Vincristine Disposition and Neurotoxicity are Unchanged in Humanized CYP3A5 Mice

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Running Title Page

Running title: Influence of CYP3A5 on vincristine disposition and toxicity

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Abbreviations: AUC, area under the curve; CYP, cytochrome P450; LC-MS/MS, liquid chromatography with tandem mass spectrometric detection; OATP, organic anion transporting polypeptide; VFH, Von Frey Hairs test; VIPN, vincristine-induced peripheral neuropathy.
ABSTRACT

Previous studies have suggested that the incidence of vincristine-induced peripheral neuropathy (VIPN) is potentially linked with CYP3A5, a polymorphic enzyme that metabolizes vincristine in vitro, and with concurrent use of azole antifungals such as ketoconazole. The assumed mechanism for these interactions is through modulation of CYP3A-mediated metabolism, leading to decreased vincristine clearance and increased susceptibility to VIPN. Given the controversy surrounding the contribution of these mechanisms, we directly tested these hypotheses in genetically-engineered mouse models with a deficiency of the entire murine Cyp3a locus [Cyp3a(-/-) mice] and in humanized transgenic animals with hepatic expression of functional and non-functional human CYP3A5 variants. Compared to wild-type mice, the systemic exposure to vincristine was increased by only 1.15-fold (95% CI: 0.84–1.58) in Cyp3a(-/-) mice, suggesting that the clearance of vincristine in mice is largely independent of hepatic Cyp3a function. In line with these observations, we found that Cyp3a-deficiency or pretreatment with the CYP3A inhibitors ketoconazole or nilotinib did not influence the severity and time course of VIPN, and that exposure to vincristine was not substantially altered in humanized CYP3A5*3 mice or humanized CYP3A5*1 mice compared to Cyp3a(-/-) mice. Our study suggests that the contribution of CYP3A5-mediated metabolism to vincristine elimination and the associated drug-drug interaction potential is limited, and that plasma levels of vincristine are unlikely to be strongly predictive of VIPN.
SIGNIFICANCE STATEMENT

The current study suggests that CYP3A5 genotype status does not substantially influence vincristine disposition and neurotoxicity in translationally relevant murine models. These findings raise concerns about the causality of previously reported relationships between variant CYP3A5 genotypes or concomitant azole use with the incidence of vincristine neurotoxicity.
Introduction

The dose-limiting side effect of many common anti-cancer drugs, including vincristine, is peripheral neuropathy (Carozzi et al., 2015; Madsen et al., 2019; Li et al., 2020). Vincristine is a core chemotherapeutic agent for pediatric patients with acute lymphoblastic leukemia (Den Boer et al., 2003; Diouf et al., 2015), of whom approximately 78% will develop vincristine-induced peripheral neuropathy (VIPN) (Smith et al., 2013). This debilitating toxicity not only affects quality of life, but often leads to treatment delays, dosage reduction, or even treatment withdrawal (Loprinzi et al., 2020). Although the mechanisms underlying VIPN remain incompletely understood, prior studies have documented racial differences in the incidence of VIPN (Diouf et al., 2015), and germline variation in certain genes predisposes to an increased risk of this side effect (Sims, 2016; Skiles et al., 2018).

Previous in vitro studies have indicated that vincristine undergoes CYP3A-mediated metabolism, with the polymorphic enzyme CYP3A5 contributing substantially to the intrinsic clearance of vincristine (Dennison et al., 2006; Dennison et al., 2007; Dennison et al., 2008). The notion that vincristine undergoes selective metabolism by CYP3A5 has sparked interest in the identification of genetic markers and combinatorial regimens that may be associated with impaired vincristine metabolism, decreased clearance, and that result in an increased incidence and severity of VIPN (Mora et al., 2016). However, previous studies evaluating the relation between vincristine pharmacokinetics and VIPN have remained inconclusive, with some (Smith et al., 2013; van de Velde et al., 2020) but not all reports (Guilhaumou et al., 2011; Moore et al., 2011) documenting statistically significant relationships. Similarly, while the incidence of VIPN was initially reported to lower in patients who functionally express CYP3A5 (Egbelakin et al., 2011; Bosilkovska et al., 2016), a recent meta-analysis of 21 studies suggests that CYP3A5 expression status is not significantly associated with VIPN (van de Velde et al., 2020; Uittenboogaard et al., 2022). Equally controversial remains the extent to which patients concurrently treated for fungal infections with azole antifungals, such as the potent CYP3A
inhibitor ketoconazole, are at increased risk of experiencing severe VIPN (Moriyama et al., 2012; Smitherman et al., 2017; Nikanjam et al., 2018) or not (van de Velde et al., 2020; Uittenboogaard et al., 2022). In order to address these uncertainties in an unbiased and unequivocal manner, we have re-examined the contribution of CYP3A5 to the hepatic metabolism of vincristine, its pharmacokinetic profile, and VIPN in engineered mouse models with a deficiency of the entire murine Cyp3a locus and in humanized transgenic animals expressing CYP3A5.

**Materials and Methods**

**Chemicals and Reagents.** Vincristine sulfate was purchased from MedChemExpress (99.7% purity, Monmouth Junction, NJ). Vincristine internal standard, [°H₃]-vincristine sulfate (vincristine-d3), was purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Triazolam was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Human liver microsomes and NADPH regeneration systems and buffers were purchased from XenoTech, LLC (Kansas City, KS), and microsome isolation kits were obtained from Abcam (Waltham, MA).

**Animals.** In animal experiments, mice were age- and sex-matched (8-12 weeks old). The procedures were approved by the Care and Use Committee of ULAR at The Ohio State University (protocol # 2015A00000101-R2). A temperature-controlled environment with a 12-hour light cycle provided to all animals with the standard chow diet and water *ad libitum*. FVB background wild-type mice and Cyp3a-deficient [Cyp3a(-/-)] mice that lack all 8 murine Cyp3a genes on the same background strain were obtained from Taconic Biosciences (Cambridge City, IN). The generation and characterization of a secondary model [mCyp3a(-/-) mice] with and without hepatic expression of CYP3A5*1 [CYP3A5*1 mice] or CYP3A5*3 [CYP3A5*3 mice] have been described previously (Abe et al., 2017). These latter two models constitute fully humanized trans-chromosomic mice with a CYP3A cluster that includes CYP3A4, CYP3A5,
CYP3A7, and CYP3A43 in which the animals differ by a single-nucleotide polymorphism modification of CYP3A5 (g.6986G to A; *3 to *1) on the CYP3A cluster, whereas the CYP3A5*3 mice display absence of CYP3A5 protein expression in the liver and intestine. The CYP3A5*1 mice closely recapitulate the CYP3A5*1 carrier phenotype in humans, this is evidenced by the similar CYP3A5 protein expression (0.17 pmol/mg protein) in liver microsomes from CYP3A5*1 mice compared with liver microsomes from a human carrying the CYP3A5*1/*3 genotype (0.35 pmol/mg protein, BD gentest, #452136), as well as the comparable catalytic activity measured by triazolam alpha-hydroxylation (87.6 and 242.1 pmol/mg protein/min, respectively; data not shown).

**Pharmacokinetic Studies.** Pharmacokinetic studies were conducted according to a published study protocol (Leblanc et al., 2018a). Vincristine plasma concentration-time profile was assessed in wild-type and Cyp3a(-/-) mice, intra-peritoneal injections at a dose of 1 mg/kg of vincristine were administered, and blood samples at 0.08, 0.25, 0.5, 1, 2, and 4 h time points were collected from the submandibular vein or from the retro-orbital sinus vein, and cardiac puncture was performed at the terminal time-point. All blood samples were immediately centrifuged for 5 min at 11,000 rpm, and the supernatants were collected and further stored at -80°C until analysis using LC-MS/MS method. Livers were collected from the same animals at the time of final blood collection and, tissue samples were snap frozen in liquid nitrogen to prevent continuing metabolic activity. In another experiment, the pharmacokinetic profiles of vincristine in mCyp3a(-/-), CYP3A5*1, and CYP3A5*3 mice were evaluated after administered by intra-peritoneal injection at a dose of 1 mg/kg. Whole blood samples were collected at 0.08, 0.25, 0.5, 1, 2, and 4 h, and livers were collected at the final time point.

To evaluate the influence of pre-treatment with oral ketoconazole (50 mg/kg) or oral nilotinib (100 mg/kg) on the profile of vincristine (1 mg/kg) or oral triazolam (2 mg/kg), samples were collected at 0.08, 0.25, 0.5, 1, 2, and 4 h for vincristine, and at 0.08, 0.17, 0.33, 0.67, 1.33, and 2.67 h for triazolam. For these studies, ketoconazole was formulated in PEG400, nilotinib in
0.5% hydroxypropyl methylcellulose, and triazolam in ethanol and 0.9% saline (1:99). All plasma and tissue samples were stored at -80°C until further processing. All liver tissues were homogenized with 5-mm stainless steel bead (Qiagen) and processed for 4 min at 40 Hz. Finally, the beads were removed and the homogenized samples were stored at -80°C until analysis by LC-MS/MS. Liver to plasma ratio was calculated by dividing the observed concentration of vincristine in the liver and the concentration of vincristine in plasma at terminal time point.

**Microsomal Experiments.** The liver metabolism of vincristine was conducted *in vitro* using mouse and human liver microsomes as previously described (Eisenmann et al., 2021). All assays were conducted in a total volume of 100 μL. Vincristine (5 μM) was incubated in a mixture of liver microsomes (20 mg protein/mL), an NADPH regenerating system (1 mM β-NADP⁺, 1 unit/mL glucose-6-phosphate dehydrogenase, and 5 mM glucose 6-phosphate), potassium phosphate buffer (100 mM, pH 7.4), and MgCl₂ (3 mM). Before initiation of the reaction, a 5 min preincubation at 37°C was performed. The reaction was started by adding the NADPH regenerating system. The reaction mixture (40 μL) was quenched with 40 μL of methanol at 0 and 1 h time points, samples were vortex-mixed and then stored at -80°C until further processing. The percentage of vincristine metabolized in mice or human liver microsome was calculated by the percentage of changes of vincristine concentration at 1hr to 0 time point.

**Determination of Drug Concentrations.** Total vincristine was quantified using LC-MS/MS as described (Jin et al., 2021), with minor modifications. Briefly, a Vanquish UHPLC coupled with a Quantiva triple quadrupole mass spectrometer from Thermo Fisher Scientific was used for all analyses. The plasma volume of 2.0 μL per sample was injected for the separation by an Accucore aQ column (50 × 2.1 mm, dp = 2.6 μm, Thermo Fisher Scientific) tandem with a C18 AQUASIL guard cartridge (2.1 mm × 10 mm, dp =3 μm, Thermo Fisher Scientific). The autosampler chamber and the separation column were homo-temperature-controlled at 4°C and 50°C. The method run time was 2.2 min with using water with 0.1% (v/v)
formic acid as aqueous mobile phase (A) and acetonitrile with 0.1% (v/v) formic acid as organic solvent phase (B). The gradient conditions were as follows: 0-0.5 min, 10% B; 0.5-0.51 min, 10 to 50%, 0.51-1.8 min, 50% to 95% B; 1.8-1.81 min, 95% to 10% B; 1.81-2.2 min, 10% B with a flow rate was 400 μL/min. The MS with the positive voltage applied to the ESI capillary was set at 3536.36 V, and the temperature of capillary and vaporizer was 375°C and 450 °C, respectively. Argon was used as the collision gas at a pressure of 1.5 mTorr. Precursor molecular ions and product ions were recorded for quantifying vincristine (825.438→765.354), using [2H3]-vincristine as an internal standard (828.488→768.275). Results from assay validation studies revealed that the intra-day precision and inter-day precision ranged 1.87-5.20%, and the accuracy ranged 91.7-107%, with a lower limit of quantification at 2.5 ng/mL.

LC-MS/MS was also used to quantify concentrations of triazolam. The analysis was conducted using a Vanquish UHPLC aligned with an Altis triple quadrupole mass spectrometer (Thermo Fisher Scientific Co.). ACQUITY UPLC HSS T3 Column (100Å, 1.8 μm, 2.1 mm X 50 mm, Waters Co.) protected with ACQUITY UPLC BEH Shield RP18 VanGuard pre-column (130Å, 1.7 μm, 2.1 mm X 5 mm, Waters Co.). The injection volume of the sample was 3 μL. The temperature of the column was kept at 40°C, whereas the autosampler rack was kept at 4°C. The mobile phase consisted of solvent A, which was a solution of 0.1% formic acid in LC-MS grade water, and solvent B, which was a solution of 0.1% formic acid in acetonitrile: methanol, 1:3 (v:v). The total run time was 5.0 min. The following were the gradient conditions: 0.25 mL/min was the flow rate for the following intervals: 0.0–0.5 min, 45% B; 0.5–4.0 min, 45 to 95%; 4.0–4.5 min, 95% B; and 4.51 –5.0 min, 95% to 45% B. The vaporizer was adjusted to 350 °C, and the capillary temperature was 325 °C. The MS assay was configured with a positive voltage of 3154 V delivered to the ESI capillary. The collision gas utilized was argon, with a pressure of 1.5 mTorr. The mass spectrometer was set up with optimized parameters; 1 Arb for sweep gas, 10 Arb for auxiliary gas, and 50 Arb for sheath gas. Precursor molecular ions and product ions were recorded for confirmation and detection of triazolam (343.057→308.049) using [2H4]-
triazolam (347.063→312.049) as internal standards. The lower quantitative limits of quantification were 0.125 ng/mL for triazolam.

**Peripheral Neuropathy Assessment.** For toxicity studies, wild-type and Cyp3a(-/-) mice received a single intra-peritoneal injection of vincristine diluted in normal saline at a dose of 1 mg/kg that was given either alone or 30 min after with a single oral dose of ketoconazole, as described previously (Li et al., 2023). The hind paw flinching behaviors after mechanical stimulation by a Von Frey Hairs (VFH) filament was used to assess the development of mechanical allodynia at baseline, and at 24 h after drug administration. Before start of the experiment, mice were housed individually and acclimated to the VFH assessing apparatus for several days. Baseline data were collected before dosing. VFH was performed in quiet room according to a previously described method (Chiorazzi et al., 2018; Leblanc et al., 2018b; Huang et al., 2020) by two individuals blinded to the genotype and treatment modalities of the groups. Briefly, mice were on a wire mesh and loosely restrained with plexiglass compartment, prior to the testing, at least 1 hour acclimation was performed. For behavioral testing, a blunt filament (0.5 mm diameter) was used perpendicularly at the plantar surface of hind paw for constant force with 2–5 seconds. When a hind paw withdrawal observed spontaneously, the stimulus was stopped and the gram force of the pressure to withdrawal was recorded. This procedure was assessed 3 times after conducting to the mid-plantar of hind paw with an interval of several seconds. Values for each individual mice were then averaged to generate mean threshold response, an upper cutoff limit of 6 g was set to prevent tissue damage.

**Data Analysis.** All data represent the mean ± SEM, unless specified otherwise. Peripheral neuropathy data were analyzed as described (Huang et al., 2020; Li et al., 2023). All individual experiments were conducted replicates for at least two independent occasions. Unpaired two-sided Student’s t test and Welch’s correction method was employed for comparisons between two groups. A one-way ANOVA and Dunnett’s test were performed for the comparisons that over than two groups. All behavioral tests were evaluated using two-way
ANOVA with either Tukey’s or Bonferroni’s post hoc test, across different time-points and groups. $P < 0.05$ was used as the statistical cutoff across all analysis. The fold-changes of plasma AUC and liver-to-plasma ratio with 95% confidence intervals (95% CI) between each genotype mice were calculated by R4.3 *effectsize* package (Hedges et al., 1999).

**Results**

**Pharmacokinetics of Vincristine in Cyp3a-Deficient Mice.** To evaluate the hypothesis that CYP3A-mediated vincristine metabolism contributes substantially to vincristine clearance and VIPN, we initially tested the changes of vincristine pharmacokinetics in genetically engineered mouse models with a deficiency of the entire murine *Cyp3a* locus. In our study, the systemic exposure of vincristine was elevated in Cyp3a(-/-) mice by only 1.15-fold (95% CI: 0.84–1.58) compared to wild-type mice, suggesting that the clearance of vincristine in mice is largely independent of hepatic Cyp3a metabolism (Fig. 1A; Table 1). Furthermore, the differences in liver to plasma ratios of vincristine between mouse genotypes were relatively modest (1.33-fold, 95% CI: 0.75–2.35) and did not reach statistical significance ($P>0.05$) (Fig. 1B). In line with these observations, we found that the CYP3A inhibitor ketoconazole did not influence plasma levels of vincristine and had no influence on vincristine-induced neurotoxicity irrespective Cyp3a genotype (Fig. 1C and 1D; Table 1).

**Influence of CYP3A Inhibition on Vincristine Pharmacokinetics.** We recently found that pretreatment with the tyrosine kinase inhibitor nilotinib, administered orally immediately before vincristine, effectively protects against VIPN through a mechanism that involves inhibition of neuronal drug uptake (Li et al., 2023). Since nilotinib displays potent CYP3A inhibitory properties similar to those reported for ketoconazole (Zhang et al., 2015) and is itself sensitive to concurrent use of strong CYP3A4 inducers or inhibitors (Tanaka et al., 2011), we next evaluated the influence of nilotinib as an alternate modulator of metabolism on the disposition of vincristine. These studies indicated that nilotinib pretreatment did not substantially affect the
pharmacokinetic profile of vincristine (Fig. 2A; Table 2) or the liver to plasma ratios of vincristine (Fig. 2B). However, pretreatment with either nilotinib or ketoconazole was associated with significantly increased plasma concentrations of the Cyp3a prototypic probe triazolam, indicating that the selected doses were sufficient to inhibit the Cyp3a metabolic pathway in mice (Fig. S1).

**Pharmacokinetics of Vincristine in Humanized CYP3A5 Mice.** Evidence from *in vitro* studies has indicated that CYP3A5 contributes to 75% of the intrinsic clearance of vincristine (Dennison et al., 2006; Dennison et al., 2007; Dennison et al., 2008). In order to evaluate the *in vivo* contribution of human CYP3A5 variants to vincristine disposition, mice with humanized transgenic expression of CYP3A5*1 (CYP3A5 expressor) or CYP3A5*3 (CYP3A5 non-expressor) with similar catalytic activities in microsomes to human were used (Abe et al., 2017). The functional similarity of these models toward vincristine was confirmed in preliminary validation studies using mouse and human liver microsomes indicating that extent of vincristine metabolism in samples from wild-type mice was significantly lower than that observed in samples from human liver microsomes with a confirmed CYP3A5*1/*1 (high activity) genotype (*P*<0.01) (Fig. S2A). The fraction of vincristine that was metabolized under the same conditions was increased in samples from humanized CYP3A5*1 mice compared with humanized CYP3A5*3 mice and human liver microsomes with confirmed CYP3A5*3/*3 (no activity) genotypes (Fig. S2B). These data are consistent with the previously reported *in vitro* data that the extent of metabolism of vincristine is greater for CYP3A5 high expressors than low expressors (Dennison et al., 2007).

Our *in vivo* results revealed that the plasma exposure to vincristine was only modestly altered in humanized CYP3A5*3 mice (1.13-fold change, 95% CI: 0.98–1.29) or in humanized CYP3A5*1 mice (0.83-fold change, 95% CI: 0.70–0.97) compared to mCyp3a(-/-) mice (Fig. 2C). These results imply that the contribution of CYP3A5-mediated metabolism to vincristine clearance in a predictive, non-human model is much smaller than what has been predicted from
in vitro studies. In line with this thesis, pretreatment with nilotinib did not affect the pharmacokinetic profile of vincristine in mCyp3a(-/-) or in humanized CYP3A5*1 and CYP3A5*3 mice (Fig. 2D; Table 2).

Discussion

In the current study, we directly tested the hypothesis that CYP3A-mediated vincristine metabolism is a critical determinant of vincristine clearance and VIPN in genetically engineered knockout and humanized transgenic mouse models. The collective results suggest that the clearance of vincristine in mice is largely independent of endogenous hepatic Cyp3a function in mice, and that transgenic overexpression of human CYP3A5*1 or CYP3A5*3 minimally affects the systemic exposure to vincristine. Importantly, we found that genetic deficiency or pharmacological inhibition of these enzymes did not exacerbate vincristine-induced sensitivity to mechanical stimuli, a common marker of peripheral neurotoxicity.

Early evidence for the thesis that CYP3A mediates the metabolism of vincristine was derived from the observation that vincristine inhibits the metabolism of the structurally-related Vinca alkaloids vinblastine and vindesine in human liver microsomes (Zhou-Pan et al., 1993). Further in vitro studies have revealed that, in contrast to the metabolism of most CYP3A substrates, the biotransformation of vincristine by CYP3A5 is considerably more efficient than that observed with CYP3A4, and this has led to the thesis that CYP3A5 genotypes may contribute to interindividual variability in the systemic exposure to vincristine (Dennison et al., 2006; Dennison et al., 2007; Dennison et al., 2008). Initially, research explored racial ancestry as a contributor to vincristine pharmacokinetic based on the notion that functional CYP3A5 expression is more common in livers of African Americans than other world populations (Kuehl et al., 2001; Roy et al., 2005). Although altered vincristine clearance and susceptibility to VIPN in African American patients has been documented in some studies (Renbarger et al., 2008; Egbelakin et al., 2011; Skiles et al., 2018), this racial connection to vincristine phenotypes
remains uncertain and could not be replicated in other studies (Uittenboogaard et al., 2022; van de Velde et al., 2022). In order to address the controversial contribution of CYP3A-mediated metabolism to VIPN, we performed pharmacokinetic and function studies in Cyp3a-deficient mice that lack all 8 murine CYP3A genes, and in a humanized mice expressing functional and non-functional CYP3A5 variants (Abe et al., 2017; Jin et al., 2021).

Beyond genetic predisposition, the concomitant administration of drugs with CYP3A-inhibitory properties has been speculated to potentially exacerbate vincristine neurotoxicity due to drug-drug interaction potential. In this context, it is noteworthy that, during chemotherapy induction, patients are often at increased risk of fungal infections due to neutropenia and corticosteroid administration and receive prophylactic treatment with azole antifungals (Baden et al., 2016). Given continued reports of severe neurotoxicity with the combination of vincristine and antifungals (van Schie et al., 2011; Madsen et al., 2019), and in view of the notion that both triazole and imidazole antifungal agents inhibit CYP3A4/CYP3A5-mediated metabolism, impaired vincristine clearance has been proposed as the main reason contributing to this severe side effect (Moriyama et al., 2012; Madsen et al., 2019).

It is worth noting that the azole antifungals itraconazole and ketoconazole are both dual inhibitors of CYP3A-mediated metabolism and ABCB1 (P-glycoprotein)-dependent efflux transport (Venkatakrishnan et al., 2000; Gubbins and Heldenbrand, 2010). In contrast, the antifungal fluconazole inhibits CYP3A but not ABCB1 (Gubbins and Heldenbrand, 2010) and does not appear to alter the incidence and severity of vincristine-induced neurotoxicity in patients (Smitherman et al., 2017; Madsen et al., 2019). A potential connection of ABCB1-mediated efflux and vincristine toxicity is supported by the previous finding that variants in the ABCB1 gene are correlated with VIPN in some populations (Pozzi et al., 2021). Interestingly, studies involving physiology-based pharmacokinetic modeling of vincristine (Pilla Reddy et al., 2021) and the use of knockout mice (Wang et al., 2010) have suggested that pharmacological inhibition or genetic deficiency of ABCB1 is associated with negligible increases in the plasma
concentrations of vincristine. Although further validation studies are required under ABCB1-deficient conditions and to determine the potential for drug-drug-gene interactions (Malki and Pearson, 2020), this collective prior work implies that modulation of ABCB1 in target cells within the peripheral nervous system can affect the cellular retention of vincristine and downstream toxic events without altering systemic clearance (Stage et al., 2021). This thesis is consistent with our finding thatazole antifungals such as ketoconazole did not affect the pharmacokinetic profile of vincristine, and that exposure to vincristine was also not substantially altered in humanized CYP3A5*3 mice or humanized CYP3A5*1 mice compared to Cyp3a(-/-) mice. Consistently, we found that pretreatment of mice with nilotinib, a tyrosine kinase inhibitor that displays inhibitory effects on CYP3A (Zhang et al., 2015) and has neuroprotective properties (Li et al., 2023), also did not negatively influence vincristine elimination. This is in line with model-based predictions (Pilla Reddy et al., 2021) for other tyrosine kinase inhibitors with CYP3A-inhibitory properties, such as acalabrutinib (Han et al., 2021), that indicate the lack of drug-drug interaction liabilities with vincristine.

In conclusion, this study suggests that the contribution of CYP3A5-mediated vincristine elimination and the associated drug-drug interaction potential are much smaller than held previously, and that plasma levels of vincristine are unlikely to be strongly predictive of VIPN. These findings raise concerns about the causality of previously reported relationships between variant CYP3A5 genotypes or concomitant azole use with the incidence of VIPN and their dependence on altered drug disposition profiles.
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Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper and are available on request from the corresponding author.

Authorship Contributions

Participated in research design: Baker, Cavaletti, Sparreboom, and Hu.

Conducted experiments: Y. Li, Drabison, Xu, Ahmed, Eisenmann, and Jin.

Contributed new reagents or analytic tools: Kazuki, Kobayashi, and Fujita.

Performed data analysis: Y. Li, J. Li, and Hu.

Wrote or contributed to the writing of the manuscript: Y. Li, Sparreboom, and Hu.
References


Footnotes

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**Figure Legends.**

**Figure 1.** Pharmacokinetics of Vincristine in Cyp3a-Deficient Mice. (A) Plasma concentration–time profiles of vincristine (1 mg/kg; i.p.) in wild-type and Cyp3a(-/-) mice. \( P > 0.05, n = 12-14 \) per group), (B) Liver to plasma ratio of vincristine (1 mg/kg; i.p.) in wild-type mice or Cyp3a(-/-) mice, \( n = 4-5 \) per group). (C) Plasma concentration-time profile of vincristine (1 mg/kg) in wild-type or Cyp3a-deficient mice pretreated for 30 min with vehicle or ketoconazole (50 mg/kg; p.o.), \( P > 0.05, n = 6-9 \) per group). (D) Mechanical allodynia baseline and after 24 h in wild-type mice or Cyp3a-deficient receiving i.p. injections of vincristine at a dose of 1 mg/kg, mice were pre-treated with vehicle or ketoconazole (50 mg/kg; p.o.) 30 min before vincristine injection, \( n = 6 \) per group). All data presented represent the mean ± SEM.

**Figure 2.** Influence of Nilotinib on Vincristine Pharmacokinetics in Cyp3a-deficient Mice and humanized transgenic CYP3A5 Mice. (A) Plasma concentration-time profile, (B) liver to plasma ratio of vincristine (1 mg/kg) in wild-type or Cyp3a-deficient mice pretreated for 30 min with vehicle or nilotinib (100 mg/kg) \( P > 0.05, n = 3-5 \) per group). Plasma concentration-time profile of vincristine (1 mg/kg) in CYP3A5*1, CYP3A5*3, and Cyp3a-deficient mice, pretreated for 30 min with (C) vehicle or (D) nilotinib (100 mg/kg) \( P < 0.05 \) in AUC mCyp3a(-/-) + Nilotinib vs. CYP3A5*1/mCyp3a(-/-) + Nilotinib, \( n = 4-5 \) per group). All data presented represent the mean ± SEM.
Table 1. Influence of Cyp3a genotype and ketoconazole on pharmacokinetic parameters of vincristine in mice.

<table>
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<th>Genotype</th>
<th>Treatment group</th>
<th>N</th>
<th>( C_{\text{max}} ) (ng/mL)\textsuperscript{a}</th>
<th>( \text{AUC}_{0-4\text{h}} ) (ng( \times )h/mL)\textsuperscript{a}</th>
<th>( t_{\text{max}} ) (h)\textsuperscript{a}</th>
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<td>725 ± 46.0</td>
<td>804 ± 96.2</td>
<td>0.196 ± 0.0323</td>
<td>1.81 ± 0.210</td>
</tr>
<tr>
<td>Wild-type</td>
<td>PEG400</td>
<td>7</td>
<td>566 ± 32.8</td>
<td>508 ± 50.3</td>
<td>0.155 ± 0.0337</td>
<td>1.86 ± 0.150</td>
</tr>
<tr>
<td>Cyp3a(-/-)</td>
<td>PEG400</td>
<td>9</td>
<td>638 ± 33.2</td>
<td>599 ± 74.1</td>
<td>0.222 ± 0.0440</td>
<td>1.87 ± 0.340</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Ketoconazole</td>
<td>7</td>
<td>799 ± 82.5</td>
<td>583 ± 53.2</td>
<td>0.226 ± 0.0239</td>
<td>1.82 ± 0.502</td>
</tr>
<tr>
<td>Cyp3a(-/-)</td>
<td>Ketoconazole</td>
<td>6</td>
<td>673 ± 72.8</td>
<td>608 ± 94.9</td>
<td>0.139 ± 0.0352</td>
<td>2.46 ± 0.503</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data represent mean and SEM.
N/A, not applicable.

Abbreviations: \( C_{\text{max}} \), peak plasma concentration; \( \text{AUC}_{0-4\text{h}} \), area under the plasma concentration-time curve between time zero and 4 hours; N, number of mice per group.
Table 2. Influence of Cyp3a genotype and nilotinib on the pharmacokinetic parameters of vincristine in mice.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment group</th>
<th>N</th>
<th>$C_{\text{max}}$ (ng/mL)$^a$</th>
<th>AUC$_{0-4h}$ (ng×h/mL)$^a$</th>
<th>$t_{\text{max}}$ (h)$^a$</th>
<th>$t_{1/2}$ (h)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>HPMC</td>
<td>5</td>
<td>540 ± 37.5</td>
<td>484 ± 54.6</td>
<td>0.116 ± 0.0334</td>
<td>1.98 ± 0.161</td>
</tr>
<tr>
<td>Cyp3a(-/-)</td>
<td>HPMC</td>
<td>6</td>
<td>601 ± 39.3</td>
<td>558 ± 67.7</td>
<td>0.264 ± 0.0545</td>
<td>2.09 ± 0.470</td>
</tr>
<tr>
<td>Wild-type</td>
<td>Nilotinib</td>
<td>5</td>
<td>455 ± 14.7</td>
<td>552 ± 80.4</td>
<td>0.083 ± 0.0000</td>
<td>2.05 ± 0.401</td>
</tr>
<tr>
<td>Cyp3a(-/-)</td>
<td>Nilotinib</td>
<td>7</td>
<td>514 ± 69.1</td>
<td>516 ± 70.9</td>
<td>0.202 ± 0.0308</td>
<td>2.04 ± 0.247</td>
</tr>
<tr>
<td>mCyp3a(-/-)</td>
<td>HPMC</td>
<td>5</td>
<td>539 ± 40.0</td>
<td>572 ± 16.9</td>
<td>0.183 ± 0.0409</td>
<td>2.46 ± 0.208</td>
</tr>
<tr>
<td>CYP3A5*1/mCyp3a(-/-)</td>
<td>HPMC</td>
<td>4</td>
<td>439 ± 52.7</td>
<td>471 ± 36.1</td>
<td>0.208 ± 0.0418</td>
<td>1.58 ± 0.204</td>
</tr>
<tr>
<td>CYP3A5*3/mCyp3a(-/-)</td>
<td>HPMC</td>
<td>4</td>
<td>696 ± 79.8</td>
<td>644 ± 41.2</td>
<td>0.167 ± 0.0482</td>
<td>1.77 ± 0.154</td>
</tr>
<tr>
<td>mCyp3a(-/-)</td>
<td>Nilotinib</td>
<td>5</td>
<td>543 ± 22.4</td>
<td>606 ± 21.3</td>
<td>0.166 ± 0.0834</td>
<td>2.43 ± 0.147</td>
</tr>
<tr>
<td>CYP3A5*1/mCyp3a(-/-)</td>
<td>Nilotinib</td>
<td>5</td>
<td>488 ± 47.5</td>
<td>511 ± 11.6</td>
<td>0.183 ± 0.0409</td>
<td>1.38 ± 0.096</td>
</tr>
<tr>
<td>CYP3A5*3/mCyp3a(-/-)</td>
<td>Nilotinib</td>
<td>5</td>
<td>582 ± 22.2</td>
<td>585 ± 14.6</td>
<td>0.233 ± 0.0764</td>
<td>1.89 ± 0.316</td>
</tr>
</tbody>
</table>

$^a$Data represent mean and SEM.

*Abbreviations:* $C_{\text{max}}$, peak plasma concentration; AUC$_{0-4h}$, area under the plasma concentration-time curve between time zero and 4 hours; N, number of mice per group.
Figure 1

**A**

Plasma concentration of Vincristine (ng/mL) over time (h)

- **Wild-type**
- **Cyp3a(−/−)**

**B**

Liver to Plasma Ratio

- Wild-type
- Cyp3a(−/−)

**C**

Plasma concentration of Vincristine (ng/mL) over time (h)

- Wild-type + Vehicle
- Wild-type + Ketoconazole
- Cyp3a(−/−) + Vehicle
- Cyp3a(−/−) + Ketoconazole

**D**

Paw withdrawal threshold (%Δ from baseline)

- Baseline
- Day 1

Legend:
- Wild-type + Vehicle
- Cyp3a(−/−) + Vehicle
- Wild-type + Ketoconazole
- Cyp3a(−/−) + Ketoconazole
Figure 2

A. Graph showing plasma concentration of vincristine (ng/mL) over time (h) for different groups:
- **Wild-type + Vehicle**
- **Wild-type + Nilotinib**
- **Cyp3a(-/-) + Vehicle**
- **Cyp3a(-/-) + Nilotinib**

B. Bar graph showing liver to plasma ratio for different groups:
- **Wild-type + Vehicle**
- **Wild-type + Nilotinib**
- **Cyp3a(-/-) + Vehicle**
- **Cyp3a(-/-) + Nilotinib**

C. Graph showing plasma concentration of vincristine (ng/mL) over time (h) for different groups:
- **mCyp3a(-/-)**
- **CYP3A5*1/mCyp3a(-/-)**
- **CYP3A5*3/mCyp3a(-/-)**

D. Graph showing plasma concentration of vincristine (ng/mL) over time (h) for different groups:
- **mCyp3a(-/-) + Nilotinib**
- **CYP3A5*1/mCyp3a(-/-) + Nilotinib**
- **CYP3A5*3/mCyp3a(-/-) + Nilotinib**
Supplementary Information

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Vincristine Disposition and Neurotoxicity are Unchanged in Humanized CYP3A5 Mice

Yang Li, Yasuhiro Kazuki, Thomas Drabison, Kaoru Kobayashi, Ken-ichi Fujita, Yue Xu, Yan Jin, Eman Ahmed, Junan Li, Eric D. Eisenmann, Sharyn D. Baker, Guido Cavaletti, Alex Sparreboom, and Shuiying Hu

Division of Pharmaceutics and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH (Y.L., T.D., Y.X., Y.J., E.A., E.D.E., S.D.B., A.S., S.H.); Department of Chromosome Biomedical Engineering, School of Life Science, Faculty of Medicine, Tottori University, Japan (Y.K.); Chromosome Engineering Research Center, Tottori University, Japan (Y.K.); Chromosome Engineering Research Group, The Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, Japan (Y.K.); Department of Biopharmaceutics, Meiji Pharmaceutical University, Tokyo, Japan (K.K.); Division of Cancer Genome and Pharmacotherapy, Department of Clinical Pharmacy, Showa University School of Pharmacy, Tokyo, Japan (K.F.); Experimental Neurology Unit and Milan Center for Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy (G.C.); Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy (G.C.); Division of Outcomes and Translational Sciences, College of Pharmacy & Comprehensive Cancer Center, The Ohio State University, Columbus, OH (J.L., S.H.)
Plasma concentration–time profiles of triazolam (2 mg/kg) in wild-type mice pretreated for 30 min with vehicle, nilotinib (100 mg/kg; p.o.) (A), or ketoconazole (50 mg/kg; p.o.) (B), (n = 4-5 per group). All data presented represent the mean ± SEM.
Vincristine Clearance in Liver Microsomes. (A) Vincristine clearance in wild-type mice liver microsome and human liver microsome with CYP3A5*1/*1 genotype. (B) Vincristine clearance in humanized CYP3A5*1 and CYP3A5*3 mice liver microsome, and human liver microsome with CYP3A5*3/*3 genotype. **P < 0.01. All data presented represent the mean ± SEM, n = 4-6 per group.