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Metabolic Profiles of Pomalidomide in Human Plasma Simulated with Pharmacokinetic Data in Control and Humanized-liver Mice

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Abstract

1. Pomalidomide has been shown to be potentially teratogenic in thalidomide-sensitive animal species such as rabbits. Screening for thalidomide analogs devoid of teratogenicity/toxicity—attributable to metabolites formed by cytochrome P450 enzymes—but having immunomodulatory properties is a strategic pathway towards development of new anticancer drugs.
2. In this study, plasma concentrations of pomalidomide, its primary 5-hydroxylated metabolite, and its glucuronide conjugate(s) were investigated in control and humanized-liver mice. Following oral administration of pomalidomide (100 mg/kg), plasma concentrations of 7-hydroxypomalidomide and 5-hydroxypomalidomide glucuronide were slightly higher in humanized-liver mice than in control mice.
3. Simulations of human plasma concentrations of pomalidomide were achieved with simplified physiologically-based pharmacokinetic models in both groups of mice in accordance with reported pomalidomide concentrations after low dose administration in humans.
4. The results indicate that pharmacokinetic profiles of pomalidomide were roughly similar between control mice and humanized-liver mice and that control and humanized-liver mice mediated pomalidomide 5-hydroxylation *in vivo*. Introducing one aromatic amino group into thalidomide resulted in less species differences in *in vivo* pharmacokinetics in control and humanized-liver mice.

Introduction

Pomalidomide (Elkinson and McCormack, 2013), a second generation amino analog of thalidomide, has been recently approved for refractory multiple myeloma in the United States, Canada, Europe, and Japan (Jones et al., 2016). Pharmacokinetic analysis of [¹⁴C]-pomalidomide after a single oral dose in healthy male subjects has been reported (Hoffmann et al., 2013). Pomalidomide was reportedly neither an inducer nor inhibitor of cytochromes P450 (P450) *in vitro* (Kasserra et al., 2015), but it was mainly metabolized by human P450

Declaration of interest

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3A4 to 5-hydroxypomalidomide (Figure 1) (Chowdhury et al., 2014). No glutathione conjugates of pomalidomide were detected in *in vitro* assays (Chowdhury et al., 2014). A useful application of TK-NOG mice (Hasegawa et al., 2011; Yamazaki et al., 2012) with transplanted human hepatocytes (humanized-liver mice) was reported for the metabolism of the first generation drug thalidomide (Yamazaki et al., 2012). Although the *in vivo* metabolic profile of thalidomide was slightly affected by endogenous mouse P450 enzymes responsible for transforming rodent specific 5'-hydroxythalidomide (Yamazaki et al., 2012), the *in vivo* oxidative metabolism of thalidomide was previously re-investigated in TK-NOG mice substituted with human liver cells harboring *P450 3A5*1* (Nishiyama et al., 2015) for successful simulation of human plasma concentrations of primary 5-hydroxylated metabolites of thalidomide. Screening for thalidomide analogs devoid of teratogenicity/toxicity attributable to its metabolites, especially those formed by auto-induced P450 3A enzymes (Yamazaki et al., 2013; Murayama et al., 2014), but having immunomodulatory properties is a strategic pathway towards development of new anticancer drugs.

In this study, human plasma concentrations of oxidized pomalidomide metabolites (including glucuronide conjugates of 5-hydroxypomalidomide) were simulated using a simplified physiologically-based pharmacokinetic (PBPK) model with data from control and humanized-liver mice to study the effect of introduction of an aromatic amino group into thalidomide on the *in vivo* pharmacokinetics.

Materials and methods

In vivo metabolic studies

Chemicals were from sources reported previously (Murayama et al., 2014; Chowdhury et al., 2014). 5-Hydroxypomalidomide was synthesized in a manner similar to 5-hydroxythalidomide, which was previously reported (Yamamoto et al., 2009). Male control and humanized-liver TK-NOG mice (20–30 g body weight) (Yamazaki et al., 2016a; Nishiyama et al., 2015) were used in this study. In humanized-liver mice, >95% of the liver cells were estimated to have been replaced with human hepatocytes (BPI-187266, Biopredic, France) in TK-NOG mice, as judged by measurements of human albumin concentrations in plasma. Blood samples (~30 µL) were collected 0.5–24 h with heparinized syringes after single oral doses of pomalidomide by gavage (100 mg/kg, 10 µL sodium carboxymethyl cellulose solution per g body weight, Selleck Chemicals, Houston, TX) administered to five animals of two groups. After treatment of the plasma with one-tenth volume of ice-cold 60% (w/v) HClO₄, the aqueous supernatant was centrifuged at $2 \times 10^3 g$ for 10 min at 4 °C and analyzed using LC-MS. The use of animals for this study was approved by the Ethics Committees of the Japan Central Institute for Experimental Animals and Showa Pharmaceutical University.

LC-MS/MS analyses of pomalidomide, 7- or 5-hydroxypomalidomide, and the 5-hydroxypomalidomide glucuronide conjugate were performed according to previously reported methods (Hoffmann et al., 2013; Kasserra et al., 2015) with minor modifications. An API4000 tandem mass spectrometry (AB Sciex, Framingham, MA) equipped with Analyst software was operated in the electrospray negative ionization mode, directly coupled to a liquid chromatography 10A System (Shimadzu, Kyoto, Japan) equipped with an

octadecylsilane (C18) column (XBridge, 3.5 μm , 2.1 mm $\times$ 150 mm, Waters, Tokyo, Japan). LC conditions were as follows: Solvent A contained 0.1% HCO_2H in acetonitrile and Solvent B contained 0.1% HCO_2H in H_2O (v/v). The following gradient program was used, with a flow rate of 0.25 mL min^{-1} : 0–2 min, linear gradient from 0% A to 25% A (v/v); 2–4 min, hold at 25% A; 4–10 min, linear gradient from 25% A to 95% A; 10–14 min, hold at 95% A; 14–15 min, linear gradient to 0% A; 15–20 min, hold at 0% A. The temperature of the column was maintained at 40 $^{\circ}\text{C}$. Samples (10 μL) were infused with an auto-sampler. The retention times of 5-hydroxypomalidomide glucuronide conjugate, 7- and 5-hydroxypomalidomide, and pomalidomide were 5.9, 6.6, 7.0, and 8.1 min, respectively. The mass spectrometer was tuned using commercially available pomalidomide and synthesized 5-hydroxypomalidomide. Pomalidomide was quantified using the m/z 272 $\rightarrow$ 161 transition of pomalidomide and 7- and 5-hydroxypomalidomide were measured using the m/z 288 $\rightarrow$ 176 transitions of 7- and 5-hydroxypomalidomide, on the basis of a standard curve of 5-hydroxypomalidomide. The 5-hydroxypomalidomide-glucuronide conjugate was monitored using the m/z 464 $\rightarrow$ 176 transition, on the basis of a standard curve of pomalidomide. Statistical analysis for the plasma concentrations of pomalidomide and its metabolites in control and humanized-liver mice was done using two-way analysis of variance (ANOVA) with Bonferroni post tests (Prism, GraphPad Software, La Jolla, CA).

Estimation of plasma concentrations using PBPK models

The simplified PBPK model consisted of a chemical receptor (gut) compartment, a metabolizing (liver) compartment, and a central compartment and was set up as described elsewhere (Nishiyama et al., 2015; Utoh et al., 2016; Yamazaki et al., 2016b). Chemical properties of pomalidomide and the primary metabolite (Table 1) were estimated as described elsewhere (Nishiyama et al., 2015; Yamazaki et al., 2016b). To define similar simplified human PBPK models, values (of k_a , V_1 , and $CL_{h,int}$) shown in Table 2 in the human PBPK model were estimated using a scaling strategy from control and humanized-liver mice to humans as described elsewhere (Nishiyama et al., 2015; Yamazaki et al., 2016b), without consideration of an interspecies factor between *in vitro* liver clearances. Briefly, parameters that represent physiological hepatic blood flow rates (Q_h) in mice (0.160 L/h) and humans (96.6 L/h) were used. Values of the fraction absorbed (F_a) $\times$ intestinal availability (F_g) were estimated by fitting curves to plasma concentrations at several sampling points for mice and humanized mice; $F_a \times F_g$ was assumed to be 1.0 for humans (Nishiyama et al., 2015). Subsequently, final parameter values and SDs for the absorption rate constant (k_a), the volume of the systemic circulation (V_1), and the hepatic intrinsic clearance ($CL_{h,int}$) were calculated to give the best fit to measured blood substrate concentration values (Shida et al., 2015; Shida and Yamazaki, 2016; Utoh et al., 2016). Finally, in a similar way to that previously carried out for mouse systems of differential equations were set up and solved to determine the concentrations in each compartment in humans.

Results and Discussion

Pomalidomide and its primary (7- and 5-hydroxypomalidomide) and secondary metabolites (5-hydroxypomalidomide glucuronide conjugate) (Hoffmann et al., 2013) were measured

(Fig. 2) by LC-MS/MS analysis in plasma samples obtained from control and humanized-liver mice following oral administration (100 mg/kg). Pomalidomide was detected 30 min following oral administration (Fig. 2A). Plasma concentrations of 7-hydroxypomalidomide were higher in humanized-liver mice than control mice (Fig. 2B). Plasma concentrations of the 5-hydroxypomalidomide were similar in two mouse systems, but the secondary metabolite 5-hydroxythalidomide-gluconide conjugate(s) (Fig. 2D) was produced at significant levels in humanized-liver mice. A corresponding molecular ion for the putative 5-hydroxypomalidomide-glutathione conjugate was not seen in the preliminary study (not shown).

Based on the present *in vivo* experiments (Fig. 2), the kinetic parameters for pomalidomide disposition in control and humanized-liver mice were calculated. By solving the equations for simplified PBPK models, *in silico* plasma concentration curves after virtual administration of pomalidomide were created in control (Fig. 3A) and humanized-liver (Fig. 3B) mice. Using an allometric scaling method (Nishiyama et al., 2015; Yamazaki et al., 2016b), human PBPK models for pomalidomide and its combined primary 5-hydroxypomalidomide metabolites were set up (Fig. 3C). The available reported human plasma data (Hoffmann et al., 2013), obtained after administration of a single low dose of 2 mg pomalidomide to subjects, could be reasonably estimated after virtual oral administration of pomalidomide to humans in two simplified human PBPK models (Fig. 3C), which were set up based on mouse and humanized-liver mouse PBPK models. The key pharmacokinetic human parameters ($C_{\max}$ and AUC_{0-24} values), taken from reported actual means with considerable standard deviations in 8 individual subjects (Hoffmann et al., 2013), were consistent with the predicted human virtual $C_{\max}$ and AUC_{0-24} values extrapolated from the two investigated animal systems with PBPK modeling, supporting the relatively good fitting results (within roughly two-fold ranges of predictions in the current system).

This study demonstrates that pharmacokinetic profiles of pomalidomide were roughly similar between control and humanized-liver mice (Fig. 2A). Plasma levels of 5-hydroxypomalidomide were comparable in control and humanized-liver mice (Figs. 3A and 3B). Chimeric mice produced similar metabolite profile of pomalidomide to that seen humans (Hoffmann et al., 2013). At this moment, the reason why glucuronide metabolite formation and 7-hydroxy metabolite were higher in humanize mouse was not known.

In the case of thalidomide (reported previously (Hasegawa et al., 2011; Yamazaki et al., 2012)), rodent specific 5'-hydroxythalidomide and the human product 5-hydroxythalidomide were detected; moreover, the primary 5-hydroxy metabolite was further oxidized to reactive metabolite(s) trapped *in vivo* by GSH (Yamazaki et al., 2012; Nishiyama et al., 2015) and non-specific proteins (Yamazaki et al., 2016a) in humanized-liver mice.

In conclusion, the results indicate that introducing one aromatic amino group into thalidomide—to yield pomalidomide—resulted in less species differences in *in vivo* pharmacokinetics in control and humanized-liver mice, which can be extrapolated to metabolic profiles in human situations by PBPK modeling.

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Fig. 1.

Metabolic pathway of pomalidomide. Other minor metabolites (hydrolysis product and *N*-acetylated product) have been reported (Hoffmann et al., 2013).

Fig. 2. Plasma concentrations of pomalidomide (A), 7-hydroxypomalidomide (B), 5-hydroxypomalidomide (C), and 5-hydroxypomalidomide-glucuronide conjugate (D) in control mice (open circles) and humanized-liver mice (solid circles) after oral administration of pomalidomide (100 mg/kg). Results are expressed as mean values ($\pm$ SD) obtained with five mice (** $p < 0.01$ and * $p < 0.05$, two-way ANOVA with Bonferroni post tests).

Fig. 3.

Plasma concentrations of pomalidomide (circles, broken lines) and the sum of 5-hydroxy pomalidomide metabolites containing 5-hydroxythalidomide-glucuronide conjugate (squares, solid lines) measured in control mice (A, gray line) and humanized-liver mice (B, black line) and estimated in humans (C) after oral administration of a single doses of pomalidomide (2 mg for subjects) by PBPK models (shown in gray and black), based on control mice (gray lines) and humanized-liver mice (black lines) models. Circles and squares in (C) show reported human plasma concentrations of pomalidomide and the sum of 5-hydroxypomalidomide metabolites, repetitively, after a single oral administration of pomalidomide (Hoffmann et al., 2013).

Table 1

Chemical properties of pomalidomide and its primary metabolite.

Parameter	Symbol	Pomalidomide	5-Hydroxy-pomalidomide
Molecular weight	MW	273	289
Octanol-water partition coefficient	logP	−0.189	−0.672
Plasma unbound fraction	$f_{u,p}$	0.900	0.858
Blood to plasma concentration ratio	R_b	0.719	0.755
Liver to plasma concentration ratio	$K_{p,h}$	0.731	0.706

The same plasma unbound fraction, blood-to-plasma ratio, and liver to plasma ratio were used for mouse and human (Shida et al., 2015; Shida and Yamazaki, 2016; Utoh et al., 2016).

Table 2
Experimental and calculated parameters for PBPK models of pomalidomide disposition.

Parameter	Symbol (unit)	Mouse	Humanized liver mouse	Human, from mouse	Human, from humanized liver mouse
Absorption rate constant	k_a (1/h)	2.04 ± 0.30	1.47 ± 0.20	1.52	1.10
Fraction absorption $\times$ intestinal availability	$F_a \cdot F_g$	1	1	1	1
Volume of systemic circulation for pomalidomide	$V_{l_substrate}$ (L)	0.578 ± 0.053	0.634 ± 0.062	171	185
	$V_{l_substrate}$ (L/kg body weight)	23.1	25.4	2.44	2.64
Hepatic intrinsic clearance for pomalidomide	$CL_{h_int_substrate}$ (L/h)	0.0890 ± 0.0106	0.0807 ± 0.0107	8.92	8.08
	$CL_{h_int_substrate}$ (L/h kg body weight)	3.56	3.23	0.127	0.115
Metabolic ratio to primary metabolite	mRatio	0.148	0.221	0.148	0.221
Volume of systemic circulation for primary metabolite	$V_{l_primary_metabolite}$ (L)	0.505	0.294	151	93.7
Hepatic intrinsic clearance for primary metabolite	$CL_{h_int_primary_metabolite}$ (L/h)	0.0449	0.0339	4.50	3.40

Values in parentheses are standard deviations

Table 3

Summary of pharmacokinetic parameters of taken form literature and calculated after virtual oral administration of pomalidomide (2 mg/kg) to humans

Drug		$C_{\max}$	AUC_{0-24}
		<i>ngEQ h mL⁻¹</i>	<i>ngEQ h mL⁻¹</i>
Pomalidomide	Literature	11.0 ($\pm$ 2.5)	135 ($\pm$ 45)
		<i>ng mL⁻¹</i>	<i>ng h mL⁻¹</i>
	Human, from mice	12.7	187
	Human, from humanized mice	11.7	188
		<i>ngEQ h mL⁻¹</i>	<i>ngEQ h mL⁻¹</i>
Metabolite	Literature	-	20.5 ($\pm$ 12.9)
		<i>ng mL⁻¹</i>	<i>ng h mL⁻¹</i>
	Human, from mice	1.2	22.2
	Human, from humanized mice	2.4	45.5

Values in parentheses are standard deviations taken form literature (Hoffmann et al., 2013).