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Pyridoxic Acid as Endogenous Biomarker of Renal Organic Anion Transporter Activity: Population Variability and Mechanistic Modeling to Predict Drug–Drug Interactions

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ABSTRACT

Pyridoxic acid (PDA) was suggested as a potential endogenous biomarker to assess in vivo renal organic anion transporter (OAT) 1 and 3 activity. Here, we first investigated the population variability in the plasma baseline levels of PDA using data from five independent studies (conducted/supported by Pfizer), and subsequently developed mechanistic physiologically based pharmacokinetic (PBPK) model to assess its effectiveness in biomarker-informed drug–drug interaction (DDI) predictions. Meta-analysis suggested that the inter-individual variability in PDA plasma concentration was ~40% across all five studies ($n = 71$ subjects). While sex-dependent differences were not evident, the baseline plasma PDA levels were significantly higher (38%, $p < 0.05$) in White males compared to Japanese males. Correspondingly, the amount of PDA excreted in urine and renal clearance were significantly higher ($p < 0.05$) in Japanese males (1.5- and 2.2-fold, respectively), compared to White males. A PBPK model considering relative activity factor-based scaling of in vitro transport data indicated > 80% contribution by OAT3 to the renal clearance of PDA. The baseline plasma concentrations across multiple studies were recovered by the model; and using in vitro inhibition potency data, the model predicted effect of OAT inhibitors (probenecid, ritlecitinib and tafamidis) on PDA pharmacokinetics. Furthermore, DDIs with OAT3 object drug, furosemide, were well-predicted by the biomarker-informed PBPK model. PDA data and the modeling approach indicated lack of clinically-relevant OAT inhibition with ritlecitinib and tafamidis. Overall, this study presents PDA as a reliable biomarker to assess OAT3-mediated renal DDIs with moderate inter-subject and inter-study variability.

1 | Introduction

Kidney plays a critical role in the elimination of drugs and involves processes such as glomerular filtration, active secretion and reabsorption [1]. Solute carrier (SLC) transporters such as organic anion transporters 1 and 3 (OAT1/3), organic cation transporter 2 (OCT2) facilitate the active renal secretion of a variety of small molecule drugs [2]. OAT activity can be modulated by inhibitor drugs potentially causing drug–drug interactions (DDIs) in the clinic [3]. As per the guidance documents by regulatory agencies like the U.S. Food and Drug Administration (FDA), European Medicine Agency (EMA) and more recently International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) M12 guidance, it is required to study a new chemical entity (NCE) as a precipitant of various drug transporters, including OAT1/3 [4–6].

In order to access a NCE as an inhibitor of OAT1/3, in vitro transporter inhibition assays are conducted, followed by static

Summary

- What is the current knowledge on the topic?
  - Pyridoxic acid (PDA) has been proposed as an endogenous biomarker for OAT1/3 function and can be used for renal DDI assessment.
- What question did this study address?
  - Characterize the population variability in PDA pharmacokinetics and evaluate co-variates such as sex and race. Develop a mechanistic PBPK model to enable biomarker-informed OAT1/3-mediated DDI predictions.
- What does this study add to our knowledge?
  - PDA shows consistent baseline plasma levels with moderate inter-individual and inter-study variability. Differences in pharmacokinetic parameters were noted between Whites and Japanese, which is associated with corresponding change in renal clearance. Mechanistic PBPK model informed with in vitro data suggests that PDA pharmacokinetics is sensitive to modulation in OAT3 activity, and also described the effects of inhibitor drugs.
- How might this change drug discovery, development, and/or therapeutics?
  - Our analysis of data from five independent studies present PDA as a reliable and robust biomarker and its plasma levels are well associated with inter-subject variability in OAT3 function and sensitive to transport inhibition. A mechanistic PBPK model for PDA was developed and verified to support quantitative prediction of OAT3-mediated DDIs of the investigation drugs.

DDI predictions. However, this approach is conservative and can lead to false positive predictions which can trigger unnecessary clinical DDI studies with probe drug substrates [7]. The ICH M12 guidance encourages alternative approaches such as use of endogenous biomarkers in interaction risk assessments. Additionally, physiologically based pharmacokinetic (PBPK) modeling can be used for predicting the DDI potential of NCE [8]. In the past decade, endogenous substrates of SLC transporters have been discovered which can be utilized as biomarkers for predicting transporter-mediated DDIs [9]. Endogenous metabolites such as pyridoxic acid (PDA, a catabolite of vitamin B_6), kynurenic acid, taurine, glycochenodeoxycholate-3-sulphate, and 6β-hydroxycortisol have been proposed as potential biomarkers of OAT1/3-mediated active secretion [10–13]. Shen et al. observed that the administration of a probenecid (an OAT1/3 inhibitor) considerably increased plasma concentration of PDA with a corresponding decrease in its renal clearance (CL_R) [13]. These findings demonstrated clinical utility of PDA as a surrogate for prediction of OAT1/3-mediated DDI of NCEs during early clinical drug development.

PDA data from controlled clinical studies is generally limited. Further, plasma concentration of PDA was suggested to show high inter-individual variability arising from various intrinsic and extrinsic factors [13–15]. It is therefore pertinent to characterize the population variability (inter-subject and inter-study) in PDA levels in order to position it as a reliable biomarker. Additionally, biomarker-informed PBPK modeling is emerging as a valuable strategy for DDI risk assessment in early drug development [16–18]. Population-PK and semi-mechanistic PBPK models of PDA have recently been reported, however, these are primarily based on top-down fitting with limited model verification [17, 19].

Here, we analyzed data from five independent studies (conducted/supported by Pfizer Inc.) measuring PDA plasma and/or urine concentrations to assess population variability and the association with sex and race. Furthermore, a PBPK model was developed and verified using these clinical data. In the process, we carried out in vitro transporter assays to determine the contribution of OAT1, OAT2, and OAT3 to the renal disposition of PDA. The generated in vitro intrinsic clearance (CL_{int}) data were scaled to in vivo values using relative activity factor (RAF) approach and then used for the development of a mechanistic PBPK model. The model was further utilized to predict in vivo OAT1/3 inhibition by probenecid and other OAT inhibitor drugs, ritlecitinib and tafamidis.

2 | Material and Methods

2.1 | Pyridoxic Acid (PDA) Clinical Dataset

PDA data from five clinical studies were collated. The clinical data included three DDI studies wherein the effect of probenecid, ritlecitinib or tafamidis on plasma concentration of PDA was monitored. In Study 1, abrocitinib, an OAT3 substrate, was either given alone as a single dose (Arm A), or with twice daily dose of probenecid (Arm B) and PDA plasma concentrations were measured in both the arms ($n = 12$; 7 male, 5 female; and 6 Whites, 6 African American) [20]. Studies 2 and 3 included two periods each, wherein rosuvastatin was either given alone or with putative OAT1/3 inhibitors. In study 2, PDA levels were measured in the absence and presence of ritlecitinib in 12 participants (10 male, 2 female; 8 Whites, 3 Hispanic/Latino, 1 African American) [21]; whereas in Study 3, PDA data were obtained in 12 Whites (11 male and 1 female) in the control and tafamidis-treated arms. Study 4 included 12 Japanese males, wherein metformin was administered to the study participants [22]. Study 5 was a Phase I study involving single or multiple ascending doses of an investigational drug (PF-X) and included 23 male participants (16 Whites, 5 Asian, 1 African American and 1 Hispanic/Latino). Details of drug dosing, sample collection, demographic information are provided in Table 1.

2.2 | Population Variability and Statistical Analysis

Statistical analysis was performed using GraphPad Prism (v10.2; San Diego, CA). Inter-study area under the plasma concentration-time profile from 0 to 24 h ($AUC_{0\text{-}24h}$) values were compared using Kruskal-Wallis test followed by Dunn's multiple comparison test. To study the association of race with PDA $AUC_{0\text{-}24h}$ or maximal plasma concentration (C_{max}), Kruskal-Wallis test followed by Dunn's multiple comparison test was used. Sex-dependent differences in PDA plasma concentration

TABLE 1 | Demographic, dosing and sample collection information of the five clinical studies measuring pyridoxic acid (PDA).

Study #	Study 1	Study 2	Study 3	Study 4	Study 5
Number of participants	12	12	12	12	23
Sex (*n*)	Male (7), female (5)	Male (10), female (2)	Male (11), female (1)	Male (12)	Male (23)
Race/ethnicity (*n*)	White (6), African American (6)	White (8), Hispanic/Latino (3), African American (1)	White (12)	Japanese (12)	White (16), Asian (Others, 4), Japanese (1), African American (1), Hispanic/Latino (1)
Age range (years)	32–61	23–54	24–56	23–38	20–52
Co-administered drug	Probenecid	Ritlecitinib	Tafamidis	—	—
Study period and drug dose (mg)	A. 200 mg abrocitinib B. 1000 mg probenecid, BIDx3 days; 200 mg abrocitinib (on Day 2)	A. 10 mg rosuvastatin B. 200 mg ritlecitinib, QDx10 days; 10 mg rosuvastatin (on Day 8)	A. 10 mg rosuvastatin B. Days 1–2: 61 mg tafamidis BID; Days 3–6: 61 mg tafamidis QD; Day 7: 61 mg tafamidis and 10 mg rosuvastatin	500 mg metformin	Phase I clinical study of Pfizer NCE (PF-X)
Sample collection	Plasma (0–24 h) A. Day 1 B. Day 2	Plasma (0–24 h) A. Day 1 B. Day 8	Plasma (0–24 h) A. Day 1 B. Day 7	Plasma (0–24 h) Urine (0–24 h)	Plasma (0–24) Urine (0–12 h)

Abbreviations: BID, twice a day; NCE, new chemical entity; QD, once a day.

and differences in urine levels of PDA (amount excreted in urine and CL_R) between Whites and Japanese were assessed using Mann–Whitney test. PDA plasma concentration at individual time-points, C_{max}, and $AUC_{0\text{-}24h}$, with and without the inhibitor, were compared using paired *t*-test. $p < 0.05$ was considered statistically significant.

2.3 | Transporter Uptake and Inhibition Studies and In Vitro–In Vivo Translation of Secretory Clearance

Details pertaining to the materials, in vitro uptake assays using transfected HEK293 cells, LC–MS/MS based quantification of analytes and in vitro-in vivo translation to estimate secretory clearance are mentioned in the supplementary file and Table S1.

2.4 | Mechanistic PDA PBPK Model Development and Verification

PBPK modeling was carried out using Simcyp Simulator (v23; Certara, Sheffield, UK). Best practices were followed in developing the PBPK models of PDA and other object and precipitant drugs in healthy population [23]. A full PBPK model of PDA was developed using the biomarker module, wherein the synthesis rate of 0.32 μmol/h (with 32% variability) was added in the model based on a previously reported value estimated using popPK model (Table S2) [19]. PDA is moderately bound to plasma proteins, with fraction unbound in plasma ($f_{u,plasma}$) value of 0.092 [13].

The tissue-to-plasma partition coefficients were predicted using Method 2 (Rodgers & Rowland method), with perfusion limited distribution for all organs (except kidney). For kidney, a permeability-limited mechanistic kidney model (MechKiM) was used. In the MechKiM, passive basolateral and apical diffusion clearance (CL_{PD}) of 0.017 μL/min/10^6 PTC was added based on literature reported data [17]. Our in vitro studies showed limited cellular accumulation in HEK293-WT cells and thus CL_{PD} could not be reliably measured. Basolateral uptake was associated with OATs; wherein, the $CL_{int,OAT1}$, $CL_{int,OAT2}$, and $CL_{int,OAT3}$ (in μL/min/10^6 PTC) values estimated from the in vitro PDA uptake studies along with RAF values were used to capture the overall uptake clearance (Table S2). Apical efflux of PDA in PTC was added via MRP4 transporter [12]. Assuming that basolateral uptake of PDA by OATs is the rate-limiting step, MRP4-mediated CL_{int} value

was set very high ($CL_{int,MRP4}$ = 1000 μL/min/10^6 PTC). Other model assumptions included: no partitioning in the red blood cells (blood to plasma ratio of 0.55), no metabolism (based on literature reports suggesting the renal excretion is the predominant elimination pathway of PDA) [24, 25], and no active reabsorption back into the blood. The baseline plasma PDA concentrations were simulated after a 6 h time to reach steady state. The generic virtual population (50% females) and study-specific virtual populations were used to simulate baseline plasma and urine levels. The model predicted C_{max}, $AUC_{0\text{-}24h}$, and CL_R values were compared with clinically observed datasets and the model simulated plasma and urine profiles were visually inspected for good fit with the literature reported data.

2.5 | PBPK Analysis of PDA Modulation by OAT Inhibitors

PBPK models of inhibitors (probenecid, ritlecitinib and tafamidis) were developed and verified, and the details are mentioned in supplementary file and Tables S3–S5. The effect of these three inhibitor drugs on PDA was then simulated individually. For the effect of probenecid on PDA, model simulations were compared across three independent studies with different probenecid dosage regimen (two literature reports [12, 13] and one in-house Study 1). As for ritlecitinib and tafamidis, data from Study 2 and Study 3 were used to compare with the model simulated plasma concentration-time profiles. A previously published PBPK model of furosemide (probe substrate of OAT3) was optimized and used to predict OAT-mediated DDIs (Table S6). The sensitivity of PDA baseline and interaction models to the input values of various parameters was investigated. For the baseline PDA model, parameters such as CL_{PD} and $CL_{int,MRP4}$ were increased and decreased by 100- to 1000-fold and the effect of this change on $AUC_{0\text{-}24h}$ and CL_R was assessed. Further, in the case of interactions, sensitivity of OAT3 inhibitory constant (K_i) values was studied.

The model predicted C_{max}, $AUC_{0\text{-}24h}$, CL_R, C_{max} ratio, AUC ratio, and CL_R ratio values were compared with clinically observed interaction datasets. PBPK model performance was evaluated by the predicted-to-observed ratio of PK parameters and presented as *R*-value. A R-value between 0.8 and 1.25 (25% error) was assumed as acceptable model performance [26].

3 | Results

3.1 | Population Variability in PDA Baseline Levels Across Studies

Individual subject data from five independent studies were evaluated for intra-study and combined population variability. The predose (0 min) plasma concentration of PDA across the five studies ranged from 1.6 to 9.2 ng/mL with mean value of 3.3 ng/mL. Similarly, the population mean baseline $AUC_{0\text{-}24h}$ was 70.9 ng•h/mL, with individual study-specific values of 86.7 ± 47.4, 70.5 ± 24.3, 64.2 ± 21.2, 55.1 ± 14.9, and 74.6 ± 15.7 ng•h/mL for studies 1–5, respectively (Table S7). The overall variability in the predose plasma concentration and $AUC_{0\text{-}24h}$ was 44% and 38%, respectively. Intra-individual variability in plasma PDA concentration across 0–24 h was low (16% CV) and ranged from 7% to 39% (Figure S1). Comparison of the predose plasma concentration of PDA with baseline $AUC_{0\text{-}24h}$ revealed statistically significant linear relationship, suggesting that predose concentration can potentially be used as a surrogate of the baseline levels in control arm (Figure S2a). While the mean baseline plasma concentrations (across 0–24 h) were overlapping for studies 1–3 and 5, the baseline plasma levels of study 4 participants were consistently low across all the time-points (Figure 1a). The $AUC_{0\text{-}24h}$ values from Study 5 (~70% White males) were significantly higher (35% higher) compared to Study 4, which consisted of all Japanese males (Figure 1b). Analysis of the population variability in plasma concentration revealed non-significant sex-dependent differences in the $AUC_{0\text{-}24h}$ and C_{max} values of PDA (Figure 1c). Similarly, the sex-dependent differences were also not evident between White males and females ($p > 0.05$) (Figure S2b). Comparison of the race-dependent differences highlighted that the average $AUC_{0\text{-}24h}$ values were 38% higher in White males than in Japanese males, with values of 78.2 ± 28.2 and 56.5 ± 15.2 ng•h/mL, respectively ($p < 0.05$; Figure 1d, Table S7). Analysis of the urine samples from Study 4 and 5 revealed that the amount of PDA excreted unchanged in urine was 35% lower in White males compared to Japanese males (Figure 1e, Table S8). PDA CL_R values in White and Japanese males were 250.1 ± 48.2 and 546.4 ± 121.8 mL/min, respectively (Figure 1e, Table S8), suggesting ~54% lower CL_R in White males ($p < 0.05$). While the average $AUC_{0\text{-}24h}$ and C_{max} values of PDA were ~ 30% lower in Hispanic/Latino compared to Whites, statistical significance was not reached ($p > 0.05$) (Figure 1d, Table S7).

3.2 | Effect of OAT Inhibitors on PDA Pharmacokinetics

Administration of multiple oral doses of probenecid (Study 1) significantly increased the $AUC_{0\text{-}24h}$ and C_{max} of PDA (upto 3-fold) (Figures 2a and S3a, Table S7). Probenecid significantly affected PDA plasma concentrations across all time-points from 0 to 24 h ($p < 0.0001$). Ritlecitinib administration (Study 2) showed no significant change in the $AUC_{0\text{-}24h}$ and C_{max} of PDA (Figures 2b and S3b, Table S7) [21]. On the other hand, tafamidis administration showed a significant increase in PDA plasma concentration at only a few time-points (2, 4, and 8 h), and resulted in an overall marginal increase in the mean $AUC_{0\text{-}24h}$ (~1.2-fold) and C_{max} (~1.3-fold) (Figures 2c and S3c, Table S7). PDA predose plasma concentration showed significant correlation with its AUC ratio ($r = 0.8$, $p < 0.05$) and C_{max} ratio ($r = 0.7$, $p < 0.05$) in the presence of probenecid (Figure 2d). Correlation was also significant ($p < 0.05$) in case of tafamidis administration, but not with ritlecitinib (Figure 2d). Similar inverse relationships between AUC or C_{max} ratio and the baseline AUC of PDA were also evident (Figure S3d). These findings suggest that PDA predose plasma concentrations or baseline AUC is a good predictor of individual variability in OAT activity.

3.3 | OAT-Mediated Renal Secretion of PDA

The cellular accumulation of PDA was significantly higher in OAT1- and OAT3-transfected HEK293 cells compared to

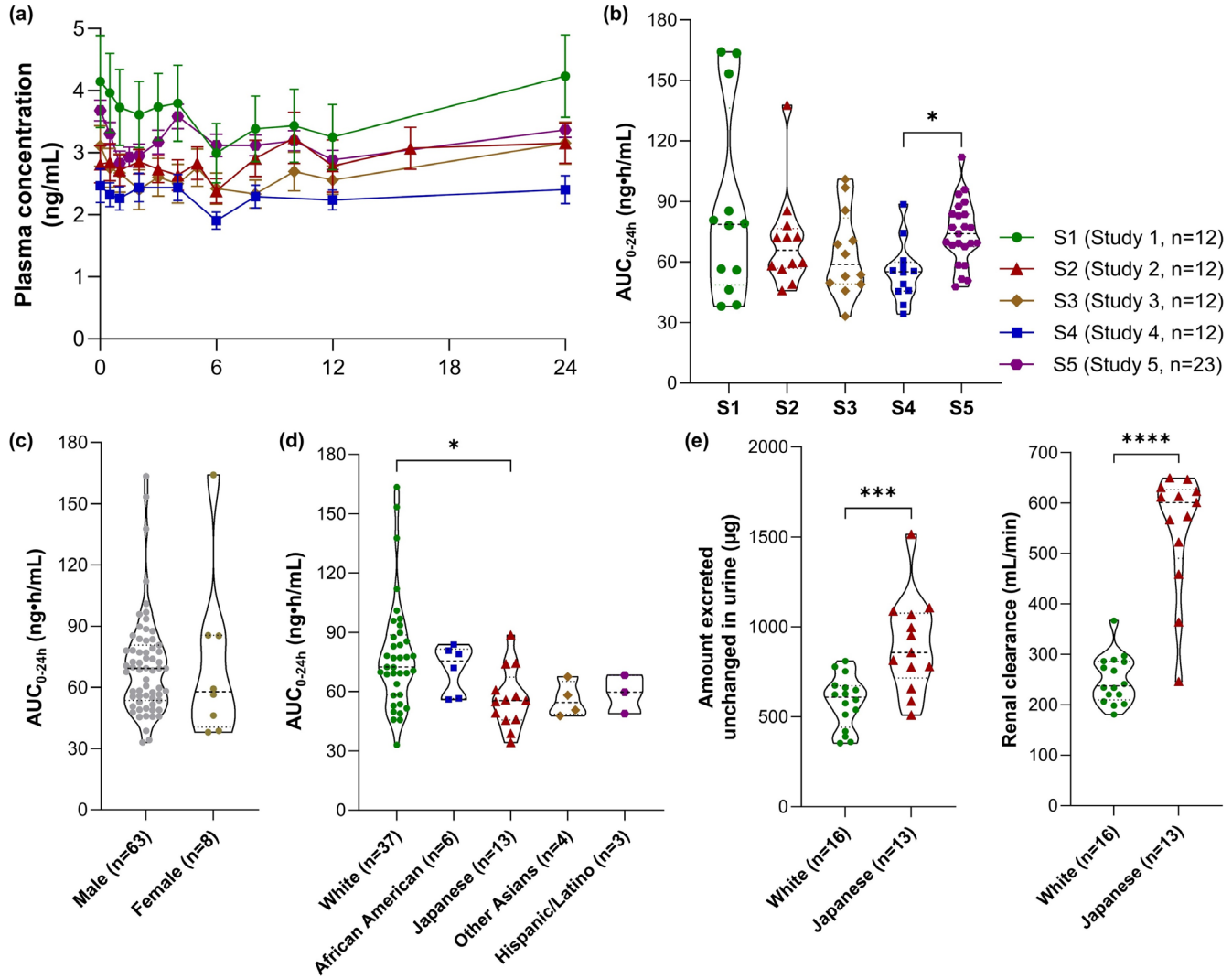

FIGURE 1 | Pharmacokinetic profile and population variability in pyridoxic acid (PDA) levels. (a) Average baseline plasma concentration-time profiles of PDA in five studies (S1–S5). Comparison of the $AUC_{0\text{-}24h}$ values between different (b) studies; (c) sexes; and (d) races (males only); and (e) comparison of amount excreted unchanged in urine and renal clearance (CL_R) between Whites and Japanese males. Error bars indicate standard error mean values. The truncated violin plots indicate the range, median, 25th and 75th percentiles of pharmacokinetic endpoints. Statistical comparisons between different studies and different races were carried out by Kruskal-Wallis test followed by Dunn's multiple comparison test. Two group comparisons (sex-dependent differences and comparison of amount excreted unchanged in urine and CL_R between White and Japanese) were conducted using Mann–Whitney test. *$p < 0.05$; ***$p < 0.001$; and ****$p < 0.0001$.

wild-type HEK293 cells. After fitting the uptake data to a two-compartment model, the CL_{pass} and $CL_{int,OAT1}$, $CL_{int,OAT2}$, and $CL_{int,OAT3}$ values were estimated to be 3.9, 51.3, 5.4, and 117 µL/min/mg protein, respectively (Figure 3a). The active uptake clearance values were then derived by scaling the in vitro data (3.31, 0.35, and 7.55 mL/min/kg via OAT1, OAT2, and OAT3, respectively) using physiological scalars and previously established relative activity factors. Based on the in vitro-in vivo scaling, the contribution of glomerular filtration, OAT1-, OAT2- and OAT3-specific active secretion to the total CL_R of PDA was estimated to be 5%, 7%, 6% and 82%, respectively (Figure 3b). This presents OAT3 as the major driver in the clearance and thus pharmacokinetics of PDA. In vitro studies showed a concentration-dependent inhibition of OAT1- and OAT3-mediated uptake of PDA by probenecid, with IC_{50} values of 15.1 ± 1.1 and 3.2 ± 0.16 µM, respectively (Figure 3c,d).

3.4 | PDA PBPK Model Verification

A PBPK model developed in a healthy volunteer population using in vitro transport data and previously estimated synthesis rate predicted the contribution of glomerular filtration, OAT1, OAT2, and OAT3-mediated clearance of as 3.3%, 6.2%, 6.5%, and 83.9%, respectively, to the overall PDA clearance. The plasma baseline PDA concentrations predicted by the model ranged from 3.2–3.8 ng/mL, with a mean value of 3.5 ng/mL (Figures 4a and S4, Table 2). The developed model predicted the plasma concentration-time profiles from the four in-house studies (Study 1–3, 5) and additionally two literature reported studies [12, 13], and the predicted $AUC_{0\text{-}24h}$ and C_{max} values were within 25% error (*R*-value 0.8–1.25) of the observed values in most cases (Figure S4, Table 2). The majority of the individual subject datapoints across the time-course from the four in-house studies were within the 95% confidence

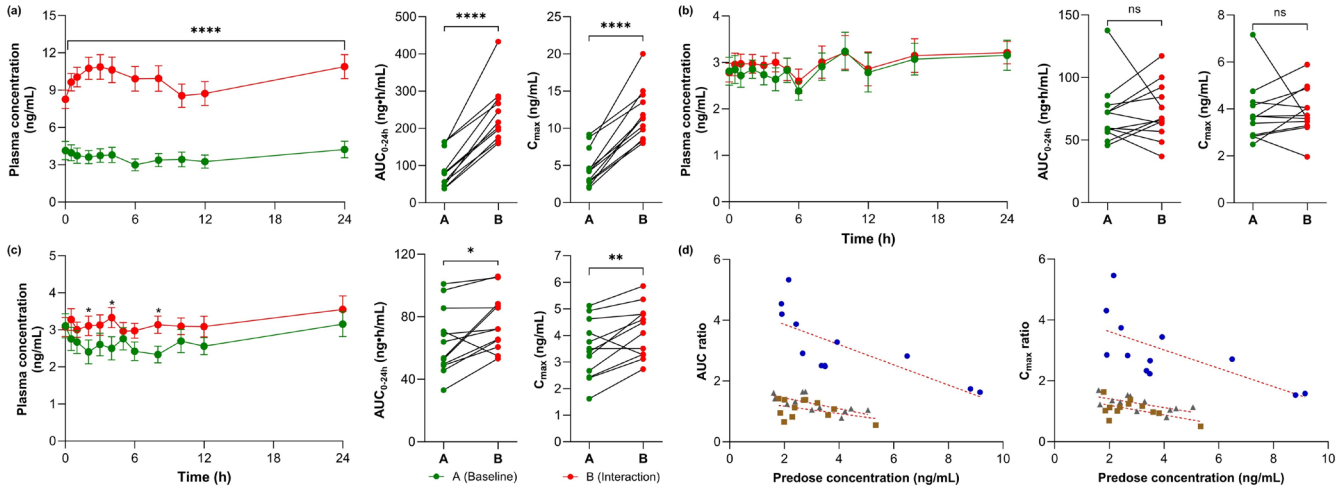

FIGURE 2 | Pharmacokinetic profiles of pyridoxic acid (PDA) in the absence and presence of inhibitors. Average plasma concentration-time profiles, and individual $AUC_{0\text{-}24h}$ and C_{max} of PDA in the absence (green closed circles) and presence (red closed circles) of (a) probenecid; (b) ritlecitinib; and (c) tafamidis. (d) Correlation of predose PDA concentration with PDA AUC and C_{max} ratios on interaction with probenecid (blue closed circles), ritlecitinib (brown closed squares), and tafamidis (gray closed triangles). Error bars indicate standard error mean values. Individual time-points, $AUC_{0\text{-}24h}$, and C_{max} were compared between baseline (absence of inhibitor) and interaction (presence of inhibitor) groups by paired t-test. *$p < 0.05$; **$p < 0.01$; and ****$p < 0.0001$. ns: Non-significant.

interval of the simulated profiles. Model predictions deviated the most in comparison to the observed data from Shen et al. [13], in which case a much larger inter-individual variability was reported (33-fold). While the model slightly overestimated the cumulative amount of PDA excreted unchanged in urine (Figure S5a–c), the predicted CL_R values were withing 25% of the observed data (Table 2). PDA $AUC_{0\text{-}24h}$ and CL_R values were not sensitive to changes in either the CL_{PD} or $CL_{int,MRP4}$ values (Figures S6a–c).

3.5 | Model-Based Prediction of the Effect of Inhibitor Drugs on PDA Pharmacokinetics

For simulating interactions, a previously published probenecid PBPK model was directly adopted, which well-recovered the plasma concentrations of probenecid across a range of single and multiple oral dosing regimens (Figure S7). Similarly, the ritlecitinib PBPK model developed based on a recently reported model [27] was able to capture the observed plasma concentration data (Figure S8a). For tafamidis, a top-down model was developed by optimizing the K_a, f_a, and oral CL values; and the optimized model successfully described the plasma concentration time profiles (Figure S8b).

Model simulation of the effect of multiple oral doses probenecid (1000 mg, BID) showed an increase in mean $AUC_{0\text{-}24h}$ values of PDA (~3-fold), with a corresponding decrease in mean CL_R (70%) (Table 2). The model successfully captured the plasma concentration-time profile of PDA following probenecid dosing within 95% confidence interval values (Figure 4b). The R-value (predicted-to-observed ratio) were 1.0 and 1.2 for AUC and C_{max} ratios. Similarly, simulation of the effect of single oral dose of probenecid (1000 mg) produced about a 2.3-fold increase in $AUC_{0\text{-}24h}$, a 2.9-fold increase in C_{max}, and a 60% decrease in CL_R. While the model successfully predicted the plasma concentration-time profiles (Figure 4c), the cumulative amount excreted unchanged in urine was overpredicted in this specific case (Figure S5d). Nevertheless, the predicted pharmacokinetic endpoints are within 2-fold of the observations. Probenecid at 500 mg multiple-doses increased the PDA plasma concentrations by ~3-fold, which was well captured by the model (Figure 4d). The R-values for AUC, C_{max} and CL_R ratios were 0.9, 0.9, and 1.8, respectively.

Ritlecitinib (200 mg multiple dose), with $C_{max,u}/IC_{50}$ values of 0.03 and 0.11 for OAT1 and OAT3, respectively, triggered regulatory cut-off criteria but showed no impact on PDA exposure. Consistently, the model predicted minimal changes in PDA plasma exposure (R-value 0.9) (Figure 4e, Table 2). Similarly, potential inhibition of OAT3 ($C_{max,u}/IC_{50} = 0.12$) by tafamidis did not translate to a meaningful change in the model predicted PDA plasma concentrations and agreed with the observations (Figure 4f, Table 2). On sensitivity analyses, a ± 100-fold change in probenecid K_i values yielded PDA AUC ratio in the range of 1.02–4.69, while the in vitro K_i used as is produced good predictions. On the other hand, PDA AUC ratios were fairly insensitive to OAT3 K_i values for ritlecitinib and tafamidis (Figure S6d–f). These results show that the current PDA PBPK model was able to accurately predict both the presence and absence of OAT-mediated DDIs.

3.6 | Biomarker-Informed Prediction of Furosemide-Probenecid DDI

An optimized furosemide PBPK model captured well the elimination profile as depicted by the intravenous plasma and urine data (Figure S9 and Table S9). A first order absorption model captured the observed oral plasma and urine data at doses of 40 and 80 mg (Figure S9 and Table S9). Using the furosemide substrate model

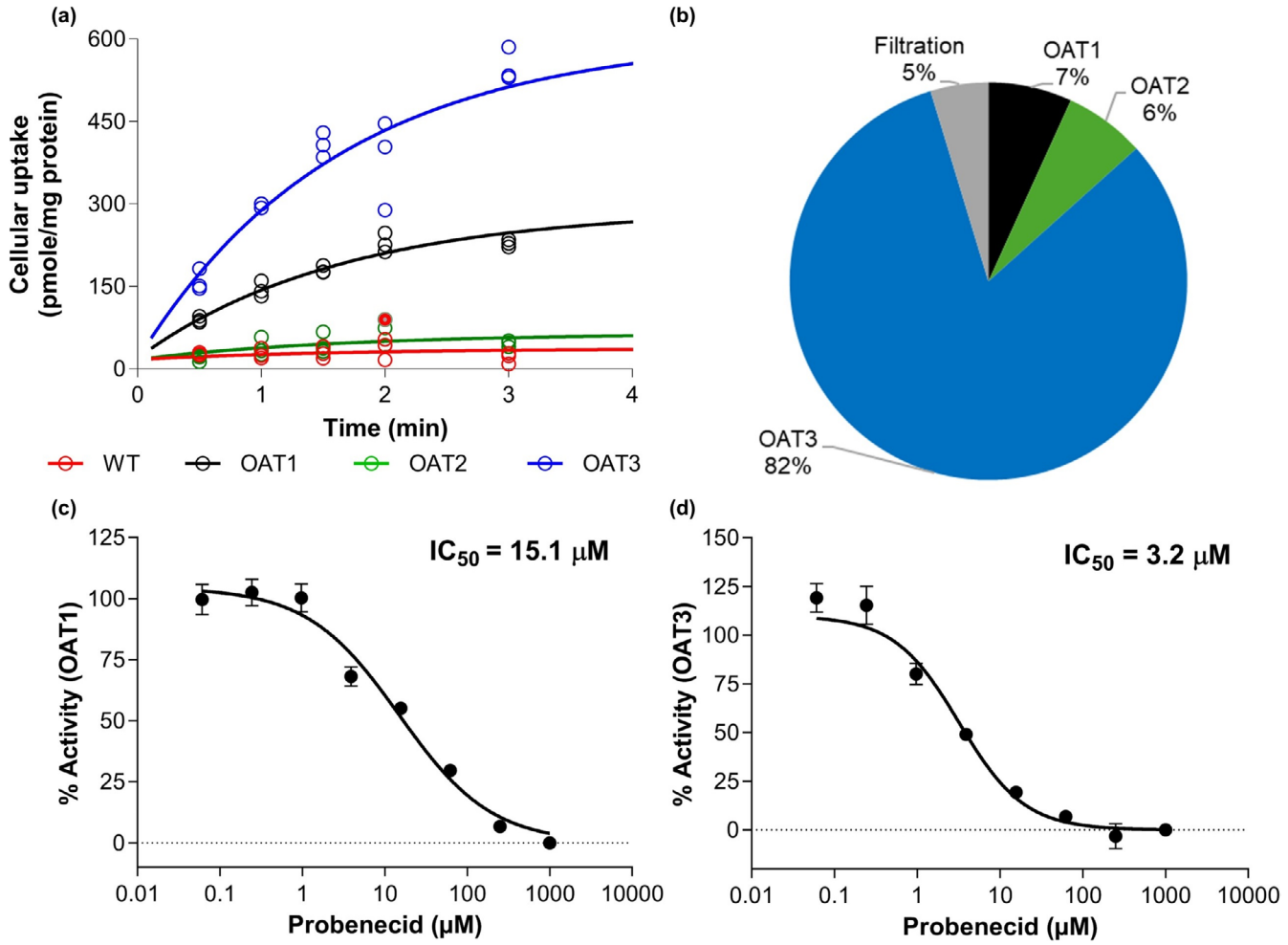

FIGURE 3 | Pyridoxic acid (PDA) in vitro uptake, f_t calculation, and inhibition of uptake by probenecid. (a) Time-dependent uptake of PDA by wild-type, OAT1-, OAT2-, or OAT3-transfected cells as represented by red, black, green and blue open circles, respectively. (b) Fractional contribution of glomerular filtration, OAT1, OAT2 and OAT3 to the overall renal clearance of PDA calculated by IVIVE of in vitro time-dependent uptake data. Percentage of remaining activity of (c) OAT1- and (d) OAT3-mediated uptake of PDA in the presence of different concentrations of probenecid. IC_{50}: Inhibitor concentration required to inhibit the transporter-mediated uptake by 50%.

and the probenecid model verified with PDA datasets, DDIs under different dosing conditions were simulated. Intravenous and oral plasma exposures of furosemide increased, and the CL_R decreased, following oral administration of probenecid (Figure 5, Table S10). Model predictions are generally consistent with the observed $AUC_{0\text{-}24h}$ and CL_R changes across multiple reports (Table S10).

4 | Discussion

Our meta-analysis of endogenous PDA plasma levels and urinary clearance data from about 70 subjects across five independent studies yielded valuable insights into the population variability of this biomarker. Firstly, PDA plasma baseline concentrations generally showed small inter-subject and inter-study variability with limited inter-day fluctuations, which is an important characteristic required for a reliable biomarker [9]. Secondly, PDA levels are not influenced by sex but were found to be associated with ethnicity, with significantly lower baseline plasma concentrations and a correspondingly higher CL_R in Japanese males compared to White males. Third, a significantly linear relationship between PDA baseline levels and change in its AUC or C_{max} in the presence of inhibitor drugs depicts that PDA plasma levels can be used as a surrogate of individual OAT activity. A PBPK model developed based on mechanistic in vitro data effectively described PDA pharmacokinetics in the absence and presence of inhibitor drugs. Collective data and mechanistic modeling positions PDA as a reliable and robust biomarker to assess OAT3-mediated renal DDIs in particular (study workflow provided in Figure S10).

PDA, an end-product of vitamin B6 metabolism, is formed in the liver from pyridoxal by the action of aldehyde dehydrogenase or aldehyde oxidase [24, 32]; and it is thought to be mostly excreted unchanged in the urine through active renal secretion mediated by OATs. PDA plasma levels can be perturbed by differences in either synthesis or elimination rate which can be affected by various intrinsic factors (e.g., age, sex, race, kidney health status, other underlying disease state, etc.) and extrinsic factors (e.g., diet, vitamin B6 deficiency/supplementation, exercise, coadministered drugs, etc.) [14, 15, 25, 33–39]. High variability (~33-fold) in predose PDA plasma concentrations reported by Shen et al.

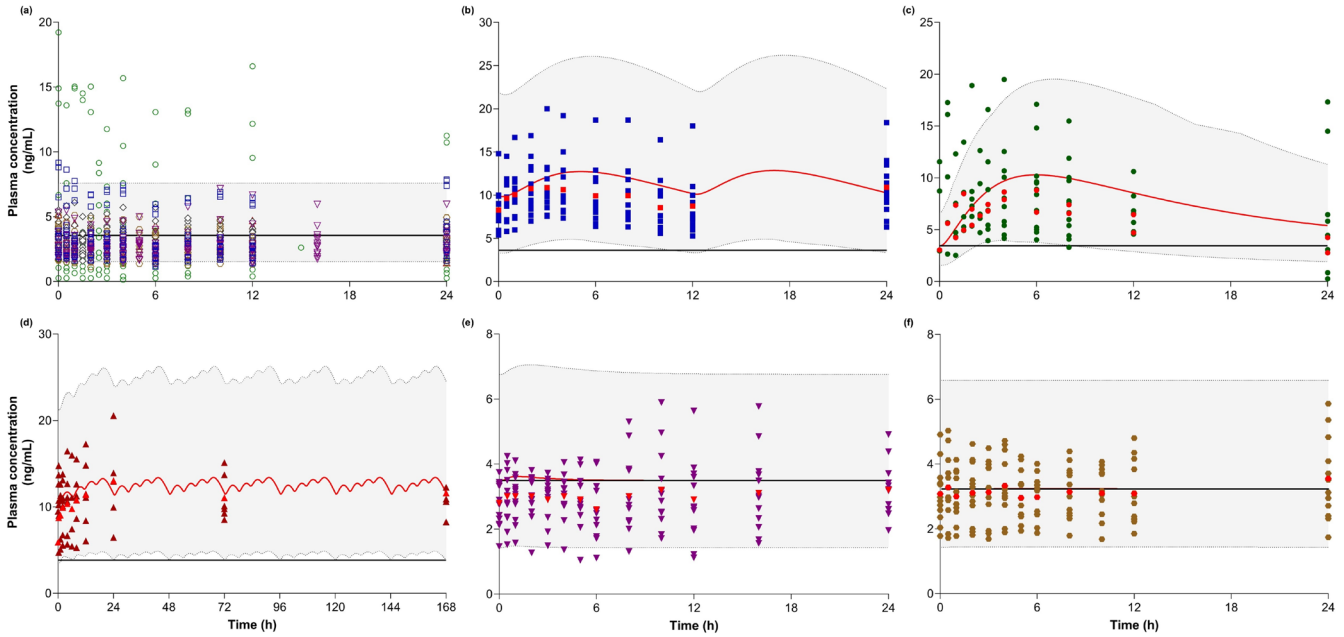

FIGURE 4 | Pyridoxic acid (PDA) model predictions in the absence and presence of inhibitors. (a) Model predicted baseline PDA plasma concentration (solid black line) in a generic virtual population with 50% females. Model predicted interaction profile of PDA (solid red line) in the presence of (b) 1000 mg multiple oral doses of probenecid (Study 1); (c) 1000 mg single oral dose of probenecid (Shen et al. [13]); (d) 500 mg multiple oral doses of probenecid (Willemin et al. [12]); (e) 200 mg multiple oral doses of ritlecitinib; and (f) 61 mg multiple oral doses of tafamidis. Predicted plasma concentration-time profiles of PDA at baseline and in the presence of an inhibitor are represented by solid black and red lines, respectively, with gray shaded area representing the 5th and 95th percentile of population levels in the (a) baseline and (b–f) interaction scenarios. Open symbols in (a) represent baseline clinical PDA data with individual simulations based on the clinical study design shown in Figure S4. Closed symbols in (b–f) represent interaction data of PDA in the presence of different inhibitors.

[13] is likely driven by multiple factors specific to the population (Indian) in which the study was conducted. However, variability in PDA predose plasma concentrations and $AUC_{0\text{-}24h}$ across our five studies is much lower (< 6-fold), suggesting a consistent signal with reasonable population variability to enable DDI risk assessment strategy in early clinical studies wherein the subject numbers are typically small. In a National Health and Nutrition Examination survey conducted by the National Center for Health Statistics, association of serum PDA levels with demographic and lifestyle variables was assessed. While age (20 to > 60 years) was significantly associated with PDA concentrations, sex-dependent differences were non-significant [33]. This is in line with the present results, wherein it was found that the sex-associated differences were minimal. The survey also found significant race-dependent differences between non-Hispanic black versus non-Hispanic white and Mexican American versus non-Hispanic white [33]. While we did not find significant differences in PDA plasma levels between Whites and African Americans, the differences were significant between Whites and Japanese. While dietary differences between White males and Japanese males can potentially drive the plasma PDA level differences, the CL_R data suggested that plasma concentration differences are likely driven by the difference in elimination kinetics between Whites and Japanese. The observed ~2.2-fold higher CL_R of PDA in Japanese could potentially be due to differences in the abundance or activity of renal transporters between the two populations. We further mined the literature on other OAT1/3 substrates (furosemide, adefovir, famotidine, rosuvastatin) to assess the race differences in their CL_R (Table S11) [40–45]. However, data were limited to make a direct comparison between Whites and Japanese. Additional controlled studies are warranted to understand the mechanisms involved in the observed race-dependent differences in PDA CL_R. A detailed comparison of the kidney proteome and plasma metabolome between Whites and Japanese is warranted. Some potential mechanisms which can lead to these differences are: (i) Race-dependent differences in the uptake of PDA by another basolateral transporter; (ii) Reduced reabsorption of PDA in Japanese by another transporter located on the apical membrane; and (iii) Substrate-dependent inhibition of PDA uptake by OAT1/3 in only Whites due to certain plasma metabolite(s) with race-dependent differences. In spite of multiple variables defining PDA pharmacokinetics, a good correlation between predose concentrations and baseline $AUC_{0\text{-}24h}$ across collective 71 participants implies that predose levels from the treatment group can be used to quantify OATs modulation, without requiring a control group (Figure S2a).

Probenecid administration, followed by untargeted metabolomics of plasma and urine samples leads to changes in the metabolome and can be utilized to identify OAT1/3 biomarkers. Such studies have been carried out in preclinical models (rats, cynomolgus monkeys) and humans and identified metabolites belonging to various pathways (steroids, tryptophan metabolites, tyrosine metabolites, bile acid conjugates, glucuronides, fatty acids, etc.) as OAT1/3 substrates [13, 46–49]. In a study conducted in cynomolgus monkeys, probenecid-mediated inhibition of renal OAT1/3 increased PDA plasma concentration with a corresponding decrease in CL_R [48]. Similarly, clinical studies (single and multiple dose probenecid administration) also showed 3- to 4-fold increase in plasma PDA concentration and 70%–85% decrease in CL_R [12, 13]. In our study,

TABLE 2 | Observed versus model predicted pharmacokinetic parameters of pyridoxic acid (PDA) at baseline and in the presence of inhibitors (interaction).

Study (Inhibitor)	Observed or predicted	Study arm	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	CL_R (mL/min)	AUC ratio	C_{max} ratio	CL_R ratio
Study 1 (Probenecid)	**Model predicted**	**Baseline**	86.9 (39.2–181.7)	3.6 (1.6–7.6)	263.6 (190.9–349)			
		Interaction	280.4 (100.6–578.8)	12.9 (4.9–26.2)	81.5 (58.8–133.6)	3.2	3.6	0.31
	Observed	**Baseline**	86.7 ± 47.4	4.6 ± 2.5	—			
		R-value	***1.00***	***0.78***	—			
		Interaction	235.4 ± 76	11.9 ± 3.4	—	3.2	3	—
		R-value	***1.19***	***1.08***	—			
Shen 2019 [13] **(Probenecid)**	**Model predicted**	**Baseline**	82.8 (37.4–160.1)	3.5 (1.6–6.7)	276.7 (216.7–365)			
		Interaction	186.4 (67.1–374.8)	10.3 (4–19.5)	121.7 (90–200)	2.3	2.9	0.44
	Study 1	**Baseline**	46 ± 53.7	3.3 ± 4.6	226.7 ± 268.3			
		R-value	***1.8***	***1.06***	***1.22***			
		Interaction	116 ± 151	9.3 ± 11.5	65 ± 55	3.1	3.1	0.37
		R-value	***1.61***	***1.11***	***1.87***			
	Study 2	**Baseline**	57.9 ± 75.7	3.6 ± 4.5	221.7 ± 221.7			
		R-value	***1.43***	***0.97***	***1.25***			
		Interaction	152 ± 174	10.1 ± 10.2	67.6 ± 41.2	3.2	3.4	0.4
		R-value	***1.23***	***1.02***	***1.80***			
Willemin 2021 [12] **(Probenecid)**	**Model predicted**	**Baseline**	92.2 (42.5–181.7)	3.8 (1.8–7.6)	248.7 (191.1–322.3)			
		Interaction	291.4 (104.5–581.3)	13.4 (4.9–26.3)	78.2 (59–127)	3.2	3.5	0.31
	Observed	**Baseline**	71.8 ± 21.3	3.6 ± 0.8	328.3 ± 203.3			
		R-value	***1.28***	***1.06***	***0.76***			
		Interaction	271 ± 93	13.7 ± 4.1	53.3 ± 25	3.7	3.7	0.17
		R-value	***1.08***	***0.98***	***1.47***			

(Continues)

TABLE 2 | (Continued)

Study (Inhibitor)	Observed or predicted	Study arm	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	CL_R (mL/min)	AUC ratio	C_{max} ratio	CL_R ratio
Study 2 (Ritlecitinib)	**Model predicted**	**Baseline**	83.6 (34.3–162)	3.5 (1.4–6.8)	268.5 (214.1–350.3)			
		Interaction	84 (34.4–163.5)	3.6 (1.5–7.1)	267.2 (212.1–349.3)	1	1	1
	Observed	**Baseline**	70.5 ± 24.3	3.8 ± 1.3	—			
		R-value	***1.19***	***0.92***	—			
		Interaction	72.8 ± 22.6	3.9 ± 1	—	1.1	1.1	—
		R-value	***1.15***	***0.92***	—			
Study 3 (Tafamidis)	**Model predicted**	**Baseline**	77.3 (34.2–157)	3.2 (1.4–6.6)	282.2 (233.1–309.3)			
		Interaction	77.4 (34.4–157.5)	3.2 (1.4–6.6)	281.8 (232.3–307.5)	1	1	1
	Observed	**Baseline**	64.2 ± 21.2	3.5 ± 1.1	—			
		R-value	***1.20***	***0.91***	—			
		Interaction	76.3 ± 18	4.2 ± 1	±	1.2	1.2	—
		R-value	***1.01***	***0.76***	—			
Study 5	**Model predicted**	**Baseline**	82.8 (37.4–160.1)	3.5 (1.6–6.7)	276.7 (216.7–365)			
	Observed	**Baseline**	79.8 ± 20.7	4.6 ± 1.9	230.2 ± 54.2			
		R-value	***1.04***	***0.76***	***1.20***			

Note: AUC, C_{max}, and CL_R ratios were calculated as ratio of the value in the presence of inhibitor/absence of inhibitor.
Abbreviations: $AUC_{0\text{-}24h}$, area under the plasma concentration-time profile from 0 to 24h; C_{max}, maximal plasma concentration; CL_R, renal clearance; R-value: ratio of predicted to observed value.

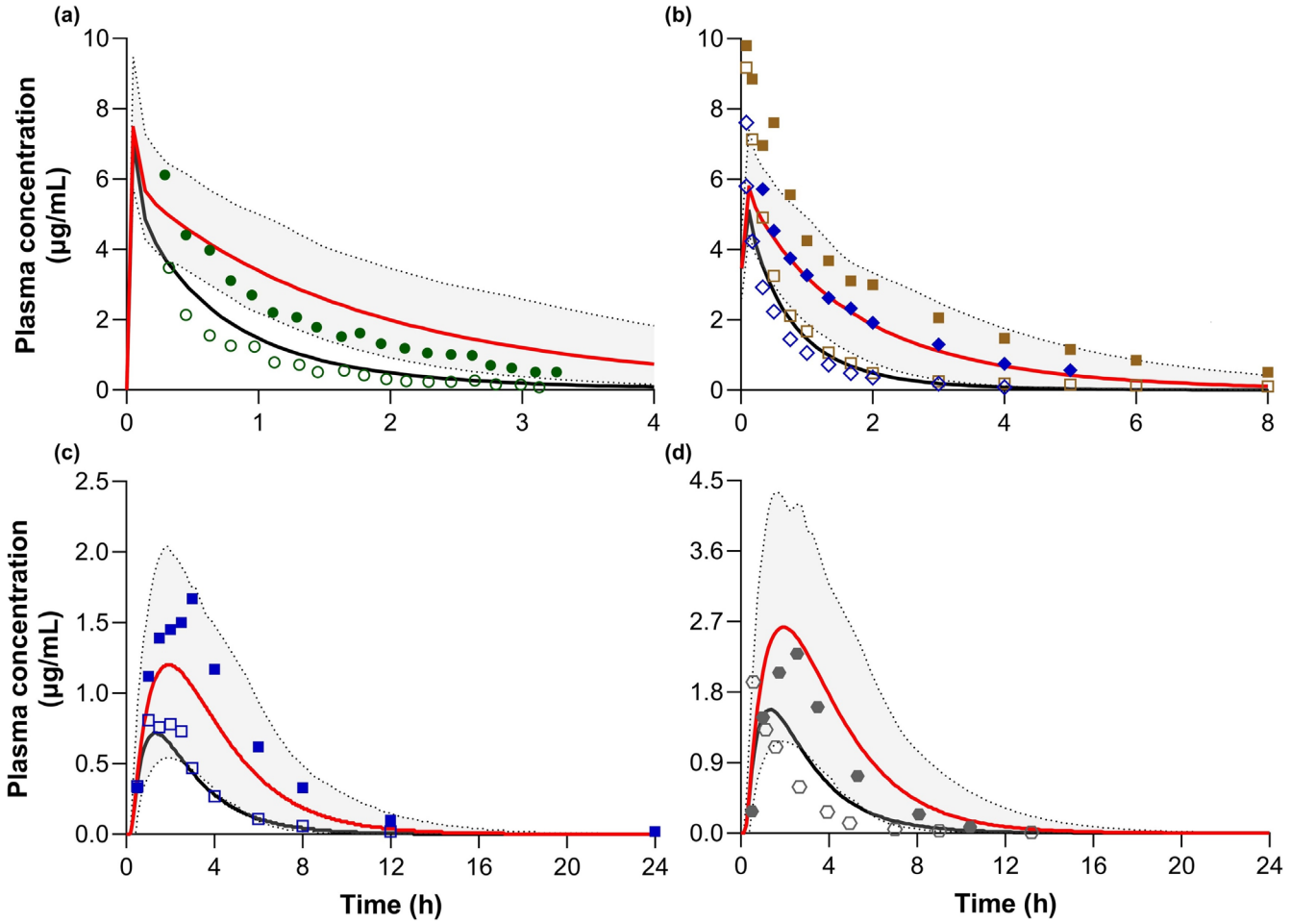

FIGURE 5 | Furosemide model simulations in the absence and presence of probenecid. Model predicted furosemide plasma concentrations in absence (solid black line) and the presence (solid red line) of probenecid wherein, (a) 40 mg intravenous bolus dose of furosemide +500 and 1000 mg multiple oral doses of probenecid [28]; (b) 40 mg intravenous bolus dose of furosemide +1000 mg multiple oral doses of probenecid [29, 30]; (c) 40 mg oral dose of furosemide +1000 mg single oral dose of probenecid [13]; and (d) 80 mg oral dose of furosemide +1000 mg single oral dose of probenecid [31] were administered. Gray shaded area depicts the 5th and 95th percentile of population levels of furosemide in the presence of probenecid. Open and closed symbols represent literature data of furosemide in the absence and presence of probenecid, respectively.

multiple doses of probenecid yielded similar results with 3-fold increase in plasma concentration of PDA across all time-points. Other inhibitor drugs with potential to inhibit OAT1/3 based on $C_{max,u}/IC_{50}$ ratios, showed either no-change (ritlecitinib) or significant but marginal (< 25%, tafamidis) change in PDA plasma concentration in the clinic, highlighting the practical utility of PDA in DDI risk assessment of investigational drugs in the early clinical development.

PDA is moderately bound to plasma proteins ($f_{u,plasma}$: 0.08–0.1) and the estimated contribution of glomerular filtration (~5%) is small compared to its total CL_R [12, 13]. PDA plasma concentration has been reported to be high in both Oat1 or Oat3 knockout mice [50, 51]. Further, based on in vitro transporter uptake assays, PDA was found to be a substrate of OAT1, OAT2, and OAT3 [12]. However, fractional contribution of each transporter to the overall renal elimination of PDA is unknown. We found that OAT3 is the major driver for PDA renal secretion ($f_t = 0.82$), compared to OAT1 and OAT2 ($f_t < 0.1$) and these results are supported by Tan et al. [52] Additionally, probenecid is 5-fold more potent as an inhibitor for OAT3 versus OAT1. Overall, the marked change in PDA pharmacokinetics in the presence of probenecid is better described by the PBPK model informed with these in vitro data. It is noteworthy that probenecid produces similar magnitude of effect as noted with PDA on the CL_R of OAT3-selective probe substrates, furosemide and benzylpenicillin (~75% decrease), but a lesser effect on OAT1-selective probes like adefovir and tenofovir (~40% decrease) [53]. PDA $AUC_{0\text{-}24h}$ and C_{max} ratios following probenecid and tafamidis treatment showed a significantly negative correlation with predose plasma concentrations (as well as baseline $AUC_{0\text{-}24h}$), highlighting that the predose plasma PDA levels are a sensitive predictor of renal OAT3 function in individual subjects (Figure 2d).

Measurement of biomarker plasma concentrations during phase I studies, combined with PBPK modeling approaches, can be valuable in assessing the potential for DDI caused by new investigational drugs, thus obviating the need to carry out a dedicated clinical DDI study with a probe substrate [16–18]. A PBPK model of PDA, parametrized based on top-down fitting of the baseline pharmacokinetics has been reported previously [17]. The model therefore does not isolate

the contribution of different OATs to PDA elimination and describe the probenecid effects via optimized OAT1/3 inhibition potency. In the current model developed using healthy subjects, a PDA synthesis rate of 0.32 μmol/h was used. This value was estimated by Ahmad et al. by pop-PK modeling of PDA plasma and urine data [19]. Mechanistic prediction of PDA elimination included input of the generated RAF-based CL_{int} values. The mechanistic middle-out PDA PBPK model described in this report was able to accurately predict the baseline plasma concentrations across multiple studies (four current and two published reports [12, 13]). Using probenecid, ritlecitinib and tafamidis as inhibitors, the model was able to predict the plasma concentration of PDA on OAT inhibition. This highlights the utility of the model for prospective prediction of OAT-mediated DDI potential of NCE. While the model overpredicted the cumulative amount of PDA excreted unchanged in urine, the predicted CL_R values were within 2-fold of clinically observed values. PDA uptake was found to be the rate-limiting step in elimination, wherein the sensitivity analysis of MRP CL_{int} values revealed minimal change in its plasma and urine levels. However, in vitro studies designed to generate efflux parameter estimates could potentially improve the amount excreted in urine as predicted by the model. In the published models [17, 19], around 80% contribution to PDA elimination was attributed to renal route and the remaining 20% to non-renal route in order to recover the effect of probenecid on PDA pharmacokinetics. In the current model, non-renal elimination of PDA was not considered due to lack of any evidence. Based on the estimated f_t values and probenecid in vitro IC_{50} values (obtained using PDA as a probe substrate), it was determined that OAT3-mediated uptake accounts for ~80% of total clearance with OAT1, OAT2 and glomerular filtration accounting for the remaining contribution. Parameter sensitivity analysis revealed that the model was insensitive to changes in either CL_{PD} or $CL_{int,MRP4}$ values. While the interaction model showed ~5-fold change in AUC ratios upon changing probenecid K_i values, the change in AUC ratio was minimal (20%) on changing K_i values of ritlecitinib and tafamidis. The mechanistic model developed here can not only be useful in biomarker-informed DDI risk assessment but may also prove valuable in understanding inter-individual variability in OAT3 function in healthy subjects as well as in specific populations such as pregnancy, renal impairment, pediatric and geriatric population.

While this is the first study comparing the $AUC_{0\text{-}24h}$ of PDA in 71 human study participants, the sample size distribution is not even for comparing population variability. The number of females is low ($n = 8$) compared to males ($n = 63$) and needs further confirmation for drawing conclusive results. Since the precursor of PDA is derived from diet, effect of different diets, gastrointestinal absorption of pyridoxine, PDA synthesis etc. on fluctuations in PDA plasma concentrations needs to be investigated. In Study 4, Japanese males were administered metformin, which can lead to marginal change in urine pH (Detal 0.33 pH) [54]. Change in urine pH can affect the passive reabsorption of various urinary metabolites. The low passive permeability of PDA (hydrophilic and charged molecule) suggests a minor contribution of passive reabsorption to the overall CL_R. Therefore, the effect of metformin administration of PDA passive reabsorption is likely minimal.

5 | Conclusions

We evaluated population variability in PDA pharmacokinetics across five independent studies and noted generally consistent baseline plasma levels with < 40% overall variability. Interestingly, differences in PDA plasma levels and CL_R were noted between Whites and Japanese males. A PBPK model parameterized based on in vitro data and clinical inputs accurately recovered PDA plasma levels in the control groups as well in the presence of inhibitor drugs. Collectively, our analysis presents PDA as a reliable and robust endogenous biomarker to assess OAT3-specific activity.

Author Contributions

Aarzoo Thakur, Sumathy Mathialagan, Emi Kimoto, and Manthena V.S. Varma wrote the manuscript. Aarzoo Thakur, Emi Kimoto, and Manthena V.S. Varma designed the research. Aarzoo Thakur, Sumathy Mathialagan, Emi Kimoto, and Manthena V.S. Varma performed the research. Aarzoo Thakur, Sumathy Mathialagan, Emi Kimoto, and Manthena V.S. Varma analyzed the data.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.