DMD Fast Forward. Published on January 8, 2018 as DOI: 10.1124/dmd.117.078824
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Clinical Pharmacokinetics and the Impact of Genetic Polymorphism on a CYP2C19 Substrate, BMS-823778, in Healthy Subjects

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Running title: Clinical Pharmacokinetics of BMS-823778 and the Impact of Genetic Polymorphism

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Number of text pages = 17
Number of words in abstract = 225
Number of words in introduction = 584
Number of words in discussion = 1072
Number of tables = 6
Number of figures = 7
Number of references = 24
Supplemental file = 1

Abbreviations: 11β-HSD1: 11β-hydroxysteroid dehydrogenase 1; AUC: area under curve; T-HALF: half-life; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LLOQ: lower limit of quantitation; SAD: single ascending dose; MAD: multiple ascending dose; AI: accumulation index; CLR: renal clearance; CLT: total clearance; EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer; AE: adverse event; BMI: body mass index.

Abstract

BMS-823778 is a potent and selective inhibitor of 11β-HSD1, an enzyme that regulates tissue specific intracellular glucocorticoid metabolism and a compelling target for the treatment of metabolic diseases. Metabolism of BMS-823778 was mainly mediated by polymorphic CYP2C19 with minor contribution from CYP3A4/5 and UGT1A4. Clinical PK of BMS-823778 was first investigated in healthy volunteers after single and multiple ascending doses. BMS-823778 was rapidly absorbed after oral dose, and systemic exposure at steady state increased proportionally to the dose. Large inter-subject variability in BMS-823778 exposure was likely due to polymorphism of metabolic enzymes. The impact of genetic polymorphism of CYP2C19, UGT1A4 and CYP3A5 on BMS-823778 PK was assessed in healthy Chinese and Japanese subjects as well as in human ADME study, where all subjects were genotyped either before or post treatment. There was a clear trend of high exposure and low clearance in CYP2C19 PMs comparing to EM or IM subjects. Impact of UGT1A4 or CYP3A5 polymorphism on BMS-823778 PK was statistically not significant in CYP2C19 EM and IM subjects. However in a subject with predicted CYP2C19 PM phenotype, the PK of BMS-823778 was affected significantly by UGT1A4 polymorphism. Overall, BMS-823778 was safe and well tolerated in healthy subjects following single or multiple oral doses. The PK of BMS-823778 was characterized with rapid absorption and the systemic clearance directly correlated with the genetic polymorphism of CYP2C19.

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1. Introduction

11β-hydroxysteroid dehydrogenases 1 (11β-HSD1) plays a central role in regulating tissue specific intracellular glucocorticoid metabolism and mediates in situ production of glucocorticoid cortisol as an alternative path besides the classical hypothalamic-pituitary-adrenal (HPA) axis (Morgan and Tomlinson, 2010; Anagnostis et al., 2012; Morgan et al., 2014). 11β-HSD1 is mainly expressed in liver, pancreas and adipose tissues which catalyzes inter-conversion between biologically inert cortisone and active cortisol. Several lines of evidence indicated that reducing cortisol generation from cortisone in tissues by inhibition of 11β-HSD1 had the potential to be an efficacious treatment for type-2 diabetes, dyslipidemia and obesity (Morton, 2010; Sooy et al., 2010; Anderson and Walker, 2013; Morentin Gutierrez et al., 2015).

BMS-823778 is a potent and selective inhibitor of 11β-HSD1, with an in vitro IC_{50} value of ~3 nM (Li et al., 2014; Furlong et al., 2016). As part of clinical development, the pharmacokinetics (PK), safety and tolerability of BMS-823778 were characterized in several clinical studies. First, a combined single/multiple ascending dose study (SAD/MAD) was conducted in healthy volunteers. Upon completion of the study, it was evident that PK variability across subjects was large, indicating potential involvement of polymorphic metabolizing enzymes. This prompted reaction phenotyping study which demonstrated that metabolism of BMS-823778 was mainly catalyzed by a cytochrome P450 2C19 (CYP2C19), with minor contribution from CYP3A4/5 and UGT1A4 (Cheng et al., 2017).

The CYP2C enzymes are clinically important enzymes which metabolize approximately 20% of all pharmaceutical drugs. CYP2C19 metabolizes S-mephenytoin (the prototypical substrate), proton-pump inhibitors such as omeprazole, diazepam, and the platelet inhibitor clopidogrel (Goldstein, 2001; Lee et al., 2002; Mega et al., 2009) and exhibits several genetic

polymorphisms with large phenotypic inter-individual variability in metabolism. Individuals can be categorized as extensive (EMs), intermediate (IMs) and poor metabolizers (PMs) based on metabolism of mephenytoin. Thus, genetic polymorphisms of CYP2C19 have the potential to significantly influence the pharmacokinetics (PK) and pharmacodynamics of many drugs in clinical use, which could result in adverse drug effects or therapeutic failure (Desta et al., 2002; Wojnowski and Kamdem, 2006; Jiang et al., 2015).

Even though CYP3A5 and UGT1A4 played a minor role compared to CYP2C19 in the clearance of BMS-823778, their contribution could increase significantly in subjects devoid of CYP2C19 functionality. A number of clinical studies have suggested that PK of substrates of CYP3A5 or UGT1A4 could be altered in subjects with polymorphic enzymes (Staatz et al., 2010; Reimers et al., 2016). UGT1A4 is involved in the glucuronidation of many drugs including lamotrigine, tamoxifen and clozapine (Ehmer et al., 2004). Substrate-dependent enzyme activity has been shown with different genetic variants (namely *2 and *3) of UGT1A4 (Reimers et al., 2016). Thus, in additional to the polymorphism of CYP2C19, the impact of UGT1A4 and CYP3A5 polymorphism on the PK of BMS-823778 needs to be evaluated in clinical, particularly in subjects with CYP2C19 variations.

A single dose human ADME study with [^{14}C]BMS-823778 in CYP2C19 EM and PM subjects was reported previously where higher exposure of BMS-823778 was observed in PMs than in EMs (Cheng et al., 2017). The current studies describe PK of BMS-823778 in healthy volunteers following single or multiple ascending doses, as well as further assessment of polymorphic impact in healthy Chinese and Japanese subjects with various genotypes of CYP2C19, UGT1A4 and CYP3A5.

Materials and Methods

All clinical studies were conducted in accordance with Good Clinical Practice, as defined by the International Council for Harmonization (ICH) and in accordance with the Declaration of Helsinki. The protocol, amendments, and consent forms were approved by the Institutional Review Board/Independent Ethics Committee prior to initiation of each study at their respective sites. In each clinical study, safety assessments included medical review of adverse events (AEs), vital sign measurements, ECGs, physical examinations, and clinical laboratory tests. Administration of each higher dose was contingent upon acceptable safety and tolerability of the previous dose panel.

Genotyping for CYP2C19, CYP3A5 and UGT1A4 was performed on the DMET microarray using the DMET Plus Premier pack kits as described previously (Cheng et al., 2017). Plasma and urine concentrations of BMS-8237778 were determined with validated LC-MS/MS methods reported previously (Furlong et al., 2016). Details about the methods including internal standard, method of extraction, HPLC column and mobile phase, monitored m/z transitions, within- and between-day variability were described therein.

SAD/MAD Study in Healthy Volunteers.

This was a randomized, placebo-controlled, double-blind, single (Part A) and multiple (Part B) ascending dose study to assess the safety, tolerability, and PK of BMS-823778 in healthy volunteers. A total of 104 healthy male subjects (102 Caucasians and 2 non-Caucasians, ages 18-55 years, body mass index [BMI] of 18 to 29 kg/m^2) participated in the study. Eight subjects were assigned to each of 8 sequential dose panels (0.1, 0.5, 2, 5, 12, 25, 50 and 100 mg) in Part A. In each panel, subjects were randomly assigned to receive a single oral dose of BMS-823778 (N = 6) or placebo (N = 2) on Day 1 as an oral solution (0.1-0.5 mg) or capsule

formulation (2-100 mg). The oral solution was formulated in a vehicle comprised of 50% (v/v) OraSweet SF in water. Blood and urine samples for PK analysis were collected up to 96 hours after dosing in the 0.1-5 mg dose panels. In 12-100 mg dose panels, blood samples were collected up to 168 hours post-dose and urine samples were collected up to 120 hours post-dose.

In part B, eight healthy male subjects were assigned to each of 5 sequential dose panels (0.5, 2, 5, 12, and 25 mg) and each subject was administered a once daily oral dose of BMS-823778 (N = 6) or a matched placebo (N = 2) as an oral solution (0.5 mg) or capsule formulation (2-25 mg) for 14 days. Subjects were kept in-house up to Day 21 for safety evaluations and PK sampling. Serial blood samples were collected following dosing on Day 1 for 24 hours and on Day 14 for up to 168 hours, and 24-hour urine samples were collected following dosing on Days 1 and 14 to characterize the PK profile of BMS-823778. Additional blood samples were collected prior to dosing on Days 2, 6, 8, 10, 12 and 14 to assess for achievement of steady-state. Each blood sample was collected into a pre-labeled tube containing K_2EDTA as the anticoagulant and was centrifuged for 15 min at approximately 1000 g to harvest plasma.

Clinical PK Study in Healthy Chinese Subjects

This was a double-blind, placebo-controlled, single- and multiple-dose study to assess safety, tolerability and PK in healthy Chinese subjects. Twenty healthy male Chinese subjects (age 20 to 55 years, BMI 19 to 25 kg/m^2) were randomly assigned to each of 2 dose panels (2 mg and 15 mg) and were administered a single oral dose of BMS-823778 (N = 15) or placebo (N = 5) as a capsule formulation on Day 1. Blood and urine samples were collected for PK analysis over 96 hours post-dose. Beginning on Day 6, subjects received oral doses of study medication once daily for 12 days. Blood and urine samples were collected for PK analysis for 24 hours after dosing on Day 17. Trough blood samples were also collected predose on Days 7, 9, 11, 13, and

15. Detailed procedures for blood collection and plasma harvesting were the same as in SAD/MAD study. Genotyping was performed retrospectively for all subjects who participated in this study. Therefore, the criteria did not pertain to a genotype assessment in advance.

Clinical PK Study in Healthy Japanese Subjects

This was a randomized, placebo-controlled, double-blind, multiple ascending dose study to assess the safety, tolerability, and PK of BMS-823778 in healthy Japanese subjects. The study consisted of 3 dose panels (2, 12 and 25 mg) and within each panel, 8 subjects were assigned randomly in a double-blinded fashion to receive daily oral doses of either BMS-823778 or matching placebo as a capsule formulation for 14 days in a ratio of 3:1. Serial blood samples and urine samples were collected up to 24 hours following the first dose on Days 1 and up to 168 hours after the last dose on Day 14. In addition, trough (pre-dose) samples were collected on Days 6, 8, 10, and 12. Detailed procedures for blood collection and plasma harvesting were the same as in the SAD/MAD study. Genotyping was performed retrospectively for all subjects who participated in this study.

Human ADME Study with [^{14}C]BMS-823778

Details of human ADME study with genotyping have been described previously (Cheng et al., 2017). Briefly, 14 healthy male subjects were placed into 3 groups based on their predicted CYP2C19 phenotype: group 1 (EMs, n = 7); group 2 (PMs, n=3,); and group 3 with bile collection from 3-8 hours post dose (2 EMs and 1 PM). Each subject received a single oral solution dose of 10 mg [^{14}C]BMS-823778 containing 80 μCi of radioactivity. Blood, urine, feces and bile samples were collected for PK and metabolite analysis. Methods for radioactivity measurement and metabolite analysis have been reported previously (Cheng et al., 2017).

Data Analysis

All subjects in the 0.1 and 0.5 mg dose panels in SAD and all subjects from 0.5 mg MAD group at day 1 had insufficient number of plasma and urine data which were above the LC/MS/MS assay lower limit of quantification (LLOQ; 0.2 ng/mL for the plasma assay and 1 ng/mL for the urine assay), and therefore, neither plasma nor urine PK parameters have been reported for these dose panels. Likewise, urine PK parameters for subjects in 2 mg SAD dose panel are not reported because of low urine concentration.

For the dose panels in SAD/MAD where subjects did have enough data above the LLOQ, as well as the Chinese and Japanese studies, pharmacokinetics of BMS-823778 were derived from plasma concentration versus time and urinary excretion data. Individual subject PK parameter values were derived by non-compartmental analysis (NCA) using the program Kinetica (Version 5, Thermo Fisher Scientific) and summarized by descriptive statistics. Plasma concentration data from all subjects who received BMS-823778 were included in the PK analysis. Pre-dose concentrations and concentrations prior to the first quantifiable concentration that were below LLOQ were set to "zero" for the purpose of calculating PK parameters, but were treated as "missing" for the calculation of summary statistics. The PK parameters Cmax, Cmin, and Tmax were recorded directly from experimental observations. Using no weighting factor, the terminal log-linear phase of the concentration-time curve was identified by least-square linear regression of at least three data points. The single dose half-life (T-HALF) of the terminal log-linear phase was calculated as $\ln 2/\lambda$, where λ is the absolute value of the slope of the terminal log-linear phase. The area under the concentration-time curve from time zero to the last quantifiable plasma concentration AUC(0-T) and the area under the plasma concentration-time curve over one dosing interval AUC(TAU) were calculated by mixed log-linear trapezoidal

summations. Area under the plasma BMS-823778 concentration-time curve from zero extrapolated to infinity, AUC(INF), was calculated by log- and linear-trapezoidal summations over the collection period, with the last quantifiable plasma concentration being divided by λ and the product added to the total area. CLT/F was calculated as Dose/AUC(TAU). CLR/F was calculated as amount of unchanged BMS-823778 recovered in urine/AUC(0-T). Accumulation index (AI) was calculated as AUC(TAU) on Day 14/AUC(TAU) on Day 1.

Additional statistical analysis was performed with GraphPad Prism (Version 7.03, GraphPad Software, Inc., La Jolla, CA) using Unpaired Student t Test for two group comparisons, and Ordinary one-way ANOVA with Dunnett's Multiple Comparison Test for multiple groups ($n \geq 3$). Differences were considered significant when $P < 0.05$. The dose proportionality of BMS-823778 was assessed using a power model, assuming a linear relationship between log-transformed pharmacokinetic exposure parameter (AUC(INF) or AUC(TAU)) and log-transformed dose: $\log(AUC) = \alpha + \beta\log(dose)$. The proportionality constant (β) and its corresponding 90% confidence interval (CI) were estimated from data fitting. Proportionality was concluded if the 90% confidence interval for β was contained completely within 0.8- 1.25 (P. Smith et al., 2000).

Results

SAD/MAD study

Mean plasma concentration vs time plots after a single dose of BMS-823778 are illustrated in Figure 1, and the PK parameters from non-compartmental analysis are summarized in Table 1. PK samples were collected for 96 hours post dose for 0.1 - 5 mg dose panels and sampling time was increased to 168 hours starting at 12 mg dose after it was found that T-HALF was longer than predicted. The drug was rapidly absorbed after oral administration, with median Tmax values ranging from 1 to 4 hours. The T-HALF ranged from ~32 to 50 hours at doses deemed to provide a reliable estimate of this parameter (12 to 100 mg where blood samples were collected for 168 hours after dosing). Dose proportionality was analyzed with a power model, and representative figures are illustrated in Figure S1 (Supplemental Information). The primary analysis suggested a slightly more than dose-proportional increase in exposure based on the data collected in all dose panels, including the 2 and 5 mg doses panels of BMS-823778 in which the % extrapolated AUC was >20% that of the AUC(0-T) and thus the AUC(INF) of BMS-823778 at these doses may not be adequately characterized. Additional dose-proportionality analysis was conducted excluding the 2 and 5 mg BMS-823778 dose panels and results indicated that AUC(INF) increased dose-proportionally ($\beta = 1.08$, 90% Cl 0.95-1.20) in the dose ranging from 12 to 100 mg. Less than 1% of the orally administered dose of unchanged BMS-823778 was recovered in the urine. The mean renal clearance of BMS-823778 was estimated to be <1 mL/min at all doses studied. Metabolites of BMS-823778 in circulation or urine samples were not measured in this study.

Mean plasma concentration vs time plots of BMS-823778 following once-daily administration for 14 days are illustrated in Figure 2 and PK parameters are summarized in Table

2. Exposure of BMS-823778 increased dose-proportionally in the tested dose range (0.5-25 mg, β = 1.04, 90% Cl 0.96-1.16) on day 14. There appeared to be considerable overlap in the Cmax and AUC(TAU) values between the 12 and 25 mg doses on day 14 and the mean T-HALF appeared to be shorter (~35 hours) at the 25 mg dose relative to lower doses (ranging from 65 to 68 hours). BMS-823778 accumulated in plasma with mean AI values of ~21.3, 7.3, 4.8 and 3, respectively, for the 2, 5, 12 and 25 mg doses of BMS-823778. Trough plasma concentrations (C24h) of BMS-823778 appeared to reach steady state levels by approximately 12 days.

PK variability was assessed based on the coefficients of variants (CV) of main PK parameters including Cmax, AUC(INF)/AUC(TAU) and CLT/F, as well as the range of these parameters in each dose panel. CV values were moderate to high, with high value reaching above 70%. Individual exposures of subjects in SAD and MAD are illustrated in Figure 3. In the same dose panel, there was 2-4 fold difference between low and high AUC(INF) or AUC(TAU). In addition, individual exposures at steady state in each dose panel appeared to be segregated into low and high (or low, medium and high) groups (Figure 3B).

Secondary peaks were observed in the concentration-time profiles of BMS-823778 at approximately 24 hours post dose in both SAD and MAD (Figure 1 & Figure 2), presumably due to the enterohepatic recirculation of the parent or metabolites.

PK Study in Chinese Population

Mean BMS-823778 PK parameters following 2 or 15 mg oral doses to healthy Chinese subjects are summarized in Table 3. The absorption of BMS-823778 was rapid with peak concentrations observed approximately 1.5 to 3 hours after dosing. Mean Cmax and AUC(TAU) on Day 17 of the 15 mg dose group were ~8-fold higher than those from 2 mg dose group, indicating an approximate dose-proportional increase of steady-state exposure. Urinary recovery

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of unchanged BMS-823778 over a 24-hour interval on Day 17 was generally below 1% of the dose in both groups.

To investigate the effect of polymorphisms on the PK of BMS-823778, all subjects participated in the study were genotyped for CYP2C19, UGT1A4 and CYP3A5 after the treatment. Out of 29 participants who received doses of BMS-823778, 9 and 19 subjects were identified as CYP2C19 EMs and IMs, respectively. Only 1 subject was identified as a poor metabolizer (CYP2C19 *2/*3). No subject in this study had the CYP2C19*17 allele (ultra-metabolizer).

The PK of BMS-823778 in Chinese subjects was further analyzed according to their predicted CYP2C19 phenotype. Representative plasma concentration-time profiles of BMS-823778 at 15 mg on day 1 and day 17 are illustrated in Figure 4 and mean CLT/F values of BMS-823778 are tabulated in Table 4 by treatment group and predicted CYP2C19 phenotype. In general, low clearance and high exposure of BMS-823778 were associated with CYP2C19*2 and *3 variations in Chinese subjects. CLT/F values of BMS-823778 in CYP2C19 EM subjects in both groups on day 17 were significantly higher than IM subjects (1.7-fold, $p = 0.004$) and were ~3.6-fold higher than the PM subject.

PK Study in Japanese Population

Similar to the Chinese study, all Japanese subjects were genotyped retrospectively for CYP2C19, CYP3A5 and UGT1A4 polymorphism. Mean PK parameters grouped based on dose levels are summarized in Table 5. BMS-823778 accumulated in plasma upon once-daily dosing with mean AI values of approximately 21.2, 4.17, and 4.25 for the 2, 12, and 25 mg groups, respectively. Both Cmax and AUC(TAU) increased in a dose proportional manner in the dose range of 2 to 25 mg on Day 14 ($\beta = 1.09$, 90% Cl 0.90-1.21). Less than 1% of the orally

administered dose of unchanged BMS-823778 was recovered in the urine and the mean renal clearance of BMS-823778 was approximately 0.2 mL/min.

The pharmacokinetic results were also examined by treatment group and predicted CYP2C19 phenotype. Among a total of 24 healthy Japanese subjects, 6, 15 and 2 subjects were identified as CYP2C19 EMs, IMs and PMs, respectively. Genotype was not identified in 1 subject due to the failure in DNA isolation. No subjects had the CYP2C19*17 allele. Representative plasma concentration-time profiles of BMS-823778 at 12 mg on day 1 and day 14 are illustrated in Figure 5, and mean CLT/F values of BMS-823778 are tabulated in Table 6 by treatment group and predicted CYP2C19 phenotype. Similar to the Chinese study, high clearance and low exposures were observed in subjects with predicted CYP2C19 EM phenotype, comparing to IM and PM subjects. Mean CLT/F value in CYP2C19 EM subjects across dosing groups was 1.7-fold ($p = 0.003$) higher than IMs, and 3.8-fold ($p < 0.001$) higher than PMs. When examined by predicted CYP2C19 phenotype, it appeared that steady state concentrations of BMS-823778 were reached by Day 10 of daily dosing for EMs and IMs. However for PMs, steady state was not reached at the end of the study.

Dose-proportionality analyses were conducted by CYP2C19 predicted phenotype to evaluate the relationship between overall PK data and CYP2C19 phenotype. Results indicated that the Day 14 AUC(TAU) increased largely in a dose proportional manner in the dose range of 2-25 mg in subjects with EM ($\beta = 1.14$, 90% Cl 0.94-1.19), IM ($\beta = 1.12$, 90% Cl 0.99-1.25) or PM ($\beta = 0.99$, 90% Cl 0.56-1.43) CYP2C19 phenotype.

Impact of Polymorphisms of UGT1A4 and CYP3A5 on BMS-823778 PK

Genetic variations of CYP3A5 and UGT1A4 were observed in CYP2C19 EM and IM subjects in both Chinese and Japanese studies, but not in CYP2C19 PM subjects. As illustrated

in Figure 6, there were no statistically meaningful differences in CLT/F of BMS-823778 between Chinese subjects with wild type and polymorphic UGT1A4 ($p = 0.28$) or CYP3A5 ($p = 0.34$). Similar results were observed in the Japanese study where polymorphism of UGT1A4 and CYP3A5 had no statistically significant impact on BMS-823778 PK in 2C19 EM or IM subjects (Figure S2, Supplemental Information). For the human ADME study, concentration-time profiles of total radioactivity (TRA) and BMS-823778 following a single dose of [^{14}C]BMS-823778 in healthy CYP2C19 EM or PM male subjects have been reported previously (Cheng et al., 2017). AUC(INF) of TRA and BMS-823778 were 2.3-fold ($p < 0.001$) and 5.3-fold ($p < 0.001$) higher in PMs than in EMs, respectively. Further analysis of BMS-823778 PK was performed based on the genotype of UGT1A4 and a scatter plot of AUC(INF) versus corresponding UGT1A4 genotype is illustrated in Figure 7. The AUC(INF) values in subjects with wild type or polymorphic UGT1A4 were comparable in CYP2C19 EM group. However in CYP2C19 PM group, AUC(INF) was ~50% higher in a subject with UGT1A4*1/*2 genotype than the mean AUC(INF) of CYP2C19 PM subjects with wild type UGT1A4. Comparing to CYP2C19 EM subjects, there was ~11 fold increase in exposure in the subject with both predicted CYP2C19 PM phenotype and polymorphic UGT1A4.

Safety and Tolerability Assessment

BMS-823778 was well-tolerated in healthy volunteers. As summarized in Table S1-4 (Supplemental Information), no deaths, severe AEs, or AEs resulting in discontinuation were reported in these studies following a single or multiple doses of BMS-823778. The investigator and the medical monitor for this study did not identify any clinically-relevant safety issues. Most AEs were mild and considered by the Investigator to be not related to study drug. There were no clinically-significant findings or trends in laboratory, vital signs, ECG, or PE assessments.

Discussion

BMS-823778 is a potent and selective inhibitor of 11β-HSD1. The studies described in this paper aimed to test safety, tolerability and PK in healthy volunteers, as well as to evaluate polymorphic impact of metabolizing enzymes on the PK of BMS-823778. The first-in-human study for BMS-823778 was a combined SAD/MAD study in healthy male subjects with no genotyping for metabolizing enzymes because the enzymes involved in the metabolism of BMS-823778 had not been identified prior to the initiation of the study. BMS-823778 was slowly cleared from human following oral absorption with the mean T-HALF ranging from ~32 to 50 hours. Both Cmax and AUC(TAU) at 25 mg overlapped significantly with those from 12 mg dose panel in MAD, with shorter mean T-HALF and greater mean CLT/F at the high dose. This is unlikely due to CYP induction since BMS-823778 is not a CYP3A4 inducer (Details of CYP induction are included in Supplemental Information), and the relatively high clearance at 25 mg was not observed in the Japanese study. One possible explanation is that more CYP2C19 EMs could have been recruited in the 25 mg dose panel in MAD which increased the mean total clearance.

A dose proportionality assessment with the power model indicated that the exposure of BMS-823778 increased proportionally with respect to dose from 12-100 mg in SAD and at steady state in MAD from 0.5-25 mg. The linear PK at steady state was also observed in the Japanese study, regardless of CYP2C19 predicted phenotype. There appeared a dose dependent AI in MAD study (~21.2, ~7.3, ~4.8 and ~3 fold at the doses of 2, 5, 12 and 25 mg, respectively) due to the relatively low exposure at low doses after the first dose of BMS-823778. The reason for the dose-dependent AI is unknown. One possibility is the existence of high-affinity low-capacity binding sites (Wright et al., 2013) for BMS-823778. Extensive tissue distribution of

BMS-923778 to these binding sites limits the exposure of BMS-823778 at low doses. This capacity limited distribution could be overcome at higher dose levels or when steady state is reached. Since dose-proportionality was observed at steady-state regardless of CYP2C19 genotype, and safety and efficacy are likely driven by the steady state exposure, non-linear PK on day 1 is not clinically significant.

Secondary peaks in the concentration-time profiles were observed in both SAD and MAD, indicating the possible involvement of the biliary route in the excretion of BMS-823778 and/or its metabolites that were recycled to parent in the gastrointestinal tract. This is consistent with the results from human ADME study where significant amount of parent and direct glucuronide metabolite were observed in bile (Cheng et al., 2017). Enterohepatic recirculation may contribute to the observed relatively flat terminal phase of the concentration-time profile and longer than expected elimination T-HALF.

High variability of PK was evident based on the large CV% of the major PK parameters and significant difference of BMS-823778 exposure between subjects in the same dose panel, which prompted the investigation on the polymorphism in the clearance of BMS-823778. Even though age, gender, body weight, etc. of the participants can also contribute to the variability, that fact that individual exposure at steady state in each dose panel segregated into low and high groups suggested that polymorphic enzymes might be involved. A subsequent reaction phenotyping study demonstrated that in vitro metabolism of BMS-823778 was mediated mainly by polymorphic CYP2C19, with minor contribution from CYP3A4/5 and UGT1A4 (Cheng et al., 2017). Therefore, all subjects in the Chinese and Japanese PK studies as well as in the human ADME study were genotyped for CYP2C19, CYP3A5 and UGT1A4 either post treatment or

prior to dose administration, to understand the impact of genetic polymorphisms on the PK of BMS-823778 across different populations.

In both Chinese and Japanese studies, genotyping was performed retrospectively after the treatment. Plasma PK of BMS-823778 in Chinese and Japanese populations was characterized by rapid absorption and slow elimination, similar to the MAD study. Cmax and AUC(TAU) at steady state increased largely proportionally to the dose within the tested dose range. Comparing the exposure of BMS-823778 across different populations, mean AUC(TAU) of BMS-823778 at steady state in Chinese and Japanese subjects were comparable at the same dose level, and were much higher than these in MAD study where the majority of participants were Caucasians. This is similar to a previous report where oral clearance of omeprazole in Caucasian was approximately 2-fold higher than in Asian (Feng et al., 2015).

An exploratory assessment of PK versus genotype suggested that mean CLT/F of BMS-823778 directly correlated to the predicted CYP2C19 phenotype. Statistically higher clearance, shorter terminal T-HALF and lower exposure of BMS-823778 were observed in CYP2C19 EM subjects than in IM or PM subjects. The results demonstrated that CYP2C19 metabolism was an important BMS-823778 elimination pathway in humans and polymorphism contributed to the inter-individual variability in exposure in clinical studies. Therefore, obtaining CYP2C19 genotype information and sparse PK sampling for BMS-823778 in future Phase 2b studies are recommended to further characterize the pharmacokinetics of BMS-823778 and understand implications of CYP2C19 genotype across populations.

Genetic variants of CYP3A5 and UGT1A4 were identified in the CYP2C19 EM or IM groups in both Chinese and Japanese subjects, but not in the CYP2C19 PM group. Analysis of PK and genotype demonstrated that CYP3A5 and UGT1A4 polymorphisms did not have a

statistically meaningful impact on the PK of BMS-823778 in CYP2C19 EM or IM subjects. This is probably due to the fact that CYP3A5 and UGT1A4 metabolism pathways were minor compared to CYP2C19 pathway. In the ADME study, a few subjects in CYP2C19 EM group were identified as UGT1A4 genetic variants whose systemic exposures to BMS-823778 were within the range of subjects with UGT1A4*1/*1 genotype. A single CYP2C19 PM subject with UGT1A4*1/*2 genotype (an intermediate metabolizer genotype for some substrates) had the highest individual BMS-823778 systemic exposure (Figure 7). While caution is needed in interpreting these findings due to the small number of subjects, the data did suggest that BMS-823778 is a substrate affected by UGT1A4 polymorphism in subjects who are devoid of CYP2C19 activity.

In summary, BMS-823778 was safe and well tolerated in healthy subjects following single or multiple oral doses. BMS-823778 was rapidly absorbed after oral administration with slow clearance and long terminal T-HALF. Plasma exposure and clearance correlated well with the genetic variants of CYP2C19 where subjects with predicted EM phenotype had statistically higher clearance of BMS-823778 than those with polymorphism. CYP3A5 and UGT1A4 polymorphisms did not have statistically meaningful impact on BMS-823778 PK in subjects with predicted CYP2C19 EM or IM phenotype. However, limited data suggested that the exposure of BMS-823778 might be affected significantly by UGT1A4 polymorphisms in subjects with no CYP2C19 activity. Further study is needed to characterize the influence of CYP3A5 and UGT1A4 polymorphisms on the PK of BMS-823778 in CYP2C19 poor metabolizers, as well as PK in subjects with CYP2C19 *17 variation. A clear understanding of the influence of all genotypic variations on the PK of BMS-823778 is needed especially since different ethnic groups have different levels of variation.

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Authorship Contribution

Participated in research design: Hansen, Iacono

Conducted experiments and data analysis: Gong, Hansen, Iacono

Wrote or contributed to the writing of the manuscript: Gong, Hansen, Iacono

Figure Legend

Figure 1. Mean plasma concentration-time profiles of BMS-823778 following a single oral dose at 2-100 mg dose range. Subjects in the 0.1 mg and 0.5 mg dose panels had insufficient numbers of plasma samples whose concentrations were above LLOQ of the bioanalytical assay.

Figure 2. Mean plasma concentration-time profiles of BMS-823778 on day 14 following daily doses at 0.5, 2, 5, 12 and 25 mg dose levels.

Figure 3. Individual and mean exposure of BMS-823778 in subjects following single or multiple doses of BMS-823778: A, AUC(INF) in SAD panels (2-100 mg); and B, AUC(TAU) on day 14 in MAD panels (0.5-25 mg).

Figure 4. Representative mean concentration-time profiles of BMS-823778 in healthy Chinese subjects with different predicted CYP2C19 phenotype following (A) a single dose of BMS-823778 at 15 mg, and (B) daily doses at 15 mg for 12 days

Figure 5. Representative mean concentration-time profiles of BMS-823778 in healthy Japanese subjects with different predicted CYP2C19 phenotype on (A) Day 1, and (B) Day 14 following daily oral doses of BMS-823778 at 12 mg.

Figure 6. Individual and mean CLT/F of BMS-823778 in healthy Chinese subjects grouped according to (A) UGT1A4 ($p = 0.28$ when *1/*3 compared to *1/*1) or (B) CYP3A5 genotype ($p = 0.34$ when *1/*3 compared to *1/*1)

Figure 7. Individual AUC(INF) of BMS-823778 in healthy subjects with different UGT1A4 genotype following a single oral dose of 10 mg [^{14}C]BMS-823778

DMD Fast Forward. Published on January 8, 2018 as DOI: 10.1124/dmd.117.078824
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Table 1: Summary statistics of BMS-823778 pharmacokinetic parameters in healthy subjects following a single ascending dose of BMS-823778 in the dose range of 0.1-100 mg.

Treatment Group	Cmax (ng/mL) Mean [N] (CV)	Tmax (h) Median [n] (Min-Max)	AUC(0-T) (ng.h/mL) Mean [N] (CV)	AUC(INF) (ng.h/mL) Mean [N] (CV)	T-HALF (h) Mean [N] (SD)	CLT/F (mL/min) Mean[N] (CV)
0.1 mg	NA	NA	NA	NA	NA	NA
0.5 mg	NA	NA	NA	NA	NA	NA
2 mg	1.98[6] (73)	3.25[4] (2.0-6.0)	109[6] (54)	NA NA	NA NA	NA NA
5 mg	19.0[6] (22)	2.50[6] (1.0-4.0)	612[6] (47)	NA NA	NA NA	NA NA
12 mg	84.7[6] (14)	1.25[6] (0.6-2.0)	2221[6] (34)	2389[6] (36)	50.4[6] (6.09)	83.7[6] (41)
25 mg	206[6] (19)	1.48[6] (0.5-3.0)	5610[6] (20)	5837[6] (21)	40.3[6] (5.77)	71.4[6] (21)
50 mg	483[6] (12)	1.00[6] (1.0-2.5)	12031[6] (41)	12826[6] (48)	43.6[6] (14.5)	65.0[6] (46)
100 mg	990[6] (29)	0.74[6] (0.5-2.5)	23912[6] (39)	24544[6] (40)	31.7[6] (6.56)	67.9[6] (34)

Notes: PK parameters were estimated with a non-compartment method. PK parameters were not calculated for subjects in the 0.1 mg and 0.5 mg dose panels due to insufficient numbers of plasma samples above bioanalytical LLOQ; AUV(INF), T-HALF and CLT/F in the 2 and 5 mg dose panels were not estimated because the % extrapolated AUC for the calculation of AUC(INF) was >20% of the AUC(0-T) values.

NA: not applicable

N: number of subjects with evaluable PK data

CV: percent coefficient of variation

SD: standard deviation

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Table 2: Summary statistics of BMS-823778 pharmacokinetic parameters on Day 1 and Day 14 in healthy subjects following daily doses of BMS-823778 for 14 days in the dose range 0.5-25 mg

Treatment Group	Study Day	Cmax (ng/mL) Mean[N] (CV)	Tmax (h) Median[N] (Min-Max)	AUC(TAU) (ng.h/mL) Mean[N] (CV)	T-HALF (h) Mean [N] (SD)	CLT/F (mL/min) Mean[N] (CV)
0.5 mg	1	NA	NA	NA	NA	NA
	14	6.81[6] (38)	3.01[6] (1.5-4.0)	119[6] (45)	NA	70.2[2] (21)
2 mg	1	1.21[6] (67)	14.9[6] (3.0-23.9)	260[5] (52)	NA	NA
	14	34.2[5] (32)	2.00[5] (1.5-2.5)	555[5] (40)	67.0[5] (12.1)	60.0[5] (35)
5 mg	1	18.7[6] (19)	1.75[6] (1.0-4.0)	252[6] (17)	NA	NA
	14	114[6] (55)	2.25[6] (1.5-4.0)	1847[6] (72)	65.1[6] (45.1)	45.1[6] (17)
12 mg	1	81.4[6] (8)	1.75[6] (1.0-2.5)	967[6] (11)	NA	NA
	14	276[6] (40)	1.50[6] (1.0-2.5)	4608[6] (52)	68.2[6] (37.1)	43.4[6] (43)
25 mg	1	184[6] (24)	1.00[6] (1.0-3.0)	1999[6] (22)	NA	NA
	14	405[6] (28)	1.50[6] (0.5-2.0)	5854[6] (38)	34.7[6] (9.11)	71.2[6] (21)

Notes: PK parameters were estimated with a non-compartment method. Day 1 PK parameters were not calculated for subjects in 0.5 mg dose panel due to insufficient numbers of plasma samples above bioanalytical LLOQ

NA: not applicable

N: number of subjects with evaluable PK data

CV: percent coefficient of variation

SD: standard deviation

Table 3: Summary statistics of BMS-823778 pharmacokinetic parameters in healthy Chinese subjects following a single (Day 1) or multiple daily doses (Day 17) of BMS-823778 at 2 or 15 mg.

Treatment Group	Study Day	Cmax (ng/mL) Mean[N] (CV)	Tmax (h) Median[N] (Min-Max)	AUC(TAU) (ng.h/mL) Mean[N] (CV)	AUC(INF) (ng.h/mL) Mean [N] (CV)	CLT/F (mL/min) Mean [N] (CV)	T-HALF (h) Mean [N] (SD)
2 mg	1	3.66 [15] (37)	3.00 [15] (1.00, 24.00)	201 [15] (35)	NA	NA	NA
	17	57.2 [14] (33)	2.00 [14] (0.50, 4.00)	965 [14] (40)	NA	38.3 [14] (45)	NA
15 mg	1	157 [14] (19)	2.00 [14] (0.50, 3.00)	4978 [14] (26)	4951 [8] (20)	50.5 [8] (32)	30.1 [8] (4.18)
	17	463 [12] (34)	1.50 [12] (1.00, 3.00)	7827 [12] (41)	NA	33.5 [12] (28)	NA

Notes: PK parameters were estimated with a non-compartment method

AUC(INF), T-HALF, and CLT/F were excluded from the summary if AUC extrapolated >20%

NA: not applicable;

N: number of subjects with evaluable PK data

CV: percent coefficient of variation

SD: standard deviation

Table 4: Total clearance (CLT/F) of BMS-823778 after the last dose in Chinese subjects by predicted CYP2C19 phenotype and treatment group

Dose Level	CLT/F (mL/min) Mean [N] (CV)		
	CYP2C19 EM*	**CYP2C19 IM**	**CYP2C19 PM**
2 mg (N=14)	56.7 [5] (45)	28.1 [9] (22)	NA
15 mg (N=12)	45.2 [3] (19)	31.5 [8] (16)	13.9 [1]

Note:

N: number of subjects with evaluable PK data

NA: not applicable

SD: standard deviation

* P = 0.004 when CLT/F values of EM subjects in both dose groups were compared to IM subjects

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Table 5. Summary statistics for BMS-823778 pharmacokinetic parameters from the Japanese study by treatment group and study day.

Treatment Group	Study Day	Cmax (ng/mL) Mean [N] (CV)	Tmax (hour) Median [N] (min-max)	AUC(TAU) (ng.h/mL) Mean [N] (CV)	CLT/F (mL/min) Mean [N] (CV)	T-half (h) Mean [N] (SD)
2 mg	1	2.98[6] (42)	3.25[6] (2.0-6.0)	44.6[6] (42)	NA	NA
	14	57.4[6] (58)	1.75[6] (1.0-3.0)	944[6] (72)	35.3[6] (44)	81.8[6] (68.8)
12 mg	1	108[6] (17)	1.75[6] (1.0-3.0)	1521[6] (18)	NA	NA
	14	375[6] (49)	1.50[6] (1.0-3.0)	6348[6] (61)	31.5[6] (54)	64.6[6] (39.2)
25 mg	1	272[6] (16)	1.75[6] (1.0-2.5)	3593[6] (19)	NA	NA
	14	931[6] (46)	1.50[6] (0.5-2.0)	15276[6] (43)	27.3[6] (34)	67.5[6] (33.2)

Notes: PK parameters were estimated with a non-compartment method

NA: not applicable

N: number of subjects with evaluable PK data

CV: percent coefficient of variation

SD: standard deviation

Table 6: Total clearance (CLT/F) of BMS-823778 after the last dose in Japanese subjects by predicted CYP2C19 phenotype and treatment group

Dose Level	CLT/F (mL/min) Mean [N] (CV)		
	CYP2C19 EM*	**CYP2C19 IM****	**CYP2C19 PM**
2 mg (N = 6)	55.51[2] (10)	37.85[3] (31)	12.50[1]
12 mg (N = 6)	58.47[2] (15)	33.13[2] (24)	16.68[2] (29)
25 mg (N = 5)	38.61[2] (18)	26.14[2] (15)	13.94[1]

Note:

N: number of subjects with evaluable PK data

NA: not applicable

* P = 0.003 when CLT/F values of EMs across dose groups were compared to these of IMs; $p < 0.001$ when CLT/F values of EMs were compared to these of PMs.

** $p < 0.001$ when CLT/F values of IMs were compared to these of PMs

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Figure 1.

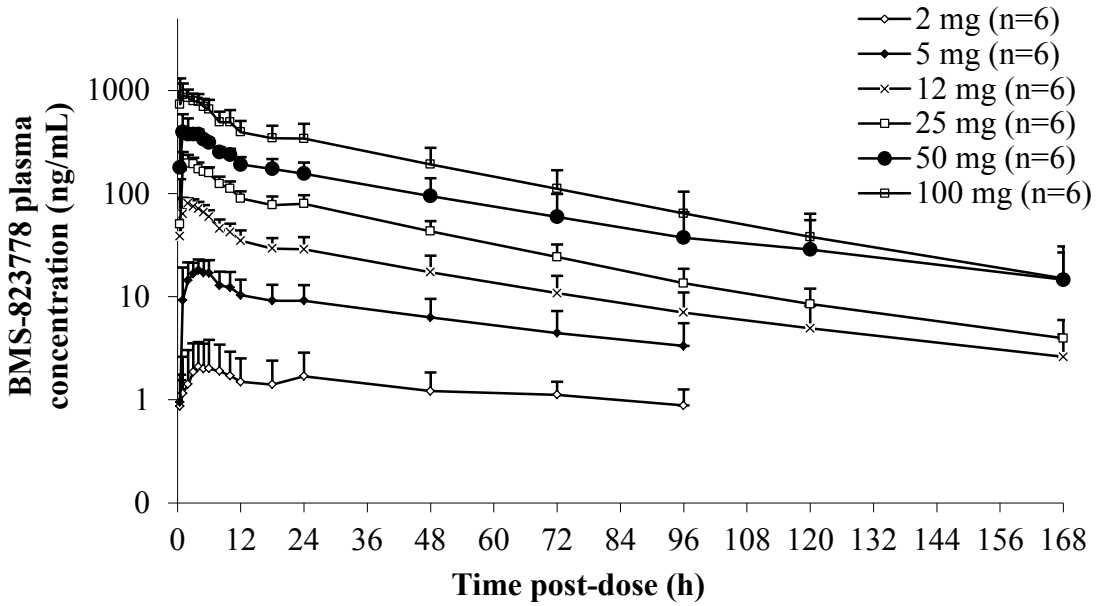

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Figure 2

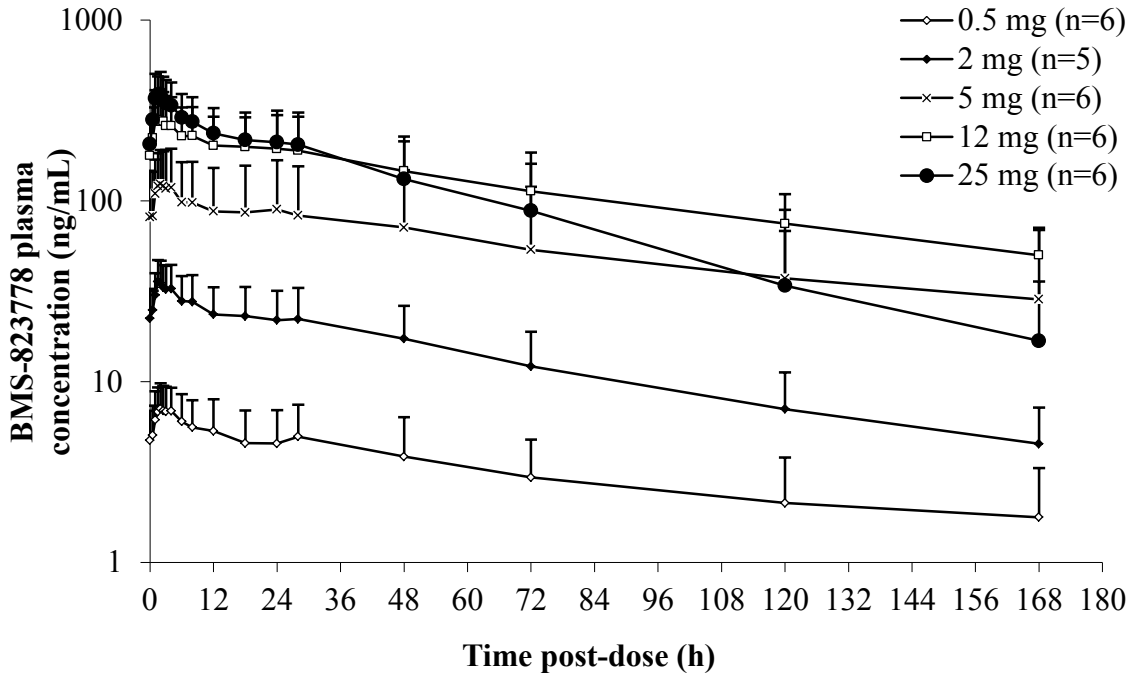

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Figure 3.

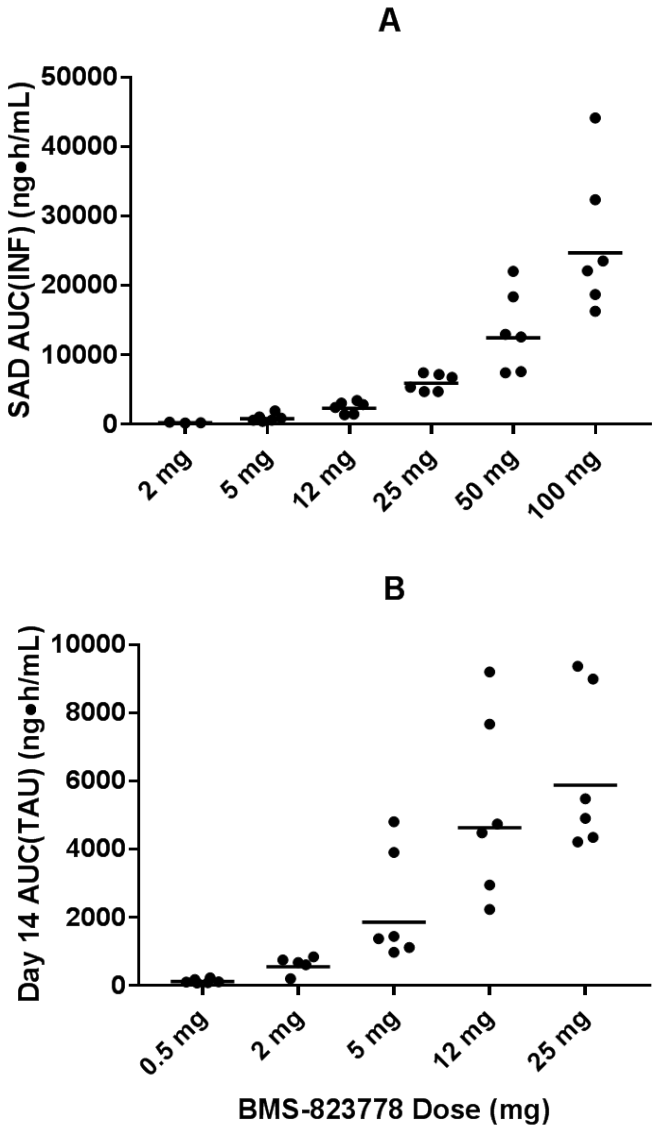

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Figure 4.

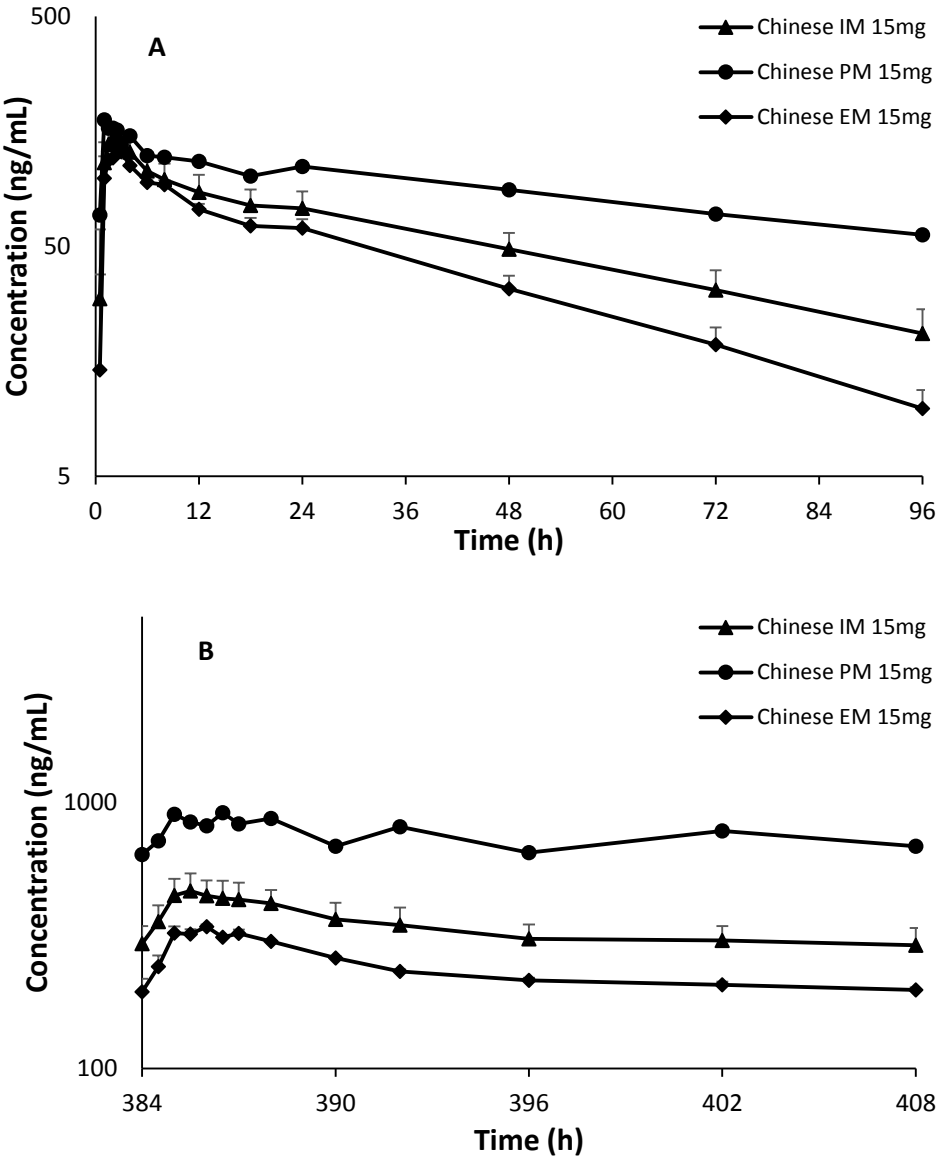

Figure 5

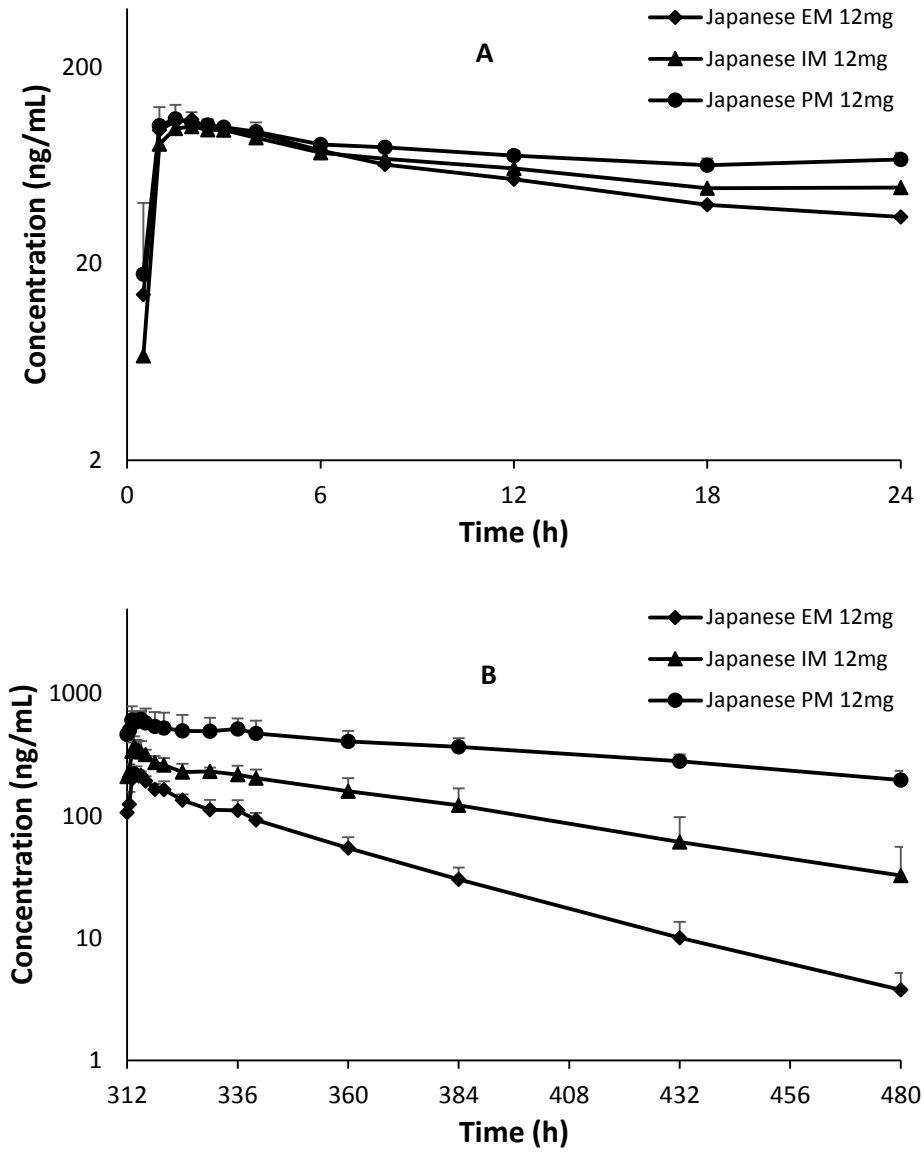

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Figure 6.

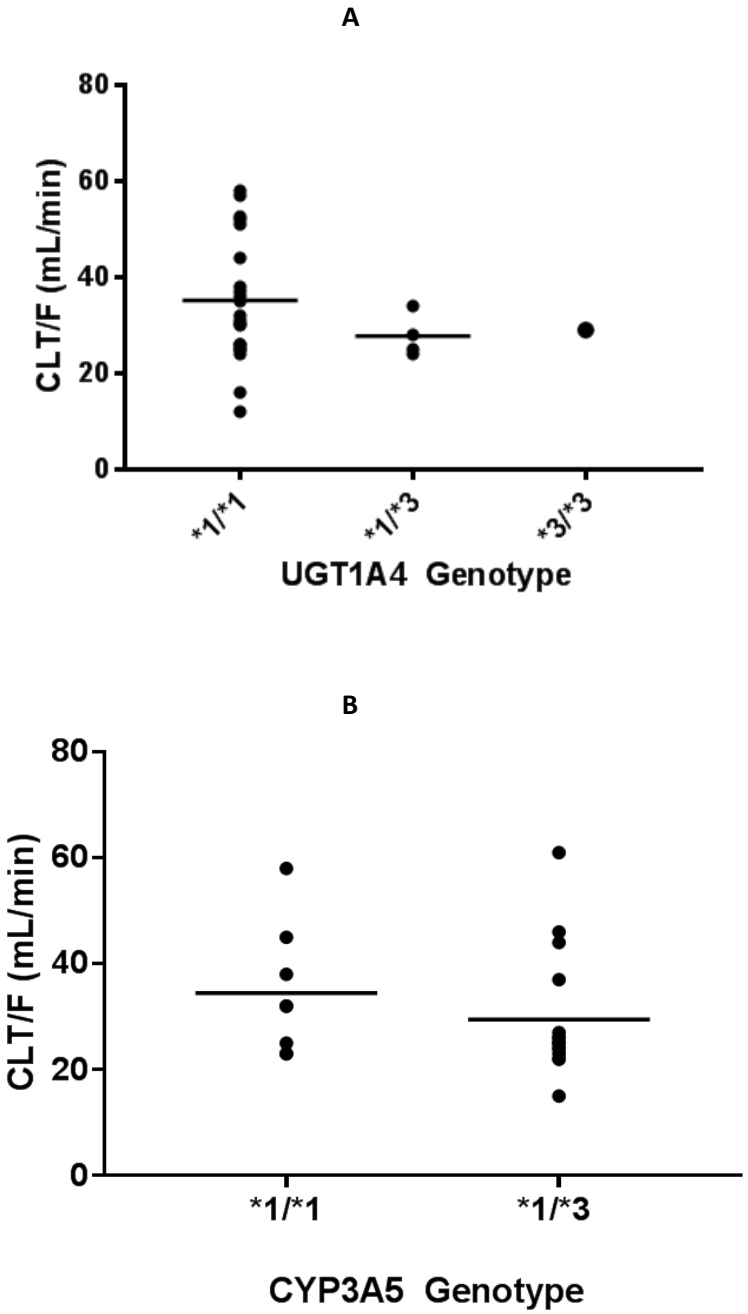

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Figure 7.