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A minimal physiologically-based pharmacokinetic model with a nested endosome compartment for novel engineered antibodies

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Abstract

We proposed here a minimal physiologically-based pharmacokinetic (mPBPK) model for a group of novel engineered antibodies in mice and humans. These antibodies are designed with altered binding properties of their Fc domain with neonatal Fc receptor (FcRn) or the Fab domain with their cognate targets (recycling antibodies) in acidic endosomes. To enable simulations of such binding features in the change of antibody pharmacokinetics and its target suppression, we nested an endothelial endosome compartment in parallel with plasma compartment based on our previously established mPBPK model. The fluid-phase pinocytosis rate from plasma to endothelial endosomes was reflected by the clearance of antibodies in FcRn dysfunctional humans or *FcRn*-knockout mice. The endosomal recycling rate of FcRn-bound antibodies was calculated based on the reported endosomal transit time. The nonspecific catabolism in endosomes was fitted using pharmacokinetic data of a human wide type IgG₁ adalimumab in humans and B21M in human *FcRn* (*hFcRn*) transgenic mice. The developed model adequately predicted the pharmacokinetics of infliximab, motavizumab, and an Fc variant of motavizumab in humans and the pharmacokinetics of bevacizumab, an Fc variant of bevacizumab, and a recycling antibody PH-IgG1 and its non-pH dependent counterpart NPH-IgG1 in *hFcRn* transgenic mice. Our proposed model provides a platform for evaluation of the pharmacokinetics and disposition behaviors of Fc-engineered antibodies and recycling antibodies.

Keywords

mPBPK model; pharmacokinetics; FcRn; endosome; recycling antibody

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Conflict of interest

F. Rode is an employee of Novo Nordisk, Denmark.

Introduction

Therapeutic monoclonal antibodies (mAbs), mostly based on immunoglobulin G (IgG), have become a group of fast-growing therapeutic modalities due to their long serum persistence and versatile target binding (1). FcRn could protect IgG from lysosome degradation and contributes to its serum persistence (2). Once taken up by endothelial cells *via* nonspecific fluid-phase pinocytosis along with other serum proteins, IgG is sorted to intracellular acidic endosomes where IgG can bind FcRn (3). FcRn salvages bound IgG from lysosomal degradation and returns IgG to the cell surface where FcRn releases IgG back to circulation (3). Because of FcRn salvage, IgG displays a long half-life (19–22 days) in humans (3). Deficiency of functional FcRn, such as in rare familial hypercatabolic hypoproteinemia syndrome (4, 5) or in *FcRn*-knockout mice (6), results in low plasma concentrations and short half-life of IgG.

The Fc portion of IgG can bind FcRn with a high affinity at acidic pH but with a relatively weak affinity at physiological pH (3, 7). To improve the efficacy of IgG-based therapeutics, the Fc portion of IgG could be engineered to enhance IgG-FcRn binding affinity in the acidic endosomes so as to prolong IgG circulation and thereby reduce doses or dosing frequency (7). However, each antibody molecule can, in theory only neutralize one antigen molecule during its lifespan, regardless of its half-life and binding affinity (1). When bound to a soluble target, the antibody, as well as the antibody-target complex, could be protected by FcRn from lysosomal degradation. Its cognate targets could also be protected along with the recycled antibodies, resulting in high antibody-mediated target accumulation (1). Thereby, to neutralize targets of great abundance and fast turnover, high dose or dosing frequency is often required (1). To prolong target neutralization and allow each antibody have multiple chances binding to its cognate targets, recycling antibodies, antibodies with pH-dependent target binding have been developed (1, 8). These engineered antibodies, with comparable target binding at physiological pH, can largely release its cognate targets in acidic endosomes during endosome recycling, which enables target lysosomal degradation and meanwhile saves the antibody for another round of target binding (1).

To facilitate development of such novel engineered antibodies, we adapted our original mPBPK model by extending an endothelial endosome compartment in parallel with the plasma compartment, where the binding dynamics of antibody-FcRn and antibody-target are explicitly defined to enable mechanism-based predictions of the enhanced antibody half-life and target neutralization. This model can be potentially applied to guide the design of Fc-engineered or recycling antibodies, selection of therapeutic doses, and interspecies translations.

Methods

Digitized pharmacokinetic data

Pharmacokinetic data for mAbs are digitized from the literature using WebPlot Digitizer v3.11 (Austin, Tx): adalimumab (9) and infliximab (10) in rheumatoid arthritis (RA) patients, radiolabeled human IgG in a familial hypercatabolic hypoproteinemia patient (4) motavizumab and its YTE variant in healthy humans (11), B21m in *hFcRn* Tg32

homozygous transgenic mice at a dose of 2 mg/kg (12), bevacizumab and its Xtend variant in *hFcRn* Tg276 heterozygous transgenic mice at a dose of 2 mg/kg (13), and two human IgG1 mAbs against human soluble interleukin 6 receptor (hsIL6-R) in *hFcRn* Tg32 homozygous transgenic mice, one pH-independent (NPH-IgG1) and one pH-dependent (PH-IgG1) at a dose of 2 mg/kg (14). hsIL6-R was co-injected at a dose of 50 µg/kg (14). All antibodies and hsIL6-R were given intravenously.

Model structure

Our previously published mPBPK model for mAbs inherited the essence of a full PBPK model by taking a reductionist approach to lump peripheral tissues into two groups based on their vascular structure and permeability (15, 16). The tight tissues include muscle, skin, adipose, and brain. All the other organs are grouped as the leaky tissues. The mPBPK model has been successfully applied in the analysis of the pharmacokinetics and disposition features of many conventional mAbs (15, 16). Here we proposed to add an endothelial endosomal compartment that represents endosomes within the systemic vascular endothelial cells. The model structure is shown in Fig. 1, where A, T, ATC, FcRnA, and FcRnATC represent free antibody, free soluble antigen target, antibody-target complex, FcRn-antibody complex, and FcRn-antibody-target complex, respectively. The model is described by differential equations (1) to (15). The subscript *p*, *l*, *2*, *L*, and *e* stand for plasma, tight tissues, leaky tissues, lymph, and endosome, respectively. The definition, value, and source for the model parameters are summarized in Table 1.

In the model, ATC was assumed to follow the same disposition processes as A: intravenously administered antibody is distributed into peripheral tissues mainly through convection, limited in interstitial space, and recollected by lymphatic system back to plasma (15). As many soluble targets (e.g., IL-β, TNF-α) in antibody therapeutics, tissue targets are assumed to be able to achieve instant distribution equilibrium with plasma targets provided their small sizes and high vascular permeability. For simplicity, only plasma clearance of target (CL_{p_T}) was defined in the model. The target is synthesized at a zero-order rate constant k_{syn} , which is a product of antigen baseline concentration IC C_{p_T} and CL_{p_T} .

Both antibodies and its cognate targets are taken up nonspecifically by pinocytosis from the plasma into the endothelial endosomal compartment at a rate of CL_{up} . FcRnA and FcRnATC are recycled back to plasma at a rate of CL_{rec} . A, T, and ATC are degraded in lysosomes at a rate of CL_e . None endosome-independent clearance for antibody was considered as before (17), while clearance of target is a sum of endosome-dependent clearance (CL_{up}) and endosome-independent clearance ($CL_{p_T} - CL_{up}$) such as kidney filtration (for proteins smaller than the glomerular filtration cutoff of 70 kDa (18)). The dynamics of antibody-target binding is characterized by association constant (k_{on}) and dissociation constant (k_{off}) in plasma, and association constant (k_{eon}) and dissociation constant (k_{eoff}) in endothelial endosomes. The dynamics of antibody-FcRn binding in acidic endosomes is characterized by association constant (kI_{on}) and dissociation constant (kI_{off}). ATC was assumed to have the same FcRn binding constants as free antibody and FcRnA has the same target binding affinity as free antibody.

Model equations**Plasma (V_p)**

$$\begin{aligned} dC_{p_A}/dt = & -k_{on} \cdot C_{p_A} \cdot C_{p_T} + k_{off} \cdot C_{p_ATC} - (1 - \sigma_1) \cdot L_1 \cdot C_{p_A}/V_p - (1 - \sigma_2) \\ & \cdot L_2 \cdot C_{p_A}/V_p - \\ & CL_{up} \cdot C_{p_A}/V_p + CL_{rec} \cdot FcRnA/V_p \quad IC(C_{p_A}) = Dose/V_p \end{aligned} \quad (1)$$

$$\begin{aligned} dC_{p_T}/dt = & k_{syn}/V_p - k_{on} \cdot C_{p_A} \cdot C_{p_T} + k_{off} \cdot C_{p_ATC} - (CL_{p_T} - CL_{up}) \cdot C_{p_T}/V_p - \\ & CL_{up} \cdot C_{p_T}/V_p \\ IC(C_{p_T}): & = \text{varied (see Table 1)} \end{aligned}$$

(2)

$$\begin{aligned} dC_{p_ATC}/dt = & k_{on} \cdot C_{p_A} \cdot C_{p_T} - k_{off} \cdot C_{p_ATC} - (1 - \sigma_1) \cdot L_1 \cdot C_{p_ATC}/V_p - (1 - \sigma_2) \\ & \cdot L_2 \cdot C_{p_ATC}/V_p - \\ & CL_{up} \cdot C_{p_ATC}/V_p + CL_{rec} \cdot FcRnATC/V_p \quad IC(C_{p_ATC}) = 0 \end{aligned}$$

(3)

Tight tissues (V_1)

$$\begin{aligned} dC_{1_A}/dt = & (1 - \sigma_1) \cdot L_1 \cdot C_{p_A}/(K_p \cdot V_1) - (1 - \sigma_L) \cdot L_1 \cdot C_{1_A}/(K_p \cdot V_1) \quad IC(C_{1_A}) \\ = & 0 \end{aligned}$$

(4)

$$\begin{aligned} dC_{1_ATC}/dt = & (1 - \sigma_1) \cdot L_1 \cdot C_{p_ATC}/(K_p \cdot V_1) - (1 - \sigma_L) \cdot L_1 \cdot C_{1_ATC}/(K_p \cdot V_1) \quad IC \\ (C_{1_ATC}) = & 0 \end{aligned}$$

(5)

Leaky tissues (V_2)

$$\begin{aligned} dC_{2_A}/dt &= (1 - \sigma_2) \cdot L_2 \cdot C_{p_A} / (K_p \cdot V_2) - (1 - \sigma_L) \cdot L_2 \cdot C_{2_A} / (K_p \cdot V_2) \quad IC(C_{2_A}) \\ &= 0 \end{aligned}$$

(6)

$$\begin{aligned} dC_{2_ATC}/dt &= (1 - \sigma_2) \cdot L_2 \cdot C_{p_ATC} / (K_p \cdot V_2) - (1 - \sigma_L) \cdot L_2 \cdot C_{2_ATC} / (K_p \cdot V_2) \quad (7) \\ IC(C_{2_ATC}) &= 0 \end{aligned}$$

Lymph (V_L)

$$\begin{aligned} dC_{L_A}/dt &= (1 - \sigma_L) \cdot L_1 \cdot C_{1_A} / V_L + (1 - \sigma_L) \cdot L_2 \cdot C_{2_A} / V_L - L \cdot C_{L_A} / V_L \quad (8) \\ IC(C_{L_A}) &= 0 \end{aligned}$$

$$\begin{aligned} dC_{L_ATC}/dt &= (1 - \sigma_L) \cdot L_1 \cdot C_{1_ATC} / V_L + (1 - \sigma_L) \cdot L_2 \cdot C_{2_ATC} / V_L - L \cdot C_{L_ATC} / V_L \\ IC(C_{L_ATC}) &= 0 \end{aligned}$$

(9)

Endothelial endosome compartment (V_e)

$$\begin{aligned} dC_{e_A}/dt &= -k_{\text{eon}} \cdot C_{e_A} \cdot C_{e_T} + k_{\text{eoff}} \cdot C_{e_ATC} - k_{l_{\text{on}}} \cdot C_{e_A} \cdot \text{FcRn} + k_{l_{\text{off}}} \cdot \text{FcRnA} \\ &+ CL_{\text{up}} \cdot C_{p_A} / V_e - CL_e \cdot C_{e_A} / V_e \quad IC(C_{e_A}) = 0 \end{aligned}$$

(10)

$$\begin{aligned} dC_{e_T}/dt &= -k_{\text{eon}} \cdot C_{e_A} \cdot C_{e_T} + k_{\text{eoff}} \cdot C_{e_ATC} - k_{\text{eon}} \cdot C_{e_T} \cdot \text{FcRnA} + k_{\text{eoff}} \cdot \text{FcRnATC} + \\ &CL_{\text{up}} \cdot C_{p_T} / V_e - CL_e \cdot C_{e_T} / V_e \quad IC(C_{e_T}) = 0 \end{aligned} \quad (11)$$

$$\begin{aligned} dC_{e_ATC}/dt = & k_{eon} \cdot C_{e_A} \cdot C_{e_T} - k_{eoff} \cdot C_{e_ATC} - k_{l_on} \cdot C_{e_ATC} \cdot FcRn + k_{l_off} \cdot FcRnATC + \\ & CL_{up} \cdot C_{p_ATC}/V_e - CL_e \cdot C_{e_ATC}/V_e \quad IC(C_{e_ATC}) = 0 \end{aligned} \quad (12)$$

$$\begin{aligned} dFcRn/dt = & - k_{l_on} \cdot C_{e_A} \cdot FcRn + k_{l_off} \cdot FcRnA - k_{l_on} \cdot C_{e_ATC} \cdot FcRn + k_{l_off} \cdot FcRnATC + \\ & CL_{rec} \cdot (FcRnA + FcRnATC)/V_e \quad IC(FcRn) = FcRntotal \end{aligned} \quad (13)$$

$$\begin{aligned} dFcRnA/dt = & k_{l_on} \cdot C_{e_A} \cdot FcRn - k_{l_off} \cdot FcRnA - k_{eon} \cdot C_{e_T} \cdot FcRnA + k_{eoff} \cdot FcRnATC - \\ & CL_{rec} \cdot FcRnA/V_e \quad IC(FcRnA) = 0 \end{aligned} \quad (14)$$

$$\begin{aligned} dFcRnATC/dt = & k_{l_on} \cdot C_{e_ATC} \cdot FcRn - k_{l_off} \cdot FcRnATC + k_{eon} \cdot C_{e_T} \cdot FcRnA - k_{eoff} \cdot FcRnATC - \\ & CL_{rec} \cdot FcRnATC/V_e \quad IC(FcRnATC) = 0 \end{aligned}$$

(15)

Model development and simulation

The model was developed based on parameters that were either directly obtained in the literature or optimized by analyzing the literature data. All parameters were summarized in Table 1. The processes and results in optimization of several model parameters (CL_{up} , CL_{rec} , and CL_e) have been detailed in Results. The model was developed for both humans and mice, with species-specific physiological parameters. Once developed, the model was applied to predict the pharmacokinetics of a variety of engineered antibodies, as well as the dynamics of their cognate targets in both humans and mice. Simulations were performed using Berkeley Madonna v8.3.18 (Berkeley, CA). Model predictions were overlaid with experimental observations to evaluate model predictive performance. Exported data from Berkeley Madonna was plotted using GraphPad Prism v6.0 (La Jolla, CA).

Results

Model development for humans

In our previous mPBPK model, the parameters specific to each mAb are σ_1 , σ_2 , and CL_p (15, 16). Here we used the reported average σ_1 and σ_2 (16) in our previous paper (Table 1). In addition, we introduced three parameters to describe the kinetics of trafficking endosomes, including CL_{up} , CL_{rec} , and CL_e . The nonspecific fluid-phase pinocytosis from

plasma to endothelial endosomal space CL_{up} in humans was estimated by multiplying the first-order rate constant of human IgG in a familial hypercatabolic hypoproteinemia patient (Fig. 2a, 0.269 day^{-1}) (4) with the volume of distribution at steady state (V_{ss}) of human wild type IgG₁ adalimumab in humans (5.508 L) (19). Because this patient lacks functional FcRn to recycle antibody back to the plasma (5), the elimination of antibody from the plasma is reflected in CL_{up} . CL_{rec} was calculated based on the endosomal transit half-life of 8 min (20) and the total volume of endosomal space in vascular endothelial cells, which is estimated as 0.005% of body weight (70 kg for human) (9).

The systemic clearance (CL_{p_T}) of a soluble target, TNF- α , in RA patients was estimated according to the results in a clinical study of exogenous TNF- α in tumor patients (half-life 13 min was used) (21). The zero-order synthesis rate (k_{syn}) of TNF- α in 70 kg human was calculated as a product of reported baseline concentration in RA patients (22) and CL_{p_T} . Non-endosome mediated clearance of antigen was calculated by subtracting CL_{up} from CL_{p_T} . Adalimumab and FcRn binding in endothelial endosomes and adalimumab and TNF- α binding in plasma were characterized by literature reported association constants and dissociation constants, which were both measured by surface plasmon resonance (Table 2). We assumed that the binding affinity of adalimumab to TNF- α in acidic endosomes was equal to the binding affinity at pH 7.4, given that adalimumab was not engineered to display pH-dependent target binding.

With the developed model and literature-derived parameters (Table 1 and 2), CL_e was obtained by fitting the pharmacokinetic data of adalimumab in RA patients at a dose of 5 mg/kg (9). The model was then applied to predict the pharmacokinetic profiles of adalimumab at four other doses. As shown in Fig. 2b, the model sufficiently predicted the pharmacokinetic profiles of adalimumab at all dose levels.

Predictions of infliximab pharmacokinetics in RA patients

We tested if the model can characterize the pharmacokinetics of another anti-TNF- α antibody with distinct target and FcRn binding properties. Infliximab is a chimeric IgG₁ against TNF- α . We parameterized the model with the reported FcRn and TNF- α binding kinetic parameters for infliximab (Table 2). Fig. 3 shows the overlap of observed and model predicted pharmacokinetic profiles of infliximab in RA patients. The developed model adequately predicted the pharmacokinetic profiles of infliximab at doses between 5 and 20 mg/kg.

Predictions of motavizumab and its Fc variant pharmacokinetics in healthy humans

We tested if the model can predict the extended circulation of a Fc-engineered variant of motavizumab in healthy humans. Motavizumab is a human IgG₁ against respiratory syncytial virus. A motavizumab variant with mutations in three amino acids (M252Y/S254T/T256E [YTE]) in the Fc region, referred to as YTE, showed 10-fold higher FcRn binding affinity at pH 6 with extended half-life in non-human primates and humans (11, 23). The same FcRn binding constants were assumed for motavizumab and adalimumab considering both antibodies are human IgG₁ wild type. As YTE is known with 10-fold higher affinity than motavizumab in acidic endosomes, $0.1 \times k_{eoff}$ was assumed for YTE

(Table 2). As shown in Fig. 4, the model predictions agreed well with the observed pharmacokinetics of motavizumab and YTE in healthy subjects at doses of 0.3, 3, 15, and 30 mg/kg.

Model development for *hFcRn* transgenic mice

hFcRn transgenic mice has become a common preclinical model for pharmacokinetic study of mAbs (24, 25). To enable simulations in *hFcRn* transgenic mice, the same model was updated with mouse-specific physiological parameters (Table 1). Most parameters were obtained from the literature. Similarly to human model, we estimated the CL_{up} in mice using the reported clearance of a murine IgG₁ 7E3 in *FcRn* knockout mice (72.5 mL/day/kg) (26). CL_e was optimized using the pharmacokinetic data of a human wild type IgG₁ B21M in *hFcRn* Tg32 homozygous transgenic mice (Fig. 5 and Table 1).

Predictions of bevacizumab and its Fc variant pharmacokinetics in *hFcRn* transgenic mice

Bevacizumab is a humanized anti-VEGF IgG₁ for treatment of colorectal, lung, breast, and renal cancers (13). A double substituted Fc variant of bevacizumab, which was referred to as Xtend, exhibited decreased *hFcRn* equilibrium dissociation constant at pH 6 from 2460 nM to 218 nM (13). Parameterizing the mice model with the reported *hFcRn* binding kinetics for bevacizumab and Xtend (Table 2), the model successfully predicted the extended circulation half-life of Xtend in comparison to bevacizumab in *hFcRn* Tg276 heterozygous transgenic mice (13) (Fig. 6).

Predictions of a recycling antibody in *hFcRn* transgenic mice

The model has shown to adequately predict the extended pharmacokinetic profiles of Fc-engineered antibodies in both humans and *hFcRn* transgenic mice. Next, we tested if our model could capture the benefit of a recycling antibody PH-IgG₁ in target suppression in comparison to its pH-independent counterpart NPH-IgG₁. Both antibodies were co-injected with the human antigen hsIL6-R into *hFcRn* Tg32 homozygous transgenic mice (14). These two antibodies exhibited similar plasma profiles. As designed, the recycling antibody resulted in much lower target plasma concentrations, indicating less antibody-mediated target accumulation and that antibody-bound antigens could be largely dissociated in endosomes for subsequent lysosomal degradation.

Prediction of hsIL6-R profiles in *hFcRn* transgenic mice was conducted similarly to the TNF- α case in humans. Since each antibody binds two hsIL6-R (14), the target concentration was divided by two in the model equations. Systemic clearance of free target was obtained from a pharmacokinetic study in healthy mice (8). This clearance was divided into endosome-dependent clearance (CL_{up}) and endosome-independent clearance ($CL_{p,T} - CL_{up}$). We assumed that none antigens were released from antibody-antigen complex in endosomes for NPH-IgG₁, while all antigens became unbound for PH-IgG₁. Target dissociation constant in endosomes was assigned 0 (no dissociation) for NPH-IgG₁ and an artificial high value 1000 day⁻¹ for pH-IgG₁ (full dissociation). The target association constants in endosomes for both antibodies were set equal to those measured at pH 7.4 (14). As shown in Fig. 7, the model was able to recapture the enhanced antigen clearance of PH-IgG₁ in comparison to its non-pH dependent counterpart NPH-IgG₁. Unexpectedly, the

model under-predicted the terminal concentration of antigen for NPH-IgG₁, which might be due to a saturable mechanism that significantly retained the targets at low concentrations, which was not included in our model assumptions. The terminal half-life of hsIL6-R (37.8 kDa) in healthy mice was reported to be 4.36 h (5), much longer than the half-life of another small protein TNF- α (17 kDa, 3 min in mice (6)).

Theoretical simulations of engineered antibodies with enhanced FcRn binding and pH-dependent target binding

The effect of enhanced FcRn binding at acidic environment on the pharmacokinetics of adalimumab and circulating TNF- α were simulated in RA patients by up-adjusting the association constant or down-adjusting the dissociation constant up to 1,000-fold. As shown in Fig. 8a and b, plasma concentrations of free adalimumab were increased and plasma concentrations of free TNF- α were decreased accordingly. An increase of the association constant to FcRn has almost equivalent effect as a decrease of the same fold of dissociation constant on the plasma profiles of adalimumab and circulating TNF- α .

The effect of reduced target binding in acidic endosomes on clearance of adalimumab and TNF- α was also simulated. In contrast to FcRn binding, TNF- α suppression showed a dramatic difference between the decrease of association rate and increase of dissociation rate. As shown in Fig. 8b and c, increase of the dissociation constant ke_{off} was more effective on target suppression than decrease of the association constant ke_{on} . Predicted plasma profiles of free adalimumab remained the same regardless of target binding rate constants.

Discussion

We developed an mPBPK model extended with an endothelial endosome compartment to enable simulations of newly developed novel antibodies that are engineered to modify either FcRn or target binding properties in acidic endosomes. The endosome compartment represented the endosomal spaces within systemic vascular endothelial cells (28), which was thereby nested in parallel with the plasma compartment instead of inside the peripheral tissue compartments. Antibody transportation into the endothelial endosome compartment was assumed to negligibly contribute to the peripheral distribution of antibodies and all FcRn-bound antibodies would be recycled back to plasma. This assumption is supported by the fact that the endosomal transit is much faster (half-life 8 min (20)) than the peripheral distribution of mAbs and the amount of mAbs in the tiny volume of endosomes is not significant at any moment of time in comparison to the total amount of antibody in the system (15, 29). This is also supported by the results of Ng et al. showing that the model performance would decrease if the endosome compartment were nested within the tissue compartment of a two-compartment mammary model (29). Thus, the endosome compartment was incorporated in parallel with the plasma compartment in our extended mPBPK model to account for catabolism of mAbs inside endothelial cells.

Additionally, our model is developed based on the following assumptions: 1) Antibody-target complex follows the same distribution kinetics as free antibody, including same pinocytosis rate into endothelial endosomes, equal vascular reflection coefficients, and no

additional target-mediated degradations. 2) Binding to target does not interfere with the binding kinetics of antibody to FcRn. 3) All FcRn-bound antibodies could be recycled back to plasma. 4) All free antibody, antibody-target complex, and antigens are trapped in late endosomes for subsequent lysosomal degradation. These model assumptions limited the current model mainly applied to engineered antibodies for soluble targets not for simulations of trans-membrane targets. Antibodies for trans-membrane targets are often known to elicit target-mediated endocytosis and degradation, also known as target-mediated drug disposition. Trans-membrane targets are often involved in tissue (or cells) specific pinocytosis and degradation other than the universal nonspecific pinocytosis for antibodies and soluble targets in our current model assumption. In addition, the values of CL_{up} were obtained based on the clearance of wild-type IgG in *FcRn*-deficient human and mice. The current CL_{up} values would not support precise predictions of antibodies pharmacokinetics that have appreciable differences than wild-type IgG. Influence by strong charge-mediated nonspecific binding or charge-induced endocytosis should be specifically considered in order to define an antibody specific CL_{up} . Endogenous IgG was also not considered in the current model but the available FcRn concentrations have already taken into considerations the competition from endogenous IgG (28,30). Saturation of FcRn could occur when IgG is injected at doses up to 200–400 mg/kg (IVIG) (31), at which conditions the model should be upgraded by consideration of the competitive binding to FcRn by nonspecific IgG. Gradual endosomal acidification was also not defined in the current model primarily considering that a continuous change of pH-dependent binding kinetics parameters is usually not available. In this regard, Chen et al. divided the endosomal space into five successive sub-compartments ranging from pH 7.4 to pH 6.0 and equally allocated the total endosomal transit time into these sub-compartments to approximate the pH and time-dependent endosomal trafficking of antibodies (7). Antibody-FcRn binding equilibrium in each brief endosomal compartment was not achieved in the catenary model of Chen, et al. Compared to an equilibrium PBPK model, the catenary model predicted moderate change in antibody half-life with improved FcRn binding at acidic conditions and agreed well with preclinical observations. Considering the experimentally measured antibody FcRn binding constants at a series of acidic conditions are not available, we only defined the endosomal compartment at pH 6.0. Similar to the catenary model, antibody and FcRn binding equilibrium was also not assumed and FcRn binding constants k_{on} and k_{off} were separately evaluated in this model. The developed model sufficiently predicted the extended half-life of Fc-engineered variants of bevacizumab and motavizumab, which showed 10-fold higher FcRn binding affinity compared to their wild-type counterparts.

Majority of the model parameters were either directly obtained in the literature or derived from literature data. Only parameter CL_e was model optimized using the pharmacokinetic data of a human wild type IgG₁ adalimumab for humans and B21M for *hFcRn* transgenic mice. The developed model successfully predicted the pharmacokinetics of adalimumab and infliximab, which have distinct FcRn and TNF- α binding properties. The CL_e value for mice is 9.8-fold lower than the allometric scaled value from 70 kg humans. This difference may be partly attributed to inaccurate estimations of FcRn expression and lack of quantitative knowledge in the endosome trafficking and degradation in humans and *hFcRn* transgenic mice. Nonetheless, the model for *hFcRn* transgenic mice well predicted the

pharmacokinetics of bevacizumab and its Fc-engineered Xtend variant and adequately captured the plasma profiles of a recycling antibody and its target in *hFcRn* transgenic mice.

In predictions of the pharmacokinetics of motavizumab and its YTE variant in humans, we found that when adjusting CL_e from 0.24 L/h to a lower value 0.069 L/h, the pharmacokinetic profiles of motavizumab and YTE could be also reproduced with a calculated kI_{off} based on reported K_D values (23) for both antibodies and kI_{on} assumed as the same as our model IgG₁ adalimumab (supplemental 1). This might be associated with either the population difference (RA patients vs. healthy human), or the drift of surface plasmon resonance measurements at various experimental settings (33, 34). Regardless, with another source of FcRn binding parameters, our extended mPBPK model could well predict the plasma profiles of motavizumab and YTE by only adjusting CL_e to a lower value. Correlation between FcRn binding affinity and lysosomal degradation has also been reported by others (28, 29), highlighting the importance of accurate measurements of FcRn binding affinity in predictions of antibody pharmacokinetics.

Indeed, antibody half-life predicted by the model is a function of FcRn expression, FcRn binding affinity, CL_{up} , CL_{rec} , and CL_e , where CL_{up} and CL_{rec} could be estimated based on the data in *FcRn* knockout mice (1) and using data from *in vitro* endocytosis studies (2). The FcRn expression, FcRn binding affinity, and CL_e are parameters that are inter-reliant in prediction of antibody half-life, but the precise values of these parameters are not readily obtainable from the literature, particularly in the absence of accurately measured antibody-FcRn binding constants. Herein the developed modeling platform is mainly to provide theoretical assessments of the design benefit of novel engineered antibodies in comparison to their wild-type counterparts. In this model, we assumed the mFcRn expression level was 40 μ M in wild type mice (3) and the same level of FcRn was assumed in hFcRn transgenic mice. B21M showed similar half-life in Tg32 homozygous, Tg32 heterozygous, and Tg276 homozygous mice and slightly shorter half-life in Tg276 heterozygous mice (4), which implied that the former three lines may have higher hFcRn expressions than the latter. Once more accurate measurements of FcRn expression are available, the model could be advanced to consider specific mice strain.

Our model simulations support that enhanced FcRn pH-dependent binding can efficiently extend mAb half-life and augment target suppression. The FcRn binding affinity could be enhanced by either increase of association constant or decrease of dissociation constant. Of note, the additional benefit by an increase in the FcRn binding affinity gradually decreased and reached a plateau once most antibodies in the endosomes bound to FcRn. One assumption in our model that endosomal antibody degradation (CL_e) and FcRn binding affinity remained unchanged after bound to its cognate target is supported by the observed similar clearance of NPH-IgG₁ and PH-IgG₁ (14). As designed, recycling antibody, which could effectively dissociate its cognate targets during endosome trafficking, exhibits dramatic benefit in target suppression. The higher benefit was noticed by increase of the target dissociation constant than decrease of the association constant. This is mainly because the target is present primarily in the antibody-bound form in endothelial endosomes (supplemental 2), and hence the degradation of antigens in endosomes is primarily constrained by its dissociation rate. A ten-fold stepwise increase of the dissociation constant

shortened the target dissociation half-life from 89 min to 8.9 min (10 fold), 0.89 min (100 fold), and 0.089 min (1000 fold). The largest change in target suppression was noticed when the dissociation half-life was modified from 8.9 min to 0.89 min, suggesting that dissociation half-life would have a significant impact on target suppression when it approximates to endosomal transit time. If the antibody target dissociation half-life in endothelial endosomes is much higher than endosomal transit half-life, more vigorous modulations of the antibody-target binding in endosomes are warranted.

Conclusions

In this study, we developed an mPBPK model extended with an endothelial endosome compartment to enable explicit characterizations of the binding kinetics of antibody to FcRn and antibody to its cognate targets. The model has been applied to adequately reproduce the pharmacokinetics of novel engineered antibodies with altered FcRn or target binding profiles in acidic endosomes. This model has demonstrated high potential to guide the design of Fc-engineered or recycling antibodies, simulations of the designed benefits, and interspecies translations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. mPBPK model with a nested endosomal compartment for simulation of engineered antibodies

Fig. 2. Model development for human. **(a)** Pharmacokinetics of human IgG in rheumatoid arthritis (RA) patients with functional FcRn and in a familial hypercatabolic hypoproteinemia patient with mutant FcRn. **(b)** Model predictions of adalimumab pharmacokinetics in RA patients. Symbols are observations and curves are model predictions

Fig. 3. Model predictions of infliximab pharmacokinetics in rheumatoid arthritis patients. Symbols are observations and curves are model predictions

Fig. 4. Model predictions of motavizumab and its Fc variant YTE pharmacokinetics in healthy humans at a dose of 0.3 mg/kg (**a**), 3 mg/kg (**b**), 15 mg/kg (**c**), and 30 mg/kg (**d**). Note that the FcRn binding affinity was assumed to be equal to adalimumab for motavizumab (both are IgG1 wild type) and 10-fold higher than adalimumab for YTE. Symbols are observations and curves are model predictions

Fig. 5.
Model fitting of CL_e to B21M pharmacokinetics in *hFcRn Tg32 homozygous* transgenic mice

Fig. 6. Model predictions of bevacizumab and its Fc variant Xtend pharmacokinetics in *hFcRn* **Tg276 heterozygous** transgenic mice. Symbols are observations and curves are model predictions

Fig. 7.
Model predictions of pharmacokinetics of anti-hsIL6-R antibody and hsIL6-R in *hFcRn*
Tg32 homozygous transgenic mice. (a) A conventional antibody NPH-IgG1; (b) A
recycling antibody PH-IgG1. Symbols are observations and curves are model predictions

Fig. 8.

Simulated effect of enhanced FcRn binding and target dissociation in endosomes on plasma profiles of adalimumab and its cognate target TNF- α . **(a, b)** Ten-fold stepwise increase of adalimumab binding affinity to FcRn at pH 6 by either increasing association constant $k_{1_{on}}$ (a) or decreasing dissociation constant $k_{1_{off}}$ (b) up to 1,000-fold. **(c, d)** Ten-fold stepwise decrease of adalimumab-TNF- α binding affinity at pH 6 by either decreasing the association constant $k_{e_{on}}$ (c) or increasing the dissociation constant $k_{e_{off}}$ (d) up to 1,000-fold. Solid lines and dotted lines show the pharmacokinetics of adalimumab and TNF- α , respectively. Solid arrows and dash-dot arrows show the change of direction for adalimumab and TNF- α , respectively. Black: 1x; red: 10x; blue: 100x; brown: 1,000x

Table 1

Model parameters for 70 kg humans and 20 g mice

Parameters	Definition	Human Value (Source)	Mice Value (Source)
Physiological parameters			
V_p	Plasma volume	2.6 L (15)	0.85 mL (36)
K_p	Fraction of ISF for IgG1 distribution		0.8 (15)
V_I	ISF volume in tight tissue	10.14 L (15)	2.83 mL (36)
V_2	ISF volume in leaky tissue	5.46 L (15)	1.52 mL (36)
σ_1	V_1 vascular reflection coefficient	0.945 (15)	0.98 (37)
σ_2	V_2 vascular reflection coefficient	0.697 (15)	0.449 (37)
V_L	Lymph volume	5.2 L (15)	1.6 mL (36)
L	Lymph flow	0.121 L/h (15)	2.88 mL/day (36)
L_1	V_1 lymph flow	0.0399 L/h (15)	0.950 mL/day (36)
L_2	V_2 lymph flow	0.0810 L/h (15)	1.930 mL/day (36)
σ_L	Lymphatic reflection coefficient		0.2 (15)
Antigen parameters (TNF- α for human model, sIL6-R for mice model)			
$IC(C_{p_T})$	Baseline antigen concentration in plasma	0.276 pM (22)	31.12 nM (14)
CL_{p_T}	Clearance of antigen	8.316 L/h ^a	25.344 mL/day (8)
k_{syn}	Antigen synthesis constant	2.299 pmol/h ^b	0
Endosome parameters			
V_e	Endothelial endosome volume	0.005% of body weight (9)	
CL_{up}	Endothelial cell pinocytosis rate	0.0617 L/h ^c	1.450 mL/day (26)
CL_{rec}	Endosomal recycling rate	0.0182 L/h ^d	0.125 mL/day ^d
CL_e	Catabolism rate in endosome	0.24 L/h ^e	1.295 mL/day ^e
$FcRntotal$	Endosomal FcRn concentration	49.8 μ M (28)	40 μ M (30)

^a calculated by $CL_{p_T} = 0.693/(13/60) \times V_p$ where 13 is the half-life of TNF- α in human in min (21)

^b calculated by $k_{syn} = CL_{p_T} \times IC(C_{p_T})$

^c calculated by the product of the first order clearance rate constant of IgG in a familiar hypercatabolic hypoproteinemia patient (4) and the volume of distribution at steady state assumed same as adalimumab in rheumatoid arthritis patients (19)

^d $CL_{rec} = 0.693/(8/60) \times V_e$ where 8 is the endosomal transit half-life in min (20)

^e Model fitted

Table 2

mAb binding kinetic parameters in plasma and endosome used in the proposed model

Target	Antibody	k_{on} $M^{-1}s^{-1}$	k_{off} s^{-1}	K_D nM	Source
hFcRn	Adalimumab	2.41E+05	0.162	672	(38)
	Infliximab	2.09E+05	0.152	727	(38)
	Motavizumab	Assume same as adalimumab			
	YTE ^a	2.09E+05	0.0152	72.7	
	B21M	Assume same as adalimumab			
	Bevacizumab	1.21E+05	0.297	2460	(13)
	Xtend	2.55E+05	0.0556	218	(13)
	NPH-IgG1	Assume same as adalimumab			
	PH-IgG1	Assume same as adalimumab			
TNF- α	Adalimumab	7.20E+05	1.30E-04	0.183	(39)
	Infliximab	1.70E+06	2.40E-04	0.138	(39)
shIL6-R	NPH-IgG1	3.20E+05	4.50E-04	1.4	(14)
	PH-IgG1	4.50E+05	4.60E-04	1	(14)

^aMotavizumab YTE variant showed 10-fold higher binding affinity to hFcRn than wild-type motavizumab (23). We assume the 10-fold higher binding affinity is presented as 10-fold lower dissociation constant