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ARTICLE

Pharmacokinetic/Pharmacodynamic Modeling of the PDE4 Inhibitor TAK-648 in Type 2 Diabetes: Early Translational Approaches for Human Dose Prediction

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TAK-648 is a PDE4 inhibitor with demonstrated preclinical antidiabetic properties. Our objective was to develop a translational pharmacokinetic/pharmacodynamic (PK/PD) model for human type 2 diabetes (T2D) dose prediction using HbA_{1c} results from a db/db mouse study. Estimated parameters in combination with tPDE4i values calculated for the clinical roflumilast dose of 500 μg were used to translate preclinical effects of TAK-648 to required exposure in humans. A first-in-human study with single TAK-648 doses of 0.05–0.85 mg in healthy volunteers yielded mean maximum TAK-648 concentrations (Cmax) and area under the curve (AUC) values from 0.62–11.9 μg/L and 4.58–93.8 μg*h/L, respectively. Based on the performed pharmacokinetic/pharmacodynamic analysis and clinical PK results, clinical efficacy would be expected at a daily dose of 0.1 mg, which is well within the investigated clinical dose range. This result significantly enhanced the confidence in TAK-648 for type 2 diabetes treatment and underlines the necessity of translational approaches in early preclinical phases.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ PDE4 inhibitors have demonstrated antidiabetic properties in both preclinical and clinical setting, with tPDE4 inhibition being related to effects.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ This analysis aimed at predicting a therapeutic exposure and dose for the development compound TAK-648 that would lead to clinically relevant glucose-lowering properties in humans.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ This study presents first-in-human PK and safety results for TAK-648. Combined with the presented preclinical modeling and simulation approach, TAK-648 is predicted to have clinically relevant HbA_{1c} lowering properties similar to roflumilast at a dose of 0.1 mg.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ The results enable a rational dose planning of future clinical studies with TAK-648 and provide reassurance that therapeutic drug concentrations can be reached at doses well tolerated in humans.

PDE4 inhibitors have primarily been investigated in inflammatory disorders, such as asthma and chronic obstructive pulmonary disease.[1] However, recent evidence suggests that PDE4 plays an essential role in glucose and fat metabolism.[2–5] Moreover, in a diabetic mouse model, the PDE4 inhibitor roflumilast, which is approved for chronic obstructive pulmonary disease treatment, has demonstrated significant antidiabetic efficacy.[6] In addition to the evidence presented in db/db mice, roflumilast also significantly reduced HbA_{1c} levels in a clinical setting.[7] Taken together, PDE4 inhibitors present a highly attractive option for the treatment of T2D.

The compound TAK-648 is a selective PDE4 inhibitor, which has been characterized for the T2D indication using a preclinical 4-week db/db mouse model. In this model, TAK-648 significantly improved glucose tolerance and the blood-glucose surrogate marker, HbA_{1c}. The glucose-lowering effects were accompanied by a substantial improvement of pancreatic islet morphology, a reduction of food and water intake, as well as a slight reduction in bodyweight in comparison with control animals. Overall, the results suggested TAK-648 to be a promising drug candidate in the T2D indication.

In order to rationally proceed with development, it was desirable to anticipate the dose at which TAK-648 would show HbA_{1c} lowering properties in humans. The accurate estimation of efficacious doses and the successful transition from preclinical development into the clinic is one of the key challenges of a compound during drug development.[8] To achieve this goal, information about efficacious

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concentrations in humans would be required, as well as human PK properties. Given the preclinical pharmacological data alone, this translation into the clinic would hardly be possible, as often, preclinical mouse studies in pharmacology lack information about drug exposure, and it is not known if the efficacious concentrations in mice can directly be translated to humans. The importance of human PK and efficacious dose projections has been recognized.[9] An approach toward rational prediction of efficacious human doses, eventually using physiologically based modeling for human PK prediction in the absence of observed clinical drug exposure, has been presented in the literature.[10] In this approach, the preclinical exposure–response is characterized, together with an adjustment for interspecies differences in potency between animals and humans. Using the translational medicine paradigm, the publication underlines the requirement to use available clinical data to feed back into the development of preclinical compounds in order to refine their predictions.

The aim of this work was to provide a quantitative, translational framework in order to bridge the db/db mouse model to the clinical patient population. For this, TAK-648 was investigated head-to-head in a tailored PK/PD study in db/db mice against the clinical comparator roflumilast and its active metabolite roflumilast-*N*-oxide. The results were to be combined with observed clinical PK to predict a human therapeutic TAK-648 dose in T2D.

METHODS

Pharmacokinetic/pharmacodynamic study in db/db mice

Db/db mice were treated once daily with vehicle (4% methocel), TAK-648, roflumilast or the active metabolite roflumilast-*N*-oxide, and PK and the PD measure HbA_{1c} were assessed over 28 days. More study details are provided as **Supplementary Material**.

Pharmacokinetic analysis in db/db mice

Five separate PK models were developed (i.e., for TAK-648, as well as both roflumilast and its metabolite after both roflumilast and roflumilast-*N*-oxide dosing, respectively). All parameters were estimated using the nonlinear mixed-effects modeling technique (version 7, ICON Development Solutions, Ellicott City, MD) with first-order conditional estimation with interaction. This methodology is well suited for the mixed data set of PK and PD animals, as the major strength of the population analysis approach is that useful information can also be extracted from sparse data by having the complete data set available for analysis, yet retaining the information obtained from each animal.[11] Control files were parameterized in terms of clearances, volumes, and the appropriate absorption parameter(s). Between-subject variability (BSV) in model parameters was described using an exponential error term:

$$P_{ki} = \theta_k \cdot e^{\eta ki} \quad (1)$$

where P_{ki} denotes the value of the parameter k from the individual i (individual parameter). All model parameters were assumed to follow a log-normal distribution and were thus modeled using an exponential error term. The θ_k is the typical value of the population parameter k and η_{ki} is the difference between the natural logarithm of P_{ki} and θ_k. The random-effects parameter η was assumed to be symmetrically distributed with zero mean and a variance of ω^2. Model improvement was judged based on diagnostic plots (if necessary with appropriate stratification) and decrease in objective function (superior over a nested model if $P \leq 0.05$).

The PK analysis provided apparent clearance (CL) and relative bioavailability (F1) estimates for each individual animal. From these parameter estimates, individual steady state AUC was calculated for each PD animal according to the following equation:

$$\mathrm{AUC}\,[\mu g^{*}h/L] = \mathrm{F1}^{*}\mathrm{Dose}\,[\mu g/kg]\,/\mathrm{CL}\,[L/h/kg]\,. \quad (2)$$

AUC estimates were, in turn, used to determine total PDE4 inhibition (tPDE4i) of the respective compounds for all PD animals. The tPDE4i relates the average free concentration of a compound in plasma to its *in vitro* IC_{50} of PDE4 inhibition, and is an exposure surrogate allowing for the consideration of parallel contribution of parent and metabolite to the overall effect. It is calculated as follows[12]:

$$tPDE4i_x = \frac{AUC_x \cdot f_{u,mouse,x}}{IC_{50,x} \cdot \tau} \quad (3)$$

with x being a placeholder for the respective compound, f_u corresponding to the unbound fraction in mouse plasma *in vitro*, IC_{50} corresponding to the compound concentration resulting in 50% PDE4 inhibition *in vitro*, and τ corresponding to the dosing interval. If two active compounds were present in the systemic circulation at the same time (i.e., roflumilast and roflumilast-*N*-oxide), their tPDE4i values were added to obtain information about their combined effect.[12]

Pharmacokinetic/pharmacodynamic analysis

The combined individual tPDE4i values were used as a measure of exposure in the subsequent PK/PD model. HbA_{1c} levels on days -1, 14, and 28 from vehicle and treatment groups were used as the PD readout, and were related to individual tPDE4i values using the following equation:

$$HbA_{1c} = H_0 + slope \cdot \left(1 - \frac{E_{max} \cdot tPDE4i}{tPDE4i + PDE_{50,x}}\right) \cdot DAY \quad (4)$$

with H_0 corresponding to the estimated HbA_{1c} on day -1, slope corresponding to the steepness of HbA_{1c} increase over time, E_{max} corresponding to the maximum possible treatment effect, DAY corresponding to the treatment day (day -1 defined as DAY = 0), and PDE_{50} being a compound-specific tPDE4i leading to 50% of E_{max}. The final PK/PD model was evaluated in a visual predictive check by simulating new individual concentration-time profiles based on 200 simulations from the original data set and the parameter estimates from the final model. The final model code is provided as **Supplementary Material**.

Prediction of therapeutic exposure in humans

The tPDE4i in animals alone cannot be used to make predictions for the human situation. A reason for this is that

concentration measurements in plasma represent a surrogate for the target concentrations. Concentrations at the target differ from those in systemic circulation. Consequently, the tPDE4i value calculated based on systemic concentrations is also only a surrogate for the tPDE4i at the target site. Two assumptions were made about the relation between compounds and tPDE4i, respectively:

- A difference between two compounds' tPDE4i values observed in mice (which can be explained by, e.g., different distribution properties to the target site) to the same extent would also be present in humans (i.e., the relation between two compounds' tPDE4i values would remain the same). This might be explained by the compound properties, which would remain the same, independent of the species to which the compound is given.
- Relative differences between tPDE4i values required for efficacy in mice and humans are independent of the compound administered. This might be explained by different sensitivity of the species to a class of compounds (i.e., this assumption would represent species properties).

Assuming these two assumptions hold true, the tPDE4i required for efficacy in humans can be calculated based on the rule of proportion.

Practically, the PDE_{50} values obtained from the PK/PD model were linked via the tPDE4i value of 1.03 calculated for the clinically effective roflumilast dose of 500 μg[12] to determine the required tPDE4i value of TAK-648 in humans, using the following equation:

$$\text{required tPDE4i}_{\text{TAK-648,human}} = \frac{\text{PDE50}_{\text{TAK-648,mouse}} \cdot \text{tPDE4i}_{\text{rofiumilast+rofiumilast-N-oxide,human}}}{\text{PDE50}_{\text{rofiumilast+rofiumilast-N-oxide,mouse}}} \quad (5)$$

After the required tPDE4i value was calculated, it was used to determine the AUC in plasma that is necessary to reach the respective tPDE4i value. For this, Eq. 3 was rearranged as follows:

$$\text{AUC}_{\text{TAK-648,human}} = \frac{\text{tPDE4i}_{\text{TAK-648,human}} \cdot \text{IC}_{50,\text{TAK-648}} \cdot \tau}{\text{f}_{\text{u,TAK-648,human}}} \quad (6)$$

TAK-648 first-in-human study

The study was a phase I, randomized, double-blind, placebo-controlled, safety, tolerability, and PK study of escalating single oral TAK-648 doses in healthy male and female subjects. It was conducted in compliance with the Institutional Review Board regulations stated in Title 21 of the United States Code of Federal Regulations, Part 56, Good Clinical Practice regulations and guidelines, and all applicable local regulations. Written informed consent was provided by all subjects prior to study participation. The maximum human recommended starting dose was derived using the methodology described in the US Food and Drug Administration Guidance "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers." Inclusion and exclusion criteria are presented as **Supplementary Material**.

Study design

Approximately 40 healthy male and female subjects, aged 18–55 years, inclusive, were planned for enrollment with 8 subjects planned (6 randomized to TAK-648 and 2 randomized to placebo) for each cohort. The study consisted of 5 cohorts: 0.05 mg, 0.15 mg, 0.35 mg, 0.7 mg, and 0.85 mg. Subjects were randomized to either TAK-648 or placebo within each cohort. Each cohort was dosed sequentially in escalating order with either TAK-648 oral dose solution or placebo for TAK-648 oral dose solution. A minimum of 7 days separated dosing in each cohort to allow for review of safety and tolerability data prior to dose escalation.

End points

The primary end points were the percentages of subjects who: (i) had at least one treatment-emergent adverse event; (ii) had markedly abnormal criteria for safety laboratory tests at least once after dosing; (iii) had markedly abnormal criteria for vital signs measurements at least once after dosing; and (iv) had at least one occurrence of postdose severe hypoglycemia. Secondary end points were PK parameters maximum measured TAK-648 concentration (C_{max}), time from dosing to C_{max} (t_{max}) and the TAK-648 AUC.

Pharmacokinetic sampling and analysis

Blood samples for PK analysis of TAK-648 in plasma were collected predose (within 30 min prior to dosing) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h postdose. Plasma concentrations of TAK-648 were measured using high-performance liquid chromatography with tandem mass spectrometry. The validated concentration range for the assay was 0.010–10.0 μg/L. PK parameters of TAK-648 were determined from concentration-time profiles for all evaluable subjects using Phoenix WinNonlin version 6.3 (Pharsight, Cary, NC). Actual sampling times were used in all computations involving sampling times.

Human dose prediction

The required AUC calculated in Eq. 6 was translated into the required human dose using the observed PK parameters from the TAK-648 first-in-human study.

RESULTS

Pharmacokinetic analysis in db/db mice

TAK-648 pharmacokinetic

The final TAK-648 PK model for db/db mice was a one-compartment model with first-order absorption and elimination. Differences in absorption and half-life were observed between single and multiple dosing, which were empirically described with separate clearance and KA values for day one of treatment. In addition, separate absorption rates were included for each dose group. The absorption rate decreased with increasing doses. Absorption half-lives at steady state were 74 min, 85 min, and 168 min for the dose groups 1 mg/kg, 3 mg/kg, and 10 mg/kg, respectively. The typical clearance was estimated to be 2.97 L/h. Bioavailability was fixed to a typical value of 100%, however, individual

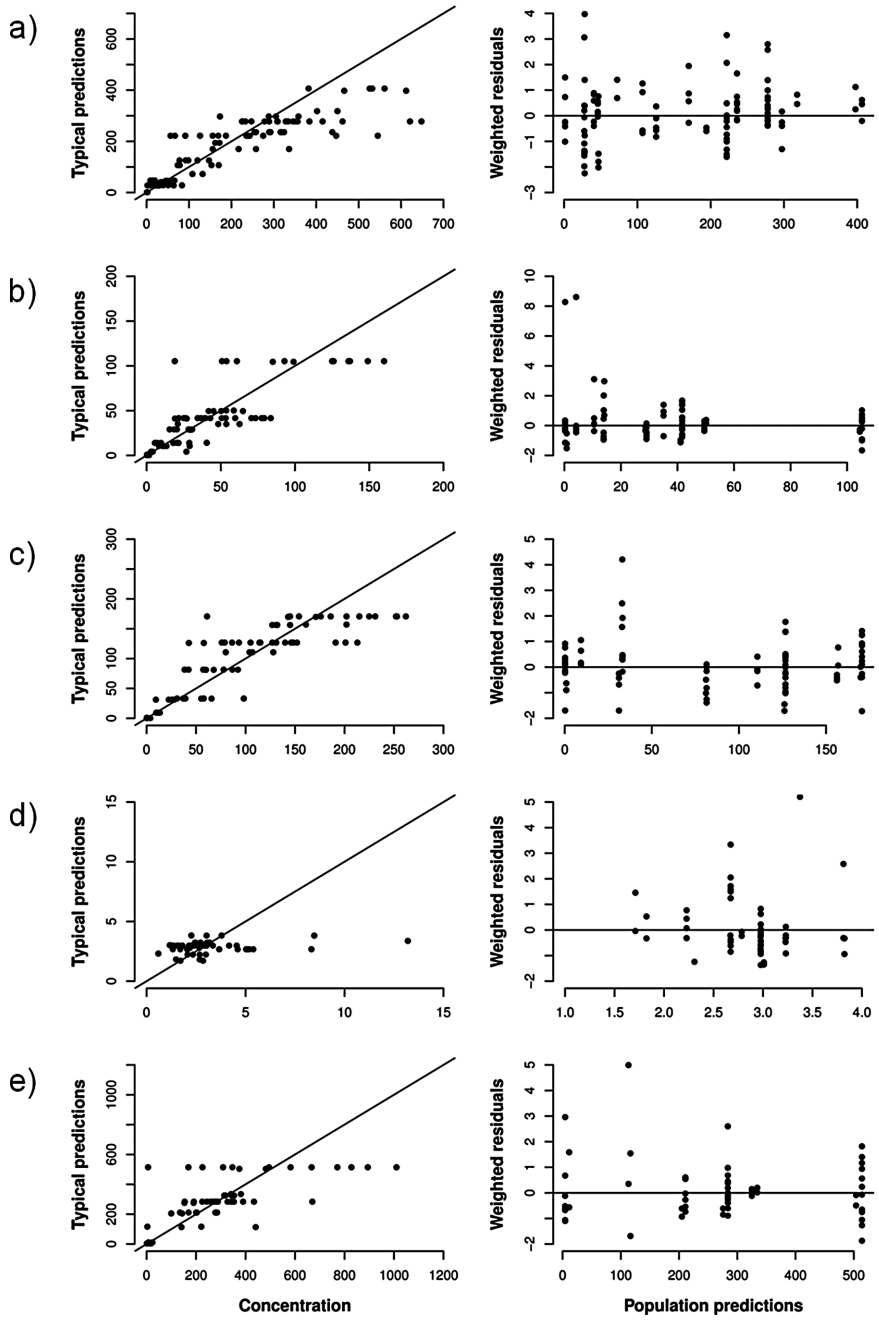

Figure 1 Goodness-of-fit plots for final pharmacokinetic models. Column 1 shows observations against population predictions, column 2 shows weighted residuals against population predictions. **(a)** TAK-648, **(b)** roflumilast observations after roflumilast dosing, **(c)** roflumilast-N-oxide observations after roflumilast dosing, **(d)** roflumilast observations after roflumilast-N-oxide dosing, and **(e)** roflumilast-N-oxide observations after roflumilast-N-oxide dosing.

values differed from each other as a result of the associated BSV with a coefficient of variation of 19%. Goodness-of-fit plots are presented in **Figure 1a**. An overview of the resulting exposures and calculated tPDE4i values is presented in **Table 1**.

Pharmacokinetic of roflumilast and roflumilast-*N*-oxide

Some results of the PK analysis of roflumilast and roflumilast-*N*-oxide have already been published.[6] The PK of both roflumilast and roflumilast-*N*-oxide after roflumilast and roflumilast-*N*-oxide dosing, respectively, in db/db mice was best described by two-compartmental model structures with first-order absorption and elimination, and BSV on the apparent clearance. Bioavailability was fixed to a typical value of 100% in all cases. Apparent roflumilast clearance was estimated to be 2.34 L/h and 41.8 L/h for roflumilast and roflumilast-*N*-oxide dosing, respectively, whereas apparent roflumilast-*N*-oxide clearance was estimated to be 3.52 L/h

Table 1 Area under the curve and corresponding total PDE4 inhibition values for pharmacodynamic animals in the db/db mouse study

	Roflumilast		Roflumilast *N*-oxide		Complete
Administered compound and dose per day	**AUC (μg*h/L)**	**tPDE4i$_{rof}$**	**AUC (μg*h/L)**	**tPDE4i$_{rofNO}$**	**tPDE4i**
Roflumilast, 0.3 mg/kg	136	0.744	86.9	0.731	1.49
	(104–178)	(0.568–0.974)	(83.7–90.2)	(0.704–0.759)	(1.27–1.74)
Roflumilast, 1 mg/kg	408	2.23	275	2.31	4.55
	(363–459)	(1.98–2.51)	(268–282)	(2.25–2.37)	(4.25–4.86)
Roflumilast, 3 mg/kg	1,201	6.56	853	7.18	13.8
	(1,170–1,233)	(6.39–6.73)	(844–863)	(7.10–7.26)	(13.5–14.0)
Roflumilast *N*-oxide, 3 mg/kg	68.6	0.375	2,633	22.1	22.5
	(57.5–81.8)	(0.314–0.447)	(2,500–2,772)	(21.0–23.3)	(21.4–23.7)
	TAK-648				
TAK-648, 1 mg/kg	337	–	–	–	0.486
	(321–351)				(0.466–0.506)
TAK-648, 3 mg/kg	976	–	–	–	1.41
	(897–1063)				(1.29–1.53)
TAK-648, 10 mg/kg	3,257	–	–	–	4.70
	(2,817–3,765)				(4.06–5.43)

AUC, Area under the curve; tPDE4i, total PDE4 inhibition.
The tPDE4i values were calculated using f_u and IC_{50} values of 20.0% and 5.77 μg/L for TAK-648, 3.7% and 0.282 μg/L for roflumilast, and 12.7% and 0.629 μg/L for roflumilast-*N*-oxide, respectively. The tPDE4i$_{rof}$ and tPDE4i$_{rofNO}$ correspond to the separate tPDE4 inhibition of roflumilast and roflumilast-*N*-oxide in the circulation, whereas the total value considers the contribution of both compounds and was calculated from the individual combinations of tPDE4i$_{rof}$ and tPDE4i$_{rofNO}$. The column with complete tPDE4i also presents the tPDE4i values for TAK-648. Animals received TAK-648 (1, 3, or 10 mg/kg/day), roflumilast (0.3, 1, or 3 mg/kg/day), or roflumilast-*N*-oxide (3 mg/kg/day) once daily for 28 days. Data are presented as geometric means (68% range).

and 1.09 L/h. These clearance values correlate with the slightly lower AUC of roflumilast-*N*-oxide after roflumilast dosing compared with the parent roflumilast (67% of parent), as well as the only marginal exposure of roflumilast after roflumilast-*N*-oxide dosing (2.6% of roflumilast-*N*-oxide). Goodness-of-fit plots are presented in **Figure 1b–e**. An overview of the resulting exposures and calculated tPDE4i values is presented in **Table 1**.

Pharmacokinetic/pharmacodynamic analysis

In the developed PK/PD model, systems parameters, i.e. maximum possible effect E_{max}, individual HbA_{1c} value on day -1 (H_0) or steepness of HbA_{1c} increase over time (slope), were assumed to be the same between compound. In contrast, PDE_{50} values represented compound properties and were thus estimated separately. One PDE_{50} value was estimated for the joint effect of roflumilast and roflumilast-*N*-oxide, and another PDE_{50} value was estimated for TAK-648. The model performed well in describing the HbA_{1c} time course for all compounds and dose groups, as demonstrated in the visual predictive check in **Figure 2**. The final parameter estimates are presented in **Table 2**. All parameters were estimated precisely. Based on the final parameters, HbA_{1c} values in the control group would linearly increase by 0.138% per day, and the maximum effect that might be reached with the investigated PDE4 inhibitors would be to keep HbA_{1c} levels at their starting levels ($E_{max} = 1$). Overall, variability between the animals was low to moderate in the systems parameters H_0 and SLOP with 12.7% and 21.4%, respectively. The variability in the tPDE4i value required to achieve the half-maximum effect (PDE_{50}) was high. Based on the estimated PDE_{50} values of the respective compounds, TAK-648 and roflumilast doses were calculated that would be required to reach a comparable half-maximum effect in db/db mice. This half-maximum effect in db/db mice would be reached with a dose of 2.3 mg/kg TAK-648 and 5.9 mg/kg roflumilast.

Prediction of therapeutic exposure in humans

After having obtained the PDE_{50} estimates for TAK-648 and roflumilast, the TAK-648 tPDE4i required to achieve comparable efficacy, as seen with 500 μg roflumilast in humans, was calculated using Eq. 5.

The required tPDE4i was translated into a required AUC, using Eq. 6 and a human fraction unbound of 0.667, resulting in a predicted efficacious AUC of 8.74 μg*h/L.

TAK-648 first-in-human study

Subject disposition and demographics

A total of 39 subjects were randomized, and all 39 subjects completed the study. A summary of demographic and baseline characteristics is presented in **Table 3**.

Safety and tolerability results

Overall, TAK-648 was well tolerated in healthy human subjects. Few events occurred, and the majority was considered by the investigator to be not related to the study drug. The gastrointestinal adverse events of abdominal pain and vomiting, which occurred in a single subject in the 0.35 mg dose group, was considered by the investigator to be related to the study drug; however, the time to onset was ~60 h after the administration of the study drug, which extended beyond the time of the last measurable TAK-648 concentration. In addition, two subjects (one in the 0.15 mg and the other in the 0.85 mg dose group) had blood pressure and pulse measurements that met protocol criteria for

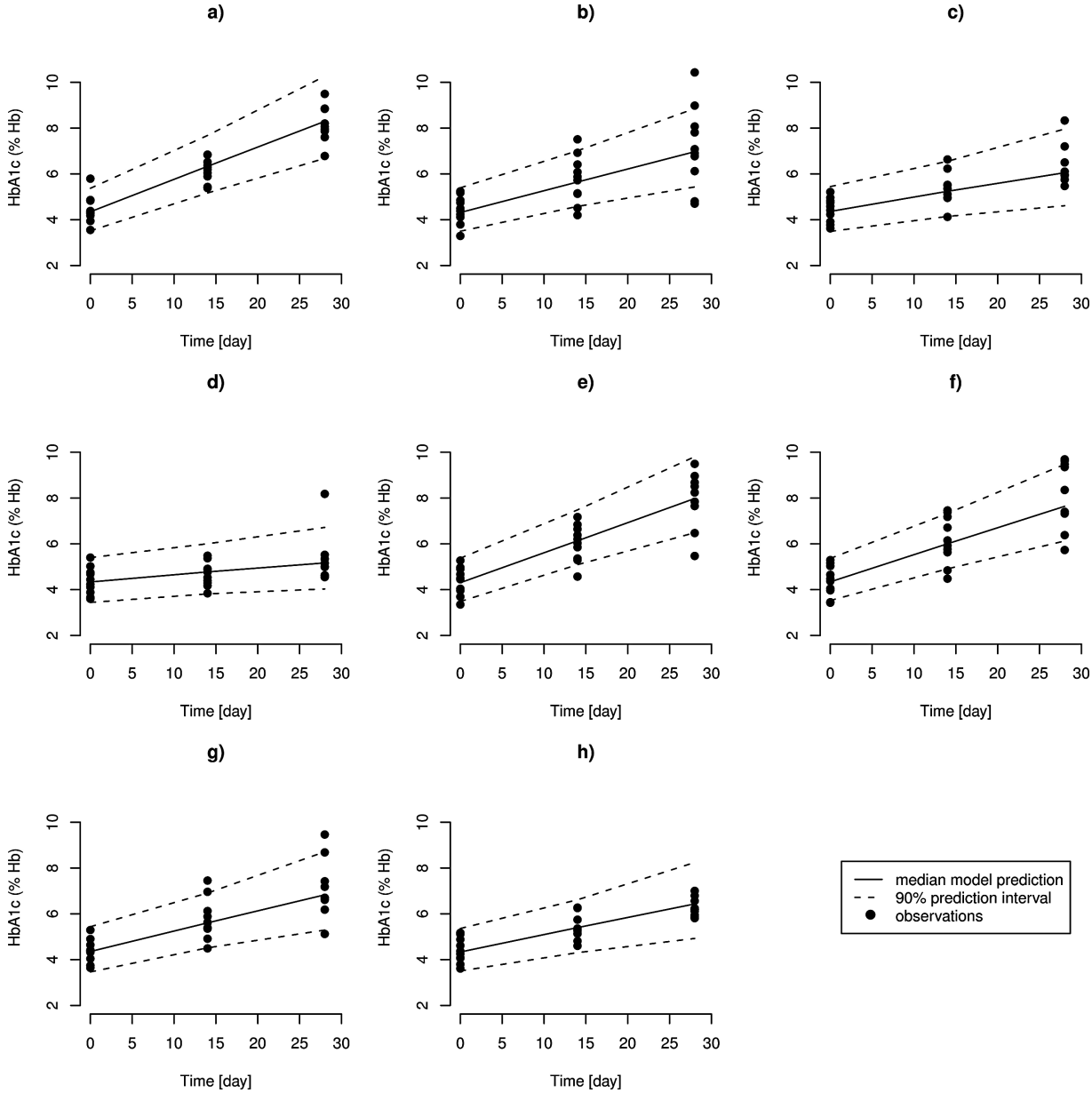

Figure 2 Evaluation of the final pharmacokinetic/pharmacodynamic (PK/PD) model via visual predictive check, considering doses of **(a)** vehicle, **(b)** 0.3 mg/kg roflumilast, **(c)** 1 mg/kg roflumilast, **(d)** 3 mg/kg roflumilast, **(e)** 3 mg/kg roflumilast-N-oxide, **(f)** 1 mg/kg TAK-648, **(g)** 3 mg/kg TAK-648, and **(h)** 10 mg/kg TAK-648 once daily for 28 days and HbA_{1c} as PD readout. Filled circles correspond to measured values of HbA_{1c}. The solid and dashed lines represent the median model prediction and the 90% prediction interval, respectively. The PK/PD model well captured the HbA_{1c} time course over all dose groups.

orthostatic hypotension; however, both were neither symptomatic nor associated with hypotension or tachycardia. In addition, these two cases were isolated and did not show a dose-dependent pattern. No hypoglycemic, neurologic, or psychiatric events were observed in any of the subjects receiving a single TAK-648 dose.

Pharmacokinetic results

Linear and log-linear plots of mean TAK-648 plasma concentration vs. time data following oral administration of a single 0.05, 0.15, 0.35, 0.7, or 0.85 mg TAK-648 dose to healthy subjects are presented in **Figure 3**, and calculated PK parameters are presented in **Table 4**. TAK-648 was rapidly absorbed following the administration of a single oral dose of 0.05, 0.15, 0.35, 0.7, or 0.85 mg; the median time of maximum plasma concentration (t_{max}) values ranged from 1–1.5 h. Mean C_{max} values increased with increasing dose from 0.05–0.85 mg, with the exception at the highest dose, in which mean C_{max} values at 0.85 mg were similar but less than those following the 0.7 mg dose. Mean AUC values increased in a near dose-proportional manner with increasing dose. Mean TAK-648 terminal elimination half-life values ranged from ~6–14.5 h, and seemed to increase with increasing dose. However, there were an increasing number of measurable

Table 2 Parameter estimates of the final pharmacokinetic/pharmacodynamic model

Model parameter	Unit	Estimate	RSE %
H_0	[%]	4.35	1.25
slope	[%/DAY]	0.138	4.80
E_{max}	[–]	1.00	6.27
$PDE_{50,TAK-648}$	[–]	1.10	31.0
$PDE_{50,roflumilast(-N-oxide)}$	[–]	27.1	25.2
Between-subject variability			
BSV_H_0	[CV%]	12.7	11.8[a]
BSV_slope	[CV%]	21.4	30.2[a]
BSV_PDE_{50}	[CV%]	79.8	44.5[a]
Residual variability			
Proportional	[CV%]	3.87	29.3[a]

BSV, between-subject variability; CV%, coefficient of variation; E_{max}, maximum effect; H_0, estimated HbA_{1c} on day −1; PDE_{50}, PDE4 inhibition leading to half-maximum effect; RSE, relative standard error; slope, steepness of HbA_{1c} increase over time.
[a]Standard error given on the variance scale.

TAK-648 plasma concentrations in the terminal portion of the plasma concentration-time profiles as the dose increased, which contributed to the characterization of the longer terminal elimination half-life values at the higher doses.

Human dose prediction

Based on the calculated PK parameters from the TAK-648 first-in-human study (**Table 4**), the required AUC of 8.74 μg*h/L translates into a predicted therapeutic human dose of 100 μg.

DISCUSSION

Pharmacokinetic/pharmacodynamic analysis

The analysis presented in this paper was based on a tailored PK/PD study in db/db mice. The study aimed at providing a within-study comparison of TAK-648 – a promising compound characterized for T2D treatment – with the clinical comparator roflumilast, for which antidiabetic efficacy has been clinically demonstrated.[7] In general, it was attempted to obtain as much information about the PK of TAK-648, roflumilast, and roflumilast-*N*-oxide in db/db mice as possible, despite the limited blood volume obtainable from this species. Consequently, different animals from one treatment group were sampled at different time points, and also varied within animals between days. Furthermore, PK samples from the PD animals on day 20 of treatment were taken at the same time points as those of the PK animals on days 14 and 20 of treatment. This approach allowed for a direct comparison of PK between PK and PD animals, and, given similar PK at these time points, assured the transferability of PK information to the sparsely sampled PD animals via the population PK analysis approach. The PK of all compounds could be well described. Minor trends might be visible at the highest TAK-648 concentration. Given the sparse nature of the sampling, these were attributed to random variability. They were accepted, as they were not considered to largely influence individual AUC estimates. The developed PK models were used to calculate AUC values at steady state for each of the PD animals. Via the use of tPDE4i as a surrogate measure for exposure, the derived AUC values were used in the subsequent development of the PK/PD model. The approach of developing population PK models to derive steady-state AUC values was considered appropriate, as the models were expected to give a more robust estimate of the animals' AUC values. In addition, they yielded exposure data for each individual animal, although each of the PD animals provided only two PK samples.

In principle, one might argue that the PD end point HbA_{1c} should have been related directly to the concentration-time profiles described by the compartmental models, instead of using the derived PK parameter AUC. This approach might carry the advantage of being able to discriminate between different shapes of PK profiles and their effect on PD end points. However, it has been demonstrated that tPDE4i is a suitable measure to describe the relation between exposure and PD effect.[12] Consequently, for this early development project, the model simplification via AUC and tPDE4i was considered to be sufficient to obtain an estimate of human efficacious dose. The final analysis was based on individual animal data. For roflumilast and roflumilast-*N*-oxide treatment, the contribution of both parent and metabolite were considered in the analysis. This was done by calculating a tPDE4i value resulting from the presence of both

Table 3 Summary of demographic and baseline characteristics

Characteristic	Pooled placebo[a] *N* = 10	TAK-648 0.05 mg *n* = 6	TAK-648 0.15 mg *n* = 6	TAK-648 0.35 mg *n* = 6	TAK-648 0.7 mg *n* = 6	TAK-648 0.85 mg *n* = 5
Mean age (SD), y[b]	36.2	39.0	32.5	37.5	37.0	45.0
	(11.10)	(13.21)	(11.08)	(9.07)	(9.78)	(11.51)
Sex, no. (%)						
Men	8 (80.0)	5 (83.3)	5 (83.3)	5 (83.3)	2 (33.3)	3 (60.0)
Women	2 (20.0)	1 (16.7)	1 (16.7)	1 (16.7)	4 (66.7)	2 (40.0)
Mean weight (SD), kg	77.7	76.1	70.0	75.6	67.1	76.1
	(14.33)	(12.39)	(9.32)	(12.86)	(4.36)	(12.79)
Mean BMI (SD), kg/m^2[c]	24.8	24.3	24.5	25.4	25.3	26.0
	(3.03)	(3.60)	(2.42)	(2.37)	(2.73)	(2.26)

BMI, body mass index.
[a]Pooled placebo consists of subjects randomized to placebo from all cohorts
[b]Age at the date the informed consent was signed
[c]BMI was calculated at screening.

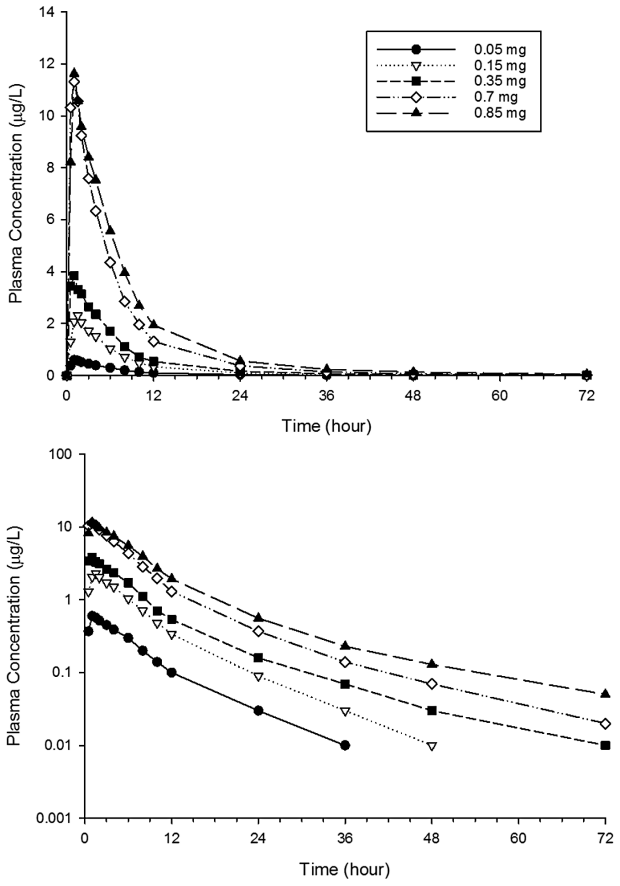

Figure 3 Mean plasma concentration-time profiles of TAK-648 (linear and log-linear) following a single 0.05, 0.15, 0.35, 0.7, or 0.85 mg oral dose of TAK 648.

compounds. After roflumilast treatment, the contribution of both compounds to the overall effect is very balanced, as exposures and respective tPDE4i values are very similar (see **Table 1)**. When roflumilast-*N*-oxide is administered, almost no roflumilast is formed (i.e., the effect is mostly a result of roflumilast-*N*-oxide). This result very nicely reflects the human situation, in which most of the tPDE4i results from the formed metabolite roflumilast-*N*-oxide. The visual predictive check showed that tPDE4i values were well suited to describe the HbA_{1c} time course after vehicle treatment as well as after treatment with different doses of TAK-648, roflumilast, and roflumilast-*N*-oxide. The combined exposure measure tPDE4i nicely correlates with the increasing effect on HbA_{1c} (0.3 mg/kg roflumilast, $<$1 mg/kg roflumilast, $<$3 mg/kg roflumilast, and $<$3 mg/kg roflumilast-*N*-oxide). The correlation was also apparent for the different TAK-648 dose strengths. However, the absolute values of tPDE4i required for the same effect on HbA_{1c} in db/db mice differed between the compounds. So far, the source of this difference has not been identified. A reason might be that plasma concentrations only represent a surrogate for the site of action, and that, due to differences in distribution properties, different compounds might require different tPDE4i values for the same effect. Further investigations would be required to finally explain the difference. For this analysis, however, it has been considered as a viable approach to assume that, whatever the difference between the compounds is in animals, the same would be true in humans, as it was expected that this difference would relate to compound properties.

TAK-648 first-in-human study

The phase I study was performed to evaluate the safety, tolerability, and the PK profile of TAK-648 in healthy male and female subjects following a single oral dose. A dose of 0.05 mg was chosen as a conservative starting point for dosing in this study, and predicted to provide an exposure (AUC) in humans that would be $>$150-times lower than the exposure in dogs at the no observed adverse effect level. Although the actual exposure following the 0.05 mg dose (4.58 μg*h/L) was higher than predicted (0.59 μg*h/L), the drug was well tolerated at the starting dose and had a favorable tolerability profile following single oral doses up to 0.85 mg. The few adverse events that occurred did not display dose dependence, and the majority of those were considered by the investigator to be not related to the study drug. As a result, a maximum tolerated dose was not achieved in this study. Following a single 0.05–0.85 mg TAK-648 dose, absorption was rapid, with median t_{max} values ranging from 1–1.5 h across the range studied. Although the PK analyses were based on small separate groups of five or six treated individuals at each dose level, the results suggested that TAK-648 exposure increased in proportion to dose within the dose range tested. Overall, the results from this study suggested that TAK-648 had favorable PK and tolerability profiles, and was suitable for evaluation in additional clinical studies.

Human dose prediction

The therapeutic dose prediction has so far not been validated with clinical data. It represents the first step of an

Table 4 Summary of plasma pharmacokinetic parameter estimates of TAK-648 following a single 0.05, 0.15, 0.35, 0.7, or 0.85 mg oral dose of TAK-648

Treatment	t_{max} (h)	C_{max} (μg/L)	AUC_{last} (μg*h/L)	AUC_{∞} (μg*h/L)	$t_{1/2z}$ (h)
0.05 mg ($n = 6$)	1.0 (0.5–2.0)	0.62 (40)	4.45 (51)	4.58 (50)	6.01 (27)
0.15 mg ($n = 6$)	1.5 (1.0–1.5)	2.33 (34)	16.61 (28)	16.81 (28)	8.43 (30)
0.35 mg ($n = 6$)	1.0 (0.5–1.5)	4.00 (40)	27.93 (34)	28.27 (34)	11.66 (41)
0.7 mg ($n = 6$)	1.0 (0.5–1.5)	11.85 (22)	75.28 (34)	75.77 (34)	13.50 (22)
0.85 mg ($n = 5$)	1.0 (1.0–1.5)	11.71 (53)	92.66 (32)	93.84 (33)	14.53 (25)

AUC, area under the curve; C_{max}, peak plasma concentration; t1/2, terminal elimination half-life; t_{max}, time of maximum plasma concentration.
Data for t_{max} are given as the median value with the range in parenthesis; all other data are given as the mean with the percent coefficient of variation (CV%) in parenthesis.

iterative learn and confirm process, which can use more sophisticated animal or human data for model refinement as it comes along in development. Even if some model adjustments will be required at a later stage, this data driven knowledge accumulation may be an advantage, as acquired knowledge might be used for potential follow-up development compounds.

It should be mentioned that the current dose prediction is a point estimate that does not consider uncertainty in PK/PD model parameters or human exposure. Further model refinements should thus also integrate uncertainty through generation of n replicate sets of parameter estimates[13] and subsequent repeated dose prediction using, for example, the nonlinear mixed-effects modeling covariance matrix. This way, a specific dose can be associated with a respective probability of achieving target efficacy – an additional helpful tool to make informed team decisions.

Predictions based on preclinical data should use all available information, preferably including data from clinical comparators. The approach described in this paper, therefore, is considered the preferred method to enable a rational dose planning of early clinical studies. The validation of the approach of using tPDE4i values for human dose prediction and comparing with roflumilast will need more time, as it will require human efficacy data from a phase II trial.

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