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RESEARCH PAPER

Systems Pharmacology Modeling of Drug-Induced Modulation of Thyroid Hormones in Dogs and Translation to Human

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ABSTRACT

Purpose To develop a systems pharmacology model based on hormone physiology and pharmacokinetic-pharmacodynamic concepts describing the impact of thyroperoxidase (TPO) inhibition on thyroid hormone homeostasis in the dog and to predict drug-induced changes in thyroid hormones in humans.

Methods A population model was developed based on a simultaneous analysis of concentration-time data of T_4, T_3 and TSH in dogs following once daily oral dosing for up to 6-months of a myeloperoxidase inhibitor (MPO-IN1) with TPO inhibiting properties. The model consisted of linked turnover compartments for T_4, T_3 and TSH including a negative feedback from T_4 on TSH concentrations.

Results The model could well describe the concentration-time profiles of thyroid hormones in dog. Successful model validation was performed by predicting the hormone concentrations during 1-month administration of MPO-IN2 based on its *in vitro* dog TPO inhibition potency. Using human thyroid hormone turnover rates and TPO inhibitory potency, the human T_4 and TSH concentrations upon MPO-IN1 treatment were predicted well.

Conclusions The model provides a scientific framework for the prediction of drug induced effects on plasma thyroid hormones concentrations in humans via TPO inhibition based on results obtained in *in vitro* and animal studies.

KEY WORDS inter-species extrapolation · pharmacokinetics and pharmacodynamics · T_3 · T_4 · Thyroperoxidase · TSH

ABBREVIATIONS

$AUC_{0\text{-}24}$	Area under the plasma concentration-time curve from time 0–24 h after dosing
C_{ss}	Steady state plasma concentration of the MPO inhibitor
DIT	Diiodotyrosine
DRUG	Function to describe the drug-induced inhibition of T_4 production
FEED1	Influence of T_4 on TSH production
FEED2	Influence of T_4 on TSH turnover
fr	The fraction of T_4 that undergoes peripheral conversion to T_3
Fraction	The fraction of T_3 converted from T_4
HPT	Hypothalamic-pituitary-thyroid
IC_{50}	Concentration which produces 50% of maximum inhibition of TPO
I_{max}	Maximal inhibition of TPO production
kin_{T3}	Zero-order production rate of T_3
kin_{T4}	Zero-order production of (the precursor of) T_4
kin_{TSH}	Zero-order production of (the precursor of) TSH
k_{T3}	First-order rate constant of elimination of T_3
k_{T4}	First-order rate constant of elimination of T_4
k_{TSH}	First-order rate constant of elimination of TSH

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LC-MS/MS	Liquid chromatography with mass spectrometry detection
LLOQ	Lower limit of quantification
MIT	Monoiodotyrosine
MPO	Myeloperoxidase
MPO-IN1	MPO inhibitor 1
MPO-IN2	MPO inhibitor 2
n	Number of transit compartments
NF1	Slope factor of *FEED1* relationship
NF2	Slope factor of *FEED2* relationship
NF3	Slope factor in *STIM* function
PKPD	Pharmacokinetic-pharmacodynamic
rT_3	Non-active reverse T_3
STIM	Function to describe TSH influencing the production of T_4
T_3	Triiodothyronine
$T_{3,BL}$	Baseline of T_3 in plasma
T_4	Thyroxine
$T_{4,BL}$	Baseline of T_4 in plasma
TPO	Thyroperoxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
TSH_{BL}	Baseline of TSH in plasma

INTRODUCTION

The hypothalamic-pituitary-thyroid (HPT) axis, composed by the hypothalamus, pituitary and thyroid glands, is part of the endocrine system and regulates metabolic pathways and many physiological functions in the body (1–3). The thyroid stimulating hormone (TSH) secreted by the anterior pituitary has an essential role in controlling the thyroid axis. Hypothalamic thyrotropin-releasing hormone (TRH) stimulates the pituitary to produce TSH, which in its turn stimulates synthesis and secretion of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). These thyroid hormones are involved in cell differentiation during development and are also important for maintaining thermogenic and metabolic homeostasis (3). The production of TRH and TSH are regulated via a negative feedback mechanism by concentrations of thyroid hormones, i.e. decreased serum concentrations of free T_4 and T_3 stimulate TSH production and increase the TRH-mediated stimulation of TSH, whereas increased serum concentrations of free T_4 and T_3 decrease TSH and inhibit TRH-mediated stimulation of TSH (3–5).

Thyroperoxidase (TPO) is involved in the formation of T_3 and T_4 in the follicular cell of the thyroid gland. The TPO enzyme oxidizes iodide to a reactive iodinating species that results in the iodination of tyrosine residues on the thyroglobulin protein. TPO-catalyzed phenolic free radical coupling of the resultant monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues, followed by pinocytosis and proteolysis, yields T_4 or T_3 (1,3). In addition, T_4 is peripherally converted by deiodinases to T_3 or to the non-active reverse T_3 (rT_3) in the liver and kidney (6). The fraction of T_3 produced peripherally from T_4 and also the rate of turnover of these hormones varies between species (6–9).

In drug discovery and development, new chemical entities are routinely tested in toxicological studies. Many different physiological and histopathological functions are evaluated, including drug effects on the thyroid system. Since TSH, T_4 and T_3 are necessary for many physiological functions in the body, it is important to quantitatively understand drug-induced effects on thyroid hormones in order to prospectively predict potential thyroid hormone effects in man based on preclinical observations. A systems pharmacology model could be of value, when based on the thyroid hormone physiology and the concepts of the drugs pharmacokinetics and drug-induced enzyme inhibition (10). Such a model, once developed, could be instrumental to describe the thyroid hormone changes on the basis of the exposure of the drug, but also to explain and to predict hormone concentrations under new conditions, such as in humans, and for other compounds. In addition, if the mechanism underlying the observed thyroid effects is known (such as TPO inhibition), then an established quantitative understanding of the relationship between *in vitro* and *in vivo* potency may reduce the need for *in vivo* safety testing in the drug discovery process.

Throughout the years, different mathematical models of the thyroid-pituitary axis have been proposed. Many of the models have been developed based on human thyroid hormone data (7,11–15). Most of the nonclinical models are involving the metabolism of iodide or dietary iodide intake (8,16–18). The recent years these models have become more physiological in nature (19–22) but sometimes also rather complex, by including many parameters (11,23,24). However, to our knowledge nobody has previously proposed a thyroid hormone model that can describe drug-induced changes on interrelationship and time-courses of TSH, T_4 and T_3.

In the present investigation, the first aim was to develop a systems pharmacology model based on hormone physiology, available mathematical models and pharmacokinetic-pharmacodynamic (PKPD) concepts, to describe the impact of TPO inhibition on thyroid hormone homeostasis in the dog. The second aim was to predict thyroid hormone profiles in humans based upon inter-species differences in hormone degradation rates and *in vitro* IC_{50} values for TPO inhibition. To this end, we developed the model based on TSH, T_4 and T_3 and plasma exposure data upon repeated administration of the myeloperoxidase (MPO) inhibitor MPO-IN1 in the dog. The model validity was tested with another MPO inhibitor (MPO-IN2) in dog. Interspecies extrapolation was investigated by prediction

of MPO-IN1 induced thyroid hormone changes in human. The mechanistic explanation for thyroid hormone changes was that these MPO inhibitors also inhibited (with less potency) TPO, there is a homology between these enzymes (25). MPO is implicated in the pathogenesis of diseases with an inflammatory component and therefore MPO inhibitors are of interest as potential treatment for example for rheumatoid arthritis, artherosclerosis, chronic obstructive pulmonary disease, multiple sclerosis and Parkinson's disease (26). Understanding and ability to predict the thyroid hormone changing potential of MPO inhibitor drug candidates could aid the development of treatments for such diseases.

MATERIALS AND METHODS

Animals

Male and female Beagle dogs were used in a toxicological evaluation of MPO-IN1 and MPO-IN2 (1 and 6-month toxicity studies). The dogs were 10 to 16 months old in the 1-month studies with a body weight of 11 to 17 at start of dosing. In the 6-month study, the dogs were 6 to 8 months old and weighted 7–10 kg at start of the study. Different dog suppliers were used, Kennel Rååhöjden, Örkelljunga, Sweden (MPO-IN1, 1-month study), Marshall Farms, NY, USA (MPO-IN2, 1-month study) and Bantin and Kingman Limited, Hull, UK (MPO-IN1, 6-month study). The dog protocols were approved by Animal Ethics Committee, Stockholm, Sweden. The dogs were housed under standard conditions in single pens with access to an exercise area with others of the same sex and group, for major part of each day. In the studies with MPO-IN1, the dogs were fed in the afternoon with CXD diet (Leo Pharmaceutical, Denmark (1-month studies) or Harlan Limited, UK (6-month study)). In the study with MPO-IN2, the dogs were fed approximately 1 h before dosing and had access to food for approximately 2 h after dosing. Water was freely available.

Study Design

Dogs were given once daily oral doses of MPO-IN1 or MPO-IN2 or vehicle, by gavage, for up to 1- or 6-months. Information on dosing regimen is given in Table I. MPO-IN1 was administered as a solution, consisting of 0.3 M Meglumine (1-month study) or purified water adjusted to pH10.3 (6-month study). MPO-IN2 was formulated in a vehicle containing 0.25% Xanthan gum, 0.025% sodium docusate and 10% citric buffer (100 mM), adjusted to pH 6.0–6.5. Serial blood samples for analysis of parent compound (8–10 blood samples/occasion) were taken after a single dose and at the last day of the treatment period. In order to confirm exposure in the dogs dosed with MPO-IN1, single samples were also taken on day 14 in the 1-month study and at weeks 4, 8 and 24 in 6-months study. Blood samples were drawn from the jugular, cephalic or saphenous vein into tubes with spray dried EDTA as anticogulant. Plasma was prepared within 0.5–1 h after sampling by centrifugation (1500 g, 10 mins at approximately 4°C) and subsequently transferred to Nunc cryotubes and then stored at -20°C or below until bioanalysis.

The total plasma concentrations of the compounds were determined, using validated bioanalytical methods followed by reversed-phase liquid chromatography and electrospray tandem mass spectrometry as described in more detail by Nilsson (27). Stable isotope-labelled internal standards were used for both compounds. The lower limit of quantification (LLOQ) was 0.010 µmol/L. Quality control samples were well within acceptance criteria (<15%). The unbound plasma concentrations of both compounds were determined after ultrafiltration (Ultrafree-MC Centrifugal Filter Unit, Millipore, Billerica, USA) at pH7.4 and 37°C

Pre-treatment and pre-dose blood samples (taken early in the morning before the daily dose) for analysis of TSH, total T_3 and/or total T_4 were regularly sampled during the treatment period (see Table I for details on sampling days). Total T_4 and TSH were measured in dog serum using analyzer IMMULITE 1000, with reagents (Canine Total T_4 and Canine TSH) and controls (K9TCM: Canine Thyroid Control Module) supplied by Siemens Medical, Solutions & Diagnostics (NY, USA). Total T_3 was determined using an electrochemiluminescence assay performed on the Elecsys 2010 (Roche, USA). LLOQs were 6.4 nmol/L, 0.3 nmol/L and 0.03 ng/ml for T_4, T_3 and TSH respectively.

TPO Iodination Assay

The inhibition of human TPO by test compounds was assayed as follows: test compounds were serially diluted in 100% dimethyl sulfoxide (10 concentrations, 2 dilutions/log unit, in duplicate) and subsequently transferred to the assay plate where diluted 1:10 in assay buffer (20 mM sodium phosphate pH6.5). Human TPO (24 nM, 1.73 U/mL, RSR Limited, UK) and H_2O_2 (100 µM in assay buffer with 3 mM KI) were added and the reaction mixture was incubated for 10 min at room temperature. The reaction was terminated by the addition of catalase (7.5 µg/ml). After 5 min incubation at room temperature, 1 mM tetramethyl benzidine (in 200 mM sodium-acetate buffer pH5.4) was added and absorbance measured at 650 nm in an absorbance plate reader (Spectramax, Molecular Devices, USA). For measurement of background response, human TPO was replaced with assay buffer.

Table I Compound, Study duration, Sampling Occasion of Hormones, Daily Dose, no. of Animal, and Mean (SD) Steady State Plasma Concentrations (C_{ss}), on the Last Day of Dosing in the Dog Toxicity Studies

Cmpd	Duration (Months)	Sampling occasion of T_4, T_3 and TSH	Daily Dose (μmol/kg)	No. of animals and gender	C_{ss} (μmol/L)
MPO-IN1	1	PT, Day 1, 4, 8, 14, 28, 31[a], 35[a], 42[a], 57[a]	0[b]	6 M+6 F	-
			15	3 M+3 F	7.2 (1.2)
			90	3 M+3 F	39 (17)
			700[b]	6M[c]+6 F	280 (95)
	6[d]	PT, Week 4, 13, 27, 31[a], 40[a]	0[b]	7 M+7 F	-
			15	4 M+4 F	6.9 (0.77)
			60	4 M+4 F	31 (7.2)
			250[b]	7 M+7 F	150 (38)
MPO-IN2	1[e]	PT, Day 4, 14, 21, 28, 29[a], 31[a], 36/37[a], 57[a]	0[b]	6 M+6 F	-
			90	3 M+3 F	1.1 (0.27)
			300	3 M+3 F	8.5 (2.9)
			700[b]	6 M+6 F	23 (9.5)

PT Pre-treatment measurements (1–2 pre-treatment measurements were done)

[a] Only taken from recovery animals

[b] 2-3 recovery animals included per sex

[c] In one dog the dosing was discontinued and the recovery period was started after 2 weeks of dosing

[d] Only TSH and T_4 were sampled in this study

[e] The exposure was 26, 42 and 55% lower on the last day as compared to the first day of dosing of 90, 300 and 700 μmol/kg, respectively

Inhibition of the canine TPO was assayed as follows. The compounds were serially diluted in 100% DMSO (10 concentrations, 2/log unit, in duplicate) and finally transferred to the assay plate where diluted 1:5 in assay buffer (20 mM phosphate buffer (Na_2HPO_4/ KH_2PO_4), pH7.4). Canine TPO (21 nM, final concentration in reaction mixture) and guaiacol (20 mM) were added. The reaction was started by addition of H_2O_2 (300 μM) and absorbance was measured for 3 min at 450 nm in an absorbance plate reader (Spectramax, Molecular Devices). Enzymatic activity was determined as the initial rate (slope of the initial 40 s) of the increase in absorbance.

Pharmacokinetics Analysis

For each individual, the pharmacokinetics, in terms of area under the plasma concentration-time curve from time zero to 24 h after dosing ($AUC_{0\text{-}24}$), was calculated on the last day of dosing, by means of the linear up-log down method using WinNonlin (V 4.1, Pharsight Corporation, USA). The steady state plasma concentrations (C_{ss}) were obtained by dividing $AUC_{0\text{-}24}$ with 24. Mean C_{ss} ± SE exposure is summarized in Table I. For dogs dosed with MPO-IN2, the average plasma exposure was between 26 and 55% lower on the last day of treatment as compared to the first day of treatment. For each dog, the C_{ss} was calculated at the same time point as thyroid hormones measurements assuming a linear decline in average C_{ss} between day 1 and day 28.

Modeling of Drug-Induced Changes in Thyroid Hormones Concentrations

The pharmacokinetic-pharmacodynamic (PKPD) analysis was based on individual steady state plasma concentrations and thyroid hormone concentrations from all dogs. A physiologically based feedback PKPD model describing the effect of the TPO enzyme inhibitors on thyroid hormones was developed based on data from MPO-IN1 and a simplification of detailed models described in literature (19,20) and is shown schematically in Fig. 1.

The production of TSH in plasma (TSH_p) is described by a transit compartment model (28), assuming that TRH stimulates TSH-producing cells in the anterior pituitary gland (preTSH), which through single transfer rate constant, k_{TSH} and *n*-compartments, results in the release of TSH in plasma (Eqs. 1–3).

$$\frac{dpreTSH(1)}{dt} = kin_{TSH} \cdot FEED1 - k_{TSH} \cdot preTSH(1), \quad (1)$$

$$\frac{dpreTSH(n)}{dt} = k_{TSH} \cdot preTSH(n-1) - k_{TSH} \cdot preTSH(n), \quad (2)$$

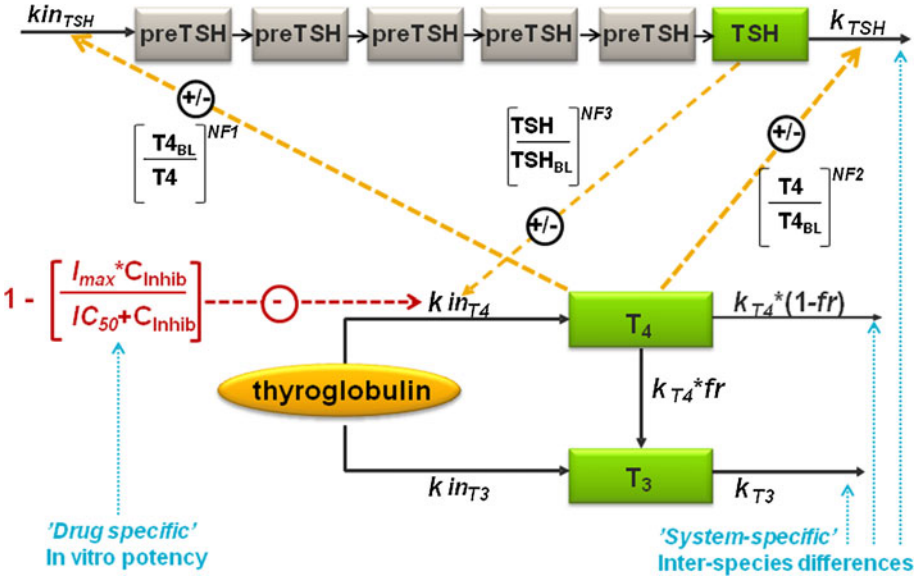

Fig. 1 Proposed feedback model of thyroid hormone homeostasis and drug mediated inhibition of TPO. Hormone interactions are shown with dashed lines, where +/− indicate a positive or negative interaction following decreased or increased hormone concentrations. Reduced concentrations of T_4 stimulate production of TSH and lower the elimination of TSH. Increased concentrations of TSH stimulate production of T_4. Green-shaded compartments are those where thyroid hormone observations were available in plasma. TSH_{BL}, $T_{4,BL}$, $T_{3,BL}$ are baseline (steady state plasma concentrations) of the thyroid hormones. The drug-specific parameter potency (IC_{50}) was different between compounds. The system-specific parameters such as first-order elimination rate constants of TSH, T_4 T_3 (K_{TSH}, K_{T4} and K_{T3}) and the fraction of T_4 that is conversed to T_3 (fr) were different between species (dog and human). I_{max} is the maximal inhibition of T_4. *NF1* and *NF2* are the slope factor of the relationship describing the influence of T_4 on TSH production and elimination. *NF3:* the slope factor of the relationship describing the influence of TSH on T_4 production. kin_{TSH}, kin_{T4} and kin_{T3}: are the zero-order elimination rate constants of TSH, T_4 and T_3.

$$\frac{dTSH_p}{dt} = k_{TSH} \cdot preTSH(n) - k_{TSH} \cdot TSH_p \cdot FEED2. \quad (3)$$

kin_{TSH} is zero-order production of the precursor of TSH in the pituitary gland and k_{TSH} is the first-order rate constant of elimination of TSH from the $(n\text{-}1)^{th}$ compartment to the n^{th} compartment. In the model development an analysis was done with number of compartments between 1 and 10, for which $n=6$ transit compartments gave the best fit as judged from model diagnostics.

TSH production is regulated by the plasma T_4 by allowing decreased concentrations of T_4 to stimulate the preTSH production via the following:

$$FEED1 = \left[\frac{T_{4BL}}{T_4}\right]^{NF1}, \quad (4)$$

where $T_{4,BL}$ is the baseline of T_4 in plasma and *NF1* is the slope factor of this relationship.

It was described in literature that decreased concentrations of T_4 also could lower the turnover of TSH_p (21), which was described following:

$$FEED2 = \left[\frac{T_4}{T_{4,BL}}\right]^{NF2}, \quad (5)$$

in which *NF2* is the slope factor.

The production and loss of T_4 in plasma are described with the following equation,

$$\frac{dT_4}{dt} = kin_{T4} \cdot [STIM \cdot DRUG] - k_{T4} \cdot T_4, \quad (6)$$

where kin_{T4} is the zero-order production rate of T_4 and k_{T4} is the first-order elimination rate of T_4. MPO-IN1 and MPO-IN2 are assumed to inhibit the production of T_4 via inhibition of TPO, and is described by the following I_{max} equation ($DRUG$)

$$DRUG = 1 - \left[\frac{I_{\max} \cdot C_{ss}}{IC_{50} + C_{ss}}\right], \quad (7)$$

in which I_{max} corresponds to the maximal inhibition of TPO, IC_{50} is the plasma concentration of the compounds which produces 50% of maximum inhibition of TPO; and C_{ss} is the steady state plasma concentration of the MPO inhibitor.

Increased concentrations of TSH_p stimulate the production and release of T_4 into blood ($STIM$), described by the following equation

$$STIM = \left[\frac{TSH}{TSH_{BL}}\right]^{NF3}, \quad (8)$$

where TSH_{BL} is the baseline of TSH in plasma and *NF3* is the slope factor.

T_3 is produced from thyroglobulin but also peripherally derived via conversion from T_4 following:

$$\frac{dT_3}{dt} = k_{T4} \cdot T_4 \cdot fr + kin_{T3} - k_{T3} \cdot T_3, \quad (9)$$

where *fr* is the fraction of T_4 that undergoes peripheral conversion to T_3, kin_{T3} is the zero-order production rate of T_3 in the thyroid and k_{T3} is the first-order rate constant of T_3 loss from plasma. $T_{3,BL}$ is the baseline value at $t=0$ for T_3. The fraction of T_3 converted from T_4 was calculated using the following equation:

$$Fraction = \frac{k_{T4} \cdot T_4 \cdot fr}{k_{T4} \cdot T_4 \cdot fr + kin_{T3}}. \quad (10)$$

The individual pre-treatment measurements of T_4, T_3 and TSH were used to initialize the respective compartments. Thyroid hormone plasma concentrations below the lower limit of quantification (LLOQ) were set to LLOQ/2. For TSH, T_4 and T_3, the samples below LLOQ were 1, 7 and 1% respectively of the total number of observations.

Modeling Procedures

Modeling of the dog hormone data was performed using the nonlinear mixed-effect software package Nonmem (Version VII, Globomax, Md., USA). Model development and selection was based on physiological understanding of the thyroid hormone pituitary axis, comparison between various structural and error models based on objective function values, visual inspection using basic goodness-of-fit plots, precision in parameter estimates and assessment of parameter correlation.

The inter-individual variability was modeled for TSH_p, T_4, T_3 and IC_{50}, using an exponential equation:

$$P_i = \theta_1 \cdot \exp(\eta_i), \quad (11)$$

where P_i represents the individual estimate, θ the population estimate for parameter P and η_i the random deviation of P_i from P. The values of η_i are assumed to be independently normally distributed with mean zero and variance ω^2.

The residual error in the thyroid hormone concentrations was characterized by a proportional error model:

$$Y_{ij} = Y'_{ij} \cdot (1 + \varepsilon_{ij}), \quad (12)$$

where Y_{ij} represents the j^{th} observation for the i^{th} individual predicted by the model. Y'_{ij} represents the predicted value of TSH, T_4 or T_3, and ε_{ij} accounts for the residual deviation of the model-predicted value from the observed value. The values of ε were assumed to be independently normally distributed with mean 0 and variance σ^2. The ADVAN6 TOL5 subroutine and the first-order estimation method (FOCE interaction) were used to estimate the population θ, ω^2, and σ^2 unless stated otherwise. Individual parameter estimates were obtained in a Bayesian post hoc step.

Within- and Cross-Species (Human) Predictions

The developed systems pharmacology model for thyroid hormone modulation was used for extrapolation within the same species for different compounds. Prediction of thyroid effects of MPO-IN2 in the dog was done by changing the drug-specific parameter, IC_{50} (see Fig. 1). To this end, the *in vitro* potency on dog TPO, was compared to the estimated *in vivo* IC_{50} in dogs. The *in vitro/in vivo* ratio was used to translate *in vitro* potencies for MPO-IN2 to a predicted *in vivo* potency. Together with pharmacokinetic information of MPO-IN2 in dogs, simulations were performed to predict the thyroid concentrations upon treatment with MPO-IN2.

For translation to human, the systems parameters such as the turnover rates of the thyroid hormones and the fraction of T_3 converted from T_4 were changed to literature values for human. The *in vitro* potency for MPO-IN1 in the human TPO assay was taken, and corrected for human plasma protein binding (assuming IC_{50u} *in vivo* equal to IC_{50} *in vitro*). Based on the human pharmacokinetics, a prediction was made of TSH and T_4 over time for a low and high dose in the 21-day multiple ascending dose clinical study. The human trial was a placebo controlled, single-center, randomized within dose panel, double-blind study with the aim to determine the safety and tolerability of MPO-IN1 following multiple ascending oral doses. Eight healthy male and non-fertile female volunteers aged 18 to 65 years were given an oral dose of 150 or 325 mg MPO-IN1 or placebo t.i.d. for 21 days. Plasma samples were taken for analysis of MPO-IN1 and also for analysis of total T_4, free T_4, free T_3 and TSH.

RESULTS

Thyroid Hormones in the Dog

Mean thyroid hormone concentration-time profiles after repeated administration of MPO-IN1 for 1 month are shown in Fig. 2. A dose-dependent change in hormone concentrations was observed. After administration of the highest dose, 700 μmol/kg, of MPO-IN1 (C_{ss} approximately 280 μmol/L), the T_4 concentrations rapidly decreased, with a marked inhibition already observed within 4 days after start of dosing. From days 4 to 28, T_4 concentrations were markedly reduced, with many observations below the LLOQ. Following the end of treatment, a rebound was seen in T_4 concentrations (the concentrations were higher than those observed at pre-treatment), which returned to normal levels within 3 days after end of dosing. At the same dose,

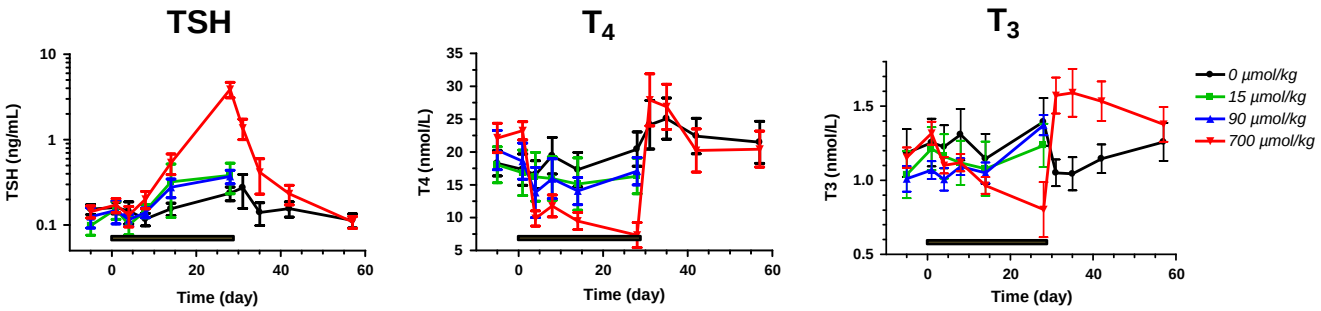

Fig. 2 Mean ± SE T_4, T_3 and TSH plasma concentrations over time after oral dosing of MPO-IN1 at doses of 15, 90, 700 µmol/kg in dogs. Values below the LLOQ were taken as ½LLOQ in the calculations of the mean. Only pre-dose observations from each measured day are shown. The horizontal bar indicates direction of treatment.

the T_3 concentrations tended to decrease during treatment. After end of treatment the T_3 plasma concentrations increased above those for the control group, and remained higher for up to 14 days. In contrast to T_4 and T_3, the TSH concentrations increased during treatment, and on day 28 they were 29-fold higher as compared to the pre-dose value on day 1. The TSH concentrations had partially returned to baseline 3 days after cessation of treatment (day 31 after start of dosing) and full recovery was observed at the end of the 1-month recovery period (day 57). The drug-dependent effect on TSH and T_4 profiles was of similar shape and size in the 6 months study.

Modeling of Drug-Induced Changes in Thyroid Hormones Concentrations

Pre-dose morning measurements of thyroid hormones and TSH concentrations were included in the model development. The number of parameters was reduced by fixing the half-lives of T_4 and T_3 to known physiological values, i.e., 16.6 and 7.8 h, respectively (30), which were comparable to those reported by Belshaw (8). Individually observed data and model predicted visual predictive check based on population parameter estimates after administration of MPO-IN1 for 1-month and 6-months are shown in Figs. 3 and 4, respectively. Population parameter estimates are listed in Table II. A population value for the baseline of T_4 was estimated, whereas it was fixed for T_3 and TSH to the mean value. Inter-individual variability for all baselines was estimated.

The model could well describe the thyroid hormone and TSH profiles. It captured the rapid decline in T_4 concentrations, followed by the low sustained plasma concentrations during treatment and the rebound following end of treatment. In the 1-month study, the maximum effect on T_3 in plasma was slightly underestimated at the end of dosing at the highest dose. The large increase in TSH concentrations and decline after stop of treatment was captured. The basal concentrations of T_4 and TSH were estimated to be 19% lower in males as compared to females and 43% higher for the 700 µmol/kg dose group. The half-life of TSH in plasma was estimated to 2.0 days (Table II). The potency (IC_{50}) of TPO inhibition was estimated at 33 µmol/L, for which the corresponding free concentration (14 µM, with free fraction of 0.44) was seven fold higher than the potency for TPO enzyme inhibition *in vitro* (2 µM, estimated from dog thyroid microsomes). The fraction of T_3 formed from T_4 was calculated to 32% (see Eq. 10).

Model Validation

The validity of the developed model was tested by predicting the drug induced changes in thyroid hormone concentrations upon dosing by the follow-up compound MPO-IN2. Both compounds had similar potency of TPO inhibition *in vitro* (2 µmol/L). By correcting the *in vitro* IC_{50} of TPO inhibition for the *in vitro-in vivo* ratio (7-fold differences) observed for MPO-IN1, and the unbound fraction for MPO-IN2 (0.54), the IC_{50} of TPO inhibition *in vivo* for MPO-IN2 was estimated to be 27 µmol/L (total exposure). Using the systems parameters describing hormone turnover rates and fraction of T_3 converted from T_4, from the PKPD modeling of MPO-IN1, and the predicted IC_{50} of 27 µmol/L for TPO inhibition for MPO-IN2, the hormone profiles after administration of different doses of MPO-IN2 (given in the 1-month toxicity study) were simulated taking into account a linear decrease of C_{ss} exposure between Day 1 and Day 28. The observed hormones concentration-time profiles for the highest dose (700 µmol/kg) and the mean prediction and its 95% prediction interval are shown in Fig. 5. Baselines concentrations of the predictions were fixed on the observed mean baseline values observed in this study. In a subsequent analysis, the IC_{50} for MPO-IN2 in the dog was estimated at 24 µM, being close to the predicted IC_{50} (data not shown).

Translational Prediction of Human Thyroid Hormone Concentrations

For MPO-IN1, human hormone profiles were predicted by changing the turnover rates of the thyroid hormones to

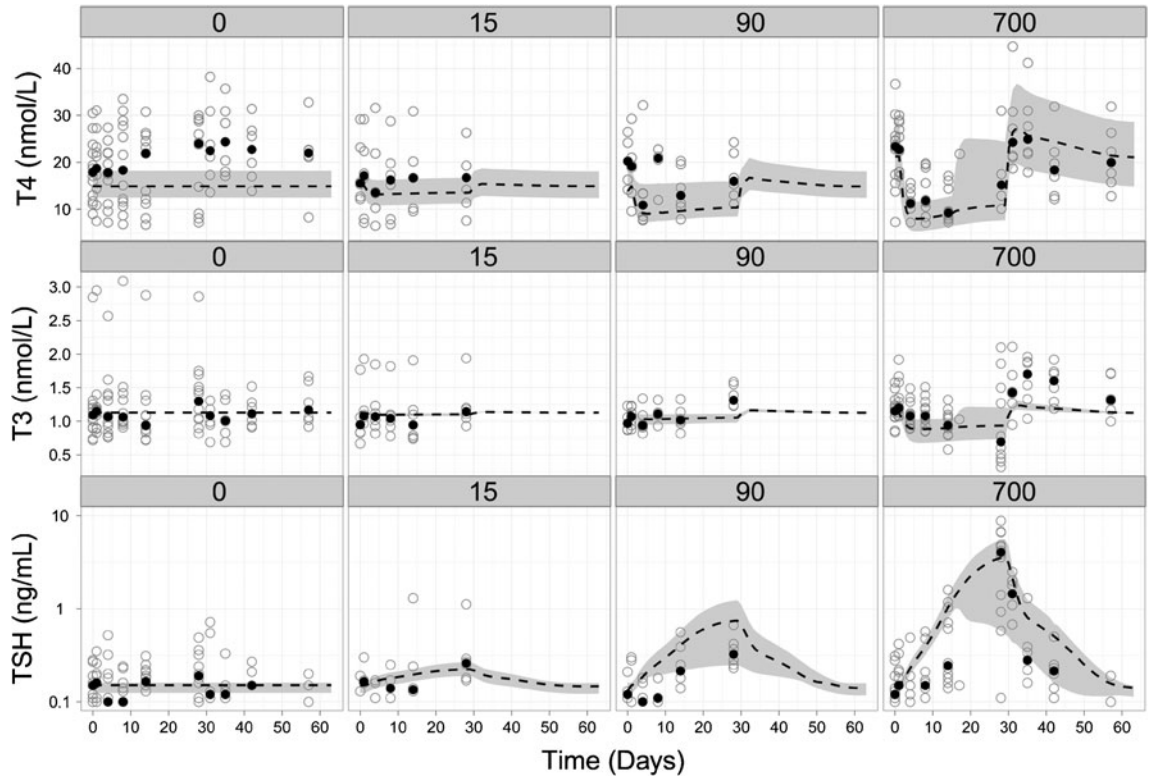

Fig. 3 Visual Predicted Check plots for T_4, T_3 and TSH based on the uncertainties in population parameter estimates after 1 month oral dosing of MPO-IN1 at doses of 0, 15, 90, 700 μmol/kg. Dashed line: Median of predictions, closed circles: median of observations, open symbols: individual observations, Shaded area = 95% prediction interval. Note: The uncertainty in the inter- and intra-individual variation was excluded from the VPC predictions and values below the LLOQ were excluded in the figure.

literature values ($t^1/_2$ for T_4: 168 h and $t^1/_2$ for T_3: 24 h (21)). The fraction T_4 converted to T_3 was set at 72% (31). The IC_{50} for TPO inhibition in human was predicted to be 28 µM. This was based on a human free fraction of 0.18 and an *in vitro* potency of 4.9 µM for MPO-IN1 in the human TPO assay assuming IC_{50u} *in vivo* equal to IC_{50} *in*

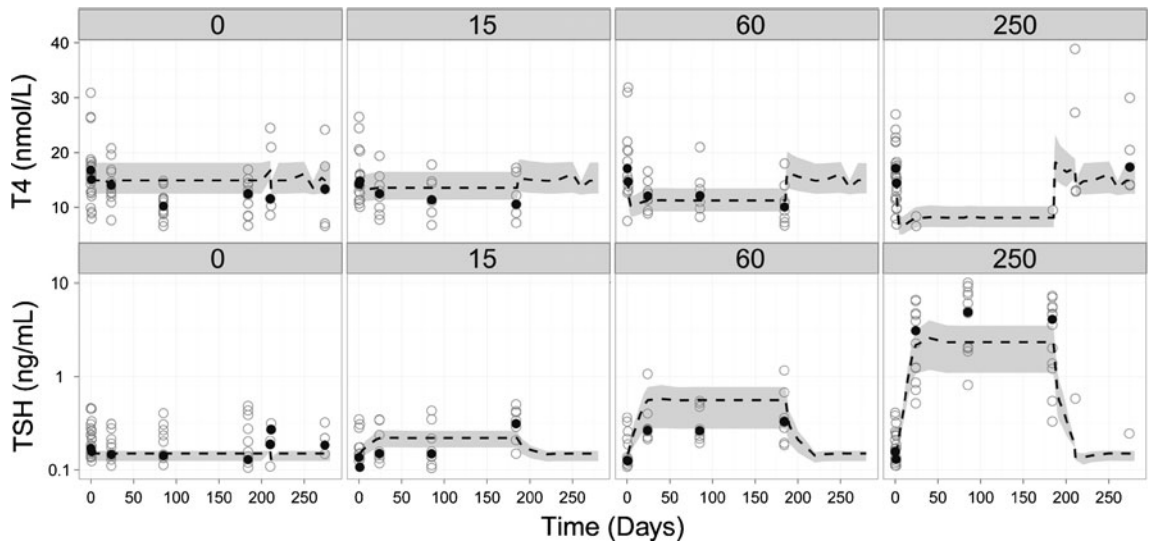

Fig. 4 Visual Predicted Check plots for T_4 and TSH based on the uncertainties in population parameter estimates after 6 month oral dosing of MPO-IN1 at doses of 0, 15, 60, 250 μmol/kg. Dashed line: Median of predictions, closed circles: median of observations, open symbols: individual observations, Shaded area = 95% prediction interval. Note: The uncertainty in the inter- and intra-individual variation was excluded from the VPC predictions and values below the LLOQ were excluded in the figure.

Table II Estimated Population Parameters Following Administration of MPO-IN1 to the Dog. The Intra-Individual Variability was Estimated at 31, 22 and 54% for T_4, T_3 and TSH, Respectively. Precision of the Estimates is Expressed as RSE in Parentheses. TSH_{BL}, $T_{4,BL}$, $T_{3,BL}$ are Baseline Concentrations of TSH, T_4 and T_3 in Plasma. k_{TSH}, k_{T4} and k_{T3}: are the First-Order Elimination Rate Constants of TSH, T_4 and T_3. *Fr* is the Fraction of T_4 that Undergoes Peripheral Conversion to T_3. IC_{50} is the Plasma Concentration which Produces 50% of Maximum Inhibition of TPO. I_{max} is the Maximal Inhibition of TPO. *NF1* and *NF2* are the Slope Factor of the Relationship Describing the Influence of T_4 on TSH Production and Elimination. *NF3:* the Slope Factor of the Relationship Describing the Influence of TSH on T_4 Production

Parameter	Unit	Estimate	Inter-individual variability (%)
TSH_{BL}	ng/mL	0.16 (fixed)	48
$T_{4,BL}$	nmol/L	16.7 (0.052)	27
$T_{3,BL}$	nmol/L	1.13 (fixed)	23
k_{TSH}	1/Days	0.35 (0.068)	-
k_{T4} [a]	1/Days	1 (fixed)	-
k_{T3} [a]	1/Days	2.1 (fixed)	-
fr [b]		0.040 (0.27)	-
I_{max}		0.740 (0.04)	-
IC_{50}	μmol/L	33.2 (0.12)	100
NF1		2.53 (0.076)	-
NF2		1.9 (0.18)	-
NF3		0.11 (0.25)	-

[a] Based on literature value (29), where $t_{½}$ of T_4 = 16.6 h and $t_{½}$ of T_3 = 7.8 h

[b] Fraction of T_3 formed from T_4 = 32% (Eq. 10)

[c] $T_{4,BL}$ was estimated to be 19% lower for males than females and 43% higher for the 700 μmol/kg dose group

vitro. This assumption was made based on the differences between the human and dog TPO assay. With description of human pharmacokinetics, the prediction was made of TSH and T_4 over time for 150 and 325 mg during the 21-day multiple ascending dose clinical study. The model could predict the modest effect on T_4 and TSH after inhibition of TPO enzyme as shown in Fig. 6. Total T_3 was not measured in the human study for which reason the validity of the model on T_3 profiles could not be tested. In Fig. 6 also the upper and lower limit of the normal range of the hormones is shown as horizontal dotted lines (32).

DISCUSSION

In this study, a systems pharmacology model based on hormone physiology and pharmacokinetic-pharmacodynamic concepts was developed to describe the effect of TPO inhibition on the thyroid hormone pituitary-axis in the dog. The model was used to predict thyroid hormone profiles in humans based upon inter-species differences in hormone degradation rates and *in vitro* TPO IC_{50} values for a clinically tested MPO inhibitor.

The PKPD model was developed based on the physiology and the production and metabolism of T_4 and T_3 hormones and TSH, including their feedback mechanisms. Different structure models and feedback mechanisms were tested during the model development phase, with the final model selected based on goodness of fit, model diagnostics, Nonmem objective function value and scientific plausibility. Our model could successfully predict the shape of hormone concentration profiles of T_4, T_3 and TSH in dogs after administration of different doses of a TPO enzyme inhibitor (MPO-IN1). The model did however slightly underestimate the maximal decrease in T_3 concentrations. The validity of our model was confirmed by prediction of the effect on T_4, T_3 and TSH in dog plasma for a follow-up compound (MPO-IN2) on the basis of measured average plasma concentrations of MPO-IN2 and *in vitro* IC_{50} for TPO inhibition. The estimated IC_{50s} for TPO inhibition were comparable for MPO-IN1 and MPO-IN2, but due to lower exposure of MPO-IN2 in the toxicity studies, the inhibitory effect of MPO-IN2 on thyroid hormones was smaller than for MPO-IN1.

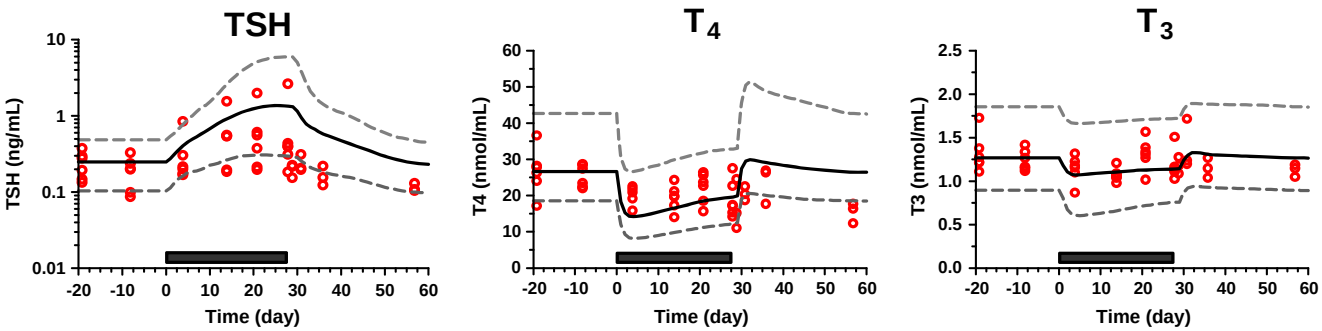

Fig. 5 Simulated and individual observed concentration-time profiles of T_4, T_3 and TSH in plasma after repeated dosing of 700 μmol/kg of MPO-IN2 for 1 month to male dogs. 5th- and 95th-percentiles following 250 simulations (including intra- and inter-individual variabilities) are shown as dashed lines and median are shown as solid line. Symbols indicate individual observations. The horizontal bar indicates the duration of treatment.

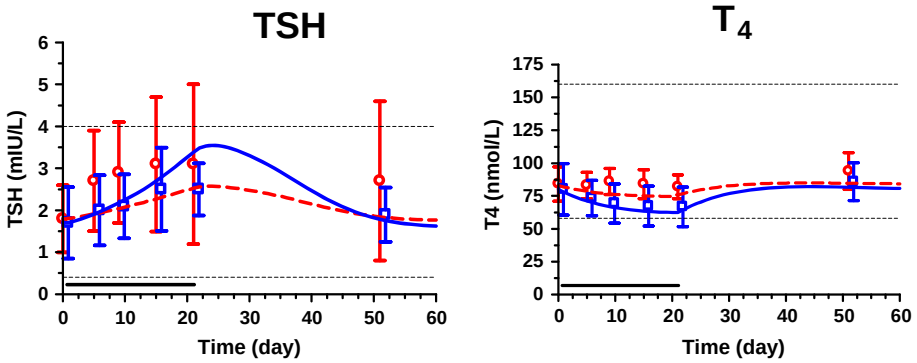

Fig. 6 Time-courses of TSH and T_4 in plasma after repeated dosing of MPO-IN1 in humans for 21 days. Squares and solid line show 325 mg dose, whereas circles and dashed line show 150 mg dose. Observed data are plotted as mean ± SD and simulations as lines. The horizontal dotted black lines represent the upper and lower limits of the normal range of TSH and T_4 concentrations, respectively.

The good agreement between the observed human T_4 and TSH concentrations and the predictions based on extrapolation of the systems model and the *in vitro* TPO inhibition is encouraging. Similar agreement was shown for prediction of clinical thyroid changes of MPO-IN2 (data not shown). For purpose of evaluation of safety for patients, it is important in a next step to estimate the actual human IC_{50} for TPO inhibition and the associated variability based on human data. However, getting a full understanding of the patient variability in both systems and drug specific parameters might need the inclusion of other data on kinetics of T_4 and TSH from other (literature) sources as well as data from multiple (high) dose levels in humans. With such refined model, better informed decisions on clinical trial designs and the risk for need of rescue medication or treatment of hyperthyroidism.

Several researchers have through the years reported various mathematical models of the thyroid-pituitary axis of different complexities in both humans and animals (11–15,17,22,33,34). Also a number kinetic models were developed to describe the metabolism of iodide or dietary iodide intake or kinetics of the thyroid hormones (7–9,16,17,35). In recent years the models have become more physiological in nature and circadian variations in TSH hormone have also been included (19,22). Many models are complex, including several compartments and parameters on a molecular level and are often used for simulations of the HPT axis under different disease states. In general, these models were not fitted to observed hormone-time data, instead they were developed based on parameter estimates derived from the literature and/or by changing parameters and comparing the simulations with the observed data (11,22–24,33). Conversely, Eisenberg *et al.* (19,20) did include both the pharmacokinetics of levothyroxine (L-T_4) and physiological human data of HPT axis in the development of their model, which consisted of TSH as well as T_3 and T_4 in plasma and tissues pools. One great advantage was that they fitted their model to the plasma concentration-time data of thyroid hormones and TSH and also validated it against several independent clinical datasets. Also their model was used for simulation of different treatment combinations of T_3 and T_4 in central hypothyroidism (21).

To our knowledge, our feedback model is the first to describe and predict drug-induced modulation of thyroid hormones. It should be noted that the model did not aim to include all mechanisms previously described, but rather be simplified (limited) towards the rate-limiting steps in the thyroid hormone feedback system. In our model, T_4 was considered to be the hormone contributing to the production and release of TSH. It was also assumed that T_4 affected the elimination of TSH in plasma, which is consistent with the results that the degradation of TSH is decreased in extreme primary hypothyroid patients (21). The feedback consisted of an exponential model, based on total plasma concentrations of T_4 in relation to its baseline, since free concentrations of thyroid hormones are normally not being measured in the nonclinical toxicology studies. Therefore it was assumed that the relationship between total and free plasma concentrations were the same over time. Some authors suggest that the negative feedback from plasma free T_3 is greater than that for free T_4 (6,12,33). According to Eisenberg *et al.* (19,20) half of the effect on TSH secretion in steady state is due to T_4 in plasma and the rest is due to T_3 in plasma. Feedback mechanisms from both T_3 and T_4 were tested in the model development phase but there were problems to estimate these two feedbacks simultaneously. In addition, the results from the modeling were not improved when only including feedback on TSH from T_3 or when estimating similar effect on feedback from T_3 and T_4. It is known that blood T_4 is converted to rT_3 and T_3 peripheral tissues like the liver, which in its turn produces T_3 (12). This is in line with the concept that from plasma, the prohormone T_4 is locally taken up and tissue-specifically converted to T_3 to interact with the thyroid hormone receptors in the target cells of the hypothalamic and pituitary regions involved in the negative feedback of the HPT-axis (4,36). In the current study, the fraction of T_3 formed from T_4 in dogs was estimated to 32%, which is in agreement with the 37% value reported by Belshaw *et al.* (8).

Six transit compartments were included in our model to describe the delayed increase in TSH concentrations during treatment. Following inhibition of the TPO enzyme our model predicted a delay of 1 day before a 10% increase in TSH plasma concentrations was observed. A time-delay of thyroidal secretion into human plasma was also included by Eisenberg *et al.* (19) in their model, which was estimated to 8 h. The turnover of TSH (K_{TSH}) was estimated as no value for dog could be found in literature. The calculated corresponding half-life was 2 days. This was considerably longer than the t½ for TSH reported in humans, i.e. 30–55 min (13,21). There are differences in the physiology of thyroid hormones in dog and humans. In the dog, the total plasma concentrations of T_3 and T_4 are lower, the free fractions of T_3 and T_4 are higher and the turnover rate of these hormones are more rapid as compared to human (8,9). Based on this we would expect the half-life of TSH to be much shorter than our predicted value. Therefore, it would be of interest to study the half-life of TSH in dogs and include this in the model. It appears likely that the observed k_{TSH} in the model reflects the (longer) half-life of some intermediate process in the TSH production/release pathway rather than the half-life of TSH itself.

Some sources of variability have not been included in the analysis. For instance, nominal sampling times of thyroid hormones were used as the actual times were not available. In addition, although daily diurnal changes were observed in both TSH and T_4 concentrations in the current study, this was not included in the final model. The main aim for the model was to describe and predict time-course of hormone concentrations over a period of at least 1 month, and therefore daily changes of hormone concentrations were considered of less interest. Therefore, only samples were included taken at the same time point during the day for all animals. The dogs given MPO-IN1 vomited occasionally close to dosing which resulted in a larger variability in exposure both within and between dogs. This day-to-day variability in exposure was not taken into account in the modeling, since only individual steady state plasma concentrations calculated on the last day of dosing were used.

In conclusion, a systems pharmacology model of the thyroid-pituitary axis after administration of a TPO enzyme inhibitor has been proposed. The model could successfully describe and explain the concentration-time profiles of the TSH, T_4 and T_3 in dogs for two MPO inhibitors that also display TPO inhibition. Based on data from *in vitro* TPO assays, the model has been shown useful for prediction of hormone profiles in both dogs and human. In addition, the model has been used for optimization of study design (doses, thyroid hormone sampling times, prediction of thyroid hormone concentration-time profiles) non-clinically and clinically for MPO-IN2. It has also been used in follow-up programs for *in silico* screening of new compounds based on TPO enzyme inhibition, unbound fraction and estimated human exposure to support the selection of compounds.

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