Ligand accumulation counteracts therapeutic inhibition of receptor systems

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Abstract: Targeting receptor systems by competitive inhibition is the objective of various protein 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 that is due to the fact that inhibition influences the ligand concentration in the microenvironment. The results suggest that the microenvironment may have an influence on the success of drug treatment with competitive inhibitors, e.g., for therapeutic proteins in cancer therapy.

Keywords: therapeutic proteins, cell surface receptors, receptor inhibition

1. INTRODUCTION

Proteins drugs such as monoclonal antibodies, growth factors and cytokines have been a major focus of research and development activities in the pharmaceutical industry over the past years (Meibohm, 2007). Their significant therapeutic potential results from their ability to bind with high affinity to specific targets such as receptors or cell surface proteins. Receptor binding often results in subsequent internalization and eventually 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). Normally, 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 blockage of receptor activation by inhibitory drugs, 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 (e.g., (Shankaran et al., 2007, 2006)) or on the drug-receptor interaction (e.g., (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, yielding important new insight.

2. MODEL DESCRIPTION

Our proposed model to study the inhibition of receptor activation by therapeutic proteins is based on a well-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 account for the drug-receptor interaction, which has been studied in pharmacokinetics based on target-mediated drug-disposition models (Mager, 2006). Our model is shown in Fig. 1.

In the model, both ligand L and drug D are present in the extracellular space (with volume V). The ligand enters

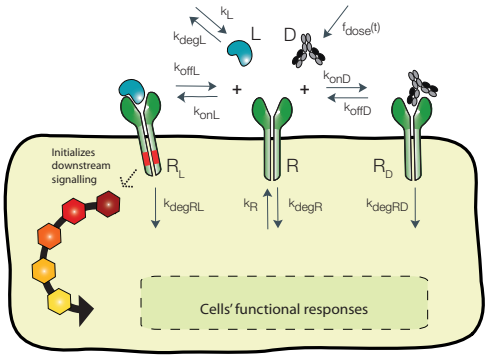

Fig. 1. Natural ligand and inhibitor drug competing for receptor binding.

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 $R_L(t)$ 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 simulation purposes only, we used experimentally determined parameter values for the epidermal growth factor receptor (EGFR), see Table 1, and assumed that the drug-related parameters were identical to the parameters of the natural ligand.

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)	(1/min)
k_{degRL}	0.15[b]	(1/min)	k_{degRD}	0.15	(1/min)

Since prior to drug application, the receptor system was assumed to be in steady-state, we used steady-state values of $2 \cdot 10^5$ receptors per cell (EGFR expression level in human mammary epithelial cells (Shankaran et al., 2007) and a ligand concentration of 10 ng/ml (Goldstein et al., 1995)) to determine the parameters k_L and k_R (with molecular weight 133.07 kD for EGF). The total drug dose was chosen as Dose =10 μg/ml (Goldstein et al., 1995). The volume V was $4 \cdot 10^{-10}$ 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 (black dashed line).

We identify two phases in Fig. 2: 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 rate constant k_{degL}. For the highest clearance rate constant $k_{degL} = 0.01$/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. Ligand accumulation is a consequence of the drug binding to the receptor such that less ligand is bound to the receptor and subsequently 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.

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 consider

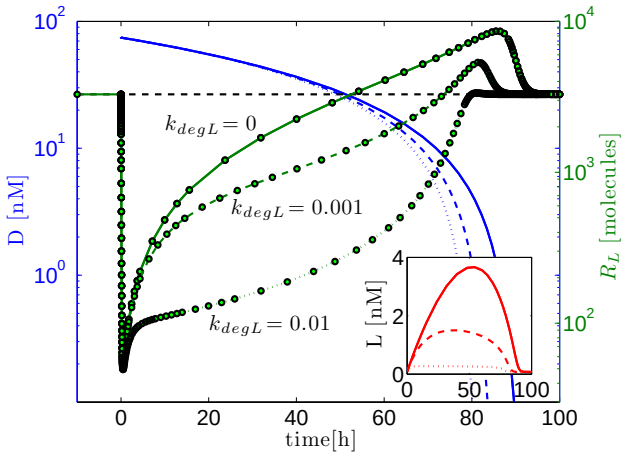

Fig. 2. Dynamic response of the number of active receptors (with circle markers) and drug concentration (without markers) after bolus dose for different ligand clearance rates k_{degL}. Inset: Ligand accumulation in the microenvironment of the cell over time.

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

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. To prevent the induction phase, one dosing strategy could be to administer a follow-up dose each before the induction phase starts. As can be inferred from Fig. 3, this is a feasible strategy, but possibly at the cost of a larger induction phase after the final dose 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.

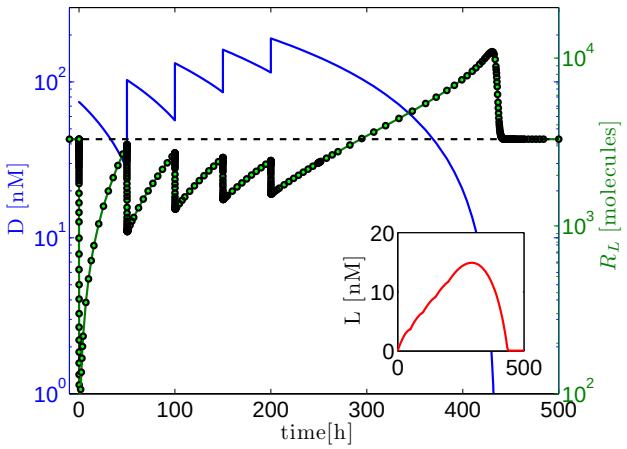

Fig. 3. Dynamic response of the number of activated receptors (solid line with circles) and drug concentration (solid line) after multiple bolus doses for $k_{degL} = 0$. Inset: Ligand accumulation in the microenvironment of the cell over time.

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. 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 rewrite the system of ODEs (1) in terms of the deviations of the species from their steady-state values. We define these incremental variables as

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

The resulting system of ODEs in terms of the incremental state vector

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

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

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

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

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

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

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

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

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

which finally implies the claimed result $E = 0$. 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

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

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

5. DISCUSSION AND CONCLUSION

Our analysis suggests that the effect of receptor antagonistic drugs could be negatively affected by the ligand accumulation in the microenvironment of the target cells.

The results show that the response of the receptor system to the drug might 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 lost activation resulting from the inhibition of the system. 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. Importantly, the counteracting effect cannot be reduced by altering the affinity of the drug or the dosing scheme, since it is independent from any drug related parameters; it is a structural property of the considered receptor class.

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 resembles those that typically arise in Control Engineering (Seron et al., 1997). The study of fundamental limitations is an extensive field of research in Control Engineering (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 an important role in the design of dosing regimes.

It is known that the microenvironment of cells in solid tumors can have a crucial influence on the success of radiotherapy (Vaupel, 2004). Our analysis suggests that the microenvironment may also influence the treatment of cancer with therapeutic proteins used as receptor inhibitors.
An analysis of the implications for *in vitro* as well as *in vivo* situations, is currently in preparation.

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