area ratio.

Results

Fourteen subjects were enrolled and received study drug. The baseline demographics and characteristics of the subjects are given in Table 1. One subject withdrew consent prior to dosing on day 5. This subject was included in safety assessments because he had been dosed with study medications, but he was excluded from PK and PD evaluation data sets due to incomplete data.

Safety and tolerability

No deaths, serious AEs or discontinuations due to AEs were reported during the study. Eight AEs occurred in six subjects. All the AEs were determined to be of mild intensity by the investigator. The most common AE was headache, which occurred in one subject (7.1%) following administration of saxagliptin 5 mg alone, one subject (7.1%) following administration of rifampicin 600 mg once daily alone and three subjects (23.1%) following co-administration of saxagliptin 5 mg and rifampicin 600 mg. One subject reported

Table 1

Baseline demographics and characteristics

	All subjects†
Age (years) Mean (SD)	33 (7)
Gender, *n* (%)	
Male	13 (93)
Female	1 (7)
Race, *n* (%)	
White	2 (14)
Black/African American	11 (79)
Other	1 (7)
Weight (kg) Mean (SD)	81.7 (10.0)
BMI (kg m^{-2}) Mean (SD)	26.3 (2.7)

†*N* = 14. BMI, body mass index; SD, standard deviation.

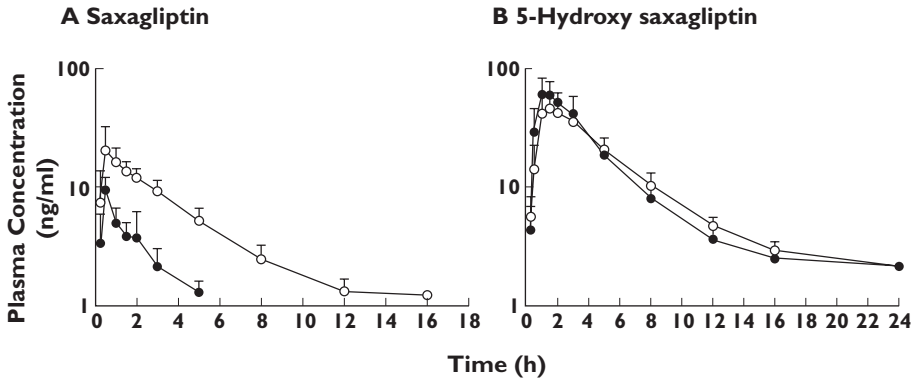

Figure 2

Mean (+SD, n = 13) plasma concentration–time profiles for (A) saxagliptin and (B) 5-hydroxy saxagliptin (major active metabolite of saxagliptin) following administration of a single oral dose of saxagliptin 5 mg to healthy subjects with and without rifampicin (600 mg once daily for 6 days). Saxagliptin 5 mg (—○—); Saxagliptin 5 mg + rifampicin 600 mg (—●—)

Table 2

Summary of saxagliptin pharmacokinetic parameters following administration of a single oral dose of saxagliptin 5 mg to healthy subjects with and without rifampicin (600 mg once daily for 6 days)

Pharmacokinetic parameter	Saxagliptin 5 mg (n = 13) (reference)	Saxagliptin 5 mg with rifampicin 600 mg (n = 13) (test)	Test : reference ratio (%)	90% CI
C_{max} (ng ml^{-1}) Geometric mean (CV%)	20 (47)	9 (25)	47	38, 57
t_{max} (h) Median (min, max)	0.48 (0.48, 2.02)	0.48 (0.48, 1.98)	NC	NC
$AUC_{0-\infty}$ (ng·h ml^{-1}) Geometric mean (CV%)	73 (26)	17 (24)	24	21, 27
$t_{1/2}$ (h) Mean (SD)	3.02 (0.64)	1.70 (0.57)	NC	NC
Urinary recovery (%) Mean (SD)	17 (4)	4 (1)	NC	NC
CL_R (ml min^{-1}) Geometric mean (CV%)	180 (29)	197 (15)	NC	NC

$AUC_{0-\infty}$, area under the plasma concentration–time curve from time 0 to infinity; CI, confidence interval; CL_R, apparent renal clearance; C_{max}, maximum plasma concentration; CV%, coefficient of variation %; NC, not calculated; $t_{1/2}$, terminal elimination half-life; t_{max}, time to reach the C_{max}.

abdominal pain and one other subject reported pain and swelling at the venipuncture site following administration of rifampicin 600 mg. No clinically significant changes or trends were observed for ECGs, vital signs, clinical laboratory tests or physical examinations.

Effect of rifampicin on the PK of saxagliptin

The mean plasma concentration–time profiles of saxagliptin and 5-hydroxy saxagliptin are shown in Figure 2. PK parameter estimates for saxagliptin and 5-hydroxy saxagliptin are summarized in Table 2 and Table 3, respectively.

Co-administration of rifampicin with saxagliptin substantially reduced saxagliptin exposure. When saxagliptin 5 mg was co-administered with rifampicin 600 mg, the geometric means for C_{max} and $AUC_{0-\infty}$ of saxagliptin decreased by 53% (point estimate 0.47, 90% CI 0.38, 0.57) and 76% (point estimate 0.24, 90% CI 0.21, 0.27), respectively, relative to those observed following administration of saxagliptin 5 mg alone. The 90% CIs for the ratios of population geometric means, with and without rifampicin, were entirely below the pre-specified no-effect interval of 80% to 125% recommended by the US [20, 21] and the European Union [22] regulatory guidances for the conduct of *in vivo* drug interaction studies for C_{max} and $AUC_{0-\infty}$. Consistent with the decrease in saxagliptin exposure was a reduction in the $t_{1/2}$ of saxagliptin from 3.02 h to 1.70 h upon co-administration of saxagliptin with rifampicin. The t_{max} of saxagliptin was unaffected by rifampicin, and no substantial changes in the renal clearance values of saxagliptin were observed when saxagliptin was administered with or without rifampicin treatment.

Table 3

Summary of 5-hydroxy saxagliptin (major active metabolite of saxagliptin) pharmacokinetic parameters following administration of a single oral dose of saxagliptin 5 mg to healthy subjects with and without rifampicin (600 mg once daily for 6 days)

Pharmacokinetic parameter	Saxagliptin 5 mg (n = 13) (reference)	Saxagliptin 5 mg with rifampicin 600 mg (n = 13) (test)	Test : reference ratio (%)	90% CI
C_{max} (ng ml^{-1})				
Geometric mean (CV%)	49 (25)	68 (21)	139	123, 156
t_{max} (h)				
Median (min, max)	1.50 (1.00, 2.98)	1.02 (0.98, 2.98)	NC	NC
$AUC_{0-\infty}$ (ng·h ml^{-1})				
Geometric mean (CV%)	258 (17)	266 (19)	103	97, 109
$t_{1/2}$ (h)				
Mean (SD)	4.32 (0.65)	3.89 (0.98)	NC	NC
Urinary recovery (%)				
Mean (SD)	24 (5)	26 (3)	NC	NC
CL_R (ml min^{-1})				
Geometric mean (CV%)	77 (24)	80 (20)	NC	NC
Metabolite : parent ratio				
Geometric mean (CV%)	3 (24)	15 (26)	NC	NC

$AUC_{0-\infty}$, area under the plasma concentration–time curve from time 0 to infinity; CI, confidence interval; CL_R, apparent renal clearance; C_{max}, maximum plasma concentration; CV%, coefficient of variation %; NC, not calculated; metabolite : parent ratio, (5-hydroxy saxagliptin $AUC_{0-\infty}$: saxagliptin $AUC_{0-\infty}$) × (315.42/331.42); $t_{1/2}$, terminal elimination half-life; t_{max}, time to reach the C_{max}.

In contrast to the parent compound saxagliptin, the PK profile of 5-hydroxy saxagliptin was not dramatically affected by rifampicin co-administration. When saxagliptin 5 mg was co-administered with rifampicin 600 mg, the geometric means for C_{max} increased by 39% (point estimate 1.39, 90% CI 1.23, 1.56) without change in the $AUC_{0-\infty}$ (point estimate 1.03, 90% CI 0.97, 1.09) of 5-hydroxy saxagliptin, relative to those observed following administration of saxagliptin 5 mg alone. The 90% CIs for the ratios of population geometric means, with and without rifampicin, were within the 80% to 125% no-effect interval for $AUC_{0-\infty}$ of 5-hydroxy saxagliptin. However, the 90% CI for the ratio of population geometric means, with and without rifampicin, extended above the no-effect interval for C_{max} of 5-hydroxy saxagliptin.

There was a 5-fold increase in the metabolite : parent ratio when saxagliptin was co-administered with rifampicin compared with saxagliptin alone. No substantial changes in renal clearance values or t_{max} and $t_{1/2}$ of 5-hydroxy saxagliptin were observed when saxagliptin was administered with and without rifampicin treatment. The geometric mean for saxagliptin total active moiety exposure ($AUC_{0-\infty}$) decreased by 27% (point estimate 0.73, 90% CI 0.66, 0.81) when saxagliptin was co-administered with rifampicin compared with when saxagliptin was administered alone.

Effect of rifampicin on the PD profile (plasma DPP-4 activity) of saxagliptin

The mean plasma DPP-4 activity–time (percentage change from baseline, where baseline equals DPP-4 activity at pre-dose) profiles for saxagliptin administered with and without rifampicin treatment are shown in Figure 3. PD parameter estimates for plasma DPP-4 activity are summarized in Table 4. The geometric mean maximum inhibition of DPP-4 activity (%I_{max}) remained unchanged (point estimate 1.00, 90% CI 0.98, 1.03) and the overall inhibition of DPP-4 activity over the dosing interval (i.e. the $AUEC_{0-24}$) was also unchanged (point estimate 0.94, 90% CI 0.90, 0.97) when saxagliptin was administered with rifampicin relative to that observed following administration of saxagliptin 5 mg alone.

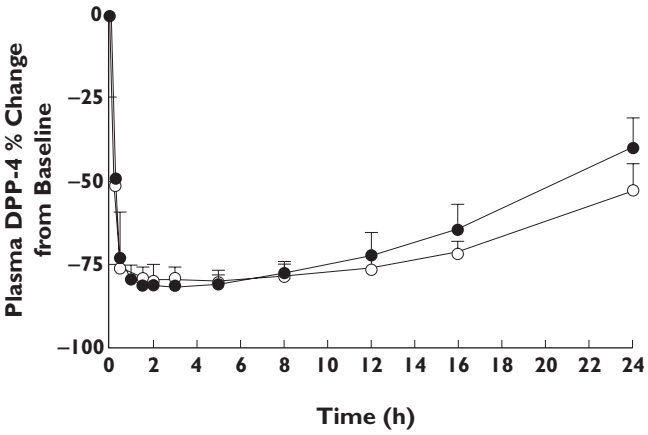

Figure 3

Mean (+SD, n = 13) plasma DPP-4 activity (change from baseline expressed as a negative value of DPP-4 % inhibition) time profiles following administration of a single oral dose of saxagliptin 5 mg to healthy subjects with and without rifampicin (600 mg once daily for 6 days). Saxagliptin 5 mg (—○—); Saxagliptin 5 mg + rifampicin 600 mg (—●—)

Table 4

Summary of plasma DPP-4 % inhibition pharmacodynamic parameters following administration of a single oral dose of saxagliptin 5 mg to healthy subjects with and without rifampicin (600 mg once daily for 6 days)

Pharmacodynamic parameter	Saxagliptin 5 mg (n = 13) (reference)	Saxagliptin 5 mg with rifampicin 600 mg (n = 13) (test)	Point estimate (90% CI) Ratio of geometric means test : reference
%I_{max} Mean (SD)	83.07 (3.50)	83.22 (2.14)	1.00 (0.98, 1.03)
t_{max} (effect) (h) Median (min, max)	2.00 (1.00, 8.00)	2.00 (1.00, 12.00)	
$AUEC_{0–24}$ (% inhibited·h) Mean (SD)	1711.61 (71.82)	1603.97 (111.20)	0.94 (0.90, 0.97)
$t_{1/2}$ (effect) (h) Mean (SD)	25.85 (10.64)	14.46 (4.23)	

$$\text{DPP-4 \% inhibition} = \frac{[(\text{DPP-4 activity at time 0 h} - \text{DPP-4 activity at time } t) \times 100]}{\text{DPP-4 activity at time 0 h}}$$

$AUEC_{0–24}$, area under the DPP-4 % inhibition (effect)–time curve from time 0 to the last measured time point 24 h post-dose; CI, confidence interval; %I_{max}, peak DPP-4 % inhibition; t_{max} (effect), time to reach peak DPP-4 % inhibition; $t_{1/2}$ (effect), apparent effect terminal half-life.

Table 5

Peak area ratios for saxagliptin metabolites from the pooled urine samples following administration of a single oral dose of saxagliptin 5 mg to healthy subjects with and without rifampicin (600 mg once daily for 6 days)

Analyte in urine	Structural description of analyte in urine	Peak area (counts) Saxagliptin 5 mg (reference)	Peak area (counts) Saxagliptin 5 mg with rifampicin 600 mg (test)	Peak area ratio Test : reference	Transition Multiple reaction monitoring
Saxagliptin	Parent	5 390 000	1 290 000	0.24	316.0/180.0
D1	Degradant	8 080	2 080	0.26	316.0/288.0
M1	Mono-hydroxy	64 300	50 500	0.79	332.0/196.0
M2 (5-hydroxy saxagliptin)	Mono-hydroxy (major metabolite)	3 820 000	3 460 000	0.91	332.0/196.0
M3	Mono-hydroxy	394 000	206 000	0.52	332.0/196.0
M5	Di-hydroxy	31 100	130 000	4.18	348.0/212.0
M8	Di-hydroxy	34 700	73 300	2.11	348.0/212.0
M13	Mono-hydroxy	3 460	4 030	1.16	332.0/304.0
M16	Mono-hydroxy	31 600	35 000	1.11	332.0/196.0
M17	Di-hydroxy	15 700	40 400	2.57	348.0/212.0
M19	Gluc-mono-hydroxy	2 870	3 220	1.12	508.0/332.0
M27	Mono-hydroxy-decyanated	14 200	55 300	3.89	321.0/196.0
M40	Gluc-mono-hydroxy	1 340	1 360	1.01	508.0/332.0
M45	Sulfate-parent	2 930	673	0.23	396.0/298.0
M46	Gluc-parent	14 300	3 080	0.22	492.0/316.0
M49	Di-hydroxy	10 400	32 600	3.13	348.0/212.0

Relative changes in the amount of metabolites in the pooled urine samples

Peak areas for parent saxagliptin and each metabolite in the two pooled urine samples from saxagliptin with and without rifampicin are summarized in Table 5. Also summarized in Table 5 is the peak area ratio for each metabolite in the two treatments. Peak area of each metabolite during saxagliptin and rifampicin co-administration treatment was divided by peak area of that metabolite during treatment with saxagliptin alone. With rifampicin there was a small (2- to 5.5-fold) increase in formation of several minor dihydroxy metabolites and a glucuronide conjugate of saxagliptin. However, the concentrations of these metabolites in the urine samples were minor compared with saxagliptin or 5-hydroxy saxagliptin.

Discussion

Safety and tolerability

The types of AEs and their frequency reported by healthy subjects were not unusual for short-term studies in healthy subjects, and did not show a clear difference for the two treatments (saxagliptin administered with and without rifampicin). This result suggests that a single dose of saxagliptin 5 mg was generally safe and well tolerated when co-administered with rifampicin.

Pharmacokinetics

Co-administration of rifampicin with saxagliptin decreased the systemic exposure of saxagliptin (53% and 76%, respective decrease in C_{max} and $AUC_{0–\infty}$ values) and

increased the C_{max} of its pharmacologically active major metabolite, 5-hydroxy saxagliptin, by 39%, consistent with an increase in CYP3A-mediated metabolism. However, co-administration of rifampicin with saxagliptin did not change the overall systemic exposure ($AUC_{0-\infty}$) to 5-hydroxy saxagliptin. While the percentage of saxagliptin dose recovered in urine was lower when rifampicin was administered with saxagliptin, there were no changes in the renal clearance of either saxagliptin or 5-hydroxy saxagliptin when saxagliptin was administered with or without rifampicin. The total active moieties (molar summations of saxagliptin exposure parameter with one-half the molar exposure parameter for 5-hydroxy saxagliptin, factoring in the 2-fold higher inhibition constant (K_i) value of 5-hydroxy saxagliptin towards *in vitro* DPP-4 inhibition at 37°C [5, Bristol-Myers Squibb/AstraZeneca, unpublished data]) $AUC_{0-\infty}$ was reduced by 27% on co-administration of saxagliptin with rifampicin. In line with the PK findings for the total active moieties, the maximum inhibition of DPP-4 activity (%I_{max}) and the overall inhibition of DPP-4 activity over the dosing interval, as measured by $AUEC_{0-24}$, remained unchanged when saxagliptin was administered with rifampicin, compared with when it was administered alone.

The decrease in systemic exposure to saxagliptin when saxagliptin is co-administered with rifampicin can be attributed mainly to induction of CYP3A4/5-mediated metabolism of saxagliptin by rifampicin. This conclusion is supported by the observation of a higher C_{max} of 5-hydroxy saxagliptin, indicating induced first-pass metabolism when saxagliptin is co-administered with rifampicin. Metabolic induction is also evidenced by the 5-fold increase in metabolite : parent $AUC_{0-\infty}$ ratio. Metabolic induction of saxagliptin also resulted in a decrease in the $t_{1/2}$ of saxagliptin from 3.02 h when saxagliptin was administered alone to 1.70 h when saxagliptin was co-administered with rifampicin.

When co-administered with saxagliptin, rifampicin did not alter renal clearance of either saxagliptin or 5-hydroxy saxagliptin, indicating that rifampicin did not induce renal transporters that may be involved in active renal secretion of saxagliptin. However, the amount of total administered dose recovered in urine (i.e. percentage urinary recovery of saxagliptin + 5-hydroxy saxagliptin) was lower when rifampicin was co-administered with saxagliptin (30%) compared with when saxagliptin was administered alone (41%). Thus, a fraction of the saxagliptin dose when co-administered with rifampicin is not accounted for in urine. Renal clearance of saxagliptin and 5-hydroxy saxagliptin is not altered by rifampicin; the implication of this finding is that a fraction of the saxagliptin dose in the systemic circulation (as measured by saxagliptin and 5-hydroxy saxagliptin PK) is also not accounted for. This is likely the source of the lower than expected impact of rifampicin on 5-hydroxy saxagliptin $AUC_{0-\infty}$.

A number of possible explanations exist for these findings. If the fraction of saxagliptin metabolized to 5-hydroxy saxagliptin was increased by rifampicin, the less than expected increase in the systemic AUC of 5-hydroxy saxagliptin may have been due to increased clearance of 5-hydroxy saxagliptin by non-renal pathways. Hence, the observation of decreased systemic exposure of saxagliptin without a subsequent larger increase in the AUC of 5-hydroxy saxagliptin than was observed could be explained by the hypothesis that rifampicin may have induced non-renal elimination pathways of 5-hydroxy saxagliptin, such as biliary/intestinal secretion of 5-hydroxy saxagliptin [Bristol-Myers Squibb/AstraZeneca, unpublished data]. *In vivo* animal experiments indicate that oral bioavailability of 5-hydroxy saxagliptin is very poor, suggesting that there is minimal likelihood of substantial intestinal resorption of 5-hydroxy saxagliptin secreted via bile or intestinal secretion. The increased biliary/intestinal secretion hypothesis in the rifampicin-induced state is consistent with the observation of a 39% increase in the C_{max} of 5-hydroxy saxagliptin (larger fraction metabolized by first pass), but no increase in its AUC (increased non-renal clearance).

When saxagliptin is administered alone, 5-hydroxy saxagliptin undergoes minimal further metabolism. The possibility that rifampicin may have induced alternate metabolic pathways, potentially catalyzing further metabolism of 5-hydroxy saxagliptin or catalyzing conversion of saxagliptin to metabolites other than 5-hydroxy saxagliptin, was tested using leftover urine samples from both treatment periods to identify whether the extent of formation of previously known minor metabolites had increased. Urine samples from both treatments were analyzed by LC-MS/MS and peak area ratios of each metabolite were determined (peak area of each metabolite during saxagliptin and rifampicin co-administration treatment was divided by peak area of that metabolite during saxagliptin alone treatment). Results of this analysis indicated that there was a small (2- to 5.5-fold) increase in formation of several minor dihydroxy metabolites and a glucuronide conjugate of saxagliptin. However, due to the comparatively much lower concentrations of these metabolites *vs.* saxagliptin or 5-hydroxy saxagliptin in the urine samples, the overall small magnitude increase in peak areas of these minor metabolites was not considered sufficient evidence for induction of alternate metabolic pathways of saxagliptin or 5-hydroxy saxagliptin.

Apart from its induction effect, there is experimental evidence to suggest that rifampicin also inhibits certain uptake transporters [15–19]. Clinically relevant drug–drug interaction studies have implicated rifampicin in inhibiting the OATP-1–mediated liver uptake of substrate drugs such as bosentan [16], atorvastatin [17] and atrasentan [15]. Similarly, rifampicin has also been shown to mediate drug–drug interactions via inhibition of MRP2 transport [19]. Even though saxagliptin has a low *in vitro* permeability, oral absorption is high, indicating that one or more as yet unidentified uptake transporters may facilitate oral absorption of saxagliptin. Rifampicin, however, may reduce

the oral bioavailability of saxagliptin by inhibiting these putative uptake transporter(s). This hypothesis is also supported by the previously discussed reduction in the amount of total dose recovered in urine (percentage urinary recovery of saxagliptin + 5-hydroxy saxagliptin) when saxagliptin was co-administered with rifampicin.

The decrease in systemic exposure to saxagliptin when saxagliptin is co-administered with rifampicin might also be explained, in part, by induction of P-gp–mediated efflux of saxagliptin in the intestine by rifampicin. This effect may be one reason for not observing subsequent larger increases in systemic exposures to 5-hydroxy saxagliptin. However, *in vitro* experiments indicate that saxagliptin is only a weak substrate of P-gp, and although induction of P-gp–mediated decreases in systemic availability of saxagliptin by rifampicin cannot be ruled out, this appears to be an unlikely mechanism for these observations [Bristol-Myers Squibb/AstraZeneca, unpublished data].

In summary, metabolic clearance of saxagliptin was increased by co-administration with rifampicin and the metabolic product was primarily 5-hydroxy saxagliptin. Systemic exposure to 5-hydroxy saxagliptin, as measured by AUC, was practically unchanged by rifampicin co-administration, whereas an increase in 5-hydroxy saxagliptin AUC was anticipated. Approximately 11% less of the saxagliptin dose was recovered in urine when saxagliptin was administered with rifampicin compared with when saxagliptin was administered alone, and there were no changes in renal clearance of the two analytes. The effects of rifampicin on 5-hydroxy saxagliptin AUC may be due to an increase in the excretion or clearance of 5-hydroxy saxagliptin or a decreased absorption of saxagliptin from the gastrointestinal tract. These findings for 5-hydroxy saxagliptin may be due to the impact of rifampicin on transporters, suggesting that the results of this study may not be generalizable to all CYP3A inducers, which may or may not affect the same transporters as rifampicin.

Pharmacodynamics

Inhibition of DPP-4 activity is the mechanism of action of saxagliptin. The DPP-4 enzyme actively converts the key insulinotropic hormone GLP-1 from intact GLP-1 to an inactive form and is responsible for the short half-life of intact GLP-1 *in vivo*. Inhibition of DPP-4 has been shown to increase GLP-1 exposure, resulting in increased postprandial insulin secretion and an improved glycaemic profile in patients with type 2 diabetes. Thus, inhibition of DPP-4 is the first step in the antidiabetic activity of saxagliptin, making it a reliable biomarker for the clinical effect of saxagliptin. Saxagliptin, via inhibition of DPP-4, increases concentrations of endogenous intact GLP-1, and thus potentiates its physiological actions, augmenting postprandial insulin secretion and improving the overall glycaemic profile in patients with type 2 diabetes.

Despite the higher metabolite : parent ratio resulting from rifampicin co-administration over a 24 h period, there was no meaningful impact on plasma DPP-4 inhibitory activity following a 5 mg dose of saxagliptin with rifampicin compared with the same dose of saxagliptin administered alone. This finding was consistent with the difference in saxagliptin's total active moiety exposure, suggesting that the total active moieties may be a reasonable surrogate from which to estimate overall DPP-4 inhibition when metabolite : parent ratios change due to disease or co-administered drugs.

Based on these results, it appears that 5-hydroxy saxagliptin plays an important role in plasma DPP-4 inhibition in humans. Presumably, the relative changes in the 5-hydroxy saxagliptin : saxagliptin ratio observed in plasma following co-administration of saxagliptin with rifampicin are paralleled in the tissues (i.e. gut and liver), where DPP-4 inhibition is thought to be important in terms of efficacy. Overall, on the basis of plasma DPP-4 activity, a dose adjustment for saxagliptin when co-administered with rifampicin appears unnecessary.

In conclusion, co-administration of saxagliptin with the strong CYP3A inducer rifampicin was generally safe and well tolerated. During rifampicin co-administration, as expected with induction of CYP3A4/5, there was a decrease in systemic exposure to saxagliptin (C_{max} and $AUC_{0-\infty}$), with a corresponding increase in C_{max} of the major pharmacologically active metabolite, 5-hydroxy saxagliptin. Overall systemic exposures to the total active moieties showed slight reductions, and there was no change in the maximum DPP-4 inhibition or $AUEC_{0-24}$ for saxagliptin when saxagliptin was co-administered with rifampicin. This lack of change in PD effect (plasma DPP-4 activity) is consistent with the observed 27% reduction in systemic exposure to the total active moieties of saxagliptin following a 5 mg dose, and is thus not considered clinically meaningful. Based on these findings, it is not necessary to adjust the 5 mg saxagliptin dose when it is co-prescribed with rifampicin. Since the PK of saxagliptin are linear over a wide dose range (2.5–400 mg), the lack of dose adjustment for saxagliptin when co-administered with rifampicin also applies to the 2.5 mg saxagliptin dose. The observation of lack of substantial change in saxagliptin PD and the recommendation for no saxagliptin dosage adjustment with rifampicin co-administration made from data gathered in healthy subjects in this study are directly applicable to the target patient population, since the PK and PD (plasma DPP-4 activity) characteristics of saxagliptin are similar in healthy subjects compared with subjects with type 2 diabetes.

Competing Interests

This study was funded by Bristol-Myers Squibb Company and AstraZeneca. All authors are employees and shareholders of Bristol-Myers Squibb Company.

Statement of approval

All of the authors have participated in developing this manuscript and approve of the final draft for submission.

The authors would like to acknowledge the analytical and technical support provided by Tandem Labs (West Trenton, NJ) and ICON Laboratory (Farmingdale, NY) as well as in-house support provided by Xiaohui Xu, PhD, and Fizal Nabbie for oversight of the bioanalytical aspects and the DPP-4 enzyme assay, respectively. We thank investigator John Coumbis, MD, and the research staff at the Bristol-Myers Squibb Clinical Research Center (Hamilton, NJ) for the conduct of the study, Yi-Hao Yu, MD, PhD, for his medical opinion in the development of the study protocol and Ramaswamy Iyer, PhD, and Lisa Christopher, PhD, for their guidance in conducting the urinary metabolite profiling. Editorial and technical assistance was provided by Quintiles Medical Communications, Parsippany, NJ.

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