Published in final edited form as:

J Pharmacokinet Pharmacodyn. 2016 June ; 43(3): 291–304. doi:10.1007/s10928-016-9472-2.

Minimal physiologically-based pharmacokinetic (mPBPK) model for a monoclonal antibody against interleukin-6 in mice with collagen-induced arthritis

Xi Chen^{1,3}, Xiling Jiang³, William J. Jusko¹, Honghui Zhou^{2,3}, and Weirong Wang³

³Biologics Clinical Pharmacology, Janssen R&D, 1400 McKean Road, Spring House, PA 19447, USA

Abstract

Therapeutic monoclonal antibodies (mAb) targeting soluble inflammatory cytokines exert their pharmacological effects in rheumatoid arthritis through binding and neutralizing free cytokines in target tissue sites. Therefore suppression of free cytokines in such sites directly relates to the magnitude of therapeutic response. Although the interrelationships between mAb and cytokines have been examined in the systemic circulation, less is known about the interaction of mAb and cytokines in inflamed joints. In the present study, the interplay between the mAb, CNTO 345, and its target IL-6 in serum as well as ankle joint synovial fluid were characterized in collagen-induced arthritic mice. A minimal physiologically-based pharmacokinetic model with target-mediated drug disposition (TMDD) features in serum and ankle joint synovial fluid was developed for the assessment of the TMDD dynamics of CNTO 345 and IL-6. Our model indicates that TMDD kinetics in ankle joints differ greatly from that in serum. The differences can be attributed to the limited tissue distribution of CNTO 345 in ankle joint synovial fluid, the significant rise of the IL-6 baseline in ankle joint synovial fluid in comparison with serum, and the relative time-scales of elimination rates between CNTO 345, free IL-6 and CNTO 345-IL-6 complex in serum and ankle joint synovial fluid.

Keywords

Monoclonal antibody; Soluble cytokine; Minimal PBPK; Tissue distribution; Target-mediated drug disposition in serum and synovial fluid

Introduction

Soluble cytokines, such as TNF- α , IL-1 and IL-6, play pivotal roles in the pathogenesis of a variety of inflammatory diseases including rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) [1, 2]. Development of monoclonal antibodies (mAb) that target and

Correspondence to: Weirong Wang.

¹Present Address: Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, NY, USA

²Present Address: Quantitative Science, Janssen R&D, Spring House, PA, USA

Electronic supplementary material: The online version of this article (doi:10.1007/s10928-016-9472-2) contains supplementary material, which is available to authorized users.

inhibit these cytokines has gained remarkable therapeutic success [3]. Soluble cytokines serve as mediators during the initiation and propagation of inflammatory responses. They are systemically and locally secreted by tissue cells, macrophages and mast cells. Through autocrine and paracrine mechanisms, they promote inflammatory responses in target tissues [4]. Therapeutic monoclonal antibodies exert their pharmacological effects by specifically binding to the soluble cytokines in the target tissue sites, neutralizing them, and blocking their pro-inflammatory activity.

The binding process and subsequent disposition of mAbs and their targets have been well characterized with target-mediated drug disposition (TMDD) kinetics [5–7]. In a previous study, the binding and disposition kinetics of siltuximab and IL-6 in plasma was characterized with a pharmacokinetic (PK)/target engagement (TE) model via simultaneous fitting of total siltuximab, and free and total IL-6 concentration profiles in cynomolgus monkeys [8]. However, the interplay of mAb and its target is likely to be different in various tissue sites. One of the reasons is that mAb have limited tissue distribution. Thus the exposure of mAb in tissue interstitial spaces is much less than that in plasma. Also, the baseline concentrations of many soluble cytokines are elevated in the inflamed tissues [9, 10]. Moreover, the elimination of cytokines in plasma is believed via catabolism, while in tissues, lymphatic drainage might be their dominant egress pathway.

As the suppression of free cytokines in target tissues directly correlates with the efficacy of therapeutic mAbs, it is important to examine the tissue distribution of mAbs as well as their interactions with target cytokines therein to make reliable assessment of pharmacological responses. However, little is known about the binding and disposition kinetics of mAbs and soluble cytokines in tissue sites.

The second-generation minimal physiologically-based pharmacokinetic (mPBPK) model introduced by Cao and Jusko [11] provides a modeling approach to assess tissue distribution of mAb. The mPBPK model inherits the essential components of mAb pharmacokinetics from full PBPK models. The model includes plasma, lymph, and two lumped tissue spaces (tight or leaky) based on their vascular endothelial structure (continuous or fenestrated) connected in an anatomical manner. Para-vascular convection and lymph drainage are assumed to be the dominant mechanisms for mAbs movement from plasma to tissue sites and return to plasma. Tissue interstitial space is assumed the major extravascular distribution space. Moreover, the mPBPK model has the flexibility to incorporate additional tissue compartments when measurements are available for tissues of interest. More importantly, the mPBPK model has the feasibility to implement TMDD kinetics in both plasma and tissue compartments [12]. Such models can serve to examine the interrelationship between mAb and its target entities either in the systemic circulation or in tissues of interest.

The collagen-induced arthritis (CIA) mouse model is the most commonly studied autoimmune model of rheumatoid arthritis. Compared to healthy mice, CIA mice resemble human rheumatoid arthritis with inflamed ankle joint and paw edema. A mechanistic PK/PD study was recently conducted to assess the binding and disposition kinetics of a mAb and its soluble target IL-6 in the systemic circulation and, more importantly, in target tissue sites in CIA mice [13]. Here, an mPBPK modeling approach incorporating TMDD was applied to

quantitatively assess the kinetics and interrelationship between CNTO 345 and IL-6 in both serum and joint lavage fluid.

Materials and methods

Test articles

A rat anti-mouse IL-6 mAb, CNTO 345, was produced at Janssen R&D, LLC (Spring House, PA, USA) and used in the study.

Animal study design and sample collection

The CIA mice were randomly assigned to 3 groups ($n = 42 \times 3$) and given single intravenous (IV) bolus injections of CNTO 345 at doses of 0.3, 3, and 30 mg/kg. Another group of healthy controls ($n = 42$) was given an IV bolus dose of 30 mg/kg CNTO 345. Serial blood samples were collected at various time points via retro-orbital bleeding (at 8 h, and 2, 5 and 10 days) and terminally via cardiac puncture (at 2 h, 1, 3, 7, 14 and 21 days). Ankle joint lavage samples were collected at the terminal time points with flush using 100 μ L of PBS containing 0.1 % BSA for each joint. The fluid samples were then collected and pooled from both hind ankles. To determine IL-6 baseline concentrations, blood and joint lavage samples were obtained from another group of CIA and healthy mice ($n = 7 \times 2$) without mAb dosing.

Bioanalytical methods

The concentrations of CNTO 345 in serum and joint lavage were quantified by a fluorescence-based immunoassay on the GyrolabTMxP platform (Gyros AB, Uppsala, Sweden). The lower limits of quantification (LLOQ) for serum and joint lavage CNTO 345 concentrations were 20 and 2 ng/mL. The total and free IL-6 in serum and joint lavage fluid were determined using an electrochemiluminescence-based immunoassay (ECLIA) using the Meso Scale Discovery (MSD[®]) platform (Meso Scale Discovery, Rockville, MD, USA) with LLOQs of 27.4 and 8.23 pg/mL for total and free IL-6 concentrations in serum and 27.4 and 6.17 pg/mL for total and free IL-6 concentrations in joint lavage samples. The details of the bioanalytical assays were described in a separate publication [13].

Pharmacokinetics of CNTO 345 and its interplay with IL-6 in serum and joint

The mPBPK modeling approach with TMDD [11] was applied to characterize the pharmacokinetics of CNTO 345 and its interplay with IL-6 in serum and joint fluid. A stepwise modeling strategy was adopted for model development as illustrated in Fig. 1. The serum concentrations of CNTO 345 were used to describe the pharmacokinetics of mAb with the second-generation mPBPK model in the first step (Fig. 1a). Then more model complexities were incorporated stepwise by including additional datasets (Fig. 1b–d). An ‘ankle joint synovial fluid’ tissue compartment was incorporated to characterize the distribution of CNTO 345 to the ankle joint in Step III (Fig. 1c). The interactions between CNTO 345 and IL-6 in serum and ankle joint were examined with the TMDD feature [12] in serum and ankle joint synovial fluid in Step II and Step IV (Fig. 1b, d). Parameters estimated in the prior step were fixed for model fitting in each subsequent step.

Step I: Serum pharmacokinetics of CNTO 345

Serum concentration profiles of CNTO 345 in healthy control (30 mg/kg) and CIA mice (0.3, 3 and 30 mg/kg) were examined. The model includes serum, lymph, and two lumped tissue compartments (leaky and tight based on vascular endothelium structures) connected in an anatomical manner (Fig. 1a). CNTO 345 was assumed to be eliminated from serum. The model was described by:

$$\frac{dC_s}{dt} = \frac{C_{lymph} \cdot L - C_s \cdot L_1 \cdot (1 - \sigma_1) - C_s \cdot L_2 \cdot (1 - \sigma_2) C_s \cdot CL_s}{V_s} \quad (1)$$

$$C_s(0) = \frac{Dose}{V_s}$$

$$\frac{dC_{tight}}{dt} = \frac{C_s \cdot L_1 \cdot (1 - \sigma_1) - L_1 \cdot (1 - \sigma_L) \cdot C_{tight}}{V_{tight}} \quad (2)$$

$$C_{tight}(0) = 0$$

$$\frac{dC_{leaky}}{dt} = \frac{C_s \cdot L_2 \cdot (1 - \sigma_2) - L_2 \cdot (1 - \sigma_L) \cdot C_{leaky}}{V_{leaky}} \quad (3)$$

$$C_{leaky}(0) = 0$$

$$\frac{dC_{lymph}}{dt} + \frac{L_1 \cdot (1 - \sigma_L) \cdot C_{tight} + L_2 \cdot (1 - \sigma_L) \cdot C_{leaky} - C_{lymph} \cdot L}{V_{lymph}} \quad (4)$$

$$C_{lymph}(0) = 0$$

where C_s is the total concentration of CNTO 345 in serum, C_{tight} and C_{leaky} are concentrations of CNTO 345 in interstitial fluid (*ISF*) in two types of lumped tissues categorized by continuous and fenestrated vascular endothelium, and C_{lymph} is the concentration of CNTO 345 in lymph. The V_{tight} ($0.65 \text{ ISF } K_p$, where K_p is the available fraction of *ISF* for antibody distribution) and V_{leaky} ($0.35 \text{ ISF } K_p$) are *ISF* volumes of the two lumped tissues [11]. The V_{lymph} is lymph volume, which is assumed equal to blood volume. The L is total lymph flow rate and L_1 and L_2 account for 1/3 and 2/3 of the total lymph flow. The σ_1 and σ_2 are vascular reflection coefficients for leaky and tight tissues. The σ_L is the lymphatic capillary reflection coefficients and is assumed to be 0.2. Mixed linear and Michaelis–Menten (MM) clearance was assumed to account for nonlinear

pharmacokinetics of CNTO 345 in CIA mice. Since healthy controls received one single dose, linear pharmacokinetics was assumed for simplicity.

For CIA mice:

$$CL_s = CL_{CIA} + \frac{V_{max}}{K_m + C_s} \quad (5a)$$

For healthy control mice:

$$CL_s = CL_{ctrl} \quad (5b)$$

where CL_{CIA} and CL_{ctrl} represent the linear clearances in CIA and healthy control animals, and the V_{max} and K_m terms account for the nonlinear elimination.

Step II: TMDD of CNTO 345 with IL-6 in serum

The interplay of CNTO 345 and IL-6 in serum was characterized using TMDD kinetics (Fig. 1b). Serum concentration–time profiles of total CNTO 345 as well as free and total IL-6 in CIA mice (0.3, 3 and 30 mg/kg) were assessed. Due to significant suppression of the free IL-6 at the highest dose (30 mg/kg), the free IL-6 concentrations were all below the LLOQ (BLQ) during the early time points. Therefore only concentrations of free IL-6 after day 14 in CIA mice at 30 mg/kg were included in model fitting. The IL-6 concentrations in control mice were BLQ and free IL-6 concentrations remained BLQ following CNTO 345 administration for most time points, therefore data from healthy mice were excluded. The model was described as follows:

$$\begin{aligned} \frac{dC_s}{dt} &= \frac{C_{lymph} \cdot L - C_{free} \cdot (L_1 \cdot (1 - \sigma_1) + L_2 \cdot (1 - \sigma_1) + CL_s)}{V_s} - k_{int} \cdot AR \\ C_s(0) &= \frac{Dose}{V_s} \end{aligned} \quad (6)$$

$$\begin{aligned} \frac{dC_{tight}}{dt} &= \frac{C_{free} \cdot L_1 \cdot (1 - \sigma_1) - C_{tight} \cdot L_1 \cdot (1 - \sigma_L)}{V_{tight}} \\ C_{tight}(0) &= 0 \end{aligned} \quad (7)$$

$$\frac{dC_{leaky}}{dt} = \frac{C_{free} \cdot L_2 \cdot (1 - \sigma_2) - C_{leaky} \cdot L_2 \cdot (1 - \sigma_L)}{V_{leaky}} \quad (8)$$

$$C_{leaky}(0) = 0$$

$$\frac{dC_{lymph}}{dt} = \frac{C_{tight} \cdot L_1 \cdot (1 - \sigma_L) + C_{leaky} \cdot L_2 \cdot (1 - \sigma_L) - C_{lymph} \cdot L}{V_{lymph}} \quad (9)$$

$$C_{lymph}(0) = 0$$

$$\frac{dR_{total}}{dt} = k_{syn} - k_{deg} \cdot (R_{total} - AR) - k_{int} \cdot AR \quad (10)$$

$$R_{total}(0) = 0$$

where C_{free} is the free CNTO 345 concentration in serum, the R_{total} and AR are the total IL-6 and the CNTO 345-IL-6 complex concentrations in serum, k_{syn} and k_{deg} are the rate constants for IL-6 biosynthesis and degradation in serum, and k_{int} refers to the elimination rate constant of CNTO 345-IL-6 complex in serum.

The formed CNTO 345-IL-6 complex is also supposed to circulate through plasma and tissues. However, CNTO 345-IL-6 complex accounted minimally to total CNTO 345 concentrations across the entire sampling time and all dose groups (less than 5 % as indicated by the ratio of free and total CNTO 345 concentration, see supplemental materials). In our model, tissue distribution of CNTO 345-IL-6 complex was not included for simplification purpose.

Assuming quasi-equilibrium condition, C_{free} can be described as [7]:

$$C_{free} = 0.5 \cdot ((C_s - K_{ss} - R_{total}) + \sqrt{(C_s - K_{ss} - R_{total})^2 + 4 \cdot C_s \cdot K_{ss}}) \quad (11)$$

The K_{ss} is the steady-state constant defined as:

$$K_{ss} = \frac{k_{int} + k_{off}}{k_{on}} \quad (12)$$

where the k_{on} and k_{off} refer to the CNTO 345-IL-6 association and dissociation rate constants. AR can be described as:

$$AR = \frac{R_{total} \cdot C_{free}}{K_{ss} + C_{free}} \quad (13)$$

Step III: Distribution of CNTO 345 to the ankle joint

The distribution kinetics of CNTO 345 in ankle joint was characterized by incorporating an ‘ankle joint synovial fluid’ compartment (Fig. 1c). Together with Eqs. 6–13, the model addition was:

$$\frac{dC_{sf}}{dt} = \frac{C_{free} \cdot L_{joint} \cdot (1 - \sigma_{joint}) - C_{sf} \cdot L_{joint} \cdot (1 - \sigma_L)}{V_{joint}} \quad (14)$$

$$C_{sf}(0) = 0$$

where C_{sf} is the concentration of CNTO 345 in ankle joint synovial fluid, L_{joint} and V_{joint} are lymphatic flow and volume of synovial fluid in a single ankle joint. The quantity of CNTO 345 in joint synovial fluid is minimal. Therefore the impact of CNTO 345 outflow from ankle joint on serum CNTO 345 is negligible and was not included.

The volume of synovial fluid in the mouse ankle joint could not be found in the literature. The volume of synovial fluid (V_{joint}) of a single ankle joint was estimated in healthy mice based on the relationship between V_{joint} , articular cartilage surface area (AC), and synovial membrane surface area (SM) as reported previously [14]:

$$AC:SM:V_{joint} = 100 \text{ cm}^2:200 \text{ cm}^2:2 \text{ ml} \quad (15)$$

The AC and SM values in mouse ankle joints were obtained from the literature [15]. In CIA mice, it is commonly assumed that inflammation produces leakier synovial membranes and retention of synovial fluid. However, there is no quantitative correlation between synovial fluid volume in healthy and inflamed joints. We assumed that the synovial fluid volume is increased by twofold in the inflamed joints (see “Discussion” section).

Concentration profiles of CNTO 345 in joint lavage in healthy controls (30 mg/kg) and CIA mice (3 and 30 mg/kg) were applied for model fitting. As anticipated, most of the CNTO 345 concentrations in joint lavage in 0.3 mg/kg dose group in CIA mice were BLQ, and they were not used for modeling fitting. The joint lavage sampling procedure caused dilution of CNTO 345 concentration. Therefore the concentration of CNTO 345 in joint lavage (C_{jl}) was:

$$C_{jl} = \frac{C_{sf}}{dil} \quad (16)$$

where the dilution factor is: $dil = V_{buffer}/V_{joint}$

The V_{buffer} is the total volume of buffer (100 μ L for one single ankle joint) used for rinsing the ankle joint capsule.

Step IV: TMDD of CNTO 345 with IL-6 in ankle joint

The interplay of CNTO 345 and IL-6 in ankle joints was characterized with a TMDD feature (Fig. 1d). Joint lavage concentration profiles of total CNTO 345 at 3 and 30 mg/kg doses, free IL-6 at 0.3 and 3 mg/kg doses, and total IL-6 at 0.3, 3 and 30 mg/kg doses in CIA mice were used for model fitting. Together with Eqs. 6–14, the model was described as:

$$\frac{dC_{sf}}{dt} = \frac{C_{free} \cdot L_{joint} \cdot (1 - \sigma_{joint}) - C_{free_j} \cdot L_{joint} \cdot (1 - \sigma_L) - k_{int} \cdot AR_j \cdot V_{joint}}{V_{joint}} C_{sf}(0) = 0 \quad (17)$$

$$\frac{dR_{total_j}}{dt} = k_{syn_j} - k_{deg_j} \cdot (R_{total_j} - AR_j) - k_{int_j} \cdot AR_j \quad (18)$$

$$R_{total_j}(0) = 0$$

where C_{free_j} is the free CNTO 345 concentration in the joint synovial fluid, R_{total_j} and AR_j are the total IL-6 and the CNTO 345-IL-6 complex concentrations in the joint synovial fluid, k_{syn_j} is the rate constant for IL-6 biosynthesis, k_{deg_j} is the degradation rate constant of IL-6 in the ankle joint, and k_{int_j} refers to the elimination rate constant of CNTO 345-IL-6 complex in joint synovial fluid. The remaining symbols are the same as previously described.

Assuming quasi-equilibrium condition, the free CNTO 345 concentration in ankle joint synovial fluid was described as:

$$C_{free_j} = 0.5 \cdot \left((C_{joint} - K_{ss_j} - R_{total_j}) + \sqrt{(C_{joint} - K_{ss_j} - R_{total_j})^2 + 4 \cdot C_{joint} \cdot K_{ss_j}} \right) \quad (19)$$

where K_{ss_j} is the steady-state constant in ankle joint defined as:

$$K_{ss_j} = \frac{k_{int_j} + k_{off}}{k_{on}} \quad (20)$$

The k_{on} and k_{off} refer to the association and dissociation rate constants of CNTO 345 and IL-6. The CNTO 345-IL-6 complex concentration in joint synovial fluid (AR_j) was described as:

$$AR_j = \frac{R_{total_j} \cdot C_{free_j}}{K_{ss_j} + C_{free_j}} \quad (21)$$

Owing to dilution of synovial fluid during joint lavage process, the binding equilibrium between CNTO 345 and IL-6 will shift. A portion (α) of mAb-IL-6 complex will dissociate to reach a new equilibrium condition. This was described as:

$$\begin{aligned} & \frac{1}{dil} \cdot (C_{free_j} + \alpha \cdot AR_j) \cdot \frac{1}{dil} \cdot (R_{total_j} - AR_j + \alpha \cdot AR_j) \quad (22) \\ & = \frac{1}{dil} \cdot K_D \cdot (AR_j - \alpha \cdot AR_j) \end{aligned}$$

Solving Eq. 22,

$$\begin{aligned} & \alpha \\ & = \frac{-(R_{total_j} - AR_j + C_{free_j} + dil \cdot K_D) + \sqrt{(R_{total_j} - AR_j + C_{free_j} + dil \cdot K_D)^2 + 4 \cdot AR_j \cdot (dil - 1) \cdot K_D}}{2 \cdot AR_j} \end{aligned} \quad (23)$$

where K_D is the in vitro equilibrium dissociation constant:

$$K_D = \frac{k_{off}}{k_{on}} \quad (24)$$

Joint lavage sampling is considered an in vitro process. Therefore K_D instead of K_{ss} was applied.

The free concentration of IL-6 in joint lavage samples (R_{free_jl}) is:

$$R_{free_jl} = \frac{R_{total_j} - AR_j + \alpha \cdot AR_j}{dil} \quad (25)$$

The total concentration of IL-6 in joint lavage samples (R_{total_jl}) is:

$$R_{total_jl} = \frac{R_{total_j}}{dil} \quad (26)$$

Model fitting and analysis

Model fittings were performed with NONMEM version 7.2 (ICON Development Solutions, Ellicott City, MD, USA) using naïve pooling of data. Since data were from serial destruction and being pooled together, between-subject variability (IIV) was not considered and the omega matrix was fixed to zero. The first-order (FO) method was used. The variance model used is

$$V_i = (\sigma_a + \sigma_p \cdot Y_i)^2 \quad (27)$$

where V_i is the variance of the i th observation, σ_a and σ_p are additive and proportional variance model parameters, Y_i is the i th model prediction. Measurements BLQ were treated as missing values. Model performance was evaluated by goodness-of-fit plots and objective function values (OFV). A nonparametric bootstrap was conducted with 100 replicates in order to obtain uncertainty in the parameter estimates. The R version 2.15.3 (Free Software Foundation, Boston, MA, USA) was used for producing graphs.

Results

CNTO 345, free and total IL-6 concentrations in serum and joint lavage samples

The Step I to Step IV model fitting results, as well as the observed total CNTO 345, and free and total IL-6 concentration versus time profiles in serum and joint lavage samples are shown in Figs. 2, 3, 4 and 5.

CNTO 345 exhibited higher clearance in CIA mice compared to healthy controls (Fig. 2). Non-compartmental analysis showed that CNTO 345 exhibited nonlinear pharmacokinetics in CIA mice and the clearance also decreased ~twofold when the dose of CNTO 345 increased from 0.3 to 30 mg/kg. The ratios of CNTO 345 concentrations in joint lavage and serum ranged between 0.01 and 1 % (compare Figs. 2, 4). Since the preparation process of joint lavage involved considerable dilution of synovial fluid, the actual CNTO 345 concentration in synovial fluid would be much higher.

The baseline IL-6 concentrations in serum were at least tenfold elevated in CIA mice compared with healthy controls (IL-6 was not detectable in healthy controls). Interestingly, the baseline concentrations of IL-6 in serum and joint lavage samples were in a similar range (compare Figs. 3, 5), indicating that IL-6 baseline concentrations in inflamed joints are much higher than that in serum, considering the significant dilution during joint lavage preparation.

Following the dosing of CNTO 345 at 0.3, 3 and 30 mg/kg, the duration and extent of the free IL-6 suppression were dose-dependent in both serum and joint lavage samples (Figs. 3,

5). Substantial accumulation of total IL-6 was observed in serum (Fig. 3). In contrast, the accumulation of total IL-6 in joint lavage samples was minimal (Fig. 5).

Pharmacokinetics of CNTO 345 and its interplay with IL-6 in serum and joints

Serum pharmacokinetics of CNTO 345 (Step I)—Model-fitted serum concentrations of CNTO 345 are overlaid with observed concentrations in healthy and CIA mice in Fig. 2. Diagnostic plots are provided in the supplemental materials. The parameter estimates and the relative standard errors (RSE) are listed in Table 1. Uncertainty in parameter estimates was calculated by performing a nonparametric bootstrap as listed. Overall, the model was able to capture the serum concentration profiles of CNTO 345 reasonably well. The linear clearance of CNTO 345 in CIA mice is two- to threefold higher compared to healthy controls (0.453 vs. 0.187 mL/day) and the MM elimination accounts for ~50 % of the total clearance in CIA mice (V_{max}/K_m is 0.657 mL/day). Importantly, the MM elimination mechanism of CNTO 345 in CIA mice does not appear to be related to the TMDD of CNTO 345 with IL-6, since the total IL-6 concentration remain orders of magnitude lower than that of CNTO 345. The vascular reflection coefficient of tight tissue (σ_1) was fixed to 0.98 to obtain precise parameter estimates [11]. The estimated vascular reflection coefficient of leaky tissue (σ_2) values in healthy and CIA mice are 0.449 and 0.334, suggesting enhanced tissue distribution in CIA mice.

TMDD of CNTO 345 with IL-6 in serum (Step II)—The TMDD feature was incorporated into the serum compartment to simultaneously fit the serum concentration–time profiles of total CNTO 345, and free and total IL-6. Model parameters estimated in step I were fixed in step II. The model fitted curves were then overlaid with the measured concentration–time profiles in Fig. 3. The model was able to describe the concentration time courses of CNTO 345, and free and total IL-6 in serum reasonably well. The estimated elimination rate constant (k_{int}) for CNTO 345-IL-6 complex is 6.91 day⁻¹, which translates to a clearance of 5.87 mL/day. The serum clearance of the CNTO 345-IL-6 complex is ~fivefold greater than that of the free CNTO 345 ($CL_{CIA} + V_{max}/K_m$ is 1.11 mL/day). The estimated baseline concentration of IL-6 is 0.0029 pmol/mL (74 pg/mL) in serum of CIA mice. The degradation rate constant (k_{deg}) of IL-6 in serum is 835 day⁻¹, indicating rapid turnover dynamics (half-life of IL-6 is approximate 1.2 min). The estimated k_{deg} of IL-6 in mice serum is consistent with previous studies with cynomolgus monkeys (k_{deg} is 232 day⁻¹) and rats (calculated k_{deg} is 333 day⁻¹ based on half-life of 3 min) [8, 16]. The elimination rate constants of the IL-6 and CNTO 345-IL-6 complex differ greater than 100-fold (835 vs. 6.91 day⁻¹). The much slower elimination rate of the CNTO 345-IL-6 complex leads to the build-up of complex concentrations and accumulation of total IL-6 following CNTO 345 treatment. The estimated K_{ss} is 0.0703 nM, which is in accordance with the in vitro K_D value (0.015 nM).

Distribution of CNTO 345 to the ankle joint—Ankle joint synovial fluid concentrations of CNTO 345 were used to characterize the tissue distribution of CNTO 345 to ankle joints in healthy and CIA mice. Parameters estimated in step I and II were fixed in step III. The uptake and clearance of CNTO 345 were governed by convection and lymph drainage in the ankle joint. The model-fitted concentration curves were overlaid with

measured CNTO 345 concentration profiles in joint lavage at all doses in Fig. 4. Overall, the observed profiles were well-captured with the model. For the lowest dose group (0.3 mg/kg), although most of the concentration measurements fall BLQ and were not included in model fitting, model simulation was able to describe the trend of the concentration profile. The estimated joint vascular reflection coefficients in healthy and CIA mice are 0.868 and 0.734 (σ_{j_ctrl} and σ_{j_CIA}). This suggests that permeability of CNTO 345 is increased in inflamed ankle joints compared with normal ankle joints. The estimated lymph flow rates in normal and inflamed ankle joints are 0.0024 and 0.0128 mL/day (L_{joint_ctrl} and L_{joint_CIA}), which are in agreement with the calculated lymph flow rate from the literature (0.004 and 0.008 mL/day derived from albumin half-life (3.9 h) in joints of rabbits using an allometric exponent factor of 0.25) [17]. The lymph flow rate in joint was calculated from the half-life of albumin in joint synovial fluid, using a method introduced by Levick [18]. Briefly, following an intra-articular bolus dose of radio-labeled albumin, the loss of albumin (m_{alb}) in synovial fluid follows mono-exponential decay as:

$$\frac{dm_{alb}}{dt} = -k \cdot m_{alb} = -\frac{0.693}{t_{half}} \cdot m_{alb} \quad (28)$$

The albumin half-life in mouse (t_{half_mouse}) is then scaled from rabbit (t_{half_rabbit}):

$$T_{half_mouse} = T_{half_rabbit} \times \frac{BWT_{rat}^{0.25}}{BWT_{rabbit}} \quad (29)$$

Because the dominant elimination pathway of macromolecules in the joint capsule is lymphatic drainage (L_{joint}), the L_{joint} is:

$$L_{joint} = -k \cdot V_{joint} = -\frac{0.693}{t_{half}} \cdot V_{joint} \quad (30)$$

TMDD of CNTO 345 with IL-6 in ankle joints (step IV)—Finally, the TMDD feature was incorporated into the tissue compartment of the ankle joint synovial fluid to simultaneously fit the concentration profiles of total CNTO 345, and free and total IL-6 in joint lavage samples from CIA mice. Parameters estimated from Step I to III were fixed in step IV. The model-fitted curves were overlaid with the measured concentration–time profiles in Fig. 5. In general, the observed profiles of all the components were well characterized with the model. The elimination rate constant of CNTO 345-IL-6 complex in joint synovial fluid (k_{int_j}) is 21.6 day⁻¹. This translates to a clearance of CNTO 345-IL-6 complex of 0.0129 mL/day, which is extremely close to the estimated ankle joint lymph flow (0.0128 mL/day), suggesting that elimination of CNTO 345-IL-6 complex is likely through lymph drainage. The estimated baseline concentration of IL-6 is 0.251 pmol/mL (6.52×10^3 pg/mL) at the joint in CIA mice, which is 100-fold higher compared to serum (0.0029 pmol/mL). The fold difference of IL-6 baseline in serum and synovial fluid in CIA mice is in

agreement with the observations of baseline IL-6 in serum (79.9 ± 60.5 pg/mL) and joint synovial fluid (15134 ± 17263 pg/mL) in RA patients [10]. The degradation rate constant of IL-6 in joint (k_{deg_j}) is 52.6 day^{-1} , which translates to a clearance in ankle joint of 0.0317 mL/day . In contrast to the serum situation, the elimination rate constants of free IL-6 and CNTO 345-IL-6 complex in the joint are similar (52.6 vs. 21.6 day^{-1}). As a result, minimal accumulation of total IL-6 in joint lavage fluid samples was observed. The estimated K_{ss_j} in joints is 0.425 nM , which is greater than K_{ss} in serum (0.0703 nM). Assuming the k_{off}/k_{on} value in both serum and joints equals the in vitro K_D (0.015 nM), the increase in K_{ss_j} can almost be attributed entirely to the higher elimination rate constant of CNTO 345-IL-6 complex in joint (52.6 vs. 6.91 day^{-1} in serum).

Discussion

In summary, the developed mPBPK model with TMDD features in serum and ankle joint synovial fluid well characterized the pharmacokinetics of CNTO 345 and its interplay with soluble cytokine IL-6 in serum and ankle joints.

Modeling was formed in a step-wise manner. The overall datasets includes both CNTO 345 and IL-6 concentrations in serum, as well as in joint lavage, which convey information of CNTO 345 pharmacokinetics and target engagement with IL-6 with various degrees of variabilities. Fitting datasets sequentially will lead to estimation of CNTO 345 serum pharmacokinetics parameters first with less uncertainty. These parameters are then fixed to drive the estimation of other parameters related to CNTO 345 ankle joint distribution and target engagement, which have greater uncertainty. Also, the objective of this modeling effort was to infer knowledge about the CNTO 345 kinetics for targeting binding and disposition in serum as well as in synovial tissue. Having all parameters simultaneously estimated led to identifiability issue with some of the parameters, which are quite important to understand CNTO 345 targeting binding and disposition.

Our model indicates that the dynamics of the interaction between CNTO 345 and IL-6 in serum and ankle joints differ greatly. This likely to be attributed to: (1) limited tissue distribution of CNTO 345 in ankle joint synovial fluid; (2) higher IL-6 baseline in ankle joint synovial fluid in comparison with serum; and (3) significant differences in the relative time-scales for the elimination rates of free CNTO 345, free IL-6 and CNTO 345-IL-6 complex, which are the three players in the TMDD system, in systemic circulation and ankle joint synovial fluid.

Extravasation of mAbs in tissues is predominantly governed by convection and the efficiency of convection is dependent on the fluid flow rate and the permeability of the vasculature. Comparison of the σ_2 values between CIA mice and normal control mice (0.334 vs. 0.449) confirmed that inflammation can cause increased vascular permeability and therefore enhanced mAb tissue distribution.

CNTO 345 shows enhanced tissue distribution to the ankle joint synovial fluid in CIA mice compared with healthy controls. The estimated vascular reflection coefficients (σ_{j_ctrl} and σ_{j_CIA}) suggest twofold increase of vascular permeability in CIA mice. This corresponds

well with our previous study showing that inflammation causes increased vascular permeability and enhanced tissue distribution of etanercept in arthritic paws in CIA rats [19].

The estimate of σ_j for the ankle joint is highly dependent on the volume of synovial fluid and the dilution factor used for the model-fitting. Firstly, the procedure for sampling joint lavage fluid does not enable accurate estimation of dilution of synovial fluid. Secondly, the volume of ankle joint synovial fluid in mice is not directly available from literature. We have derived the ankle joint synovial fluid volume in healthy mice based on the relationship between synovial membrane surface area (SM) and synovial fluid volume [14]. To validate the relationship, knee joint SM values from various species reported in the literature were used to calculate the knee joint synovial fluid volumes and compared with the measured values from publications. As Table 2 shows, the calculated synovial fluid volumes are consistent with the measured values. In CIA mice, inflammation leads to increased vascular permeability and fluid retention in synovial tissue. The increase of synovial fluid volume has an upper limit due to space constraint within the joint capsule (cannot exceed tenfold in ankle joint). Therefore the fold-change of synovial fluid in CIA mice ankle joint should be between 1 and 10. A 'twofold' increase was assumed for modeling, and the assumption lead to L_{joint} estimates that are physiologically relevant. The L_{joint} estimates would differ if a different fold-change was assumed, but not too significantly within the one to tenfold as specified above. Regardless, this will not change any of our major conclusions.

IL-6 is highly expressed in inflamed joints and the baseline concentration of IL-6 in ankle joint synovial fluid is approximately 100-fold higher than in the systemic circulation. This is a result of both strong local production of IL-6 in the inflamed tissue, and its slower clearance from the joint than in serum. IL-6 was shown to be produced and functions locally in the joints of RA patients [20]. Upon stimulation by TNF- α and IL-1, IL-6 is produced in large amounts by synovial fibroblast cells [21]. Notably, given the small volume of ankle joint synovial fluid, the amount of IL-6 being produced in ankle joint is insignificant to the whole body.

The relative time-scale differences among the elimination rates of CNTO 345, free IL-6 and CNTO 345-IL-6 complex differs profoundly in ankle joint synovial fluid in comparison with serum. In serum, the clearances of CNTO 345 and CNTO 345-IL-6 complex are approximately 100-fold slower compared to free IL-6. This would lead to a significant accumulation of total IL-6 upon CNTO 345 treatment. In contrast, the clearances of CNTO 345 and CNTO 345-IL-6 are on the same time-scale with free IL-6 in joints, and thus minimal accumulation of total IL-6 was observed. The accumulation of total IL-6 would limit the duration of free IL-6 suppression as it dissociates. Moreover, the turnover rate of IL-6 is ~tenfold faster in serum than in joint synovial fluid, which indicates that the production rate difference between serum and joint (>tenfold) is less than that in their baselines (>100-fold). All these factors contribute to the relatively long duration of free IL-6 suppression despite the lower mAbs concentrations in the joint.

Our model made no assumptions about the elimination mechanisms of free IL-6 and CNTO 345-IL-6 complex in serum and ankle joint synovial fluid and simple first-order elimination rate constants were assigned. Interestingly, the model parameter estimates allow us to

speculate the likely elimination pathways for free IL-6 and CNTO 345-IL-6 complex from serum and joints. In serum, the estimated clearance of CNTO 345-IL-6 complex in serum is much closer to the clearance of CNTO 345. Elimination of CNTO 345 in serum is believed via catabolism. The rapid turnover rate of free IL-6 is consistent with the rapid elimination of small soluble cytokines by liver and kidney. In ankle joint synovial fluid, the removal of free CNTO 345 was assumed to be via lymph flow. Interestingly, the estimated clearance of the CNTO 345-IL-6 complex is extremely close to the lymph flow, which indicates that lymph drainage also account for the elimination of CNTO 345-IL-6 complex. Free IL-6 clearance from the joint is ~2.5-fold higher than lymph flow, indicating that both lymph drainage and local degradation contributed to its elimination. But the local degradation of free IL-6 is significantly lower comparing to what happens systemically.

With the joint lavage sampling procedure, the ankle joint synovial fluid samples are highly diluted. Dilution will cause dissociation of the CNTO 345-IL-6 complex and the binding equilibrium would be shifted [22]. Therefore, in our model development, the dissociation of the CNTO 345-IL-6 complex due to dilution was quantitatively accounted for. Since many analytical approaches require dilution, careful interpretation of such data is needed. Misleading characterization of the TMDD dynamics would occur if such dilution effect on the ligand binding equilibrium was not considered.

The developed mPBPK model with TMDD provided decent performance in describing the concentration profiles of CNTO 345, and free and total IL-6 in serum and ankle joint lavage. However, there are limitations with the model assumptions. The model assumes that the production of IL-6 for the full time course of CIA disease progression is the same. However, since the expression of IL-6 is correlated with disease severity [13], this assumption may not hold true all the time, especially over long-term treatment. Also, the model assumes the same synovial fluid volume for inflamed ankle joints for all CIA mice, which can only be an approximation.

In conclusion, the interrelationships of CNTO 345 and IL-6 in serum and joints differ greatly. The difference can be attributed to limited tissue distribution of CNTO 345, significant rise of the baseline IL-6 in joint synovial fluid, and the time-scales among the eliminations rates of CNTO 345, IL-6, and the complex. Simple extrapolation of free cytokine suppression in tissues assuming similar dynamics of TMDD of mAb and cytokine in serum and tissues would lead to errant conclusions. Our modeling efforts to decipher the interplay of CNTO 345 and IL-6 in the systemic circulation and inflamed joints shed some light on possible mechanisms of TMDD of mAb and soluble cytokines in joint synovial fluid, which helps in better projection of free cytokine suppression in inflamed joints. The quantitative interrelationships between mAb and soluble cytokines obtained in mice, incorporating additional information about the expression and dynamics of soluble cytokines in RA patients, could possibly allow translation into humans and better predictions of therapeutic responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Thomas McIntosh, Rajitha Doddareddy and Elayne Dell for the bioanalytical work that makes this manuscript possible, and Drs. Donald L. Heald, Chao Han and Zhenhua Xu for scientific discussion and critical review of the manuscript. Dr. William J Jusko and Xi Chen were supported by NIH Grant GM24211

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Fig. 1.

Scheme of mPBPK models with TMDD in serum and ankle joint synovial fluid developed step-wise for the characterization of distribution of CNTO 345 and interrelationship of CNTO 345 and IL-6 in serum and ankle joint synovial fluid. **a** Basic second-generation mPBPK model used for the characterization of serum pharmacokinetics of CNTO 345 in Step I, **b** mPBPK model with TMDD in serum for the characterization of the interplay between CNTO 345 and IL-6 in serum in Step II, **c** mPBPK model including ankle joint tissue compartment for the characterization of distribution of CNTO 345 to the ankle joint in Step III, and **d** mPBPK model with TMDD in ankle joint tissue compartment for the characterization of the interplay between CNTO 345 and IL-6 in ankle joint synovial fluid in Step IV

Fig. 2. Serum CNTO 345 concentrations versus time profiles in CIA mice (0.3, 3 and 30 mg/kg) and healthy controls (30 mg/kg). Circles are serum CNTO 345 concentrations, curves depict model fittings, and dashed lines mark the LLOQ. Number of measurements BLQ is listed

Fig. 3. Serum concentrations versus time profiles of free and total IL-6, and CNTO 345 in CIA mice at doses of 0.3, 3 and 30 mg/kg. *Circles* are serum concentrations, *curves* depict model fittings, and *dashed lines* mark the LLOQ. *Number* of measurements BLQ is listed

Fig. 4. Joint lavage CNTO 345 concentrations versus time profiles in CIA mice (0.3, 3 and 30 mg/kg) and healthy controls (30 mg/kg). Circles are joint lavage CNTO 345 concentrations, curves depict model fittings, and dashed lines mark the LLOQ. Number of measurements BLQ is listed

Fig. 5.

Joint lavage concentrations versus time profiles of free and total IL-6, and CNTO 345 in CIA mice at doses of 0.3, 3 and 30 mg/kg. *Circles* are joint lavage concentrations, *curves* depict model fittings, and *dashed lines* mark the LLOQ. *Number* of measurements BLQ is listed

Table 1

Summary of model parameters and estimates

Parameter	Definition	Estimate	RSE (%)	Bootstrap, median (95% CI)
Step I: mAb PK in serum				
σ_1	Vascular reflection coefficient of tight tissue	0.98	Fixed	NA
σ_{2_ctrl}	Vascular reflection coefficient of leaky tissue in healthy mice	0.449	9.0	0.440 (0.336–0.528)
σ_{2_CIA}	Vascular reflection coefficient of leaky tissue in CIA mice	0.334	14	0.330 (0.206–0.445)
CL_{ctrl} (mL/day)	Linear serum clearance in healthy mice	0.187	4.0	0.183 (0.147–0.201)
CL_{CIA} (mL/day)	Linear serum clearance in CIA mice	0.453	6.0	0.442 (0.354–0.497)
V_{max} (pmol/day)	Capacity constant	5.38	69	6.59 (1.94–39.4)
K_m (nM)	Affinity constant	8.19	89	9.39 (1.16–74.4)
Step II: TMDD in serum				
k_{deg} (1/day)	Degradation rate constant of IL-6 in serum	835	26	848 (502–1487)
k_{int} (1/day)	Elimination rate constant of mAb-IL-6 complex	6.91	14	6.86 (4.76–9.49)
K_{ss} (pmol/mL)	Quasi-equilibrium binding constant	0.0703	22	0.0656 (0.0415–0.0982)
$IL-\sigma_{bl}$ (pmol/mL)	Baseline concentration of serum IL-6 in CIA mice	0.0029	20	0.0029 (0.0018–0.0037)
Step III: mAb PK in ankle joint				
σ_{joint_ctrl}	Vascular reflection coefficient in healthy mice	0.868	2.0	0.868 (0.827–0.907)
σ_{joint_CIA}	Vascular reflection coefficient in CIA mice	0.734	4.0	0.731 (0.677–0.784)
L_{joint_ctrl} (mL/day)	Lymphatic flow rate in healthy mice	0.0024	33	0.0024 (0.0012–0.0087)
L_{joint_CIA} (mL/day)	Lymphatic flow rate in CIA mice	0.0128	51	0.0109 (0.0033–2.91)
Step IV: TMDD in ankle joint				
k_{deg_j} (1/day)	Degradation rate constant of IL-6	52.6	44	63.1 (8.13–8000)
k_{int_j} (1/day)	Elimination rate constant of the mAb-IL-6 complex	21.6	33	20.9 (3.34–868)
K_{ss_j} (pmol/mL)	Quasi-equilibrium binding constant	0.425	94	0.323 (0.073–72.5)
$IL-\sigma_{bl_j}$ (pmol/mL)	Baseline concentration of IL-6 in CIA mice	0.251	26	0.234 (0.097–0.356)
mPBPK parameters				
V_s (mL) ^a	Serum volume	0.85		
ISF (mL) ^a	Total tissue interstitial space volume	4.35		
V_{lymph} (mL) ^a	Lymph volume	1.53		
L (mL/day) ^b	Total lymph flow rate	2.88		
V_{joint_ctrl} (μl) ^c	Single ankle joint synovial fluid in healthy mice	0.3		
V_{joint_CIA} (μl) ^d	Single ankle joint synovial fluid in CIA mice	0.6		

^aPhysiological parameter values obtained from [23]. Assumed 25 g body weight^bTotal lymphatic flow allometrically scaled from human (2.9 L/day) with exponent factor 0.74^cSynovial fluid volume of mouse ankle joint in healthy mice estimated from mouse ankle joint synovial membrane values [14, 15]^dSynovial fluid volume of mouse ankle joint was assumed to increase by twofold in CIA mice compare to healthy mice

Table 2

Comparison of the calculated and observed synovial fluid volumes across species

Species	Body weight (kg)	Knee joint SM (cm ²)	Refs.	Calculated V _{joint} (mL)	Observed V _{joint} (mL)	Refs.
Human	70	277	[24]	1.39	1.1 (0.13–3.5)	[25]
	70	200	[15]	1.00	0.5–4.0	[26]
Dog	16	39	[27]	0.195	0.05–0.2	[28]
					0.24	[29]
Rabbit	3.5	16	[30]	0.0571	0.032	[31]