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Strain-Dependent Variability of Early Discovery Small Molecule Pharmacokinetics in Mice: Does Strain Matter?

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Running Title: Effect of Mouse Strain on Pharmacokinetic Parameters

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Abbreviations: ADME, absorption distribution metabolism and elimination; BALB/c, Bagg Albino; CV, coefficient of variation; CL, clearance; CL_{int}, intrinsic clearance; C_{max}, maximum plasma concentration observed in vivo; DMEM, Dulbecco's modified eagle medium; DMSO, dimethyl sulfoxide; HPMC, Hydroxypropyl methylcellulose; IS, internal standard; IV, intravenous; LogD, partition coefficient of a molecule between octanol and buffer at pH 7.4; PD, pharmacodynamics; PK, pharmacokinetics; PO, oral; $t_{1/2}$, half-life; TK, toxicokinetic; T_{max}, time to reach C_{max}; Tox, toxicology; V_{ss}, volume of distribution at steady state.

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Abstract

Drug discovery programs routinely perform pharmacokinetic (PK) studies in mouse to prioritize lead compounds based on anticipated exposure-efficacy and exposure-toxicity relationships. Due to logistical and/or technical issues, the strain of mouse in early discovery PK studies may not always match the strain in toxicity or efficacy studies. This elicits the question: do appreciable strain-dependent differences in PK parameters exist to an extent that would warrant conducting PK studies in a strain that matches efficacy and toxicity models? To understand the impact that strain may have on PK parameters, we selected 8 marketed drugs with well characterized absorption, distribution, metabolism, and excretion properties and diverse structures to perform PK studies in three common mouse strains (BALB/c, C57BL/6, and CD-1). Some statistical strain-dependent differences were observed, however, we found good general agreement of PK parameters between strains: 88%, 100%, 75%, 76%, 94% and 88% of compounds were within two-fold across strains for CL, V_{ss}, $t_{1/2}$, C_{max}, T_{max} and F, respectively. Overall, we recommend that an approach using a single strain of mouse is appropriate for discovery screening PK studies, provided that proper caution is exercised.

Significance Statement

The mouse strain in discovery PK studies may not match the strain in efficacy and tox studies. Currently, there is gap in the literature addressing if differences in PK parameters across mouse strains exist such that multiple PK studies are warranted. The results from this study indicated that the PK properties of clinically used drugs between mouse strains are within an acceptable range such that single strain PK is appropriate.

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Introduction

In drug discovery, preclinical pharmacokinetic (PK) studies are critical to the process of optimizing hits to leads and leads to clinical candidates. From an absorption, distribution, metabolism and excretion (ADME) perspective, PK studies can inform liabilities for parameters such as clearance, volume of distribution, half-life and bioavailability and may serve as a differentiating factor to prioritizing suitable molecules with optimal ADME properties for pipeline advancement. Furthermore, preclinical PK studies are fundamental to understand the exposure driven pharmacodynamic and toxicological effects of a drug candidate.

Preclinical PK studies are routinely performed at an early stage of a discovery program for numerous drug leads which may result in a high cost and resource burden. To partially mitigate the resource burden, rodent models are typically the first preclinical PK studies conducted; these studies are relatively less expensive and provide rapid measurement of PK parameters. In an ideal case, the rodent strain used for PK studies would match both the pharmacodynamic (PD) and toxicology (Tox) model for each drug target of interest as this would ensure consistency of drug metabolism enzymes and transporters abundances, physiology related to drug excretion and distribution and, thereby bolster confidence in the translation of PD and toxicokinetic (TK) exposure-effect models. However, given the logistical challenges (e.g. maintenance of multiple strain colonies, coordinating dosing events and resources for bioanalytical analysis), early discovery PK is often measured in a single strain that does not necessarily match the PD model and/or the Tox strain. Furthermore, the in vitro liver preparations (microsomes, S9 and hepatocytes) that are used for primary screening are also typically derived from a single strain, most commonly (in our experience) CD-1. With this in mind, we asked the question, specifically as it relates to mouse: do empirical strain-dependent differences in PK parameters exist to the extent which would warrant matching the PK strain with the PD and Tox strain in early drug discovery?

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Studies addressing the question of PK variability associated with strain selection are extremely sparse in the scientific literature. MacCallum and Odds observed a statistically higher exposure of in BALB/c mice relative to DBA/2 for itraconazole (MacCallum and Odds, 2002). McCarthy et al. compared the pharmacokinetics of the psychostimulant cocaine in CD-1 and C57BL/6 mice with chronic dosing and reported higher cocaine exposure in C57BL/6 mice compared to CD-1 mice (McCarthy et al., 2004). To date, there has not been a controlled, systematic evaluation of the effect of mouse strain on PK using a diverse set of compounds in commonly used mouse strains (BALB/c, C57BL/6 and CD-1).

In this study, our central hypothesis was that based on similarities of known physiological parameters, it is not expected that appreciable differences in PK between mouse strains would be found. To test this, we selected 8 marketed drugs with well characterized properties and diverse structures (Figure 1). The compounds were chosen to represent diverse drug space, accounting for several factors including molecular weight (200 – 650 Daltons), compound class (acid, base, neutral and zwitterion), lipophilicity (logD -4.6 to 5.9), and clearance mechanism (metabolism, unchanged drug excretion, renal or biliary excretion) as shown in Table 1. We performed both intravenous (IV) and oral (PO) dosed PK studies in three of the most commonly used strains for preclinical mouse experiments: Bagg Albino c (BALB/c), C57 black 6 (C57BL/6), and CD-1. Herein we discuss the implications and estimated risk associated with using a single mouse strain for all PK studies in early drug discovery.

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Materials and Methods

Chemicals and Materials. Eight commercial drugs (Figure 1) were selected to represent diverse drug space and clearance mechanism in human (Table 1). Test compounds (amiodarone, captopril, chlorpromazine, dexamethasone, fluvastatin, levofloxacin, naproxen, and propranolol) for in vitro clearance experiments were obtained as a 10 mM solution in dimethyl sulfoxide (DMSO) from Selleck

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Chemicals (Houston, TX). Suspended CD-1 (male, pooled, n =18, lot# BVU), C57BL/6 (male, pooled, n =50, lot # BSE), and BALB/c (male, pooled, n =45, lot # CYG) primary hepatocytes and INVITROGRO™ HT media were obtained from Bioreclamation IVT (Baltimore, MD). Compound solids (amiodarone, captopril, chlorpromazine, dexamethasone, fluvastatin, levofloxacin, naproxen, and propranolol) for in vivo testing were obtained from Amgen's internal compound library. DMSO, acetonitrile, hydroxypropyl methylcellulose (HPMC) and formic acid were obtained from Sigma Aldrich (St. Louis, MO). Dulbecco's modified eagle medium (DMEM) was purchased from Gibco (Dublin, Ireland). All other reagents were analytical quality or better. Male CD-1, C57BL/6 and BALB/c mice were obtained from Envigo (Huntingdon, United Kingdom).

Measurement of Pharmacokinetic Parameters in Mouse. Mice were housed in groups at an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility. Animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition. All research protocols were reviewed and approved by the Amgen Institutional Animal Care and Use Committee. Mice (CD-1, C57BL/6 and BALB/c; Envigo, US/Netherlands; 8 -12 weeks old; male) were housed in individual ventilated caging (IVC) system on an irradiated corncob bedding (Envigo Teklad 7097). Lighting in animal holding rooms was maintained on 12:12 hr light:dark cycle, and the ambient temperature and humidity range was at 68 to 79 F and 30 to 70%, respectively. Animals had ad libitum access to irradiated pelleted feed (Envigo Teklad Global Rodent Diet- soy protein free extruded 2020X) and reverse-osmosis (RO) chlorinated (0.3 to 0.5 ppm) water via an automatic watering system. Cages were changed biweekly inside an engineered cage changing station. For IV dosing, compounds were formulated in DMSO at a concentration of 2 mg/mL. Using the appropriate volume, compounds were administered IV at a final dose of 1 mg/kg via femoral vein cannula. For PO dosing, compounds were formulated in 1% Tween 80, 2% HPMC, 97% water at a concentration of 0.5 mg/mL. The PO formulations were solution except for dexamethasone which had suspension formulation. Using

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the appropriate volume, compounds were administered PO at a final dose of 5 mg/kg. Compounds were dosed to three animals per group. Blood was collected at 0.083 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8, 24 hours post-dosing. At each timepoint, approximately 20 µL of blood was collected serially through saphenous vein (alternatively from each leg for consecutive timepoints) from each subject. Samples were collected in K_2EDTA tubes and placed on wet ice until centrifuged (at 4˚C, 10 minutes at 13000 rpm, within 1 hour of collection) to separate plasma. Plasma samples were separated into polypropylene tubes / 96- deep well plates and were transferred immediately after separation to freezer (-70˚C) up to 24h (or until analysis). Plasma concentrations were determined using the calibration curves prepared in plasma matrix for each analyte, briefly: 10 µL of calibration standards, blank, blank+ internal standard (IS) and study samples were transferred to a 0.6 mL 96 deep well plate. 150 µL of IS solution (0.1 µg/mL verapamil in acetonitrile) was added to all the samples except for the two blank samples. 150 µL of acetonitrile was added to both of the blank samples. All samples were vortexed for 10 min and centrifuged at 3200 g for 10 min. Approximately 125 µL of the supernatant was transferred to a 96-deep well plate. Analytes were measured using LC-MS/MS as described previously (Barr et al., 2019). Pharmacokinetic parameters were obtained for each individual animal via non-compartmental analysis of the data in Watson LIMS 7.5 (ThermoFisher Scientific). Individuals that had greater than 20% extrapolation of area under the curve were excluded from the analysis.

Determination of Clearance in Primary Suspended Mouse Hepatocytes. Test compounds were obtained as a 10 mM stock in DMSO and subsequently diluted to a working solution of 50 µM in 1:1 acetonitrile: water. Cryopreserved hepatocytes were thawed at 37 °C and gently rinsed by inversion with 50 mL of invitroGRO HT media. The cell suspension was centrifuged at room temperature for 3 minutes at 65 g. The resultant supernatant was discarded, and cells were resuspended in DMEM media (prewarmed to 37 °C) to have a final density of 0.5 million live cells/mL. 800 µL of the cell suspension was dispensed into wells in a 96-well plate. Incubations were initiated by addition of 8 µL of compound

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working solution described above, resulting in a final concentration of 0.5 µM substrate with 0.5% v/v acetonitrile. At 0, 10, 30, 60, 90, and 120 min reactions were stopped by addition of 300 µL of acetonitrile containing 0.1% v/v formic acid and 1 µM tolbutamide as IS. Upon incubation termination, plate wells were capped to avoid evaporation. Upon completion of all timepoints, sample plates were vortexed at room temperature for 10 minutes and subsequently centrifuged at 3220 g for 20 min at 4 °C. Supernatants were transferred to a separate plate for analysis. Analytes were measured using LC-MS/MS as described previously (Barr et al., 2019). Incubations were performed in duplicate for each analyte. In vitro clearance (CL_{int}) was calculated from the parent compound depletion in each incubation (eq. 1).

$$CL_{int} = -slope\ of\ \ln(\%drug\ remaining)\ vs\ time \times \frac{mL\ incubation}{million\ cells}\ \mu L \cdot min^{-1} \cdot mil\ cells^{-1} \quad (1)$$

Statistical Analyses. To test for statistical differences in parameters measured between different mouse strains, a single factor analysis of variance (ANOVA) test was performed in Microsoft Excel. A p-value of 0.05 or less was used as a threshold for statistical significance. Groups without enough data for a proper ANOVA analysis were denoted with a p-value of not determined (ND).

Results

Compounds Were Selected for Diversity. All 8 test compounds are marketed drugs with well characterized properties and diverse structures (Figure 1). The compounds were selected to represent diverse drug space, accounting for several factors including molecular weight, compound class, lipophilicity, and clearance mechanism in human (Table 1). Overall, the physicochemical properties were

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represented as follows: molecular weight varied from approximately 200-650 Daltons, all charge states (acidic, basic, neutral, and zwitterionic) were represented, and LogD ranged from low to high lipophilicity (-4.6 to 5.9). Multiple primary clearance mechanisms were represented by the group, including urinary excretion, biliary excretion, phase I metabolism by various cytochrome P450s (CYPs 1A2, 2C8, 2C9, 2D6 and 3A) and phase II metabolism by UGTs.

Measurement and Cross-strain Comparison of PK in Mouse. Concentration vs time profiles for compounds dosed IV and PO are shown in Figure 2 and Figure 3, respectively. IV clearance (CL), volume of distribution at steady state (V_{ss}), half-life ($t_{1/2}$), and oral bioavailability (F) were all determined by non-compartmental analysis. Maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were based on observed plasma concentration time profile. For each parameter, the data across three mouse strains were compared in multiple ways. First, a single factor ANOVA was performed to assess the statistical significance of differences between groups; in this way, the analysis is used to test the null hypothesis that the average value of the observed parameter is the same for all strains. For the purposes of this analysis, a p-value less than or equal to 0.05 was used as the threshold for statistical significance. Second, the coefficient of variation (CV) was also calculated as a measure of the data dispersion between strains. Thirdly, a simple fold-comparison was made using CD-1 as the comparator strain; this was calculated by taking the measured parameter in each strain (either C57BL/6 or BALB/c) and dividing it by the corresponding value in CD-1.

Clearance values for different test compounds ranged from 0.061 to 7.4 L/hr/kg. Using an ANOVA analysis of CL, a p-value of less than 0.05 was not observed for 50% of compounds. Percent CV between strains was less than 50% for 88% of compounds. Clearance for BALB/c relative to CD-1 was within two-fold for 100% of compounds tested, and clearance for C57BL/6 relative to CD-1 was within two-fold for 75% of compounds tested. Overall, clearance for either BALB/c or C57BL6 was within two-fold of CD-1 in

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88% of the cases. A detailed summary of the CL data across multiple mouse strains is provided in Table 2.

Volume of distribution values for different test compounds ranged from 0.22 to 27 L/kg. Using an ANOVA analysis of V_{ss}, a p-value of less than 0.05 was not observed for 75% of compounds. Percent CV between strains was less than 50% for 100% of compounds. V_{ss} for BALB/c relative to CD-1 was within two-fold for 100% of compounds tested, and V_{ss} for C57BL/6 relative to CD-1 was within two-fold for 100% of compounds tested. Overall, V_{ss} for either BALB/c or C57BL6 was within two-fold of CD-1 in 100% of the cases. A detailed summary of the V_{ss} data across multiple mouse strains is provided in Table 3.

Half-life values for different test compounds ranged from 1.4 to 12 hr. Using an ANOVA analysis of $t_{1/2}$, a p-value of less than 0.05 was not observed for 63% of compounds. Percent CV between strains was less than 50% for 75% of compounds. Half-life for BALB/c relative to CD-1 was within two-fold for 100% of compounds tested, and half-life for C57BL/6 relative to CD-1 was within two-fold for 50% of compounds tested. Overall, half-life for either BALB/c or C57BL6 was within two-fold of CD-1 in 75% of the cases. A detailed summary of the half-life data across multiple mouse strains is provided in Table 4.

Oral bioavailability values for different test compounds ranged from 1.3 to 110%. Using an ANOVA analysis of F, a p-value of less than 0.05 was not observed for 63% of compounds. Percent CV between strains was less than 50% for 63% of compounds. F for BALB/c relative to CD-1 was within two-fold for 88% of compounds tested, and F for C57BL/6 relative to CD-1 was within two-fold for 88% of compounds tested. Overall, F for either BALB/c or C57BL6 was within two-fold of CD-1 in 88% of the cases. A detailed summary of the F data across multiple mouse strains is provided in Table 5.

C_{max} values for different test compounds ranged from 0.024 to 9.6 µM. Using an ANOVA analysis of C_{max}, a p-value of less than 0.05 was not observed for 63% of compounds. Percent CV between strains was less than 50% for 63% of compounds. C_{max} for BALB/c relative to CD-1 was within two-fold for 88% of

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compounds tested, and C_{max} for C57BL/6 relative to CD-1 was within two-fold for 63% of compounds tested. Overall, C_{max} for either BALB/c or C57BL6 was within two-fold of CD-1 in 76% of the cases. A detailed summary of the C_{max} data across multiple mouse strains is provided in Table 6.

T_{max} values for different test compounds ranged from 0.25-2 hr. Because T_{max} is a categorical variable, the data is not suitable for statistical analysis, and therefore ANOVA analysis or CV calculations were not performed. T_{max} for BALB/c relative to CD-1 was within two-fold for 100% of compounds tested, and T_{max} for C57BL/6 relative to CD-1 was within two-fold for 88% of compounds tested. Overall, T_{max} for either BALB/c or C57BL6 was within two-fold of CD-1 in 94% of the cases. A detailed summary of the T_{max} data across multiple mouse strains is provided in Table 7.

Assessment of Inter-day Variability of PK Parameters. For determining typical inter-day experimental variability, chlorpromazine was selected as an exemplar compound. PK was assessed using different animal cohorts across three days in both CD-1 and C57BL/6 (Table 8). Overall, an ANOVA analysis of CL, V_{ss}, half-life, C_{max}, and F afforded p-values greater than 0.05 across inter-day studies in both CD-1 and C57BL/6 strains. Inter-day CV for these parameters ranged from 8.2 to 27%.

In Vitro Hepatocyte Clearance. Hepatocyte CL_{int} ranged from <2.5 to 310 µL/min/million cells. Using an ANOVA analysis of $t_{1/2}$, a p-value of less than 0.05 was not observed for 88% of compounds. Percent CV between strains was less than 50% for 88% of compounds. CL_{int} for BALB/c relative to CD-1 was within two-fold for 88% of compounds tested, and CL_{int} for C57BL/6 relative to CD-1 was within two-fold for 88% of compounds tested. Overall, CL_{int} for either BALB/c or C57BL6 was within two-fold of CD-1 in 88% of the cases. A detailed summary of the CL_{int} data across multiple mouse strains is provided in Table 9.

Discussion

Rodent PK screening is a paramount tool in early small molecule drug discovery that is used for two primary reasons: 1) to understand ADME related liabilities for molecules and 2) to understand the

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exposure-efficacy and exposure-toxicity relationships. Due to efforts (such as a sustainable approach to reduce, replace and recycle resources) to streamline preclinical PK workflows or resource constraints, it is often the case that the strain in a screening PK experiment may not match the strain in a PD or Tox study. This risk is particularly prevalent in mouse studies, in which the selection of PD model strain is often quite variable, depending largely on the therapeutic area of study, and may even change over the course of a discovery program. Currently, we have identified a gap in the available body of literature: there are no publications addressing the issue of whether an appreciable strain-dependent PK difference exists in mouse. To address this, we designed a study using a panel of diverse commercial drugs and systematically evaluated the IV and PO PK in BALB/c, C57BL/6, and CD-1 mice. To our knowledge, this is the first study of its kind.

Analysis of the impact of mouse strain on drug exposure is quite limited in the broader scientific literature. Studies often involve a single compound and were not designed to capture critical PK parameters that are used to inform decisions on drug discovery teams. For example, McCarthy et al. compared the pharmacokinetics of the psychostimulant cocaine in CD-1 and C57BL/6 mice with chronic dosing and reported higher cocaine exposure in C57BL/6 mice compared to CD-1 mice (McCarthy et al., 2004). Guo et al. evaluated warfarin metabolism in 13 different inbred strains of mice, including BALB/c and C57BL/6 following a single IP dose (Guo et al., 2006). The group found substantial differences in formation of certain warfarin metabolites, however, parent warfarin area under the plasma concentration-time curve (AUC) was within 2.1-fold for all strains (Guo et al., 2006). In another study, cotinine and nicotine were independently dosed to groups of DBA/2 and C57BL/6 mice; the resulting PK parameters (AUC, CL/F, t1/2, and C_{max}) for both strains were within two-fold for parent nicotine and cotinine, despite seeing a strain-dependent difference in metabolite formation for each compound (Siu and Tyndale, 2007). When taken together, these studies suggest that although certain metabolic

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pathways may be affected by strain, the elimination of a compound is generally governed by multiple pathways and, as a result, absolute exposure remains relatively consistent between strains.

When selecting compounds in this study to compare PK parameters across mouse strains, we sought to cover as much chemical and ADME space as feasible. We chose compounds with a wide variation in lipophilicity, charge state, chemical functionality, and molecular weight. We also selected compounds that undergo various routes of elimination including phase I metabolism (CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP3A), phase II metabolism (UGTs), urinary elimination, and biliary elimination in human. The limitations here are recognized, as the sample set is relatively small and, as such, not all elimination routes are covered. Additionally, the routes of elimination were based on available human data rather than mouse, so we recognize that there may be some interspecies differences. Despite these caveats, the group of compounds used in this study represents a representative cross section of leads that may arise in drug discovery.

We analyzed the data multiple ways to understand the variability of data between strains. By far the most stringent of metrics was statistical analysis using ANOVA. Using this test, we found that there was not a p-value less than 0.05 for 50, 75, 63, 63, and 63% of compounds in observed CL, V_{ss}, $t_{1/2}$, $C_{max,}$ and F, respectively. Chlorpromazine was an outlier, with strain-dependent differences observed in CL, V_{ss} and $t_{1/2}$. Second, we used percent CV as a metric for the dispersion of parameters. When applying this metric, we found that for 88, 100, 75, 63, 75, and 63% of compounds the CV fell within 50% for CL, V_{ss}, $t_{1/2}$, C_{max}, T_{max}, and F, respectively. Understanding the statistical significance of variability is important, however, given the inherent variability of PK in animals and the limited number of mice used within each study (n=3), basing decisions entirely on statistical approaches seems overly prescriptive. As a more empirical and straightforward approach we also used a two-fold cutoff to look for strain-dependent differences in PK parameters. In our experience, tolerance for PK variability in early discovery, often referred to as screening PK, is generally acceptable if parameters are within two-fold

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(see discussion below). Using this tolerance for variability, we found that parameters were within two-fold for 88%, 100%, 75%, 76%, 94% and 88% of compounds for CL, V_{ss}, $t_{1/2}$, C_{max}, T_{max}, and F, respectively.

Screening PK experiments are carried out in a relatively high-throughput fashion at relatively low doses, and, as a result, there is an expectation for appreciable variability. These experiments are often used to guide the prioritization of molecules, and the values derived from these experiments are treated cautiously, rather than absolutely. To assess variability in PK parameters, we ran an IV/PO study using chlorpromazine dosed to cohorts (n=3) of CD-1 and C57BL/6 animals on three separate days. The data showed little variability; percent CV was less than 30% for all parameters measured and there was no case where we observed a p-value less than 0.05 for ANOVA analysis of PK parameters. Although we generally observed little inter-day variability in PK parameters for a single compound (chlorpromazine), the scientific community generally accepts two-fold variability in PK parameters from in vivo studies (FDA, 2003; Abduljalil et al., 2014; Daublain et al., 2017) acknowledging diversity in subjects (animal or human), variability in drug metabolism enzymes and transporters expression due to genetics, diet and environmental factors (Yang et al., 2013; Tracy et al., 2016) as well as possible experimental errors. The FDA guidance on Pharmacokinetics in Patients with Impaired Hepatic Function (FDA, 2003) recommends using a confidence interval approach (2-fold or greater increase in AUC) rather than statistical significance to indicate PK differences in hepatically impaired patients. Primary literature to support a 2-fold threshold in preclinical species is sparse, however authors in a recent publication used a two-fold cutoff when considering tolerable intra-animal and inter-animal screening PK variability (Daublain et al., 2017). In their analysis of a large dataset (~17,000 compounds) of observed inter-animal PK for all typical preclinical animals (mouse, rats, dogs, and monkeys) combined the authors observed the variability was within two-fold for approximately 80% and 60% of all IV and PO dosing events, respectively (Daublain et al., 2017). These data show that for the same compound, within the same dosing event, the PK variability can be somewhat high, however it generally falls within two-fold. In this study, using a 2-fold

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tolerance for variability, we found that parameters were within two-fold for 88%, 100%, 75%, 76%, 94%, 88% of compounds for Cl, V_{ss}, $t_{1/2}$, C_{max}, T_{max} and F, respectively, supporting the idea that single strain PK is acceptable for discovery PK screening.

Finally, to see if in vivo strain-dependent differences in CL could be observed in vitro, we measured the hepatocyte CL_{int} in each strain. Using CD-1 as the comparator strain, we found 88% of compounds had hepatocyte CL_{int} values that fall within two-fold for both C57BL/6 and BALB/c. Interestingly, the outlier compound was chlorpromazine, which was the compound with the most observed strain-dependent CL in vivo. Looking further, we found the rank ordering for chlorpromazine hepatocyte CL_{int} (BALB/c > C57BL/6 > CD-1) did not match the CL in vivo (BALB/c > CD1 > C57BL/6). Based on this, hepatocyte CL_{int} did not appear to be predictive of CL differences in vivo, which implies that multiple strain hepatocyte CL_{int} screening may be of little utility on its own without additional data. Perhaps scaling intrinsic hepatocyte clearance to systemic clearance by incorporating strain-specific plasma protein binding, hepatocyte binding, and physiological scaling factors would provide more utility towards predicting inter-strain differences in clearance. This exercise is a subject of future work.

Overall, the in vitro results in this study were consistent with previous work. Richmond et al measured microsomal clearance for 96 model compounds using liver microsomes obtained from BALB/c, C57BL/6 and CD-1 mice. The clearance values were binned into either low, medium or high clearance categories, and the authors found that approximately 95% of compounds had no strain-dependent differences in the categorical interpretation of clearance (Richmond et al., 2010). Lofgren et al. also showed enzyme activity between CD-1 and C57BL/6 microsomes was within two-fold for twelve of thirteen cytochrome P450 probe substrates tested (Lofgren et al., 2004). Similarly, the authors from another study found a lack of strain dependence of coumarin 7-hydroxylase activity in microsomes prepared from eight different strains of mouse, including BALB/c and C57BL/6 (van Iersel et al., 1994). Although it appears the clearance is largely unaffected by strain for most compounds, it should be noted that specific

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examples of strain-dependent differences have been shown in enzyme kinetics, metabolite formation, enzyme abundance, and enzyme inducibility (Siu and Tyndale, 2007; Wang et al., 2019).

In summary, we measured PK for 8 small molecules in three strains of mice. Some statistical strain-dependent differences were observed, however, we found good general agreement of PK between strains: 88%, 100%, 75%, 76%, 94%, and 88% of compounds were within two-fold across strains for CL, V_{ss}, $t_{1/2}$, C_{max}, T_{max}, and F, respectively. Overall, we recommend that an approach using a single strain of mouse is appropriate for discovery screening PK, if caution is exercised. The data suggest that testing hepatocyte CL_{int} across strains may not be predictive of in vivo CL on its own. Therefore, in cases where the PK strain does not match the PD or Tox strain, we recommend to periodically spot-check major strain-dependent differences within a chemical series to mitigate risk of PK/PD or PK-Tox translational errors.

Authorship Contributions

Participated in research design: Barr, Rock, Wahlstrom, Dahal

Conducted experiments: Tran

Contributed new reagents or analytic tools: N/A

Performed data analysis: Barr, Tran, Dahal

Wrote or contributed to the writing of the manuscript: Barr, Rock, Wahlstrom, Dahal

References

Abduljalil K, Cain T, Humphries H, and Rostami-Hodjegan A (2014) Deciding on success criteria for predictability of pharmacokinetic parameters from in vitro studies: an analysis based on in vivo observations. *Drug Metab Dispos* **42:**1478-1484.

Barr JT, Lade JM, Tran TB, and Dahal UP (2019) Fraction Unbound for Liver Microsome and Hepatocyte Incubations for All Major Species Can Be Approximated Using a Single-Species Surrogate. *Drug Metab Dispos* **47:**419-423.

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Daublain P, Feng KI, Altman MD, Martin I, Mukherjee S, Nofsinger R, Northrup AB, Tschirret-Guth R, Cartwright M, and McGregor C (2017) Analyzing the Potential Root Causes of Variability of Pharmacokinetics in Preclinical Species. *Mol Pharm* **14:**1634-1645.

FDA (2003) Guidance for Industry: Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling.

Guo Y, Weller P, Farrell E, Cheung P, Fitch B, Clark D, Wu SY, Wang J, Liao G, Zhang Z, Allard J, Cheng J, Nguyen A, Jiang S, Shafer S, Usuka J, Masjedizadeh M, and Peltz G (2006) In silico pharmacogenetics of warfarin metabolism. *Nat Biotechnol* **24:**531-536.

Hartvig P, Simonsson B, Oberg G, Wallin I, and Ehrsson H (1988) Inter- and intraindividual differences in oral chlorambucil pharmacokinetics. *Eur J Clin Pharmacol* **35:**551-554.

Lofgren S, Hagbjork AL, Ekman S, Fransson-Steen R, and Terelius Y (2004) Metabolism of human cytochrome P450 marker substrates in mouse: a strain and gender comparison. *Xenobiotica* **34:**811-834.

MacCallum DM and Odds FC (2002) Influence of grapefruit juice on itraconazole plasma levels in mice and guinea pigs. *J Antimicrob Chemother* **50:**219-224.

McCarthy LE, Mannelli P, Niculescu M, Gingrich K, Unterwald EM, and Ehrlich ME (2004) The distribution of cocaine in mice differs by age and strain. *Neurotoxicol Teratol* **26:**839-848.

Ngo L, Yoo HD, Tran P, Cho HY, and Lee YB (2017) Population pharmacokinetic analysis of rebamipide in healthy Korean subjects with the characterization of atypical complex absorption kinetics. *J Pharmacokinet Pharmacodyn* **44:**291-303.

Richmond W, Wogan M, Isbell J, and Gordon WP (2010) Interstrain differences of in vitro metabolic stability and impact on early drug discovery. *J Pharm Sci* **99:**4463-4468.

Siu EC and Tyndale RF (2007) Characterization and comparison of nicotine and cotinine metabolism in vitro and in vivo in DBA/2 and C57BL/6 mice. *Mol Pharmacol* **71:**826-834.

Tracy TS, Chaudhry AS, Prasad B, Thummel KE, Schuetz EG, Zhong XB, Tien YC, Jeong H, Pan X, Shireman LM, Tay-Sontheimer J, and Lin YS (2016) Interindividual Variability in Cytochrome P450-Mediated Drug Metabolism. *Drug Metab Dispos* **44:**343-351.

van Iersel M, Walters DG, Price RJ, Lovell DP, and Lake BG (1994) Sex and strain differences in mouse hepatic microsomal coumarin 7-hydroxylase activity. *Food Chem Toxicol* **32:**387-390.

Wang S, Chen L, Wang Q, He Z, Chen S, Zhang H, Li H, Guo P, Li Q, Zhang R, Xing X, Zeng X, Lin W, Xiao Y, Dong G, Ma L, Gurram N, Zhang A, Chen W, and Li D (2019) Strain differences between CD-1 and C57BL/6 mice in expression of metabolic enzymes and DNA methylation modifications of the primary hepatocytes. *Toxicology* **412:**19-28.

Yang L, Price ET, Chang CW, Li Y, Huang Y, Guo LW, Guo Y, Kaput J, Shi L, and Ning B (2013) Gene expression variability in human hepatic drug metabolizing enzymes and transporters. *PLoS One* **8:**e60368.

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Figure Legends

Figure 1. Chemical structures of compounds used in this study.

Figure 2. Plasma concentration-time profiles of compounds in CD1, C57BL/6 and BALB/c mice following a 1 mg/kg IV dose (black triangle CD-1, blue square C57BL/6 and green circle BALB/c). Markers and error bars represent the mean and standard deviation, respectively, for three subjects.

Figure 3: Plasma concentration-time profiles of the compounds in CD1, C57BL/6 and BALB/c mice following 5 mg/kg oral dose (black triangle CD-1, blue square C57BL/6 and green circle BALB/c). Markers and error bars represent the mean and standard deviation, respectively, for three subjects.

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Tables

Table 1. Physicochemical properties of compounds and primary elimination route in human

Compound	MW	Class	pKa[a]	LogD (at pH 7.4)	Primary route of elimination
Amiodarone	645.3	Acidic	6.6	5.9	Hepatic metabolism (CYP3A and 2C8) and biliary excretion of metabolites[b]
Captopril	217.3	Zwitterion	3.7, 9.8	-2.8	Urinary excretion of unchanged drug (40-50%) + disulfide dimer or cysteine conjugate[c]
Chlorpromazine	318.9	Basic	9.3	3.2	Metabolism (oxidative) and urinary excretion of metabolites (43-65%)[d]
Dexamethasone	392.5	Neutral	-	-4.6	Hepatic metabolism (CYP3A) and urinary excretion of metabolites (65%)[e]
Fluvastatin	411.5	Acidic	4.5	-2.2	Hepatic metabolism (CYP2C9) and biliary elimination (~90%)[f]
Levofloxacin	361.4	Zwitterion	6.2, 8.7	-0.39	Urinary excretion of unchanged drug (~87%)[g]
Naproxen	230.3	Acidic	4.2	0.35	Urinary excretion of glucuronide metabolite (66-92%)[h]
Propranolol	259.4	Basic	9.4	0.79	Metabolism (oxidative - CYP1A2 & 2D6 and glucuronidation)[i]

[a]https://pubchem.ncbi.nlm.nih.gov
[b]https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/018972s047lbl.pdf
[c]https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/018343s084lbl.pdf
[d]https://genesight.com/wp-content/uploads/2017/05/Chlorpromazine-Thorazine-FDA-Label.pdf
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[g]https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/020634s045,020635s048,021721s031lbl.pdf
[h]https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020353s028lbl.pdf
[i]https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/016418s080,016762s017,017683s008lbl.pdf

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Table 2. Measured CL parameters and the inter-strain comparisons for eight compounds.

Compound	Average CL ± SD(L/hr/kg)			p-value	%CV	BALB/c ÷ CD-1	C57BL/6 ÷ CD-1
	BALB/c	C57BL/6	CD-1				
Amiodarone	1.2 ± 0.040	0.73 ± 0.061	1.4 ± 0.20	0.0017*	30	0.87	0.53
Captopril	0.32 ± 0.087	0.21 ± 0.088	0.44 ± 0.080	0.049*	35	0.73	0.48
Chlorpromazine	7.4 ± 1.2	1.7 ± 0.46	4.5 ± 0.46	0.00039*	63	1.7	0.38
Dexamethasone	1.0 ± 0.24	1.1 ± 0.46	1.3 ± 0.38	0.65	12	0.78	0.87
Fluvastatin	2.7 ± 0.0071	1.6 ± 0.071	2.2 ± 0.34	0.027*	25	1.2	0.75
Levofloxacin	3.9 ± 0.29	3.7 ± 0.34	4.4 ± 1.1	0.46	9.4	0.88	0.84
Naproxen	0.073 ± 0.0085	0.061 ± 0.0094	0.065 ± 0.0050	0.26	8.9	1.1	0.93
Propranolol	4.0 ± 1.2	3.1 ± 0.86	4.5 ± 0.46	0.45	18	0.88	0.69

*Indicates p-value of 0.05 or less found by a one-way ANOVA test

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Table 3. Measured V_{ss} parameters and the inter-strain comparisons for eight compounds.

Compound	Average V_{ss} ± SD (L/kg)			p-value	%CV	BALB/c ÷ CD-1	C57BL/6 ÷ CD-1
	BALB/c	C57BL/6	CD-1				
Amiodarone	18 ± 2.8	10 ± 0.45	15 ± 4.4	0.036*	29	1.2	0.67
Captopril	1.6 ± 0.020	1.6 ± 0.68	2.2 ± 0.78	0.42	20	0.72	0.72
Chlorpromazine	27 ± 4.7	18 ± 3.0	15 ± 4.9	0.031*	32	1.8	1.2
Dexamethasone	2.8 ± 0.42	4.1 ± 3.7	4.6 ± 2.1	0.69	24	0.62	0.89
Fluvastatin	4.1 ± 1.1	5.0 ± 1.4	4.1 ± 1.3	0.71	12	1.0	1.2
Levofloxacin	8.3 ± 3.8	7.4 ± 1.9	8.5 ± 2.0	0.87	7.4	0.97	0.87
Naproxen	0.22 ± 0.024	0.26 ± 0.028	0.22 ± 0.024	0.14	10	0.97	1.2
Propranolol	9.4 ± 2.4	11 ± 2.4	6.2 ± 5.1	0.12	27	1.5	1.7

* Indicates p-value of 0.05 or less found by a one-way ANOVA test

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Table 4. Measured $t_{1/2}$ parameters and the inter-strain comparisons for eight compounds.

Compound	Average $t_{1/2}$ (hr)			p-value	%CV	BALB/c ÷ CD-1	C57BL/6 ÷ CD-1
	BALB/c	C57BL/6	CD-1				
Amiodarone	12 ± 2.1	11 ± 0.30	9.9 ± 1.3	0.46	8.5	1.2	1.1
Captopril	5.2 ± 1.9	6.5 ± 2.6	4.9 ± 2.4	0.68	15	1.0	1.3
Chlorpromazine	3.1 ± 1.1	9.1 ± 3.4	2.8 ± 1.1	0.020*	71	1.1	3.2
Dexamethasone	2.5 ± 0.39	2.3 ± 1.1	4.0 ± 3.3	0.56	32	0.62	0.57
Fluvastatin	2.8 ± 0.67	4.5 ± 0.40	2.2 ± 0.70	0.039*	38	1.3	2.1
Levofloxacin	2.7 ± 1.7	2.6 ± 1.9	2.5 ± 1.7	1.0	2.5	1.1	1.0
Naproxen	2.8 ± 0.21	5.7 ± 1.1	2.6 ± 0.44	0.0030*	47	1.1	2.2
Propranolol	1.9 ± 0.47	4.4 ± 2.2	1.4 ± 0.85	0.079	63	1.3	3.2

*Indicates p-value of 0.05 or less found by a one-way ANOVA test

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Table 5. Measured F parameters and the inter-strain comparisons for eight compounds.

Compound	Average F (%)			p-value	%CV	BALB/c ÷ CD-1	C57BL/6 ÷ CD-1
	BALB/c	C57BL/6	CD-1				
Amiodarone	31 ± 2.3	22 ± 2.0	38 ± 8.8	0.028*	27	0.80	0.57
Captopril	40 ± 5.5	57 ± 37	45 ± 18	0.69	18	0.90	1.3
Chlorpromazine	25 ± 13	7.1 ± 1.1	17 ± 1.1	0.065	53	1.5	0.44
Dexamethasone	99 ± 12	107 ± 4.4	99 ± 15	0.64	4	1.0	1.1
Fluvastatin	19 ± 1.7	70 ± 2.8	37 ± 4.7	4.0E-06*	61	0.52	1.9
Levofloxacin	57 ± 9.2	38 ± 4.0	48 ± 3.0	0.022*	20	1.2	0.80
Naproxen	89 ± 11	99 ± 6.4	92 ± 26	0.84	5	0.97	1.1
Propranolol	1.2 ± 0.61	5.2 ± 0.19	6.9 ± 4.0	0.061	65	0.19	0.74

*Indicates p-value of 0.05 or less found by a one-way ANOVA test

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Table 6. Measured C_{max} parameters and the inter-strain comparisons for eight compounds.

Compound	Average C_{max} (µM)			p-value	%CV	BALB/c ÷ CD-1	C57BL/6 ÷ CD-1
	BALB/c	C57BL/6	CD-1				
Amiodarone	0.32 ± 0.078	0.33 ± 0.033	0.24 ± 0.11	0.38	17	1.3	1.4
Captopril	9.6 ± 2.2	7.6 ± 4.7	6.1 ± 1.2	0.42	23	1.6	1.2
Chlorpromazine	0.19 ± 0.066	0.082 ± 0.0072	0.17 ± 0.056	0.095	39	1.1	0.48
Dexamethasone	3.1 ± 0.79	5.0 ± 0.84	1.7 ± 0.28	0.0028*	51	1.8	2.9
Fluvastatin	1.0 ± 0.16	2.6 ± 0.51	1.2 ± 0.16	0.0020*	54	0.83	2.2
Levofloxacin	0.49 ± 0.11	0.49 ± 0.051	0.67 ± 0.3	0.46	19	0.73	0.73
Naproxen	88 ± 7.6	97 ± 15	90 ± 13	0.68	5.2	1.0	1.1
Propranolol	0.024 ± 0.0043	0.18 ± 0.0093	0.13 ± 0.084	0.021*	72	0.18	1.4

*Indicates p-value of 0.05 or less found by a one-way ANOVA test

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Table 7. Measured T_{max} parameters and the inter-strain comparisons for eight compounds.

Compound	Average T_{max} (hr)			%CV	BALB/c ÷ CD-1	C57BL/6 ÷ CD-1
	BALB/c	C57BL/6	CD-1			
Amiodarone	1 (0.5 - 1)	1 (0.5 - 1)	2 (0.5 - 4)	43	0.50	0.50
Captopril	0.5 (0.5 - 1)	2 (0.5 - 2)	0.5 (0.5 - 2)	87	1.0	4.0
Chlorpromazine	0.5 (0.5 -0.5)	1 (1 -1)	0.5 (0.5 -1)	43	1.0	2.00
Dexamethasone	1 (1 - 1)	0.25 (0.25 - 0.5)	1 (0.5 - 6)	58	1.0	0.3
Fluvastatin	0.25 (0.25 - 0.25)	0.5 (0.25 - 0.5)	0.25 (0.25 - 0.25)	43	1.0	2.0
Levofloxacin	0.5 (0.5 -0.5)	1 (1 - 1)	0.5 (0.5 - 1)	43	1.0	2.0
Naproxen	0.25 (0.25 - 0.25)	0.5 (0.25 - 0.5)	0.25 (0.25 - 0.5)	43	1.0	
Propranolol	1 (0. 5- 2)	1 (1 - 1)	1 (0.5 - 1)	0	1.0	1.0

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Table 8. Chlorpromazine PK parameters for studies performed on three separate days.

		Study 1	Study 2	Study 3	p-value	%CV
CD-1	CL(L/hr/kg)	4.5 ± 0.46	5.5 ± 0.56	4.4 ± 0.72	0.11	13
	V_{ss} (L/kg)	15 ± 4.9	13 ± 2.3	12 ± 6.2	0.78	10
	$t_{1/2}$ (hr)	2.8 ± 1.1	2.3 ± 0.48	2.7 ± 1.5	0.85	10
	C_{max} (µM)	0.17 ± 0.056	0.12 ± 0.015	0.20 ± 0.015	0.075	25
	T_{max} (hr)	0.5 (0.5 - 1)	1 (0.5 - 2)	1 (0.5 - 1)	ND	ND
	F (%)	17 ± 1.1	18 ± 3.2	21 ± 5.9	0.40	11
C57BL/6	CL (L/hr/kg)	1.7 ± 0.46	1.6 ± ND	2.6 ± 1.1	0.44	29
	V_{ss} (L/kg)	18 ± 3.0	21 ± ND	19 ± 4.9	0.76	9.1
	$t_{1/2}$ (hr)	9.1 ± 3.4	12 ± ND	7.1 ± 4.3	0.56	27
	C_{max} (µM)	0.082 ± 0.0072	0.075± 0.012	0.11 ± 0.025	0.07	21
	T_{max} (hr)	1 (1 - 1)	0.5 (0.5 - 1)	0.5 (0.5 - 1)	ND	ND
	F (%)	7.1 ± 1.1	10 ± 3.9	10 ± 2.2	0.38	19

ND = not determined

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Table 9. Inter-strain comparison for hepatocyte CL_{int} across 3 mouse strains and 8 compounds.

	Hepatocyte CL_{int} (µL/min/mil cells)						
	BALB/c	C57BL/6	CD-1	p-value	%CV	BALB/c ÷ CD1	C57BL/6 ÷ CD1
Amiodarone	15 ± 0.44	29 ± 7.5	26 ± 0.43	0.10	32	0.59	1.1
Captopril	<2.5 ± ND	<2.5± ND	<2.5± ND	ND	ND	1.0	1.0
Chlorpromazine	16 ± 5.2	7.0 ± 0.055	<2.5 ± ND	0.070	79	6.2	2.8
Dexamethasone	6.7 ± 0.35	6.2 ± 1.2	4.8 ± 2.4	0.52	17	1.4	1.3
Fluvastatin	87 ± 1.1	48 ± 2.6	68 ± 0.31	0.00041*	28	1.3	0.71
Levofloxacin	4.1 ± 0.64	5.0 ± 0.44	6.5 ± 2.0	0.30	23	0.64	0.77
Naproxen	19 ± 3.3	8.5 ± 2.8	13 ± 1.6	0.058	40	1.5	0.66
Propranolol	310 ± 23	250 ± 86	160 ± 5.8	0.24	31	1.9	1.6

*Indicates p-value of 0.05 or less found by a one-way ANOVA test; ND = not determined.

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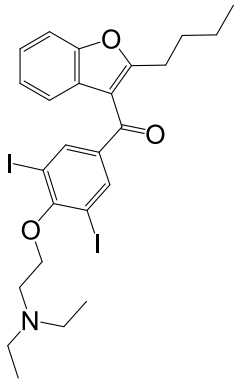
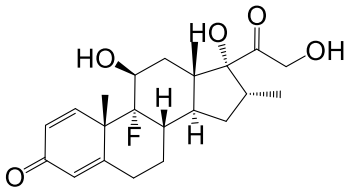


amiodarone



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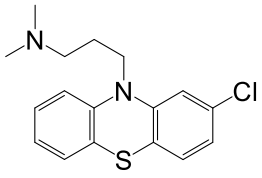

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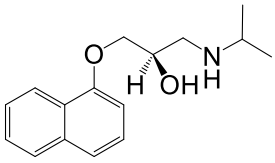

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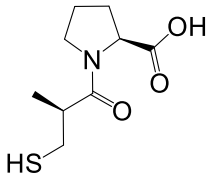
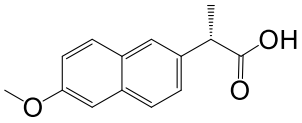
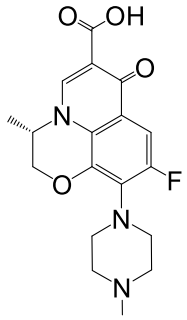


captopril



naproxen



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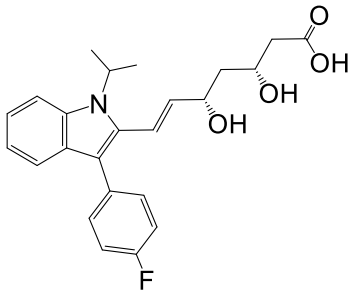

fluvastatin

Figure 1

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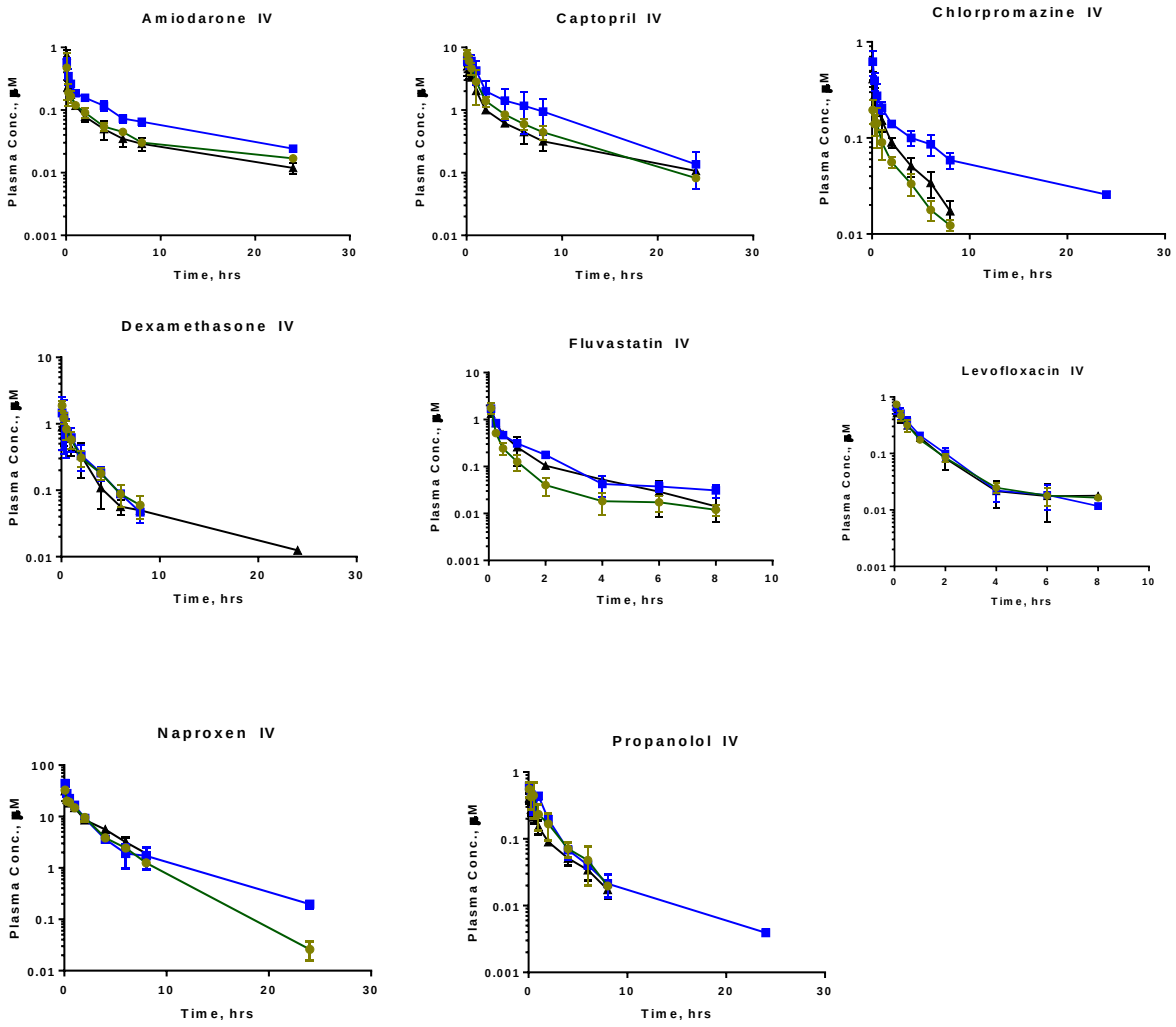

Figure 2

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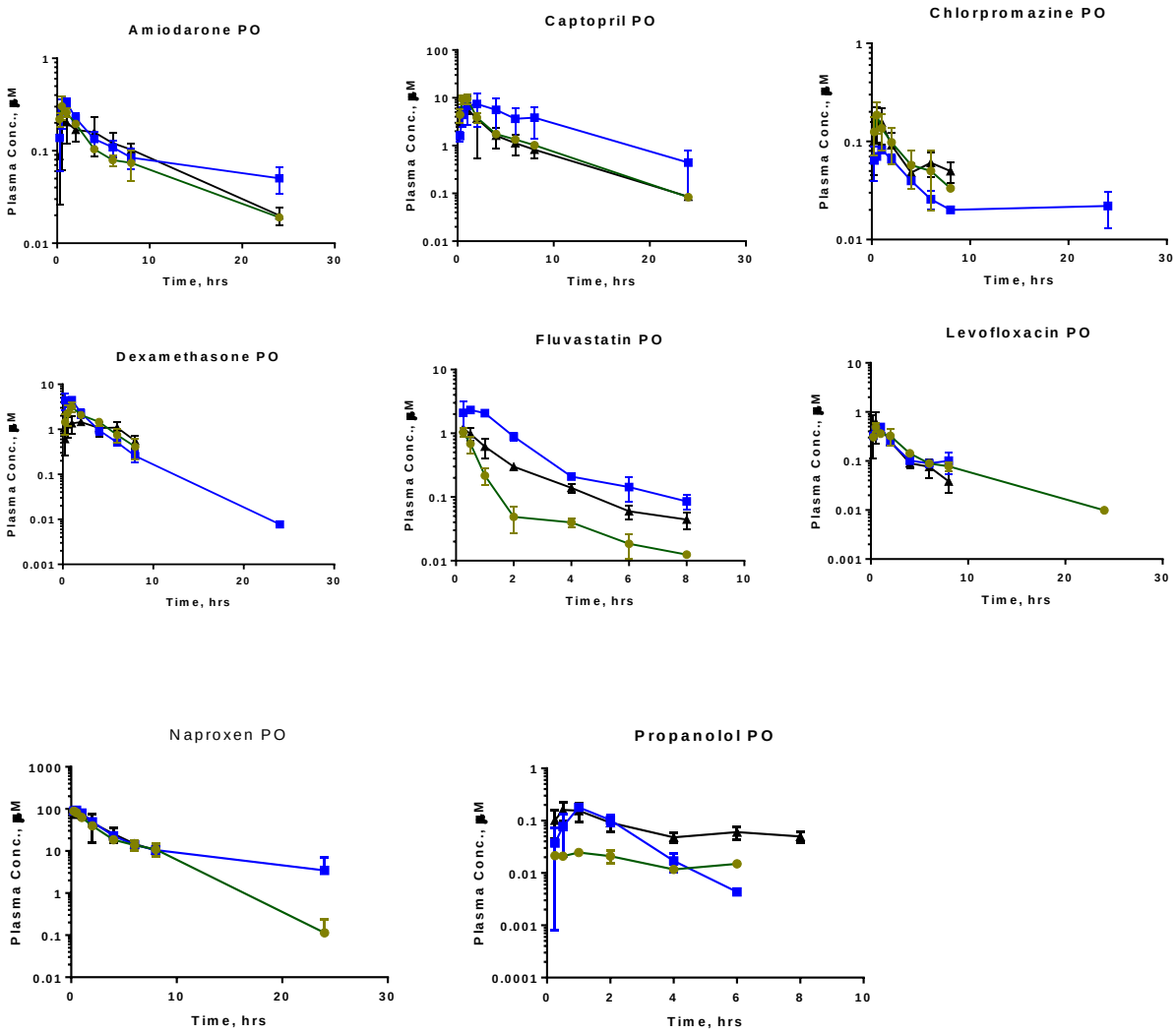

Figure 3

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