Prediction of gastric pH-mediated drug exposure using physiologically-based pharmacokinetic modeling: A case study of itraconazole

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Abstract

Abnormal gastric acidity, including achlorhydria, can act as a significant source of variability in orally administered drugs especially with pH-sensitive solubility profiles, such as weak bases, potentially resulting in an undesirable therapeutic response. This study aimed to evaluate the utility of physiologically-based pharmacokinetic (PBPK) modeling in the prediction of gastric pH-mediated drug exposure by using itraconazole, a weak base, as a case. An itraconazole PBPK model was developed on the mechanistic basis of its absorption kinetics in a middle-out manner from a stepwise in vitro-in vivo extrapolation to in vivo refinement. Afterward, an independent prospective clinical study evaluating gastric pH and itraconazole pharmacokinetics (PKs) under normal gastric acidity and esomeprazole-induced gastric hypoacidity was conducted for model validation. Validation was performed by comparing the predicted data with the clinical observations, and the valid model was subsequently applied to predict PK changes under achlorhydria. The developed itraconazole PBPK model showed reasonable reproducibility for gastric pH-mediated exposure observed in the clinical investigation. Based on the model-based simulations, itraconazole exposure was expected to be decreased up to 65% under achlorhydria, and furthermore, gastric pH-mediated exposure could be mechanistically interpreted according to sequential variation in total solubility, dissolution, and absorption. This study suggested the utility of PBPK modeling in the prediction of gastric pH-mediated exposure, especially for drugs whose absorption is susceptible to gastric pH. Our findings will serve as a leading model for further mechanistic assessment of exposure depending on gastric pH for various drugs, ultimately contributing to personalized pharmacotherapy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Abnormal gastric acidity, including achlorhydria, is a significant source in variability in drugs especially with pH-dependent solubility profiles, such as weak bases,

potentially resulting in an undesirable therapeutic response. Physiologically-based pharmacokinetic (PBPK) modeling is expected to mechanistically quantify drug exposure in terms of gastric pH, however, there is still limited experience with its application.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study evaluated the utility of PBPK modeling in the quantitative prediction of gastric pH-mediated drug exposure by using itraconazole, a weak base, as a case.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The valid PBPK model based on its mechanistic absorption kinetics adequately predicted gastric pH-mediated itraconazole exposure. This study suggests the utility of PBPK modeling in the prediction of gastric pH-mediated exposure, particularly for drugs sensitive to gastric pH.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

Our finding may facilitate further assessment of gastric pH-mediated exposure for various drugs by serving as a leading model and ultimately contribute to personalized pharmacotherapy.

INTRODUCTION

In normal stomach acidity, gastric pH is maintained between 1.0 and 3.0 via the secretion of gastric acid composed of hydrochloric acid from the parietal cells in the stomach in response to stimulation by factors, such as food.[1,2] However, when gastric acid secretion is impaired, the stomach becomes hypochlorhydric or achlorhydric wherein there is low or absent secretion of hydrochloric acid, resulting in an increase in gastric pH.[3] Abnormal gastric acidity, including hypochlorhydria or achlorhydria, is usually caused by underlying diseases and/or intake of acid-reducing agents (ARAs) and is more likely to occur in the elderly given atrophic gastritis, whose prevalence increases with age, is associated with reduced gastric acid secretion.[4–10] Notably, due to the accelerated aging population worldwide, hypo−/achlorhydria in the elderly has become more frequent and is emerging as a clinically important issue.[11] Furthermore, as ARAs, including proton pump inhibitors (PPIs), have been more prevalently used recently, the incidence of ARA-induced hypo−/achlorhydria and the resultant gastric pH elevation are presumably increasing.[12]

Variation in gastric pH is a significant source of variability in the absorption of orally administered drugs, in particular, weak bases and weak acids exhibiting pH-dependent solubility profiles.[13] The influence of gastric pH on drug absorption is mostly prominent in poorly soluble and highly permeable weak base drugs belonging to the Biopharmaceutics Classification System (BCS) class II, particularly with low pK_a values, for which sufficient gastric acidity is a prerequisite for optimal in vivo dissolution

and absorption.[14] Several clinical studies reported that low gastric acidity was associated with impaired and variable absorption of BCS class II weak base drugs, including ketoconazole, atazanavir, and dipyridamole.[13,14] This altered absorption kinetic is known to be primarily ascribed to the diminished solubility and thus the slow and incomplete dissolution of such drugs in high gastric pH conditions, which may ultimately result in a loss of efficacy.[13,14] Given the possibility of an undesirable therapeutic response due to gastric pH elevation, it is imperative to evaluate potential gastric pH-mediated changes in pharmacokinetics (PKs) for drugs especially showing pH-dependent solubility profiles. Moreover, quantifying exposure in terms of gastric pH can aid in guiding optimal pharmacotherapy for drugs especially with pH-dependent solubility properties.

Gastric pH-mediated drug exposure can be investigated through a dedicated clinical study with an ARA, which constitutes a mainstay framework but is cost-intensive and time-consuming.[15] Because gastric pH is likely to be variably elevated according to the types and dosing regimens of ARAs as well as the genetic polymorphisms of enrolled subjects,[16–18] this framework is not feasible for quantitatively assessing drug exposure with regard to gastric pH. In addition, diverse physiological changes observed in the geriatric population, including decreased gastrointestinal (GI) motility, cannot be comprehensively considered under this framework, limiting its application to the elderly's hypo−/achlorhydria.

Physiologically-based pharmacokinetic (PBPK) modeling is a mechanism-based approach that integrates both drug-specific and physiological characteristics.[19] Of note, because it allows the simulation of drug absorption

kinetics in each GI segment on the mechanistic basis of the drug's physicochemical properties, such as solubility and dissolution, PBPK modeling is expected to quantitatively evaluate variability in the absorption and exposure of drugs in terms of gastric pH.[20] To date, there is still limited experience with the use of the PBPK modeling framework for predicting gastric pH-mediated drug exposure, albeit it has been applied to evaluate the liability of enzyme/transporter-mediated drug–drug interactions or drug exposure in special populations.[21]

Itraconazole, a BCS class II weak base ($pK_a = 3.7$), is a triazole antifungal agent for the treatment of a broad spectrum of fungal infections.[22] Itraconazole is ionized at a very low pH, such as in gastric juices, but is practically insoluble at a neutral pH, such as in water.[23] On account of its extremely poor and pH-dependent solubility profile, itraconazole absorption, particularly in a capsule formulation, has been observed to be decreased under pharmacologically induced gastric hypoacidity, although to be maximal after food intake.[23] Considering that gastric pH acts as a highly influential factor for its absorption, itraconazole (Sporanox capsule) was chosen as a case drug for this study.

The aim of this study was to evaluate the utility of the PBPK modeling framework in the quantitative prediction of gastric pH-mediated drug exposure through a robust approach, including a prospective clinical investigation for model validation, by using itraconazole as a case drug.

METHODS

The workflow for the PBPK modeling approach is outlined in Figure 1. PBPK modeling and simulation were conducted using Simcyp Simulator version 20.0 (release 1; Certara). Modeling with in vitro experimental data was performed using Simcyp In Vitro data Analysis toolkit version 4.0 (release 1; Certara). Information on clinical studies used in PBPK modeling and simulation is summarized in Table S1. Model workspace files are available at the following link: https://members.certara.co.uk/Simcyp/CustomerRepository.

PBPK model development

PBPK model for itraconazole

A PBPK model describing the mechanistic absorption kinetics with the Advanced Dissolution, Absorption, and Metabolism model was constructed for itraconazole by a middle-out approach. The PBPK model was initially built based on the pre-validated "'itraconazole fed capsule" compound file in the Simcyp library, further developed based on in vitro data, and refined based on in vivo clinical data. Details of PBPK model development of itraconazole are given in Section 1 of the Supplementary Methods, and the final PBPK model input parameters for itraconazole are presented in Table S2.

PBPK model for hydroxy-itraconazole

A minimal PBPK model with a single adjusting compartment was constructed for hydroxy-itraconazole based on the pre-validated "OH-itraconazole" compound file in the Simcyp library. The final PBPK model input parameters for hydroxy-itraconazole are presented in Table S3.

Clinical evaluation for model validation

An independent prospective clinical study (i.e., current prospective study; Table S1) was conducted in
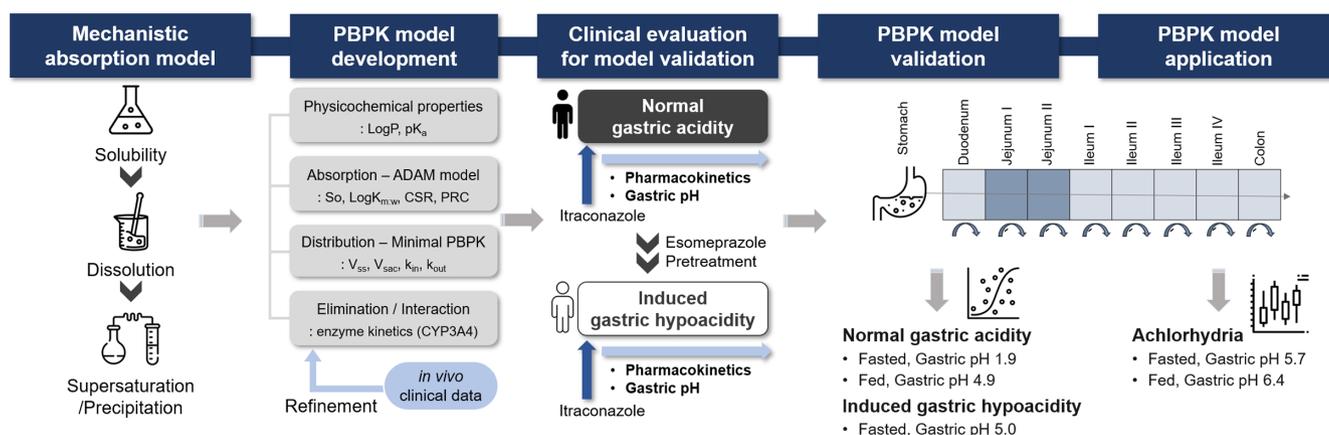

FIGURE 1 Workflow for the physiologically-based pharmacokinetic (PBPK) modeling approach.

12 healthy Korean male subjects to validate the predictive performance of the developed PBPK model (NCT04942652). This study evaluated gastric pH and itraconazole PK profiles under three different conditions: at fasted state under normal gastric acidity (i.e., itraconazole alone [fasted]), at fed state under normal gastric acidity (i.e., itraconazole alone [fed]), and at fasted state under pharmacologically induced gastric hypoacidity (i.e., itraconazole + esomeprazole [fasted]; Figure S1). Details of the methods for the current prospective study are given in Section 3 of the Supplementary Methods.

PBPK model validation

The developed PBPK model for itraconazole and hydroxy-itraconazole was evaluated for its predictive performance under normal gastric acidity and induced gastric hypoacidity using clinical data from the current prospective study and legacy study 2 (Table S1). Each simulation was performed in the "healthy volunteers" population in the Simcyp library using a trial design adapted to the reference study (Table S4). For populations with normal gastric acidity, gastric pH (coefficient of variation [CV]) was reflected as 1.9 (18.4%) for fasted conditions and 4.9 (22.0%) for fed conditions, which were the observed physiological values in the absence of esomeprazole in the current prospective study. For populations with induced gastric hypoacidity, fasted gastric pH (CV) was reflected as 5.0 (38.3%), which was the observed value after pretreatment with esomeprazole in the current prospective study. The performance of the PBPK model was considered acceptable if the observed plasma concentration-time profiles were within the 90% predicted concentrations (5th to 95th percentile range) and if the predicted to observed ratios of the maximum plasma concentration (C_{max}) and the area under the concentration-time curve (AUC) as well as the predicted to observed ratios of C_{max} ratio and AUC ratio (i.e., R value) were contained within the two-fold range.

PBPK model application

Prediction of gastric pH-mediated drug exposure

The validated final PBPK model for itraconazole and hydroxy-itraconazole was applied in virtual achlorhydric populations, which represent the worst scenario for abnormal gastric acid secretion, to predict gastric pH-mediated exposure in fasted or fed conditions. The PK profiles of itraconazole and hydroxy-itraconazole were simulated with the same dosing regimen as in the current prospective study, and the predicted gastric pH-mediated exposure was evaluated in achlorhydric populations compared to populations with normal gastric acidity. Each simulation was performed in the "healthy volunteers" population in the Simcyp library using a trial design adapted to the current prospective study (Table S4). Virtual achlorhydric populations were designed by setting an achlorhydria frequency of 100%, which resulted in gastric pH (CV) was adjusted to 5.7 (8.9%) for fasted conditions and 6.4 (11.5%) for fed conditions.

Mechanistic interpretation of gastric pH-mediated drug exposure

Sensitivity analyses were conducted by changing gastric pH in the range from 1 to 7 to explore the impact of gastric pH on the absorption and systemic exposure of itraconazole. Gastric pH-mediated itraconazole exposure was mechanistically interpreted in terms of gastric pH, solubility, dissolution, and absorption based on the final PBPK model-based simulations.

RESULTS

PBPK model development

The initial itraconazole PBPK model (i.e., model 1), which was generated by the stepwise in vitro-in vivo extrapolation (IVIVE), overpredicted the observed plasma itraconazole exposure at fasted state under normal gastric acidity in legacy study 1. After optimizing the absorption parameters, diffusion layer model scalar and particle radius, the first refined PBPK model (i.e., model 2) was able to capture the observed plasma concentration-time profiles of itraconazole (Figure S2; Table S5).

Thereafter, model 2 was evaluated for its predictability for gastric pH-mediated PK variability and underpredicted the observed plasma itraconazole exposure at fasted state under induced gastric hypoacidity in legacy study 2. After applying bile salt micelle to water partition coefficient ($K_{m:w}$) values estimated separately for fasted and fed states, the second refined PBPK model (i.e., model 3) was improved for its performance for gastric pH-mediated exposure and was selected as the final PBPK model for itraconazole (Figure S3; Table S5).

Clinical evaluation for model validation

Gastric pH profiles

Under fasted conditions without any pretreatment, the mean gastric pH was in the range of 1 to 3, with an arithmetic mean [range] value for the 0.4-h median pH of 1.9 [1.4–2.5], representing the normal gastric acidity of the enrolled subjects (Figure 2a; Figure S4). The 6-day pretreatment with once-daily esomeprazole 40 mg resulted in a substantial gastric pH elevation. The mean gastric pH was consistently maintained above 4, with an arithmetic mean [range] value for the 0.4-h median pH of 5.0 [1.7–7.2], suggesting that gastric hypoacidity was pharmacologically induced in the majority of subjects (Figure 2a; Figure S4). Meanwhile, intake of a high-fat meal temporarily increased the mean gastric pH more than 4 and then returned to the preprandial level, and an arithmetic mean [range] value for the 1.5-h median pH was 3.2 [2.0–6.2] (Figure 2a; Figure S4).

Pharmacokinetic profiles

When administered at fasted state after 6-day esomeprazole pretreatment, the plasma concentrations and systemic exposure of itraconazole and hydroxy-itraconazole were overall lower compared to when administered alone
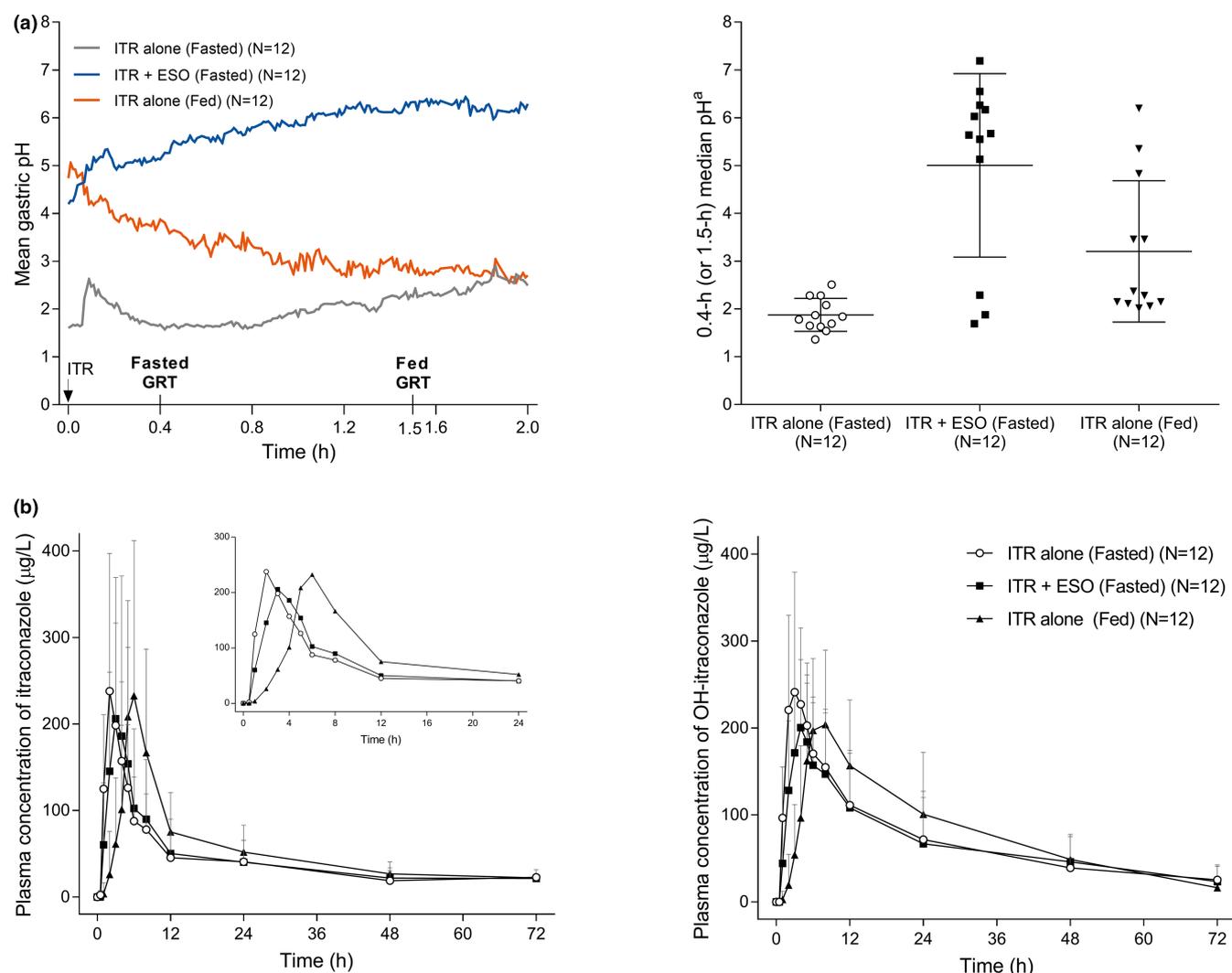

FIGURE 2 Gastric pH and pharmacokinetic profiles in the current prospective study. (a) Arithmetic mean gastric pH-time profiles up to 2 h (left panel) and individual median pH values during gastric residence time (right panel), and (b) arithmetic mean plasma concentration-time profiles of itraconazole (left panel) and hydroxy-itraconazole (right panel) following a single oral administration of itraconazole 200 mg alone or with esomeprazole 40 mg in fasted condition, or alone in fed condition. In the right panel of (a), the horizontal lines, error bars, and symbols denote the means, standard deviations, and observed values, respectively. In (b), the error bars denote the standard deviations. ITR, itraconazole; ESO, esomeprazole; GRT, gastric residence time. [a]0.4-h median pH for "ITR alone (Fasted)" and "ITR + ESO (Fasted)"; 1.5-h median pH for "ITR alone (Fed)."

at fasted state (Figure 2b; Table S6). Gastric hypoacidity induced by esomeprazole led to a slight reduction in itraconazole bioavailability, thereby decreasing C_{max} and the AUC from 0 to last measurable timepoint (AUC_{last}) of itraconazole by 15% and 13%, respectively (Table S6). When administered alone at fed state compared to at fasted state, itraconazole absorption was delayed, and the systemic exposure of itraconazole and hydroxy-itraconazole was marginally higher (Figure 2b, Table S6). Intake of a high-fat meal somewhat raised itraconazole bioavailability, thereby increasing AUC_{last} of itraconazole up to 14% (Table S6).

The currently observed impact of gastric pH elevation and food on itraconazole PKs was in line with a tendency that was previously reported, whereas the corresponding magnitude was overall lower than that in other clinical studies.[24–30] In view of that, we robustly validated the developed PBPK model using clinical data from not only the current prospective study but also the other previous clinical study (i.e., legacy study 2).

PBPK model validation

Under normal gastric acidity

The developed PBPK model successfully recovered the observed plasma concentration-time profiles of itraconazole and hydroxy-itraconazole at both fasted and fed states under normal gastric acidity (Figure 3a,b; Table S7). The predicted C_{max} and AUC of itraconazole and hydroxy-itraconazole were within 0.5 to 2-fold of the observed values, demonstrating good predictive performance under normal gastric acidity (Table 1). Moreover, the magnitude of C_{max} and AUC fold change laid within the predefined criteria for the R value, thereby validating the predictability for the observed positive food effect of itraconazole (Table 1).

Under induced gastric hypoacidity

The developed PBPK model could reasonably capture the observed plasma concentration-time profiles of itraconazole and hydroxy-itraconazole at fasted state under induced gastric hypoacidity, although it tended to be slightly underpredicted (Figure 3c; Table S7). The predicted to observed ratios as well as R values for C_{max} and

AUC of itraconazole and hydroxy-itraconazole were within the two-fold range, except for those of itraconazole in the current prospective study (Table 1). The predictive performance of the PBPK model for gastric pH-mediated itraconazole exposure was considered acceptable based on the highly variable PKs of itraconazole and the adequate prediction for legacy study 2 data, albeit deviated from the predefined criteria in the case of itraconazole data from the current prospective study.

PBPK model application

Prediction of gastric pH-mediated drug exposure

In achlorhydric populations, the predicted fraction absorbed (f_a) of itraconazole was reduced by 49% at fasted state and by 32% at fed state, compared with populations with normal gastric acidity, suggesting the impaired absorption of itraconazole according to gastric pH elevation (Table 2). Accordingly, the predicted the AUC from zero to infinity (AUC_{inf}) of itraconazole was decreased up to 65% at fasted state and 46% at fed state under achlorhydria (Table 2).

Mechanistic interpretation of gastric pH-mediated drug exposure

Changes in gastric pH had a more pronounced impact on itraconazole PKs at fasted state than at fed state, indicating less sensitivity to gastric pH due to bile salt-enhanced solubility after food intake. Under fasted conditions, f_a, C_{max}, and AUC of itraconazole showed drastic reductions with increasing gastric pH from 1 to 3 and remained relatively constant at higher gastric pH (Figure 4). In contrast, under fed conditions, the corresponding parameters of itraconazole showed mild reductions with increasing gastric pH from 1 to 5, with greater values compared to under fasted conditions (Figure 4).

Gastric pH alteration led to sequential variations in the total solubility, dissolution, and absorption of itraconazole. Regardless of food intake, gastric pH elevation under achlorhydria decreased total solubility in the stomach, followed by slower and lower dissolution

FIGURE 3 Predicted versus observed plasma concentration-time profiles of itraconazole (left panel) and hydroxy-itraconazole (right panel) following a single oral administration of itraconazole 200 mg (a, b) in fasted or fed condition under normal gastric acidity, or (c) in fasted condition under induced gastric hypoacidity. The solid lines and dashed lines denote the predicted arithmetic mean concentration-time profiles and the 5th–95th percentile of the total simulation by using trial designs adapted to the current prospective study, respectively. The symbols and error bars denote the observed arithmetic mean concentrations and the corresponding standard deviations obtained from clinical studies, respectively.

(a) Normal gastric acidity - Fasted, Gastric pH 1.9
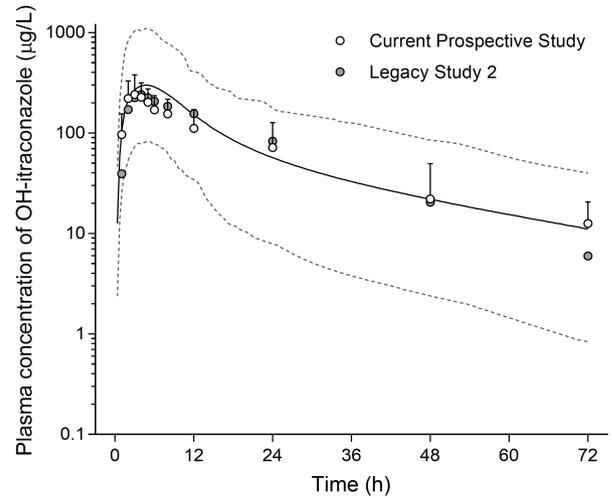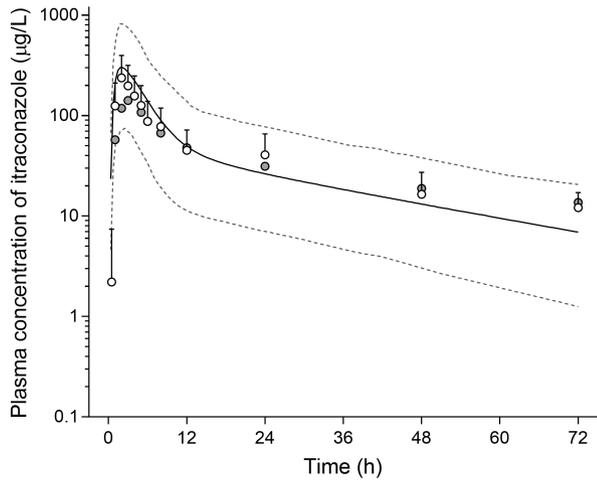
(b) Normal gastric acidity - Fed, Gastric pH 4.9
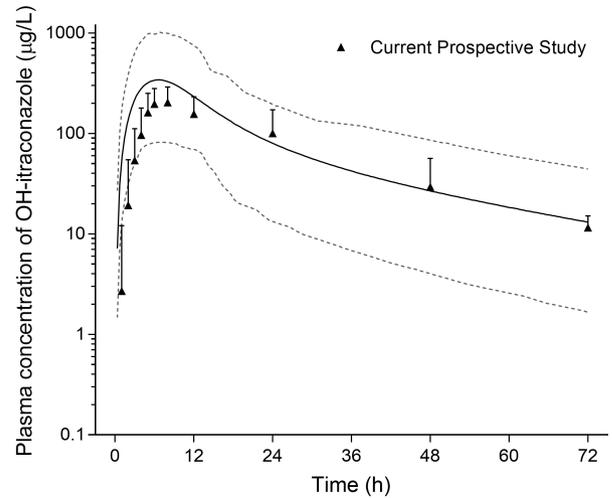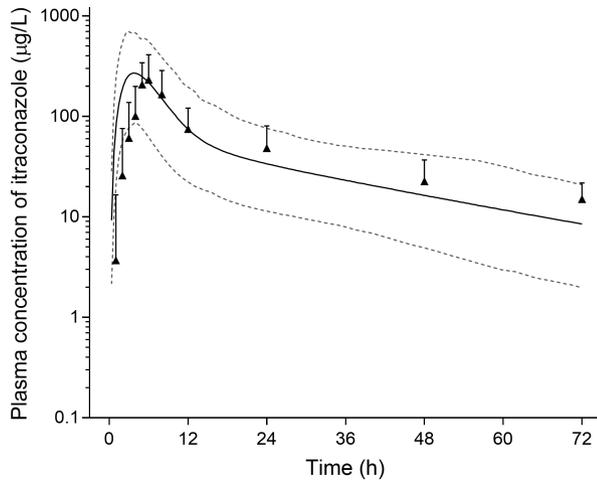
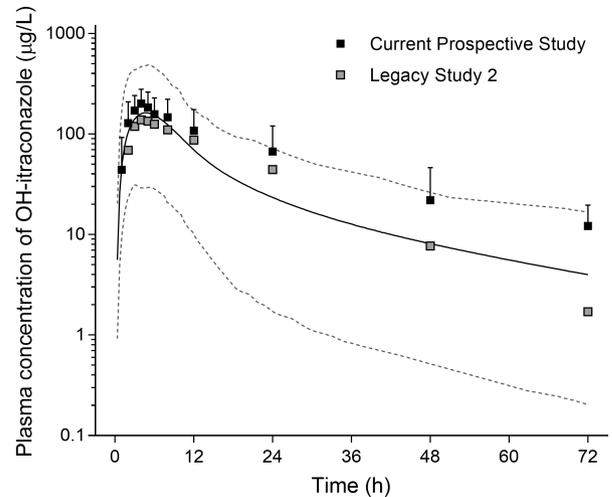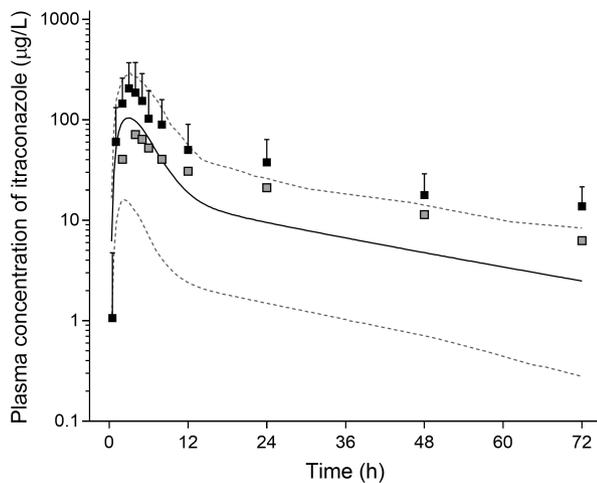**(c) Induced gastric hypoacidity - Fasted, Gastric pH 5.0**

TABLE 1 Predicted and observed pharmacokinetic parameters of itraconazole and hydroxy-itraconazole following a single oral administration of itraconazole 200 mg under normal gastric acidity or pharmacologically induced gastric hypoacidity.

Condition	Gastric pH	C_{max} (μg/L)			Pred. $C_{max}R$[a]	Obs. $C_{max}R$[a]	R value (C_{max})	AUC (h*μg/L)[b]			Pred. AUCR[a]	Obs. AUCR[a]	R value (AUC)
		Pred.	Obs.	Ratio				Pred.	Obs.	Ratio			
Itraconazole													
Normal gastric acidity													
Fasted[1]	1.9	237.0	222.4	1.07	–	–	–	2182.0	2030.3	1.07	–	–	–
Fasted[2]	1.9	249.4	124.9	2.00	–	–	–	1728.5	1163.5	1.49	–	–	–
Fed[1]	4.9	227.3	219.3	1.04	0.96	0.99	0.97	2794.4	2313.5	1.21	1.28	1.14	1.12
Gastric hypoacidity													
Fasted + ESO[1]	5.0	77.5	188.3	0.41	0.33	0.85	0.38	764.3	1773.3	0.43	0.35	0.87	0.40
Fasted + RAN[2]	5.0	86.4	59.7	1.45	0.36	0.48	0.76	644.4	621.0	1.04	0.30	0.53	0.56
Hydroxy-itraconazole													
Normal gastric acidity													
Fasted[1]	1.9	221.2	249.6	0.89	–	–	–	3810.2	3170.4	1.20	–	–	–
Fasted[2]	1.9	231.3	234.5	0.99	–	–	–	3009.2	3231.3	0.93	–	–	–
Fed[1]	4.9	272.1	205.5	1.32	1.23	0.82	1.50	5184.9	3392.7	1.53	1.36	1.07	1.27
Gastric hypoacidity													
Fasted + ESO[1]	5.0	119.7	195.1	0.61	0.54	0.78	0.69	1717.4	2620.6	0.66	0.45	0.83	0.54
Fasted + RAN[2]	5.0	130.1	134.0	0.97	0.59	0.57	1.03	1500.8	1802.2	0.83	0.39	0.56	0.70

Note: (1) and (2) refer to the current prospective study and legacy study 2, respectively.

C_{max} and AUC are expressed as geometric mean.

Abbreviations: AUC, area under the concentration-time curve; AUCR, geometric mean ratio for AUC; C_{max}, maximum plasma concentration; Obs., observed; Pred., predicted; Ratio, predicted to observed C_{max} or AUC; $C_{max}R$, geometric mean ratio for C_{max}; ESO, esomeprazole; R value, ratio for predicted to observed $C_{max}R$ or AUCR; RAN, ranitidine.

[a]$C_{max}R$ and AUCR indicate "Fed," "Fasted + ESO," or "Fasted + RAN" to "Fasted" in each study.

[b]AUC_{0-72h} for (1) and AUC_{0-24h} for (2).

TABLE 2 Predicted gastric pH-mediated exposure of itraconazole and hydroxy-itraconazole following a single oral administration of itraconazole 200 mg under achlorhydria compared to normal gastric acidity.

Condition	Gastric pH	Pred. f_a	Pred. C_{max} (µg/L)	Pred. AUC_{inf} (h*µg/L)	Predicted fold decrease[a]		
					f_aR	$C_{max}R$	AUCR
Itraconazole							
Fasted							
Achlorhydria	5.7	0.23	77.5	836.6	0.51	0.33	0.35
Normal gastric acidity	1.9	0.45	237.0	2412.4	–	–	–
Fed							
Achlorhydria	6.4	0.39	112.3	1668.1	0.68	0.49	0.54
Normal gastric acidity	4.9	0.57	227.3	3116.1	–	–	–
Hydroxy-itraconazole							
Fasted							
Achlorhydria	5.7	–	118.8	1808.7	–	0.54	0.44
Normal gastric acidity	1.9	–	221.2	4130.2	–	–	–
Fed							
Achlorhydria	6.4	–	185.7	3431.5	–	0.68	0.61
Normal gastric acidity	4.9	–	272.1	5639.9	–	–	–

Note: f_a, C_{max}, and AUC_{inf} are expressed as geometric mean.

Abbreviations: AUC_{inf}, area under the concentration-time curve from 0 to infinity; AUCR, geometric mean ratio for AUC_{inf}; C_{max}, maximum plasma concentration; $C_{max}R$, geometric mean ratio for C_{max}; f_a, fraction absorbed; f_aR, geometric mean ratio for f_a; Pred., predicted.

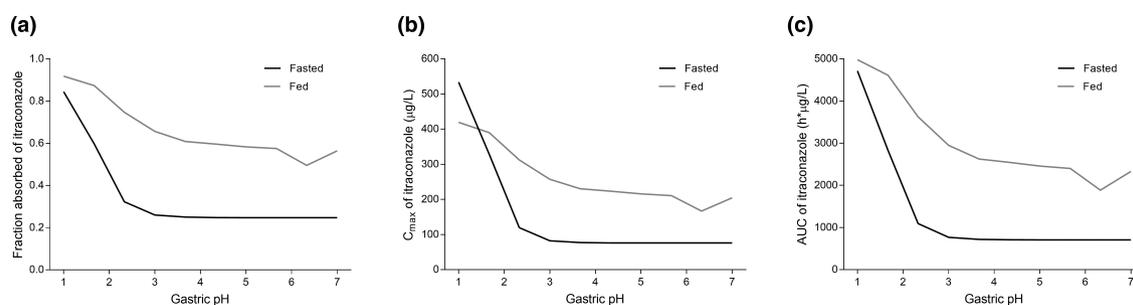[a]Ratio of "Achlorhydria" to "Normal gastric acidity."

FIGURE 4 Impact of gastric pH on (a) fraction absorbed (f_a), (b) C_{max}, and (c) AUC of itraconazole. AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration.

kinetics, and markedly reduced absorption in the duodenum and jejunum (Figure 5). Meanwhile, higher total solubility in the duodenum and upper jejunum owing to bile salt secretion stimulated by food resulted in greater absorption at fed state than at fasted state (Figure 5).

DISCUSSION

Stepwise IVIVE-informed PBPK modeling has been increasingly attempted for BCS class II and i.v. drugs which

may exhibit highly variable absorption profiles depending on the GI physiology, resulting in better mechanistic predictions.[31–33] Accordingly, we adopted this strategy for the mechanistic absorption modeling of itraconazole, a BCS class II weak base. A meticulous process of calculating or estimating, and verifying the absorption parameters based on in vitro data, including pH-solubility and dissolution profiles proceeded, thereby providing confidence in applying the parameters to the PBPK model. The absorption parameters were allowed to be translated into solubility, dissolution, and absorption
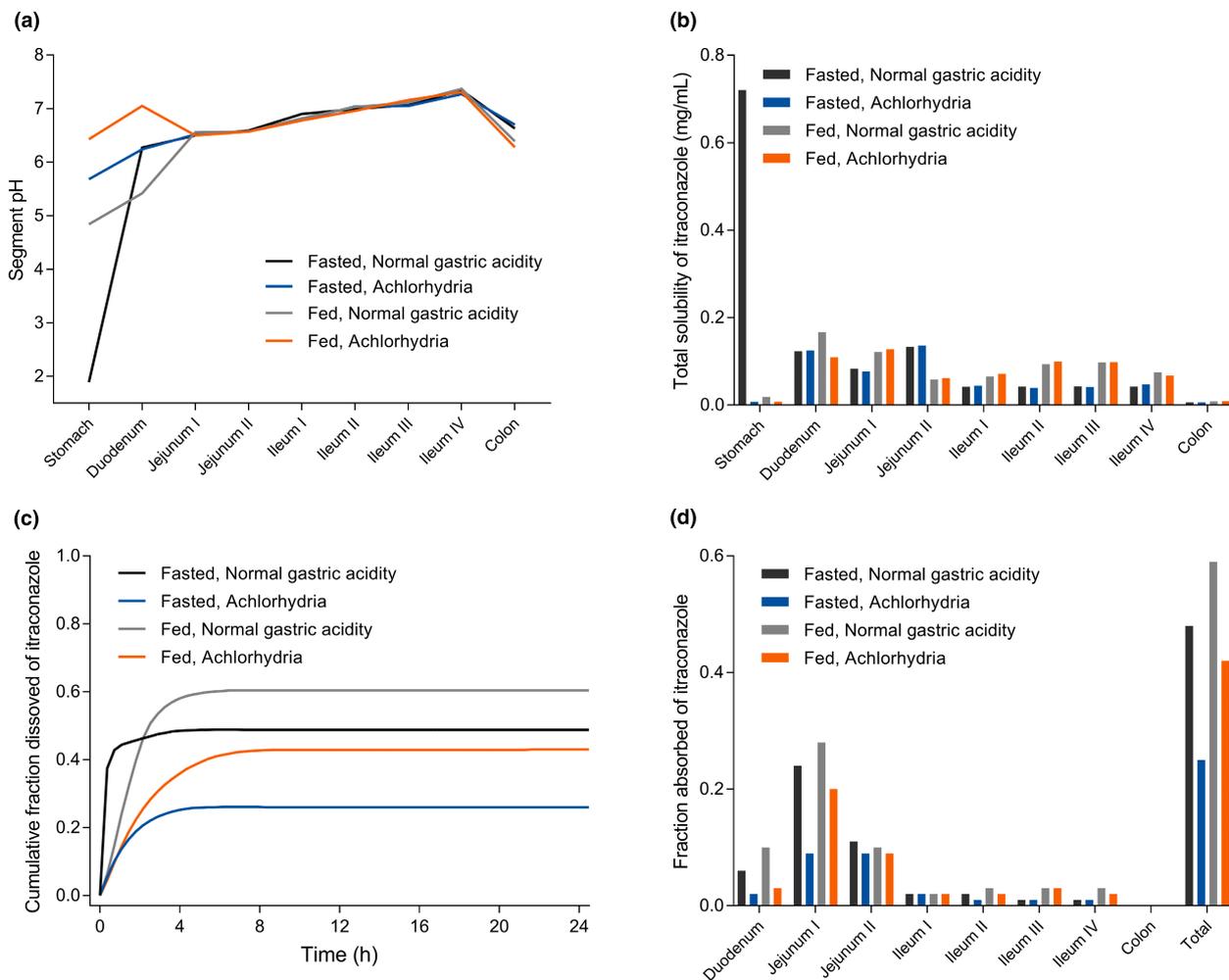
(a)

(b)

(c)

(d)

FIGURE 5 Predicted arithmetic mean (a) segment pH, (b) total solubility, (c) cumulative fraction dissolved, and (d) fraction absorbed (f_a) of itraconazole following a single oral administration of itraconazole 200 mg under achlorhydria or normal gastric acidity.

kinetics in each GI segment on the basis of physiological factors, such as luminal pH and bile acid concentrations. Consequently, an itraconazole absorption model capable of considering the effects of gastric pH as well as food in a mechanistic manner was built via the stepwise IVIVE strategy and incorporated into the initial PBPK model (i.e., model 1).

Meanwhile, the initial PBPK model generated by only IVIVE showed several limitations in its predictability, and we could improve its performance by model refinement from two perspectives, one for PKs at fasted state under normal gastric acidity and the other for gastric pH-mediated exposure. In fact, mechanistic absorption modeling involved complexity, such as limited in vitro data and differences in experimental values between the literature. This complexity might be ascribed to the extremely low solubility of itraconazole, with the resulting effects on the limitation of quantification, etc. It shows that IVIVE-informed PBPK modeling for predicting gastric pH-mediated drug exposure requires consistent and

sufficient in vitro data, clinical evaluation, and, if necessary, model refinement based on in vivo data.

During model refinement process, we chose $K_{m:w}$ values estimated separately for fed and fasted states based on in vitro data to account for bile salt-mediated solubility. The single $K_{m:w}$ estimated regardless of food status was first used for model simplicity, but this value seemed not to reflect well bile salt effect in the intestine, especially at fasted state. Accordingly, we applied the separately estimated $K_{m:w}$ to the model and improved the performance for gastric pH-mediated exposure. Because bile salts can significantly enhance the absorption of poorly soluble lipophilic drugs, $K_{m:w}$ can serve as an important parameter to explain the gastric pH effect on PKs with PBPK modeling. Moreover, the $K_{m:w}$ estimated separately for food condition based on in vitro data may better the model's predictability for gastric pH-mediated drug exposure.

Our approach included a prospective clinical investigation for model validation in particular, allowing us to evaluate whether the effects of gastric pH and food were

reflected in the PBPK model properly. Unexpectedly, the extent of exposure changes by gastric hypoacidity or food intake observed in the current prospective study was lower than that in other clinical studies.[24-30] Because gastric hypoacidity was sufficiently induced by esomeprazole in most subjects, we judged that these results might have been attributed to the extremely highly variable itraconazole PKs, with an intra-individual CV of ~45% for $C_{\max}$,[34] rather than issues during the study. These large variable PK properties could not be statistically considered under the one-sequence crossover design with a small sample size and seems to have complexly influenced the current findings of no clear trends in individual exposure levels as well as the absence of a significant correlation between systemic exposure and gastric pH (Figures S5 and S6). Regardless, our independent prospective clinical study identified results consistent with a trend previously reported, and therefore, we utilized these clinical observations for further PBPK model validation.

Above all, it is meaningful in that our prospective clinical investigation continuously monitored 24-h gastric pH under both normal gastric acidity and gastric hypoacidity and assessed itraconazole exposure with relation to directly measured gastric pH. To the best of our knowledge, this was the first attempt to perform such a study.

The developed PBPK model was elaborately validated based on the observed PK and physiological characteristics (i.e., gastric pH) in the current prospective study. In general, the predicted PK profiles were comparable to clinical PK data from the current prospective study, however, itraconazole concentrations under induced gastric hypoacidity were slightly underpredicted, leading to overprediction for gastric pH-mediated exposure decreases. This disparity was deemed probably due to the highly variable PKs of itraconazole, and data from only one controlled clinical study with a small sample size could not be representative of the general population.[35] Hence, further validation was performed using clinical data from the other clinical study (i.e., legacy study 2). As a result, the observed PK profiles and exposure changes under induced gastric hypoacidity in the legacy study 2 were adequately predicted, thus validating the predictability for gastric pH effect on PKs. These results strengthened our confidence in applying the validated final itraconazole PBPK model to predict exposure according to gastric pH in relation to food status.

We assumed that the final itraconazole PBPK model could quantitatively predict gastric pH-mediated exposure under fed conditions by mechanistically integrating each predictability for the effect of gastric pH or food, albeit without validation by clinical data at fed state under induced gastric hypoacidity. When applying the final PBPK model under achlorhydria, itraconazole exposure was expected to be decreased even at fed state, similar to at fasted state, resulting in an AUC ratio (achlorhydria/normal) of 0.54, which was comparable with the previous clinical study result (i.e., R value of 1.54).[26] This consistency between the observed and simulated results indicate that our modeling strategy brought out reasonable predictive performance of the itraconazole PBPK model for gastric pH-mediated exposure reflecting up to food status.

Sensitivity analyses apparently showed that gastric pH elevation impaired itraconazole absorption irrespective of food status. Specifically, the corresponding sensitivity was lower at fed state than at fasted state as higher total solubility in the duodenum and upper jejunum due to bile salt solubilization after food intake caused greater absorption. It underpins the current clinical findings that, albeit not statistically significant, the correlation between itraconazole exposure and gastric pH was weakly negative at fasted state but extremely weakly positive at fed state because the effect of gastric pH on its absorption was diluted by food intake (Figure S6).

Furthermore, we mechanistically interpreted our prospective clinical study results with the final itraconazole PBPK model. Because the administration of PPIs has been found to delay gastric emptying, reduce gastric volume, and increase gastric bile concentration,[36-38] we hypothesized that the other esomeprazole-induced physiological changes besides gastric pH elevation may have influenced the observed itraconazole exposure under induced gastric hypoacidity. Indeed, a minor delay in the time to reach to $C_{\max}$ of itraconazole from 2.00 h to 3.00 h was observed in the presence of esomeprazole (Table S6), which was speculated as a manifestation of gastric emptying slowed by esomeprazole. To test this hypothesis, additional sensitivity analyses were performed to explore the impact of these potential physiological factors on the extent of itraconazole absorption. At a gastric pH of 5 (i.e., induced gastric hypoacidity), the increased gastric residence time resulted in a modest increase in itraconazole absorption, whereas changes in gastric volume and bile concentration had no impact on itraconazole absorption (Figure S7). These results imply the possibility that an esomeprazole-induced delay in gastric emptying may have countervailed the effect of gastric pH elevation on itraconazole PK under pharmacologically induced gastric hypoacidity, supporting the lower degree of exposure changes observed in our prospective clinical study.

The prediction of gastric pH-mediated drug exposure through the PBPK modeling framework has the following significance. First, it can serve as a tool for the preliminary exploration of the potential gastric pH-mediated PK variability only with in vitro data under the early development phase. Based on physiological and drug-specific parameters, PBPK modeling can

elaborately screen drugs that need to conduct clinical studies with ARAs and prospectively predict the extent of gastric pH effect on PKs in advance, additionally guiding formulation selection and future clinical study designs.[39–41] The prospective prediction of gastric pH-mediated drug exposure with PBPK modeling seems challenging to completely replace clinical PK studies for gastric pH effect so far, whereas it is important to continuously generate the relevant evidence through case studies, as in our study. Second, it can provide personalized pharmacotherapy in populations with gastric hypoacidity in clinical settings, such as the elderly, even comprehensively reflecting their physiological factors. With regard to itraconazole, because of its exposure-response relationship with relatively narrow therapeutic range,[42] optimal dosing regimens may be recommended by using the final itraconazole PBPK model, whereas validation with multiple-dose clinical data is needed for appropriate utilization.

A limitation of our study is that the current prospective study showed insignificant PK changes by gastric pH elevation or food intake owing to highly variable itraconazole PKs, and thus it was challenging for model validation with the current prospective study data. Nevertheless, successful model validation with the legacy study 2 data provided confidence in the model's predictability for gastric pH-mediated exposure.

In conclusion, this study suggested the utility of PBPK modeling in the quantitative prediction and mechanistic interpretation of gastric pH-mediated exposure, especially for drugs whose absorption is susceptible to gastric pH. Our findings will serve as a leading model for further mechanistic assessment of exposure depending on gastric pH for various drugs, ultimately contributing to personalized pharmacotherapy.

AUTHOR CONTRIBUTIONS

E.Y. and S.L. wrote the manuscript. E.Y. and S.L. designed the research. E.Y., K.-S.Y., and S.L. performed the research. E.Y. and S.L. analyzed the data.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.