

ORIGINAL ARTICLE

Interaction Between Domperidone and Ketoconazole: Toward Prediction of Consequent QTc Prolongation Using Purely *In Vitro* Information

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We aimed to investigate the application of combined mechanistic pharmacokinetic (PK) and pharmacodynamic (PD) modeling and simulation in predicting the domperidone (DOM) triggered pseudo-electrocardiogram modification in the presence of a CYP3A inhibitor, ketoconazole (KETO), using *in vitro*–*in vivo* extrapolation. *In vitro* metabolic and inhibitory data were incorporated into physiologically based pharmacokinetic (PBPK) models within Simcyp to simulate time course of plasma DOM and KETO concentrations when administered alone or in combination with KETO (DOM+KETO). Simulated DOM concentrations in plasma were used to predict changes in gender-specific QTcF (Fridericia correction) intervals within the Cardiac Safety Simulator platform taking into consideration DOM, KETO, and DOM+KETO triggered inhibition of multiple ionic currents in population. Combination of *in vitro*–*in vivo* extrapolation, PBPK, and systems pharmacology of electric currents in the heart was able to predict the direction and magnitude of PK and PD changes under coadministration of the two drugs although some disparities were detected.

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During the drug development process, thousands of potential candidates are screened, to identify various indicators of likely pharmacological side effects. Based on *in vivo* toxicity testing and subsequent preclinical experiments, many of these candidates may not survive and thus progress to the drug development phase. The severity of expected side effects is also a determinant of progression. A study investigating recent drug withdrawals revealed that majority were withdrawn from the market due to their detrimental cardiotoxic effects.¹ One of the prominent clinical cardiotoxic effects observed was the ventricular arrhythmia, a characteristic of Torsades de Pointes. This arrhythmia can degenerate to ventricular fibrillation resulting in death if there is no immediate medical intervention.² Torsades de Pointes is characterized by the pronounced prolongation of the QT interval, which on a body surface electrocardiogram (ECG), is measured from the beginning of the QRS complex to the end of the T wave. This qualitative relationship between QT prolongation and risk of Torsades de Pointes is treated as a surrogate of proarrhythmic risk of drugs, and therefore the ICH E14 recommends “testing the effects of new agents on the QT interval as well as the collection of cardiovascular adverse events”.²

Physiologically, QT interval is defined as the duration of ventricular depolarization and repolarization and is inversely related to heart rate, i.e., the faster the heart rate the shorter the QT interval.³ Taking this into consideration, any measurement of the QT interval requires correction with respect to the heart rate at the time of the ECG reading by applying mathematical formulas such as Bazett's or Fridericia's. The outcome is represented as the QTc interval.³ QTc prolongation

can effect from the inhibition of human ether-a-go-go related gene (*hERG*) channel, which is responsible for the rapid component (I_{Kr}) of the delayed-rectifier potassium current, involved in the cardiomyocytes membrane repolarization.⁴ However, disruption of some other ionic currents may also contribute to QTc prolongation (e.g., the depolarizing current I_{Na} and the repolarizing currents I_{CaL} and I_{Ks}).^{5,6}

A closer look at the drug withdrawals related to cardiotoxic effects¹ demonstrates the fact these incidences were not purely the effect of the drug on its own but a combination of the effects from the drug and some other underlying condition such as concomitant administration with other drugs. Investigation of the various permutations of these conditions in a clinical setting is an impossible task. However, recently developed, sophisticated mathematical models of heart can be used for studying the influence of drugs together with any covariates related to the study population on cardiac physiology. Their use allow for testing various effects of drugs on pathways that may lead to cardiac effects. These models consist of the mechanism-based nodes, describing the physiology of single cardiac cells and they require information on the interaction of a drug candidate with each component of the pathway.⁷ Alternative minimal models may be used to answer specific questions, which require less detailed information and are therefore potentially more practical in the early drug development stage.^{8–10} Demonstrating applicability and limitations of such models is highly desirable for expansion of the systems pharmacology approach in the area of assessing cardiotoxicity to overcome the limited conditions under which the clinical TQT (thorough QT/QTc)

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studies are performed (where many permutations of real-life scenarios cannot be tested despite the use of some arbitrary supratherapeutic concentrations as a conservative safety margin). The current study provides such a case for the cardiotoxicity output expected from a combined administration of domperidone (DOM) and KETO where there are potential pharmacokinetics (PK) and pharmacodynamics (PD) interaction combinations.

DOM, a peripheral dopamine antagonist, is rapidly and almost completely absorbed after oral administration, with a reported oral bioavailability in the range of 13–23%.¹¹ This low bioavailability may be the result of the poor solubility of DOM at an alkaline pH,^{12,13} and its extensive first pass metabolism, as it is extensively metabolized, through multiple pathways, by CYP3A, with some contributions from CYP1A2, 2D6, and 2C19.¹⁴ DOM is believed to be a low-risk alternative to cisapride, as a gastroprokinetic agent, after the latter was withdrawn in many markets due to its cardiotoxic potential. However, early studies of high-dose DOM showed QTc prolongation and arrhythmias as side effects.¹⁵ Additionally, recent reports have shown that DOM prolongs cardiac repolarization by blocking the rapid component of the delayed-rectifier potassium current (I_{Kr}) in a concentration-dependent manner with an *in vitro* IC_{50} value of 162 and 57 nmol/l measured in *hERG*-transfected Chinese hamster ovary cells and HEK293 cells, respectively.^{16,17} Due to this potential side effect of DOM at increased plasma levels, coadministration of DOM and KETO is either not recommended or contraindicated as studies in healthy volunteers have shown a threefold increase in the exposure of DOM, in the presence of KETO, possibly due to the inhibition of CYP3A4-mediated metabolism; therefore, alternative drug combinations are often suggested.¹⁸

This DOM-KETO coadministration study provided the opportunity to establish the usefulness, as well as limitations, of *in vitro*–*in vivo* extrapolation modeling and simulation in predicting the extent of the proarrhythmic potency of CYP (cytochrome P450) enzyme substrates in the presence of inhibitors. The interactions are mechanistically modeled at the level of PK as well as PD.

RESULTS

Validation of PK simulations

The DOM compound file was validated using nine sets of observed clinical data, of which 80% of the trial simulations were able to reasonably capture the summary PK parameters viz C_{max} , area under the curve, and T_{max} . Although some *in vitro* parameters were fitted based on a test study, the use of mechanistic physiologically based pharmacokinetic (PBPK) models allowed the *in vivo* extrapolation of these parameters and subsequent successful prediction of PK parameters, in comparison to the validation study set. The results for the compound file validation are presented in the **Supplementary Materials** online section (**Supplementary 1; Supplementary Tables S1–S4; Supplementary Figures S1–S4**).

The steady-state (day 7) predicted DOM (10 mg, four doses daily, at 4 h intervals) concentration–time profile (Mean \pm SD) superimposed with observed data, with and without coadministration of CYP3A inhibitor KETO (200 mg every 12 h), for

men and women and KETO alone, is presented in **Figures 1** and **2** respectively. A summary of comparison of observed and simulated PK parameters is presented in **Table 1**.

QTc simulations and Δ QTc analysis

Obtained results are presented separately for women and men cohorts for all studied scenarios. QTcF and Δ QTc values for DOM alone, KETO alone, and DOM + KETO combination are presented in **Figures 3** and **4**.

Predicted individual Δ QTc values calculated as the individual difference between baseline QTcF and QTcF derived from the simulated ECG signal after the drugs ingestion are presented in **Figure 5**.

DISCUSSION

PK–PD modeling and simulation is widely used to describe the factors affecting the QT interval and drug influence on the QT interval duration.³ The methods applied can be listed among the top-down approaches. In these cases, the necessary elements of the analysis are either clinical or preclinical *in vivo* data, which can be further correlated with the clinical end points (i.e., QTc/ Δ QTc/QRS/ Δ QRS). The models covariates include drug triggered, *in vitro* measured currents inhibition, drug concentration, sex, age, etc.³ Once established, models can be further used for various scenarios testing, dose, and dosing optimization. The main obstacle of such an approach is the need of operating at the clinical data level (top-down approach) what narrows usability which is limited to the tested drug.

There are additional approaches that can help to understand the drug effects on the heart which involve computational models to simulate the drugs action and their influence on the electrical activity of the heart. The relatively simplest methods focus on single cardiomyocyte, however, these suffer from lack of direct correlation with much more complex clinical reality. The most sophisticated whole-heart modeling approaches offer a full insight into the organ level effects.⁷ The major concern with their practical and wide implementation lies paradoxically in the complexity of the utilized models and methods which need specialized knowledge, a wide range of assumptions and significant computational resources. In the current work, we propose and validate a case study using bottom-up approach, which allows for a reliable and mechanistic insight into the electrophysiological phenomena on the left ventricular wall level (one-dimensional simulation). This method is computationally efficient enough to run simulations at the population level.

DOM and KETO coapplication was chosen as the basis for this case study. Such treatment is not a recommended combination, due to the potential proarrhythmic side effects of DOM at increased plasma levels, which we were able to assess using a PBPK model-based approach.^{15,18} The PBPK model, used to simulate the kinetics of DOM, was able to recover its plasma concentration profile after an intravenous dose and various single oral doses (see **Supplementary Materials** online) within twofold of the observed values. The fasted simulations of an oral tablet dosing were underpredicted in some cases (**Supplementary Materials** online). This under prediction may be an artifact of using the reported mean observed profile data

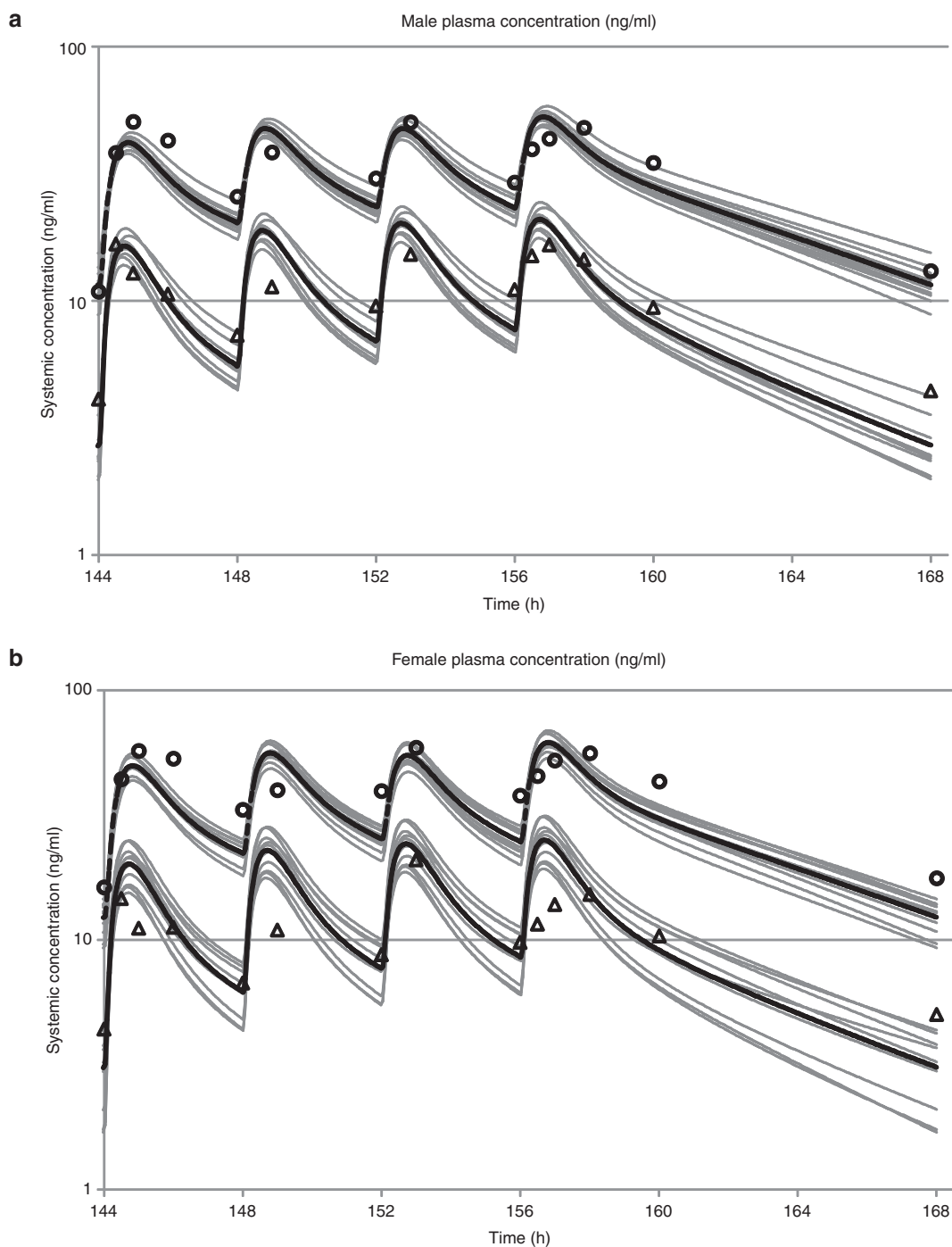


Figure 1 Predicted steady-state (day 7) systemic concentration of domperidone (10 simulated trials: grey solid lines; mean profile without inhibitor: black solid line; with inhibitor: black dotted line) superimposed with observed data (without inhibitor: triangles; with inhibitor: circles) in healthy (a) men and (b) women volunteers.

for comparison, or the small number of subjects ($n = 5$) might have low small-intestinal pH and thus have a high *in vivo* solubility of DOM, which is not representative of the general population. Also, the *in vitro* solubility data for DOM was from a single reference and may not necessarily capture the actual observed variability in *in vivo* solubility. The PBPK model was also able to recover the steady-state plasma levels after multiple dosing with and without the coadministration of KETO (Figure 1).

The predicted increase in the exposure of DOM with KETO (area under the curve ratio) was 2.8 ± 0.4 -fold, which correlated well with the observed 3.5-fold increase.¹⁵ Data for competitive and time-dependent inhibition of CYP3A4 were included in the DOM compound file, but this had minimal impact on the amount of active CYP3A4 in the liver or gastrointestinal tract, due to the high K_i , K_{app} , and K_{inact} values (Supplementary Table S6 online).

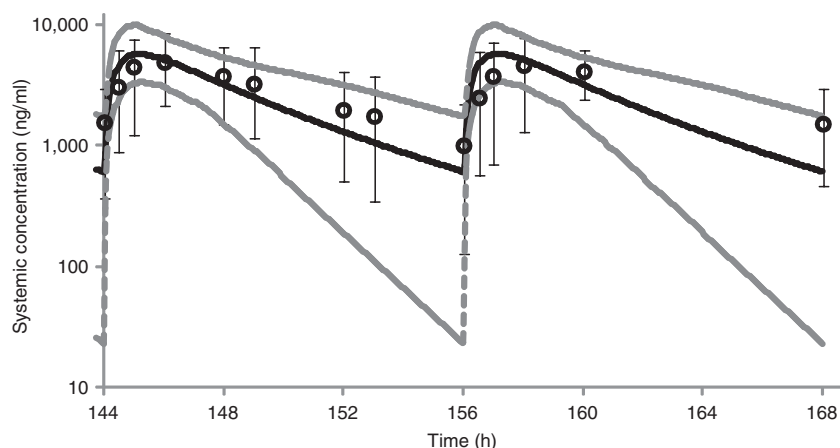


Figure 2 Predicted systemic concentrations of ketoconazole over time (10 simulated trials: solid lines; mean profile: black line; 5th and 95th percentile profile: grey dotted lines) and superimposed with observed data (open circles) in healthy volunteers, for an oral dose of 200 mg.

Table 1 Observed (Obs) vs. Simcyp-simulated (Sim) PK parameters for single DOM (40 mg), KETO (200 mg) dosage, and DOM+KETO combination

Dose	C_{max} (ng/ml)	SD	Area under the curve (ng* h/ml)	SD	T_{max} (h)	SD/range
Obs DOM	23.5	7.4	249.0	65.3	9.0	0.5–14
Sim DOM	23.3	2.6	260.2	38.0	12.7	0.04
Obs DOM+KETO	67.9	21.1	878.1	267.7	5.0	0.5–14.1
Sim DOM+KETO	56.7	4.4	737.8	71.0	12.8	0.03
Obs KETO	5,690.0	1,760.0	70,400.0	2,810.0	2.1	0.5–24.0
Sim KETO	5,371.4	435.4	56,160.8	5,016.3	5.5	6.22

DOM, domperidone; KETO, ketoconazole.

The apparent Caco-2 permeability and the phys-chem data resulted in a two- to threefold under prediction of C_{max} and incorporation of only CYP3A4 metabolism data for DOM resulted in an under prediction of clearance.^{19,20} So, the model was fit to the observed clinical data for a 20 mg oral dose, and the values for effective jejunal permeability (P_{eff} , man), intrinsic hepatic clearance, and renal clearance were estimated, using the parameter estimation module within Simcyp.^{21,22} For a complete mechanistic PK model, these *in vitro* values need to be revisited and also the role of transporters in the absorption of DOM needs to be evaluated.

One of the study assumptions states that the multiple drug triggered ionic currents inhibition is a sum of their inhibiting potency alone (additive effect). Such an assumption does not necessarily have its reflection in the human clinical settings as the interaction is supposed to be more complex as presented in the *in vitro* studies.²³ Moreover, there are reports suggesting different current reactions regarding the drugs dosing order.²⁴ The results suggest a partial additive effect, not a clear synergism although what has to be emphasized is that both of the cited studies were done in the *in vitro/ex vivo* animal settings and the situation can be considerably different in the complex *in vivo* situation.

The most commonly used surrogate of the drug proarrhythmic potency is the *in vitro* measured I_{Kr} current inhibition. Such an approach is fully reasonable as the majority of drugs that were shown to have torsadogenic potency in man are I_{Kr} (*hERG*) blockers. As a result, most efforts in examining the safety of new compounds have concentrated on assessing their effects on the *hERG* channel. There are, however, other

channels of which drug triggered blockades are known to be connected with the risk of the Torsades de Pointes. The most important is *KCNQ*, responsible for the I_{Ks} potassium current occurrence. Some of the known torsadogenic drugs, including terfenadine, are potent I_{Ks} current inhibitors *in vitro*.^{25,26} This is consistent with the statement that torsadogenic compounds are *hERG* blockers although not all *hERG* blockers are necessarily dangerous.⁸ It is likely that the change in ECG is an interplay between various channels inhibition. In general, it is postulated that the I_{Ks} current is one of the most overlooked sources of cardiovascular liability in drug safety assessment.⁵ Other ionic currents that are postulated to play significant role in the drug safety profile include peak sodium (I_{Na}) and late calcium (I_{CaL}) currents.²⁷

In the current study, information about the I_{Ks} and I_{CaL} currents inhibition were added to the *in vitro* measured I_{Kr} current modification data. As it was not possible to assess the I_{Ks} and I_{CaL} IC_{50} prediction accuracy, the imperfection of the prediction can be caused by the imperfect assessment of the drug triggered alteration of the slow delayed rectifier potassium current and late calcium current. One of the main methodological assumptions of the current study accounted for is the simple summation of the *in vivo* PD effect of concomitantly given drugs and the analysis of the DOM and KETO interaction cannot be effectively completed without considering the same. Clinical effect under prediction observed in the results, which is more prominent in men in comparison to women is probably a consequence of the improperly coupled effects of the two tested drugs. In the most likely scenario, the DOM influence on the I_{Ks}

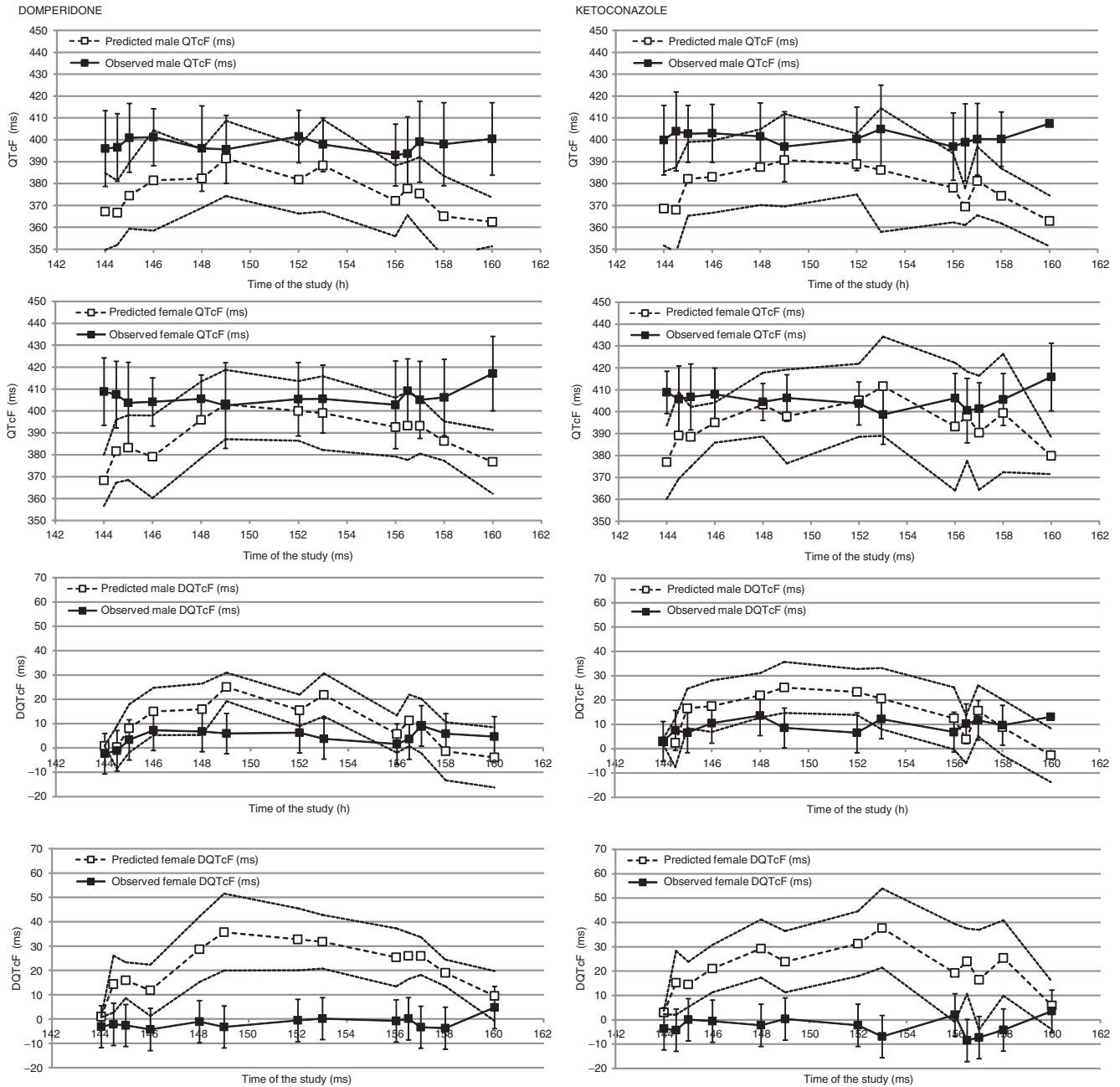


Figure 3 Observed (black squares) and predicted (empty squares) QTcF and DQTcF values (\pm SD) in domperidone single and in ketoconazole single scenarios.

current was underpredicted, and the total *in vivo* effect of the two given drugs does not simply sum up. The largest under predictions are seen after the first and the last dose. This could be due to the under prediction of the combined ionic current disruption at the lowest concentration, if we assume that the nonlinear concentration–inhibition relationship is not fully capturing the steepness (or shallowness) of the true relationship. Cardiac Safety Simulator was also able to differentiate between genders in the drug-triggered QTcF modification although Δ QTcF values were over predicted for females what suggests that the baseline value prediction was imperfect (Table 2 and Figure 5). However,

it should be noted that the simulated vs. observed Δ QTcF values were calculated in different ways due to the inability of simulations to create the placebo effects and relying only on baseline correction (see Methods).

The observations suggest that additional differentiation between sexes at the cardiac cell level are needed to improve the predictability.²⁸ On the other hand, the simulation results stay in agreement with other literature sources which point out female gender as a risk factor for the drug-triggered heart electrophysiology modification.²⁹ Taking it altogether, one may suggest that the observed higher male susceptibility was specific for this particular study population only.

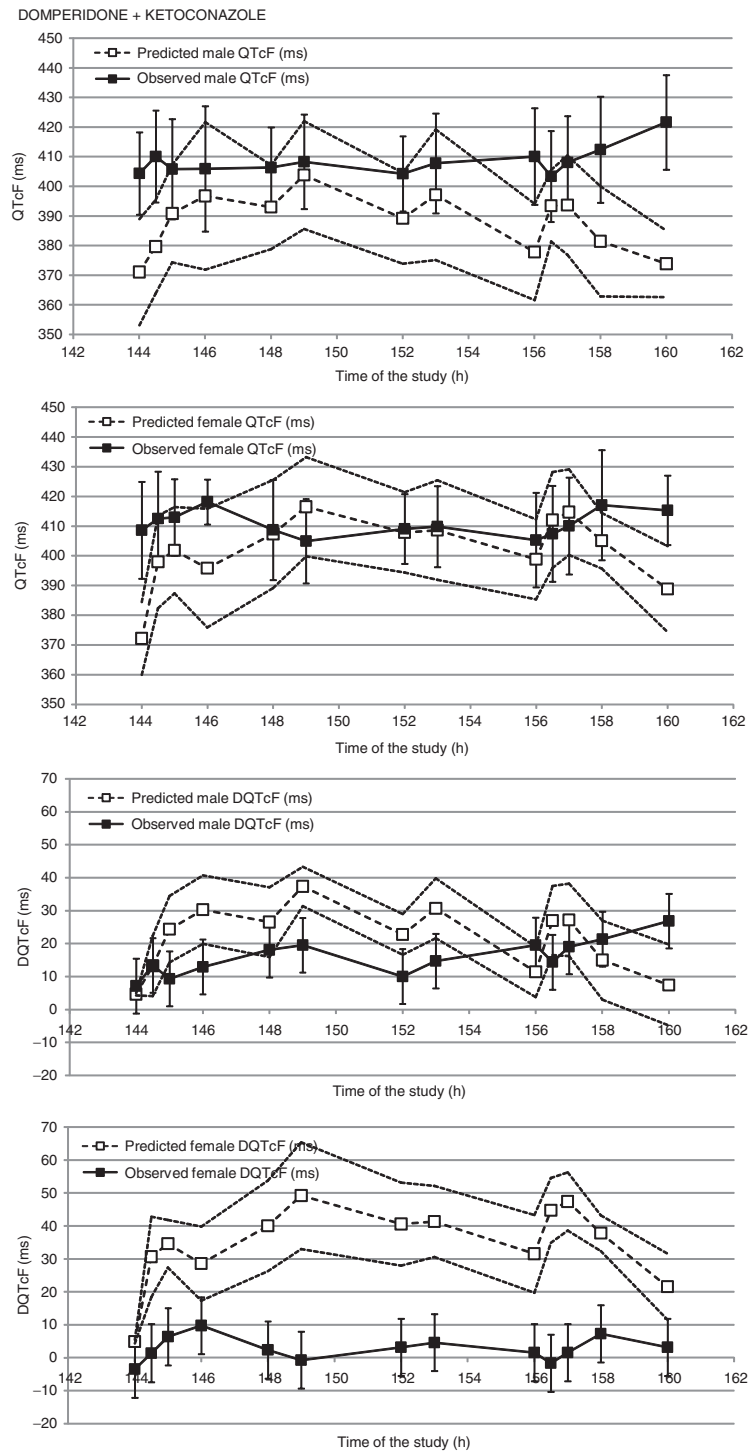


Figure 4 Observed (black squares) and predicted (empty squares) QTc and DQTcF values for domperidone plus ketoconazole therapy (\pm SD) in domperidone with ketoconazole scenario.

This may highlight the well-known concept of repeatability of single clinical studies as discussed below.

The requirement for repeated clinical studies and possibility of using confirmatory studies (in Learn–Confirm cycle) have been the subject of much debate.³⁰ Ability to avoid repeat clinical study depends on whether there is consistency between mechanistically derived expectations (what we have

learnt independently) and the observations made. The inconsistency between predictions and observations does not necessarily question the modeling outcome, but it may highlight specific conditions in the clinical study which made it deviate from expectations. In the current case, in contrast to the observations by Boyce *et al.*¹⁵, the simulated Δ QTc values clearly suggest that significant cardiac risk connected not only with

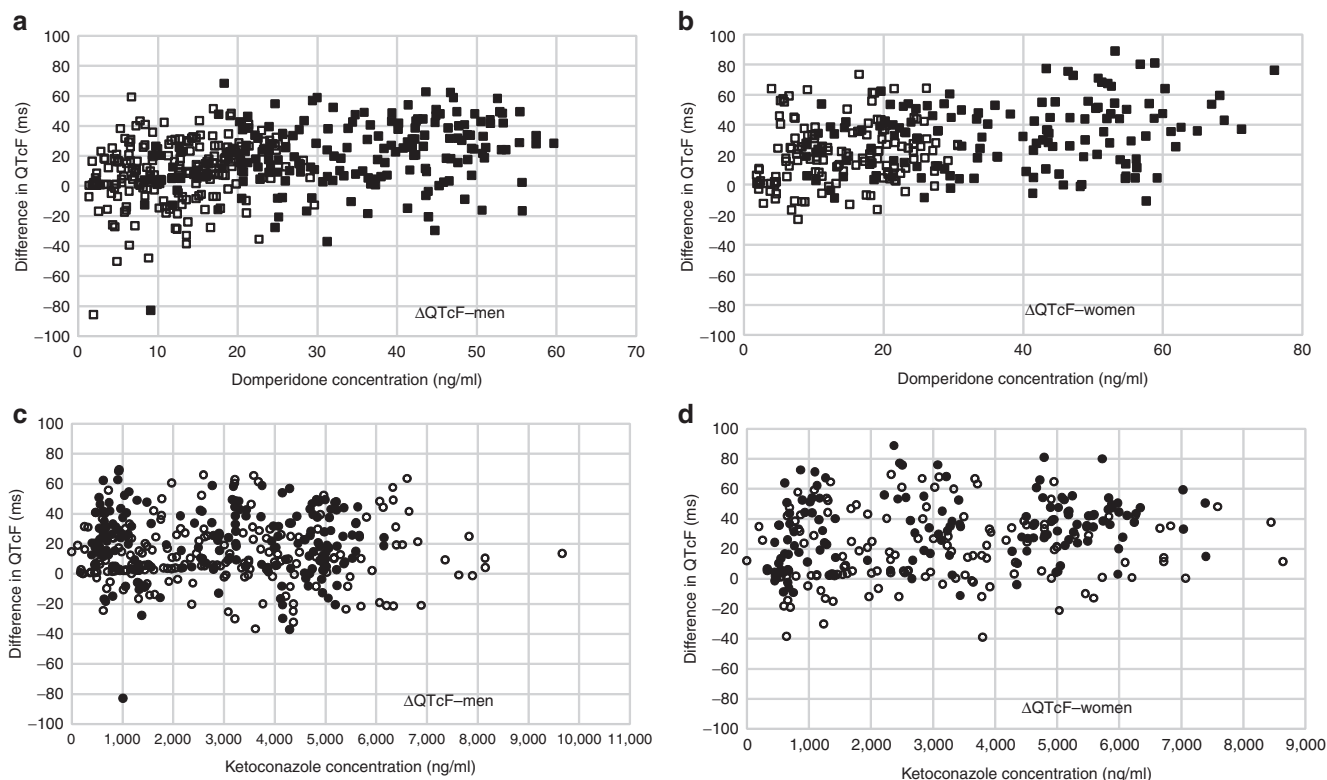


Figure 5 Concentration– Δ QTcF relation. Relation between plasma domperidone (a,b) and ketoconazole (c,d) concentration and difference in QTcF from baseline (Δ QTcF), in men (a,c) and women (b,d) on single and combination therapy at steady state (day 7 of dosing). Domperidone alone (empty squares); domperidone in combination (black squares); ketoconazole alone (empty circles); ketoconazole in combination (black circles).

the studied drug combination but also with the single DOM. While the first conclusion one may draw would be related to the shortcomings of the model, evidence from other literature supports the outcome of the modeling as recent reports suggest that DOM carries significant cardiac risk and should be withdrawn from the market.^{31,32} The simulation results, from the risk assessment point of view, suggest potential risks.^{33,34} It is in this line that the European Medicines Agency initiated reviewing the DOM via the Belgian Medicines Agency.³⁵

The main aim of the clinical trial run was to establish the DOM and KETO combination safety margin. The simulation confirmed many of the clinical trial findings—the PK interaction between DOM and KETO resulted in the QT prolongation and such combination is not recommended (Table 2).

CONCLUSIONS

Combination of mechanistic PBPK-Tox modeling and simulation tools (Simcyp and Cardiac Safety Simulator) was able to recover the PK and toxicological effect of DOM administered as a single drug and its combination with the pharmacokinetically and pharmacodynamically interacting drug (KETO), through simulations largely based on collated *in vitro* data. Generating such *in vitro* data on the properties of a drug and its impact on the cardiac action potential currents, at the drug discovery stage, could be beneficial. By simulating the interaction in virtual populations, extreme cases can also be evaluated by identifying

the characteristics of the most susceptible individuals. QTc predictions for more than one drug rely substantially on the total effect calculations. It is therefore crucial to work on the models describing such effects. This study, despite of the disparities between the observed and predicted values, highlights the potential of using model-based drug development and simulation as a cardiac safety assessment tool, which has to be thoroughly validated before being routinely utilized for the drug development process. The strength and weakness of the proposed approach lies in the combination of various modeling and simulation techniques including quantitative structure -activity relationship (QSAR), PBPK, PD, and systems biology. Uncertainty in the unknown and estimated parameters has possibly contributed to the mismatch between clinical observations and the predicted values yet the general conclusions on the drugs triggered ECG modifications can be made. Such systematic deviation has not been observed in other simulations and studies including recently published work where the cardiac effect expressed as the QTc value of six central nervous system drugs was predicted using the Cardiac Safety Simulator.³⁶ Hence this mismatches could be attributed to lack of full knowledge of drug-specific data and/or special conditions in the clinical study, which made them different to other more representative cases. A potential solution to this challenge can be implementation of the middle-out approaches where available preclinical and clinical data can be used to improve predictability of the models.

Table 2 Cardiac Safety Simulator–simulated Δ QTcF values for single DOM (40 mg) and DOM+KETO combination (mean \pm 95% CI)

Time of the study (h)	Domperidone			Ketoconazole			Combination		
	Predicted male Δ QTcF (ms)	95% CI		Predicted male Δ QTcF (ms)	95% CI		Predicted male Δ QTcF (ms)	95% CI	
144	0.83	0.69	0.97	2.97	1.85	4.09	4.69	2.57	6.81
144.5	0.29	-8.49	9.08	2.41	-7.64	12.46	7.44	-1.42	16.29
145	8.04	-1.84	17.91	16.55	8.45	24.66	16.71	7.85	25.57
146	15.01	5.28	24.74	17.48	6.90	28.06	23.03	13.38	32.69
148	15.90	5.37	26.43	21.92	12.82	31.01	22.34	11.78	32.90
149	25.06	19.20	30.93	25.17	14.65	35.70	34.17	28.44	39.91
152	15.45	8.96	21.94	23.34	13.91	32.76	21.70	14.53	28.87
153	21.82	13.01	30.62	20.59	8.03	33.15	30.07	21.03	39.11
156	5.70	-2.01	13.41	12.50	-0.23	25.23	11.52	3.33	19.71
156.5	11.31	0.69	21.93	3.85	-5.95	13.65	20.55	9.96	31.13
157	9.01	-2.16	20.17	15.62	5.17	26.07	19.21	8.47	29.95
158	-1.39	-13.35	10.57	8.80	-2.64	20.23	7.80	-3.90	19.51
160	-3.92	-16.27	8.42	-2.66	-13.67	8.34	3.37	-9.01	15.74
168	-0.66	-14.64	13.33	2.80	1.76	3.84	3.50	-11.36	18.36
Time of the study (h)	Predicted female Δ QTcF (ms)	95% CI		Predicted female Δ QTcF (ms)	95% CI		Predicted female Δ QTcF (ms)	95% CI	
144	1.02	0.75	1.30	3.00	2.30	3.69	2.52	2.21	2.83
144.5	14.35	2.68	26.02	15.21	2.09	28.33	25.30	13.11	37.50
145	15.95	8.56	23.34	14.55	5.27	23.82	28.43	21.30	35.55
146	11.79	1.23	22.34	21.07	11.34	30.80	23.20	12.33	34.06
148	28.69	15.24	42.13	29.26	17.35	41.17	36.18	22.40	49.96
149	35.69	19.87	51.51	23.87	11.28	36.45	42.00	25.93	58.08
152	32.74	19.97	45.50	31.26	17.93	44.60	36.08	23.23	48.93
153	31.79	20.73	42.84	37.69	21.43	53.94	34.66	23.59	45.73
156	25.36	13.43	37.30	19.26	-0.86	39.38	27.25	15.30	39.20
156.5	25.95	16.38	35.52	24.01	10.55	37.46	37.72	28.09	47.35
157	25.91	18.11	33.71	16.45	-4.05	36.96	39.41	31.04	47.78
158	18.97	13.50	24.43	25.42	9.90	40.94	30.94	25.15	36.72
160	9.49	-0.77	19.74	5.87	-3.94	15.69	16.76	6.38	27.14
168	-0.54	-5.21	4.13	3.05	2.35	3.75	0.90	-3.82	5.63

CI, confidence interval; DOM, domperidone; KETO, ketoconazole.

METHODS

PK simulations

Simcyp (v11.1) platform was used for the PK simulations. A Simcyp library compound file for DOM was developed using physiochemical information from the PubChem compound database and the DrugBank.^{19,37} Drug parameters such as the blood to plasma ratio (B/P), fraction unbound in plasma (f_u), and the volume of distribution (V_{ss}) were predicted using inbuilt models in Simcyp v11.1.^{38–41} A solubility–pH profile was obtained from Zhang *et al.*¹² to predict the *in vivo* solubility. The physiochemical absorption and distribution parameters are summarized in **Supplementary Table S5** online.

Using Baculovirus–insect cell–expressed recombinant CYPs, Ward *et al.*¹⁴ established that DOM is metabolized through three different pathways mainly catalyzed by CYP3A4. Chang *et al.*⁴² reported that DOM exhibits mechanism-based inhibition of CYP3A4 and also acts as a competitive inhibitor resulting in a clinically significant increase in the exposure of CYP3A4 substrates. The metabolism and inhibition model parameters are summarized in **Supplementary Table S6** online.

Initial compound file validation against clinical data showed that in the absence of detailed information on the supersaturation state following stomach emptying to intestine and the gut wall permeability, the model underpredicted the drug absorption and the elimination profile (data not shown). So, subsequently, the observed clinical data²¹ were fitted by obtaining optimal values for the intestinal permeability, intrinsic clearance, and renal clearance using the parameter estimation module within Simcyp. The final values used during the compound file validation and simulation studies were 4.596×10^{-4} cm/s, 37.6 μ l/min/ 10^6 hepatocytes, and 2.55 l/h, respectively.

The KETO compound file was taken from the Simcyp compound database, with the modification of CL_{po} of 8.3 l/h to account for the dose-dependent elimination kinetics of KETO.⁴³ The DOM compound file was validated against published clinical data from various sources for both intravenous and oral dosing conditions under fasted and fed states, based on the outputs for a simulated virtual population.^{11,21,44}

To assess the proarrhythmic potency of DOM (alone and in the presence of KETO), the plasma concentration values, at the same time points as reported by Boyce *et al.*¹⁵ were

recorded from the Simcyp outputs and used as input parameters for the Cardiac Safety Simulator platform (v1.0) to simulate the drug-induced QTcF (Fridericia correction) interval change.^{45,46}

The *in vitro* I_{Kr} ionic current inhibition data for both drugs were taken from the literature, and the I_{Ks} and I_{CaL} currents inhibition were predicted with the Cardiac Safety Simulator built-in QSAR models.^{17,47,48} The QSAR models were used as it was hypothesized that DOM and KETO can also inhibit currents other than I_{Kr} . As the QSAR models utilize *in vitro* setting as the independent parameters, it was assumed that the parameters mimic the literature-derived information for the *hERG* channels inhibition for DOM and KETO respectively. Chemical structures encoded as the sdf files were downloaded from PubChem resources. The full set of Cardiac Safety Simulator input parameters are presented in **Supplementary Table S7** online.

Population-dependent parameters (age, sex, and heart rate) were set up to mimic the clinical trial settings (24 individuals consisting of 14 men and 10 women; age 18–39; weight range: 53.8–98.8 kg). In the original study, 1 of the 24 healthy individuals was Afro-Caribbean; in our study, we assumed that all 24 were North European Caucasian individuals. To account for potential statistical sampling issues and population variability, each clinical study was simulated 10 times, where the size of study in each trial was the same as the clinical study, and it was assumed that all individuals completed the study.

The DOM and KETO interaction was based on the clinical study design by Boyce *et al.*¹⁵, wherein the effects of DOM and KETO, alone and in combination, on the heart rate corrected QT interval in healthy volunteers were assessed. The population PK behavior was predicted in healthy volunteers, however since Boyce *et al.* studied the gender differences in the effects of DOM, this effect was also analyzed for female only and male only virtual populations.^{39,45,46}

ECG simulations

The Cardiac Safety Simulator system was used to simulate the drugs triggered ECG modification. The platform combines a physiologically based electrophysiological model of the human left ventricular cardiomyocytes (TNNP - ten Tusscher-Noble-Noble-Panfilov) and a database of human physiological, genotypic, and demographical data enabling prediction of the QT prolongation in humans based on the *in vitro* data.¹⁰ To account for the heterogeneities in ionic currents between endocardial, midmyocardial, and epicardial cells, a 1D fiber model paced at the endocardial side was used. The default 50:30:20 distribution of the endo-, mid-, and epicardial cells was used together with the diffusion coefficient equals to 0.0016 cm²/ms. The forward Euler method was used to integrate model equations. For a one-dimensional string of cells, the results are used to calculate a pseudo-ECG. A space step and a time step are, by default, set to $\Delta x = 0.01$ mm and $\Delta t = 0.01$ ms.

Drug-triggered ionic current modifications were incorporated in specific equations describing I_{Kr} , I_{Ks} , and I_{CaL} currents

by multiplying them by the inhibition factor, which was either provided or QSAR predicted and described the concentration-dependent ionic current inhibition. The inhibition factors were calculated using the Hill equation (Eq. 1).

$$\text{Inhibition factor} = \frac{1}{1 + (IC_{50}/\text{Concentration}(\mu\text{mol/l}))^n} \quad (1)$$

Simulation time was set to 10,000 ms, and during the ECG analysis, the first and last beats were withdrawn from the analysis to assure stability and avoid computation bias. The population variability effect was mimicked by applying the virtual population generator as described previously.^{46,49} The heart rate and plasma ions variability were represented in the simulation by adding circadian variation.^{49–51} The Cardiac Safety Simulator system was able to accurately recover the RR (inter-beat) intervals (data not shown).

The total ionic currents inhibition by interacting drugs was assumed to be the sum of their inhibiting potencies alone. During the ECG simulation study, the average predicted concentrations, from Simcyp outputs, at certain time points for single DOM and DOM with KETO were used. The predicted mean DOM concentrations on day 7 following administration of the drug alone, or in combination with KETO, were used as part of the input to the Cardiac Safety Simulator system. Based on these values, ionic current inhibition was calculated using the Hill equation (both parameters namely IC_{50} and “ n ” were taken from the available literature as reported in **Supplementary Table S7** online). For the I_{Ks} predicted IC_{50} value, the “ n ” parameter was assumed to be 1. To enable direct comparison, the selected time points were similar to those at which the observed QTcF values were derived. All predicted QTcF values were presented as mean and SD. For the ΔQTc , 95% CI was used as the dispersion measure to keep the data presentation method used in the original study.

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Author Contributions. S.P., H.M., M.J., and A.R.-H. wrote the manuscript. S.P. and H.M. designed the research. S.P. and H.M. performed the research. S.P. and H.M. analyzed the data.

Conflict of Interest. H.M. was and S.P. and M.J. are employees in Simcyp (a Certara Company). A.R.-H. is an employee of the University of Manchester and part-time secondee to Simcyp (a Certara Company). Associate Editor A.R.-H. was not involved in the review or decision process for this paper.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

- ✓ Due to the potential side effect, coadministration of DOM and KETO is either not recommended or contraindicated as studies have shown a threefold increase in the exposure of DOM, in the presence of KETO, possibly due to the inhibition of CYP3A4-mediated metabolism.

WHAT QUESTION DID THIS STUDY ADDRESS?

- ✓ Is it possible to simulate PK and PD effects of the concomitantly given KETO and DOM with the use of the mechanistic model at the population level?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

- ✓ The combination of mechanistic PBPK, Tox modeling, and simulation tools were able to recover the PK and toxicological effect of DOM administered as a single drug and its combination with the pharmacokinetically and pharmacodynamically interacting drug (KETO), through simulations largely based on previously collated *in vitro* data.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

- ✓ This study highlights the potential of using model-based drug development and simulation as a valuable cardiac safety assessment tool.

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