Pharmacokinetics of the potent LTB4 Inhibitor, CP-105696, in Male and Female Mice.

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Abstract

Leukotriene B4 (LTB4) is a proinflammatory mediator important in the progression of a number of inflammatory diseases. Preclinical models provide essential information regarding the mechanisms of action of tool compounds, such as CP-105696, that modulate LTB4 activity. This study focuses on understanding the pharmacokinetics of CP-105696 in mice in the context of systemic inflammation induced by a high fat diet (HFD).

Results from a single oral administration of CP-105696 suggest a half-life of 62 hours in mice fed normal chow (NC) versus 44-52 hours for the HFD with a lower apparent volume of distribution in HFD animals (0.51 – 0.66 L/kg) compared to NC fed animals (0.72 L/kg). Interestingly, daily dosing led to an unexpected saturable absorption of the compound, suggesting a threshold above which higher dosing has no beneficial biologic effect.

While CP-105696's long half-life suggests that twice weekly dosing provides sufficient target coverage if the biological effect is related to the average drug concentration, a shorter dosing interval may be a better choice if drug concentrations need to be maintained above a minimal concentration. Given that most chronic inflammatory diseases will require long-term therapies, these studies are useful in determining the optimal dosing schedules for preclinical studies using CP-105696.

Introduction

Modulation of leukotriene B4 (LTB4), an oxidized fatty acid metabolite of arachidonic acid, is of interest in multiple inflammatory disease areas including asthma, arthritis and atherosclerosis (Singh et al., 2013, Di Gennaro and Haeggstrom, 2013). LTB4 recruits proinflammatory immune cells to sites of inflammation and stimulates the production of a variety of proinflammatory cytokines and chemokines. To explore its role in pathophysiology, tool compounds that modulate the binding of LTB4 to its high affinity BLT1 G-protein-coupled receptor can be used (Goodnow et al., 2010). BLT1 is a plausible target for anti-inflammatory therapies as it is expressed in a variety inflammatory and immune cells including granulocytes, eosinophils, macrophages, differentiated T cells, dendritic cells and osteoclasts (Yokomizo, 2011). Several BLT1 antagonists have been developed and used successfully in preclinical models of disease, such as amelubant (Boehringer-Ingelheim) in mouse ear inflammation, transdermal chemotaxis in guinea pigs and neutropenia in various species (Birke et al., 2001) and CP-105696 (Pfizer) in atherosclerosis in mice (Aiello et al., 2002), ischemia and reperfusion injury in rats (Souza et al., 2002) and articular inflammation in mice (Guerrero et al., 2008). Unfortunately clinical trials have failed to replicate the success of preclinical studies, suggesting the need for further examination of these compounds' efficacy using different conditions and model systems in preclinical studies.

This study focuses on the potent, highly selective BLT1 antagonist CP-105696. When using this and other tool compounds, it is important to understand the drug's pharmacokinetic (PK) profile. In particular, we are interested in the effects that a proinflammatory high fat diet (HFD) could

have on the drug's PK profile. Therefore, this work evaluates CP-105696's PK profile in normal chow (NC) and HFD fed male and female C57Bl/6 mice.

To evaluate the PK in mice, single dose and multi-dose studies were undertaken. The data were reviewed using typical PK metrics as well as compartmental modeling methods. The results presented here provide additional information about CP-105696's in vivo pharmacokinetics, which will be helpful in designing future studies.

Materials and Methods

Animals

Seven and 14 week old C57Bl/6 male and female mice were purchased from the Jackson Laboratory. For single dose pharmacokinetics experiments, female mice were ovariectomized (OVX) at 15 weeks of age, and both male and OVX female mice (n=4, each) were fed NC (13.5% kcal from fat; Teklad Diets, WI, USA) for 3 wks. For the multi-dose pharmacokinetic experiments, half of the female mice were OVX'd at 8 weeks of age, and male, intact female and OVX female mice (n=4, each) were fed an HFD (60% kcal from fat; Research Diets Inc, NJ, USA) for 8 wks from 10 wks of age. Animals were housed in a specific pathogen-free facility and given free access to food and water. All procedures were approved by the University of California, San Diego animal care and use committee.

Pharmacokinetic studies

CP-105696 (Pfizer, San Diego, CA) was re-suspended in 0.05% tween 80 and given as an oral suspension by gavage to mice at concentrations of 35, 100 or 130 mg/kg body weight. Blood was

collected via the tail vein into EDTA coated capillary tubes (Drummond Scientific, PA, USA) and spotted onto filter paper. The filter paper was air-dried at room temperature overnight and the dried blood spots (DBS) stored with dessicant until assayed. Body weights were measured weekly.

To characterize the oral pharmacokinetics of CP-105696 in mice, animals were given a single oral dose of 35 mg/kg or 130 mg/kg CP-105696 and blood was sampled as DBS at 0.5, 1, 2, 6, 24, 72 and 96 hours post-dose in each animal. In the female high dose group, in addition to the DBS, plasma was collected at 2 hours post-dose so that the DBS samples could be converted to plasma concentrations. This study was carried out for NC-fed male and female mice.

For multi-dose pharmacokinetic studies, male, intact female and OVX female mice on an HFD were given oral doses of 130 mg/kg CP-105696 on alternating 3rd and 4th days following the initial dose for 3 weeks (dose times: 0, 72, 168, 240, 336 and 408 hours). Blood was sampled at 6, 24, 72, 74, 78, 168, 174, 240, 246, 336, 342, 408, 414 and 504 hours following the start of the study. In addition to the sparse dosing protocol, a once-daily dosing paradigm was also investigated in OVX female mice at a dose of 100 mg/kg CP-105696. PK samples were collected at 3, 7, 24 hours after the doses given on days 0, 6, 11 and 13.

Analytical Methods

CP-105696 was prepared as a 0.1 mg/mL stock solution in 50% acetonitrile/50% DMSO. CP-105696 standard at 10,000 ng/mL was prepared in liquid whole blood by addition of a 0.05 mL aliquot of 0.1 mg/mL CP-105696 into a tube containing 0.45 mL of mouse blood. Subsequent CP-105696 standards in mouse blood at 5000, 2500, 1000, 500, 250, 100 and 50 ng/mL were

prepared through serial dilution. Quality control (QC) samples were prepared at 200, 2000, 4000 and 8000 ng/mL concentrations in mouse blood. Twenty µL aliquots of standards, QC samples and blanks were spotted onto DMPK-C cards (GE Whatman, PA, USA) and allowed to air-dry overnight at room temperature. Three mm punches from standards, blanks, QC samples and study samples were placed into a 96 well plate. One hundred µL aliquot of indomethacin (internal standard (IS) at 150 ng/mL) in 60% methanol/40% water was added to each well. The plate was then vortex mixed and centrifuged for 15 min. Fifty µL aliquot of the supernatant was transferred into a second 96 well plate and diluted with 150 µL of water. A 10 µL aliquot was injected into the LC-MS/MS system.

The LC-MS/MS system consisted of a CTC analytics HTS PAL autosampler (LEAP Technologies; NC, USA), a Shimadzu LC-20AD HPLC system and an API 5500 triple stage quadrupole mass spectrometer (Applied Biosystems, NY, USA). Mobile phase A consisted of water containing 0.1% formic acid, and mobile phase B consisted of acetonitrile containing 0.1% formic acid. Both CP-105696 and IS were separated using a Waters HSS T3; 50 x 2.1 mm id , 2.5 µm column (Waters Corp., MA, USA) at a flow rate of 0.5 ml/min. A gradient elution program was utilized with the initial solvent composition held at 10% mobile phase B for 0.25 min and then linearly increased to 90% mobile phase B over 1.35 min and held at 90% mobile phase B for an additional 0.4 min. The column was then re-equilibrated at 10% mobile phase B for 0.5 min. The total run time was 2.5 min.

The MS was operated in negative ionization mode using multiple reaction monitoring (MRM). The turbo ion voltage was set to -4.5 kV and the auxiliary gas temperature maintained at 500^{0}C. High purity nitrogen was used for GS1 and GS2, curtain and collision gases. The mass resolution was set to a peak width of unit mass at half height for both Q1 and Q3. The electron multiplier

was set at - 2200 V. Declustering potential, collision energy, entrance potential and collision cell exit potential conditions were optimized for both CP-105696 and IS. The dwell time for each MRM transition was 45 ms. CP-105696 and indomethacin as IS were monitored using specific precursor ion -> product ion transitions of m/z 427.1>365.4 and 356>312, respectively.

Analyst software (AB sciex, CA, USA), version 1.5.2 was used for data acquisition and chromatographic peak integration. The peak area ratios of CP-105696 and IS were plotted as a function of the nominal concentrations of the analytes. The standard calibration curve was constructed using a weighted (1/x) linear regression. PK parameters were calculated using non-compartmental analyses and Watson[TM] Bioanalytical LIMS software version 7.2.0.03 (Thermo Fisher Scientific; MA, USA).

Pharmacokinetic Analyses

Mathematical modeling was performed using SAAM II software (The Epsilon Group, Charlottesville, VA, USA). In all cases, a naïve-pooled approach was taken and an additive error model was used. The model implemented is described in detail below.

Compartmental Model

$$\frac{dA_{Depot}(t)}{dt} = - K_a \cdot A_{Depot}(t)$$

$$\frac{dA_{central}(t)}{dt} = K_a \cdot A_{Depot}(t) - k_{el} \cdot A_{central}(t)$$

$$C_{central}(t) = \frac{A_{central}(t)}{Vol}$$

$$Plasma\ Concentration(t)\ = C_{central}(t) + error(t)$$

Initial conditions:

$$A_{Depot}(0) = Dose$$

$$A_{central}(0) = 0$$

K_a = absorption rate constant; K_{el} = elimination rate constant; A_{Depot} = Amount of drug in the absorption compartment; $A_{central}$ = Amount of drug in the central compartment; $C_{central}$ = concentration of drug in the central compartment. The measurement and biological variability was modeled using an additive error with mean zero and variance estimated in the model fitting process.

Results

The LC-MS/MS method developed for analyses of CP-105696 as DBS demonstrated a linear dynamic range between 50 ng/mL to 10,000 ng/mL. The lower limit of quantitation (LLOQ) of the assay was 0.5 ng/mL, however, due to the high concentration of CP-105696 in blood from these studies the curve range was set to a range of between 50 ng/mL – 10,000 ng/mL. The accuracy of calibration standards was within +/-20% of theoretical. The accuracy and precision (%CV) of QC samples evaluated at 4 concentrations, namely, 200, 2000, 4000 and 8000 ng/mL were within +/-15%. Over the curve DBS study samples were re-analyzed by dilution with diluent obtained from extracts of DBS containing blank mouse blood containing IS, a standard accepted practice by practioners of DBS analyses (Rahavendran et al., 2012)

The pharmacokinetic parameters for CP-105696 have been previously defined in other species, including humans (Liston et al., 1998). Using this information and back-extrapolating to mice, the terminal half-life in mice was estimated to be around 50 hours. With this knowledge, CP-105696's plasma concentration was collected at several time points over 4 days following a single oral administration of the drug at two dose levels, 35 and 130 mg/kg. To allow for serial sampling from a single animal, the DBS technology was used. This sampling technique provided blood concentrations, which were converted to plasma concentrations using the blood to plasma ratio. This ratio was estimated to be 0.5, indicating that plasma had approximately twice the amount of drug than blood. This is not surprising given that CP-105696 is highly bound to plasma proteins (Showell et al., 1996, Liston et al., 1998). All figures and calculations included here describe CP-105696's plasma concentrations. CP-105696's plasma PK profile is shown in **Figure 1** and summary metrics are reported in **Table 1**. To assess the linearity of the compound, the area under the curve (AUC) value was divided by the dose. This value was equivalent between doses in the female group (82 vs 84 for the 35 and 130 mg/kg dose groups, respectively), suggesting that the compound's plasma exposure increases linearly with dose. The male data were not equivalent between doses (50 vs 68 for the 35 and 130 mg/kg dose groups, respectively), suggesting that the compound's exposure is greater at the higher dose.

To explore this observation further and to extrapolate to different dosing paradigms, the plasma concentration data were characterized using compartmental modeling methods. A one-compartment model with oral absorption best characterized the data presented in **Figure 1**. This model was able to capture a single value for the absorption and elimination rate constants across all dose groups. Both female groups and the high dose male group were adequately described by a single volume parameter as well. However, the low dose male group's volume parameter was

estimated separately. **Figure 1** shows that a reasonable model fit to the data was achieved in all dose groups, although the model fit to the high dose male group over-estimated the plasma concentrations at the 72 and 96 hour time points compared to the fit for the low dose male group. **Table 2** summarizes the model parameters. From the model parameters derived from the single dose study, CP-105696's elimination half-life is estimated to be 62 hours and the compound has an apparent volume of distribution between 0.7 and 1.2 L/kg these data are from single dose.

Building from the information gathered in the single dose study, a multi-dose study was performed to further understand the compound's behavior when dosed repeatedly. CP-105696's long half-life makes it possible to extend the time interval between dosing events. To explore this, a twice-weekly dosing paradigm was investigated at 130 mg/kg in male, female and female OVX mice on an HFD. This was accomplished by dosing on alternating 3rd and 4th days from the previous dose (q3dq4d) for 3 weeks (a total of 6 doses). Sparse plasma concentrations were collected over the course of the study to capture the peak and trough concentrations following each dose. The maximal and minimal plasma concentration values are shown in **Figure 2A-C**. In general, the plasma concentrations were consistent between groups. A slight deviation was observed in the Cmax values of the male animals, which appear lower than those observed in the female animals, especially after the later doses. The trough (minimal) values appear consistent between all groups. Under this dosing regimen, the data illustrate that plasma concentrations will drop 2 – 3 fold from the maximal concentration before the next dose is given. Body weights remained stable in males and OVX females, however intact females initially lost body weight with CP-105696 treatment and then continued to gain weight, though maintaining a slightly lower body weight than control vehicle gavaged mice (**Figure 2D**).

The model developed to fit the single dose data does well in describing the female groups from the multi-dose study, but over-predicts the concentrations observed in males (Fig. 1). To achieve the best description of the data, the model was refit to the individual dose groups, using a Bayesian prior on the absorption parameter. The model parameters changed slightly (**Table 2**), resulting in a decrease in the apparent volume of distribution and a slight increase in the elimination rate constant, which may be reflective of differences between NC and HFD animals. However, in all cases, the 95% confidence intervals around the point estimates of the apparent volume and the elimination rate constant overlapped, suggesting that the values are not statistically different from one another.

In addition to the q3dq4d dosing schedule, a once-daily (qd) protocol was also investigated whereby a single group of OVX HFD female mice were given daily oral doses of 100 mg/kg CP-105696. Given the long half-life and frequent dosing regimen, the plasma concentrations were expected to increase significantly over the course of the study, however this did not occur. Comparing the simulated profile versus the measured data (**Figure 3A**), illustrates that reduced levels of plasma concentrations were obtained in the study, perhaps due to limitations in some aspect of absorption. Given this observation, we hypothesized that the same plasma concentrations could be achieved with a lower dose. Since the animals were still receiving drug, additional PK samples were collected after an additional 7 days of dosing at 100 mg/kg daily and then the dose was reduced to 40 mg/kg and additional PK samples were collected after the dose reduction. **Figure 3B** shows that reducing the dose from 100 mg/kg to 40 mg/kg did not limit the plasma concentrations that were achieved with this compound. Additionally, it appears that the 40 mg/kg qd dose was better tolerated than the 100 mg/kg qd dose, which resulted in a loss of body weight in OVX female mice (**Figure 3C**).

Discussion

Given the interest in LTB4-mediated biology, inhibitors such as CP-105696 are being used as tools to explore the mechanism's involvement in a variety of diseases (Di Gennaro and Haeggstrom, 2013, Singh et al., 2013). When using these inhibitors, it is important to understand their pharmacokinetic behavior to ensure that appropriate target exposures are achieved in vivo. This work describes the pharmacokinetics of CP-105696 in NC fed male and OVX female mice as well as male, intact female and OVX female given an HFD. All data presented here are total plasma concentrations. CP-105696 is known to be highly bound to plasma proteins (> 99% bound) (Showell et al., 1996, Liston et al., 1998) therefore, while high total concentrations are achieved, only a fraction is free and able to interact with its target (Smith et al., 2010).

In our study, a one-compartment model with oral absorption best described the data. This model structure has two solutions: one where the elimination rate is slow and the absorption rate is fast and another where the elimination rate is fast and the absorption rate is slow. Previous evidence collected in mice following an intravenous injection of CP-105696 suggests that a long half-life (on the order of hours) appropriately describes the pharmacokinetics of this compound (Griffiths et al., 1995). This supportive evidence lends credibility to the model parameters identified in the current study.

CP-105696 has a long half-life in mice (62 hours), which may be slightly reduced in animals on an HFD (44 – 52 hours). The apparent volume of distribution was also found to be slightly lower in HFD animals (0.51 – 0.66 L/kg) compared to NC animals (0.72 L/kg). As stated earlier, these differences are not significant when considering the uncertainty in the parameter estimates. In general, the data obtained in females was consistent between doses (single dose data) and

between intact and OVX females on the HFD (multi-dose data). The concentrations observed in the male high dose (130 mg/kg) group of the single dose study were consistent with the female data; however the low dose (35 mg/kg) male group resulted in concentrations that were lower than would have been expected. Additional studies should be conducted to better understand this observation.

The plasma concentrations obtained following once-daily (qd) dosing of 100 mg/kg CP-105696 were unexpected given our previous knowledge of the compound. It was hypothesized that a limitation in absorption was occurring following the frequent dosing. No clear evidence of saturable absorption was observed in the single dose study or with the infrequent dosing schedule (q3dq4d), suggesting that the frequency of repeat dosing is a factor in the limited absorption. If the frequent dosing at 100 mg/kg CP105696 was saturating an absorption process, then it is possible that a lower dose could provide the same plasma exposures since a lower dose may not be saturating the mechanisms involved. To explore this hypothesis, animals receiving 100 mg/kg qd doses to steady state concentrations were transitioned to doses of 40 mg/kg qd. The results supported the hypothesis and showed that plasma concentrations were maintained at the same level after the dose reduction (**Figure 3B**). Currently, the processes behind the limited absorption of CP-105696 are undefined and additional work would be needed to elucidate the mechanism.

CP-105696's long half-life allows for a more flexible dosing paradigm in pre-clinical studies, potentially one where the drug is dosed on every 3rd and 4th day per week (q3dq4d), which would be particularly helpful in long term studies. The infrequent dosing schedule will result in larger differences (2 – 3 fold) between the maximal and minimal concentration ranges following multiple doses. These differences will not be so great with more frequent dosing, such as once-daily or every other day. We did observe that the less frequent dosing (q3dq4d) at 130 mg/kg

was better tolerated than the 100 mg/kg qd dosing regimen according to body weight changes, whereby the 100 mg/kg QD was sufficient to result in a significant loss of body weight in OVX females. Whether this effect was related to the absorption issues with the 100 mg/kg qd schedule or the greater sustained plasma concentrations remains to be investigated. Further studies will also be necessary to determine whether the 40 mg/kg qd schedule, which gave the same plasma concentrations as the 100 mg/kg, would reduce the body weight loss.

When selecting doses for efficacy studies, in vitro knowledge will be used as a starting point. Typically, drug concentrations providing half-maximal biomarker modulation (e.g. EC50 values) are generated and then doses are selected that provide plasma or site-of-action concentrations at or above these levels. A potential in vitro biomarker for CP-105696 is the CD11b inhibition response observed in isolated human neutrophils (Showell et al., 1995). The EC50 value for this biomarker was determined to be 430 nM. **Figure 4** shows the minimal unbound plasma concentrations following doses of the 100 mg/kg qd and the 130 mg/kg q3dq4d dosing regimen studies compared to the EC50 value. The figure illustrates that while both regimens provide unbound plasma concentrations above the EC50 value, the qd regimen will cover 5 times the EC50 value (~ EC80), while the q3dq4d schedule may drop below levels that are 3 times the EC50 value (EC75). While the minimal plasma concentrations differ, the two dosing regimens provide similar average concentrations (qd: 2200 nM; q3dq4d: 2000 nM). If the pharmacological response is expected to be related to the average plasma concentration, then either dosing regimen should be effective. However, if coverage of the target (e.g. time over the EC80 value) is the driving factor, then the two dosing regimens investigated here may provide different responses. This will be especially true if significant coverage of the target is required,

which would support a more frequent dosing regimen that avoids the large drop in plasma concentrations observed with less frequent dosing regimens.

Conclusions

Given the potential for CP-105696's use as a tool compound to explore LTB4 biology in vivo, knowledge of its pharmacokinetic behavior in male and female mice on NC and HFD is a critical factor. Of particular importance is the limited absorption observed at frequent high doses and the tolerability of these doses in mice. The details provided in this paper give researchers a starting point to explore new dosing regimens in silico that can then be verified in vivo and used in the design and analysis of future efficacy studies.

Authorship Contribution

Participated in research design: Spilker, Visswanathan, Bagrodia, Fantin, and Ellies

Conducted experiments: Chung and Ellies

Contributed new reagents or analytic tools: Visswanathan and Gernhardt

Performed data analysis: Spilker, Chung, Visswanathan and Ellies

Wrote or contributed to the writing of the manuscript: Spilker, Chung, Visswanathan and Ellies

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Table 1. Summary PK metrics

Dose	Gender	Cmax (ug/ml)	Tmax (h)	AUC (ug*h/ml)
35 mg/kg	Male	58 (15)	7.9 (5.5)	1762 (148)
	Female	39.8 (6.3)	11 (4.5)	2860 (480)
130 mg/kg	Male	154.8 (11.7)	7.1 (4.7)	8780 (440)
	Female	218 (4.7)	3.8 (5.5)	10860 (603)

Cmax = maximal plasma concentration; Tmax = time at maximal plasma concentration; AUC = area under the time-concentration curve. Values in parentheses are SEM.

Table 2. Parameters from model fit to single dose and multi-dose PK data.

Parameter	Single Dose	Multi-Dose			Units
		Intact HFD Female	**OVX HFD Female**	**HFD Male**	
Vol (female)	0.72 (3.6)	0.51 (4.7)	0.58 (5.2)	n/a	L/kg
Vol (male)	0.72 (3.6) - 1.22 (4.4)*	n/a	n/a	0.66 (7.2)	L/kg
k_a	0.82 (8.9)	0.82 (8.8)	0.83 (8.7)	0.86 (8.6)	hr-1
k_{el}	0.011 (8.1)	0.015 (7.6)	0.013 (8.0)	0.016 (11.8)	hr-1
$T_{1/2}$	61.7	45.5	51.8	44.2	hr

Vol = apparent volume of distribution; k_a = absorption rate constant; k_{el} = elimination rate constant; $T_{1/2}$ = elimination half-life. Percent Coefficient of Variation is reported in parentheses.

*Vol from low dose male group was fit separately from other groups and resulted in a range of volumes for the male groups from the single dose study.

Figure Captions

Figure 1. Compartmental model fit to individual data following a single dose of CP105696

Compartment model fit (lines) to individual plasma concentrations following a single oral dose of CP-105696 to NC-fed C57Bl6 mice at a dose of 35 mg/kg in males (A), or females (B) or 130 mg/kg in males (C) or females (D).

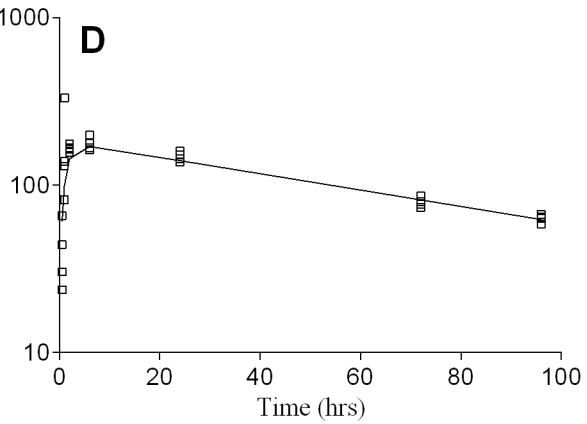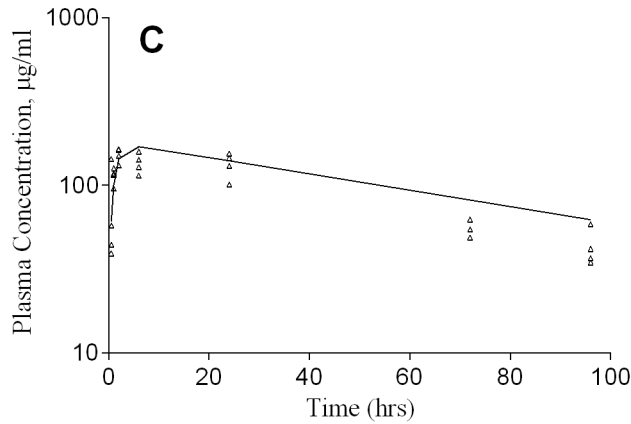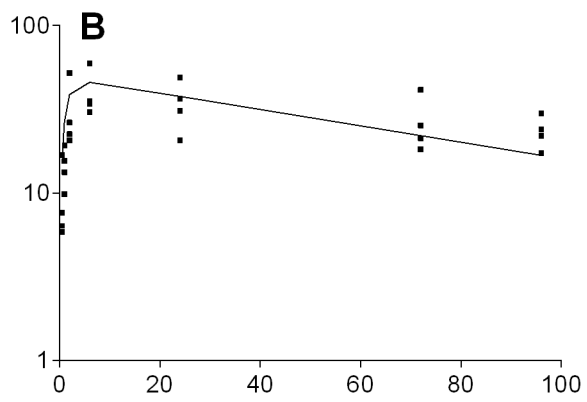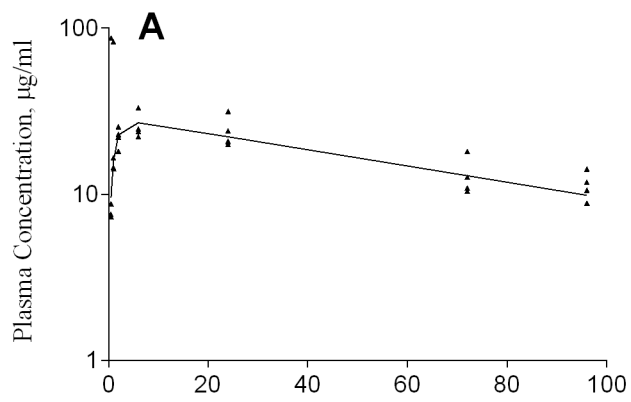**Figure 2. Plasma concentrations and body weights following 130 mg/kg q3dq4d multiple dosing of CP-105696**

A - C. Plasma concentrations following multiple oral doses of 130 mg/kg CP-105696 in HFD animals. Doses were given at 0, 72, 168, 240, 336, and 408 hours. Simulated PK profiles using model parameters derived from single dose data (dotted line); predicted concentrations from model refit to multi-dose data (solid line). (A) Intact HFD Females; (B) OVX HFD Females; (C) HFD Males. D. Body weight measurements indicated that intact females were the only group to lose weight with twice weekly treatment. The vertical dashed lines represent the dosing and PK samples shown in panels A-C. Dosing was continued to 22 weeks. Intact Females (Treated: ●; Vehicle: ○); Ovx Females (Treated: ▲; Vehicle: △); Males (Treated: ■ ; Vehicle: □). Data are presented as means ± SEM. * $p<0.05$, ** $p<0.01$.
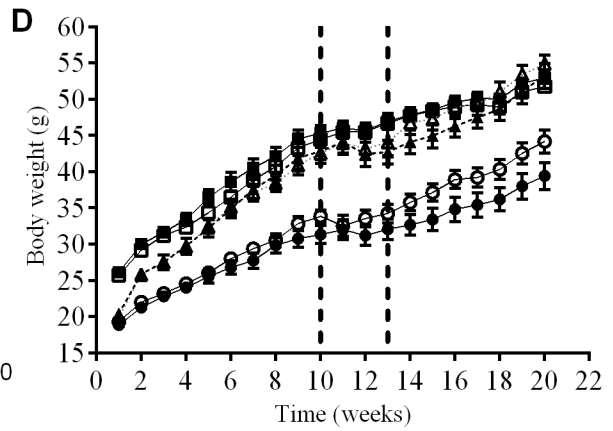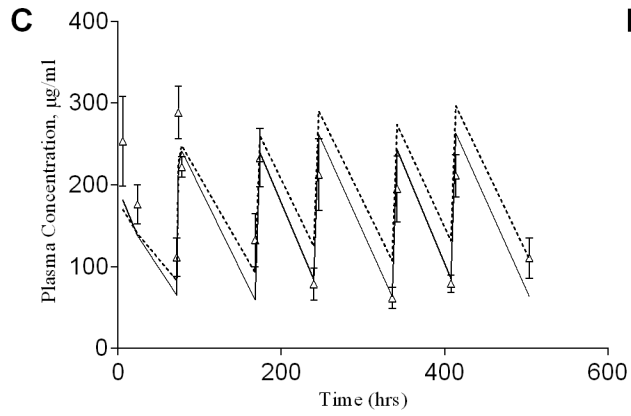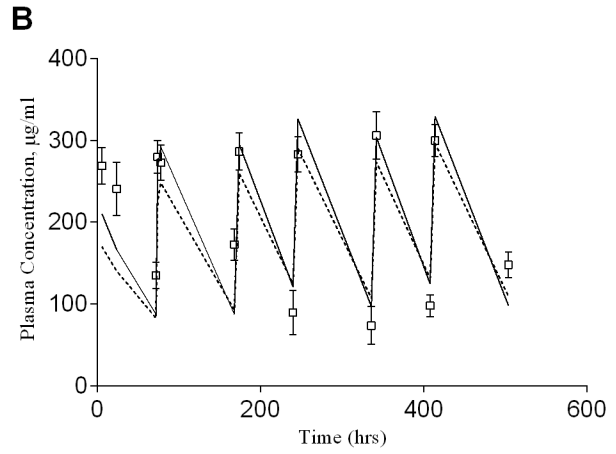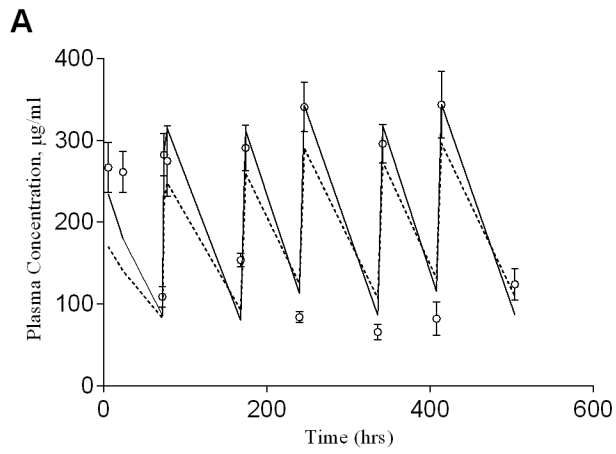
Figure 3. Plasma concentrations and body weight changes following 100 mg/kg -qd dosing and a dose reduction to 40 mg/kg.

A. Measured and simulated plasma concentrations following once-daily 100 mg/kg oral dosing of CP-105696 and a subsequent dose reduction to once-daily 40 mg/kg oral dosing of CP-105696. **B**. Body weights of OVX females fed an HFD and dosed with either 100 mg/kg or 40 mg/kg daily. The entire body weight profile is included in the insert. Data are means ± SEM * $p < 0.05$.
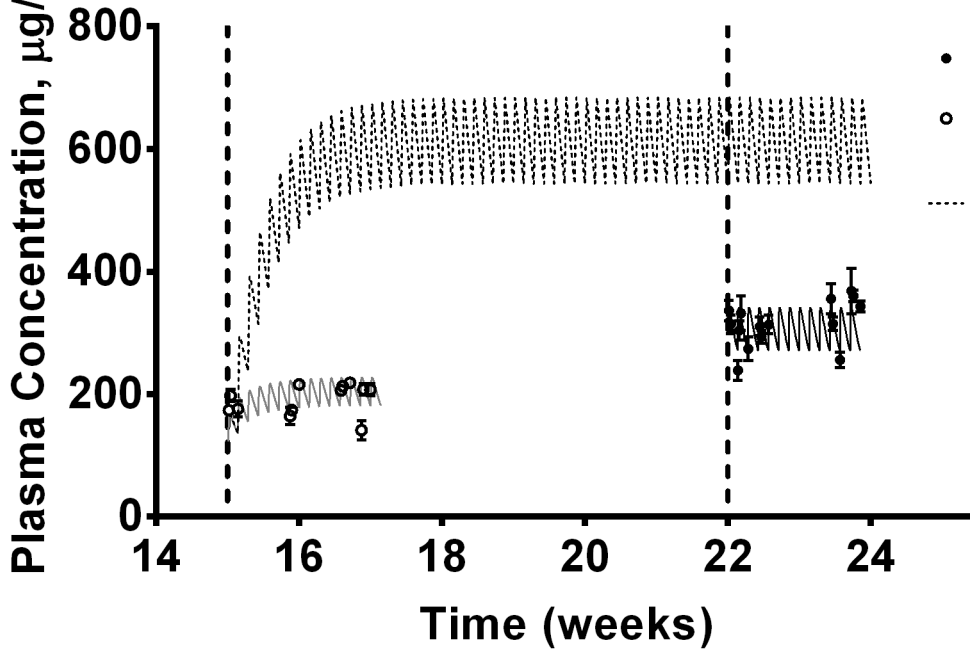
Figure 4. Comparison of minimal unbound plasma concentrations and fold over EC$_{50}$ values.

Comparison of minimal (trough) unbound plasma concentration levels and fold over the in vitro EC$_{50}$ value for inhibition of CD11b in human neutrophils. Once-daily dosing at 100 mg/kg will maintain concentrations at or above 5x the EC$_{50}$ value (~ EC$_{80}$). 130 mg/kg q3dq4d dosing results in trough values that are above the EC$_{50}$ value, but potentially below 3x the EC$_{50}$ value (EC$_{75}$).

Plasma Concentrations

- • 40 mg/kg, QD
- ∘ 100 mg/kg, QD
- ⋯ 100 mg/kg, QD (Sim)

Plasma Concentration, µg/ml

Time (weeks)

Body Weight

- △ BLT1 In
- ■ Vehicle

Body weight (g)

Time (weeks)

100 mg/kg   40 mg/kg