BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12448

The impact of co-administration of ketoconazole and rifampicin on the pharmacokinetics of apremilast in healthy volunteers

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Keywords
drug interaction, inflammatory autoimmune disorders, phosphodiesterase 4 inhibitor

Received
7 February 2014

Accepted
17 June 2014

Accepted Article Published Online
24 June 2014

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- *In vitro*, CYP3A4 has been reported to be involved in the metabolism of apremilast. However, there is no published literature on the clinical drug–drug interaction between ketoconazole and apremilast or rifampicin and apremilast.
- This study evaluated the clinical impact of CYP3A4 inhibition and induction on apremilast.

WHAT THIS STUDY ADDS

- There is no published literature on the clinical drug–drug interaction between ketoconazole and apremilast or rifampicin and apremilast. This manuscript provides evaluation of the clinical impact of CYP3A4 inhibition and induction on the pharmacokinetics (PK) of apremilast.
- CYP3A4 inhibition does not have a clinically meaningful impact on the pharmacokinetics of apremilast due to multiple metabolic pathways.
- Conversely, the effect of CYP3A4 induction by multiple oral doses of rifampicin on apremilast clearance is much more pronounced (≈3.6-fold increase).
- Apremilast clearance is more sensitive to strong CYP3A4 induction than CYP3A4 inhibition.

AIMS

Two clinical studies were conducted to determine possible drug–drug interactions between apremilast and a strong CYP3A4 inhibitor, ketoconazole, or a potent CYP3A4 inducer, rifampicin. The main objectives of these two studies were to evaluate the impact of multiple doses of ketoconazole on the pharmacokinetics of apremilast and its metabolites, and the effect of multiple oral doses of rifampicin on the pharmacokinetics of apremilast.

METHODS

These single centre, open label, sequential treatment studies in healthy subjects included two treatment periods for ketoconazole and three treatment periods for rifampicin. Apremilast was administered as a 20 mg (ketoconazole study) or 30 mg (rifampicin study) single oral dose.

RESULTS

Ketoconazole increases overall exposure (AUC(0,∞)) of apremilast by ≈36% (2827 *vs.* 2072 ng ml^{-1} h, 90% CI = 126.2, 147.5) and peak exposure (C_{max}) by 5% (247 *vs.* 236 ng ml^{-1}). Multiple doses of rifampicin increase apremilast clearance ≈3.6-fold and decrease apremilast mean AUC(0,∞) by ≈72% (3120 *vs.* 869 ng ml^{-1} h, 90% CI = 25.7, 30.4) and C_{max} (from 290 *vs.* 166 ng ml^{-1}) relative to that of apremilast given alone. A 30 min intravenous infusion of rifampicin 600 mg had negligible effects on the overall exposure (AUC(0,∞)) of apremilast (2980 *vs.* 3120 ng ml^{-1} h, 90% CI = 88.0, 104.1).

CONCLUSION

Ketoconazole slightly decreased apremilast clearance, resulting in a small increase in AUC which is probably not meaningful clinically. However, the effect of CYP3A4 induction by rifampicin on apremilast clearance is much more pronounced than that of CYP3A4 inhibition by ketoconazole. Strong CYP3A4 inducers may result in a loss of efficacy of apremilast because of decreased drug exposure.

Introduction

Apremilast is a novel, oral small molecule phosphodiesterase 4 (PDE4) inhibitor. PDE4 enzymes are the principal PDEs in immune cells, and inhibition of these enzymes modulates the production of immune-mediated pro-inflammatory cytokines such as tumour necrosis factor (TNF), interleukin (IL)-17 and IL-23, as well as anti-inflammatory mediators such as IL-10. Apremilast is presently in clinical development for the treatment of patients with several immune inflammatory conditions [1]. In phase II and III studies, apremilast has demonstrated efficacy in patients with moderate to severe plaque psoriasis, active psoriatic arthritis and Behçet's disease [2–5]. Apremilast (Otezla®, Celgene Corporation, Summit, NJ, USA) was recently approved by the U.S. Food and Drug Administration (FDA) to treat adults with active psoriatic arthritis.

Apremilast is cleared via multiple metabolic pathways, including cytochrome P450 (CYP)-mediated oxidative metabolism (and subsequent glucuronidation) and non-CYP-mediated hydrolysis. Only 3% of the apremilast dose is excreted unchanged in urine [6, 7]. *In vitro* studies using human CYP isoforms showed that CYP3A4 is capable of metabolizing [^{14}C]-apremilast at a high rate, with lower rates of metabolism observed with other isoforms, including CYP1A2 and CYP2A6 [6]. Therefore, two clinical studies were conducted to determine the impact of strong inhibition or induction of CYP3A4 on the pharmacokinetics (PK) of apremilast.

In the first study (KETO study), the primary objective was to determine the impact of CYP3A4 inhibition by ketoconazole on the PK of apremilast and its metabolites in healthy subjects. Ketoconazole is well known to be a strong CYP3A4 inhibitor as well as a MDR1 inhibitor and is commonly used to evaluate potential drug–drug interactions related to inhibition of CYP3A4 [8, 9].

In the second study (RIF study), the primary objective was to determine the impact of strong CYP induction by multiple oral doses of rifampicin on the PK of apremilast in healthy subjects. The effect of inhibition of OATP1B1, OATP1B3 and other transporters was also evaluated in this study. Rifampicin is established as a strong inducer of CYP3A4 following multiple doses [8]. In the literature, the full induction potential of CYP3A4 activity is obtained after 1 to 2 weeks of dosing with oral rifampicin 600 mg once daily. Rifampicin has also been reported to exert an inhibitory effect on MDR1 and hepatic uptake transporters, including OATP1B1 and OATP1B3, following a single dose [10, 11]. OATP1B1 and OATP1B3 are specifically expressed in the liver and may play important roles in the hepatic drug uptake [12]. Thus, co-administration of a single dose of rifampicin may lead to an increase of exposure for drugs that are substrates of these transporters [13]. *In vitro* studies have shown that apremilast is a MDR1 substrate, but not a substrate for OATP1B1 and OATP1B3. In the RIF study, the differentiation of single *vs*. multiple dose administration is used to distinguish the effects of OATP1B1 and OATP1B3 inhibition *vs*. CYP3A4 induction on the PK of apremilast.

Methods

These two studies were both single centre, open label, sequential treatment trials in healthy volunteers with two treatment periods for the KETO study and three treatment periods for the RIF study. Healthy male subjects (KETO study) and healthy male and female subjects (RIF study) between 18 and 55 years of age (inclusive), with a body mass index (BMI) between 19 and 29 kg m^{-2} (KETO study) and 18 and 33 kg m^{-2} (RIF study), were enrolled in the study. The clinical portion of the studies was conducted at Parexel (Clinical Pharmacology Research Unit at Northwick Park Hospital, Harrow, UK; KETO study) and Quintiles Phase I Services (Overland Park, KS, USA; RIF study).

In both studies, subjects successfully completed screening prior to the first study dose. Subjects received a single oral 20 mg (2 × 10 mg) dose (KETO study) and a single oral 30 mg (1 × 30 mg) dose (RIF study) of apremilast with 240 ml of water on the morning of day 1 after an overnight fast. The subjects' fast continued until 4 h after dosing. Blood and urine samples were collected for PK assessments just before dosing and through 72 h (KETO study) and 48 h (RIF study) after the single dose.

In the KETO study, a 5–7 day washout period was scheduled between day 1 of each of the two treatment periods. The washout period after the single dose apremilast administration ensured complete systemic elimination of apremilast, based on the terminal elimination half-life ($t_{1/2}$) between 5 and 6 h for apremilast. During the second treatment period, subjects received an oral 400 mg dose of ketoconazole once daily for 7 days. The ketoconazole dose was administered on day 5 with a single oral 20 mg (2 × 10 mg tablets) dose of apremilast after an 8 h overnight fast. The 20 mg dose of apremilast was selected because it was demonstrated to be safe and well tolerated in previous studies and it was also likely to be safe and well tolerated in these studies even if a drug interaction occurred with ketoconazole.

In the RIF study, after the first apremilast alone treatment period, a 4 day washout period was followed by the second single oral dose administration of apremilast 30 mg and 5 min later by a 30 min intravenous (i.v.) infusion of rifampicin 600 mg. On day 7, after the completion of the sampling for the 48 h post-dose plasma apremilast concentration determination, oral doses of rifampicin 600 mg once daily were administered for 15 days (days 7–21). On day 20, the 14th oral rifampicin dose was administered concurrently with the third single oral dose of apremilast 30 mg. The last (15th) oral dose of rifampicin was administered to ensure that steady-state concentrations were maintained during the apremilast sampling

period. A 30 mg dose of apremilast was selected to ensure that a decrease in exposure could still be measured, since there is a potential to induce elimination, which would result in decreased apremilast exposures.

The drug/molecular target nomenclature conforms to the British Journal of Pharmacology's The Concise Guide to PHARMACOLOGY 2013/14 [14]. Apremilast oral tablets were supplied by Celgene Corporation (Summit, NJ, USA) as apremilast 10 mg capsules (batch number 0293X; KETO study) and apremilast 30 mg tablets (batch number 11F2066; RIF study). Commercially available ketoconazole 200 mg oral tablets (Nizoral®, Janssen Pharmaceuticals, Titusville, NJ, USA; batch number 04KL836) were supplied by Parexel (KETO study). Commercially available rifampicin oral capsules (Rifadin™ 300 mg capsules, Sanofi-Aventis, Guildford, Surrey, UK; batch number 3083315) and rifampicin i.v. solution (RIFADIN™ containing rifampicin 600 mg; Sanofi-Aventis, Guildford, Surrey, UK; batch number 7005369) were supplied by Quintiles (RIF study).

Safety

Safety was assessed in both studies and included periodic assessments of laboratory analytes (urinalysis, clinical chemistry and haematology), vital signs, 12-lead electrocardiograms, physical examinations, adverse events (AEs) and concomitant medication use.

Ethical considerations

The clinical study protocols, informed consent documents, and appropriate study-related documents were reviewed and approved by an appropriate institutional review board or independent ethics committee. The KETO study was reviewed by the Brent Medical Ethics Committee (Park Royal, London, UK; REC reference number 05/Q0408/23), an independent ethics committee. The RIF study was reviewed by Midlands Independent Review Board (Overland Park, KS, USA). The studies were conducted in compliance with the Declaration of Helsinki, Guidelines for Good Clinical Practice, and applicable regulatory requirements. For the KETO study, a clinical trial application was submitted and Medicines and Healthcare products Regulatory Agency approval to conduct the study was obtained before the first subject was screened. All subjects provided written informed consent before the start of any study-specific procedures.

PK sampling, collection and analytical methodology

Blood samples were collected in lithium heparin tubes after each apremilast dose to determine plasma apremilast concentrations. Apremilast and its metabolites are subject to hydrolysis at physiological pH in buffer and biological matrices such as plasma and urine. However, they are stable under acidic conditions. Therefore, plasma samples were mixed with Sorenson's citrate buffer (25 mM citrate buffer, pH 1.5, KETO study) and fortified with 0.04 M citric acid (RIF study). Blood samples were collected at selected time points following dosing with apremilast: predose (time 0) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48 and 72 h (KETO study) and predose (time 0) and 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, 24, 36 and 48 h (RIF study).

Urine was collected during the KETO study after both single doses of apremilast for PK assessment. A urine sample was collected from each subject before dosing with apremilast. Total voiding urine collections were made 0–4, 4–8, 8–12, 12–24, 24–36 and 36–48 h after apremilast dosing. Aliquots of the thoroughly mixed urine were collected and mixed with equal volumes of Sorenson's citrate buffer (25 mM citrate buffer, pH 1.5) containing amastatin 20 μM and stored at −70°C until analysis.

Plasma and urine apremilast, CC-10007 (R-enantiomer of apremilast) and CC-10055 (M7) concentrations were measured using a validated liquid chromatography-mass spectrometry method conducted and validated by QPS, LLC (Newark, DE, USA). The KETO study utilized a chiral assay where apremilast and CC-10007 plasma concentrations were measured together in a two-in-one assay. For this analytical method, apremilast, CC-10007 and the internal standard (3S-cis)-(+)-tetrahydro-3,7-a-diphenylpyrrolo [2,1-b]oxazol-5(6H)-one were quantitatively extracted from 100 μl of the human plasma sample using a liquid-liquid extraction method with methyl tert-butyl ether and reconstituted with 200 μl of H_2O : methanol : formic acid/80:20:0.1 (v : v : v). The sample extract was loaded onto a CHIRAL-AGP 150 × 4.0 mm, for separation. The high performance liquid chromatography effluent was introduced into an API-4000 tandem mass spectrometer equipped with an ESI source for apremilast. Positive ions were detected in the multiple reaction monitoring mode with precursor→product ion pairs of 461.16 m/z →257.05 m/z for apremilast and CC-10007, and 280.10 m/z→160.30 m/z for the internal standard. CC-10007, the R-enantiomer of apremilast, was not detected in human plasma or urine in any quantifiable amount during the KETO study, indicating that there is no interconversion of apremilast (an S-enantiomer) to the R-enantiomer, CC-10007. Therefore, the full PK and statistical results of CC-10007 are not discussed here.

The RIF study utilized an achiral assay to measure apremilast. For this analytical method, apremilast and its internal standard CC-16305 were quantitatively extracted from 100 μl of plasma sample using a liquid-liquid extraction method with methyl tert-butyl ether and reconstituted with 200 μl of H_2O : methanol : formic acid/80:20:0.1 (v : v : v). The sample extract was loaded onto a Synergy Hydro-RP 30 × 2 mm, 4 μm (Phenomenex Inc., CA, USA) for separation. The mobile phase was composed of both H_2O : formic acid/100:0.1 (v : v) as well as MeOH : formic acid/100:0.1 (v : v). The high performance liquid chromatography effluent was introduced into an API-4000 tandem mass spectrometer equipped with an ESI source for apremilast. Positive ions were detected in the multiple

reaction monitoring mode with precursor→product ion pairs of 461.16 m/z→257.05 m/z for apremilast, and 465.16 m/z→261.05 m/z for CC-16305.

The apremilast plasma method had an assay range of 1.021 to 1021 ng ml^{-1} (KETO study) and 1 to 1000 ng ml^{-1} (RIF study) with a precision (percent coefficient of variation [%CV]) of ≤8.1% (KETO study) and ≤6.0% (RIF study) and an accuracy (percent relative error [%RE]) of 1.7% to 4.2% (KETO study) and −3.3% to 5.3% (RIF study). The apremilast urine assay used in the KETO study ranged from 1 to 10040 ng ml^{-1} with a precision (%CV) of ≤12.5% and an accuracy (%RE) of −2.4 to −0.5%. The CC-10007 plasma method used in the KETO study had an assay range of 1.004 to 1004 ng ml^{-1} with a precision (%CV) of ≤12.7% and an accuracy (%RE) of −0.7% to 3.7%. The CC-10007 urine assay used in the KETO study ranged from 1 to 1000 ng ml^{-1} with a precision (%CV) of ≤15.5% and an accuracy (%RE) of −2.0% to 3.7%.

CC-10055 (M7), the *N*-deacetylated product of non-CYP-mediated hydrolysis of apremilast, was only measured in the KETO study. The formation of this metabolite is not related to the CYP3A4 pathway. In the KETO study, it was found to be a minor metabolite with overall metabolite to parent ratios ≤5% in plasma and no detectable concentrations in urine. Therefore, the full PK and statistical results of M7 are not discussed here. The assays used to measure M7 in the KETO study were validated achiral assays. The plasma assay had an assay range of 0.3 to 300 ng ml^{-1} with a precision (%CV) of ≤10.8% and an accuracy (%RE) of 2.1% to 9.8%. The M7 urine assay had a range of 1 to 1000 ng ml^{-1} with a precision (%CV) of ≤11.7% and an accuracy (%RE) of −7.3% to 9.7%.

PK analysis and statistical methods

The PK parameters assessed for apremilast were maximum concentration (C_{max}), time to C_{max} (t_{max}), area under the concentration *vs*. time curve (AUC) from the time zero to the time of last quantifiable analyte concentration (AUC(0,*t*), calculated by linear up/log down trapezoidal summation), AUC extrapolated to infinity (AUC(0,∞)), estimate of $t_{1/2}$ in plasma, apparent oral plasma clearance, apparent volume of distribution during the terminal phase, renal clearance and percentage excreted in urine. The PK parameters were calculated using WinNonlin (version 4.0, KETO study; version 5.2, RIF study). All values that were below the limit of quantitation (BLQ) in the absorption phase were substituted by zeros, except for BLQ values between evaluable concentrations, which were substituted by a value that was half the lower limit of quantification, before the calculation of the PK parameters. The terminal BLQ values were ignored. These measures were taken to prevent an overestimation of AUC(0,*t*) and AUC(0,∞). No imputation was performed on missing PK data.

The effect of ketoconazole and rifampicin on the PK of apremilast was evaluated using an analysis of variance, carried out on log-transformed values of AUC(0,*t*), AUC(0,∞) and C_{max} of apremilast, to estimate the mean ratio of apremilast administered with and without ketoconazole or either rifampicin treatment (i.e. oral or i.v.). The model included period (treatment) and subject as the main effects. No statistically significant interaction between apremilast and either ketoconazole or rifampicin is confirmed if both the upper and lower 90% confidence interval (CI) limits for the relevant mean ratios of apremilast administered with either ketoconazole or rifampicin to apremilast alone fall within the range 80% to 125%. A clinically significant interaction was defined as the 90% CI for AUC or C_{max} falling outside the range of 50% to 200% for the KETO study. The rationale for using 50% to 200% was based on the known side effect profile of apremilast over the dose range of 10 to 100 mg, where apremilast exposure was considered safe up to 200% of the clinical values and was defined *a priori* in the KETO protocol.

Results

A total of 18 (KETO study) and 21 (RIF study) subjects were enrolled in the studies and 18 (KETO study) and 20 (RIF study) subjects received all planned doses of study drug and completed all study procedures. One subject in the RIF study was withdrawn from the study after completing the first single dose treatment of apremilast because of an AE of H1N1 influenza. Subjects enrolled in the KETO study had a mean age of 30 years (range 22–46 years), mean weight of 76.1 kg (range 64.7–94.5 kg), mean height of 178.9 cm (range 165–189 cm) and mean BMI of 23.79 kg m^{-2} (range 19.8–27.6 kg m^{-2}). Subjects enrolled in the RIF study had a mean age of 34 years (range 19–54 years), mean weight of 90.7 kg (range 57.6–112.7 kg), mean height 176.1 cm (range 161.4–190 cm) and mean BMI of 29.14 kg m^{-2} (range 22.1–32.6 kg m^{-2}). All subjects in both studies were male (97%) except for one female subject (1/39) in the RIF study. Twenty-seven subjects (69%) were White, nine (23%) were Black and three (8%) were Asian or Pacific Islander.

PK parameters

Mean plasma concentration *vs*. time curves for apremilast-treated subjects with and without ketoconazole are shown in Figure 1. Mean apremilast plasma concentrations were higher in the apremilast alone treatment period up until 3 h post dose, from which point plasma concentrations were higher with concomitant treatment with ketoconazole. These plasma concentration differences resulted in higher AUC exposures (approximately 36%) when apremilast was co-administered with ketoconazole. The PK parameters and statistical analysis are included in Table 1. Ketoconazole co-administration did not impact on the C_{max}. However, the AUC exposure increased by

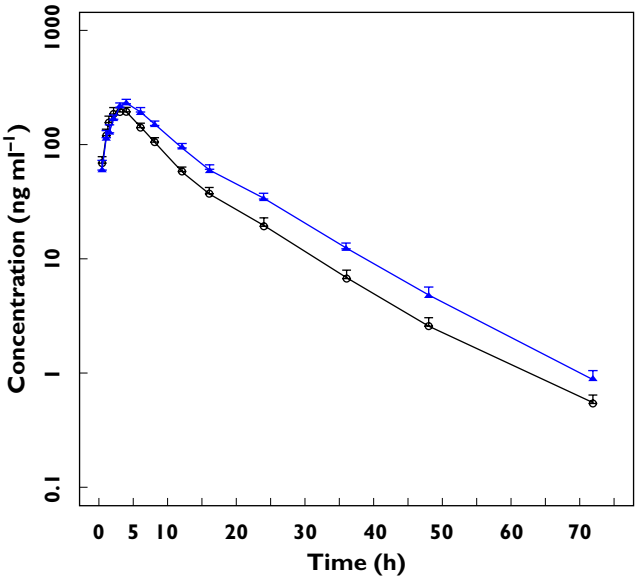

Figure 1

Mean (± SD) plasma concentration (ng ml^{-1}) of apremilast (APR) *vs.* time by treatment with and without ketoconazole (KETO) on a semi-logarithmic scale. ⊸, APR; ▲, KETO + APR

approximately 36% with co-administration of ketoconazole, which was statistically significant, as indicated by the 90% CI. The 90% CI remained within 50% to 200% (*a priori* defined in the protocol). Therefore, this increase was not considered clinically meaningful. The mean $t_{1/2}$ of apremilast was slightly longer when co-administered with ketoconazole (8.0 h for apremilast and ketoconazole *vs.* 7.6 h for apremilast alone). This small change in $t_{1/2}$ suggests that CYP3A4 inhibition by ketoconazole negligibly affects the elimination of apremilast. The apparent clearance and apparent volume of distribution of apremilast were both lower after co-administration with ketoconazole (Table 1). Overall, the fraction of apremilast excreted unchanged in the urine was low (<5%). An increase in the percentage of urinary excretion (approximately 36%) was seen when apremilast was given with ketoconazole, which was consistent with the increase in AUC when apremilast was co-administered with ketoconazole. However, there was no meaningful change in renal clearance.

Among the 20 subjects who received all planned doses of study drug in the RIF study, one subject had undetectable apremilast plasma concentrations following treatment with oral rifampicin and apremilast, and had only trace amount of apremilast exposure following treatment with i.v. rifampicin and apremilast. This subject was considered a PK outlier, and only the subject's PK profile of apremilast administered alone was included in the analyses. Inclusion or exclusion of this subject's data had no impact on the descriptive summary and overall conclusion of the effect of rifampicin on the PK of apremilast.

Table 1

Summary of apremilast plasma pharmacokinetic parameters and statistical analysis

		$AUC(0,\infty)$ (ng ml^{-1} h)	C_{max} (ng ml^{-1})	Arithmetic mean (SD) t_{max} (h)*	$t_{1/2}$ (h)	CL/F (l h^{-1})	V_z/F (l)
KETO study							
	Apremilast 20 mg with ketoconazole (n = 18)	2982.5 (955.5)	259.0 (80.5)	4.0 (1.0–6.1)	8.140 (1.7)	7.5 (2.7)	84.05 (22.0)
	Apremilast 20 mg (n = 18)	2180.0 (702.1)	245.8 (71.9)	2.5 (1.0–4.0)	7.644 (1.3)	10.2 (3.5)	110.74 (37.8)
Ratio (90% CI)†		136.4 (126.2, 147.5)	104.9 (92.2, 119.3)	0.74 (0.06, 1.10)	106.5 (100.3, 112.7)	73.3 (67.8, 79.2)	77.5 (69.8, 86.1)
RIF study							
i.v. rifampicin	Apremilast 30 mg with i.v. rifampicin (n = 19)	3110 (991)	324 (114.5)	1.25 (0.5–5.0)	7.35 (18.5)	10.5 (3.11)	115 (43.6)
	Apremilast 30 mg (n = 21)	3270 (1081)	298 (81.4)	2.0 (0.5–5.0)	8.20 (1.166)	10.0 (3.02)	119 (43.9)
Ratio (90% CI)†		95.71 (88.0, 104.1)	113.1 (103.2, 123.9)	−0.25 (−0.75, 0.00) P = 0.2656			
Oral rifampicin	Apremilast 30 mg with oral rifampicin (n = 19)	912 (290)	162 (53.2)	1.0 (0.5–5.0)	6.31 (1.667)	36.2 (11.37)	342 (177.7)
	Apremilast 30 mg (n = 21)	3270 (1081)	298 (81.4)	2.0 (0.5–5.0)	8.20 (1.166)	10.0 (3.02)	119 (43.9)
Ratio (90% CI)†		27.96 (25.7, 30.4)	56.80 (51.8, 62.3)	−0.50 (−1.00, 0.00) P = 0.1141	NC	NC	NC

$AUC(0,\infty)$, area under the concentration *vs.* time curve from time 0 to infinity; CI, confidence interval; CL/F, apparent total plasma clearance; C_{max}, maximum observed plasma concentration; i.v., intravenous; NC, not calculated; $t_{1/2}$, elimination half-life; t_{max}, time to C_{max}; V_z/F, apparent total volume of distribution. *t_{max} is summarized by median (range); statistical comparison based on median difference, 90% CI calculated using the Hollander & Wolfe method (RIF study) and the Hauschke, Steinijans and Diletti method (KETO study); the P value is from the Wilcoxon signed rank test (RIF study). †The ratio (90% CI) is based on the least-square geometric means.

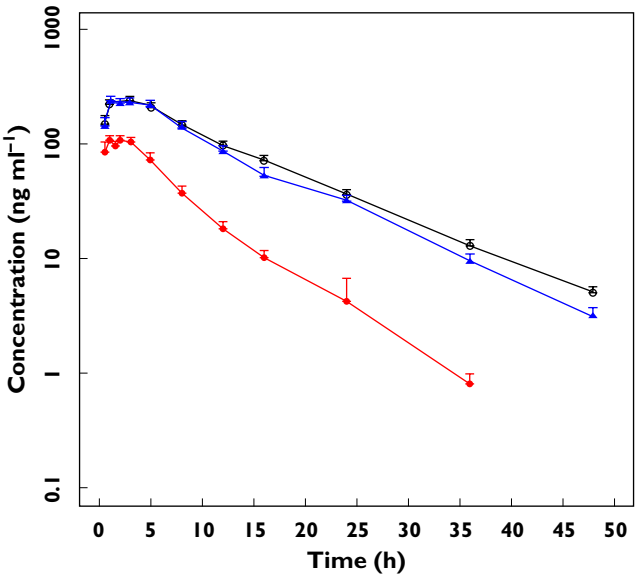

Figure 2

Mean (± SD) plasma concentration (ng ml^{-1}) of apremilast (APR) *vs*. time by treatment with and without multiple oral dose and single intravenous (i.v.) rifampicin (RIF) on a semi-logarithmic scale. ⊸, APR; ⟷, APR + i.v. RIF; ●⟷, APR + oral RIF

The mean (± SD) apremilast plasma concentration *vs*. time curves by treatment are shown in Figure 2. The mean apremilast plasma concentration profile for the apremilast alone treatment period and the apremilast co-administered with i.v. rifampicin treatment period are nearly superimposable. The PK and statistical analysis results are presented in Table 1. A 13% increase was observed in the apremilast C_{max} after administration of i.v. rifampicin. Although the 90% CI did not include 100%, it was contained entirely within the 80% to 125% limits, indicating that there was no statistically significant nor clinically meaningful difference. A significant decrease in the mean apremilast concentration was noted after administration for 2 weeks of twice daily oral rifampicin, to the extent that there was a clear separation in the mean plasma concentration when apremilast was administered alone *vs*. when it was co-administered with oral rifampicin for 2 weeks. This is further supported by the PK and statistical analysis (Table 1), which show that AUC exposures and C_{max} values of apremilast after 2 weeks of oral rifampicin were only 28% and 57%, respectively, of the apremilast alone values. Multiple doses of oral rifampicin showed a statistically significant and clinically meaningful decrease in apremilast exposure. The apremilast $t_{1/2}$ was slightly shorter and the clearance of apremilast increased approximately 3.6-fold after co-administration with multiple oral doses of rifampicin. The mean apremilast apparent volume of distribution was also higher after co-administration with multiple oral doses rifampicin.

Safety

No deaths or serious AEs were reported during either study. In the KETO study, headache and nausea were the most frequently reported AEs. In the RIF study, headache and rhinitis were the more frequently reported AEs. In both studies, only one subject prematurely discontinued the study drug because of an AE. The subject developed H1N1 influenza in the RIF study. There were no clinically meaningful changes observed in clinical laboratory analytes, vital signs, electrocardiogram intervals or physical examination findings throughout the studies.

Discussion

The main findings of these two studies are that 1) ketoconazole increases overall exposure (AUC(0,∞)) of apremilast by approximately 36% (2827 *vs*. 2072 ng ml^{-1} h) and peak exposure (C_{max}) by 5% (247 *vs*. 236 ng ml^{-1}) when co-administered with a single dose of apremilast 20 mg, although these increases are not clinically meaningful and 2) rifampicin decreases apremilast mean AUC(0,∞) by approximately 72% (3120 *vs*. 869 ng ml^{-1} h) and C_{max} by approximately 43% (290 *vs*. 166 ng ml^{-1}) when co-administered with a single dose of apremilast 30 mg. These results demonstrate that ketoconazole, which has shown to be a strong CYP3A4 inhibitor, has limited impact on the PK of apremilast, but rifampicin, and potentially other CY3A4 inducers, increases clearance of apremilast, which may negatively impact on the therapeutic efficacy of apremilast because of decreased drug exposure. The PK profiles of apremilast in the apremilast alone treatment group were consistent with the PK from previous studies [6, 15–18].

According to the U.S. FDA guidelines, a sensitive CYP isozyme substrate is defined as a drug whose plasma AUC values increase five-fold or higher when co-administered with a known CYP inhibitor or AUC ratio in poor metabolizers *vs*. extensive metabolizers is greater than five-fold [8]. Midazolam has been shown to be a typical sensitive substrate of CYP3A4. It is reported that the AUC of midazolam increases by approximately 16-fold (or by 1590%) when administered with of ketoconazole [18]. The observation that apremilast is not a CYP3A4-sensitive substrate can be explained by the data from a human [^{14}C] ADME study [10]. The [^{14}C] ADME study demonstrates that apremilast clearance *in vivo* occurs via multiple metabolic pathways, such as non-enzymatic hydrolysis, non-CYP-dependent N-deacetylation and oxidative metabolism mediated by CYP isozymes (including CYP3A4, CYP1A2 and CYP2A6), followed by glucuronide conjugation (with *O*-demethylated metabolite). Therefore, inhibiting a single metabolic pathway such as CYP3A4 would not result in a substantial decrease in metabolic clearance.

Ketoconazole, while established as a strong CYP3A4 inhibitor, has also been shown to be a MDR1 inhibitor. Despite its dual inhibition of CYP3A4 and MDR1, the treatment of multiple doses of ketoconazole 400 mg once daily resulted in only a small change in apremilast exposure (~36% and ~5% increases in AUC(0,∞) and C_{max}, respectively), which is not clinically meaningful. The results suggest that dose adjustment is not necessary if apremilast is taken with drugs which are inhibitors of CYP3A4 or P-gp.

After multiple doses, rifampicin is a very potent broad-spectrum inducer on phase 1, phase 2 metabolizing enzymes and transporters, including CYP3A4 and other CYP450 isoforms, glutathione-S-transferases and UDP-glucuronosyltransferases and transporters, and MDR1 [19, 20]. Rifampicin decreases midazolam exposure to 4% of the exposure when midazolam is administered alone [21].

The effect of induction by rifampicin on apremilast clearance observed in the RIF study is much more pronounced than that of the inhibition by ketoconazole. Apremilast clearance increased by approximately 3.6-fold when co-administered with multiple dose rifampicin in the RIF study. The results suggest that other CYP3A4 inducers may negatively impact on the therapeutic efficacy of apremilast because of decreased drug exposure.

In summary, ketoconazole decreased apremilast clearance, resulting in a small increase in AUC, which is within the safety margin. Thus, dose adjustment is not necessary when apremilast is taken with other strong CYP3A4 inhibitors. However, the effect of induction by rifampicin on apremilast clearance is much more pronounced. The results demonstrate that multiple oral doses of rifampicin caused an approximately 3.6-fold increase in apremilast clearance, resulting in a decrease in drug exposure by approximately 72%. The decrease in drug exposure may result in a loss of efficacy of apremilast. Therefore, the use of rifampicin or other strong CYP3A4 inducers with apremilast is not recommended.

Competing Interests

YL, MP and SZ are employees of the Celgene Corporation and hold stock in Celgene Corporation. YW is a former employee of the Celgene Corporation. AW is a former employee of the Celgene Corporation and holds stock in the Celgene Corporation.

The studies were sponsored by Celgene Corporation. The authors thank Prachi Wickremasingha, PharmD, who wrote the first draft of the manuscript. The authors also thank colleagues Liangang Liu, PhD and John Patin, MS, for performing the statistical analysis. Editorial assistance was provided by Peloton Advantage, Parsippany, NJ, USA and funded by the Celgene Corporation.

Authors' contributions

MP, SZ, YL, AW and YW conceived and designed the study. All authors were involved in carrying out the study and interpreting the results. All authors read, provided critical input and revisions to, and approved the final manuscript.

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