Local vs. systemic anti-TNF-α effects of adalimumab in rheumatoid arthritis: pharmacokinetic modeling analysis of interaction between a soluble target and a drug

David Stepensky

Department of Pharmacology and School of Pharmacy, Faculty of Health Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel
Office: +972-8-6477381
Fax: +972-8-6479303
E-mail: davidst@bgu.ac.il

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ABSTRACT

Objective: To assess the relative importance of local vs. systemic interaction of adalimumab with TNF-α in rheumatoid arthritis (RA), to identify localization of site of adalimumab action, and efficacy of local (intraarticular) vs. systemic adalimumab administration for treatment of RA.

Methods: The clinical and pre-clinical data on adalimumab and TNF-α disposition were analyzed using pharmacokinetic modeling and simulation approach. The disposition of adalimumab and TNF-α and the interaction between them at the individual compartments (the synovial fluid of the affected joints, central and peripheral compartments) following different routes of adalimumab administration were studied.

Results: Outcomes of modeling and simulation using the developed pharmacokinetic model indicate that adalimumab can efficiently permeate from the diseased joints to the central circulation in the rheumatoid arthritis patients. Permeability of TNF-α, that is excessively secreted in the joints, is even higher than that of adalimumab. As a result, subcutaneous, intravenous and intraarticular administration of the clinically-used doses of adalimumab (40 mg) exert similar effects on the time course of TNF-α levels at different locations in the body and efficiently deplete the TNF-α in all the compartments for prolonged period of time (8-10 weeks). At these doses, adalimumab exhibits predominantly systemic anti-TNF-α effects at the central and peripheral compartments (~93% of the overall effect) and the contribution of the local effects in the rheumatic joints is ~7% for all the studied routes, including the local intraarticular injections. The major pathway of TNF-α elimination from the synovial fluid (~77% for SC administration, and ~72% for IV and IA administration of 40 mg adalimumab) is interaction with adalimumab that reaches the joints following local or systemic administration.

Conclusions: Kinetics of adalimumab permeation to the synovial fluid (0.00422 L/hr clearance of permeation) vs. the rate of TNF-α turnover in the affected joints (1.84 pmol/h synthesis rate, and 0.877 h⁻¹ degradation rate constant) are apparently the major parameters that determine the time course of TNF-α levels in the synovial fluid and the TNF-α-neutralizing effects of adalimumab in rheumatoid arthritis patients. Outcomes of this study suggest that intraarticular administration of adalimumab is not preferable to subcutaneous or intravenous treatment. Local and systemic permeability, turnover and interactions between the drug and the target should be taken into account for optimization of use of drugs acting on soluble targets (growth factors, interferons, interleukins, immunoglobulins, etc.).

KEY WORDS: TNF-α; adalimumab; distribution of the soluble target and the drug; pharmacokinetic modeling; target-mediated drug disposition.
Drug distribution is a major pharmacokinetic process that governs the time course of drug concentrations at the site of action and of its pharmacological effects. Distribution of drugs can follow complex patterns, as a function of their physicochemical properties and ability to bind/interact with endogenous compounds. For instance, therapeutic antibodies exhibit limited permeability via physiological barriers due to their high molecular weight (approximately 150 kDa for IgG) and high hydrophilicity. Additional factors that govern the distribution of therapeutic antibodies are the cellular uptake via FcRn receptor-mediated endocytosis (for IgG1, 2 and 4 isotypes) and almost irreversible binding to the target (specific antigen) \[1, 2\]. Both FcRn receptor-mediated endocytosis and target binding are accompanied by significant clearance of the antibody and are regarded as processes of antibody disposition (and not distribution only).

Several types of pharmacokinetic models have been applied to describe the disposition of antibodies, including compartmental models \[3\], physiologically-based pharmacokinetic (PBPK) models \[4, 5\], and target-mediated drug disposition (TMDD) models \[6, 7\] that take into account capacity-limited (saturable) antibody-target binding processes. Theoretical and practical aspects of drug-target interaction have been extensively analyzed during the last decade, and modified versions of TMDD model have been introduced \[8\], such as models with rapid binding approximation \[9\], quasi-steady-state approximation, and Michaelis-Menten elimination kinetics \[10\].

All the above-mentioned models assume that the interaction between the antibody and its target takes place in a single location/compartment, usually referred to as the central compartment, that includes the bloodstream (and in some cases also the highly-perfused organs and tissues). This assumption is usually appropriate if the antibody is directed towards stationary target, such as cell surface receptors overexpressed by solid tumors (EGFR, HER2, and others), and also for lymphoma and leukemia cells that are located predominantly in the bloodstream and in the bone marrow (a highly-perfused tissue). However, increasing number of therapeutic antibodies are directed toward soluble/mobile targets that are synthesized also in the peripheral organs and tissues, and can permeate from the peripheral tissues to the central circulation and in the opposite direction. Examples of such targets and therapeutic antibodies that can bind them are: tumor necrosis factor-alpha (TNF-α) and anti-TNF-α antibodies (e.g., adalimumab for rheumatoid arthritis and psoriasis), VEGF and anti-VEGF antibodies (e.g., bevacizumab for colorectal cancer), Immunoglobulin E and omalizumab (for asthma), etc. For these cases, erroneous conclusions can be reached if the pharmacokinetic and pharmacodynamic analysis is based on assumption that interaction between the therapeutic antibody and its target takes place in the central compartment only.

This study focuses on analysis of the available data on disposition of TNF-α and adalimumab (HUMIRA®), a fully human anti-TNF-α antibody. TNF-α is transmembrane protein which is proteolytically cleaved into a soluble 17 kDa compound characterized by high tendency to form dimers or trimers \[11, 12\]. TNF-α can be secreted by several types of cells, and it is over-produced by systemic & local T cells in inflammatory conditions, such as rheumatoid arthritis, ankylosing spondylitis, psoriasis, Crohn's disease, etc. During the late 1980's and early 1990's TNF-α has been developed as a potential anti-cancer agent, but this project has been discontinued, apparently due to the pro-inflammatory and cytotoxic adverse effects. Nevertheless, pharmacokinetic data from several preclinical \[11, 13-15\] and clinical \[16, 17\] studies of TNF-α are available and indicate two-compartmental pharmacokinetic behavior and rapid elimination of TNF-α following intravenous (IV) administration (terminal t1/2 of 15-45 min in tumor patients). TNF-α levels are increased in the synovial fluid and in the serum of rheumatoid arthritis (RA) patients \[18-20\]. This stems apparently from excessive local
production of TNF-α in the affected joints of the RA patients and its permeation from the joint's synovial fluid to the central circulation.

Adalimumab (Humira) is a 148 kDa fully human antibody of the IgG1 isotype that binds and neutralizes TNF-α\textsuperscript{[21]}. Following IV administration, adalimumab exhibits two-compartmental pharmacokinetic behavior\textsuperscript{[22-24]} (terminal $t_{1/2}$ of ~14 days). Adalimumab has small volume of distribution ($V_{ss}$ of 4.7-6.0 L), but in RA patients it can efficiently permeate into the inflamed joints and its concentrations in the synovial fluid can reach 31 - 96% of the serum concentrations\textsuperscript{[21]}. These findings are consistent with reports on permeation of radio-labeled antibodies to the synovial fluid of animals and patients with rheumatoid arthritis\textsuperscript{[25, 26]}. Thus, substantial amounts of adalimumab are expected to reach the synovial fluid following systemic (subcutaneous (SC) or IV) administration. Nevertheless, local intraarticular (IA) injections of anti-TNF-α antibodies were suggested for the management of persistent inflammatory monoarthritis\textsuperscript{[27]}. It is not clear whether local adalimumab therapy is superior to the systemic administration, and several clinical reports that applied intraarticular injections of anti-TNF-α antibodies\textsuperscript{[28-30]}, including adalimumab\textsuperscript{[31]}, did not provide definitive answer to this question.

From the above-mentioned data it is evident that both TNF-α and adalimumab are present in the synovial fluid and in the serum, and hence can interact both locally and systemically. The objective of the present study was to assess the relative importance of local vs. systemic interaction of adalimumab with TNF-α in rheumatoid arthritis, to identify the localization of the site of adalimumab TNF-α-neutralizing action, and the relative efficiency of local (intraarticular) vs. systemic TNF-α-neutralizing effects of adalimumab administration in the RA patients. To this end, the existing clinical and pre-clinical pharmacokinetic data on adalimumab and TNF-α were analyzed using pharmacokinetic modeling and simulation approach. A pharmacokinetic model that describes interaction between adalimumab and TNF-α in the affected joints, the central (systemic circulation) and peripheral compartments has been developed and was applied to study the effects of individual parameters on disposition of adalimumab and TNF-α, on interactions between them at the individual compartments, and on the localization of site of action.
METHODS

The Developed Pharmacokinetic Model

The model was based on the available pharmacokinetic data on TNF-α (the target) and adalimumab (the drug). It has been assumed that the interaction between these compounds takes place in three compartments (see Fig. 1): central compartment (serum), peripheral compartment, and the joints (synovial fluid). The differential equations of the applied pharmacokinetic model were as follows:

\[
\frac{dT_C}{dt} = \frac{k_{\text{syn}}}{V_C} + \frac{k_{\text{d11}}}{V_C} \cdot T_P \cdot V_p + \frac{k_{\text{d31}}}{V_C} \cdot T_J \cdot V_J - \left( k_{\text{degr}} + k_{\text{d12}} + k_{\text{d13}} \right) \cdot T_C - k_{\text{ON}} \cdot T_C \cdot D_C + k_{\text{OFF}} \cdot D_C T_C
\]

(1)

\[
\frac{dT_P}{dt} = \frac{k_{\text{syn}}}{V_P} + \frac{k_{\text{d12}}}{V_P} \cdot T_C \cdot V_C - \left( k_{\text{degr}} + k_{\text{d12}} \right) \cdot T_P - k_{\text{ON}} \cdot T_P \cdot D_P + k_{\text{OFF}} \cdot D_P T_P
\]

(2)

\[
\frac{dT_J}{dt} = \frac{k_{\text{syn}}}{V_J} + \frac{k_{\text{d13}}}{V_J} \cdot T_C \cdot V_C - \left( k_{\text{degr}} + k_{\text{d31}} \right) \cdot T_J - k_{\text{ON}} \cdot T_J \cdot D_J + k_{\text{OFF}} \cdot D_J T_J
\]

(3)

\[
\frac{dSC}{dt} = -k_a \cdot SC
\]

(4)

\[
\frac{dD_C}{dt} = \frac{k_{\text{d10}}}{V_C} \cdot D_P - k_{\text{d11}} \cdot D_P - k_{\text{ON}} \cdot T_P \cdot D_P + k_{\text{OFF}} \cdot D_P T_P
\]

(5)

\[
\frac{dD_P}{dt} = \frac{k_{\text{d12}}}{V_C} \cdot D_C \cdot V_C - k_{\text{d11}} \cdot D_P - k_{\text{ON}} \cdot T_P \cdot D_P + k_{\text{OFF}} \cdot D_P T_P
\]

(6)

\[
\frac{dD_J}{dt} = \frac{k_{\text{d13}}}{V_J} \cdot D_C \cdot V_C - k_{\text{d31}} \cdot D_J - k_{\text{ON}} \cdot T_J \cdot D_J + k_{\text{OFF}} \cdot D_J T_J
\]

(7)

\[
\frac{dD_T C}{dt} = \frac{k_{\text{d21}}}{V_C} \cdot D_P T_P \cdot V_P + \frac{k_{\text{d31}}}{V_C} \cdot D_J T_J \cdot V_J + k_{\text{ON}} \cdot T_C \cdot D_C - \left( k_{\text{OFF}} + k_{\text{el}} + k_{\text{d12}} + k_{\text{d13}} \right) \cdot D_C T_C
\]

(8)

\[
\frac{dD_T P}{dt} = \frac{k_{\text{d12}}}{V_P} \cdot D_C T_C \cdot V_C + k_{\text{ON}} \cdot T_P \cdot D_P - \left( k_{\text{OFF}} + k_{\text{el}} + k_{\text{d21}} \right) \cdot D_P T_P
\]

(9)

\[
\frac{dD_T J}{dt} = \frac{k_{\text{d13}}}{V_J} \cdot D_C T_C \cdot V_J + k_{\text{ON}} \cdot T_J \cdot D_J - \left( k_{\text{OFF}} + k_{\text{el}} + k_{\text{d31}} \right) \cdot D_J T_J
\]

(10)

where \( T_C, T_P, \) and \( T_J \) are the concentrations of TNF-α in the central compartment, peripheral compartment, and in the synovial fluid (joints), respectively; \( D_C, D_P, \) and \( D_J \) are the concentrations of adalimumab in the same compartments; \( D_C T_C, D_P T_P, \) and \( D_J T_J \) are the concentrations of adalimumab-TNF-α complexes in the same compartments; and \( SC \) is the amount of adalimumab at the site of subcutaneous injection.

The rate constants of the applied model for adalimumab are: \( k_a \) drug absorption following SC administration; \( k_{\text{d10}} \) drug elimination from the body; \( Q_{\text{dp}} \) drug permeation between the central compartment and the joints (synovial fluid); \( Q_{\text{dp}} \) drug permeation between the central and peripheral compartments. The rate constants of the applied model for TNF-α are: \( k_{\text{syn}} \), \( k_{\text{syn}} \), \( k_{\text{syn}} \) zero-order synthesis in the central compartment, peripheral compartment, and the joints, respectively; \( k_{\text{degr}} \) target degradation; \( Q_{\text{tp}} \) target permeation between the central compartment and the joints (synovial fluid); \( Q_{\text{tp}} \) target permeation between the central and peripheral compartments. The \( k_{\text{on}} \) is the 2nd order rate constant of interaction between TNF-α and adalimumab in each one of the compartments, and the formed drug-target complex is assumed to dissociate and degrade (be internalized by the cells in the specific compartment).
with $k_{\text{off}}$ and $k_d$ rate constants, respectively. The permeation of the drug-target complex is between the central compartment and the joints (synovial fluid) and between the central and peripheral compartments is described using $Q_{\text{djt}}$ and $Q_{\text{dpp}}$ parameters, respectively. The volumes of the central compartment, peripheral compartment, and the joints are $V_C$, $V_P$, and $V_J$, respectively, and the rate constants of drug, target, and drug-target complex permeation between the compartments were expressed as: $k_{d12}=Q_{\text{dtp}}/V_C$, $k_{d21}=Q_{\text{dtp}}/V_P$, $k_{d13}=Q_{\text{djt}}/V_C$, $k_{d31}=Q_{\text{djt}}/V_J$, $k_{t12}=Q_{\text{tp}}/V_C$, $k_{t21}=Q_{\text{tp}}/V_P$, $k_{t13}=Q_{\text{tp}}/V_C$, $k_{t31}=Q_{\text{tp}}/V_J$, $k_{dt12}=Q_{\text{dtp}}/V_C$, $k_{dt21}=Q_{\text{dtp}}/V_P$, $k_{dt13}=Q_{\text{dtp}}/V_C$, $k_{dt31}=Q_{\text{dtp}}/V_J$.

Effects of several routes of drug administration were analyzed, including subcutaneous, intravenous and intraarticular injections of adalimumab ($D_{SC}$, $D_{IV}$, and $D_{IA}$ doses, respectively, see Fig. 1). The initial conditions of equations 1-3 were: $T_C = T_P = 4.47$ pM (based on the TNF-\(\alpha\) concentrations of 76 pg/ml in the serum of rheumatoid arthritis patients [19]), $T_J = 5.88$ pM (based on the TNF-\(\alpha\) concentrations of 100 pg/ml in the synovial fluid of rheumatoid arthritis patients [18]). Initial amounts of drug (equations 4-7) and drug-target complexes (equations 8-10) prior to drug administration were set to zero. Following drug administration, the initial conditions were set as follows: for subcutaneous route $SC = D_{SC} \cdot F$, for intravenous route $DC = D_{IV}/V_C$, and for intraarticular route $DJ = D_{IA}/V_J$.

**Parameter Setting**

Data on TNF-\(\alpha\) and adalimumab pharmacokinetics and their interaction were extracted from the available scientific literature (see Table I). Several parameters (including $F$, $k_a$, $k_{\text{on}}$, and $k_{\text{off}}$) were taken directly from the adalimumab prescription information and scientific publications. Other parameters were estimated based on the time course of adalimumab or TNF-\(\alpha\) concentrations reported in clinical studies ($Q_{\text{dtp}}$, $Q_{\text{d10}}$, $V_C$, $V_P$, $k_{\text{degr}}$). For this purpose, relevant graphs were digitalized and the extracted data were analyzed using PK solver 2.0 software [12] using 2-compartment PK model and 1/Y weighting. Specifically, serum concentration-time profiles of adalimumab after a single injection of 0.5, 1, 3, 5, or 10 mg/kg IV to patients ($n = 18$ for each group) with long-standing active RA were extracted from den Broeder et al. [22]. Serum concentration-time profiles of adalimumab after a single injection of 0.25, 0.5, 1, 3, or 5 mg/kg IV to patients ($n = 9$ for each group) with RA receiving concomitant methotrexate were extracted from Weisman et al. [23]. Terminal half-life of TNF-\(\alpha\) has been extracted from Mittelman et al. [17]. Other permeation parameters were estimated based on the dimensions (molecular radius) of TNF-\(\alpha\) and adalimumab and on the available clinical and preclinical data on their tissue distribution and joint permeability, specifically from the correlation between the protein molecular radius and the synovial permeability in the knee joints of patients with moderate-activity RA reported by Pejovic et al. [33], and from the kinetics of antibody accumulation in the inflamed joints in rat adjuvant arthritis model reported by Kinne et al. [26]. The synthesis rates of TNF-\(\alpha\) in the individual compartments were calculated based on the values of TNF-\(\alpha\) permeation and degradation ($Q_{\text{tj}}$, $Q_{\text{tp}}$, and $k_{\text{degr}}$), and the initial conditions of $T_C$, $T_P$ and $T_J$.

**Simulations and Data Analysis**

All simulations were executed utilizing MATLAB® 7.11 (The Mathworks, Inc., Natick, MA). The system of equations (equations 1-10) was solved using MATLAB’s ode23s command, a variable order method for solving a system of stiff differential equations. A series of simulations were carried out to examine the developed pharmacokinetic model (Fig. 1 and Table I).

In the first set of simulations, the adalimumab serum concentration vs. time data following intravenous bolus administration of different drug doses to rheumatoid arthritis patients [22],[23] were compared to the predictions based on the developed pharmacokinetic
model. In the second set of simulations, effect of route of drug administration on the time course of TNF-α and adalimumab concentrations and on the elimination of adalimumab-TNF-α complexes were analyzed using the developed pharmacokinetic model.

The sensitivity of model output to 5-fold alterations of parameter values was assessed by evaluating the percent change of area under the curve (AUC) of adalimumab and of TNF-α in the specific compartments. For TNF-α, the AUC values reflected the decline in its levels below the baseline concentrations. Contribution of the individual compartments to the overall TNF-α-neutralizing effect of adalimumab was calculated based on the cumulative amounts of adalimumab-TNF-α complexes for each compartment. Contribution of the individual pathways of TNF-α elimination from the joints (during the time period when active adalimumab concentrations were present in the body) was calculated based on the cumulative amounts of adalimumab-TNF-α complexes eliminated according to the $k_{on}$ (i.e., $k_{on}D_j$), $k_{t13}$, and $k_{degr}$ processes.

RESULTS
The developed model and simulations
The developed pharmacokinetic model (Fig. 1) was based on the available clinical, preclinical, and in vitro data for adalimumab and TNF-α and accounted for disposition and interaction between these compounds in different compartments, including the central compartment (serum), peripheral compartment, and the joints (synovial fluid). The developed model appropriately described the pharmacokinetics of adalimumab following intravenous administration for the whole range of the studied doses (see Fig. 2) and reflected target-mediated disposition of adalimumab (as can be seen from the steeper slope of the concentration vs. time curve at the concentrations below $10^4$ pM). The developed model apparently was the minimal (most parsimonious) model for assessment of local vs. systemic TNF-α-neutralizing effects of adalimumab. Attempts to use model with two compartments (the central compartment and the joints) were unsuccessful since this model could not describe appropriately the time course of adalimumab serum concentrations that was observed in the clinical studies $^{[22, 23]}$.

The time course of adalimumab and TNF-α concentrations
Simulations using the developed model indicate that following subcutaneous, intravenous or intraarticular administration of a clinically-applied doses (40 mg), adalimumab is slowly eliminated from the body (see Fig. 3a, 3c, 3e) and efficiently depletes the TNF-α in all the compartments for prolonged period of time (8-10 weeks, Fig. 3b, 3d, 3f). Within this time period, both adalimumab and TNF-α can equilibrate rather rapidly between the different compartments and pseudo-equilibrium between the adalimumab concentrations in all the compartments is achieved within 4-5 days for the subcutaneous and intravenous adalimumab administration. Equilibration time following intraarticular adalimumab administration is delayed and is achieved only 2-3 weeks after the dosing.

Outcomes of the sensitivity analysis indicate that change of SC adalimumab dose affects the local and systemic exposure to the drug (Fig. 4a and 4c) in almost proportional fashion, while the effect of adalimumab dose on the TNF-α levels in the synovial fluid and in the central compartment is moderate (Fig. 4b and 4d). The local and systemic exposure and effects of adalimumab were almost unaffected by the 5-fold alteration of adalimumab-TNF-α binding affinity or of elimination kinetics of adalimumab-TNF-α complexes (see Fig. 4). Limited changes in the exposure and effects of adalimumab were induced by the changes in adalimumab and TNF-α permeability to the synovial fluid, and by the change in the volume of the synovial fluid. Somewhat higher changes were exerted by alterations of TNF-α baseline...
concentrations in the synovial fluid and turnover rate, as expected for drugs acting by binding and neutralizing their target [34].

**Local vs. systemic TNF-α-neutralizing effects of adalimumab**

Analysis of elimination of adalimumab-TNF-α complexes indicated that contribution of the central compartment, peripheral compartment, and the joints to the TNF-α-neutralizing effect of adalimumab at the clinically-applied doses (40 mg) was approximately 50%, 43%, and 7%, respectively, and that it was not affected by the route of drug administration (see Fig. 5a). This means that local effects accounted only for 7% of adalimumab's anti-TNF-α activity, even following local intraarticular drug administration, while the remaining 93% are attributed to the systemic drug effect (at the central and peripheral compartments).

Increase in the volume of synovial fluid and in TNF-α baseline concentrations in the synovial fluid (increase of V_j and T_{j0}) increased the relative contribution of local anti-TNF-α effects of adalimumab in the joints, and vice versa (see Fig. 5b and 5c). Nevertheless, even following these changes, systemic effects of adalimumab still accounted for more than 70% of the overall anti-TNF-α activity. Changes in all the rest of the analyzed parameters, including the adalimumab dose, adalimumab-TNF-α binding affinity, elimination kinetics of adalimumab-TNF-α complexes, and adalimumab and TNF-α permeability to the synovial fluid, did not affect the relative contribution of local vs. systemic anti-TNF-α effects of adalimumab (see Fig. 5).

**Pathways of TNF-α elimination from the joints**

Analysis of the modeling outcomes indicates that in absence of adalimumab 98% of TNF-α that is produced in the joints is degraded locally and ~2% permeates to the central compartment (reflecting the k_{degr} and k_{s31} values of 0.877 h^{-1} and 0.0182 h^{-1}, respectively). During the presence of active adalimumab concentrations in the body (i.e., during the period when the synovial TNF-α concentrations are below the baseline levels), adalimumab-TNF-α binding in the synovial fluid becomes the major route of TNF-α elimination from the joints (~77% for SC administration, and ~72% for IV and IA administration of a 40-mg dose; see Fig. 6a).

Outcomes of the sensitivity analysis indicate that following 5-fold changes in values of all the studied parameters adalimumab-TNF-α binding in the synovial fluid remains the major pathway of TNF-α elimination from the joints (see Fig. 6b and 6c). Increased dose and decreased adalimumab-TNF-α and TNF-α degradation rate constants lead to enhanced contribution of adalimumab-TNF-α binding for local TNF-α elimination, as expected. On the other hand, 5-fold changes in the adalimumab or TNF-α permeation from the joints to the central circulation have limited effect on the local TNF-α elimination (see Fig. 6b and 6c). It is expected that more than 10-fold reduction of adalimumab permeation to the joints (Q_{dj}) is required in order to substantially reduce the local effects of subcutaneously-administered adalimumab in the rheumatic joints (see Fig. 7). Even if the permeation of adalimumab between the central compartment and the joints is set to zero, subcutaneously-administered adalimumab will still be able to decrease the synovial TNF-α levels by approximately 2% due to the permeation of TNF-α from the joints to the central circulation (simulation not shown).
DISCUSSION

Immobile vs. soluble targets

Disposition of biopharmaceuticals, such as therapeutic antibodies, is different as compared to the ‘classical’ small molecular weight drugs. Due to their high molecular weight, permeability of the therapeutic antibodies to the diseased tissue following systemic administration can be the major factor limiting their treatment efficiency. In many cases, the target is immobile (e.g., solid tumors expressing specific tumor antigens) and the antibody availability at the site of action is governed solely by the distribution of the drug. In some diseases, the target can be mobile, but is confined to the central compartment (e.g., lymphoma and leukemia cells), and the therapeutic effect is determined by the serum concentrations of the antibody.

On the other hand, increasing amount of compounds targeted by therapeutic antibodies are soluble/mobile. Examples of such targets are growth factors (VEGF, 45 kDa), interferons (18-22 kDa), interleukins (~17 kDa), immunoglobulins (Ig E, 188 kDa), and others. All these compounds are secreted at multiple organs and tissues, can permeate from these sites to the central circulation, and can interact with specific antibodies at multiple sites in the body. Thus, distribution of these compounds and of the antibodies will determine their relative availabilities at the individual sites of action.

Distribution of the target compounds and its effect on the pharmacokinetics and pharmacodynamics of therapeutic antibodies has been largely neglected until now. Interaction between a drug and a soluble target has been generally assumed to take place solely in the central compartment [35-38]. To the best of my knowledge, only Lowe et al. had assumed that the therapeutic antibody and the soluble target can interact in two different locations in the body (the central plasma and the tissue interstitiae) [39]. In the current study we make an additional step towards the physiologically-based TMDD model and analyze the disposition and interaction between soluble target and therapeutic antibody (adalimumab and TNF-α in rheumatoid arthritis) that take place in several locations in the body that correspond to the specific physiological location (the synovial fluid) and the compartments that represent lumped organs and tissues (the central, and peripheral compartments).

Conclusions regarding anti-TNF-α therapy

Outcomes of the modeling and simulation analysis indicate that adalimumab can efficiently permeate from the diseased joints to the central circulation in the rheumatoid arthritis patients. Permeability of TNF-α is even higher than that of adalimumab and certain amounts of TNF-α (that is excessively secreted in the rheumatic joints) can permeate to the central circulation and lead to elevated systemic TNF-α levels. As a result, of more rapid distribution kinetics as compared to the overall duration of pharmacological effects, subcutaneous, intravenous and intraarticular adalimumab administration have similar effect on the time course of TNF-α levels (see Fig. 3b, 3d, and 3f).

The above-mentioned TNF-α levels refer to the free cytokine that is biologically or immunologically active, and their comparison to the clinical data should be done with caution since the commonly-used ELISA-based assay detects both free and drug- or carrier-bound TNF-α [40]. Moreover, the correlation between the time course of TNF-α levels in the synovial fluid (or in the central circulation) and the clinical response is not clear [19] vs. [40]). Therefore, conclusions of this study are limited to the analysis of effect of adalimumab on TNF-α levels and not of the time course of anti-rheumatic responses (see below).

Outcomes of the modeling and simulation analysis indicate that adalimumab induces predominantly systemic anti-TNF-α effects following all the studied routes of administration, including the intraarticular injections (see Fig. 5a). This efficient and prolonged depletion of TNF-α levels in the tissues that form the central and peripheral compartments is not desired and can potentially lead to adverse effects. The synovial fluid represents the minor site of
adalimumab TNF-α-neutralizing activity for the studied doses of the drug (~7%, see Fig. 5a), and the major pathway of TNF-α elimination from the synovial fluid is due to adalimumab binding, and not its degradation or permeation to the central circulation (see Fig. 6a). These values (see Fig. 5 and 6) are correct for the clinically-applied dose of adalimumab (40 mg, the clinically-approved dose), and the relative magnitude of the local vs. systemic effects of adalimumab and of the drug-induced TNF-α elimination from the synovial fluid are expected to be dose-dependent (decreased effects for lower drug doses). Currently, only subcutaneous adalimumab administration has been approved for the management of rheumatoid arthritis patients [21]. However, local administration of anti-TNF-α antibodies has been claimed to be superior to the systemic therapy [27], especially for the management of persistent inflammatory monoarthritis. Outcomes of this study suggest that sufficient amounts of adalimumab can reach the synovial fluid following systemic administration, and subcutaneous, intravenous and intraarticular administration of adalimumab exerts similar anti-TNF-α effects (see Fig. 3 and 5). Thus, apart from somewhat longer duration of the TNF-α-neutralizing effect, intraarticular administration does not appear to offer substantial advantages over subcutaneous or intravenous treatment.

It should be noted that the above-presented conclusions stem from the limited amount of data on distributional behavior of adalimumab and of TNF-α, and specifically on the kinetics of their permeability from the synovial fluid to the systemic circulation (see Table I). Unfortunately, sampling of synovial fluid is not a part of regular clinical protocol for anti-TNF-α antibodies, and quantitative analysis of TNF-α (free and total concentrations, see above) and adalimumab in the synovial fluid and in serum is technically challenging. Detailed clinical and preclinical analysis of the time course of adalimumab and TNF-α concentrations following different routes of adalimumab administration is required to confirm the conclusions of this study. Moreover, correlation between the time course of TNF-α-neutralizing effects and the clinical score of disease in the RA patients warrants further investigation. It has been previously reported that the clinical effects of adalimumab in patients with moderate/severe RA (the swollen joint count, tender joint count, and numeric American College of Rheumatology criteria) correlated with serum concentrations of adalimumab [41]. It is not clear whether the pharmacological effects of adalimumab in RA derive from the TNF-α AUC values that are reported in this study, from increase in TNF-α levels above a certain threshold value, or from migration to the joints and/or activation of specific subsets of leukocytes.

In similar fashion, applicability of this study's outcomes for other diseases managed with adalimumab (such as psoriasis, ankylosing spondylitis, Crohn's disease, etc.), for other anti-TNF-α agents, including infliximab, golumimab, etanercept (51.2 kDa, a receptor fusion protein), certolizumab pegol (47.8 kDa, PEGylated Fab' fragment), for other classes of fragment antibodies or conjugates [42], and for diseases other than rheumatoid arthritis should be determined. For instance, for optimizing the anti-psoriasis therapy, permeability of the anti-TNF-α agents to the psoriatic plaques and the turnover of TNF-α in psoriatic plaques vs. systemic circulation should be analyzed, although very limited clinical data on these parameters are currently available [43, 44].

**Characteristics of the developed model**

The developed model is an extension and a special case of the target-mediated drug disposition (TMDD) model that has been developed by Mager and Jusko [7] based on a concept by Gerhard Levy [6]. According to the modeling outcomes, adalimumab exhibits target-mediated disposition at the serum concentrations below $10^4$ pM (see Fig. 2). The developed model is relatively complex, and is based on several assumptions that can not be readily verified from the available data on disposition of adalimumab and of TNF-α (see
above). It has been assumed that all the pharmacokinetic processes of adalimumab and TNF-α are linear (first-order kinetics, except of second-order association kinetics of adalimumab and TNF-α, and zero-order synthesis kinetics of TNF-α). Moreover, influence of TNF-α or adalimumab-binding factors (such as soluble TNF receptors [45], or neutralizing antibodies against adalimumab [21]) has been neglected.

In addition, the rate constants of association, dissociation, and elimination of the adalimumab-TNF-α complexes have been assumed to be equal for all the compartments (see Fig. 1). Since the modeling outcomes were insensitive to the values of these parameters (see Fig. 4), even significant deviations from this assumption would not affect the conclusions of this study. The modeling outcomes were affected, although to a low extent, by the value of the rate constant of TNF-α degradation, and significant differences in this parameter between the individual compartments can affect the modeling outcomes to a certain extent. It has been assumed also that adalimumab is degraded only in the central compartment, and some of the outcomes regarding intraarticular vs. systemic injection of adalimumab would not be valid in case that the drug can be substantially degraded also in the joints.

CONCLUSIONS
Critical analysis of the available data on the disposition of adalimumab and TNF-α using pharmacokinetic modeling and simulation approach indicates that both adalimumab and TNF-α can equilibrate rather rapidly between the synovial fluid and the serum. As a result, subcutaneous, intravenous and intraarticular administration of the clinically-used doses of adalimumab (40 mg) exert similar effects on the time course of TNF-α levels. At these doses, adalimumab exhibits predominantly systemic anti-TNF-α effects and the contribution of the local effects in the rheumatic joints is small (~7%) for all the routes, including the local intraarticular injections. On the other hand, the major pathway of TNF-α elimination from the synovial fluid is interaction with adalimumab that reaches the joints. Kinetics of adalimumab permeation to the synovial fluid vs. the rate of TNF-α turnover in the affected joints are apparently the major parameters that determine the time course of TNF-α levels in the synovial fluid and the TNF-α-neutralizing effects of adalimumab in rheumatoid arthritis patients. The same permeability vs. turnover parameters should be taken into account to optimize treatment for antibodies and other classes of biopharmaceutical drugs acting on soluble targets (e.g., growth factors, interferons, interleukins, immunoglobulins, etc.).
REFERENCES


Table I. Parameters Used in Simulations

<table>
<thead>
<tr>
<th>Set</th>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug (adalimumab antibody)</td>
<td>F</td>
<td>SC bioavailability</td>
<td>0.64</td>
<td>-</td>
<td>from adalimumab prescription information [21]</td>
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<tr>
<td></td>
<td>$k_a$</td>
<td>SC absorption rate constant</td>
<td>0.030</td>
<td>h$^{-1}$</td>
<td>from Hollensen [24]</td>
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<tr>
<td></td>
<td>$Q_{dj}$</td>
<td>Antibody permeation into joints</td>
<td>0.00422</td>
<td>L/h</td>
<td>based on analysis of kinetics of antibody permeation to synovial fluid [26]</td>
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<td>$Q_{dp}$</td>
<td>Antibody permeation into tissues</td>
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<td>L/h</td>
<td>based on analysis of PK data [22, 23]</td>
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<tr>
<td></td>
<td>$k_{d10}$</td>
<td>Drug elimination rate constant</td>
<td>0.00431</td>
<td>h$^{-1}$</td>
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<tr>
<td>Target (TNF-α)</td>
<td>$k_{synC}$</td>
<td>Target synthesis rate in central compartment</td>
<td>11.3</td>
<td>pmol/h</td>
<td>based on the values of $Q_{tj}$, $Q_{tp}$, $k_{degr}$, and initial conditions of $T_c$, $T_p$ and $T_j$</td>
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<td></td>
<td>$k_{synJ}$</td>
<td>Target synthesis rate in joints</td>
<td>1.84</td>
<td>pmol/h</td>
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<td>$k_{synP}$</td>
<td>Target synthesis rate in peripheral compartment</td>
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<td>$Q_{tj}$</td>
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<td>based on $Q_{dj}$ adjusted for molecular radius of TNF-α [33]</td>
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<td>$Q_{tp}$</td>
<td>Target permeation into tissues</td>
<td>0.0625</td>
<td>L/h</td>
<td>based on $Q_{dp}$ adjusted for molecular radius of TNF-α [33]</td>
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<td></td>
<td>$k_{degr}$</td>
<td>Target degradation rate</td>
<td>0.877</td>
<td>h$^{-1}$</td>
<td>based on Mittelman et al [17] (assuming equal degradation rate in plasma and joints)</td>
</tr>
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<td>Drug-target complex</td>
<td>$k_{on}$</td>
<td>Association constant</td>
<td>6084</td>
<td>L/pmol/h</td>
<td>from Kaymakcalan et al [46]</td>
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<td></td>
<td>$k_{off}$</td>
<td>Dissociation constant</td>
<td>0.170</td>
<td>h$^{-1}$</td>
<td>same as target degradation rate</td>
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<tr>
<td></td>
<td>$k_{el}$</td>
<td>Degradation rate of drug-target complexes</td>
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<td>h$^{-1}$</td>
<td></td>
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<td>$Q_{dtj}$</td>
<td>Drug-Target complex permeation into joints</td>
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<td>based on $Q_{dj}$ adjusted for molecular radius of adalimumab-TNF-α complex [33]</td>
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<td>$Q_{dtp}$</td>
<td>Drug-Target complex permeation into tissues</td>
<td>0.0446</td>
<td>L/h</td>
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<td>Common</td>
<td>$V_C$</td>
<td>Central volume</td>
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<td>$V_J$</td>
<td>Synovial fluid volume</td>
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<td>based on Altman et al [47]</td>
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<tr>
<td></td>
<td>$V_P$</td>
<td>Peripheral volume</td>
<td>2.53</td>
<td>L</td>
<td>based on analysis of PK data [22, 23]</td>
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</table>
Figure legends

Figure 1. The pharmacokinetic model of TNF-α and adalimumab (the target and the drug). Both compounds exist in the body in 3 separate kinetic pools (central compartment, peripheral compartment and the joints) and can form drug-target complexes and be eliminated from each one of these locations.

Figure 2. Comparison of observed and predicted serum concentrations of adalimumab following intravenous bolus administration of the drug. The symbols represent the data from the clinical trials by den Broeder et al\textsuperscript{[22]} (a) and Weisman et al\textsuperscript{[23]} (b) and the lines represent the simulations based on the developed pharmacokinetic model.

Figure 3. Time course of adalimumab and TNF-α concentrations in the individual compartments following different modes of adalimumab administration as predicted from the developed pharmacokinetic model. The data sets include subcutaneous (a, b), intravenous bolus (c, d), and intraarticular injection (e, f) of 40 mg (270 nmol) adalimumab.

Figure 4. Effect of 5-fold alterations of individual parameter values on the time course of adalimumab and TNF-α concentrations. The percent change of adalimumab AUC in the synovial fluid (a) and in the serum (c) (D\textsubscript{J} AUC, and D\textsubscript{C} AUC, respectively) are presented along with percent change of TNF-α AUC in the synovial fluid (b) and in the serum (d) (T\textsubscript{J} AUC, and T\textsubscript{C} AUC, respectively). This sensitivity analysis was based on simulations of subcutaneous administration of 40 mg (270 nmol) adalimumab (except of the first bar on each panel when 5-fold lower or higher dose was used).
Figure 5. Contribution of the individual compartments to the anti-TNF-α activity of adalimumab as predicted from the developed pharmacokinetic model. (a) The relative contribution of the individual compartments to the anti-TNF-α activity of adalimumab on the whole body level following subcutaneous (SC), intravenous bolus (IV), and intraarticular injection (IA) of 40 mg (270 nmol) adalimumab. (b) and (c) The sensitivity analysis based on simulations of subcutaneous administration of 40 mg (270 nmol) adalimumab (except of the first bar on each panel when 5-fold lower or higher dose was used): effect of 5-fold alterations of individual parameter values.

Figure 6. The relative contribution of the individual pathways of TNF-α elimination from the joints as predicted from the developed pharmacokinetic model. (a) The relative contribution of the individual pathways of TNF-α elimination from the joints following subcutaneous (SC), intravenous bolus (IV), and intraarticular injection (IA) of 40 mg (270 nmol) adalimumab. (b) and (c) The sensitivity analysis based on simulations of subcutaneous administration of 40 mg (270 nmol) adalimumab (except of the first bar on each panel when 5-fold lower or higher dose was used): effect of 5-fold alterations of individual parameter values.

Figure 7. Predicted effect of change in the antibody permeation into the joints (Qdj values) on: (a) The TNF-α AUC in the serum and in the synovial fluid (T_C AND T_j, respectively; calculated as decline in TNF-α concentrations below the baseline values), and (b) The relative contribution of the individual pathways of TNF-α elimination from the joints. These simulations assumed subcutaneous administration of 40 mg (270 nmol) adalimumab. The dotted line indicates the expected value of Qdj in the rheumatoid arthritis patients (see Table I).
Figure 1.

Drug (antibody)

Drug-Target complex

Target (TNFα)

joints (synovial fluid)

central (serum)

peripheral

Figure 2.

a. den Broeder 2002 data

b. Weisman 2003 data
Figure 3.

SC

IV

IA

Adalimumab concentration, pM

TNF-α concentration, pM

Time, days

Time, days
Figure 4.
Figure 5.

a. original values

b. 5-fold decrease

c. 5-fold increase
Figure 6.

a. original values

b. 5-fold decrease

c. 5-fold increase
Figure 7.

a. 

b.