Pharmacokinetics of primary metabolites 5-hydroxythalidomide and 5′-hydroxythalidomide formed after oral administration of thalidomide in the rabbit, a thalidomide-sensitive species

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ABSTRACT — The teratogenicity of the chemotherapeutic drug thalidomide is species-specific and affects humans, non-human primates, and rabbits. The primary oxidation of thalidomide in previously investigated rodents predominantly resulted in the formation of deactivated 5′-hydroxythalidomide. In the current study, similar in vivo biotransformations to 5-hydroxythalidomide and 5′-hydroxythalidomide were confirmed by the analysis of blood plasma from male rabbits, a thalidomide-sensitive species, after oral administration of thalidomide (2.0 mg/kg). Similar levels of thalidomide in seminal plasma and in blood plasma were detected using liquid chromatography–tandem mass spectrometry at 4 hr and 7 hr after oral doses in male rabbits. Seminal plasma concentrations of 5-hydroxythalidomide and 5′-hydroxythalidomide were also seen in male rabbits in a roughly similar time-dependent manner to those in the blood plasma after oral doses of thalidomide (2.0 mg/kg). Furthermore, the values generated by a simplified physiologically based pharmacokinetic rabbit model were in agreement with the measured in vivo blood plasma data under metabolic ratios of 0.01 for the hepatic intrinsic clearance of thalidomide to both unconjugated 5-hydroxythalidomide and 5′-hydroxythalidomide. These results suggest that metabolic activation of thalidomide may be dependent on rabbit liver enzymes just it was for cytochrome P450 enzymes in humanized-liver mice; in contrast, rodent livers predominantly mediate biotransformation of thalidomide to 5′-hydroxythalidomide. A developmental toxicity test system with experimental animals that involves intravaginal exposures to the chemotherapeutic drug thalidomide via semen should be considered in the future.

Key words: 5-Hydroxythalidomide, Seminal plasma, PBPK modeling, Human

INTRODUCTION

Among experimental animals, thalidomide [α-(N-phthalimido)glutarimide] appears not to be teratogenic in rodents (Kim and Scialli, 2011), but is notoriously teratogenic in rabbits and nonhuman primates (Calabrese and Resztak, 1998). In the 2000s, thalidomide was approved as a chemotherapeutic drug for multiple myeloma and an anti-inflammatory agent for Hansen’s disease (Palumbo et al., 2008; Nakamura et al., 2013). Pregnant rabbits orally dosed with up to 500 mg/kg thalidomide experienced changes in some natural delivery and litter parameters and limb splay in some pups (Teo et al., 2004a). Because of its known adverse effects on fetal development (Speirs, 1962), the dispensing of thalidomide is now strictly regulated in the clinical setting.

The teratogenicity of many xenobiotics depends partly on their metabolic bioactivation (Wells et al., 1997). Pretreatment of pregnant rabbits with a free radical spin-trapping agent has reportedly inhibited the teratogenicity of
thalidomide (Wells et al., 1997; Parman et al., 1999). The metabolism of thalidomide is also important for outcomes in humans, especially as its pharmacokinetics is known to be modified by other co-administered anti-cancer drugs (Chung et al., 2004b). The primary oxidative metabolic pathways of thalidomide mediated by liver cytochrome P450 enzymes were experimentally confirmed to generate aromatic hydroxylated 5-hydroxythalidomide or aliphatic hydroxylated 5′-hydroxythalidomide (Chowdhury et al., 2010, 2014). In animal species that are not sensitive to thalidomide, e.g., rats, the main metabolite in plasma is 5′-hydroxythalidomide, which is further converted to excreted conjugated forms such as 5′-hydroxythalidomide sulfate or 5′-hydroxythalidomide glucuronide (Miura et al., 2021). In contrast, in humanized-liver mice, hydroxylation of the aromatic ring occurs to form the activated primary oxidative metabolite 5-hydroxythalidomide; secondary oxidation of 5-hydroxythalidomide was found to result in nonspecific protein bindings in liver (Yamazaki et al., 2016a) and substituted conjugations with glutathione (Yamazaki et al., 2012). Consequently, primary aromatic hydroxylation of thalidomide, but not aliphatic hydroxylation, may be relevant to its toxicological and metabolic activation. Nevertheless, the detailed pharmacokinetics of thalidomide have not been determined in rabbits (Chung et al., 2004a) in terms of the concentrations of both primary metabolites, 5-hydroxythalidomide and 5′-hydroxythalidomide.

The present study’s purpose was to explore in vivo the primary oxidative metabolism of thalidomide in rabbits to clarify the extent of conversion to activated or deactivated forms. Utilizing in vivo plasma concentration–time data acquired in the rabbit, a simplified physiologically based pharmacokinetic (PBPK) model made up of four compartments, i.e., gut, liver, kidney, and central compartments, was established. Using this system, the metabolic ratios of these activated and deactivated primary metabolites of thalidomide were examined by applying the simplified rabbit PBPK models to generate 5-hydroxythalidomide and 5′-hydroxythalidomide concentration profiles. In addition, seminal plasma concentrations of thalidomide, 5-hydroxythalidomide, and 5′-hydroxythalidomide were detected using liquid chromatography–mass spectrometry 4 hr and 7 hr after oral doses (2.0 mg/kg) in male rabbits, revealing a similar time dependence to those in blood plasma. We report herein more extensive in vivo 5-hydroxythalidomide formation in rabbits (known to be thalidomide-sensitive) compared to that reported in humanized-liver mice; however, 5-hydroxythalidomide formation was not evident in rats, which are known not to be sensitive to thalidomide. These results imply that future developmental toxicity test systems with experimental animals involving intravaginal exposures via semen should be considered to reflect the parallel metabolic pathways of 5-hydroxythalidomide and 5′-hydroxythalidomide formation.

MATERIALS AND METHODS

Animals and chemicals
Male New Zealand White rabbits (16–17 weeks old, 3.0–4.5 kg, Kitayama Labes, Nagano, Japan) were used in this study. Blood samples (~400 µL) were collected from the male rabbits between 0.5 and 24 hr after a single oral dose of 2.0 mg/kg thalidomide (> 98%, CarboSynth, Compton, UK). Semen samples (~0.5 g) were also collected at 4 hr or 7 hr after oral doses. The use of experimental animals in the current study was authorized by the Ethics Committees of the National Institute of Health Sciences and BoZo Research Center. After treatment of the rabbit blood plasma and seminal plasma fractions (10 µL with equal and ninefold volumes of 25 mM citrate buffer (Na₃C₆H₅O₃·HCl, pH 1.5), respectively, and with two volumes of a mixture of methanol, acetonitrile, and formic acid (50:49:1, v/v)) containing pomalidomide as the internal standard, the resulting mixture was applied to universal polymeric reversed-phase sorbents (Oasis HLB 96-well plate, 10 mg Sorbent per well, 30 µm; Waters, Milford, MA, USA) with the aid of 0.50 mL of 25 mM citrate buffer (pH 1.5). After the solid-phase extraction sorbents were washed with 1.0 mL of water and 1.0 mL of the mixture of methanol, acetonitrile, and formic acid (140:60:1, v/v), thalidomide and its metabolites were eluted with 0.50 mL of methanol. The organic eluates were dried under a gentle nitrogen stream, and the residues were then dissolved in 10% acetonitrile in 0.1% acetic acid aqueous solution (v/v).

Quantitative liquid chromatography (LC)–tandem mass spectrometry (MS) evaluations of thalidomide, 5′-hydroxythalidomide, and 5-hydroxythalidomide levels were carried out in accordance with previously reported methods (Nishiyama et al., 2015; Shimizu et al., 2017; Miura et al., 2021) with minor adjustments. A Triple Quad 5500 tandem mass spectrometer (AB Sciex, Framingham, MA, USA) was directly coupled to an Acquity UPLC I-Class System liquid chromatograph (Waters) and operated in electrospray positive ionization mode for thalidomide and pomalidomide (internal standard) or electrospray negative ionization mode for 5′-hydroxythalidomide, 5-hydroxythalidomide, and pomalidomide (internal standard). Analyte samples (3.0 µL) were separated by a reversed-phase C₁₈ column (BetaSil C18, par-
Thalidomide PK in rabbits

ticle size: 5 μm, 4.6 × 125 mm; Thermo Fisher Scientific, Boston, MA, USA) at 25°C with a mobile phase of 50% acetonitrile in 0.1% acetic acid aqueous solution (v/v) at a flow rate of 0.50 mL/min. Thalidomide, 5'-hydroxylthalidomide, and 5-hydroxylthalidomide were quantified using the following transitions: m/z 259→186 for thalidomide (positive ion mode), m/z 273→161 for 5-hydroxylthalidomide (negative ion mode), and m/z 273→146 for 5'-hydroxylthalidomide (negative ion mode), as described previously (Yamazaki et al., 2012). For pomalidomide, the m/z 274→201 transition in positive ion mode and the m/z 272→161 transition in negative ion mode were used. For the currently employed conditions, thalidomide, 5-hydroxylthalidomide, and 5'-hydroxythalidomide levels in plasma were measurable at concentrations ≥ 0.40 ng/mL or ng/g and detectable at concentrations ≥ 0.04 ng/mL or ng/g. Authentic thalidomide-related metabolites were purchased from the previously reported sources (Miura et al., 2021).

The general procedures employed to prepare in vitro human intestinal Caco-2 monolayers were described previously (Kamiya et al., 2020b). The permeability coefficients for thalidomide were calculated from the time-dependent data of in vitro absorption from the apical to basal and basal to apical sides of Caco-2 monolayers.

**Physiologically based pharmacokinetic modeling**

A simplified physiologically based pharmacokinetic (PBPK) rabbit model made up of chemical receptor (gut), metabolizing (liver), excreting (kidney), and central compartments was established following the same methodology as that for a previous rat model. The reported in silico physicochemical parameters for thalidomide, 5-hydroxythalidomide, and 5'-hydroxylthalidomide were used (Miura et al., 2021). The reported physiological values for rabbits (Davies and Morris, 1993) were adopted, i.e., hepatic and renal volumes (V_h and V_r) of 0.10 L and 0.015 L, respectively. The renal and hepatic blood flow rates (Q_h and Q_r) in rabbits (2.5 kg body weight) were set at a common value of 10.6 L/hr for simplicity. The preliminary parameter values for PBPK modeling, i.e., the fraction absorbed × intestinal availability (F_a; F_i), the hepatic clearance (CL_h), and the renal clearance (CL_r), were generated from the constants evaluated using one-compartment models (Miura et al., 2021).

The concentrations of thalidomide and its metabolites in the blood plasma of orally treated rabbits were measured in vivo. Based on the resulting experimental pharmacokinetic data in rabbits, in vivo pharmacokinetic parameters were primarily derived from the plasma concentration–time curves using one-compartment analysis with Phoenix WinNonlin 8.2 (Pharsight, Mountain View, CA, USA). In this way, the preliminary initial values for the fraction absorbed × intestinal availability (F_a; F_i), the hepatic clearance (CL_h = Q_a·CL_h×Q_a), and the renal clearance (CL_r) for the PBPK model were obtained from the elimination constants (Miura et al., 2021, 2020). The input parameters for the PBPK model, i.e., the absorption rate constant (k_a), the volume of the systemic circulation (V_s), and the hepatic intrinsic clearance (CL_h,int) (Table 1), were calculated using simplex and modified Marquardt methods to ensure that the model’s output was in accordance with the substrate and metabolite concentrations in plasma measured in vivo in the current study. The general ratio of CL_h to CL_r, was set at 9:1 (Kamiya et al., 2019, 2020a). In the current rabbit PBPK models, the metabolic ratios to unconjugated 5-hydroxylthalidomide and to 5'-hydroxythalidomide were both estimated to be 0.01 by fitting procedures. The set of differential equations described below was used to generate in silico concentrations of the substrate and its primary metabolites (subscript m):

\[
\frac{dM}{dt} = -k_a \cdot M + \left(\frac{Q_h}{V_h} \cdot C_{tot} \cdot Q_r + \frac{Q_r}{V_r} \cdot C_{tot} \cdot Q_h\right)
\]

where \(X_a\), \(V_h\), \(V_r\), \(K_{hp}\), and \(K_{rp}\) indicate the following: the quantity of substrate in the gut compartment; the volumes of the liver and kidney; and the concentrations of substrate (metabolites) in liver, kidney, and blood, respectively.

**RESULTS AND DISCUSSION**

Thalidomide is not teratogenic in rodents, but it is teratogenic in rabbits, nonhuman primates, and humans (Calabrese and Resztak, 1998). In the current study, a low oral dose of 2.0 mg/kg thalidomide (Chung et al., 2004a) was selected for the rabbit pharmacokinetic experiments on the basis of the reported embryopathic doses for rabbits, which are known to be sensitive to thalidomide (Hui et al., 2014; Teo et al., 2004b). After oral administrations of thalidomide to rabbits, the pharmacokinetics of thalidomide and its metabolites were quantitatively investigated using LC-MS/MS analyses with authentic standard thalidomide and its primary metabolites 5-hydroxylthalidomide and 5'-hydroxythalidomide after solid-phase extrac-
Thalidomide was detected in rabbit plasma fractions 0.5 hr after oral administration. Apparent half-life of thalidomide in the clearance from rabbit plasma was calculated to be 1.9 hr, in accordance with the reported half-life of 2.2 hr (Chung et al., 2004a). In addition, two hours after single oral doses of 2.0 mg/kg thalidomide in rabbits, the maximum concentrations in blood plasma of thalidomide (330 ng/mL), 5-hydroxythalidomide (4.9 ng/mL), and 5′-hydroxythalidomide (4.6 ng/mL) were evident. (Fig. 1). After 24 hr, these concentrations in plasma samples were below the quantitative detection limits (< 0.40 ng/mL) using LC-MS/MS analyses under the present conditions.

In humans, detectable levels of thalidomide in plasma (10–350 ng/mL) and semen (10–250 ng/g) have been reported after 4 and 8 weeks of treatment in two

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**Table 1.** Experimental and final calculated parameters for rabbit one-compartment and PBPK models for thalidomide and its metabolites based on rabbit in vivo pharmacokinetic data.

| Parameter | Abbreviation (unit) | Rabbit |
|-----------|----------------------|--------|
| **Observed levels** | | |
| C<sub>max</sub> in plasma for thalidomide | ng/mL | 334 |
| C<sub>max</sub> in plasma for 5-hydroxythalidomide | ng/mL | 4.88 |
| C<sub>max</sub> in plasma for 5′-hydroxythalidomide | ng/mL | 4.61 |
| AUC in plasma for thalidomide | ng·hr/mL | 1350 |
| AUC in plasma for 5-hydroxythalidomide | ng·hr/mL | 20.4 |
| AUC in plasma for 5′-hydroxythalidomide | ng·hr/mL | 18.2 |
| **One-compartment model** | | |
| Absorption rate constant for thalidomide | 1/hr | 1.80 ± 0.41<sup>ab</sup> |
| Volume of distribution/bioavailability for thalidomide | L/kg | 3.54 ± 0.55<sup>ab</sup> |
| Oral clearance for thalidomide | L/hr/kg | 1.36 ± 0.13<sup>ab</sup> |
| **PBPK model** | | |
| Fraction absorbed × intestinal availability | F<sub>a</sub>·F<sub>g</sub> | 1 |
| Absorption rate constant | k<sub>a</sub> (1/hr) | 2.37 ± 0.07<sup>ab</sup> |
| Volume of systemic circulation for thalidomide | V<sub>1_substrate</sub> (L) | 7.08 ± 0.07<sup>ab</sup> |
| Hepatic intrinsic clearance for thalidomide | CL<sub>h,int_substrate</sub> (L/hr) | 6.08 ± 0.07<sup>ab</sup> |
| Hepatic clearance for thalidomide | CL<sub>h_substrate</sub> (L/hr) | 2.67 |
| Renal clearance for thalidomide | CL<sub>r_substrate</sub> (L/hr) | 0.28 |
| Volume of systemic circulation for 5-hydroxythalidomide | V<sub>1_5-hydroxythalidomide</sub> (L) | 2.78 ± 0.02<sup>ab</sup> |
| Volume of systemic circulation for 5′-hydroxythalidomide | V<sub>1_5′-hydroxythalidomide</sub> (L) | 4.05 ± 0.01<sup>ab</sup> |
| Hepatic intrinsic clearance for 5-hydroxythalidomide | CL<sub>h,int_5-hydroxythalidomide</sub> (L/hr) | 9.05 ± 0.02<sup>ab</sup> |
| Hepatic intrinsic clearance for 5′-hydroxythalidomide | CL<sub>h,int_5′-hydroxythalidomide</sub> (L/hr) | 3.61 ± 0.01<sup>ab</sup> |
| Hepatic clearance for 5-hydroxythalidomide | CL<sub>h_5-hydroxythalidomide</sub> (L/hr) | 1.78 |
| Hepatic clearance for 5′-hydroxythalidomide | CL<sub>h_5′-hydroxythalidomide</sub> (L/hr) | 1.84 |
| Renal clearance for 5-hydroxythalidomide | CL<sub>r_5-hydroxythalidomide</sub> (L/hr) | 0.18 |
| Renal clearance for 5′-hydroxythalidomide | CL<sub>r_5′-hydroxythalidomide</sub> (L/hr) | 0.19 |
| **Estimated values** | | |
| C<sub>max</sub> in plasma for thalidomide | ng/mL | 390 (1.2) |
| C<sub>max</sub> in plasma for 5-hydroxythalidomide | ng/mL | 4.61 (0.95) |
| C<sub>max</sub> in plasma for 5′-hydroxythalidomide | ng/mL | 3.71 (0.89) |
| AUC in plasma for thalidomide | ng·hr/mL | 1240 (0.92) |
| AUC in plasma for 5-hydroxythalidomide | ng·hr/mL | 19.5 (0.96) |
| AUC in plasma for 5′-hydroxythalidomide | ng·hr/mL | 17.3 (0.95) |

The molecular weights (258, 274, and 274), octanol–water partition coefficients (clogP: 0.528, –0.138, and 0.402), plasma unbound fractions (f<sub>u,p</sub>: 0.588, 0.615, and 0.237), and blood–plasma concentration ratios (R<sub>b</sub>: 0.893, 0.885, and 0.904) of thalidomide, 5′-hydroxythalidomide, and 5-hydroxythalidomide, respectively, were used as described previously (Miura et al., 2021). The metabolic ratios to 5-hydroxythalidomide and 5′-hydroxythalidomide in rabbits were both set to 0.01 by fitting procedures. Mean C<sub>max</sub> and AUC data (for 0–7 hr) are for a single oral dose of 2.0 mg/kg thalidomide in six male rabbits. Estimated values for virtual administrations of 2.0 mg/kg thalidomide using the newly developed PBPK rabbit model. Values in parentheses are ratios of the estimated/observed values.
of four patients taking 100 mg/day thalidomide (Teo et al., 2001). In the current rabbit experiments, the seminal plasma concentrations of thalidomide (130 and 36 ng/g) and 5′-hydroxythalidomide (4.8 and 1.7 ng/g) 4 hr and 7 hr after oral doses (Table 2) were similar to their concentrations in blood plasma under the present conditions (Fig. 1). In our preliminary experiments, after a high single oral dose of 250 mg/kg thalidomide in rabbits, the observed blood and seminal plasma concentrations of thalidomide, 5-hydroxythalidomide and 5′-hydroxythalidomide at 7 hr after administration were 10 µg/mL and 6 µg/g, 0.1 µg/mL and 0.02 µg/g, and 0.05 µg/mL and 0.06 µg/g, respectively. There was no apparent condensation of the substrate thalidomide or the primary metabolite 5′-hydroxythalidomide from blood plasma to seminal plasma, as evidenced by the similar observed blood and seminal plasma concentrations after oral administration of the main experimental dose of 2.0 mg/kg and after the preliminary dose of 250 mg/kg in rabbits; this is consistent with reported data for thalidomide (Teo et al., 2001). The observed seminal plasma concentrations of the other primary metabolite, 5-hydroxythalidomide (0.49 ng/g), after oral administration was low compared with those in blood plasma in rabbits. In this study, the in vitro apparent permeability constants for thalidomide were determined to be similar in both directions [240 nm/sec from apical to basal (Kamiya et al., 2020b) and 180 nm/sec from basal to apical], which is consistent with the reported findings of no apparent active transport for thalidomide by transporters such as P-glycoprotein (Zimmermann et al., 2006; Zhou et al., 2003). The reason for the lower concentrations of 5-hydroxythalidomide in seminal plasma than in blood plasma is not clear at present; indeed, 5-hydroxythalidomide should be further cleared from blood plasma through secondary oxidation metabolism by liver cytochrome P450 3A enzymes, as was identified in humanized-liver mice (Yamazaki et al., 2016a). In rat plasma, the conjugated metabolites of 5′-hydroxythalidomide were present at much higher concentrations than those of 5-hydroxythalidomide after oral administration of 250 mg/kg thalidomide (Miura et al., 2021).

In rabbits, the concentrations of 5′-hydroxythalidomide in plasma were similar to those of 5-hydroxythalidomide (Fig. 1). From these time-dependent concentration data, the input values of $k_a$, $V_1$, and CL$_{h,int}$ for use in rabbit PBPK models were established using fitting procedures. The coefficients of variation for these three input parameters were less than 10% in this study, as shown in Table 1. The metabolic ratios of thalidomide to 5-hydroxythalidomide and to 5′-hydroxythalidomide were set at the same value, 0.01, for the current rabbit PBPK models, based on the measured plasma concentration data. For the previously established rat PBPK models, the metabolic ratios of thalidomide to unconjugated 5′-hydroxythalidomide and 5-hydroxythalidomide, respectively, were set at 0.05 (fivefold that of the rabbit) and 0.0025 (one-quarter that of the rabbit) (Miura et al., 2021).

Table 2. Detection of thalidomide and its metabolites in seminal plasma after oral doses (2.0 mg/kg) in male rabbits.

| Time after oral administration, hr | Seminal plasma concentration, ng/g |
|-----------------------------------|----------------------------------|
|                                   | Thalidomide | 5-Hydroxythalidomide | 5′-Hydroxythalidomide |
| 4                                 | 130 ± 50    | 0.49 ± 0.09       | 4.8 ± 0.5        |
| 7                                 | 36 ± 9      | < 0.4*            | 1.7 ± 0.3        |

Three seminal plasma samples at either 4 hr or 7 hr after oral administration of thalidomide (2.0 mg/kg) were obtained from six individual male rabbits. Data are the mean and SD (n = 3) for the stated sampling points.
solutions to the equations that constitute the rabbit PBPK model generated virtual plasma concentration curves for rabbits. The modeled concentration profiles for thalidomide, 5-hydroxythalidomide, and 5-hydroxythalidomide are given in Fig. 1. The PBPK-generated output was in accordance with the empirically derived in vivo data points. Our experimentally evaluated maximum plasma concentrations (C\text{max}) and areas under the curve (AUCs) for thalidomide were reasonably comparable with previously reported findings (Chung et al., 2004a). Furthermore, the measured C\text{max} and AUC values for thalidomide, 5-hydroxythalidomide, and 5′-hydroxythalidomide and the PBPK-modeled values for single oral doses were similar and are given in Table 1; all modeled values were within 20% of the observed data.

The pharmacokinetics of the substrate thalidomide are available for multiple myeloma patients and for mice (Chung et al., 2004a). In previous experiments with humanized-liver mice, it was established that the proportions of mouse livers replaced with transplanted human hepatocytes (in immunodeficient mice) were correlated with the ratios of in vivo concentrations of 5-hydroxythalidomide to 5′-hydroxythalidomide in blood plasma 2 hr after an oral dose of 100 mg/kg thalidomide (Miura et al., 2021). Because there was no evidence of active condensation or apparent difference in observed concentrations of the substrate thalidomide or the primary metabolite 5′-hydroxythalidomide between blood plasma and seminal plasma, the current simplified PBPK modeling system was deemed also to be suitable for application to rabbits. To describe the pharmacokinetics for a range of substrates, simplified PBPK models consisting of gut, liver, and central compartments have been established for typical P450 probe drugs across animal species such as monkeys (Shida et al., 2015), dogs, minipigs (Shida and Yamazaki, 2016), marmosets, and humanized-liver mice (Utoh et al., 2016). To allow the estimation of chemical exposures in plasma and tissues (i.e., liver and kidney), our simplified PBPK models were recently successfully modified with the addition of an excreting (kidney) compartment for rats (Kamiya et al., 2021) and humans (Yanagi et al., 2021; Miura et al., 2020). Furthermore, to investigate pharmacokinetics in humans, these animal PBPK models were converted to human PBPK models by using known species allometric scaling factors to determine the input parameters (Yamazaki et al., 2016b). This simplified PBPK modeling system can also be expanded to rabbits, as is clearly indicated in this study.

With further improvements to the model, investigations using rabbit PBPK modeling would be of use to explore the toxicokinetics after large doses of thalidomide. Our preliminary observations of rabbit plasma concentrations of the substrate thalidomide and its primary metabolites 5-hydroxythalidomide and 5′-hydroxythalidomide at 7 hr after a high oral dose of 250 mg/kg could be successfully simulated using the current rabbit PBPK model with the same pharmacokinetic input parameters generated using data from the low dose of 2.0 mg/kg (Table 1). These results imply that rabbit liver function may be a determinant factor for the generation of the active primary metabolite 5-hydroxythalidomide in vivo. Rabbit liver mediated thalidomide oxidation at the aromatic ring (Yamazaki et al., 2012; Nishiyama et al., 2015), thereby leading to the reported activation of thalidomide (Calabrese and Resztak, 1998). It has also been reported that mice suffered no apparent adverse effects on receiving an oral dose of 270 mg/kg (~1 nmol) thalidomide/kg, but this oral dose was lethal for approximately half of the humanized-liver mice (Miura et al., 2021). Rabbit livers appear to be slightly different from human liver, but based on the current pharmacokinetic information, the intravaginal administration of thalidomide to female rabbits will be conducted in the future as a new approach for investigating adverse effects on fetal development.

5-Hydroxythalidomide, which results from 5-hydroxylation of thalidomide’s aromatic ring, was previously retrieved from rabbit urine, but not from rat urine (Schumacher et al., 1965). Species differences in terms of susceptibility to thalidomide teratogenicity may arise from differences in the primary biotransformation of potentially toxic substances. In the present investigation, the metabolic ratio adopted for the hepatic intrinsic clearance of thalidomide to unconjugated 5-hydroxythalidomide in rabbit PBPK modeling was 0.01, which is four times higher than that used for rats. In summary, metabolic activation of thalidomide may be dependent on rabbit liver enzymes in the same manner as that reported for cytochrome P450 enzymes in humanized-liver mice, but not for rodent livers. Because the threshold dose and exposure levels of thalidomide that result in birth defects are not known yet, male patients are advised to use barrier contraception while being treated with thalidomide.

In the future, a developmental toxicity test system combined with pharmacokinetic information on the primary metabolites in experimental animals should be considered that investigates intravaginal exposures via semen of the chemotherapeutic drug thalidomide and other related medicines.
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Conflict of interest---- The authors declare that there is no conflict of interest.

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