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Modeling and simulation of receptor-toxin-antibody interaction

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Abstract:

This paper describes a simple, kinetic model of the effect of an antibody on the binding of a toxin to its receptor. This model has been used to evaluate the contributions of antibody affinity and concentration to reduction in complex formation and enable prediction of the antibody kinetic constants and concentration required to provide a specified degree of protection. An expression for determining equilibrium time for the system has also been derived.

Based on the proposed model we analytically calculated the relative reduction in toxin-receptor complex formation a variety of input parameters (kinetic constants and concentrations). Our analytical results were validated by numerical simulations, using the COPASI tool.

Experimental validation of the model may provide a useful tool for *in vitro* selection of potentially therapeutic and prophylactic antibodies for progression to *in vivo* evaluation.

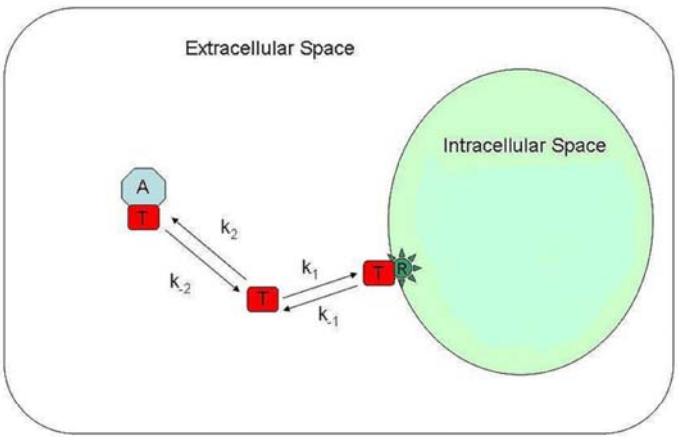

Figure 1. *Model of Receptor-Toxin-Antibody interaction.* The toxin, [*T*], binds reversibly to cell surface receptors, [*R*], with a forward rate k_1 and a reverse rate k_{-1} to form the toxin-receptor complex [*RT*]. The neutralising antibody binds to the toxin with on and off rates of k_2 and k_{-2} respectively. The antibody-toxin complex, [*AT*], remains in the extracellular space.

Keywords: Toxin, Receptor, Antibody, Ligand-receptor Complex, Kinetics, Protection factor, Equilibrium time

1. INTRODUCTION

Toxins are important potential therapeutic targets for a number of infectious diseases and many inhibitors, including antibodies, with a potential for prophylactic or therapeutic use have been developed (Albrecht et al., 2007; Rainey and Young 2004).

The dynamics of ligand-receptor-antibody systems can be described using standard competitive binding kinetics. Based on this approach we recently found (Skvortsov and Gray, 2009) that for a given receptor concentration. The protective properties of an antibody can be calculated analytically for given concentration of antibody, toxin and associated kinetic constants. This protection factor provides an important criterion for selection of an antibody for effective prophylactic and therapeutic use.

Another important parameter for the selection of antibody is the time required for the receptor-toxin-antibody system to reach equilibrium, since this parameters provides a timescale for the therapeutic effect of antibody to reach its maximum. Other conditions being equal the favorable selection of antibodies should be based on minimization of this timescale.

In the current paper we proposed a simple kinetic model to estimate these parameters. We applied the Quasi-Steady-State Approximation and extended the analytical framework initially proposed by McPherson and Zettner (1975) to include antibody. We supported our findings with numerical simulations using COPASI (Hoops et al., 2006).

2. MODEL

The kinetic model used in this study is shown in Figure 1. The rates of concentration change of each of the species shown in the model are:

$$\frac{d[RT]}{dt} = k_1[R][T] - k_{-1}[RT] \quad (1)$$

$$\frac{d[AT]}{dt} = k_2[A][T] - k_{-2}[AT] \quad (2)$$

where [*R*], [*T*] and [*A*] are the concentrations of receptor, toxin and antibody respectively, [*RT*] and [*AT*] are the concentrations of the receptor-toxin and antibody-toxin complexes, k_i are the rate constants shown in Fig.1.

This system should be supplemented with three conservations laws for [*R*], [*T*] and [*A*]:

$$R_0 = [R] + [RT] \,, \quad (3)$$

$$A_0 = [A] + [AT] \,, \quad (4)$$

$$T_0 = [T] + [RT] + [AT]. \quad (5)$$

If we introduce new variables $z = [RT]$, $y = [AT]$, then our model can be written in the form

$$\frac{dz}{dt} = k_1(R_0 - z)(T_0 - z - y) - k_{-1}z \,, \quad (6)$$

$$\frac{dy}{dt} = k_2(A_0 - y)(T_0 - z - y) - k_{-2}y \,. \quad (7)$$

3. EQUILIBRIUM OF THE CELL-SURFACE BINDING

At the equilibrium $d/dt = 0$ and (6), (7) can be reduced to

$$(R_0 - z)(T_0 - z - y) - K_1 z = 0 \,, \quad (8)$$

where

$$y = A_0 \varepsilon z / (R_0 - z(1 - \varepsilon)), \quad (9)$$

$\varepsilon = K_1 / K_2$ and $K_1 = k_{-1} / k_1$, $K_2 = k_{-2} / k_2$ are dissociation constants for the toxin binding to the receptor and antibody respectively.

Eq.(8) is a cubic algebraic equation that has a well-known closed-form analytical solution (Cardano's formula). This provides a consistent way to derive an exact solution for the proposed model. A comprehensive classification of realisable solutions of (8) will be published elsewhere. Here we present only an asymptotic approach based on reasonable assumptions that result in simple analytical expressions.

In the case without antibody $y = 0$ (i.e. $A_0 = 0$) (9) is an elementary quadratic equation that has two solutions (roots):

$$z_{1,2} = \frac{C_0}{2}\left(1 \pm \left(1 - \frac{4R_0T_0}{C_0^2}\right)^{1/2}\right). \qquad (10)$$

Under an obvious constrain $z_{1,2} \to 0$ as $T_0 \to 0$ only one solution holds:

$$z_0 = \frac{C_0}{2}\left(1 - \left(1 - \frac{4R_0T_0}{C_0^2}\right)^{1/2}\right), \qquad (11)$$

where $C_0 = R_0 + K_1 + T_0$. If we further assume $T_0 / C_0 << 1$, then we can further simplify:

$$z_0 \approx \frac{R_0T_0}{C_0}. \qquad (12)$$

From (8), (10) we can see that if $\varepsilon << 1$ then $y \approx A_0\varepsilon z / R_0$ and the solutions (11), (12) is still hold, but now with a new value of K_1:

$$K_1 \to K_1 + \varepsilon A_0. \qquad (13)$$

In the opposite case $\varepsilon >> 1$ (or, more precisely, $\varepsilon z_0 / R_0 << 1$) (11), (12) are valid , but now with the "redefined" toxin concentration

$$T_0 \to T_0 - A_0, \qquad (14)$$

and $T_0 \approx 0$ if $A_0 > T_0$.

4. ESTIMATION OF PROTECTION PROPERTIES OF ANTIBODIES

In order to universally characterize protection properties of antibodies it is convenient to introduce the following nondimentional parameter (protection factor)

$$\Psi = \frac{z(A_0 > 0)}{z(A_0 = 0)}. \qquad (15)$$

which meaning is a relative reduction of concentration of toxin-receptor complexes due to the presence of antibody.

It is evident that by definition Ψ is always within the range $0 \le \Psi \le 1$. From (12) - (15) it is readily to derive

$$\Psi = \frac{1}{1 + \varepsilon\lambda}, \qquad \text{if } \varepsilon\, T_0/R_0 << 1, \qquad (16)$$

where $\lambda = A_0/C_0$. Therefore, under condition $\varepsilon\, T_0/R_0 << 1$ Ψ is determined as a simple function of the concentrations of antibody, receptor and toxin and the ratio of the dissociation constants for the binding of the toxin to the receptor and to the antibody. In the opposite case $\varepsilon\, T_0/R_0 >> 1$

$$\Psi = 1 - A_0 / T_0 , \tag{17}$$

and $\Psi \approx 0$ if $A_0 > T_0$. In the later case, the key parameter for estimation of the protection of antibody is the ratio of antibody to toxin concentrations.

It is worth note that the vast majority of practical applications can be well described by the case (16).

5. ESTIMATION OF EQUILIBRIUM TIME

The theoretical framework for evaluation of the equilibrium time in competitive binding systems $[R]+[T] \Leftrightarrow [RT]$ was comprehensively studied by McPherson and Zettner (1975). It was shown that for such system with the rate equation (see (6) with $y = 0$)

$$\frac{dz}{dt} = k_1 (R_0 - z)(T_0 - z) - k_{-1} z \equiv az^2 + bz + c \tag{18}$$

the equilibrium time can be calculated by using the formula

$$\tau = F(a,b,c,d) , \tag{19}$$

where four parameters (a,b,c,d) are defined as

$$a = k_1 \ , \ b = -(k_1 R_0 + k_1 T_0 + k_{-1}) , \qquad c = k_1 R_0 T_0 , \quad d = f[RT]_{eq} . \tag{20}$$

Here $[RT]_{eq}$ is the equilibrium concentration as $t \to \infty$ and is given by (11) or (12), f is its fraction (for estimation of the half-time of a reaction rate we assume $f = 1/2$) and

$$F(a,b,c,d) = \frac{1}{D} \ln\left(\frac{(2ad + b - D)(b + D)}{(2ad + b + D)(b - D)} \right), \tag{21}$$

where $D = (b^2 - 4ac)^{1/2}$.

Our aim is to apply formula (19) to calculate the equilibrium time for the full system (1), (2). Based on our numerical simulation using realistic parameter values (Fig 2), we found that for a given range of input parameters (see below) the process of reaching quasi-equilibrium state in receptor-toxin binding is relatively rapid (in comparison with equilibrium process of antibody binding). This assumption will be also verified by the retrospective analysis, see below.

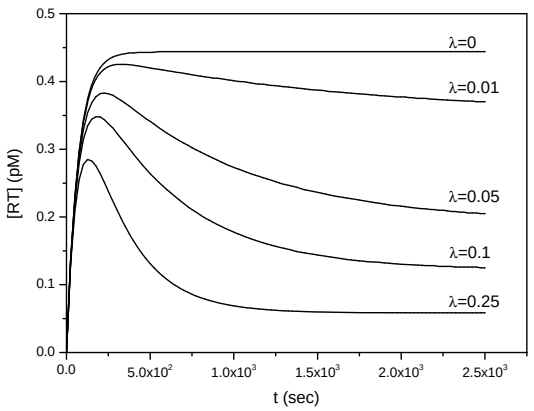

Figure 2. *Simulated effect of antibody concentration on formation of RT*. The binding curves were simulated using COPASI and the kinetic constants in Table 1. $[R]_0$ = 5nM; $[T]_0$=10pM, C_0=1.15x10^{-7}

This allows us to set $dz/dt \approx 0$ in (6) during the beginning of the antibody binding stage described by Eq (7) (Quasi-Steady-State Approximation). We can also expand the RHS of (6) near the quasi-equilibrium point ($z = z_0$, $y = 0$) and keep only the linear terms of that expansion. Therefore (6) simplifies to

$$0 = C_0 (z_0 - z) - (R_0 - z_0) y \tag{22} .$$

Now we can deduce z from this equation and plug it into the second equation (7). Therefore we arrive at

$$\frac{dy}{dt} = k_2(A_0 - y)(T_0 - z_0 - y(1-\xi)) - k_{-2}y = -\xi\frac{dz}{dt}, \quad (23)$$

where $\xi = (R_0 - z_0)/C_0 \approx R_0/C_0$.

Eqs (22), (23) describe simplified dynamics of the receptor-toxin-antibody interaction after toxin-receptor binding has reached its quasi-equilibrium state (i.e $z \approx z_0$). We can see that during this stage the concentration of toxin-receptor complexes is driven by the antibody-toxin reaction. The time derivatives *dz/dt* and *dy/dt* are proportional to each other and the coefficient of their proportionality ξ is a simple function of receptor concentration and the dissociation constants for the binding of the toxin to the receptor; it is independent on antibody properties.

It is important that now equation (23) becomes of the type (18), so the equilibrium time for the system can be calculated based on the formula (19), with

$$a = k_2(1-\xi), \quad b = -(k_2A_0(1-\xi) + k_2T_* + k_{-2}), \quad c = k_2A_0T_*, \quad d = f[AT]_{eq}, \quad (24)$$

$T_* = T_0 - z_0$ and z_0 is as above given by (11) or (12). For the low toxin concentration we can always assume that $T_* = T_0 - z_0 \approx T_0K_1/C_0$, so

$$a = k_2K_1/C_0, \quad b = -(k_2K_1(A_0 + T_0)/C_0 + k_{-2}), \quad c = k_2K_1A_0T_0/C_0. \quad (25)$$

To have a closed model we need to calculate $[AT]_{eq}$ in terms of known variables . From the general formula (9) we can write

$$[AT]_{eq} = A_0\varepsilon z/(R_0 - z(1-\varepsilon)),$$

where z_0 should be taken at the limit $t \to \infty$, i.e when the effect of antibody on toxin-receptor binding certainly should be taken into account. According to (13) ,(14) the effect of antibody can be incorporated into any expression for z_0 (11), (12) by simple substitution of new values for K_1 or T_0 . Therefore we can readily deduce two asymptotics:

$$[AT]_{eq} \approx \frac{\varepsilon A_0T_0}{C_0 + \varepsilon A_0}, \quad [AT]_{eq} \approx A_0, \quad (26)$$

which are valid for $\varepsilon T_0/R_0 << 1$ and $\varepsilon T_0/R_0 >> 1$ respectively. We can also express $[AT]_{eq}$ in terms of the protection parameter introduced above (15):

$$[AT]_{eq} \approx \frac{\varepsilon\lambda\Psi T_0A_0}{C_0 - \Psi(1-\varepsilon)T_0}, \quad (27)$$

and Ψ is defined by (16). This representation is especially useful for optimisation studies when the protection factor Ψ and the equilibrium time τ needed to be optimised concurrently.

The expressions (19), (21), (25), (26) provide a complete solution for the problem of estimation of the equilibrium time in the competitive binding system of receptor, toxin-and antibody. They establish an analytical framework for the sensitivity study, i.e. estimation of a magnitude of changes of the equilibrium time with the changes in the input parameters of the system. We can see that dependency of the equilibrium time on any of the input parameters can be extremely convoluted.

We can validate our initial assumptions about relative speed of the equilibrium processes in receptor-toxin subsystem by comparing equilibrium time estimated from (18)-(20) to one estimated from (19), (21), (25), (26) (see next section).

It is worth note that the above framework can be also applied in the opposite case (i.e. when equilibrium in receptor-toxin subsystem is much slower than in antibody-toxin subsystem) for which it needs only minor modifications. In the

later case the "driving" equation for the Quasi-Steady-State Approximation becomes equation (17) (i.e. we set $dy/dt = 0$) and the resulting expressions will look exactly the same except for the obvious circular substitutions of parameters:

$$k_1 \leftrightarrow k_2, \quad k_{-1} \leftrightarrow k_{-2}, \quad A_0 \leftrightarrow R_0.$$

6. VALIDATION OF THE MODEL: NUMERICAL SIMULATION

Testing of the model was carried out using COPASI (Hoops *et al.*, 2006) and the kinetic parameters for the binding of ricin to its receptor and its internalisation (Sandvig *et al.*, 1976) and the competition by the monoclonal antibody 2B11 (McGuinness and Mantis, 2006). The kinetic parameters used are shown in Table 1. The value of k_3 used is that determined by (Sandvig *et al.*, 1976) to be the rate of irreversible binding of ricin to HeLa cells.

Reaction	k_{on} ($[M]^{-1}s^{-1}$)	k_{-off} (s^{-1})	k_{-3} (s^{-1})
HeLa cell receptor	1.3×10^{5}	1.4×10^{-2}	3.3×10^{-5}
2B11	1.25×10^{5}	5.2×10^{-4}	

Table 1. Kinetic constants for the binding of ricin to its receptor and the monoclonal antibody 2B11

Figure 3 shows the relationship (16) between antibody concentration and the toxin/antibody and toxin/receptor dissociation constants. This plot is valid for all combinations of toxin, receptor and antibody consistent with the assumptions used to derive (16), principally $\varepsilon\, T_0/R_0 << 1$

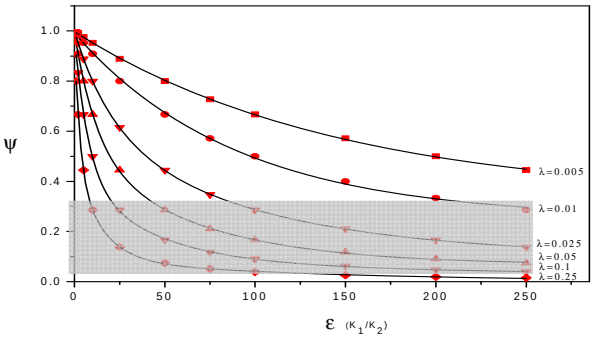

Figure 3. Ψ as a function of ε and λ. Ψ was calculated using Eq (16) (■) and by numerical simulation of the full system (1), (2)

The antibody kinetic parameters and concentration required to provide a specified degree of protection may be determined from this plot. For example, any combination of ε and λ falling in the shaded area will reduce either [*RT*] by 80%.

This, in turn, enables judgements to be made about antibody selection. For example, if an antibody concentration of $0.25C_0$ (λ=0.25) is achievable, then an antibody with an ε value of 50 will provide good protection (93% reduction in [*RT*]). If an antibody concentration of only $0.05C_0$ (λ=0.05) is achievable, then an ε value of 250 is required to achieve the same level of protection. The structure of Eq. (16) is such that, a given increase in protection (Ψ) may be achieved by either an x-fold increase in ε or an x-fold increase in λ.

The equilibrium time was estimated from the plot similar to one presented in Fig 2. For the case $\lambda > 0$ the position of the maximum on this plot approximately corresponds to a timescale of the toxin-receptor binding and the width of the bell-shape curve at the half-maximum provides a simple estimate of the equilibrium time in the presence of an antibody. Based on our simulations we found that for the system with the parameters in Fig 2. in the presence of an antibody the equilibrium time may increase significantly (i.e. from *~20* to *200 sec*) that was in a reasonable agreement with our analytical predictions (19), (21), (25), (26).

The simplified equation (22) was validated by comparing its results with the results provided by the numerical simulation of the full system (1) – (2). In order to present this comparison in a graphical form we introduced a parameter *P*

$$P = \frac{C_0(z_0 - z)}{(R_0 - z_0)y}, \qquad (28)$$

where $z_0 = T_0R_0/C_0$, and plotted its evolution over time by solving (1) – (2). Then the horizontal line $P = 1$ corresponded to the case when equation (22) holds. The results presented in Figure 4 is a typical outcome of our simulations: we observed that for a wide range of input parameters the value of P tended to 1 very quickly (i.e. during the receptor-toxin binding time). This provides a support for the Quasi-Steady-State Approximation leading to Eq.(22).

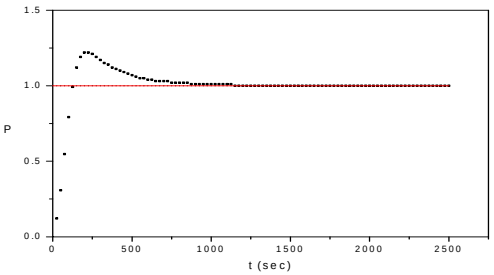

Figure 4 *Validation of the Quasi-Steady-State Approximation, see text for details :* (■) Parameter P (28) derived from the solution of the full system (1),(2); (●) Quasi-Steady-State Approximation for the long-time evolution

More comprehensive comparison of our analytical model and numerical simulations will be published elsewhere.

CONCLUSIONS

We have developed and validated a simple yet scientifically rigorous framework for the estimation of the protective effect of antibodies including the reduction of concentration of toxin-receptor complexes and the equilibrium time of toxin-receptor-antibody system (i.e. timescale when therapeutic effect of antibody reaches its maximum).

We have derived analytical expressions for the protection factor of the antibody and for the equilibrium time in terms of initial concentrations of the species and kinetic constants of the reactions. We have validated our results by numerical simulations and observed reasonable agreement.

In the presence of an antibody, using the antibody kinetic parameters in Table 1, establishment of the equilibrium value of $[RT]$ takes significantly longer than in the absence of antibody (Fig.2). Selection of antibodies with kinetic properties that minimise this time and hence reduce the potential for activation of receptor-linked processes will also improve the protective effect.

The proposed model can provide a useful tool for *in vitro* selection of potentially therapeutic and prophylactic antibodies for progression to *in vivo* evaluation.

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