Mechanisms of Subcutaneous Absorption of Rituximab in Rats

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ABSTRACT

Absorption of monoclonal antibodies (mAbs) after s.c. injection results from the interplay among several kinetic processes. The aims of this study were to investigate the absorption mechanisms of rituximab in rats by using slow s.c. infusion and coadministration with nonspecific IgG or hyaluronidase, and to evaluate the predictive performance of the pharmacokinetic model previously developed to describe the nonlinear absorption behavior of mAbs. Rituximab serum concentrations were measured after s.c. co-administration with nonspecific IgG and hyaluronidase to rats. Several dose levels and different injection sites were evaluated. For the back site, 6.5- and 2.6-fold decreases in the area under the concentration-time curve were obtained after coadministration with IgG for 1 and 10 mg/kg doses compared with administration of rituximab alone. For the abdomen, only a minor reduction in concentrations was observed. Hyaluronidase increased the rate of s.c. absorption and the bioavailability (1.9- and 1.6-fold for the back and the abdomen injection of 10 mg/kg). Our previously established pharmacokinetic model provided excellent predictions of the effect of nonspecific IgG on rituximab absorption. In conclusion, the magnitude of the effect of absorption modifiers is dependent on the site of injection and the dose level of rituximab. Pharmacokinetic profiles further support the hypothesis that neonatal Fc receptor-mediated transport is a major determinant of s.c. absorption of mAbs.

Introduction

Parenteral administration represents a major limitation to treatment with therapeutic proteins. There is a substantial interest in utilizing s.c. delivery for monoclonal antibodies (mAbs) to improve patient convenience and potentially reduce treatment costs (Aue et al., 2010; Bittner and Schmidt, 2012; Richter et al., 2012). Mechanisms of mAb absorption after s.c. administration are not fully understood but are influenced by several kinetic processes, including transport through the extracellular matrix, uptake by the blood and lymphatic capillaries, and presystemic elimination (Porter and Charman, 2000; Swartz, 2001; Kagan et al., 2007). Experimental perturbation coupled with mathematical modeling is needed to resolve the relative contribution of these components to overall mAbs absorption.

We systematically investigated several factors that influence absorption behavior of rituximab in rats (Kagan et al., 2012). The anatomic site of injection had a major impact on both the rate and extent of absorption, which is consistent with pharmacokinetic studies for other proteins (Beshyah et al., 1991; Macdougall et al., 1991; Kota et al., 2007). An inverse correlation was found between the dose level and rituximab bioavailability, and it was hypothesized to result from saturation of neonatal Fc receptor (FcRn)-mediated protective binding at the absorption site. Moreover, pharmacokinetic modeling suggested that this binding interaction might serve as one of the absorption mechanisms for mAbs.

The role of binding to FcRn in modifying the systemic half-life of mAbs is well known (Oganesyan et al., 2009; Yeung et al., 2009). This extended half-life might also translate into enhanced drug efficacy as effective drug concentrations are maintained for longer durations (Zalevsky et al., 2010). Several studies suggest that FcRn is also an important determinant in mAbs absorption. For example, the s.c. bioavailability of 7E3 IgG1 was 3-fold higher in wild-type compared with FcRn-deficient mice (Wang et al., 2008). Increased affinity of mAbs to FcRn at pH 6.0 was associated with greater s.c. bioavailability, whereas an increased affinity at pH 7.4 led to a decrease in s.c. absorption in mice (Deng et al., 2010, 2012). In contrast, no definitive effect of altering FcRn affinity on s.c. bioavailability was found for a series of IgG4 variants in cynomolgus monkeys (Datta-Mannan et al., 2012).

The movement of macromolecules through the extracellular matrix is highly restricted and might represent a rate-limiting step in protein absorption (Swartz, 2001). Coadministration of compounds that modify the local interstitial milieu might be useful for optimizing the rate and extent of protein absorption. For example, s.c. injection of albumin was shown to enhance overall bioavailability and to reduce the rate of absorption kinetics of recombinant human interferon-β (Bocci et al., 1986b). It was proposed that albumin acts as an interstitial fluid expander and thereby promotes lymphatic uptake. Similarly, the s.c. injection of recombinant human interferon-α2 with albumin or hyaluronidase increased thoracic lymph recovery 2- and 8-fold compared with injection of drug alone (Bocci et al., 1986a). Hyaluronidase has been evaluated for enhancing the s.c. delivery of fluids and several drugs (Bookbinder et al., 2006; Frost, 2007).

The first aim of this study was to investigate the absorption mechanisms of rituximab in rats by using slow s.c. infusion and coadministration with nonspecific IgG or hyaluronidase. A range of rituximab dose levels and different injection sites were evaluated. The

ABBREVIATIONS: AUC, area under the concentration-time curve; FcRn, neonatal Fc receptor; mAb, monoclonal antibody.
second aim was to evaluate the predictive performance of the pharmacokinetic model previously developed to describe the nonlinear absorption behavior of mAbs. Rituximab, a chimeric anti-CD20 IgG1, was used as a model drug (Reff et al., 1994). CD20 antigen is expressed on the surface of normal and malignant human B-lymphocytes, and rituximab is clinically used for a variety of lymphoid malignancies (Plosker and Figgitt, 2003). Rats do not express human CD20 antigen; therefore, rituximab pharmacokinetics is not affected by the antigen-antibody interaction in this species.

Materials and Methods

Animals. Male Wistar rats, weighing 350–375 g, were purchased from Harlan Laboratories, Inc. (Indianapolis, IN). Two animals were housed in each cage during the study with free access to standard food and water and were maintained on a 12/12-hour light/dark cycle. Rats were allowed to acclimate for 1 week before study initiation. This study was conducted in accordance with an approved protocol by the Institutional Animal Use and Care Committee at the University at Buffalo, State University of New York.

Experimental Procedure. Animals were divided into eight groups (n = 4–5 each) according to the route of administration, dose level, and coadministered drug (Table 1). In one group, animals received a single i.v. dose of rituximab (1 mg/kg; Rituxan, 10 mg/ml; Genentech, Inc., San Francisco, CA) and a single s.c. dose of IgG (500 mg/kg; Gammagard Liquid, human IgG 10%; Baxter Healthcare Corporation, Westlake Village, CA) at the lower back region. Intravenous injection of rituximab was followed by 200 μl of normal saline to ensure delivery of the entire dose. In six other groups, each animal received a single dose of rituximab at the dose level of 1 or 10 mg/kg by s.c. injection. In addition, each animal was given a s.c. injection of either 500 mg/kg IgG or 100 IU/kg hyaluronidase (Vitrase, ovine hyaluronidase injection, 200 USP U/ml; ISTA Pharmaceuticals, Inc., Irvine, CA). Subcutaneous injection was performed at the lower back or middle abdomen region using a 27-G needle. For the 1 mg/kg dose of rituximab, the drug was diluted 10-fold with sterile normal saline. In the last group, rituximab was administered as a 10-day constant rate infusion to a s.c. space at the back using an osmotic pump (Alzet; Durect Corporation, Cupertino, CA). The implantation and removal of the osmotic pump was performed under isoflurane anesthesia (IsoThesia; Butler Healthcare Corporation, Westlake Village, CA) at the lower back region. Intravenous injection of rituximab was followed by 200 μl of normal saline to ensure delivery of the entire dose. In six other groups, each animal received a single dose of rituximab at the dose level of 1 or 10 mg/kg by s.c. injection. In addition, each animal was given a s.c. injection of either 500 mg/kg IgG or 100 IU/kg hyaluronidase (Vitrase, ovine hyaluronidase injection, 200 USP U/ml; ISTA Pharmaceuticals, Inc., Irvine, CA). Subcutaneous injection was performed at the lower back or middle abdomen region using a 27-G needle. For the 1 mg/kg dose of rituximab, the drug was diluted 10-fold with sterile normal saline. In the last group, rituximab was administered as a 10-day constant rate infusion to a s.c. space at the back using an osmotic pump (Alzet; Durect Corporation, Cupertino, CA). The implantation and removal of the osmotic pump was performed under isoflurane anesthesia (IsoThesia; Butler Animal Health Supply, Dublin, OH).

Serial blood samples (40 μl) were obtained after drug administration (up to 5 weeks) from the saphenous vein under isoflurane anesthesia using nonheparinized microhematocrit tubes (Fisherbrand; Thermo Fisher Scientific, Pittsburgh, PA). Blood was allowed to clot at room temperature for 30–60 minutes, and serum was separated by centrifugation at 2000g for 20 minutes at 4°C. Serum was divided into aliquots and stored at −80°C until analysis.

Analytical Assay. A sandwich enzyme-linked immunosorbent assay was used to quantify rituximab concentrations in rat serum samples, as described previously (Kagan et al., 2012). Briefly, rat anti-rituximab IgG2a (clone MB2A4; AbD Serotec, Raleigh, NC) and goat anti-human IgG–peroxidase conjugate (Fc specific; Sigma-Aldrich, St. Louis, MO) were used as capturing and detection antibodies. The plate was developed using o-phenylenediamine (SigmaFast OPD; Sigma-Aldrich), and optical density was measured at 492 nm. Samples and standards were prepared at 1:100, 1:1000, or 1:10,000 dilution using phosphate-buffered saline + 1% bovine serum albumin and analyzed in duplicate. The working range of the assay was between 0.25 and 62.5 ng/ml. The calibration curves were fitted with a four-parameter logistic equation.

Pharmacokinetic Modeling. The pharmacokinetics of rituximab was evaluated first using the model structure from our previous study (Fig. 1, model A) (Kagan et al., 2012). Briefly, rituximab and nonspecific IgG are

![Fig. 1. Proposed model structures for rituximab pharmacokinetics in rats. Both models include rituximab absorption of free drug and the drug-receptor complex (Kagan et al., 2012). In model A, systemic disposition follows a standard two-compartment model with linear elimination. In model B, systemic distribution and elimination follows the model proposed by Hansen and Balthasar (2003) and is based on binding of IgG to FcRn. Further details on the parameters are available in Materials and Methods. DR, drug-receptor complex; R, free receptor; RTX, rituximab.](image-url)
introduced at the s.c. injection site ($A_{inj}$) and transferred to an absorption site by a first-order process ($k_{abs}$). At the absorption site, free antibody ($ABS_{free}$) can bind to a receptor ($R_{free}$) to form a complex ($DR = ABS_{tot} - ABS_{free}$), which was assumed to occur rapidly and characterized by the equilibrium dissociation constant ($K_D$). For simplicity, the same $K_D$ was assumed for rituximab and IgG.

In this case, the total antibody amount at the absorption site ($ABS_{tot}$) is given by the sum of the amounts of rituximab and the other IgG, and the rituximab first-order elimination ($kel$).

Systemic disposition of rituximab includes nonspecific distribution ($k_{deg}$) and first-order elimination ($kel$). The antibody-receptor complex delivers antibodies to the systemic circulation by a first-order process ($k_E$). The amount of total receptor at the absorption site was assumed to remain constant with time.

Systemic disposition of rituximab included nonspecific distribution ($k_{deg}$) and first-order elimination ($kel$), and the system of differential equations that define the model is as follows (eqs. 1–5):

$$\frac{dA_{inj}}{dt} = -k_{abs} \cdot A_{inj}$$  \hspace{1cm} (1)

$$\frac{dABS_{free}}{dt} = k_{abs} \cdot A_{inj} - \left( k_{deg} + k_{E} \right) \cdot ABS_{free} - k_{E} \cdot \left( ABS_{tot} - ABS_{free} \right)$$  \hspace{1cm} (2)

$$ABS_{free} = 0.5 \cdot \left[ \left( ABS_{tot} - R_{tot} - K_D^2 \right) \right.$$  
$$+ \sqrt{\left( ABS_{tot} - R_{tot} - K_D^2 \right)^2 + 4 \cdot K_D^2 \cdot ABS_{tot}} \left. \right]$$  \hspace{1cm} (3)

$$\frac{dC_{RTX}}{dt} = k_{a1} \cdot f_{RTX} \cdot \frac{ABS_{free}}{V_c} + k_{a2} \cdot f_{RTX} \cdot \frac{ABS_{tot} - ABS_{free}}{V_c}$$  
$$- \left( k_{E} + k_{12} \right) \cdot C_{RTX} + k_{12} \cdot A_{RTX}$$ \hspace{1cm} (4)

$$\frac{dA_{RTX}}{dt} = k_{12} \cdot C_{RTX} \cdot V_c - k_{21} \cdot A_{RTX}$$ \hspace{1cm} (5)

where $C_{RTX}$ is the rituximab concentration in the central compartment (with volume of $V_c$), $A_{RTX}$ is the amount of rituximab in the peripheral distribution compartment, and $k_{12}$ and $k_{21}$ are the first-order transfer rate constants between central and peripheral compartments. For intravenous administration, $C_{RTX}(0)$ was set equal to dose/$V_c$, and the initial conditions for eqs. 1, 2, and 5 were set to zero. For s.c. administration, $A_{inj}(0)$ was set equal to total antibody dose (1 or 10 mg/kg of rituximab with or without 500 mg/kg of nonspecific IgG), and the initial conditions for eqs. 2, 4, and 5 were set to zero.

To further evaluate the effect of IgG coadministration with rituximab, the model was modified to incorporate the FcRn-mediated endosomal recycling of antibodies at the systemic level (Fig. 1, model B) according to the model proposed by Hansen and Balthasar (2003). It was assumed that endogenous IgG does not influence the absorption kinetics of rituximab. Equations 4 and 5 were replaced by equations describing systemic disposition and elimination of rituximab (RTX), and exogenous IgG (IgG) and endogenous IgG (Endo) (eqs. 6–12):

$$\frac{dC_{RTX}}{dt} = k_{a1} \cdot f_{RTX} \cdot \frac{ABS_{free}}{V_c} + k_{a2} \cdot f_{RTX} \cdot \frac{ABS_{tot} - ABS_{free}}{V_c}$$  
$$- k_{deg} \cdot C_{RTX} + k_{ret} \cdot C_{E,F} \cdot (1 - f_a)$$ \hspace{1cm} (6)

$$\frac{dC_{RTX}}{dt} = k_{up} \cdot C_{RTX} - k_{int} \cdot C_{E,F} \cdot (1 - f_a) - k^E_{deg} \cdot C_{E,F} \cdot f_a$$ \hspace{1cm} (7)

$$\frac{dC_{IgG}}{dt} = k_{a1} \cdot f_{IgG} \cdot \frac{ABS_{free}}{V_c} + k_{a2} \cdot f_{IgG} \cdot \frac{ABS_{tot} - ABS_{free}}{V_c}$$  
$$- k_{deg} \cdot C_{IgG} + k_{ret} \cdot C_{E,F} \cdot (1 - f_a)$$ \hspace{1cm} (8)

$$\frac{dC_{Endo}}{dt} = k_{up} \cdot C_{Endo} - k_{int} \cdot C_{E,F} \cdot (1 - f_a) - k^E_{deg} \cdot C_{E,F} \cdot f_a$$ \hspace{1cm} (9)

$$\frac{dC_{Endo}}{dt} = k_{enj} \cdot k_{up} \cdot C_{Endo} + k_{ret} \cdot C_{E,F} \cdot (1 - f_a)$$ \hspace{1cm} (10)

where $C$ and $C_E$ represent antibody concentration in central and endosomal compartments. Antibodies enter the endosomal compartment with a first-order rate constant ($k_{enj}$), where they can bind to the FcRn receptor with the affinity ($K_D^2$). For simplicity, the same affinity is assumed for rituximab and endogenous and exogenous IgG. The total antibody concentration is calculated as $C_{tot} = C_{RTX} + C_{IgG} + C_{Endo}$, and $f_u$ is the unbound fraction. In the endosomal compartment, free antibodies are degraded with a first-order process ($k_{deg}$), and bound antibodies are returned to the central compartment with a first-order rate constant ($k_{ret}$).
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process \( k_{inj} \). For i.v. administration, \( C_{ENDO}^{RXN} \) was set equal to dose/\( V_h \), \( A_{END} \) was set to zero for groups that did not receive IgG and to 500 mg/kg for groups that received IgG, and the initial conditions for eqs. 7, 8, and 9 were set to zero. For s.c. administration, \( A_{END} \) was set equal to the total antibody dose (1 or 10 mg/kg of rituximab with or without a 500 mg/kg dose of nonspecific IgG), and the initial conditions for eqs. 6, 7, 8, and 9 were set to zero. The initial condition for eq. 10 is given by the steady-state concentration of endogenous antibodies \( C_{SS}^{ss} \), and the initial condition for eq. 11 is defined as shown in eq. 13:

\[
C_{ENDO}^{RXN} = \frac{C_{SS}^{ss} \cdot k_{ret} \cdot (1 - f_{unss}) + k_{EE} \cdot f_{unss}}{k_{uu}}
\]

where \( f_{unss} \) is the unbound fraction of endogenous antibodies at steady state.

The production rate of endogenous antibodies is a secondary parameter:

\[
k_{uu} = k_{uu}^{d} \cdot f_{unss} - C_{ENDO}^{RXN}
\]

The fixed values for \( k_{uu}^{d} \), \( R_{SS}^{SS} \), and \( C_{SS}^{ss} \) were obtained from the literature (Hansen and Balthasar, 2003).

Data Analysis. A standard noncompartmental analysis was performed for each individual rituximab concentration-time profile using Phoenix WinNonlin 6.1 software (Pharsight, Mountain View, CA). The maximum plasma drug concentration \( C_{max} \) and time to reach \( C_{max} (T_{max}) \) were obtained directly from the experimental data. Terminal half-life, area under the concentration-time curve from time zero to infinity (AUC, calculated by linear trapezoidal method), mean residence time, volume of distribution at steady state \( V_{ss} \), and clearances were calculated. The bioavailability after s.c. administration was calculated by dividing individual AUC values by the mean AUC after i.v. administration of the same dose level. Profiles and parameters were compared with pharmacokinetic data in rats from a previous study, in which rituximab was administered without absorption modifiers by i.v. and s.c. routes (Kagan et al., 2012).

Pharmacokinetic parameters between more than two groups were compared using a one-way analysis of variance, followed by a Tukey multiple comparisons test where appropriate. Comparisons between two groups were conducted using the two-tailed t test. A P value of less than 0.05 was considered statistically significant. Data are presented as mean ± S.D., unless stated otherwise.

Initially, serum pharmacokinetic profiles of rituximab after s.c. administration of rituximab with IgG were simulated using model A, and parameters were fixed to previously estimated values (Kagan et al., 2012). The effects of coadministration with hyaluronidase and slow s.c. infusion were subsequently evaluated by fitting the model and estimating only the parameters that were hypothesized to be affected by these modes of administration. Finally, the effect of coadministration with IgG on rituximab pharmacokinetics was evaluated by fitting and simulation using model B.

Model fitting and parameter estimation were performed using MATLAB R2008a software (The MathWorks, Natick, MA) and the maximum likelihood method. The variance model was defined as \( \text{VAR}_i = (\sigma_i + \sigma_j Y(\theta, t_i)) \), where \( \text{VAR}_i \) is the variance of the \( i \)th data point, \( \sigma_i \) and \( \sigma_j \) are the variance model parameters, and \( Y(\theta, t_i) \) is the \( i \)th predicted value from the pharmacokinetic model. The goodness of fit was assessed by system convergence, Akaike information criterion, estimator criterion value for the maximum likelihood method, and visual inspection of residuals and fitted curves.

Results

The mean serum concentration-time profiles of rituximab injected s.c. at the back (1 and 10 mg/kg) and abdomen (10 mg/kg) regions in combination with 500 mg/kg of nonspecific IgG are presented in Fig. 2. The corresponding parameters obtained by noncompartmental analysis are listed in Supplemental Tables 1 and 2. Pharmacokinetic profiles of rituximab administered s.c. without IgG for both injection sites are shown for comparison [data from Kagan et al., (2012)]. For the back injection site, coadministration with IgG resulted in significantly lower serum rituximab concentrations compared with administration of the drug alone. This effect was more pronounced for the low dose compared with the high dose, with 6.5- and 2.6-fold decreases in the AUC, respectively. For the abdomen injection site, coadministration with IgG affected rituximab serum concentrations to a lesser extent (around a 5% decrease in the AUC). Rituximab concentration-time profiles simulated with model A using previously estimated parameters (Table 2) (Kagan et al., 2012) were in excellent agreement with the observed data, with the exception of initial time points for the abdomen site (Fig. 2C). This discrepancy might be related to a higher total volume of injection when rituximab is given with IgG because a similar effect of volume on \( C_{max} \) was observed for injection at the foot site (Kagan et al., 2012).

TABLE 2

Final pharmacokinetic model-estimated parameters

<table>
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<tr>
<th>Parameter</th>
<th>Units</th>
<th>Model A</th>
<th>%CV</th>
<th>Model B</th>
<th>%CV</th>
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</table>

%CV, percent coefficient of variation; N/A, not applicable.

* Fixed to values previously published by Kagan et al. (2012).

* Fixed to values previously published by Hansen and Balthasar (2003).
The mean serum concentration-time profiles of rituximab injected s.c. at the back (1 and 10 mg/kg) and abdomen (10 mg/kg) regions in combination with 100 U/kg of hyaluronidase are presented in Fig. 3. The corresponding parameters obtained by noncompartmental analysis are also listed in Supplemental Tables 1 and 2. Pharmacokinetic profiles of rituximab administered without hyaluronidase for both injection sites are shown for comparison [data from Kagan et al., (2012)]. Coadministration with hyaluronidase resulted in an enhanced absorption of rituximab. For injection at the back, the increase in serum concentrations was higher for the 10 mg/kg dose compared with 1 mg/kg (1.9- and 1.2-fold increase in AUC, respectively). The extent of absorption was greater from the abdomen versus the back injection site. However, for the 10 mg/kg dose, hyaluronidase had a greater relative effect for the back compared with the abdomen (1.9- and 1.6-fold increase in AUC). The observed data were well captured using model A, and the absorption rate constant for free rituximab (in the presence of hyaluronidase) was estimated while other parameters were fixed to previously obtained values (Table 2).

The mean serum concentration-time profile of rituximab administered as a 10-day s.c. infusion at the back (40 mg/kg) is presented in Fig. 4. The corresponding parameters obtained by noncompartmental analysis are listed in Supplemental Table 3. Pharmacokinetics of rituximab after s.c. bolus injection (40 mg/kg) is shown for comparison [data from Kagan et al. (2012)]. Infusion of rituximab resulted in slower absorption and an overall greater bioavailability (2.5-fold increase in AUC). Infusion concentration-time profiles were simulated using model A and previously estimated parameters. The shape of the pharmacokinetic profile obtained by using $k_{a1}$ that was estimated for rituximab given alone (0.063 day$^{-1}$) was consistent with the data, but the magnitude of the effect was underpredicted (Fig. 4, long dashed line). However, a greater rate constant value (which was estimated for rituximab administered with hyaluronidase, $k_{a1} = 0.373$ day$^{-1}$) resulted in a simulation that was in a good agreement with the observed data (Fig. 4, short dashed line). Simulations indicate that surgical implantation of the osmotic pump might have disrupted the normal structure of the s.c. space.

Fig. 5 shows mean serum concentration-time profiles of rituximab after i.v. injection (10 mg/kg) in combination with 500 mg/kg of IgG injected s.c. at the back, along with i.v. profiles for rituximab given alone [data from Kagan et al. (2012)]. The corresponding parameters obtained by noncompartmental analysis are listed in Supplemental Table 4. The concentrations of rituximab administered with IgG were only slightly less than the corresponding concentrations when rituximab was given alone (statistically significant from day 2 to 21). Model B was used to characterize the data and results of fitting and simulation are presented in Fig. 5. A simulation using parameters from Hansen and Balthasar (2003) was unable to capture the data, because the half-life was significantly underestimated (data not shown). Model B was used to simultaneously fit all three i.v. data sets (with $K_D$, $K_{int}$, and $C_{ss}$ fixed to previously published values), and a satisfactory fit was obtained (Fig. 5). Finally, model B was used to re-fit all s.c. data sets for rituximab given alone. The model fitted profiles superimposed with those obtained with model A (not shown) and estimated absorption parameters were similar to prior estimates (Table 2).

Simulations were performed to illustrate the relative contribution of the absorption pathways to rituximab s.c. bioavailability after various modes of administration (Fig. 6). For rituximab given alone, receptor-mediated transport accounts for absorption of approximately 57, 22, and 9% of the dose for 1, 10, and 40 mg/kg dose levels, respectively. Coadministration with IgG almost completely abrogated this pathway, whereas hyaluronidase enhanced the absorption of unbound rituximab.

**Discussion**

In this study, a therapeutic antibody was coadministered with hyaluronidase or excess amount of nonspecific IgG to quantitatively assess the role of FcRn binding and interstitial properties on s.c. absorption. Administration of test antibodies with an excess amount of IgG (such as IVIG formulations) has been used to study the contribution of FcRn-mediated protection from endosomal degradation on systemic elimination of mAbs. Coadministration of IgG (1 g/kg i.v.) led to an increase in clearance of 7E3 mAb in wild-type mice but not in FcRn-deficient animals (Hansen and Balthasar, 2002). Our data show that a 500 mg/kg dose of IgG given s.c. has only a minor effect on systemic distribution and elimination of rituximab. This can be
attributed to the relatively small contribution of s.c. IgG to the overall serum concentration of antibodies. A low bioavailability is predicted by the model for a 500 mg/kg s.c. dose, whereas the endogenous IgG concentration in rats is substantially greater (1.67–15.5 g/l or 11–103 μM) (al-Bander et al., 1992; Salauze et al., 1994). Simulations using model B suggested that doses greater than 2 g/kg of i.v. IgG would be required to significantly affect pharmokinetics of rituximab (data not shown). Similar absorption parameters were estimated by both models (Table 2); thus, for simplicity, model A was used for further evaluation of the effect of mode of administration on s.c. absorption of rituximab.

The s.c. bioavailability of rituximab in rats decreases with increasing dose levels (Kagan et al., 2012). This is consistent with the hypothesis that the binding to a receptor can provide protection from degradation at the level of the absorption site, and the developed mathematical model further supported this assumption. Other studies suggest that altering affinity to FcRn might alter the bioavailability of mAbs (Deng et al., 2010, 2012), and s.c. bioavailability appears lower in FcRn-deficient mice compared with wild-type animals (Wang et al., 2008). In this work, rituximab injected s.c. with an excess amount of nonspecific IgG showed decreased absorption for both evaluated dose levels, and further corroborates the hypothesis of FcRn-mediated absorption of mAbs. Moreover, the extent of absorption modulation was successfully predicted by our pharmacokinetic model for different dose levels and injection sites.

Transport of macromolecules through the interstitium at the s.c. space is dependent on physical and electrostatic interactions with the components of the matrix (e.g., fibrous collagen network and glycosaminoglycans) (Porter and Charman, 2000; Swartz, 2001). Hyaluronan, one of the glycosaminoglycans in the hypodermis, has a rapid turnover rate, and hyaluronidase enzymes can be used to disrupt the glycosaminoglycan matrix in a reversible manner (Frost, 2007). Recombinant human hyaluronidase (rhPH20) increases the dispersion of locally injected drugs (Bookbinder et al., 2006), has been tolerated well in clinical studies (Pirrello et al., 2007), and is being investigated for the s.c. delivery of large fluid volumes that might be required for mAb formulations (Frost, 2007; Bittner and Schmidt, 2012). An increase in rituximab bioavailability was observed after coadministration of hyaluronidase, which was in good agreement with theoretical expectations (Fig. 3). The observed data were successfully captured by model A using parameters obtained from fitting to the pharmokinetics of rituximab given alone, except for the absorption rate constant of free drug ($k_{a1}$), which was estimated separately for each injection site. Hyaluronidase administration resulted in an increase in $k_{a1}$ of around 6- and 11-fold for the back and abdomen sites (Table 2), which might be attributed to a difference in morphology of the s.c. space between the two anatomic locations. Simulations show that the fraction of rituximab absorbed through the linear pathway increases more than 3-fold compared with rituximab given alone (Fig. 6). Hyaluronidase not only appears useful for increased volumes of injected mAb formulations, but also affects the rate of absorption and increases bioavailability.

Rituximab absorption decreases with increasing dose levels, and its bioavailability after a bolus s.c. injection of 40 mg/kg at the back was only 18% compared with 69% for a 1 mg/kg dose (Kagan et al., 2012). It was hypothesized that extending the duration of delivery time (i.e., controlled release) would prevent saturation of the absorption mechanism and thereby enhance drug uptake. The pharmokinetic simulation for a s.c. infusion of a 40 mg/kg dose over a 10-day period using parameters obtained for rituximab administered alone predicted that serum concentrations should be comparable with or higher than those after bolus injection after approximately 6 days of the infusion (Fig. 4, long dashed line). According to the model, the bioavailability would have been enhanced by 34%, mostly due to increase in transport of the bound rituximab (Fig. 6, simulation for infusion with $k_{a1} = 0.063$). Interestingly, the observed enhancement of absorption was greater than that predicted by the model. Although the exact reason for this is unknown, one potential explanation is that the surgical procedure required for implantation of the osmotic pump may have disrupted the natural structure of the s.c. space (similar to the effect of hyaluronidase). To support this hypothesis, another simulation of rituximab kinetics that included the absorption rate of free rituximab in the presence of hyaluronidase was performed. The simulated profile is in a good agreement with the observed data (Fig. 4, short dashed line), and the amount of rituximab absorbed by both pathways increases (Fig. 6, simulation for infusion with $k_{a1} = 0.373$).
The pharmacokinetic models in this study are based on the assumption that the test antibody and endogenous and exogenous IgG share the same binding affinity to FcRn. This simplifying assumption has been used elsewhere (Hansen and Balthasar, 2003; Garg and Balthasar, 2007) and allows for the implementation of a rapid-binding solution for the ligand-receptor binding interaction, where the unbound and bound drug fractions can be obtained by solving a quadratic equation (see eqs. 3 and 12). However, competition among exogenous and endogenous IgG to FcRn (due to differences in binding affinities) might lead to a more complex absorption and distribution behavior, especially when human mAbs are evaluated in animal species. Moreover, the rates of association and dissociation of IgG to/from FcRn are pH dependent. Studies with mutant variants of wild-type IgG have clearly shown that a balance between binding at pH 6 and 7.4 is important for optimization of pharmacokinetic behavior (Deng et al., 2010, 2012; Datta-Mannan et al., 2012).

The translation of preclinical pharmacokinetics of peptides and proteins after s.c. administration to clinical practice will require further research to identify sources of interspecies differences in drug absorption. In this study, the relative contribution of absorption pathways in rituximab pharmacokinetics in rats was evaluated. Multiple factors could influence human drug absorption, including clinically relevant dose levels and injection sites, interspecies differences in the structure of s.c. space and antibody-FcRn interactions, and the presence of a therapeutic target for mAbs (McDonald et al., 2010; Richter et al., 2012). For example, s.c. absorption of golimumab was similar from three injection sites (upper arm, abdomen, and thigh) in humans (Xu et al., 2010). However, the development of a meaningful structural model and identification of species-specific parameters might result in a model-based framework for predicting human pharmacokinetics of s.c. administered peptide and protein therapeutics from preclinical studies.

In conclusion, mechanisms of s.c. absorption of mAbs in rats were tested by the coadministration of exogenous nonspecific IgG and a modifier of the interstitial matrix. The magnitude of the effect of absorption modifiers was dependent on the site of injection and the dose level of rituximab. These data further support the hypothesis that FcRn-mediated transport is a major determinant of s.c. absorption of mAbs. The mathematical model of rituximab absorption demonstrated good predictive performance and may be further used for developing s.c. delivery strategies for mAbs.

Fig. 6. Simulated percent of the rituximab dose absorbed as free (solid line) or bound (dashed line) drug after s.c. administration at the back using model A and associated parameters (Table 2).
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Authorship Contributions

Participated in research design: Kagan, Mager.

Conducted experiments: Kagan.

Performed data analysis: Kagan, Mager.

Wrote or contributed to the writing of the manuscript: Kagan, Mager.

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