Poster: T2116

The effect of IgG-FcRn binding stoichiometry on simulated mAb half-life using a minimal human PBPK model

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ABSTRACT

Purpose: Existing PBPK models for monoclonal antibodies (mAbs) use 1:1 stoichiometry of IgG to FcRn binding. However, in a variety of species binding of IgG to FcRn has a 1:2 stoichiometry [1]. *In vitro* studies have shown that modification of wild type IgG to remove one FcRn binding site (hdFc) results in proteins with different intracellular fates, with more hdFc being degraded in the lysosome, suggesting a role for avidity effects in FcRn-mediated IgG transport [2]. A modeling study was performed to integrate these biological findings into a minimal human PBPK model.

Methods: A minimal human PBPK model accounting for competition at FcRn between endogenous IgG and mAbs and either 1:1 (left panel) or 1:2 (right panel) IgG:FcRn binding stoichiometry was developed in Matlab (version R2012a) (Figure 1). The effect of changing the affinity of binding of mAb to FcRn on mAb elimination half-life was investigated assuming either 1:1 or 1:2 stoichiometry.

Results: Figure 2 shows the simulated relationship between mAb-FcRn affinity and elimination half-life. In the 1:2 model, if the two binding sites are identical and independent, then $K_{d1} = K/2$and $K_{d2} = 2K$ (where K= equilibrium dissociation constant for binding to individual sites). Co-operativity in binding is positive if K_{d2} is < $2K$ and negative if $K_{d2}$is > $2K$. Under conditions of negative co-operativity, the sensitivity of simulated mAb half-life to FcRn affinity is reduced (figure 2B). Interestingly, a similar relationship between fold change in mAb- FcRn affinity and half-life was obtained by Chen & Balthasar [3] using a PBPK model where the endosome is divided into 5 sub-compartments with different pH values and within each compartment mAb-FcRn binding was modeled using on and off rates and 1:1 stoichiometry [3].

Conclusions: Adapting a PBPK model for mAbs to account for 1:2 IgG to FcRn stoichiometry leads to a model that under conditions of negative co-operativity is less dependent on changes in FcRn K_D and provides further potential to predict PK of mAbs.

PURPOSE

- Existing PBPK models for monoclonal antibodies (mAbs) use 1:1 stoichiometry of IgG to FcRn binding.
- However, in a variety of species binding of IgG to FcRn has a 1:2 stoichiometry [1].
- *In vitro* studies have shown that modification of wild type IgG to remove one FcRn binding site (hdFc) results in proteins with different intracellular fates, with more hdFc being degraded in the lysosome, suggesting a role for avidity effects in FcRn-mediated IgG transport [2].
- A modeling study was performed to integrate these biological findings into a minimal human PBPK model.

METHODS

A minimal human PBPK model accounting for competition at FcRn between endogenous IgG and mAbs and either 1:1 (left panel) or 1:2 (right panel) IgG:FcRn binding stoichiometry was developed in Matlab (version R2012a) (Figure 1). . All of the organs are lumped into a single tissue compartment, which is further divided into three sub-compartments consistent with a previously published model structure for mAbs [4]

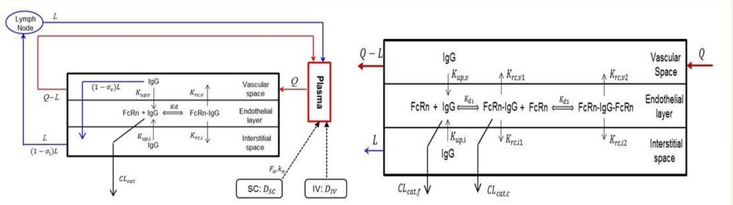

Figure 1 The model structure of the minimal PBPK model with 1:1 binding (left) and 1:2 binding (right)

- The model parameters are listed in table 1 and 2.
- Recycling of the 1:1 IgG:FcRn complex to the cell surface is known to be less efficient than for the 1:2 complex [2], thus we set $K_{rc,v1} = K_{rc,v2}/2$ and $K_{rc,i1} = K_{rc,i2}/2$.
- Specific values of recycling rates in table 2 were determined as follows. When $K_{d1} = K/2$ and $K_{d2} = 2K$ for both endogenous and exogenous IgG, the 1:2 binding mode is essentially equivalent to the 1:1 mode. Values of $K_{rc,v1}$, were fitted to give the same half-life of plasma concentration profile (21 day), endogenous IgG synthesis rate (~38 mg//kg/day), and reduction of plasma concentration when FcRn is knocked out (~9-fold) as the original minimal PBPK with 1:1 binding mode.

Table 1. Model parameters for the 1:1 binding model.

Parameters	Values
$K_{up,v}$	0.02979 (1/h)
$K_{up,i}$	0.02979 (1/h)
CL_{cat}	0.0175 (L/h)
$K_{rc,v}$	0.2999 (1/h)
$K_{rc,i}$	0.1196 (1/h)
Lymph Flow	120 (mL/h)
FcRn abundance	40 (μM)
Plasma flow (L/h)	190 (L/h)
Binding affinity of mAb to FcRn (K_D pH 6)	0.728 (μM)

Table 2. Additional Model parameters for the 1:2 binding model.

Parameters	Values
$K_{up,v}$	0.02979 (1/h)
$K_{up,i}$	0.02979 (1/h)
$CL_{cat,f}$	0.0175 (L/h)
$CL_{cat,c}$	0.0175 (L/h)
$K_{rc,v1}$	0.4216 (1/h)
$K_{rc,i1}$	0.1680 (1/h)
$K_{rc,v2}$	0.8432 (1/h)
$K_{rc,i2}$	0.3360 (1/h)

- Both the endogenous IgG and administered mAb are modeled simultaneously, with competition for binding to FcRn receptors in the endosome considered.
- To run the model considering 1:2 binding within the endosome it is necessary to solve the non linear equation (Eq 1) to obtain the free fraction of FcRn (x). In turn this value is used to calculate the unbound drug concentration using equation 2.

$$(1)\quad x = 1 - 2\beta + \beta_{en}\frac{\alpha_{en,2}x + 2\alpha_{en}}{\alpha_{en} + \alpha_{en,2}x + x^2} + \beta_{ex}\frac{\alpha_{ex,2}x + 2\alpha_{ex}}{\alpha_{ex} + \alpha_{ex,2}x + x^2}$$

$$(2)\quad f_{u,en} = \frac{\alpha_{en}}{\alpha_{en} + \alpha_{en,2}x + x^2},\ f_{u,ex} = \frac{\alpha_{ex}}{\alpha_{ex} + \alpha_{ex,2}x + x^2}.$$

where $\beta_{en} = \frac{[\mathrm{IgG}]_{total}}{[\mathrm{FcRn}]_{total}}$, $\beta_{ex} = \frac{[\mathrm{mAb}]_{total}}{[\mathrm{FcRn}]_{total}}$, $\beta = \beta_{en} + \beta_{ex}$, $\alpha_{en,1} = \frac{K_{d1}^{en}}{[\mathrm{FcRn}]_{total}}$, $\alpha_{en,2} = \frac{K_{d2}^{en}}{[\mathrm{FcRn}]_{total}}$,

$\alpha_{en} = \alpha_{en,1}\alpha_{en,2}$, $\alpha_{ex,1} = \frac{K_{d1}^{ex}}{[\mathrm{FcRn}]_{total}}$, $\alpha_{ex,2} = \frac{K_{d2}^{ex}}{[\mathrm{FcRn}]_{total}}$, $\alpha_{ex} = \alpha_{ex,1}\alpha_{ex,2}$.

When using the 1:2 binding model if the two sites are identical and independent then

$$K_{d1} = \frac{K}{2},\ K_{d2} = 2K,$$

where K is the equilibrium dissociation constant for binding to individual sites.
If K_{D2} < 2K then positive co-operativity occurs and if it is > 2K then negative co-operativity occurs.

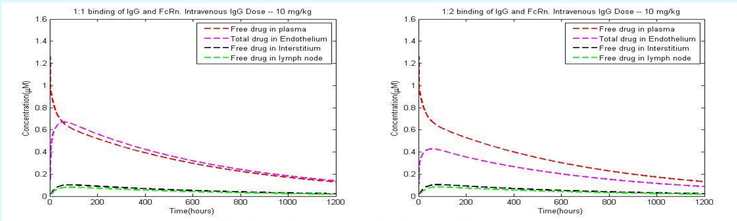

Figure 2. Concentration time profiles from the 1:1 and 1:2 binding models

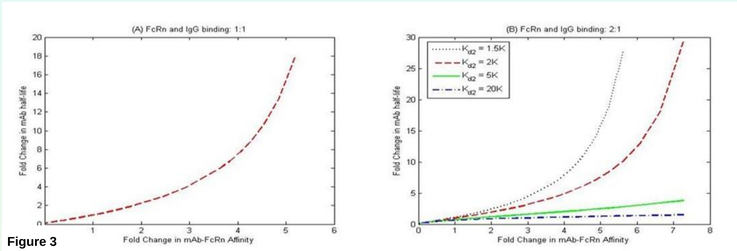

Figure 3

Figure 2 Fold change is calculated relative to the wild type IgG with $K_d = 0.728\,\mu M$ and half-life = 21 days

RESULTS

- Baseline simulations with either the 1:1 or 1:2 binding model gave a half-life of ~ 21 days and synthesis rates of endogenous IgG (~35 mg/kg/day) that are within the reported range in humans.
- The concentration profiles in plasma, endosomal and interstitial space for both models are shown in Figure 2.
- The effect of changing the affinity of mAb:FcRn binding of on mAb elimination $T_{1/2}$ was investigated assuming either 1:1 or 1:2 stoichiometry (Figure 3)
  - In the 1:1 model (Left panel) there is a marked ↑ in simulated $T_{1/2}$ as the affinity of mAb for FcRn ↑
  - In the 1:2 model (Right panel)
    - if the binding sites are assumed to be independent, the relationship between the simulated mAb half-life and FcRn affinity is similar to the 1:1 model
    - under conditions of positive co-operativity the ↑ in $T_{1/2}$ with increased affinity becomes steeper
    - under conditions of negative co-operativity, the sensitivity of simulated mAb $T_{1/2}$ to FcRn affinity is reduced
- The negative co-operativity 1:2 model gives a relationship between FcRn affinity and $T_{1/2}$ more in keeping with *in vivo* data from pre-clinical studies where the maximum ↑ in $T_{1/2}$ or ↓ in CL reported in the literature is in the 5-6 –fold range [5,6]
- A similar relationship between change in mAb- FcRn affinity and half-life was obtained by Chen & Balthasar [3] using a PBPK model where the endosome is divided into 5 sub-compartments with different pH values and within each compartment mAb-FcRn binding was modeled using on and off rates and 1:1 stoichiometry.

CONCLUSIONS

Adapting a PBPK model for mAbs to account for 1:2 IgG to FcRn stoichiometry leads to a model that under conditions of negative co-operativity is less dependent on changes in FcRn K_D and provides further potential to predict PK of mAbs.

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