Pre-clinical and clinical pharmacokinetics of PF-02413873, a non-steroidal progesterone receptor antagonist

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List of nonstandard abbreviations used in the paper:

ACAT, Advanced Compartmental Absorption Transit
CL\textsubscript{int}, intrinsic clearance
CL\textsubscript{p}, plasma clearance
CL\textsubscript{u}, unbound clearance
CL/F, oral clearance
CYP, cytochrome P450
DLM, dog liver microsomes
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FIH, first-in-human
FaSSIF, fasted state simulated intestinal fluid
FeSSIF, fed state simulated intestinal fluid
Fu, unbound fraction
HLM, human liver microsomes
Kp, tissue to plasma partition coefficient
LC, liquid chromatography
MS, mass spectrometry
PBPK, physiologically based pharmacokinetic
PK, pharmacokinetics
PR, progesterone receptor
RLM, rat liver microsomes
SSS, single species scaling
TBME, tert-butylmethylester
Abstract

The recently discovered selective non-steroidal progesterone receptor (PR) antagonist, PF-02413873 was characterized in metabolism studies in vitro, in pre-clinical pharmacokinetics in rat and dog and in an initial pharmacokinetic study in human volunteers. Clearance (CL) of PF-02413873 was found to be high in rat (84 ml/min/kg) and low in dog (3.8 ml/min/kg), consistent with metabolic stability determined in liver microsomes and hepatocytes in these species. In human, CL was low in relation to hepatic blood flow, consistent with metabolic stability in human in vitro systems, where identified metabolites suggested predominant CYP-catalysed oxidative metabolism. Prediction of CL using intrinsic CL determined in human liver microsomes (HLM), recombinant human CYP enzymes and single species scaling (SSS) from pharmacokinetic studies showed that dog SSS and HLM scaling provided the closest estimates of CL of PF-02413873 in human. These CL estimates were combined with a physiologically-based pharmacokinetic (PBPK) model to predict pharmacokinetic profiles following oral suspension administration of PF-02413873 in fasted and fed states in human. Predicted plasma concentration vs. time profiles were found to be similar to those observed in human over the PF-02413873 dose range 50 - 500mg and captured the enhanced exposure in fed subjects. This case study of a novel non-steroidal PR antagonist underlines the utility of PBPK modelling techniques in guiding prediction confidence and design of early clinical trials of novel chemical agents.
Introduction

Endometriosis and uterine fibroids are common gynaecological conditions affecting approximately 20-40% of women and characterized by chronic pelvic pain, cyclical menstrual pain, excessive menstrual blood loss and infertility. The most widely prescribed medical therapies indicated for the treatment of endometriosis are GnRH analogues and the progestin medroxyprogesterone acetate (Olive, 2003). GnRH analogues and progestins are only indicated for short term use (6 months) because they result in bone mineral density loss as well as other side effects secondary to hypoestrogenism. A body of pre-clinical and clinical data suggests the potential utility of selective progesterone receptor (PR) antagonists, acting as functional estrogen antagonists, in the treatment of endometriosis and uterine fibroids (Grow et al., 1996; Kettel et al, 1996; 1998; Fiscella et al., 2006; Baird et al., 2003). A selective progesterone antagonist would be expected to maintain estrogen at mid follicular levels hence avoiding the systemic effects of hypoestrogenism.

A selective non-steroidal PR antagonist, PF-02413873, was recently identified (Gibson et al., 2009). Pre-clinical pharmacological and toxicological characterization of PF-02413873 suggested that systemic exposure levels in human that are likely to be required to elicit the desired functional estrogen antagonism could do so with an acceptable safety margin. Therefore, its preclinical PK properties were investigated in order to assess the likelihood that its PK in human would support its progression as a potential therapeutic agent. The accurate prediction of PK in human can be a significant challenge to drug discovery scientists, and a number of approaches have been employed with varying degrees of success (Beaumont and Smith, 2009). PK
prediction methods have been described and compared in several retrospective analyses of groups of compounds (Obach et al., 1997; Ito and Houston, 2005; Jones et al., 2006a; Hosea et al., 2009) but there have been few examples reported that show a PK prediction strategy used in practice prior to initial first-in-human (FIH) clinical studies (Allan et al., 2008; Yamazaki et al., 2011). The prospective approach used in the present paper for PF-02413873 was to integrate the knowledge of its in vitro properties, including metabolic turnover and routes, with preclinical PK to determine likelihood of appropriate PK in human, by applying PK prediction methods and taking into account the weight of evidence presented in the literature. PK predictions often focus on parameters such as clearance (CL) and half-life ($t_{1/2}$) as these are determinants of dose and duration of action. However, it can also be of value to predict PK concentration vs. time profiles (Allan et al., 2008) and the potential for food intake to influence absorption, particularly where drug candidates possess high lipophilicity and consequent low solubility. The latter point is especially relevant to PF-02413873 as its neutral and lipophilic nature was a requirement for its PR binding affinity.

This paper describes the pre-clinical data obtained in in vitro and in vivo systems, the human PK prediction methods used, and the human PK of PF-02413873 subsequently determined in a FIH clinical study. Based on preclinical data, simulations of PK profiles were carried out using physiologically-based pharmacokinetic (PBPK) modelling as implemented in GastroPlus™, enabling the potential for food effects following oral dosing to also be examined. This is the first reported study of human PK of a non-steroidal PR antagonist. It additionally serves as a case example of the assessment of PK prediction, whereby the accuracy of PK prediction methods used for
PF-02413873 may be viewed in the context of retrospective assessment of published PK prediction methods over ranges of compounds.


Materials and methods

Dulbecco’s phosphate-buffered saline without CaCl₂ and MgCl₂ (PBS) was purchased from Sigma (D8537). Spectra/Por dialysis membranes (120 x 22 mm, molecular weight cut-off 12-14kDa) were purchased from Spectrum Laboratories Inc. MF3 (2mM ammonium acetate in 10% methanol, 90% water containing 0.027% formic acid) and MF4 (2mM ammonium acetate in 90% methanol, 10% water containing 0.027% formic acid) were purchased from Romil. PF-02413873 (4-[3-cyclopropyl-1-(methylsulfonylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy-2,6-dimethylbenzonitrile) was synthesised in the Chemistry Department, Sandwich Laboratories, Pfizer Global Research & Development (PGRD), and was greater than 95% pure. PF-02339955 (4-[3,5-dicyclopopyl-1-(methylsulfonylmethyl)-1H-pyrazol-4-yl]oxy-2,6-dimethylbenzonitrile) and PF-02327888 (4-[3,5-dicyclopopyl-1-(N-methylacetamide)-1H-pyrazol-4-yl]oxy-2,6-dimethylbenzonitrile) were analogues of PF-02143873 that were used as internal standards in bioanalytical assays and were also synthesised at PGRD.

logD₇.₄

logD₇.₄ was measured by a fully automated shake flask method, performed on a Hamilton Microlab STAR, that determined the partition of PF-02413873 between octanol and phosphate buffer, pH 7.4 (Stopher and McClean 1990).

Caco-2 transcellular flux
The transcellular flux of PF-02413873 across Caco-2 cell monolayers was determined in both apical to basolateral (A to B) and basolateral to apical (B to A) directions using an established method (Walker et al 2005).

**Solubility in simulated intestinal fluids**

Simulated intestinal fluids representing the fasted (FaSSIF) and fed (FeSSIF) states were prepared according to published recipes (Klein et al., 2005). FaSSIF medium was supplemented with 3 mM sodium taurocholate and 1mM lecithin, pH 6.5 and FeSSIF was supplemented with 15 mM sodium taurocholate and 9 mM lecithin, pH 6.5. To assess solubility, an excess of PF-02413873 was shaken in medium for 24 h at 37 °C, and undissolved material separated by centrifugation at 1300 rpm for 5 min. Medium concentration of PF-02413873 was determined by an HPLC method.

**Plasma protein binding**

Plasma protein binding of PF-02413873 was determined at two concentrations of PF-02413873 in rat (1.5 and 15 μg/ml), dog (1.0 and 10 μg/ml) and human (0.1 and 1.0 μg/ml) by equilibrium dialysis. In each species, pooled plasma was obtained from at least 5 donor subjects with plasma from males and females pooled separately. Equilibrium dialysis was performed using a 96-well device in which dialysis membranes (12-14 kDa cut-off) were incorporated that had been soaked in PBS for 1 h before use. Plasma samples (150 μl) were dialysed against an equal volume of PBS for 4 h at 37 °C in an atmosphere of 5% CO₂ in the device that had been sealed with a breathable membrane. At the end of the incubation, samples of plasma and buffer were removed and stored at -80 °C until analysis. Samples were matrix matched with blank reagents prior to analysis (i.e. 25 μl of blank PBS was added to 25 μl plasma samples...
and vice versa). Protein precipitation was performed by addition of 200 μl methanol containing 100 ng/ml internal standard (PF-02339955) followed by centrifugation for 15 min at 2,000 g at 6 °C. Samples of 150 μl of supernatant were dried under a stream of nitrogen at 40 °C and the residues reconstituted with 150 μl water:methanol (1:1, v/v). Following vortex mixing, samples were analysed on a SCIEX API 4000 LC-MS/MS turbo ion spray system operating in positive ion mode under multiple reaction monitoring. The elution mobile phase was 45% 10 mM ammonium formate, pH 3.5, 55% acetonitrile delivered at 1 ml/min through a Zorbax SB C8 3.5 μm 75 x 4.6 mm column. A collision energy of 25 eV was used and transitions of 360.28 to 280.20 and 386.16 to 306.16 were monitored for PF-02413873 and internal standard, respectively. The unbound fraction (Fu) of PF-02413873 was calculated by dividing the concentration in buffer by the concentration in plasma.

Blood partitioning

The blood:plasma ratio of PF-02413873 was determined following incubation of PF-02413873 (1 μg/ml) in rat, dog and human blood for 3 h. After incubation, 50 μl aliquots were removed and the remaining blood was centrifuged at 2000 g for 10 min, following which, 50 μl aliquots of plasma were removed. Concentrations of PF-02413873 in whole blood and plasma were determined using the LC-MS/MS method described below. Separate calibration lines were determined using blank blood and plasma containing known added amounts of PF-02413873.

Metabolic turnover in in vitro systems

The in vitro stability of PF-02413873 was studied in microsomes (BD Gentest, San Jose, CA, USA) and cryopreserved hepatocytes (InVitro Technologies, Baltimore,
MD, USA) from rat, dog and human liver and in human recombinant CYP isozymes (CYPs 1A2, 2C9, 2C19, 2D6 and 3A4; PanVera, Madison, WI, USA). All incubations were performed at a concentration of PF-02413873 of 1 μM. Liver microsome preparations were diluted to yield 0.5 μM cytochrome P450 and recombinant CYP isozymes were used at 0.1 μM. Incubations (1.3 ml) contained 50 mM KPO₄ buffer, pH 7.4 and 1 mM MgCl₂ and were supplemented with a reducing equivalent regenerating system (isocitrate/isocitrate dehydrogenase). The assay cocktail was incubated at 37 °C for 15 min before the addition of NADP⁺ (1 mM) to initiate reactions. Samples (100 μl) were removed at time intervals up to 60 min after addition of NADP⁺ and the reaction quenched by addition of each aliquot to 200 μl acetonitrile containing fluconazole (1 μM) as an internal standard. Cryopreserved rat, dog and human hepatocytes were defrosted and washed according to the supplier’s instructions. Incubations were carried out at a concentration of PF-02413873 of 1 μM with 0.5 x 10⁶ viable hepatocytes/ml in 50 μl Williams E medium. Incubations were carried out for 3 h at 37 °C in a CO₂ incubator and reactions terminated at time intervals with 200 μl acetonitrile containing fluconazole (1 μM) as an internal standard.

Samples (80 μl) from microsomal and hepatocyte incubations were analysed by LC-MS/MS using a Sciex API2000 mass spectrometer (Perkin-Elmer Sciex). The API2000 was operated in positive ion mode using the Turboionspray® interface and data were acquired in the multiple reaction monitoring (MRM) mode using argon as the collision gas. Chromatography was performed using a 2.1 mm C18 Opti-lynx special column with a pore diameter of 40 μm (Optimize Technologies Inc.) and mobile phases A (2mM ammonium acetate, pH 3 in 90% water, 10% methanol) and B (2mM ammonium acetate, pH 3 in 10% water, 90% methanol). A step gradient of 0-10s 100% A; 11-78s
100% B and 79-116s 100% A was used with a flow rate of 1 ml/min. Flow was split (1:5) post-column before entering the mass spectrometer. Ratios of peak areas of PF-02413873 to internal standard were calculated and the natural logarithm of peak area ratio plotted against time. The apparent first order rate constant taken from the slopes of the plots were used to calculate apparent intrinsic clearance (CL\text{int,app}), and normalized per mg microsomal protein or per 10^6 hepatocytes.

**Microsomal binding**

A human liver microsomal incubation was prepared as described above but without addition of NADP\(^+\), and left at room temperature overnight to inactivate metabolizing enzymes. PF-02413873 was added to the mixture to a final concentration of 1 \(\mu\)M and 220 \(\mu\)l used for equilibrium dialysis against blank buffer mix in a rapid equilibrium device (RED) (Waters et al., 2008). Following incubation at 37 °C for 4 h, samples of microsomal incubation (15 \(\mu\)l) and buffer (45 \(\mu\)l) were added to 120 \(\mu\)l of ice-cold acetonitrile containing internal standard. Concentrations of PF-02413873 in microsomal and buffer compartments were then assessed using LC-MS/MS.

**Identification of metabolites of PF-02413873 in vitro**

Experiments investigating routes of metabolism were conducted using the same liver microsome and hepatocyte preparations used to study metabolic turnover. Microsomal and hepatocyte incubations were conducted with PF-02413873 at an initial concentration of 1 \(\mu\)M. Liver microsomal protein concentration was adjusted to 0.25 \(\mu\)M of total P450, and hepatocyte incubations performed at 0.5 x 10^6 viable hepatocytes/ml. Incubations were conducted in a water bath at 37 °C and in the case of liver microsomes, were performed in the presence and absence of NADPH.
appropriate time-points, dependent on previously measured half-lives, reactions were terminated by addition of five volumes of cold acetonitrile. Terminated samples were centrifuged and the supernatant removed and evaporated to dryness under a stream of nitrogen. Dried samples were stored frozen until analysis.

Qualitative analysis to identify potential metabolites of PF-02413873 was conducted by HPLC-MS/MS. The HPLC aqueous mobile phase component of the binary gradient consisted of 0.1% v/v formic acid in water (mobile phase A), and the organic component consisted of 0.1% v/v formic acid in acetonitrile (mobile phase B). An Agilent 1100 binary pumping system was used to control the mobile phase gradient at 95% A for the first minute, changing linearly to 2% A at 8 min followed by 1 min held at 2% A, then back to 95% A over 0.1 min and held for 4 min. The gradient was delivered to a 2.1 x 100 mm Sunfire C18 3 µm analytical column via a CTC HTS Pal autosampler. The flow rate was set at 200 µl/min and was introduced into the source of the mass spectrometer (Waters Q-ToF Premier) with no post-column split. The mass spectrometer was coupled to the HPLC via a Z-Spray interface with a desolvation temperature of 350 °C, a source temperature of 120 °C and a cone voltage of 25 V. Leucine-enkephalin was introduced into the ion source via a lock-spray inlet to provide a standard for accurate mass calibration. Positive-ion MS and MS/MS experiments were performed with the TOF operated in V-mode and data acquisition performed in centroid mode.

Animal experimentation

All studies involving animals were conducted in compliance with UK national legislation and subject to local ethical review. At all stages, consideration was given to
experimental refinement, reduction in animal use and replacement with *in vitro* techniques.

**PK in the rat**

Male Sprague Dawley rats weighing approximately 250 g were used in the following studies. At least 48 h before compound administration, animals were surgically prepared by the placement of a cannula in the jugular vein. Throughout the study animals were housed in stock boxes with access to food (RM1 rodent diet, Special Diet Service) and water *ad libitum*. PF-02413873 was dissolved in 20% PEG in saline to a final concentration of 0.2 mg/ml. 2 ml/kg was dosed either intravenously via the tail vein or orally by gavage to give a final dose of 0.4 mg/kg. Separate groups of rats (n=3) were used for each dose route. Following dosing by either route, blood (approximately 175 μl) was withdrawn via the jugular vein catheter at various times into heparin coated tubes. The cannula was flushed with heparinised saline (10 units/ml) after each sample. Plasma was prepared by centrifugation at 2,000 g and the samples were then stored at -20 °C prior to analysis.

**PK in the dog**

One female and one male dog weighing approximately 12 kg were used for the intravenous and oral studies conducted 1 week apart. Prior to the studies, the dogs were given food and water *ad libitum*. For both intravenous and oral administration, PF-02413873 was dissolved in DMSO to a concentration of 10 mg/ml, then diluted with PEG 200 to 0.5 mg/ml and finally with saline to a final concentration of 0.1 mg/ml in 1% DMSO 20% PEG 200 in saline. Intravenous administration was by infusion via the
saphenous vein of 2 ml/kg over 15 min to give a final dose of 0.2 mg/kg and oral administration was via gavage at 2 ml/kg to give a final dose of 0.2 mg/kg.

On the day of dosing, the dogs were placed in slings and remained there for the first 6 hours following dosing. This allowed blood samples (approx. 2 ml) to be collected from the saphenous vein by means of indwelling catheters. After this period, the dogs were returned to their pens and samples were obtained from the cephalic vein by venepuncture. After sampling, blood was centrifuged at 2,000 g and plasma was removed into separate Eppendorf tubes which were stored at -20 °C until analysis.

**PF-02413873 analysis in rat and dog plasma samples**

PF-02413873 was extracted from 50 μl aliquots of rat or dog plasma using a specific liquid-liquid extraction and HPLC method with mass spectrometric detection. Samples were prepared by the addition of 1 ml borate buffer, pH 10 containing 10 ng of internal standard PF-02327888, followed by 2 ml tert-butylmethylester (TBME). Samples were vortex mixed, centrifuged at 2,000 g for 5 min and 1.8 ml of the TBME layer removed and blown to dryness under nitrogen at 40 °C. The residues were reconstituted in 300 μl of MF3 and 180 μl was injected into an LC-MS/MS system for quantitation of analyte. In each assay, standard calibration lines were constructed using known amounts of PF-02413873 added into 50 μl aliquots of blank plasma from the appropriate species.

Samples were analysed using a Sciex API 4000 with TurboIonSpray Interface in positive ion multiple reaction monitoring mode with collision energy of 25 eV. The MRM transitions monitored were 360 to 280 for PF-02413873 and 358 to 278 for
PF-02327888. Each sample was injected sequentially into the LC-MS/MS system with a CTC PAL autoinjector. The mobile phase (flow rate 3 ml/min) was split 5:1 post-column using an Acurate™ flow splitter.

Clinical study in human volunteers

The randomized, double-blind, third party open, placebo controlled dose-escalation study was conducted in healthy male subjects. The protocol was reviewed and approved by an independent ethics committee and all subjects gave written informed consent. PF-02413873 was administered orally as a micronized suspension. Subjects were divided into two cohorts: Cohort 1 received 4 escalating doses (0.5, 5, 50 and 150 mg) with randomised placebo insertion in the fasted state plus one dose (150 mg) in the fed state; Cohort 2 received 3 doses (500, 1500 and 3000 mg) with randomised placebo insertion in the fed state. Subjects in the fed state received a standard high fat high calorie breakfast. Serial blood samples for determination of plasma concentrations of PF-02413873 were collected for up to 120 h in Cohort 1 and up to 240 h in Cohort 2. Standard non-compartmental PK analysis was performed on plasma concentration data.

Analysis of clinical PK samples

Plasma samples were analysed with a validated assay using PF-02339955 as an internal standard. PF-02413873 and internal standard were extracted from 100 µl of human plasma with a solid phase method extraction using. Thawed plasma samples were briefly mixed and centrifuged at 2,000 g for 10 min at room temperature. 100 µl of each sample was mixed with 100 µl methanol containing 200 ng/ml PF-02339955. Following preconditioning of 15 mg OMIX C18 tips (Tomtec Quadra) with 2x150 µl
of methanol followed by 150 µl water, the samples were loaded onto the C18 micropipette tips. The tips were washed with 2x150 µl water and the analytes eluted with 2x75 µl methanol. Eluates were evaporated under nitrogen at 40 ºC, reconstituted with 200 µl of MF5 and shaken for 10 min. 50 µl of each reconstituted sample was injected onto an LC-MS/MS system containing a 100 x 4.6 mm Onyx Monolithic column and analyte measured by MS/MS detection in a API 3000 triple quadrupole mass spectrometer operating in positive ion MRM mode. The lower and upper limits of quantification of this assay were 1 ng/ml and 500 ng/ml PF-02413873, respectively.

Prediction methods

Clearance (CL) prediction from allometric single species scaling (SSS)

PK in rat and dog were used to predict CL in human using the following equation (Hosea et al., 2009):

\[
\text{human } \text{CL}_u = \text{animal } \text{CL}_u \times \left( \frac{\text{human BW}}{\text{animal BW}} \right)^{0.75},
\]

where BW is body weight (kg), assumed to be 0.25 for rat, 12 for dog and 70 for human and CL\textsubscript{u} is the unbound plasma CL with units of ml/min. Then, plasma CL (CL\textsubscript{p} , ml/min/kg) in human is given by:

\[
\text{human } \text{CL}_p = \frac{\text{human } \text{CL}_u \times \text{Fu}}{\text{human BW}}
\]

CL prediction from in vitro metabolism

Using human liver microsomal turnover, prediction of in vivo intrinsic CL and, subsequently, hepatic CL was carried out using physiologically based scaling factor and the “well-stirred” liver model as described in the literature (Ito and Houston, 2005; Obach et al., 1997). It was assumed that human liver contains 45 mg/g of microsomal protein and that there are 21 g liver per kg human body weight.

Dose prediction
Doses of PF-02413873 predicted to be required to achieve a given exposure were calculated from the relationship, assuming 100% absorption:

\[ \text{Dose} = \frac{\text{CL}_p}{1 - \left(\frac{\text{CL}_b}{\text{Q}}\right)} \times \text{AUC}_{\text{eff}} \]

where \( \text{Q} \) = human hepatic blood flow; \( \text{BPR} \) = blood:plasma ratio; \( \text{CL}_b \) = blood \( \text{CL} \), calculated as \( \frac{\text{CL}_p}{\text{BPR}} \); \( \text{AUC}_{\text{eff}} \) = target AUC in plasma at steady state.

Prediction of plasma concentration versus time profiles in GastroPlus™

The PBPK i.v. (disposition) and oral simulations were performed within the GastroPlus™ software (Simulations Plus Inc., Lancaster, CA) using version 5.0. These models have been described in detail previously (Jones et al., 2009). The choice of GastroPlus™ software offered the potential to incorporate biorelevant solubility and simulate PK profiles under fed and fasted conditions. The PBPK model was composed of several compartments corresponding to the different tissues of the body which are connected by the circulating blood system. Each compartment was defined by a tissue volume and a tissue blood flow rate which is specific for the species of interest. Each tissue was assumed to be perfusion rate limited. The liver was considered to be the only site of elimination. Tissue to plasma partition coefficients (Kps) and intrinsic CL (\( \text{CL}_{\text{int}} \)) predicted from pre-clinical data were required as input to this model. The Kp values were predicted from physicochemical and in vitro inputs using methods described by Rodgers and Rowland (2006). In brief, these equations account for partitioning of unionised drug into neutral lipids and neutral phospholipids, dissolution of drug in tissue water and interactions with extracellular protein.

The ‘Advanced Compartmental Absorption Transit’ (ACAT) model within GastroPlus™ was used to predict the rate and extent of oral absorption in human and preclinical species. The ACAT model has been described in detail previously (Agoram
et al., 2001) and is based on the CAT model described by Yu and Amidon (1999). The main input parameters for the absorption prediction were: solubility, permeability, particle size, LogD and dose (Table 1).

The following PBPK prediction strategy was followed as proposed by Jones et al. (2006a):  
1. validation of i.v. disposition prediction in rat and dog  
2. validation of oral absorption prediction in dog  
3. simulation of disposition and absorption in human
Results

Log$D_{7.4}$ of PF-02413873

The geometric mean log$D_{7.4}$ of PF-02413873 was found to be $3.4 \pm 0.03$ (S.E.M., $n = 6$), consistent with its nature as a lipophilic, neutral (non-ionizable) compound.

Validation of analytical methods

The PF-02413873 rat plasma assay displayed linearity of signal with concentration over a calibration range of $0.2 – 1000$ ng/ml. Inaccuracy (6 replicate samples) was 1.4, 10.9, 2.8 and 10.7% at 1, 20, 100 and 200 ng/ml, respectively and the imprecision at the same concentrations was 3.1, 8.7, 1.3 and 8.3%. The dog plasma assay displayed linearity of signal with concentration over a calibration range of $0.2 – 1000$ ng/ml. Inaccuracy (6 replicate samples) was 1.7 and 3.7% at 5 and 500 ng/ml, respectively and the imprecision at the same concentrations was 11.7 and 2.9%. The clinical assay was linear over a calibration range of $1 – 400$ ng/ml and had an inaccuracy at 1, 3, 250 and 400 ng/ml of 7, 7, 2.8 and 0.5%, respectively. At the same concentrations, the imprecision was 6.1, 3.9, 2.6 and 6.4%.

Plasma protein binding and blood:plasma ratio

In rat, dog and human plasma, no concentration-dependence of binding or differences between male and female were evident across a concentration range of $1.5$ and $15$ μg/ml (rat), $1$ and $10$ μg/ml (dog) and $0.1$ and $1.0$ μg/ml (human) (data not shown). Therefore a single value of unbound fraction (Fu) was used for each species based on the overall mean of the data across concentration and sexes. Thus, the Fu of PF-02413873 in rat, dog and
human plasma was 0.042, 0.035 and 0.031 respectively. The blood:plasma ratios of PF-02413873 in rat, dog and human were 0.93, 0.82 and 0.61, respectively.

**In vitro metabolism by liver microsomes and recombinant CYPs and microsomal binding**

In rat liver microsomes, the apparent intrinsic CL (CL\text{int,app}) of PF-02413873 was >500 \(\mu\text{l/min/mg protein} (t_{1/2} < 2 \text{ min})\). In dog and human microsomes, CL\text{int,app} values were significantly lower than in rat, at 36 and 29 \(\mu\text{l/min/mg protein} \) respectively (\(t_{1/2} = 25 \text{ and } 30 \text{ min} \) respectively). The unbound fraction of PF-02413873 in human liver microsomes was 0.78. In the recombinant CYP experiments, turnover of PF-02413873 was only detectable in the presence of rCYP3A4. The CL\text{int,app} value (1.27 \(\mu\text{l/min/pmol P450} \)) calculated for rCYP3A4 was subsequently scaled to human liver, using the appropriate relative activity factor (RAF) and intersystem extrapolation factor (CL\text{int ISEF}) (Youdim et al., 2008). The scaled rCYP3A4 CL\text{int} value was 25.9 \(\mu\text{l/min/mg protein} \).

**In vitro metabolism by hepatocytes**

In cryopreserved rat and dog hepatocytes, CL\text{int,app} of PF-02413873 was >500 and 20 \(\mu\text{l/min/10}^6 \text{ cells} \) respectively (\(t_{1/2} < 2 \text{ and } 35 \text{ min} \) respectively). In human hepatocytes, the turnover of PF-02413873 (\(t_{1/2} > 120 \text{ min} \) ) was too low to allow an accurate measurement (<6 \(\mu\text{l/min/10}^6 \text{ cells} \)).

**Metabolite profiles in in vitro systems**

The main metabolites of PF-02413873 formed by liver microsomes and hepatocytes are shown in the scheme in Figure 1. Following incubation with human liver microsomes, the major metabolite of PF-02413873 resulted from mono-oxidation of one of the methyl groups on the dimethylbenzonitrile moiety (M1). Further minor products of mono-
oxidation of the methyl and/or cyclopropyl substituents of the pyrazole ring were also detected (M2 and M3), along with products of di-oxidation of both ring systems (M4, and M9. The mono-oxidised metabolite M3 was the only product formed after incubation of PF-02413873 in human hepatocytes. In rat and dog liver microsomes and hepatocytes, a similar range of mono-oxidised (M1, M2, M3) and di-oxidised products (M4 and M9) were found. The only product of phase 2 conjugation reactions that was found was a glucuronide in dog hepatocytes, a minor product that was most likely the result of conjugation to a hydroxylated metabolite (data not shown).

PK in rat

The PK of PF-02413873 were determined following intravenous administration at a single dose of 0.4 mg/kg in male rats (Figure 2, PK parameters in Table 2). The mean elimination half-life was 1.1 h and plasma CL (CLp) was 84 ml/min/kg. Following oral dosing, plasma concentrations of PF-02413873 were below the level of quantitation. Assuming that hepatic blood flow in the rat is 70 ml/min/kg (Boxenbaum, 1980), the lack of exposure following oral administration is consistent with the CLp observed following intravenous administration, i.e. CLp > hepatic blood flow.

PK in dog

The PK of PF-02413873 was determined following intravenous administration at a single dose of 0.2 mg/kg in dogs. The mean elimination half-life was 7.6 h, CLp was 3.8 ml/min/kg and Vss was 2.5 L/kg. The plasma concentrations following oral dosing of PF-02413873 are shown in Figure 3, and the PK parameters in Table 3. In contrast to rat, the plasma exposure and calculated PK parameters demonstrated high oral bioavailability of PF-02413873 in the dog and, furthermore, were consistent with complete absorption.
PK in male volunteers

The PK profiles of PF-02413873 in Cohort 1 (doses of 0.5 – 150 mg) are shown in Figure 4, the PK profiles in Cohort 2 in Figure 5 and the derived PK parameters for both cohorts in Table 4. In subjects that were fasted, exposure increased slightly less than proportionally with dose between 5 and 150 mg. Exposure at 0.5 mg was too low in relation to the limit of quantitation to allow satisfactory calculation of all PK parameters. At 150 mg, dosing in fed subjects increased AUC_{inf} by approx. 60% in comparison with fasted subjects, with a corresponding 76% increase in C_{max} and delay in T_{max} from 3 to 6 h. In fed subjects overall, exposure increased roughly in proportion to dose between 150 and 3000 mg. The terminal half-life of PF-02413873 over the dose range varied between 28 and 44 h, with no obvious pattern of variation that reflected dose or fed or fasted states. Oral CL ranged between approximately 4 and 7 ml/min/kg, with the lower values reflecting higher AUC obtained per unit dose in the fed state.

Prediction of human PK

The predictions of the V_{ss} of PF-02413873 in rat, dog and human using the tissue composition equations developed by Rodgers and Rowland are shown in Table 5. These equations provided accurate estimates of V_{ss} in rat and dog. When the K_{p} values provided by this prediction method were combined with the observed CL as inputs, simulation of the intravenous concentration versus time profiles in rat and dog provided good approximations to the observed data. Therefore, the Rodgers and Rowland (2006) equations were used for PBPK simulation in human.
The ACAT model was able to accurately simulate the dog oral plasma concentration versus time profiles using the in vitro solubility, predicted Kp values and observed intravenous CLp as inputs to the model (Figure 6). Therefore, the ACAT model was used to simulate absorption in human.

Human CLp was predicted to be 13.5 and 2.6 ml/min/kg when scaled from unbound CLp in rat and dog respectively. When in vitro CLint determined in HLM was used, the predicted human CLp was 1.0 ml/min/kg. The prediction of blood CL from rat SSS (CLp/BPR = 22 ml/min/kg) was close to hepatic blood flow in human (assumed to be 20 ml/min/kg). For this reason, CL from rat SSS was not used in subsequent simulations using GastroPlus™.

As i.v. administration was not undertaken in the clinical study, CLp predicted from preclinical data was compared with the range of oral CL (CL/F) determined in the clinical study. This demonstrated an over-prediction by rat SSS. Predictions from dog SSS and from scaling of HLM turnover were more comparable with the measured human CL/F, and broadly consistent with characterization of PF-02413873 as a low CL molecule in human. Dog SSS provided the closest estimates of Cmax and AUC observed in the clinical study and predicted an oral CL of 5.5 ml/min/kg, assuming complete absorption.

Separate preclinical pharmacological studies of PF-02413873 predicted that steady state systemic exposure (AUC0-24) of approximately 6000 ng.h/ml would be required to elicit a pharmacological effect in human (data not shown). This information was combined with CL/F predictions to calculate approximate doses of 30 mg (HLM scaling) and 85 mg (dog SSS) that would be expected to be pharmacologically active and hence inform the range to be explored in early human studies (5 – 3000 mg).
GastroPlus™ simulations of the plasma concentration versus time profile of PF-02413873 following micronised suspension doses of 50, 150 and 500 mg in human alongside the actual measured plasma concentrations are shown in Figure 7. In general, the shapes of the profiles were predicted well using the Rodgers and Rowland (2006) $K_p$ values. However, plasma exposures of PF-02413873 ($C_{\text{max}}$ and AUC) were generally over-predicted using $CL_p$ predicted from HLM (up to 4-fold) and dog PK (up to 3-fold) (Table 6), indicating a potential under-prediction of CL.

At a dose of 150 mg, using CL predicted from HLM or dog SSS, biorelevant solubility (FaSSIF and FeSSIF), together with altered physiologies in the two feeding states, GastroPlus™ predicted increases in $C_{\text{max}}$ (approx. 2-fold) and AUC (approx. 1.2-fold) with food. These compared well with observed increases in $C_{\text{max}}$ and AUC in the clinical PK study of 1.8- and 1.6-fold respectively. The model also predicted the slight reduction in dose-normalised exposure that was observed in the fasted state between 50 and 150 mg, consistent with saturation in absorption (Table 6). However, as doses increased to 500, 1500 and 3000 mg, the model predicted progressive reduction of dose-normalised exposure, whilst it remained broadly constant in the clinical study.
**Discussion**

A combination of preclinical in vitro metabolism and in vivo PK data were used to determine the likelihood that the PK of PF-02413873 in human would be suitable for its progression to clinical studies. In human in vitro systems, turnover of PF-02413873 was relatively low, so that scaling of HLM CL$_{int}$ to in vivo CL using the appropriate scaling factors and the well-stirred liver model predicted low CL in human (<20% hepatic blood flow). The PK of PF-02413873 in rat and dog demonstrated that CL in rat was high (greater than hepatic blood flow) and in dog, low (approx. 10% of hepatic blood flow). This was in keeping with metabolic stability of PF-02413873 in liver microsomes and hepatocytes obtained from these species. These differences in PK and metabolic stability between rat and dog were reflected in predictions of human CL by SSS (Hosea et al., 2009) from the two species which differed widely, such that dog predicted low CL (approx. 20% hepatic blood flow) and rat predicted high CL (similar to human hepatic blood flow).

Metabolites of PF-02413873 formed in in vitro systems in all three species were products of oxidation. Although appearance in urine of unchanged PF-02413873 was not determined in the PK studies, its physicochemical characteristics (lipophilic neutral compound) make it unlikely that renal excretion is a significant CL pathway. Therefore, the major route of CL in vivo is likely to be via oxidative metabolism catalysed by hepatic cytochrome P450 (CYP) enzymes. In the absence of quantifiable metabolite samples, rates of metabolite formation were not investigated. However, other studies that monitored disappearance of PF-02413873 showed that it was turned over by recombinant human CYPs, with CYP3A4 displaying the highest turnover of those enzymes tested. When appropriate scaling factors were applied to the data based on enzyme abundance in human
liver, a CL_{int} value for CYP3A4 turnover of PF-02413873 was calculated that was similar
to that measured in HLM. These data are consistent with CYP3A4 being the major CYP
isoform involved in metabolism of PF-02413873 in human liver. However, further studies
in vivo would be required to confirm the dominance of oxidative pathways in human and
also to understand the potential pharmacokinetic interactions between PF-02413873 and
other drugs known to be metabolised principally by CYP3A4 or that are inhibitors of this
enzyme.

Prior to constructing a model in GastroPlus™ for human PK simulation, the assumptions
of the model were tested by reference to observed rat and dog PK profiles (Jones et al.,
2006a; DeBuck et al., 2007). This validation exercise showed that the Rodgers and
Rowland equations provided the simulated profiles that most closely resembled the
observed data. The intrinsic absorption of PF-02413873 was expected to be good, based
on comparison of intravenous and oral PK determined in dog. The simulation of
absorption in the dog using the ACAT model was found to provide a good approximation
to the observed oral PK data (Figure 6(C)). As PF-02413873 possesses high lipophilicity
(logD = 3.4) and consequent low aqueous solubility (<10 μg/ml in aqueous buffer), the
formulation chosen for clinical use was a micronized suspension, intended to maximise
particle surface area and, hence, dissolution rate. Thus, the absorption model used the
particle size in the micronized formulation and solubility determined in biorelevant media
(Fassif and Fessif). Estimates of CL from HLM and dog PK were then combined with the
absorption and distribution model in GastroPlus™ and simulations of PK profiles
performed with inputs of oral suspension doses in the planned FIH study single ascending
dose range. Given the range of CL predictions, some uncertainty in the outcome of the
FIH study existed. For instance, taking a Vss of 2.6L/kg (Rogers and Rowland prediction)
effective t½ estimates of approximately 8h and 24h were calculated from CL predictions made from dog SSS and HLM scaling respectively, while conversely, prediction from rat SSS was consistent with little oral exposure due to a predicted large first pass. However, metabolic turnover rates exhibited by rat and dog liver microsomes were consistent with their respective CL determined in these species in vivo, providing some confidence that the low turnover in HLM would translate to low CL in human. Furthermore, similarity in microsomal CL_{int} in dog and human suggest that the dog, rather than rat, may be the most appropriate species for SSS of human CL of PF-02413873. Therefore, confidence in acceptability of human PK was considered to outweigh the uncertainty, supporting progression of PF-02413873 to a FIH study.

The human PK of PF-02413873 determined following oral dosing in the Phase 1 study was characterized by low CL/F (4 – 7 ml/min/kg) and terminal t½ of 28 – 44 h, characteristics compatible with high oral bioavailability and a once-daily dose that would be desirable for therapeutic administration. Of the human CL prediction methods used, SSS from rat was not found to be a good guide, greatly over-predicting observed CL. The predictions from dog SSS and scaling from HLM were much more consistent with the low oral CL exhibited by PF-02413873, in accordance with the observed metabolic turnover in liver microsomes (CL_{int} in RLM >> DLM ≈ HLM). Previous retrospective analysis of CL prediction methods suggested that if CL is principally via metabolism catalysed by CYP, HLM scaling was as predictive of oral CL as SSS based on data from rat, dog and monkey (Hosea et al., 2009). In the present study, metabolite profiling and scaling of CL_{int} catalysed by recombinant CYP3A4 were consistent with CYP-mediated CL of PF-02413873. HLM scaling correctly predicted CL to be low in relation to hepatic blood flow, although the value was just below the observed CL range. Although turnover in
HLM of lower concentrations of PF-02413873 could indicate potential underestimation of CL\textsubscript{int} at 1μM, CYP inhibition data (CYP3A4 IC\textsubscript{50} >10μM, data not shown) were consistent with a CYP3A4 Km for PF-02413873 of >1μM. Dog SSS provided the estimate of CL closest to that observed, and was a better predictive model than rat in this instance. This corresponds with the retrospective analysis of Hosea et al. which showed that dog SSS predicted oral CL within 2-fold of observed in 52% of CYP-cleared compounds, whereas HLM scaling achieved 30%. Our prospective use of pre-clinical data showed that: i) two out of three prediction methods (HLM and dog SSS) predicted low human CL; ii) CL was likely to be principally via metabolism catalysed by CYP; iii) in vitro CL\textsubscript{int} and in vivo CL correlated in dog and rat i.e. low DLM CL\textsubscript{int} and high RLM CL\textsubscript{int} predicted low dog CL and high rat CL, implying CYP mediated CL, and that low HLM CL\textsubscript{int} is likely to predict low human CL.

Dose escalation in fasted subjects provided approximately dose-proportional increases in AUC\textsubscript{inf} but less than dose-proportional increases in C\textsubscript{max}. Simulations in GastroPlus\textsuperscript{TM} displayed trends towards over-prediction of C\textsubscript{max} and under-prediction of the terminal phase half-life which could be explained by an under-prediction of Vss. This theory was confirmed by increasing the muscle Kp (largest tissue mass) resulting in a lowering of the predicted C\textsubscript{max} and a better prediction of the terminal phase (data not shown).

Provision of a standard high fat, high calorie food prior to dose administration led to an increase in exposure over fasted subjects, as demonstrated by an increase in AUC\textsubscript{inf} and C\textsubscript{max} at a dose of 150 mg. Remarkably, exposure was also found to increase largely in proportion to dose in fed subjects from 150 mg to 3000 mg. This suggests that absorption was enhanced in fed subjects, presumably as a result of changes in increased secretion of
bile acids with consequent higher dissolution rate. The increased exposure with food was captured at doses of 150 and 500 mg using the ACAT model which accounts for the increased stomach transit time, the change in stomach pH and the biorelevant solubility data that incorporates solubility differences in the presence and absence of bile salts. Although the model was unable to fully account for the approximately dose-linear exposure increase from 500 – 3000mg under fed conditions, it was able to predict that dosing PF-02413873 with food would increase exposure at the higher end of its dose range and permit extensive testing of pharmacologically relevant exposures. Other studies have demonstrated the utility of PBPK methodology and GastroPlus™, incorporating biorelevant solubility, for simulating food effects of lipophilic compounds (Jones et al., 2006b; Parrott et al., 2009), although these examples were generally weak bases. In this program, as the modelling provided evidence for a higher exposure with food, the plan for the subsequent repeat dose part of the clinical study incorporated fed subjects in order to optimize exposure for a given dose. Furthermore, the PBPK model in GastroPlus™ allowed simulation of repeat dose scenarios of PF-02413873 targeting pharmacologically relevant exposures that were also within acceptable limits established in separate preclinical safety studies (e.g. 150mg per day under fed conditions).

In conclusion, the non-steroidal PR antagonist PF-02413873 possessed low oral CL in human with a PK profile consistent with a once daily dosing schedule. Further studies are required in appropriate subjects to confirm the duration of desired PR pharmacological effects that would lead to amelioration of symptoms in gynaecological diseases. The PK characteristics, including an increase in exposure in fed subjects, were predicted well using an ACAT model in GastroPlus™ and low CL prediction from HLM scaling and dog SSS. This represents a case study that highlights the prospective use of preclinical data
combined with PBPK modelling techniques to provide predictions that guide decision making and design of early clinical trials of novel chemical agents.
Acknowledgements

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Authorship Contributions

Participated in research design: Bungay, Tweedy, Howe, Gibson and Mount.

Conducted experiments: Tweedy

Contributed new reagents or analytic tools: Gibson

Performed data analysis: Bungay, Jones

Wrote or contributed to the writing of the manuscript: Bungay, Jones, Mount
References


Figure 1: Proposed pathways of metabolism of PF-02413873 in microsomes and hepatocytes from rat, dog and human liver. Labels next to arrows denote metabolite was formed in microsomes from rat (RLM), dog (DLM) and human (HLM) and hepatocytes from rat (rHEP), dog (dHEP) or human (hHEP) liver.

Figure 2: PF-02413873 pharmacokinetics following intravenous administration in male rats (0.4mg/kg). Symbols represent the mean value from 3 animals (error bars represent S.D.).

Figure 3: PF-02413873 pharmacokinetics following intravenous (closed circles) and oral suspension (open symbols) dosing (0.2mg/kg) in dogs (n=2). Symbols represent the mean value from 2 animals.

Figure 4: PF-02413873 pharmacokinetics following oral suspension dosing in Cohort 1. Doses were 5 mg, fasted (open circles, n = 7), 50 mg, fasted (open triangles, n = 8), 150 mg, fasted (open squares, n = 7) and 150 mg, fed (closed squares, n = 7) and the error bars represent S.D..

Figure 5: PF-02413873 pharmacokinetics following oral suspension dosing in Cohort 2 (fed condition). Doses were 500 mg (circles, n=8), 1500 mg (triangles, n = 8) and 3000 mg (squares, n = 2) and the error bars represent S.D..

Figure 6: Predicted and observed plasma concentration versus time profiles of PF-02413873 following intravenous administration to rat (A), and dog (B) and oral solution administration to dog (C). Open circles represent observed data from individual
animals and the solid lines the profiles predicted from the PBPK model in GastroPlus™. Doses were 0.4 mg/kg in rat and 0.2 mg/kg in dog.

Figure 7: Predicted and observed plasma concentration versus time profiles of PF-02413873 following administration of micronized suspension to human volunteers. Symbols represent mean observed data and error bars represent S.D.. The simulated profiles from the PBPK model in GastroPlus™ are shown, using clearance predicted from dog SSS (solid lines). Doses were 50 mg under fasted conditions (A), 500 mg under fed conditions (B) and 150 mg under both fasted (open symbols, solid line) and fed (closed symbols, dotted line) conditions (C).
Table 1: Input data used in the GastroPlus PBPK model

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<tr>
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<td>FeSSIF solubility (mg/ml)</td>
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Table 2: Pharmacokinetic parameters of PF-02413873 in rat following intravenous bolus and oral solution administration. CLₚ = plasma clearance; AUCₗ₉ = area under plasma concentration vs. time profile from time 0 extrapolated to infinite time; \( t\frac{1}{2} \) = terminal half-life; Vdss = volume of distribution at steady state; MRT = mean residence time; F = oral bioavailability. Data are mean ± S.D. (n=3)

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<tr>
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<tr>
<td>Vdss (L/kg)</td>
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<td>( t\frac{1}{2} ) (h)</td>
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<td>MRT (h)</td>
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Table 3: Pharmacokinetic parameters of PF-02413873 in dogs following intravenous and oral solution administration

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Table 4: PF-02413873 pharmacokinetic parameters in human male volunteers following oral micronized suspension dosing. n = number of subjects assessed; AUC_{0-24} = area under the concentration-time curve from time 0 to 24 hours post-dose; AUC_{inf} = area under plasma concentration vs. time profile from time 0 extrapolated to infinite time; C_{max} = first incidence of maximum observed plasma concentration; T_{max} = time at which C_{max} was observed; t_{1/2} = terminal half-life; Vz/F = apparent volume of distribution associated with the terminal phase; CL/F = apparent clearance after oral administration; NC = not calculated; CV = coefficient of variation.

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<th>AUC_{0-24}</th>
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<th>T_{max}</th>
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<td>(CV%)</td>
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<td>NC</td>
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<td>(28)</td>
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<tr>
<td>5 mg</td>
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<td>148 (23)</td>
<td>191 (36)</td>
<td>19.8</td>
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<td>(36)</td>
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<td>50 mg</td>
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<td>2300 (23)</td>
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<td>5290 (21)</td>
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a geometric mean (CV%); b median (range); c arithmetic mean (CV%)
Table 5: Prediction of Vdss of PF-02413873 in rat, dog and human using tissue composition equations

<table>
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<tr>
<th>Species</th>
<th>Plasma volume of distribution at steady state (L/kg)</th>
<th>Rodgers and Rowland (2006)</th>
<th>Single species scaling</th>
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<tbody>
<tr>
<td></td>
<td>measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>5.1</td>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td>Dog</td>
<td>2.5</td>
<td>2.8</td>
<td>-</td>
</tr>
<tr>
<td>human</td>
<td>-</td>
<td>2.6</td>
<td>3.5 (rat) 2.9 (dog)</td>
</tr>
</tbody>
</table>
Table 6: Predicted and observed PF-02413873 AUC and $C_{\text{max}}$ in human following oral micronised suspension dosing

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Mean observed</td>
<td>2300</td>
<td>5290</td>
<td>8310</td>
<td>29400</td>
<td>74500</td>
<td>151000</td>
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<tr>
<td>GastroPlus - dog CLp</td>
<td>3579</td>
<td>8722</td>
<td>10745</td>
<td>32056</td>
<td>50392</td>
<td>59924</td>
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<tr>
<td>GastroPlus - HLM CLp</td>
<td>10920</td>
<td>26606</td>
<td>32780</td>
<td>97790</td>
<td>153700</td>
<td>182700</td>
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</table>

<table>
<thead>
<tr>
<th>Dose-normalised AUC_{inf} (ng.h/ml/mg)</th>
<th>50 mg</th>
<th>150 mg</th>
<th>150 mg</th>
<th>500 mg</th>
<th>1500 mg</th>
<th>3000 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean observed</td>
<td>46</td>
<td>35</td>
<td>55</td>
<td>59</td>
<td>50</td>
<td>50</td>
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<tr>
<td>GastroPlus - dog CLp</td>
<td>72</td>
<td>58</td>
<td>72</td>
<td>64</td>
<td>33</td>
<td>20</td>
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<tr>
<td>GastroPlus - HLM CLp</td>
<td>218</td>
<td>177</td>
<td>218</td>
<td>196</td>
<td>102</td>
<td>61</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>50 mg</th>
<th>150 mg</th>
<th>150 mg</th>
<th>500 mg</th>
<th>1500 mg</th>
<th>3000 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean observed</td>
<td>117</td>
<td>245</td>
<td>427</td>
<td>1420</td>
<td>3350</td>
<td>5420</td>
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<tr>
<td>GastroPlus - dog CLp</td>
<td>324</td>
<td>459</td>
<td>961</td>
<td>1817</td>
<td>2121</td>
<td>2268</td>
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<tr>
<td>GastroPlus - HLM CLp</td>
<td>440</td>
<td>652</td>
<td>1310</td>
<td>2620</td>
<td>3170</td>
<td>3448</td>
</tr>
</tbody>
</table>
Figure 1
Figure 4

PF-02413873 concentration (ng/ml) vs. time (h). The graph shows four different lines representing different conditions or groups. The y-axis represents the concentration in ng/ml, varying from 1 to 1000. The x-axis represents time in hours, ranging from 0 to 120. Each line has error bars indicating the variability or uncertainty in the measurements.
Figure 5

PF-02413873 concentration (ng/ml) vs. time (h)
Figure 7

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z