Ligand accumulation counteracts therapeutic inhibition of receptor systems

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Abstract: Targeting receptor systems by competitive inhibition is the objective of various antibody drugs in development and on the market. A variety of receptor systems also constitute a degradation mechanism for ligand and drug via endocytosis and therefore influence the microenvironment of the cell. A thorough understanding of the complex interplay between ligand kinetics, drug pharmacokinetics, and the drug effect arising from the inhibition of the receptor by competing with the natural ligand is largely missing. Based on a mathematical model of the drug-ligand-receptor dynamics we show that receptor inhibition may lead to accumulation of the natural ligand in the microenvironment of the cell, with counteracting impact on the inhibitory effect of the drug. In the absence of receptor-independent ligand degradation, we prove analytically that this counteracting effect cannot be eliminated by changing the structural properties of the drug, like the affinity, nor by changing drug dosage. It is a structural property of the type of receptor system under study. The results suggest that the microenvironment may have an influence on the success of drug treatment with competitive inhibitors, like therapeutic antibodies in cancer therapy.

Keywords: therapeutic antibodies, cell surface receptors, receptor inhibition

1. INTRODUCTION

The development of therapeutic antibodies has been a major focus of the pharmaceutical industry over the past years. Their significant therapeutic potential results from their ability to bind with high affinity to specific targets such as receptors or other cell surface proteins. Receptor binding often results in subsequent internalization and eventual degradation, which for many protein drugs is an important route of elimination (Meibohm, 2007). Receptors are promising drug targets because they transmit external signals across the cellular membrane, which are processed by downstream signalling cascades and lead to the cells' functional responses (e.g., changes in gene transcription). Alterations in the receptor's ability to transduce information can result in the development of diseases. In cancer, for example, some of these alterations result from mutations in the receptor that increase the sensitivity of the cell to growth factors (Wells et al., 1990). Growth factors are tightly controlled. After receptor activation, the growth factor molecule is cleared from the environment by receptor mediated endocytosis (RME). Local processes like autocrine and paracrine signalling as well as degradation of ligands by RME are likely to be important in the microenvironment of target cells, in particular, if the exchange with distant cells is impaired, like it is observed in solid tumors.

In this article we study the inhibition of receptors by therapeutic antibodies, and its interplay with the ligand concentration in the microenvironment of the cell. We focus on receptor systems where the ligand is internalized by RME after receptor activation. This is the case for a variety of receptor families (Backer et al., 1991; Flores-Morales et al., 2006; Hilton and Nicola, 1992), including the important receptor tyrosine kinases activated by growth factors. Existing *in silico* studies of receptor systems focus on the ligand-receptor interaction (Shankaran et al., 2007, 2006) or on drug-receptor interaction (Mager, 2006). In contrast, our analysis is based on a mathematical model that describes the time-dependent interaction of drug, ligand and the receptor system. We find that when RME has an influence on the concentration of the ligand in the microenvironment, therapeutic inhibition is counteracted by ligand accumulation. This indicates that the microenvironment of tumor cells may not only have a crucial influence on the success of radiotherapy (Vaupel, 2004), but it also potentially influences antibody based therapies.

2. MODEL DESCRIPTION

The proposed model to study the inhibition of receptor activation by therapeutic proteins is based on an established ligand-receptor interaction model (Shankaran et al., 2007, 2006; Lund et al., 1990; Wiley and Cunningham, 1981). This canonical model was extended to also include the drug-receptor interaction, which has been studied in phar-

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macokinetics based on target-mediated drug-disposition models (Mager, 2006). The studied model is shown in Fig. 1.

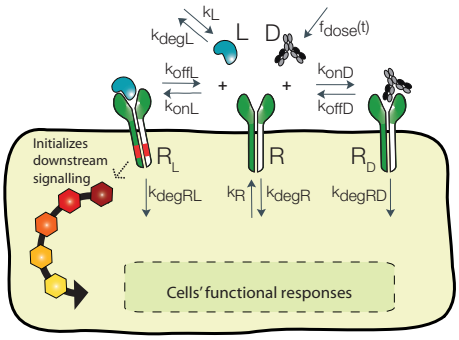

Fig. 1. The proposed model of receptor-ligand-drug interaction. The natural ligand and the drug compete for the receptor binding.

In the model, both ligand L and drug D are present in the extracellular space (with volume V). The ligand enters the extracellular space at rate k_L, and is cleared with rate constant k_{degL}. The drug enters the extra-cellular space at rate $f_{dose}(t)$. The free membrane receptor R is produced at rate k_R and internalized with the rate constant k_{degR}. Both ligand and drug reversibly bind to free receptors R with association rate constant k_{onL} and k_{onD}, respectively, and a dissociation rate constant k_{offL} and k_{offD}, respectively. The resulting ligand-receptor complex R_L and drug-receptor complex R_D are internalized by forming an endosome with the rate constant k_{degRL} and k_{degRD}, respectively.

Based on the law of mass action, the rates of change for the molecular species are given by the following system of ordinary differential equations (ODEs):

$$\begin{aligned}
\frac{\mathrm{d}\,L}{\mathrm{d}t} &= \frac{k_L}{VN_a} - \frac{k_{onL}}{VN_a}RL + \frac{k_{offL}}{VN_a}R_L - k_{degL}L,\\
\frac{\mathrm{d}\,D}{\mathrm{d}t} &= f_{dose}(t) - \frac{k_{onD}}{VN_a}RD + \frac{k_{offD}}{VN_a}R_D,\\
\frac{\mathrm{d}\,R}{\mathrm{d}t} &= k_R - k_{onL}RL - k_{onC}RD + k_{offL}R_L\\
&\quad + k_{offD}R_D - k_{degR}R,\\
\frac{\mathrm{d}\,R_L}{\mathrm{d}t} &= k_{onL}RL - k_{offL}R_L - k_{degRL}R_L,\\
\frac{\mathrm{d}\,R_D}{\mathrm{d}t} &= k_{onD}RD - k_{offD}R_D - k_{degRD}R_D.
\end{aligned} \tag{1}$$

The species L and R are expressed in [M]; R, R_L and R_D are in units [# molecules]. Division by the product of Avogadro's constant N_a and volume V ensures conversion from units [# molecules] to [M]. The non-negative drug dosing rate is given by $f_{dose}(t) = f(t) \cdot \text{Dose}$, with

$$\int_0^\infty f(t)\,dt = 1. \tag{2}$$

Different dosing regimes can be modeled by choosing $f(t)$ appropriately. For example, a bolus-dose at time $t = 0$ is represented by choosing f as a delta-distribution at $t = 0$.

Prior to any drug administration, the system is assumed to be in steady state, resulting in some number of active receptor $R_L = R_L^*$. The effect of the drug results from the inhibition of receptor activation, i.e., from the change in the number of active receptor R_L over time. Since this effect depends on the dosing function $f_{dose}(t)$, the problem can be interpreted as a control problem (Franklin et al., 2002) where $f_{dose}(t)$ acts as an external input that has to be designed to push the output $R_L(t)$ below its steady-state value. For numerical simulations, we used experimentally determined parameter values for the epidermal growth factor receptor (EGFR) and assumed that the drug-related parameters were identical to the parameters of the natural ligand (see Table 1).

Table 1. Parameter values for the EGF receptor system. [a] (Hendriks et al., 2005); [b] (Resat et al., 2003)

Constant	Value	Unit	Constant	Value	Unit
k_{onL}	2.47^a	nM	k_{onD}	2.47	nM
k_{offL}	0.24^a	1/min	k_{offD}	0.24	1/min
k_{degR}	0.02^b	1/min	k_{degL}	see Fig. 2	1/min
k_{degRL}	0.15^b	1/min	k_{degRD}	0.15	1/min

We assumed that the cell has $2 \cdot 10^5$ receptors (which is the EGFR expression level in human mammary epithelial cells (Shankaran et al., 2007)) and the ligand concentration to be 10 ng/ml (from (Goldstein et al., 1995)). Using these concentrations and the molecular weight of the ligand EGF (133.07 kD) the parameters k_L and k_R can be determined. A drug dose of 10 μg/ml was administered to the system as in (Goldstein et al., 1995). The volume V was set to $4 \cdot 10^{-10}$ l/cell (Shankaran et al., 2007).

3. RESPONSE TO DRUG ADMINISTRATION

Single bolus dose. In the following we consider the response of the receptor system to a single bolus dose of the inhibitor. Figure 2 shows the time course of the drug concentration in the microenvironment and the resulting number of active receptors R_L for different values of the ligand clearance rate k_{degL}. Following the bolus dose at time $t = 0$, the number of activated receptors drops rapidly to a much lower level. Inhibition of active receptors is due to the competition for free receptors between the natural ligand and the drug. Since binding to receptor implies internalization and degradation, the drug concentration decreases over time such that eventually the number of active receptors recovers to its unperturbed steady-state level (dashed line).

Two phases in Fig. 2 can be identified: In a first phase the number of active receptors decays below its steady-state level, resulting in an inhibition of the receptor system; in a second phase, however, the active receptors are above their steady-state, resulting in an induction of the receptor system. The extent of inhibition and induction depends on the clearance k_{degL}. For $k_{degL} = 0.01$/min, the induction phase is almost absent, whereas for $k_{degL} = 0$ the induction phase is the highest. The inset in Fig. 2 shows the increase and decline of the ligand concentration in the microenvironment of the cell. The ligand accumulation is the consequence of the drug binding to the receptor resulting in less ligand bound and degraded. For low values of k_{degL}, the extracellular ligand accumulates considerably, while for high values of k_{degL} it is cleared by the receptor-independent route.

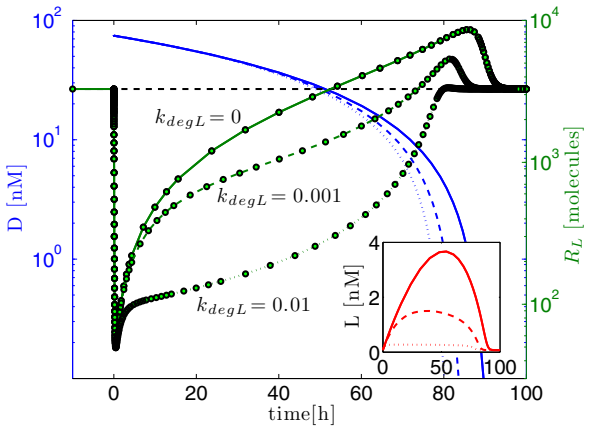

Fig. 2. Dynamic response of the number of active receptors (with circle markers) and drug concentration (without markers) after bolus dose for different ligand clearance rates k_{degL}. Inset: Ligand accumulation in the microenvironment of the cell over time.

To further understand the relation between inhibition and induction, it is useful to quantify the drug effect in a precise way. As a measure of the drug effect we define:

$$E = \int_0^\infty \big(R_L^* - R_L(t)\big)dt. \tag{3}$$

Thus, E measures the *net inhibition* as the sum of the inhibition and induction. Fig. 2 shows that small values of k_{degL} increase the induction phase and decrease the inhibition phase, implying a lower net inhibition according to eq. (3). Moreover, in the case of $k_{degL} = 0$ we numerically observe a zero net inhibition ($E = 0$), which suggests that ligand accumulation totally counteracts the drug effect.

Multiple bolus dose. A dosing strategy yo prevent the induction phase, could be to administer a follow-up dose before the induction phase starts. As can be inferred from Fig. 3, this first prevents the induction, but comes with the cost of a larger induction phase after the final dose. This is due to a longer ligand accumulation phase (see inset in Fig. 3). For $k_{degL} = 0$, numerical computations show a zero net inhibition as in the previous case.

4. THEORETICAL ANALYSIS OF NET INHIBITION

In the following we analytically show that in the limiting case when $k_{degL} = 0$, the net inhibition vanishes and this effect is independent of the parameter values. Therefore, in this scenario the extent of ligand accumulation and the resulting induction phase do not depend on the model parameters, which suggests that it is a structural property of the studied receptor system.

We assume that the unique steady state L^*, D^*, R^*, R_L^* and R_D^* is exponentially stable, which for any realistic scenario is trivially satisfied. This guarantees that the net effect E is well-defined. It is convenient to eqs. (1) in terms of the deviations of the species from their steady-state values. We define these incremental variables as

$$\bar{L}(t) = L^* - L(t), \qquad \bar{R}_D = R_D^* - R_D(t),$$
$$\bar{R}(t) = R^* - R(t), \qquad \bar{R}_L = R_L^* - R_L(t),$$
$$\bar{D}(t) = D^* - D(t).$$

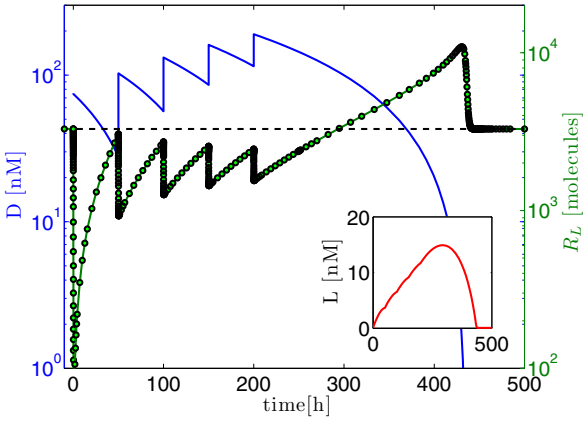

Fig. 3. Dynamic response of the number of activated receptors (solid line with circles) and drug concentration (solid line) after multiple bolus doses for $k_{degL} = 0$. Inset: Ligand accumulation in the microenvironment of the cell over time.

The resulting system of ODEs in terms of

$$\bar{x}(t) = \big[\bar{L}(t)\ \bar{D}(t)\ \bar{R}(t)\ \bar{R}_L(t)\ \bar{R}_D(t)\big]^T$$

is the given by

$$\frac{\mathrm{d}\,\bar{x}}{\mathrm{d}t} = \boldsymbol{A}\bar{x}(t) + \boldsymbol{B_{RL}}\bar{R}(t)\bar{L}(t) + \boldsymbol{B_{RD}}\bar{R}(t)\bar{D}(t) - \boldsymbol{B}f(t), \tag{4}$$

with $\bar{x}(0) = [0\ -D(0)\ 0\ 0\ 0]^T$, and where $\boldsymbol{A}$ is the Jacobian of the right hand side of (1) evaluated at the steady state (given in eq. (5)). The vectors $\boldsymbol{B_{RL}}$, $\boldsymbol{B_{RD}}$ and $\boldsymbol{B}$ are given by

$$\boldsymbol{B_{RL}} = \left[\frac{k_{onL}}{VN_a}\ 0\ k_{onL}\ -k_{onL}\ 0\right]^T,$$
$$\boldsymbol{B_{RD}} = \left[0\ \frac{k_{onD}}{VN_a}\ k_{onD}\ 0\ -k_{onD}\right]^T,$$
$$\boldsymbol{B} = [0\ 1\ 0\ 0\ 0]^T.$$

Integration of (4) from $t = 0$ to infinity gives

$$\bar{x}(\infty) - \bar{x}(0) = \boldsymbol{A}\int_0^\infty \bar{x}(t)\,dt + \boldsymbol{B_{RL}}\int_0^\infty \bar{R}(t)\bar{L}(t)\,dt + \boldsymbol{B_{RD}}\int_0^\infty \bar{R}(t)\bar{D}(t)\,dt - \boldsymbol{B}\int_0^\infty f(t)\,dt. \tag{6}$$

The stability of the system implies $\bar{x}(\infty) = 0$, and using the initial condition yields

$$\int_0^\infty \bar{x}(t)\,dt = \boldsymbol{A}^{-1}\boldsymbol{B}\cdot\text{Dose} - \boldsymbol{A}^{-1}\boldsymbol{B_{RL}}\int_0^\infty \bar{R}(t)\bar{L}(t)\,dt - \boldsymbol{A}^{-1}\boldsymbol{B_{RD}}\int_0^\infty \bar{R}(t)\bar{D}(t)\,dt. \tag{7}$$

We notice that $E = \int_0^\infty [\bar{x}(t)]_4\,dt$ and moreover,

$$\big[\boldsymbol{A}^{-1}\boldsymbol{B}\big]_4 = \big[\boldsymbol{A}^{-1}\boldsymbol{B_{RL}}\big]_4 = \big[\boldsymbol{A}^{-1}\boldsymbol{B_{RD}}\big]_4 = 0, \tag{8}$$

which finally implies $E = 0$. Hence, in absence of receptor-independent ligand clearance, the inhibition and subsequent induction phase are identical, resulting in a zero net inhibition. Since this phenomenon is independent of any

$$\boldsymbol{A} = \begin{bmatrix} -\frac{k_{onL}R^*}{VN_a} & 0 & -\frac{k_{onL}L^*}{VN_a} & \frac{k_{offL}}{VN_a} & 0 \\ 0 & -\frac{k_{onD}R^*}{VN_a} - k_{degD} & 0 & 0 & \frac{k_{offD}}{VN_a} \\ -k_{onL}R^* & -k_{onD}R^* & -k_{onL}L^* - k_{degR} & k_{offL} & k_{offD} \\ k_{onL}R^* & 0 & k_{onL}L^* & -k_{offL} - k_{degRL} & 0 \\ 0 & k_{onD}R^* & 0 & 0 & -k_{offD} - k_{degRD} \end{bmatrix}. \quad (5)$$

drug- and receptor- specific parameters, it is suggested that it is a structural feature of the considered receptor class.

5. DISCUSSION AND CONCLUSION

The effect of antibody-based therapeutics for targeting tumors is influenced by cell-level kinetic processes. One example is the binding and internalization of antibodies by the tumor cells, which limits the penetration of solid tumors (Thurber et al., 2008). In this article we identified another kinetic mechanism with the potential to compromise the effect, namely the accumulation of ligands in the microenvironment of tumor cells. Receptor trafficking can have a critical influence on the ligand concentration in the cells' environment as was shown for the EGF-EGFR system in vitro (Reddy et al., 1994).

We therefore analyzed the effect of inhibiting such a receptor system, and found that the response of the receptor system to the drug in this case can have two counteracting phases: An initial inhibitory phase and a second inductive phase. The latter is due to extracellular accumulation of the ligand, which is larger for environments where receptor-independent ligand clearance is slow. In such situations the inhibitor only postpones the activation, until the local concentration of the drug has sufficiently declined, acting as a memory of the prevented activation. In the limiting (theoretical) case when there is no receptor-independent ligand clearance, the induction of active receptors totally offsets the inhibitory response and renders a nil total drug effect. The dosing function can be regarded as an external input signal that is applied to the receptor system to control its activation. The phenomenon of counteracting ligand accumulation constitutes a "fundamental limitation" in the inhibition of the receptor system, which is independent of the parameter values and resembles those that typically arise in Control Engineering (Seron et al., 1997). The study of fundamental limitations is an extensive field of research (Franklin et al., 2002) that addresses the question how the structure of the system limits certain characteristics of every possible response to a class of inputs. Our analysis suggests that this kind of limitations can also play a role for antibody based cancer treatment.

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REFERENCES

J M Backer, S E Shoelson, E Haring, and M F White. Insulin receptors internalize by a rapid, saturable pathway requiring receptor autophosphorylation and an intact juxtamembrane region. *J Cell Biol*, 115(6):1535–45, Dec 1991.

Amilcar Flores-Morales, Christopher J Greenhalgh, Gunnar Norstedt, and Elizabeth Rico-Bautista. Negative regulation of growth hormone receptor signaling. *Mol Endocrinol*, 20(2):241–53, Feb 2006. doi: 10.1210/me.2005-0170.

Gene F. Franklin, J. David Powell, and A. Emami-Naeini. *Feedback Control of Dynamics Systems*. Prentice Hall, 4th edition, 2002.

N I Goldstein, M Prewett, K Zuklys, P Rockwell, and J Mendelsohn. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res*, 1(11):1311–8, Nov 1995.

Bart S Hendriks, Gayla Orr, Alan Wells, H Steven Wiley, and Douglas A Lauffenburger. Parsing erk activation reveals quantitatively equivalent contributions from epidermal growth factor receptor and her2 in human mammary epithelial cells. *J Biol Chem*, 280 (7):6157–69, Feb 2005. doi: 10.1074/jbc.M410491200.

D J Hilton and N A Nicola. Kinetic analyses of the binding of leukemia inhibitory factor to receptor on cells and membranes and in detergent solution. *J Biol Chem*, 267(15):10238–47, May 1992.

K A Lund, L K Opresko, C Starbuck, B J Walsh, and H Steven Wiley. Quantitative analysis of the endocytic system involved in hormone-induced receptor internalization. *J Biol Chem*, 265(26): 15713–23, Sep 1990.

Donald Mager. Target-mediated drug disposition and dynamics. *Biochem Pharmacol*, 72(1):1–10, Jun 2006. doi: 10.1016/j.bcp.2005.12.041.

Bernd Meibohm. The role of pharmacokinetics and pharmacodynamics in the development of biotech drugs. In *Pharmacokinetics and Pharmacodynamics of Biotech Drugs*. Wiley-VCH, 2007.

C C Reddy, A Wells, and Douglas A Lauffenburger. Proliferative response of fibroblasts expressing internalization-deficient epidermal growth factor (egf) receptors is altered via differential egf depletion effect. *Biotechnol Prog*, 10(4):377–84, Jan 1994.

Haluk Resat, Jonathan A Ewald, David A Dixon, and H Steven Wiley. An integrated model of epidermal growth factor receptor trafficking and signal transduction. *Biophys J*, 85(2):730–43, Jul 2003.

María M. Seron, Julio H. Braslavsky, and Graham C. Goodwin. *Fundamental Limitations in Filtering and Control*. Springer Verlag, London, 1997.

H Shankaran, H Resat, and H Steven Wiley. Cell surface receptors for signal transduction and ligand transport: A design principles study. *PLoS Comput Biol*, 3(6):e101, May 2007. doi: 10.1371/journal.pcbi.0030101.

Harish Shankaran, H Steven Wiley, and Haluk Resat. Modeling the effects of her/erbb1-3 coexpression on receptor dimerization and biological response. *Biophys J*, 90(11):3993–4009, Jun 2006. doi: 10.1529/biophysj.105.080580.

G Thurber, M Schmidt, and K Dane Wittrup. Factors determining antibody distribution in tumors. *Trends Pharmacol Sci*, 29(2): 57–61, Jan 2008. doi: 10.1016/j.tips.2007.11.004.

P Vaupel. Tumor microenvironmental physiology and its implications for radiation oncology. *Seminars in Radiation Oncology*, 14 (3):198–206, Jul 2004. doi: 10.1016/j.semradonc.2004.04.008.

A Wells, J B Welsh, C S Lazar, H Steven Wiley, G N Gill, and M G Rosenfeld. Ligand-induced transformation by a noninternalizing epidermal growth factor receptor. *Science*, 247(4945):962–4, Feb 1990.

H Steven Wiley and D D Cunningham. A steady state model for analyzing the cellular binding, internalization and degradation of polypeptide ligands. *Cell*, 25(2):433–40, Aug 1981.