Modeling the Binding Kinetics of Bispecific Antibodies under the Framework of a Minimal Human PBPK Model

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PURPOSE

There has been a revival in interest in bispecific antibodies (bsAbs) in recent years, and many have entered clinical trials [1]. In this study, a mechanistic mathematical model for bsAbs binding was developed to investigate and simulate the binding behavior of this very important class of proteins *in vitro* and *in vivo*.

METHODS

Binding between a bsAb (represented by Y in the right panel of Figure 1) and its two targets (A and B) was assumed to follow a classic non-competitive binding scheme [2]. This binding scheme, coupled with processes of target synthesis and degradation as well as processes of complex elimination, is embedded in a minimal PBPK model [3] in order to simulate the kinetic of a bsAb in the *in vivo* condition. The binding process can be treated as either quasi-equilibrium (QE), quasi-steady-state (QSS), or fully kinetic states resulting in corresponding QE, QSS, and full TMDD models. These were coded in Matlab (version R2012a). The effect of target properties (abundances, synthesis and degradation rate), the bsAb's affinities to the two targets, and the elimination rate of drug-target complexes, on i*n vitro* and *in vivo* receptor occupancy, was investigated.

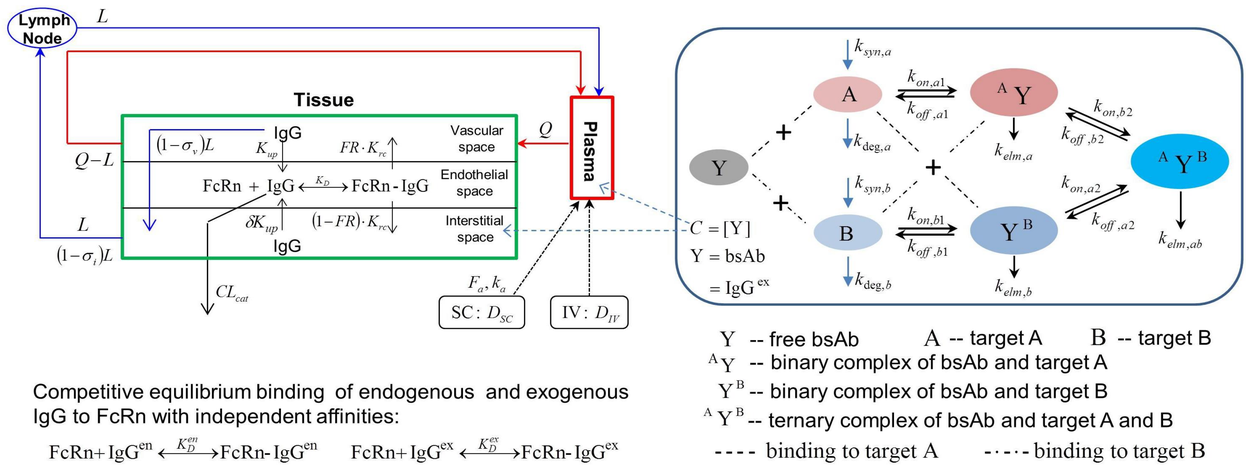

Figure 1. Schematic representation of a non-competitive binding model for bispecific antibodies embedded in the framework of a minimal PBPK model for mAbs [3].

The receptor occupancy can be defined in terms of different receptor species being occupied by bsAb, as shown below [equation 1]. However, as in the context of targeting of immune system effector cells, the meaningful occupancy for characterising the capability of recruiting effector function by a bsAb is thought to be specified by the amount of formed ternary complex and thus the levels of ternary complex were the focus of this modelling exercise. Specifically an assumption is made that the prerequisite for a pharmacological effect is that both targeted cells (through antigen and bsAb binding) and effect cells (through effect receptors and bsAb binding) are linked in a ternary complex by the bsAb and it is the amount of the formed ternary complex that determines the pharmacological outcomes.

Under the **equilibrium binding** condition (Figure 2), the level of ternary complex is determined by total drug concentration $[Y]_{total}$, total target concentrations $[A]_{total}$ & $[B]_{total}$, binding affinities of bsAb to targets (i.e., the reverse of K_A or K_B) as well as the affinity ratio α (for definition see figure 2. legend). The fraction of free drug (x) and ternary complex (y) can be calculated using a set of nonlinear equations [equation box 2] derived from equilibrium equations and conservation relations of drug and targets.

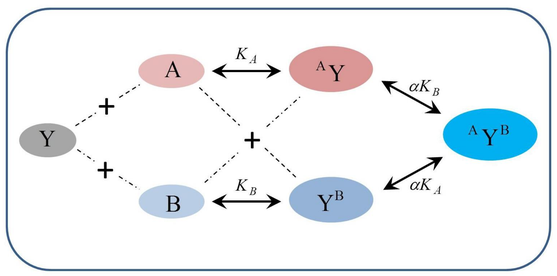

Figure 2. Equilibrium binding without target synthesis, target degradation, and elimination of drug-target complexes. K_A and K_B are equilibrium dissociation constants for antibody binding to targets A and B, respectively. α is the affinity ratio between Y and Y^B for target A, or Y and ^AY for target B (which is equal to the affinity ratio between Y and Y^B for target A according to the equilibrium binding).

$$\mathrm{RO_A}=\frac{[^{\mathrm{A}}\mathrm{Y}]}{[\mathrm{A}]_{\mathrm{total}}},\quad \mathrm{RO_B}=\frac{[\mathrm{Y}^{\mathrm{B}}]}{[\mathrm{B}]_{\mathrm{total}}},\quad \mathrm{RO_{AB}}=\frac{[^{\mathrm{A}}\mathrm{Y}^{\mathrm{B}}]}{R_M},\quad R_M=\min\{[\mathrm{A}]_{\mathrm{total}},[\mathrm{B}]_{\mathrm{total}}\}. \qquad (1)$$

$$1=x\left(1+\frac{\gamma_A-y}{\beta_A+x}+\frac{\gamma_B-y}{\beta_B+x}\right)+y,\quad \alpha y=x\frac{\gamma_A-y}{\beta_A+x}\cdot\frac{\gamma_B-y}{\beta_B+x}, \qquad (2)$$

$$x=\frac{[\mathrm{Y}]}{[\mathrm{Y}]_{\mathrm{total}}},\ y=\frac{[^{\mathrm{A}}\mathrm{Y}^{\mathrm{B}}]}{[\mathrm{Y}]_{\mathrm{total}}},\ \beta_A=\frac{K_A}{[\mathrm{Y}]_{\mathrm{total}}},\ \beta_B=\frac{K_B}{[\mathrm{Y}]_{\mathrm{total}}},\ \gamma_A=\frac{[\mathrm{A}]_{\mathrm{total}}}{[\mathrm{Y}]_{\mathrm{total}}},\ \gamma_B=\frac{[\mathrm{B}]_{\mathrm{total}}}{[\mathrm{Y}]_{\mathrm{total}}}.$$

Equations (2) can be solved numerically for a given set of parameters K_A, K_B, α , $[A]_{total}$, $[B]_{total}$, and $[Y]_{total}$, and thus all receptor occupancies defined in (1) can be calculated. The simulation based on this simple equilibrium approach are assumed to mimics the *in vitro* binding (figure 3). Inclusion of target dynamics (target synthesis, degradation and elimination of drug-target complexes) and coupling with the minimal PBPK model as shown in Figure 1, allows simulation represent the *in vivo* situation to be made.

RESULTS

Figure 3 shows the simulated receptor occupancy for a range of total bsAb concentration. The simulation is done by assuming there are no synthesis and degradation of targets, nor elimination of complexes, thus mimicking *in vitro* binding conditions. For both the cases of equal affinities and different affinities of a bsAb toward two targets the optimal occupancy can be only achieved when the bsAb concentration falls into the range between that of two targets. The bell shape of ternary occupancy shown in Figure 3 was observed in an *in vitro* experiment with a bsAb for targeting T cells to HER2-positive tumors [4]. This property is also observed in the simulation mimicking the *in vivo* conditions, presented in Figure 4.

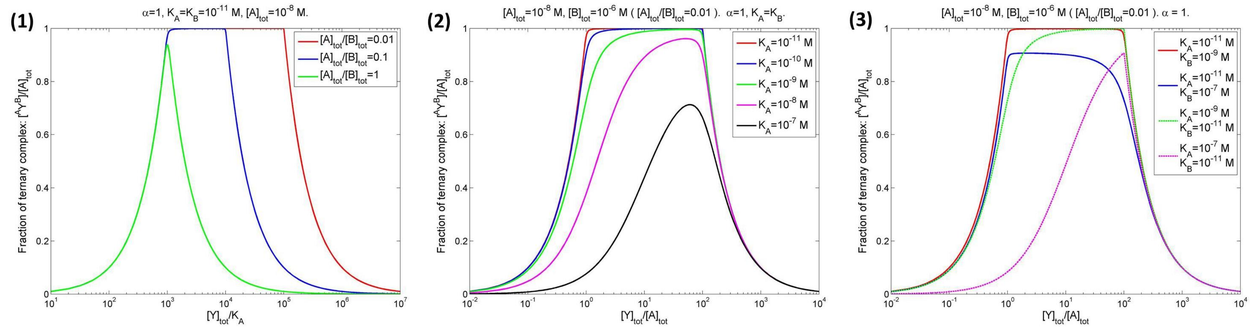

Figure 3 Simulations for the *in vitro* equilibrium non-competitive binding model without PBPK model and target dynamics. **(1)** Simulated occupancy defined in terms of ternary complex and total target A concentration. A variety of ratios of two target concentrations with $[A]_{total} \leq [B]_{total}$ is presented. **(2)** Simulated occupancy for fixed target concentrations with a range of target affinity (in all cases affinity A = affinity B). **(3)** Simulated occupancy for fixed target concentrations where the affinities to each target are varied.

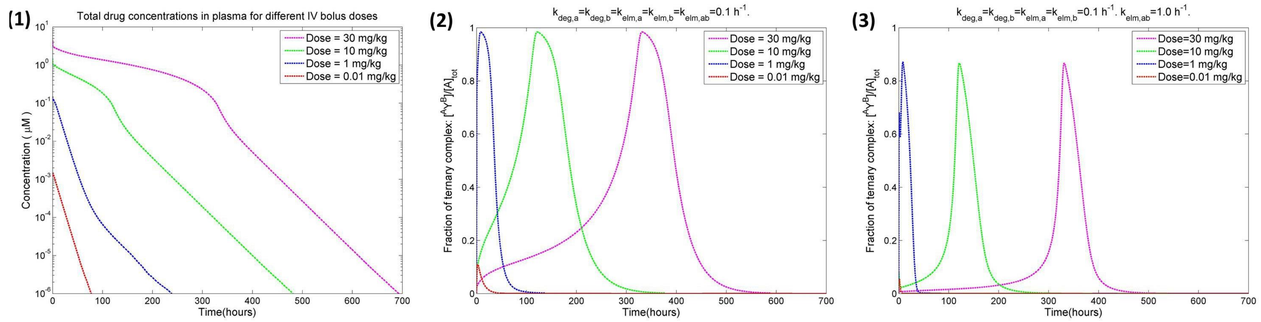

Figure 4 Simulations for the non-competitive binding with target dynamics embedded in the minimal PBPK model. Initial target levels was set to be $[A] = 0.01\mu M$, $[B] = 0.1\mu M$. $k_{on} = 100(\mu M)^{-1}h^{-1}$ and $k_{off} = 0.001\ h^{-1}$ are assumed for all binding steps. **(1)** Total drug concentrations at different doses, assuming the rates of target degradation and complex elimination are same. When the concentration temporarily falls into the range between two target levels and consequently invokes the target-mediated elimination pathway for ternary complex, resulting in a sharp decrease in the plasma profile in a short period. **(2)** The simulated occupancy corresponds to the sharp drop in plasma concentration shown in (1). **(3)** The simulated occupancy under the condition that the elimination rate of ternary complex is 10 times faster than that of binary complex.

CONCLUSION

This study shows that from a PK perspective, there is a great challenging to determine a dose regimen for a bsAb because the efficacy will be dependent on the cross-linking of two targets, and thus is determined by the receptor occupancy in terms of the ternary complex. As we demonstrate that high concentration doesn't mean a high efficacy, and instead the concentration has to be in the range between two targets to achieve an optimal occupancy. This property holds true for *in vitro* condition and may be also observed *in vivo* as shown in the *in vivo* simulation. The implications from this modelling exercise suggest that a much higher dose giving high drug concentrations relative to both target levels may postpone ternary complex formation due to insufficient occupancy in the initial phase and a lower dose might not have a response in the case the resultant concentration is much lower than that of targets or might have an immediate response when it falls into the range between two targets. In addition, a sharp and temporal drop in plasma profile is potentially an indication that the target-mediated elimination pathway through ternary complex (i.e., cell killing) is transiently in operation.

Although highlighting the utility of the model, the simulation results *in vivo* should not be generalized to bsAbs with other properties since the true outcome of the system is derived from an interplay among many factors, such as relative abundances of targets, relative affinities of bsAb to targets, turnover rates of targets, elimination rates of target complexes, etc., as well as the feedback from cell killing. In this aspect, PBPK modeling coupled with mechanistically-based binding model can have a great potential to assist in both preclinical and clinical study of bsAbs.

REFERENCES

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