Pharmacokinetics of fostamatinib, a spleen tyrosine kinase (SYK) inhibitor, in healthy human subjects following single and multiple oral dosing in three phase I studies

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- R940406 (R406) is a small molecule that is a spleen tyrosine kinase (SYK) antagonist and an inhibitor of immunoglobulin (Ig)E and IgG-mediated activation of Fc receptor signalling.
- Activity against autoimmune diseases was demonstrated in animal models.
- Fostamatinib (R788), a SYK inhibitor, is an orally dosed prodrug designed to deliver the active metabolite R406 for the treatment of rheumatoid arthritis.
- It represents a novel pathway to treat autoimmune diseases, and has demonstrated activities in early clinical trials of patients with rheumatoid arthritis.
- The pharmacokinetics (PK) of fostamatinib and its active metabolite (R406) were determined from three clinical studies, representing the initial first-in-man experience in healthy human volunteers.

WHAT THIS STUDY ADDS

- Fostamatinib demonstrates rapid and extensive conversion to R406.
- Detailed information of the PK of R406 (following fostamatinib dosing) regarding dose + time linearity, and the effect of food, is shown.
- Solid dosage forms of fostamatinib overcome the challenge of low aqueous solubility of R406.
- The PK profile of R406 could potentially allow once-daily or twice-daily oral administration of fostamatinib.

AIM

Fostamatinib (R788) is an orally dosed prodrug designed to deliver the active metabolite R940406 (R406), a spleen tyrosine kinase (SYK) inhibitor, for the treatment of rheumatoid arthritis. The objectives were to evaluate the human pharmacokinetic properties of fostamatinib and R406.

METHOD

Three clinical studies were conducted in healthy subjects: (A) A single ascending dose study for R406 with doses ranging from 80–600 mg, (B) a single- and multiple-dose study of fostamatinib in aqueous suspension, with single doses ranging from 80–400 mg and multiple doses at 160 mg twice daily and (C) a study comparing suspension and tablet of fostamatinib, with the latter tested in both fed and fasted states.

RESULTS

These studies demonstrated that when administered as a solution, R406 was rapidly absorbed. Increases in exposure were observed with doses up to 400 mg. A terminal half-life of 12–21 h was observed. Similar R406 exposure could be achieved with fostamatinib suspension and steady-state was achieved after 3–4 days following twice daily administration. Fostamatinib tablet and suspension exhibited similar R406 exposure. Upon co-administration with food, a delay in peak time and lower peak concentrations of R406 were observed but at the same time the overall exposure did not change.

CONCLUSION

Fostamatinib demonstrates rapid and extensive conversion to R406, an inhibitor of SYK. Solid dosage forms of fostamatinib overcome the challenge of low aqueous solubility of R406. The PK profile of R406 could potentially allow once daily or twice daily oral administration of fostamatinib.

Introduction

Fc receptor signalling is an important process in the immunologic response for macrophages, neutrophils and mast cells [1]. Activation of the Fc receptor results in the degradation and gene transcription of cytokines that play a critical role in inflammation. Ligand binding to the Fc receptor results in activation of intracellular immunoreceptor tyrosine-based activation motifs that interact with the non-receptor spleen tyrosine kinase (SYK) for downstream signalling [2, 3]. SYK is expressed in most haematopoietic cells and is involved in the downstream signal transduction of activated Fc receptors [1]. Therefore, inhibition of SYK represents a potential novel therapeutic target for the treatment of autoimmune diseases [4, 5], such as rheumatoid arthritis (RA).

R940406 (R406) is a SYK inhibitor [6]. In a rodent collagen-induced arthritis model, R406 was shown to produce significant improvement in histopathology, clinical scores and joint radiography, when compared with the vehicle treated group [7]. However, R406 has low aqueous solubility and thus a methylene-phosphate pro-drug of R406, fostamatinib (R788), was designed to enhance the aqueous solubility to enable its further clinical development as an oral therapeutic agent. Three clinical studies, representing the initial first-in-man experience of R406 and fostamatinib in healthy human volunteers, are reported in this manuscript. In study A, the pharmacokinetic (PK) profile of R406 was evaluated following oral administration as a solution in TPGS (d-alpha-tocopheryl-polyethylene-glycol-1000 succinate) and PG (propylene glycol). In study B, the PK profile of R406 was examined following single-dose and multiple-dose administration of fostamatinib in an aqueous suspension. In study C, R406 exposure was compared between a tablet and an aqueous suspension of fostamatinib. In addition, the effect of food on R406 exposure was evaluated for the tablet formulation. In all three studies, the tolerability of R406 and fostamatinib were also evaluated. These studies represent the initial studies wherein fostamatinib was tested in humans, and were important in providing guidance for subsequent testing of the safety and efficacy of fostamatinib in patient studies.

Methods

Ethics

The study protocol and its amendments were reviewed and approved by the local Institutional Review Boards prior to initiation of the study. Studies A and B were conducted in accordance with Medicines and Healthcare Products Regulatory Agency, the regulatory authority in the United Kingdom. Study C was conducted in accordance with current United States Food and Drug Administration (FDA) regulations. All studies were conducted in

accordance with Good Clinical Practice guidelines and ethical principles that have their origins in the Declaration of Helsinki. Patients provided written informed consent prior to undergoing any procedure for this study.

Subjects and study design

Subjects who were eligible for studies A (C-940406-001) and B (C-935788-001) were healthy adult males, between 18–45 years who had a body mass index between 19–28 kg m⁻². Subjects who were eligible for study C (C-935788-005) included healthy adult males and postmenopausal or surgically sterile females, between 40–65 years who had a body mass index of between 18–35 kg m⁻². In all studies, eligible subjects included non-smokers or smokers who smoked fewer than 10 cigarettes or equivalent per day, and were able and willing to give written informed consent.

Subjects were healthy as determined by prestudy medical history, physical examination, 12-lead electrocardiogram (ECG) and clinical laboratory test results. Subjects were ineligible for the studies if they had used any investigational drug and/or participated in any clinical trial within 2 months (C-935788-005) or 4 months (C940406-001 and C-935788-001) of first dosing.

Study design

Study A was a single centre, double-blind, randomized, placebo-controlled, single ascending dose study. Single doses of R406 besylate, formulated in a TPGS/PG (40:60) solution, were administered to 35 healthy male subjects at five dose levels (groups 1 to 5, receiving 80, 250, 400, 500 and 600 mg, respectively). Five subjects were randomized to receive R406 and one subject was randomized to receive placebo in groups 1 and 2, whereas six subjects received R406 and two subjects received placebo in groups 3, 4 and 5, respectively.

Study B was a single centre, double-blind, randomized, placebo-controlled, single ascending dose and multiple dose study in 26 healthy male subjects. The study consisted of two parts. In part I, single oral doses of fostamatinib calcium powder in an orange juice suspension (80, 250 and 400 mg) were investigated in three sequential groups of six subjects. Within each group of six subjects, one subject was randomized to receive placebo and the remaining five subjects were randomized to receive fostamatinib. A single group of eight subjects was enrolled in Part II of the study. Within this group, two subjects received the placebo and the remaining six subjects received 160 mg fostamatinib orally for 7 days. On days 1 and 7, subjects received fostamatinib or placebo once daily in the morning. On days 2 to 6, subjects received fostamatinib or placebo twice daily, with the second daily dose given 10 h after the morning dose. Fostamatinib was administered each day at 10/14 h apart, instead of every 12 h, as a convenience to the volunteers. Fostamatinib was dosed more frequently than once a day so that higher drug

exposure could be achieved in a more expedient manner to allow safety evaluation.

Study C was a single-centre, open-label, randomized, three-way crossover study in healthy subjects (10 male and eight female) to evaluate the PK of a single dose of fostamatinib sodium in a tablet formulation (with or without food), or fostamatinib calcium suspension (without food only). Each subject was randomly assigned to one of three treatment sequences (six subjects per sequence). Fostamatinib was administered as a single dose on three separate occasions (the morning of day 1 in each treatment period), with each treatment period separated by a 7-day washout. The three treatment periods were (i) fostamatinib calcium suspension in orange juice (80 mg) after an overnight fast, (ii) fostamatinib sodium tablets (3×25 mg tablets, 75 mg total dose) immediately after a high-fat/high-calorie breakfast and (iii) fostamatinib sodium tablets (75 mg) after an overnight fast. All subjects were fasted at least 10 h prior to the day 1 dose. For treatments i and iii, the study drug (tablet or powder suspension) was administered in the morning 4 h prior to lunch (breakfast was not served on this day). For treatment ii, the subjects were fed a high fat/high calorie breakfast within 30 min of dosing. The breakfast meal calorific composition was 506 kCal, 286 kCal and 124 kCal from fat, carbohydrate and protein, respectively, in accordance with FDA guidance 'Food-effect bioavailability and fed bioequivalence studies' [8].

Sample collection

In study A, blood samples were collected from each subject at predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 32, 48, 60, 72, 96 and 120 h after dosing. In Study B, blood samples were collected from each subject at predose, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 32, 48, 56, 72, 96 and 120 h after dosing in the single-dose portion of the study. For the multiple-dose portion of the study, blood samples were collected on days 1 and 7 at predose (AM), and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16 and 24 h after dosing. From day 3 to day 6, a predose (AM) sample was also collected. Additional samples were collected at 32, 48, 56, 72, 120 and 168 h after day 7 dosing. In study C, blood samples were collected from each subject at predose, and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 32, 48, 56, 72 and 144 h after dosing.

In all studies, 6 ml of blood were drawn into vacutainer tubes containing K_2EDTA and maintained on wet ice. The blood samples were centrifuged at $2750 \text{ rev min}^{-1}$ (1500 g) for 10 min at 4°C , within 30 min of collection. Plasma was decanted into prelabelled sample tubes and stored frozen (at -80°C or colder) until analysis.

Assay description

Plasma samples were analyzed to determine the concentrations of fostamatinib and its metabolite R406 using a validated liquid-liquid extraction using methyl tert-butyl ether (HPLC Grade, Fisher Scientific), turbo ion spray LC/MS/MS assays. The calibration ranges of the assay were

0.5–1000 ng ml^{-1} and 0.25–250 ng ml^{-1} , with a lower limit of quantitation of the assays at 0.5 ng ml^{-1} and 0.25 ng ml^{-1} for fostamatinib and R406, respectively. The inter-assay precision (% CV, coefficient of variation) of quality control (QC) samples was $\leq 9.6\%$ for fostamatinib and was $\leq 7.6\%$ for R406. The interassay accuracy of the QC samples ranged from -5.7% to 2.4% for fostamatinib and from -8.4% to -3.4% for R406. Dilution integrity was established up to 100 times for both analytes.

Pharmacokinetic analysis

PK parameters were calculated using non-compartmental methods (WinNonlin Professional, Pharsight Corporation, Cary, NC, USA, Version 4.0 or higher). The maximum concentrations (C_{max}) and the time to C_{max} (t_{max}) were recorded as observed values. The area under the plasma concentration time curve ($\text{AUC}(0, t_{\text{last}})$) from dosing to the last measurable concentrations (C_{last}) was calculated using the linear trapezoidal rule. $\text{AUC}(0, t_{\text{last}})$ was extrapolated to infinity ($\text{AUC}(0, \infty)$) by adding the quotient of C_{last} and λ_z (where λ_z , the apparent first-order elimination rate constant, was calculated as the log linear terminal slope). The terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. CL/F is apparent clearance ($\text{Dose}/\text{AUC}(0, \infty)$), while V_z/F is the apparent volume of distribution ($\text{CL}/F/\lambda_z$). For the multiple-dose study, the observed accumulation ratio (R_0) was calculated by dividing $\text{AUC}(0, \tau)$ (last day of dosing) with $\text{AUC}(0, \tau)$ (day 1) where $\tau = 10$ h. The linearity ratio (R_{lin}) was calculated by dividing ($\text{AUC}_{\text{ss}}/2$) with $\text{AUC}(0, \infty)$ (day 1). AUC_{ss} for twice-daily dosing was calculated by adding $\text{AUC}(0, 10 \text{ h}) + \text{AUC}(0, 14 \text{ h})$, based on fractional AUC estimates obtained from WinNonlin following day 7 dosing. Due to the long $t_{1/2}$ of R406, error would be expected when the $\text{AUC}(0, \infty)$ from day 1 of dosing was estimated, where samples were only collected up to 24 h. However, from the single-dose study, using the data from the first 24 h to calculate $\text{AUC}(0, \infty)$ (day 1, 0–24 h) only led to a $<10\%$ difference from the $\text{AUC}(0, \infty)$ (day 1, full profile). Therefore, in this study, the $\text{AUC}(0, \infty)$ on day 1 was calculated and used in the R_{lin} calculation. If there is no time-dependent change in exposure following multiple dosing, R_{lin} is expected to approximate unity.

Statistical analysis

An evaluable population (subjects who completed dosing and PK sampling in all treatment periods, provided sufficient blood samples and had no major protocol violations), together with the safety population (subjects who received any amount of study drug) were identified for this study. Baseline demographic data and safety data were analyzed based on the safety population and PK data were analyzed based on the evaluable population.

For study C, 90% confidence intervals (CIs) were constructed for the ratio of the geometric means of $\text{AUC}(0, \infty)$, t_{max} and C_{max} of fostamatinib calcium suspension (fasted) vs. fostamatinib sodium tablets (fasted) and fostamatinib

sodium tablets (fasted) vs. fostamatinib sodium tablets (fed). The CIs were constructed based on a mixed model with sequence, treatment and period as fixed factors and a random effect of subject nested in sequence on natural log-transformed parameters ($t_{\max}$, $C_{\max}$, $AUC(0,\infty)$).

Safety assessments

Safety was assessed throughout the study based on adverse events (AEs), clinical laboratory parameters (haematology, serum chemistry, urinalysis), vital signs (blood pressure and pulse rate, respiration rate, oral temperature) and 12-lead ECGs.

Results

Subjects demographics

A total of 35, 26 and 18 subjects completed studies A, B and C, respectively. The age of the volunteer population who participated in these studies ranged from 18 to 62 years and their body mass index ranged from 20 to 32 kg m⁻². A total of 72 White, 6 Black and one Asian volunteers participated in these three studies. Only male volunteers participated in studies A and B, whereas 10 male subjects and eight female subjects participated in study C.

Pharmacokinetics of fostamatinib/R406

There were only minor deviations from the scheduled PK blood collection time points. Therefore, nominal times were used for PK analyses. All plasma samples from the drug-treated subjects were analyzed and reported. For placebo-treated subjects, a limited number of samples from each subject were analyzed to confirm the lack of exposure. In those samples, no quantifiable plasma fostamatinib and R406 concentrations were detected in the R406/fostamatinib placebo subjects.

In both the single ascending dose and multiple-dose parts of study B, plasma concentrations of fostamatinib were only observed sporadically. The highest peak concentration of fostamatinib (7 ng ml⁻¹) was observed within 2 h of dosing following a 400 mg dose. No quantifiable fostamatinib was observed in any subject after this time point. In study C, only the 1 h post-dose time point was tested for fostamatinib. The highest level observed was 10.4 ng ml⁻¹ and over 75% of the samples did not exhibit any fostamatinib concentration. Based on the low and sporadic concentrations of fostamatinib observed in these studies, no PK parameter for fostamatinib was calculated.

Study A: R406 single ascending doses Average log concentrations of R406 in human plasma after single oral doses of R406 (80, 250, 400, 500 and 600 mg) are shown in Figure 1. A summary of PK parameters derived from the plasma concentration data of R406 is shown in Table 1.

Following single oral administration of R406 to human volunteers at 80–600 mg doses, R406 was rapidly absorbed

and exhibited good oral exposure. Peak plasma concentrations were observed within 1–2 h post-dose. The rapid absorption phase was followed by a slow bi-phase decline in plasma concentrations, with a $t_{1/2}$ ranging between 13–21 h across all doses. At 600 mg, R406 plasma concentrations were observed up to 120 h post-dose. There was a dose-related increase in exposure ($C_{\max}$ and AUC) when the dose increased from 80 to 400 mg. However, the exposure was essentially unchanged from 400 to 600 mg doses.

Study B, Part I: fostamatinib single ascending doses Average log concentrations of R406 in human plasma after single oral doses of fostamatinib (80, 250 or 400 mg) are shown in Figure 1. A summary of PK parameters derived from the part I plasma concentration data of R406 is shown in Table 1.

Following oral fostamatinib administration, R406 appeared rapidly in the systemic circulation. Peak plasma concentrations were observed between 1–2 h post-dose. As stated previously, only sporadic concentrations of fostamatinib were observed (<7 ng ml⁻¹). The data suggest that fostamatinib was rapidly converted to R406. The rapid absorption phase was followed by a slow bi-phase decline in plasma concentrations, with a $t_{1/2}$ ranging between 12–16 h across all doses. There was a dose-related increase in R406 exposure (both $C_{\max}$ and AUC) when the fostamatinib dose increased from 80 to 250 mg, although there was no apparent difference in R406 exposure between 250 and 400 mg.

Study B, Part II: fostamatinib multiple doses Average log concentrations of R406 in human plasma after multiple oral doses of fostamatinib at 160 mg are shown in Figure 2. A summary of PK parameters derived from the plasma concentration data of R406 are shown in Table 2. Following 7 days of multiple dosing of fostamatinib at 160 mg, there was an approximate 2–2.5-fold increase in exposure of R406 on day 7 compared with day 1. This increase is consistent with the long terminal phase half-life of R406 and the twice daily dosing regimen. The accumulation ratio of R406 averaged about 2.54 ± 0.62 . Based on trough R406 concentrations, collected prior to the morning dose from day 1 to day 7, steady-state was achieved following 3–4 days of twice daily dosing. R406 plasma concentrations were 385 ± 176 , 451 ± 190 , 624 ± 431 , 516 ± 213 , 485 ± 229 ng ml⁻¹ on days 3, 4, 5, 6 and 7, respectively. The R_{lin} of R406 averaged at 1.22 ± 0.28 and was similar to a value of unity, which suggests that the accumulation observed following multiple dosing could be predicted from single-dose exposure data and no time-dependent change in exposure was apparent.

Study C: effect of formulation and food Average log concentrations of R406 in human plasma following single-dose administration of fostamatinib, as a tablet and as an aqueous suspension, under fasted and fed (tablets only)

Figure 1

Plasma R406 concentrations (ng ml⁻¹) in healthy human volunteers after an oral administration of a single dose of 80 mg (▲), 250 mg (●), 400 mg (◻), 500 mg (✕), and 600 mg (◊) of R406 (A) and 80 mg (▲), 250 mg (◻), and 400 mg (●) of fostamatinib (B). Data shown as mean + SD

conditions are shown in Figure 3. A summary of PK parameters is shown in Table 3. Comparing the R406 exposure data following single dose administration of fostamatinib in a suspension formulation or as a tablet under fasted condition, similar PK profiles were observed. Plasma con-

centrations of R406 increased rapidly, reaching maximum concentrations within 1 h and declining thereafter, with an average half-life of about 18 h.

Plasma concentrations of R406 were lower in subjects who received 75 mg fostamatinib sodium (free acid

Table 1

PK parameters of R406 following single oral administration of R406 and fostamatinib to human volunteers

Dosing\PK parameters	$t_{\max}$ (h)	$C_{\max}$ (ng ml ⁻¹)	AUC(0,∞) (ng ml ⁻¹ h)	$t_{1/2}$ (h)	CL/F (l min ⁻¹)	V_z/F (l)
R406 80 mg (n = 5)	1.30 ± 0.27	501 ± 128	4410 ± 997	14.5 ± 3.9	0.32 ± 0.07	409 ± 175
R406 250 mg (n = 5)	1.20 ± 0.27	2030 ± 489	18 100 ± 2930	14.9 ± 3.6	0.24 ± 0.04	306 ± 94.8
R406 400 mg (n = 6)	1.50 ± 0.45	3410 ± 745	34 900 ± 14 600	17.8 ± 10.6	0.22 ± 0.09	340 ± 276
R406 500 mg (n = 5)	1.10 ± 0.22	3660 ± 713	29 600 ± 6270	20.9 ± 5.8	0.29 ± 0.06	546 ± 249
R406 600 mg (n = 6)	1.25 ± 0.27	3920 ± 888	36 600 ± 7760	12.9 ± 5.9	0.29 ± 0.09	316 ± 146
Fostamatinib 80 mg (n = 5)	1.10 ± 0.22	306 ± 46.1	3150 ± 982	14.8 ± 4.7	0.45 ± 0.13	557 ± 166
Fostamatinib 250 mg (n = 5)	1.60 ± 0.42	1140 ± 122	13 700 ± 3140	16.0 ± 2.8	0.32 ± 0.07	431 ± 82.0
Fostamatinib 400 mg (n = 5)	1.60 ± 0.42	1220 ± 317	13 400 ± 4360	12.1 ± 2.0	0.54 ± 0.15	545 ± 109

CL/F is apparent oral clearance, while V_z/F is the apparent oral volume of distribution. AUC(0,∞), the area under the plasma concentration–time curve from dosing to infinity; $C_{\max}$, maximum concentration; PK, pharmacokinetic; $t_{1/2}$, terminal elimination half-life; $t_{\max}$, the time to $C_{\max}$.

Table 2

PK parameters of R406 following multiple oral administration of fostamatinib at 160 mg to healthy normal human volunteers for 7 days

PK parameters	Day 1 (n = 6)	Day 7 (n = 6)
$t_{\max}$ (h)	1.25 ± 0.42	1.08 ± 0.20
$C_{\max}$ (ng ml ⁻¹)	747 ± 286	1530 ± 534
AUC(0,24 h) (ng ml ⁻¹ h)	5500 ± 2030	14 100 ± 6640
AUC(0,∞) (ng ml ⁻¹ h)	8250 ± 3970	19 900 ± 8750
AUC _{ss} (ng ml ⁻¹ h)	NA	19 800 ± 9350
R_o	NA	2.54 ± 0.62
R_{lin}	NA	1.22 ± 0.28
$t_{1/2}$ (h)	NA	19.9 ± 9.2

Fostamatinib was administered once daily on days 1 and 7, and twice daily from days 2–6. AUC(0,24 h), the area under the plasma concentration–time curve from dosing to the measurable concentrations at 24 h; AUC(0,∞), the area under the plasma concentration–time curve from dosing to infinity; AUC_{ss}, the area under the plasma concentration–time curve at steady-state; $C_{\max}$, maximum concentration; NA, not available; PK, pharmacokinetic; R_{lin} , linearity ratio; R_o , observed accumulation ratio; $t_{1/2}$, terminal elimination half-life; $t_{\max}$, the time to $C_{\max}$.

equivalent) tablets compared with subjects who received a 100 mg fostamatinib suspension (80 mg of free acid equivalent). Both AUC(0,∞) and $C_{\max}$ average values were about 11–14% lower in subjects who received fostamatinib tablet (6490 ± 1750 ng ml⁻¹ h and 605 ± 221 ng ml⁻¹) compared with subjects who received fostamatinib suspension (7510 ± 1560 ng ml⁻¹ h and 682 ± 187 ng ml⁻¹). The 90% CIs for the ratio of $C_{\max}$ and AUC(0,∞) for the tablet and the suspension formulations were (1.03, 1.31) and (1.11, 1.24), respectively, shown in Table 3. However, when adjusted for the dose level, the 90% CIs for the ratio of $C_{\max}$ and AUC(0,∞) were within the acceptable equivalency range of 0.80, 1.25 (0.97, 1.23) and (1.04, 1.17), respectively. The $t_{1/2}$ of R406 was similar in the three groups. These data show that the fostamatinib tablets delivered a similar amount of R406 to the systemic circulation, as compared with the suspension formulation.

After single dose administration of the fostamatinib tablet with food, R406 plasma concentrations increased slowly, reaching maximum concentrations within 2–4 h. There was a reduction in the rate of absorption, as shown by the delayed $t_{\max}$ and lower $C_{\max}$. The mean $C_{\max}$ value under fasting conditions was higher (605 ng ml⁻¹) compared with the fed condition (363 ng ml⁻¹). Correspondingly, the $t_{\max}$ was prolonged after food consumption (3.22 h) as compared with fasting conditions (1.39 h). However, the R406 exposure (AUC(0,∞) values) was similar under fasted and fed conditions (Table 3). The 90% CI for the ratio of the geometric means after fasted to fed conditions were within the acceptable equivalency range of 0.80, 1.25 for AUC(0,∞) (0.85, 0.95), but not the $C_{\max}$ (1.42, 1.81), as shown in Table 3.

Safety assessments

In general, R406 and fostamatinib appeared well-tolerated in the three clinical studies. There were no serious adverse events (AEs) reported in any of these studies. Observed AEs were generally classified as mild events, and included abdominal pain, nausea, vomiting, sore throat and postural dizziness. Although AE assessment was not the primary objective of this publication, there was no obvious relationship between incidence of AEs and increasing doses of R406 and fostamatinib, with the exception of postural dizziness, which was the most frequent treatment-emergent AE (see Table 4).

Discussion

R406 is a small molecule that is a SYK antagonist and an inhibitor of immunoglobulin (Ig)E and IgG-mediated activation of Fc receptor signalling [6]. In a rodent collagen-induced arthritis model R406 was shown to produce significant improvement in histopathology, clinical scores and joint radiography, when compared with the vehicle group [7]. Co-administration of fostamatinib with

Figure 2

Plasma R406 concentrations (ng ml⁻¹) following multiple oral administration of fostamatinib at 160 mg to healthy normal human volunteers for 7 days. Fostamatinib was administered once daily on days 1 and 7 and twice daily from days 2–6. A) day 1; B) day 7. Data presented as mean (bold line) and individual values (dash line) ($n = 6$)

methotrexate did not affect the PK of methotrexate [9], and in early clinical trials which evaluated fostamatinib in RA patients, clinical activity was shown [10–12]. In early studies, fostamatinib has shown potential clinical efficacy in patients with idiopathic thrombocytopenic purpura [13], as well as some forms of B-cell lymphoma [14]. The studies described in this report represent the initial first in man studies to examine the PK and safety profile of fostamatinib in healthy volunteers.

SYK activity in peripheral blood cells was determined in study A by measuring CD63 levels. Details of this measure-

ment have been previously reported by Braselmann *et al.* [6]. In summary, a method was developed to measure the activation of peripheral blood basophils following *ex vivo* anti-IgE stimulation. Basophils degranulate upon activation (a SYK-dependent process), an event that coincides with increased surface expression of CD63. Measuring the percentage of CD63+ basophils in blood samples obtained after R406 dosing, normalized to pre-dose samples, provides an indicator for the inhibitory effect of R406 on the SYK signalling pathway. SYK activity in peripheral blood cells was determined and a good correlation existed

Figure 3

Plasma R406 concentrations (ng ml^{-1}) in healthy human volunteers after single oral administration of 100 mg of fostamatinib suspension ($\blacksquare$) given under fasted conditions and single oral administration of 75 mg of fostamatinib tablets under fed ($\blacktriangle$) and fasted ($\circ$) conditions. Data shown as mean $\pm$ SD ($n = 18$)

Table 3

R406 PK parameters in normal human volunteers after single oral administration of 100 mg fostamatinib (calcium salt) suspension given under fasted conditions, and single oral administration of 75 mg fostamatinib (sodium salt) tablets under fed and fasted conditions ($n = 18$)

Parameter (units)	Suspension (Fasted)*	Tablet (Fasted)	Tablet (Fed)
$t_{\max}$ (h)	1.17 ± 0.24	1.39 ± 0.74	3.22 ± 1.62
$C_{\max}$ (ng ml^{-1})	682 ± 187	605 ± 221	363 ± 94
$C_{\max}$ ratio (90% CI)	$1.09 (0.97, 1.23)^{\dagger}$	$1.60 (1.42, 1.81)^{\ddagger}$	
$\text{AUC}(0, \infty)$ ($\text{ng ml}^{-1} \text{ h}$)	7510 ± 1560	6490 ± 1750	7140 ± 1760
$\text{AUC}(0, \infty)$ ratio, (90% CI)	$1.10 (1.04, 1.17)^{\dagger}$	$0.90 (0.85, 0.95)^{\ddagger}$	
$t_{1/2}$ (h)	19.0 ± 4.3	17.9 ± 4.0	18.4 ± 5.0

$\text{AUC}(0, \infty)$, the area under the plasma concentration time curve from dosing to infinity; $C_{\max}$, maximum concentration; PK, pharmacokinetic; $t_{1/2}$, terminal elimination half-life; $t_{\max}$, the time to $C_{\max}$. *Ratio adjusted for the difference in doses between the sodium tablets and the calcium suspension. Sodium tablet dosage was 75 mg. Calcium solution dosage is equivalent to 80 mg. † Ratio between fostamatinib suspension vs. fostamatinib tablet (fasted) adjusted to dose, and the associated confidence intervals (CI). ‡ Ratio between fostamatinib tablet (fasted) vs. fostamatinib tablet (fed), and the associated confidence intervals (CI).

between the plasma R406 concentrations and its pharmacodynamic effect, as measured by quantifying the CD63+ basophils following anti-IgE stimulation. The estimated EC_{50} of R406 was approximately $1 \mu\text{M}$ (470 ng ml^{-1}).

Due to the limited solubility of R406, a TPGS/PG-based formulation was used in the initial human studies. TPGS is a well-known solubility enhancer that was shown to be able to improve bioavailability of poorly soluble drugs [15]. When R406 was administered in the TPGS/PG vehicle as a solution, R406 was rapidly absorbed and exhibited good oral exposure between doses of 80–600 mg in this study. Peak plasma concentrations were observed within 1–2 h post-dose. There was a dose-related increase in exposure

($C_{\max}$ and AUC) when the dose increased from 80 to 400 mg. However, R406 exposure was essentially unchanged in doses from 400 to 600 mg, probably due to saturation in R406 absorption. With the low aqueous solubility of R406, it is possible that at high dose levels, the TPGS/PG vehicle failed to keep R406 in solution. R406 exhibited a long $t_{1/2}$, ranging between 13–21 h across all doses. The long $t_{1/2}$ of R406 could potentially allow once or twice-daily dosing. However, the poor solubility of R406 presented a barrier for its development as a therapeutic agent, especially for a solid dosage form.

The prodrug approach to overcome formulation, delivery and toxicity limitations on drugs with poor aqueous

Table 4

Summary of treatment emergent adverse events following single oral administration of R406 and fostamatinib to human volunteers

Treatment	n*	Number of events	Number (%) of subjects†	Postural dizziness [number of subjects (%)]
80 mg R406	5	3	2 (40)	1 (20)
250 mg R406	5	1	1 (20)	0 (0)
400 mg R406	6	5	4 (67)	1 (17)
500 mg R406	5	8	3 (60)	1 (20)
600 mg R406	6	8	5 (83)	5 (83)
Total R406	27	25	15 (56)	8 (30)
Placebo	8	1	1 (13)	1 (13)
80 mg fostamatinib	5	0	0	0 (0)
250 mg fostamatinib	5	1	1 (20)	1 (20)
400 mg fostamatinib	5	8	4 (80)	3 (60)
Total fostamatinib	15	9	5 (33)	4 (25)
Placebo (fostamatinib)	3	1	1 (33)	0 (0)

*Number of subjects in each treatment group. †Number (percent) of subjects reporting at least one adverse event.

solubility had been documented previously. The strategy to design a prodrug with methylene phosphate linkage was also demonstrated by Stella *et al.* [16, 17] in the design of fosphenytoin, a methylene phosphate prodrug of phenytoin. Similar to phenytoin [18], R406 exhibits low aqueous solubility, which limits the potential for oral solid dosage form development. Therefore fostamatinib, the methylene phosphate prodrug of R406, was synthesized and tested in preclinical and clinical studies. This oral prodrug was designed to be cleaved to R406 by alkaline phosphatases that are present on the apical brush border membranes of enterocytes, after which the more hydrophobic R406 could be readily absorbed [19, 20]. *In vivo* studies established that fostamatinib was essentially completely converted to R406 by phosphatases in the intestinal mucosa, such that little or no fostamatinib could be detected in the systemic circulation [21, 22].

In this current study, fostamatinib was detected in plasma sporadically and in low concentrations only. The highest peak concentration of fostamatinib was observed within 2 h of dosing, and in all subjects, no quantifiable fostamatinib was obtained after this time point. The fostamatinib prodrug appears to perform as designed, and deliver the active metabolite, R406, to the systemic circulation. There was a dose-related increase in R406 exposure when the fostamatinib dose increased from 80 to 250 mg, although there was no difference between 250 and 400 mg. The 160 mg dose from part B of the study is in line with the linearity portion of part A, AUC(0,24 h) was 2240 ± 376 , 5500 ± 2030 and 9660 ± 2050 ng ml⁻¹ h following single doses of 80, 160 and 250 mg fostamatinib, respectively. The average *t*_{1/2} ranged between 12–16 h. These exposure data are consistent with R406 data obtained in study A, where R406 was dosed using a highly organic base

formulation (TPGS-based solution). The exposure of R406 following 250 mg fostamatinib accounted for 56% (based on *C*_{max}) and 76% (based on AUC(0,∞)) of the R406 exposure obtained following 250 mg R406 dosing in study A. *C*_{max} values were 2030 ± 489 and 1140 ± 122 ng ml⁻¹, while AUC(0,∞) values were $18\,100 \pm 2930$ and $13\,700 \pm 3140$ ng ml⁻¹ h following 250 mg doses of R406 and fostamatinib, respectively. These differences approximate the theoretical differences in molecular weights of R406 and fostamatinib (R406 is approximately 65% of fostamatinib, by molecular weight). Thus it would appear that the conversion of fostamatinib to R406 is extensive, approaching complete conversion at these dose levels.

Following 7 days of multiple dosing of fostamatinib at 160 mg, there was an approximate 2–2.5-fold increase in exposure of R406 on day 7, compared with day 1. This increase is consistent with the long *t*_{1/2} of R406 and the twice daily dosing regimen. In subsequent phase II studies with RA patients, fostamatinib demonstrated efficacy following twice-daily or once-daily regimens [11]. There was no evidence of unexpected accumulation of R406 concentrations upon multiple dosing. Apparent steady-state was achieved following 3–4 days of twice-daily dosing. The steady-state trough concentrations on day 7 (485 ± 229 ng ml⁻¹) would sustain plasma concentrations higher than 1 μM (470 ng ml⁻¹), required for inhibition of CD63 surface expression [6], throughout the dosing period.

Study C was the first study in which fostamatinib sodium tablets were used in humans. Based on internal data at Rigel, the sodium salt of fostamatinib exhibited better physical properties for solid dosage form development. Since the fostamatinib sodium tablet formulation was intended to replace the fostamatinib calcium powder in bottle formulation in subsequent safety and efficacy trials in humans, exposure of R406 following fostamatinib sodium tablet and fostamatinib calcium suspension was compared. Following a single-dose administration of the two formulations of fostamatinib to fasted subjects, plasma concentrations of R406 increased rapidly, reaching maximum concentrations within about 1 h. Plasma concentrations of R406 were slightly lower when subjects received fostamatinib in the tablet formulation than when they received the suspension formulation. The 90% CI for the ratio adjusted for the difference in actual administered dose of *C*_{max} and AUC(0,∞) for the tablet and the suspension formulations demonstrated that the two formulations are essentially comparable, and the tablets of fostamatinib sodium were used in subsequent studies.

Fasting and fed conditions can affect bioavailability of solid dosage forms, and in the case of prodrugs, there may be an effect on the conversion to the active component [23]. After a single dose administration of fostamatinib tablets to fed subjects, R406 plasma concentrations increased slowly, reaching maximum levels within 3 h. The effect of the high-fat/high-calorie breakfast on R406 exposure after administration of the fostamatinib tablet was a

reduction in the absorption rate ($C_{\max}$ and $t_{\max}$) but no meaningful change in the extent ($AUC(0,\infty)$) of R406 exposure was observed. The 90% CI of the ratios of the geometric means of the $AUC(0,\infty)$ was within the acceptable equivalency range. Previously reported studies [8, 24] suggest that for some drugs, high-fat/high-calorie meal would delay the rate of absorption (lower $C_{\max}$ and longer $t_{\max}$), while not affecting the extent of absorption. This delay could be due to a delay in drug release (lower dissolution rate), or slower gastric emptying of a highly soluble drug. For fostamatinib, the delay in gastric emptying probably resulted in the change in the R406 exposure profile, since fostamatinib is a highly soluble drug and would be expected to be affected by gastric emptying. Moreover, upon multiple dosing, the impact of lower $C_{\max}$ should become less significant, especially since the drug exhibits a relatively long $t_{1/2}$, as evidenced by the low peak to trough fluctuation at steady-state.

Based on the PK results and the tolerability/safety data of this study, fostamatinib sodium tablet was subsequently used in phase II clinical studies with RA patients in which fostamatinib improved clinical outcomes of patients with RA on background methotrexate [10–12].

In summary, fostamatinib demonstrates rapid and extensive conversion to R406, an inhibitor of SYK. Solid dosage forms of fostamatinib overcome the challenge of low aqueous solubility of R406. The PK profile of R406 could potentially allow once-daily or twice-daily oral administration of fostamatinib. The data from these studies allow further investigation of the safety and efficacy of fostamatinib in patients suffering from autoimmune diseases. Fostamatinib is currently being evaluated in a series of larger phase III clinical studies as a potential oral treatment for RA.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that MB, EG, TM and DL had support from Rigel Pharmaceuticals, Inc. for the submitted work, MB, EG and DL are employees of Rigel Pharmaceuticals and stockholders of Rigel Pharmaceuticals, Inc. and there are no other relationships or activities that could appear to have influenced the submitted work. TM is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London.

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