Pharmacokinetic Model of a Glucocorticoid-Binding Antibody Fusion Protein

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This document covers the design and results of the R-based quantitative systems pharmacology model "steroid-afp-dmod-model_2019-03-06_v21.R". It was originally written as the fourth chapter of my doctoral dissertation, "Biosynthetic and pharmacokinetic approaches to improve steroid therapeutics", defended in April 2019.

Abstract

Steroid hormone analogs are clinically important, but their use is limited by severe side effects. My dissertation presented two approaches to improve steroid drugs, of which this is the second. In this chapter, I considered whether a fusion protein could carry steroids to desired cells. I developed a multi-scale pharmacokinetics model to determine which features a glucocorticoid-binding antibody fusion protein requires to deliver steroid exclusively to leukocytes. The antibody's target antigen must endocytose quickly upon protein binding, but endosomal release of steroid or antibody is not helpful. The model also showed that the fusion protein could direct endogenous cortisol to leukocytes, achieving immunosuppression without any synthetic glucocorticoid.

Attributions

The anti-inflammatory antibody fusion protein was initially designed by Dr. Jeffrey Way. We agreed it would be interesting to model its action, so I set up the system and found the relevant constants from the literature. Some initial fusion protein constructs were expressed, but their pharmacokinetically-relevant parameters were never measured. This work was entirely unpublished at the time of writing in April 2019.

Introduction

Anti-inflammatory steroids efficiently treat autoimmune conditions, but have numerous side effects due to activity in off-target tissues. These drugs mimic endogenous cortisol to bind to the glucocorticoid receptor (GR) located in the cytoplasm of leukocytes, hepatocytes, and osteoblasts. The GR then translocates to the nucleus, where it decreases immune response in

leukocytes, but triggers glucose release in hepatocytes and decreases bone growth in osteoblasts.[1] This system prepares the body for stress, increasing blood sugar for fight-or-flight while decreasing costly maintenance functions. These global effects of glucocorticoids, also known as corticosteroids, prevent their long-term use outside of local application.[2] External surfaces, such as the skin and respiratory tract, can be targeted by using formulations that prevent steroids from reaching the bloodstream or steroid derivatives that degrade quickly.[3,4] Yet many common and debilitating autoimmune diseases, such as lupus or rheumatoid arthritis, require glucocorticoids to reach cells deep inside the body.

Antibody fusion proteins, which can selectively bind desired cell types to deliver a cargo domain, have resolved these targeting problems for cytokines. The strategy taken for these 'chimeric activators' could improve many classes of therapeutics. Essentially, these drugs consist of a signaling domain fused to an antibody that exclusively binds a target cell. Mutations decrease the signaling domain affinity, so the relevant receptor only activates upon antibody binding. For example, erythropoietin signaling on red blood cell precursors alleviates anemia, but erythropoietin binding to megakaryocytes increases thrombosis.[5] To improve therapeutic index, Burrill *et al.* constructed an antibody fusion protein consisting of anti-glycophorin A, which exclusively binds hematopoietic cells, and an erythropoietin with decreased receptor affinity.[6] Similarly, Garcin *et al.* made an interferon alpha-2 with decreased affinity fused to an anti-PD-L2 nanobody, which decreases leukopenia in mice while triggering STAT1 activation in select splenocytes.[7]

Antibody-drug conjugates (ADCs) can carry small molecules to desired cell types, using covalent linkers to bind the molecule to the antibody. These face several obstacles that do not apply to the chimeric activators described above. Firstly, the small molecule component of an ADC must have a site which can be modified to attach to the antibody. The covalent linker must then be cleaved to release the drug, and the small molecule must usually enter the cell to take effect.[8] The current approved ADCs are used in cancer treatment, delivering cytotoxic compounds to tumor cells.[9] However, research into anti-inflammatory ADCs is gaining traction.[10] ADCs can deliver promiscuous kinase inhibitors, such as dasatinib or PDE4 inhibitors, exclusively to leukocytes, preventing side effects.[11,12] Antibody-glucocorticoid conjugates have been studied in recent years. An anti-CD163-dexamethasone targets macrophages and is capable of sparing hepatocytes.[13,14] A carefully-designed fluticasone derivative conjugated to an anti-CD74 antibody selectively affects B cells and not T cells, even when they are co-cultured.[15]

With these concepts in mind, Jeff Way and I envisioned a glucocorticoid-carrying antibody fusion protein. This single polypeptide would consist of an anti-leukocyte single chain variable fragment (scFv) and a glucocorticoid-binding domain (Fig. 1). Glucocorticoids could be loaded onto the fusion protein, and the protein would expose only cells expressing the relevant antigen to the small molecule inside. The non-covalent glucocorticoid attachment could allow

the protein to pick up stray steroid diffusing out of the desired cells, or even to carry endogenous cortisol to the target. While this drug is not a covalent ADC, it uses an antibody to deliver a small molecule, and thus ADC-related methods are relevant to its design.

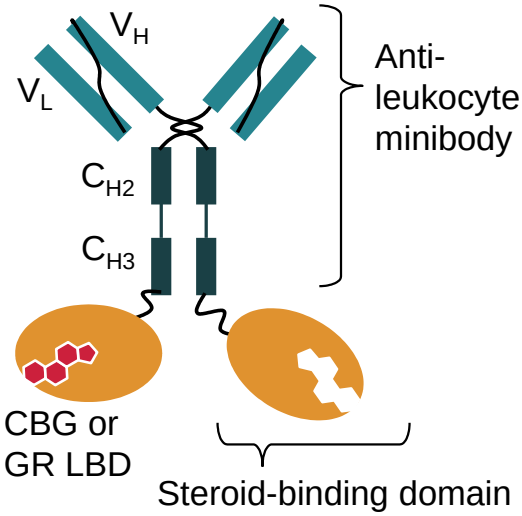

Figure 1. Schematic of a steroid targeting fusion protein.

Pharmacokinetic and molecular mechanism models can predict which features should be included in this antibody fusion protein drug. The basic design concept allows for many choices; the antibody target, the glucocorticoid, and the protein's steroid and antigen affinities could dramatically change the drug action. Modeling relevant compartment pharmacokinetics and biochemical mechanisms could direct these choices, resulting in a more effective design. Rate constants, abundances for different molecules, and compartment volumes are available from literature, which can be assembled into an ordinary differential equation (ODE) model.[16,17] Pharmacokinetic and mechanistic models of ADCs are necessarily complex. The protein component's target binding affects the distribution of different drug subspecies. The small molecule component, when freed, also has its own pharmacokinetic properties.[18] Compartment-level distributions must be integrated with subcellular compartments and processes, resulting in a multiscale systems pharmacology model for even this relatively simple system.[19]

I used the dMod package in R to model the pharmacokinetics of a glucocorticoid-binding antibody fusion protein. The protein design is distinct from traditional ADCs in that free steroid can reassociate with the protein, adding to the system complexity. This model predicts several features that would improve drug efficacy, such as targeting a fast-internalizing antigen and loading a less permeable steroid cargo. It also suggests that protein without pre-loaded steroid could be used to carry endogenous cortisol to desirable cell types.

Results

This model considers a fusion protein consisting of an anti-CD45 minibody and a glucocorticoid binding domain (Fig. 2). CD45 was selected as the antibody target because it is found on all leukocytes, and no off-target cell types, such as hepatocytes or osteoblasts.[20] The BC8 variable region was used in this initial protein design, and was chosen based on its successful delivery of radioactive compounds to CD45-expressing cancers.[21] The IgG constant fragment of the minibody enables neonatal Fc receptor recycling, and thus an extended serum

half-life.[22] The steroid-binding domain could be based on corticosteroid binding globulin (CBG) or the steroid-binding domain of GR. The model uses the steroid on- and off-rates associated with GR, as its affinities to various synthetic glucocorticoids are better characterized.[23] This protein will deliver fluticasone propionate, chosen because of its high affinity to glucocorticoid receptor and its relatively short half-life in humans.[24] A wide variety of other synthetic glucocorticoids are available, and any could theoretically be loaded into this fusion protein if different properties are required. The engineered protein will form a homodimer, with each unit binding two CD45 receptors and two glucocorticoid molecules. To simplify the model, each molecule of protein is represented as a unit capable of binding one receptor and one glucocorticoid molecule.

The R package dMod was used to generate and solve an ordinary differential equation (ODE) model, which provides a concentration over time for drug-derived molecules in on- and off-target cells.[25] The relevant cells and tissues are simplified into a system of four compartments, as is typical in pharmacokinetic models. The fusion protein begins in the 'blood' compartment and delivers steroid to 'on-target cytoplasm' via internalization into an 'on-target

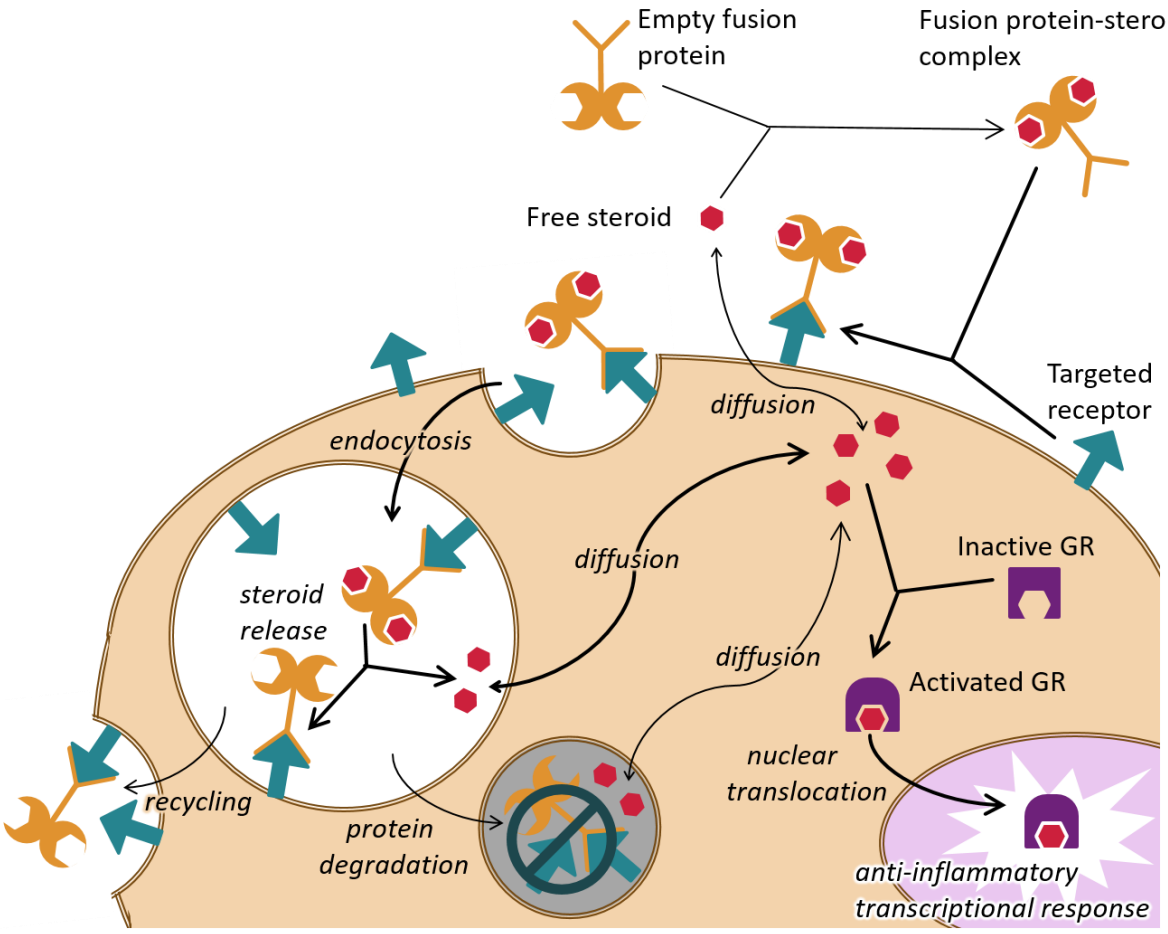

Figure 2. Schematic for relevant processes in steroid delivery via a glucocorticoid-binding antibody fusion protein.

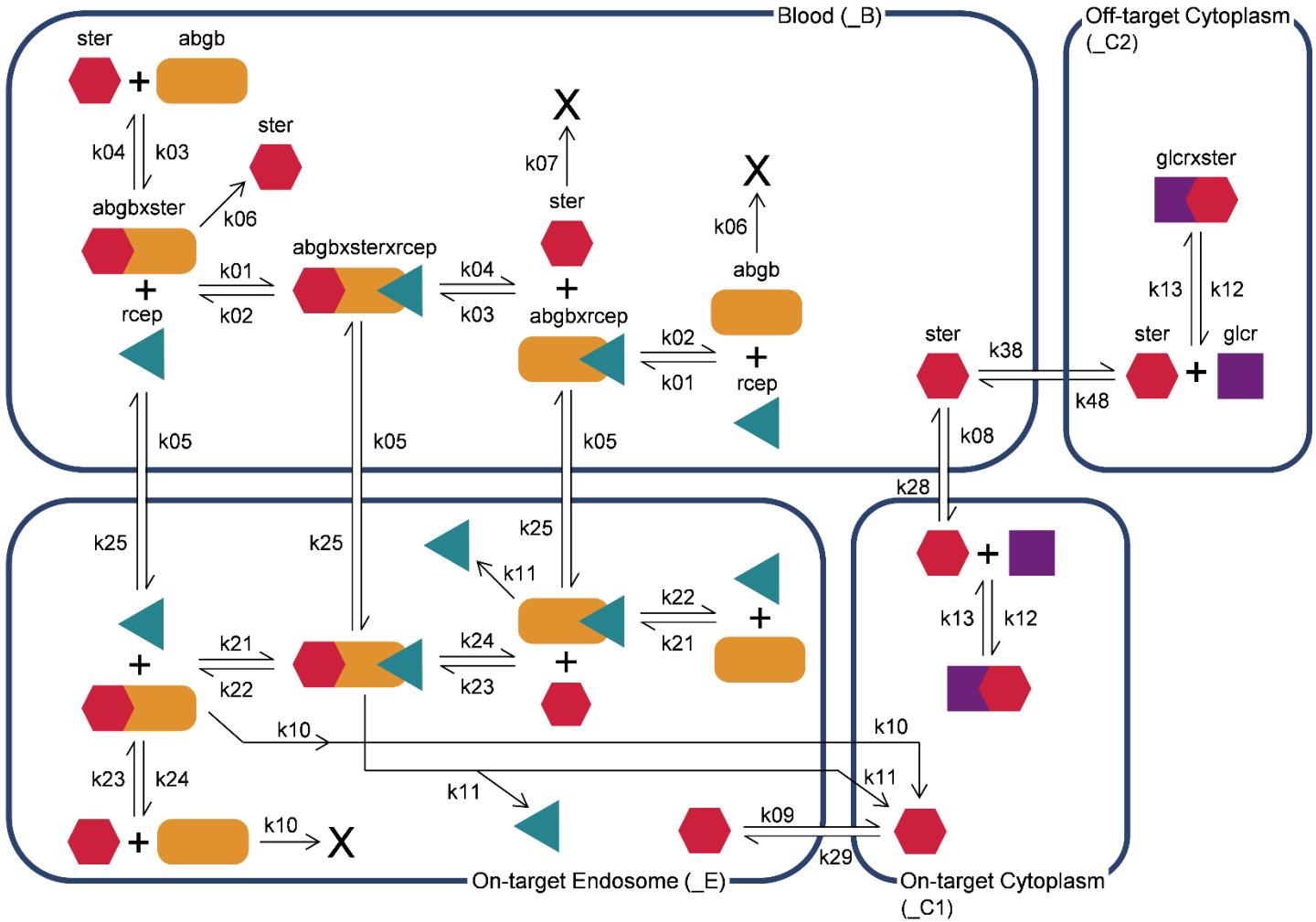

Figure 3. Diagram of the glucocorticoid-binding fusion protein pharmacokinetic model. Red hexagons represent steroid, yellow rounded rectangles represent fusion protein, teal triangles represent target receptor, and purple squares represent GR. Species may be depicted multiple times in the same compartment for clarity, but are in a shared pool in the model. A black X shows output for processes that remove all molecules involved. Labels denote the names of these species and their combinations in the code. Species names have appended suffixes denoting their compartment, as shown after the compartment name. Reversible processes are denoted by half-arrows, while irreversible degradation processes are shown as full arrows.

endosome'. Free steroid can diffuse from the 'blood' to an 'off-target cytoplasm', which has twice the volume of the 'on-target cytoplasm'. The fusion protein may bind steroid and antigen. The steroid can also bind the glucocorticoid receptor in the on- and off-target cytoplasm compartments. I used these constraints to construct the ODE model, in which eight chemical species interconvert via 39 chemical reactions with 22 rate constants (Fig. 3). The rate constant and volume parameter values have varying degrees of accuracy, internally and with respect to this application. Nevertheless, this simplified approach can suggest which aspects of a glucocorticoid-targeting antibody drug could be altered to improve its function. In the model, this efficacy is reflected in the difference in glucocorticoid receptor occupancy between on- and off-target cells.

An increased rate of target receptor endocytosis is critical to the fusion protein's ability to deliver glucocorticoid. CD45 receptor is not actively endocytosed upon antibody binding, resulting in a slow fusion protein uptake and extended half-life. Antibody fusion protein bound to CD45 spends too long in the 'blood' compartment, which allows the glucocorticoid to dissociate, diffuse across the off-target cell membrane, and bind to the off-target glucocorticoid receptor. Fortunately, there are many receptors found on leukocyte subtypes that undergo clathrin-mediated endocytosis, such as CD163, CD11a and CD74. Antibody drugs have been designed to bind these receptors, so suitable scFv sequences exist that trigger internalization and degradation.[12,13,15] Rate constants from EGFR internalization were used to represent the speed of this process, as it is well-studied. Increasing the receptor internalization rate from 5.0×10^{-4} to 5.5×10^{-3} sec^{-1} and degradation rate from 2.26×10^{-5} to 1.65×10^{-4} sec^{-1} dramatically improves the maximum on-target glucocorticoid receptor occupancy (Fig. 4A).

Histidine switching to release glucocorticoids in the endosome does not improve steroid targeting in this system. Histidine switching takes advantage of histidine protonation in the pH 5.5 environment of the endosome. Engineered proteins with correctly placed histidines will have increased dissociation rates for ligands in this compartment, potentially improving their pharmacokinetics.[26] In this system, release of antibody from the target receptor does not havemuch effect on GR occupancy, as the receptor-bound antibody degradation rate of 1.65×10^{-4} sec^{-} rate of 3.70×10^{-4} sec^{-1}. Thus it is only appropriate to consider whether histidine switching to release steroid from the fusion protein could improve targeting. Initially, the effect of a ten-fold increase in steroid-fusion protein dissociation rate was modeled. This is generous, as the largest affinity difference between pH 5.5 and pH 7.4 for a histidine switched protein is only 6.8-fold.[26] This ten-fold change made no difference in GR occupancy; a 5,000-fold increase in glucocorticoid off-rate is needed for an easily-observable change (Fig. 4B). When the dissociation rate passes 1 sec^{-1}, at an increase of 50,000 from the original rate, the occupancy behavior shifts entirely, as shown. Unfortunately, a dissociation rate increase of even 100-fold would be hard to achieve without decreasing the steroid binding at pH 7. Thus histidine switching is unlikely to improve the fusion protein performance.

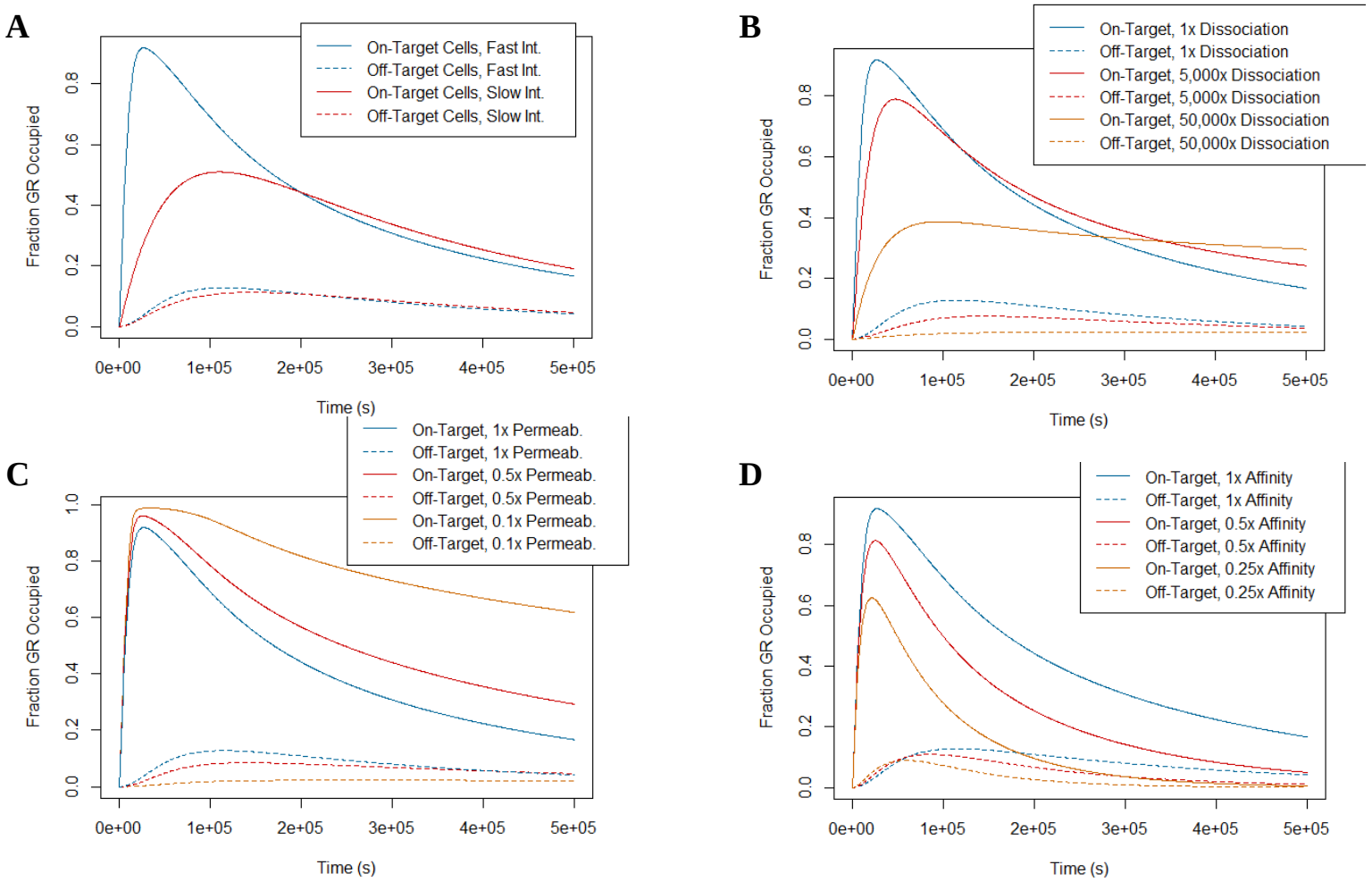

Figure 4. Simulated glucocorticoid receptor occupancy in on- and off-target tissues over time, as related to properties of the fusion protein and synthetic glucocorticoid. **A.** Increased receptor endocytosis and degradation improves the fraction of GR occupied in on-target versus off-target cells. **B.** Increased dissociation of steroid in endosome does not improve GR occupancy in on-target cells. **C.** Decreased steroid permeability, resulting in a proportional decrease in diffusion rate, increases GR occupancy in on-target cells and decreases occupancy in off-target cells. **D.** Targeted delivery decreases but is not eliminated with decreased steroid affinity, as represented by a faster steroid-fusion protein dissociation rate and a faster steroid-GR dissociation rate.

The small molecule-determined parameters of glucocorticoid receptor affinity, steroid-fusion protein affinity, and permeability can change system behavior substantially. This allows for potential improvements from a change in glucocorticoid. Decreasing steroid permeability increases targeting efficacy, such that a steroid that diffuses half as quickly increases on-target receptor occupancy (Fig. 4C). However, changing steroid parameters might necessitate selecting a molecule with less affinity for the fusion protein and the glucocorticoid receptor. Thus I considered the system tolerance to a faster off-rate from both of these binding domains. A steroid with a two-fold or even four-fold faster GR and fusion protein off-rate is capable of accumulating in on-target cells, even with the original fluticasone permeability constant (Fig. 4D). Hence this fusion protein could be loaded with different glucocorticoids if decreased permeability or other properties are required.

Fusion protein without pre-loaded steroid could use endogenous cortisol to increase on-target cell GR occupancy. This concept was considered by adding fusion protein without pre-bound steroid to a system with free cortisol in the 'blood' compartment. However, entering a 'blood' cortisol concentration of 4.14×10^{-8} M, the average in human plasma, occupied more than 60% of GR in on- or off-target cells (Fig. 5A).[27] This is likely due to the oversimplified nature of the model. In order to consider this scenario further, an initial 'blood' compartment cortisol concentration of 4×10^{-9} was used. This occupies 16% of GR in all cells when fusion protein is absent. With the assumption that total cortisol is in a steady state, the rate of free steroid degradation in the blood was set to zero. The steroid-GR on- and off-rates were adjusted for cortisol, which has a lower receptor affinity than fluticasone propionate.[28] GR occupancy in two-to-one, one-to-one, and one-to-two ratios of fusion protein to antibody are compared in Figure 5B. Increased fusion protein results in higher maximum GR occupancy and longer occupancy

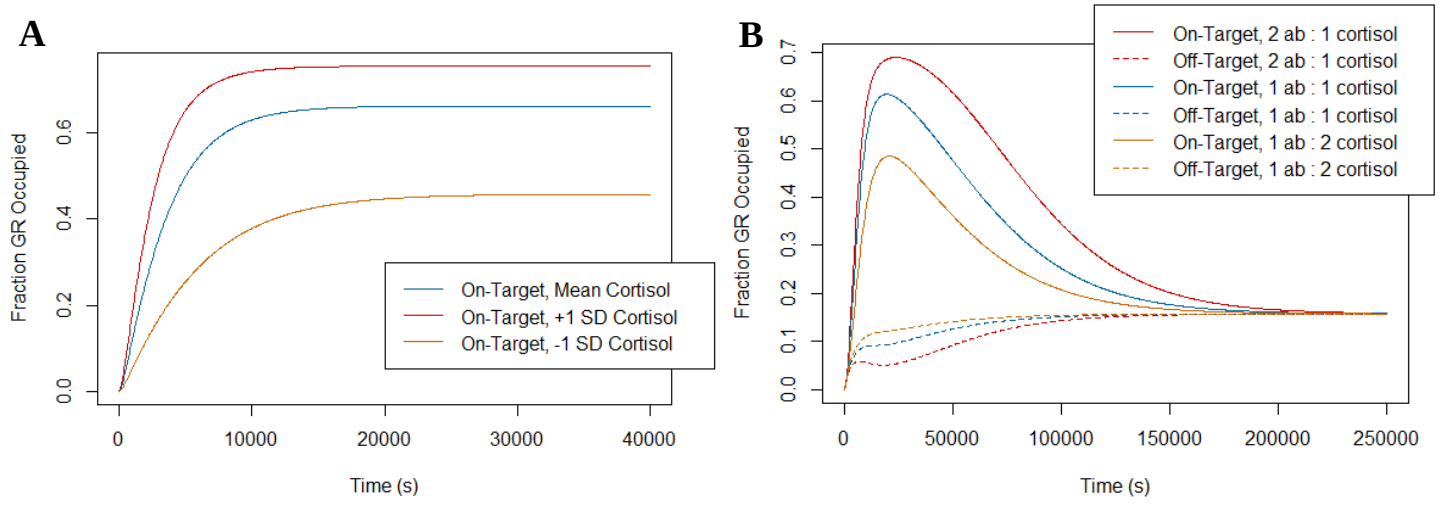

Figure 5. Simulated effect of antibody fusion protein manipulating endogenous cortisol to alter glucocorticoid receptor occupancy over time. **A.** Literature values of cortisol in blood flood GR in all cells in this model, requiring approximate lower values for downstream use. **B.** Fusion protein can carry endogenous cortisol to occupy on-target cells' GR, even when the cortisol concentration exceeds the fusion protein concentration.

difference between on- and off-target cells. While large quantities of fusion protein would be needed to bring cortisol selectively to the targeted cell type, it is theoretically feasible given these system parameters.

Discussion

This ODE model identified several important characteristics that should improve a glucocorticoid-binding antibody fusion protein. Firstly, the antigen chosen must be endocytosed quickly upon protein binding. A less-permeable glucocorticoid derivative could improve targeting, even if it had lower affinity for the receptor or the protein. Meanwhile, releasing glucocorticoid or fusion protein in the endosome via histidine switching does not increase selectivity. In addition, the fusion protein could shepherd endogenous cortisol to the antigen-bearing cell type.

The ODE model is relatively sensitive to the values of certain essential rate constants, and the available literature values might not be accurate for this system. In particular, the free steroid diffusion rate between compartments affects the GR activation cell-type specificity. This parameter depends on the surface area of the compartment, which requires accurate measures of the targeted cell type. It also requires an estimate of a permeability constant.[15,29] While there are references for all of these calculations, it is hard to ascertain if the diffusion rates are correct. In addition, the rates of target antigen endocytosis and free antibody degradation in the endosome are both rough estimates. It can be difficult to find even apparently simple characteristics, such as volume, surface area, and abundance of a given cell type. While the BioNumbers website aggregates this type of information, it was often missing the values this model required.[30]

Throughout this project, I noticed a distinct lack of software packages with support for multiscale pharmacology ODE models. The concept is not difficult, nor are the relevant mathematics, but most packages are not adapted for both separate compartments and many molecule types. The number of distinct chemical species from the fusion protein binding antigen and steroid overwhelmed MATLAB's SimBiology GUI, despite its intuitive handling of multi-compartment systems. BioNetGen's RuleBender had excellent shortcuts for indicating combinatorial chemical species, but I had trouble with compartmentalization even with expert advice.[31] In addition, the RuleBender integrator behaved oddly when working with small numbers, which are necessary when working in terms of molarity rather than molecules. After switching to R, my strongest language, I found dMod, a flexible and powerful chemical ODE generator.[25] Despite the straightforward addReaction() function, I found the documentation inaccessible. dMod also has bug in which intercompartment transfer rates must be divided by the volume of the destination compartment. I found this through trial and error when glucocorticoid failed to distribute evenly between compartments, even without other molecules in the system. Given that multi-scale pharmacokinetic models are quite easy to imagine, and the mathematics is easier still, the lack of intuitive packages is surprising.

The fusion protein modeled here was considered before a recent wave of research on antibody-glucocorticoid conjugates. Jeff Way first proposed the protein design in spring 2016, and I developed the model that September. At the time, we were only aware of the Moestrup group's 2012 paper on an anti-CD163-dexamethasone ADC.[13] In September 2016 and March 2017, the Moestrup group published further research on this drug, finding its ability to treat liver inflammation.[14,32] The aforementioned anti-CD74-fluticasone phosphonate, from a team at Merck, was published in 2018.[15] While our project halted in 2017, considerations of antibody-based methods for glucocorticoid targeting were clearly prescient.

If this project had continued, the next step would be to measure relevant biochemical and physiological constants. Once protein designs were expressed, *in vitro* interferometry experiments using the ForteBio BLItz could accurately measure rate constants for antigen and glucocorticoid binding. Rates of receptor internalization and antibody degradation after endocytosis could be measured in tissue culture by incorporating a fluorescent domain into the fusion protein. The dMod package can fit pharmacokinetic parameters, such as compartment volumes and diffusion rates, to results from *in vivo* experiments.[25] These measurements would reveal the weaknesses of the model, and accounting for these discrepancies would improve the model. The improved model could then direct new design features for the protein, creating a more efficient drug development process. Using these methods, this fusion protein could become the first non-covalent antibody-based small molecule delivery system.

Methods

The protein drug design was used to set up a pharmacokinetics compartment system for the ODE model (Table 1). The 'blood' compartment contains the total volume of blood and extracellular fluid in an average human, about 18 liters.[33] The antibody-steroid drug begins in this 'blood' compartment, as antibody drugs are typically injected. The cytoplasm of all leukocytes combined forms an 'on-target cytoplasm' compartment, which excludes the volumes of the cells' nuclei. The 'on-target cytoplasm' has a total volume of 0.059 L and a plasma membrane surface area of 13,000 dm^2, which faces the 'blood' compartment. These on-target cells have a relevant 'endosome' compartment, consisting of their pooled endosome volumes. This 'endosome' has 0.0033 L of volume and 3900 dm^2 of surface area, which separates it from the 'on-target cytoplasm'. Table 3, originally in the appendix, contains details on the calculation of these leukocyte-related values. Finally, an 'off-target cytoplasm' was defined as representing cells in which glucocorticoids have an undesirable effect. The 'off-target cytoplasm' has twice the volume and surface area of the 'on-target cytoplasm', in order to simplify comparisons. The 'off-target cytoplasm' has the same access to the 'blood' as the 'on-target cytoplasm'. There is no off-target endosome represented because without receptor, the endosome does not play a significant role in steroid distribution.

Parameter	Value	Sources	Name
Total human extracellular volume (L)	18	Davies 1993[33]	VolB
Total leukocyte cytoplasm volume in human (L)	5.9E-02	Ting-Beall 1993[34], Segel 1981[35], Schmid-Schonbein 1980[36]	VolC1
Total leukocyte endosome volume in human (L)	3.3E-03	Corlier 2015[37], Sarkar 2003[38]	VolE
Total leukocyte plasma membrane surface area in human (dm^2)	1.3E+04	Schmid-Schonbein 1980[36]	SAplasma1
Total leukocyte endosome surface area in human (dm^2)	3.9E+03	Yogurtcu 2018[39]	SAendo
Ratio of off- to on-target cells	2	N/A	OfftoOnRatio
Initial concentration of CD45 receptors in blood (M)	3.99E-09	Peyron 1991[40], Bausch-Fluck 2015[41], Matthews 1991[42]	initamts["rcep_B"]
Initial concentration of CD45 receptors in endosome (M)	6.54E-06	Peyron 1991[40], Bausch-Fluck 2015[41], Matthews 1991[42]	initamts["rcep_E"]
Initial concentration of glucocorticoid receptors in leukocytes (M)	8.99E-08	Biggin 2011[43]	initamts["glcr_C1"], initamts["glcr_C2"]
Initial concentration of antibody drug in blood (M)	1E-10	N/A	initamts["abgbxster_B"]

Table 1. Volumes, surface areas, and initial concentrations used in the ODE model of fusion protein steroid delivery. Refer to Table 3 for calculations and original literature values.

Eight chemical species are present in these compartments. Four free molecules are shown: steroid, fusion protein, antigen receptor, and glucocorticoid receptor. The fusion protein can bind or release the antigen receptor and the steroid, resulting in three more chemical species. Glucocorticoid receptor can also bind steroid, creating an occupied glucocorticoid receptor. Initial concentrations of all species are listed in Table 1. Glucocorticoid receptor is located in the cytosol of on- and off-target cells; it is assumed to be entirely free of steroid ligand at the beginning of the simulation and equally abundant in both cytosolic compartments. The ratio of occupied to total glucocorticoid receptor in a cytosol compartment is used as a proxy for steroid response.

These chemical species interconvert and move between compartments via 39 reactions, which were entered into the R package dMod.[25] The reactions use 22 rate constants, which can be found in Table 2. Some of these constants were used as-is or required only a unit conversion. For calculated values, parameter inputs and calculation methods are shown in Table 3, originally in the dissertation appendix. The code used to generate the model and compare the effects of parameter changes is in "steroid-afp-dmod-model_2019-03-06_v21.R". The chemical concentrations are in terms of molarity, volume is in liters, surface area is in dm^2, and time is in seconds. Rate constants are in sec^{-1} or $M^{-1}sec^{-1}$, as appropriate. The dMod package solves the ODE system generated by the input reactions, giving the concentrations of each species in each compartment over time.

Rate Constant	Value	Units	Source	Name
Antibody-receptor association rate	1.00E+06	$L\ mol^{-1}\ sec^{-1}$	Raman 1992[44]	k01, k21
Antibody-receptor dissociation rate	3.86E-04	sec^{-1}	Matthews 1991[42], Lin 2006[21]	k02, k22
Glucocorticoid binding domain-steroid association rate for fluticasone propionate	3.98E+04	$L\ mol^{-1}\ sec^{-1}$	Hogger 1994[23]	k03, k23
Glucocorticoid binding domain-steroid dissociation rate for fluticasone propionate	2.20E-05	sec^{-1}	Hogger 1994[23]	k04, k24
Glucocorticoid binding domain-steroid association rate for cortisol	3.76E+04	$M^{-1}\ sec^{-1}$	Eliard 1984[28]	k03, k23
Glucocorticoid binding domain-steroid dissociation rate for cortisol	7.71E-04	sec^{-1}	Eliard 1984[28]	k04, k24
Receptor internalization rate for actively endocytosed receptor	5.50E-03	sec^{-1}	Sigismund 2008[45]	k05 (fast)
Receptor externalization rate for actively endocytosed receptor	1.83E-02	sec^{-1}	Sigismund 2008[45]*	k25 (fast)
Receptor internalization rate for passively endocytosed receptor	5.00E-04	sec^{-1}	Alberts 2002[46]	k05 (slow)
Receptor externalization rate for passively endocytosed receptor	1.67E-03	sec^{-1}	Alberts 2002[46]*	k25 (slow)
Antibody drug degradation rate in blood compartment	2.01E-06	sec^{-1}	Keizer 2010[22]	k06
Steroid degradation rate in blood compartment for fluticasone propionate	3.08E-05	sec^{-1}	Allen 2013[24]	k07
Steroid degradation rate in blood compartment for cortisol	0	sec^{-1}	Assuming constant total cortisol amount	k07
Steroid diffusion rate from blood to on-target cytoplasm	1.61E-06	$M^{-1}\ sec^{-1}$	Brandish 2018[15]*	k08
Steroid diffusion rate from on-target cytoplasm to blood	4.91E-04	$M^{-1}\ sec^{-1}$	Brandish 2018[15]*	k28
Steroid diffusion rate from blood to off-target cytoplasm	6.44E-06	$M^{-1}\ sec^{-1}$	Brandish 2018[15]*	k38
Steroid diffusion rate from off-target cytoplasm to blood	9.83E-04	$M^{-1}\ sec^{-1}$	Brandish 2018[15]*	k48
Steroid diffusion rate from on-target cytoplasm to endosome	2.70E-08	$M^{-1}\ sec^{-1}$	Brandish 2018[15]*	k09
Steroid diffusion rate from endosome to on-target cytoplasm	4.83E-07	$M^{-1}\ sec^{-1}$	Brandish 2018[15]*	k29
Free antibody degradation rate in endosome	3.70E-04	sec^{-1}	Huotari 2011[47]	k10

Rate Constant	Value	Units	Source	Name
Receptor degradation rate in endosome for actively endocytosed receptor	1.65E-04	sec^{-1}	Sigismund 2008[45]	k11 (fast)
Receptor degradation rate in endosome for passively endocytosed receptor	2.26E-05	sec^{-1}	Minami 1991[48]	k11 (slow)
Steroid to glucocorticoid receptor association rate for fluticasone propionate	3.98E+04	M^{-1} sec^{-1}	Hoffer 1994[23]	k12
Steroid to glucocorticoid receptor dissociation rate for fluticasone propionate	2.20E-05	sec^{-1}	Hoffer 1994[23]	k13
Steroid to glucocorticoid receptor association rate for cortisol	3.76E+04	M^{-1} sec^{-1}	Eliard 1984[28]	k12
Steroid to glucocorticoid receptor dissociation rate for cortisol	7.71E-04	sec^{-1}	Eliard 1984[28]	k13

Table 2. Rate constants used in the ODE model of fusion protein steroid delivery. Asterisks indicate calculations more complex than unit conversions from the literature; refer to Table 3 for more information.

ID	Parameter	Value	Units	Source
A	Number of granulocytes in human body	4.55E+10	cells	Athens 1961[49]
B	Number of lymphocytes in human body	4.60E+11	cells	Trepel 1974[50]
C	Number of monocytes in human body	4.40E+09	cells	Vander 2001[51] and Alberts 2002[46]; assuming half of total population is in the blood
D	Volume of single granulocyte	300	μm^3	Ting-Beall 1993[34]
E	Volume of single lymphocyte	210	μm^3	Segel 1981[35]
F	Volume of single monocyte	240	μm^3	Schmid-Schonbein 1980[36]
G	Fraction of granulocyte volume in the cytoplasm	0.627		Schmid-Schonbein 1980[36]
H	Fraction of lymphocyte volume in the cytoplasm	0.516		Schmid-Schonbein 1980[36]
I	Fraction of monocyte volume in the cytoplasm	0.681		Schmid-Schonbein 1980[36]
J	Total leukocyte cytoplasm volume in body	5.91E-02	L	((A*D*G)+(B*E*H)+(C*F*I)) *1E-15
K	Endosome volume of single granulocyte	6.435	μm^3	Corlier 2015[37]
L	Endosome volume of single lymphocyte	6.435	μm^3	Corlier 2015[37]
M	Endosome volume of single monocyte	10	μm^3	Sarkar 2003[38]
N	Total leukocyte endosome volume in body	3.30E-03	L	((A*K)+(B*L)+(C*M))*1E-15
O	Surface area of a single granulocyte	300	μm^2	Schmid-Schonbein 1980[36]
P	Surface area of a single lymphocyte	250	μm^2	Schmid-Schonbein 1980[36]
Q	Surface area of a single monocyte	450	μm^2	Schmid-Schonbein 1980[36]
R	Total leukocyte plasma membrane surface area in body	1.31E+04	dm^2	((A*O)+(B*P)+(C*Q))*1E-10
S	Ratio of plasma membrane surface area of leukocyte to endosome surface area	0.3		Yogurtcu 2018[39] (estimate)
T	Total leukocyte endosome surface area in body	3.92E+03	dm^2	R*S
U	Apparent permeability coefficient for fluticasone propionate in lymphocytes	2.10E-09	$dm*sec^{-1}$	Brandish 2018[15]

ID	Parameter	Value	Units	Source
V	Diffusion rate formula for fluticasone propionate	See k08, k28, k38, k48, k09, and k29	sec^{-1}	U*surface area *destination compart vol
W	Formula for receptor externalization rate (assuming constant total amount on surface and endosome)	See k25	sec^{-1}	Internalization rate*(R/T)
X	Number of glucocorticoid receptors in a single macrophage	1.30E+04	molecules	Biggin 2011[43]
Y	Concentration of glucocorticoid receptors in leukocyte cytoplasm	8.99E-08	M	(X*1E15)/(F*6.022E23)
Z	Number of CD45 receptors on a single cell from a leukocyte cell line	1.00E+05	molecules	Peyron 1991[40], Bausch-Fluck 2015[41], Matthews 1991[52]
α	CD45 receptors per um^2 of membrane	3.33E+02	molecules $*um^{-2}$	Z/D
β	Concentration of CD45 receptors in blood	3.99E-09	M	(α*1E10*R)/(6.022E23*18)
γ	Concentration of CD45 receptors in endosome	6.54E-06	M	(α*1E10*T)/(6.022E23*N)

Table 3. Calculations for volumes, surface areas, initial amounts, and select rate constants used in the ODE model for glucocorticoid-binding fusion protein pharmacokinetics. Replacement of a source with equations indicate the ID letters of the relevant constants. References to 'k' numbers indicate a rate constant or set of constants that can take multiple values due to variation in inputs; this avoids showing repetitive arithmatic. All of the final 'k' values are in Table 2.

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