Regular Article

Theory Based Analysis of Anti-Inflammatory Effect of Infliximab on Crohn's Disease

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Summary: Tumor necrosis factor (TNF)-α, a primary mediator of inflammatory responses, is increased in patients with active Crohn's disease (CD) and considered to play an important role in the regulation of inflammation in CD. Infliximab (IFX) is a chimeric murine-human monoclonal IgG1 antibody that targets TNF-α and is used as a therapeutic agent for CD. Although that dosage regimen has been established through clinical trial experience, it has not been analyzed theoretically. We analyzed of sequential changes of the Crohn's disease activity index (CDAI) using a pharmacokinetic-pharmacodynamic model integrating the pharmacokinetics of IFX and turnover rate of TNF-α. The time course effects of IFX derived from the present model were matched to reported data regarding CDAI ratios, and we found that the clinical effect of IFX reached a maximum value 2 to 4 weeks after administration and was maintained for the next several weeks. Our results suggested that the standard dosage regimen of IFX is theoretically appropriate. Further, based on the results of various dosage regimens, a second administration of IFX 2 weeks after the first dose was shown to achieve remission in the early stage of active CD, when IFX was given as a repeated treatment.

Key words: tumor necrosis factor-α; Crohn's disease activity index; pharmacokinetic-pharmacodynamic model

Introduction

Crohn's disease (CD) is characterized by chronic transmural inflammation in any part of the gastrointestinal tract.[1] The major symptoms are diarrhea, abdominal pain, fistulas, and weight loss, with the peak age at onset ranging from 15 to 25 years old.

Tumor necrosis factor (TNF)-α, a primary mediator of inflammatory responses, is increased in patients with active CD[2-4] and considered to play an important role in the regulation of inflammation in CD. Infliximab (IFX) is a chimeric murine-human monoclonal IgG$_1$ antibody that targets TNF-α and is used as a therapeutic agent for CD, as it binds with a high affinity to TNF-α and neutralizes its effects.[5-7] In Japan, the recommended dose of IFX is 5 mg/kg, which is given as a single intravenous infusion for treatment of moderately to severely active CD.[8] In patients with fistulizing disease, the initial dose should be followed with an additional dose at 5 mg/kg at 2 and 6 weeks after the first infusion.[8] Most patients treated with IFX show a clinical response in approximately 2 weeks,[9] which lasts for several weeks thereafter.[10] Thus, after following for at least 2 weeks, retreatment should be considered for patients who respond and then lose responsiveness.[8] Although that dosage regimen has been established through clinical trial experience, it has not been analyzed theoretically.

In the present study, we evaluated the validity of the clinical dosage regimen of IFX using a pharmacokinetic-pharmacodynamic model by utilizing sequential changes in the Crohn's disease activity index (CDAI) scores of patients who received IFX as a therapeutic index.

Methods

Pharmacokinetic and pharmacodynamic parameters: All data regarding use of the present analyses were

collected from previously published reports. We determined the time course of serum concentration of IFX after administration of a single infusion of IFX at a dose of 5, 10, or 20 mg/kg.[11] Clinical responsiveness was evaluated by CDAI score, which is commonly used in clinical studies to determine the severity of CD activity. Values representing the sequential changes of CDAI after a single intravenous infusion of 1, 3, 5, or 10 mg/kg of IFX were obtained from CDAI scores obtained at 0, 2, 4, 8, and 12 weeks.[12] Values for sequential changes of CDAI with repeated IFX treatments were used from 2 groups of patients who responded to a single infusion of IFX within 2 weeks,[13] according to the following protocol. Following assessment of response after week 2, all patients were randomly grouped into those who received repeated infusions of 5 mg/kg of IFX at weeks 2 and 6 and then every 8 weeks thereafter until week 46 (group 1), or those who received 5 mg/kg of IFX at weeks 2 and 6 followed by a single administration of 10 mg/kg (group 2).[13]

Analysis of time courses of serum concentration of IFX: At dosage amounts ranging from 5 to 20 mg/kg, the clearance of IFX is unchanged by increasing the dose, whereas linear relationships can be observed among the dose administered, maximum serum concentration (C_{max}), and area under the curve. Therefore, in the present study, the time courses of serum concentration after a single infusion of IFX were analyzed using the following one-compartment pharmacokinetic model:

$$C_{IFX} = C_{IFX}^0 \cdot e^{-k_e \cdot t} \qquad (1)$$

where C_{IFX} (μM), C_{IFX}^0 (μM), k_e (day^{-1}), and t (weeks) represented the serum concentration of IFX, maximum serum concentration of IFX, elimination rate constant, and time after administration, respectively. To estimate the pharmacokinetic parameters of C_{IFX}^0 (μM) and k_e (day^{-1}), the serum concentration of IFX after a single infusion of IFX at a dose of 5, 10, or 20 mg/kg was simultaneously fitted to Eq. (1) using the nonlinear least squares program MLAB (Civilized Software). MLAB is a computer program whose name is an acronym for "modeling laboratory". It is a tool for experimentation with and exploration of mathematical models and for their evaluation, originally developed at the National Institutes of Health. MLAB can simultaneously fit multiple non-linear model functions, some or all of which may be implicit functions, or may even be defined by a system of differential equations.

Analysis of sequential changes of CDAI: The pharmacodynamic model derived from the pharmacokinetic profile of IFX and the turn-over rate of TNF-α is shown in **Fig. 1**. With this model, we assumed that the degree of inflammation was changed by the binding of
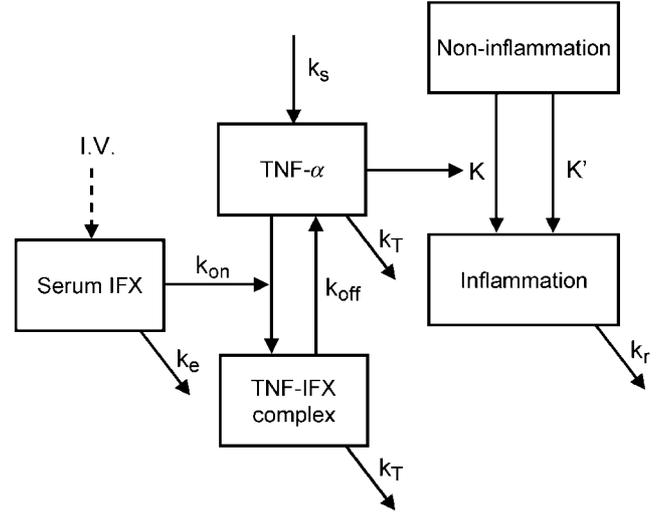
Fig. 1. Pharmacokinetic and pharmacodynamic model for anti-inflammatory effect of infliximab (IFX). IFX was shown to be eliminated at the rate constant of k_e (day^{-1}), while tumor necrosis factor (TNF)-α was generated at the rate constant of k_s (μM·day^{-1}) and eliminated at the rate constant of k_T (day^{-1}). TNF-α forms a complex with IFX at the binding rate constant of k_{on} (μM^{-1}·day^{-1}) and is dissociated from the TNF-IFX complex at a dissociation rate constant of k_{off} (day^{-1}). Inflammation occurs at the rate constants of K (day^{-1}) and K′ (day^{-1}), and is remitted *in vivo* at the rate constant of k_r (day^{-1}).

IFX to TNF-α, which served to neutralize its biological activity.

TNF-α is generated at a rate constant of k_s (μM·day^{-1}) and eliminated at a rate constant of k_T (day^{-1}). TNF-α forms a complex with IFX at a binding rate constant of k_{on} (μM^{-1}·day^{-1}) and is dissociated from the TNF-IFX complex at a dissociation rate constant of k_{off} (day^{-1}). Thus, the concentration of TNF-α and TNF-IFX complex was represented as follows:

$$\frac{dC_{TNF}}{dt} = -k_{on} \cdot C_{TNF} \cdot C_{IFX} - k_T \cdot C_{TNF} + k_s$$
$$+ k_{off} \cdot C_{TNF-IFX} \qquad (2)$$

$$\frac{dC_{TNF-IFX}}{dt} = k_{on} \cdot C_{TNF} \cdot C_{IFX} - k_T \cdot C_{TNF-IFX}$$
$$- k_{off} \cdot C_{TNF-IFX} \qquad (3)$$

$$k_{off} = k_I \cdot k_{on} \qquad (4)$$

where k_I (μM^{-1}) is the dissociation constant. The total concentration of TNF-α, C_T (μM), was expressed as $C_T = k_s/k_T$. Then, when it was defined as $C_{TNF}/C_T = X$ and $C_{TNF-IFX}/C_T = Y$, Eqs. (2) and (3) were rearranged into Eqs. (5) and (6), respectively.

$$\frac{dX}{dt} = k_{on} \cdot X \cdot C_{IFX} - k_T \cdot X + k_T + k_I \cdot k_{on} \cdot Y \qquad (5)$$

$$\frac{dY}{dt} = k_{on} \cdot X \cdot C_{IFX} - k_T \cdot Y + k_I \cdot k_{on} \cdot Y \qquad (6)$$
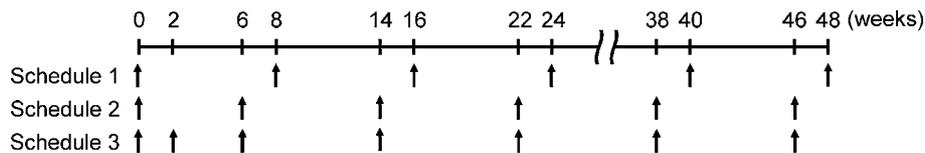
Fig. 2. Dosage intervals of infliximab.

Assuming that the inflammation caused by TNF-α at the rate constant of K (day^{-1}) was according to the proportion of the unbound TNF-α, K was determined according to Eq. (7) as follows:

$$K = K_{max} \cdot \frac{C_{TNF}}{C_{TNF-IFX} + C_{TNF}} = K_{max} \cdot \frac{X}{X+Y} \quad (7)$$

where K_{max} (day^{-1}) is K in the absence of IFX. We thought that the inflammation caused by factors other than TNF-α at the rate constant of K′ (day^{-1}), as well as inflammation caused by K and K′, would be remitted *in vivo* at the rate constant of k_r (day^{-1}). Therefore, the value of CDAI at t (weeks) (E_t) in the presence of IFX was represented as follows:

$$\frac{dE_t}{dt} = K + K' - k_r \cdot E_t \quad (8)$$

Further, the value of CDAI in the absence of IFX (E_0) was expressed by the following equation:

$$E_0 = \frac{K_{max} + K'}{k_r} \quad (9)$$

The values of the sequential changes of CDAI were represented by the ratio (CDAI ratio: ER) of CDAI at t (weeks) (Et) to E_0:

$$ER = \frac{E_t}{E_0} \quad (10)$$

To estimate k_{on}, k_T, K_{max}, K′, and k_r, data from patients administrated a single infusion or repeated infusions of IFX at the tested doses were fitted simultaneously to the above equations. The reported k_I value (0.046 nM)[6] was adopted for this study. Patients given a single infusion and those administrated repeated infusions were independently grouped by different investigators, thus, the estimated k_T values for each patient group were calculated as k_{T1} and k_{T2}, respectively.

Simulation of values for sequential changes of CDAI following administrations of IFX at various intervals: The changes of ER following repeated infusions of IFX at a dose of 5 mg/kg at various intervals **(Fig. 2)** were simulated using the estimated pharmacokinetic and pharmacodynamic parameters and assessed to determine a rational dosage regimen. For this study, the threshold of positive clinical response was defined as a decrease in CDAI score of 70 points or more and clinical
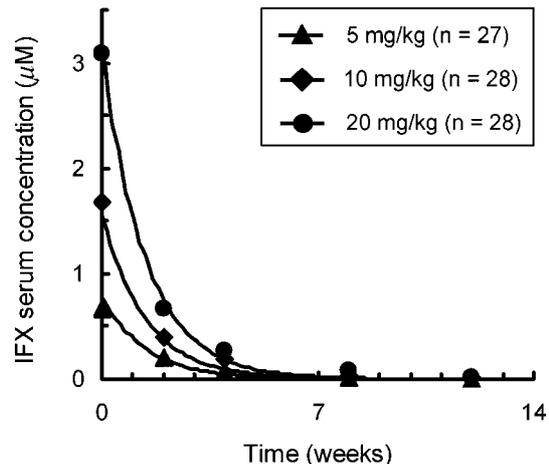

Fig. 3. Serum concentration-time profiles of infliximab (IFX) following a single infusion. Symbols represent reported data (median) from literature.[11] The regression lines were calculated simultaneously using the nonlinear least squares method.

Table 1. Pharmacokinetic parameters for infliximab

| Parameter	Estimated value
C_{max}^0 (μM)	0.778 ± 0.015
k_e (day^{-1})	0.101 ± 0.005

remission was defined by a CDAI score of less than 150 points.[12,13]

Results

Time course analysis of serum concentration of IFX: The time courses of serum concentration of IFX following a single infusion at a dose of 5, 10, or 20 mg/kg, along with the fitted curves based on the nonlinear least squares methods, are shown in **Fig. 3**. The estimated pharmacokinetic parameters of IFX are shown in **Table 1**. The fitted curves were well matched to the observed data.

Analysis of sequential changes of CDAI: The sequential changes of CDAI ratio (ER) following a single infusion or repeated infusions of IFX and the fitted curves based on the simultaneous nonlinear least squares methods are shown in **Figs. 4 and 5**. The estimated pharmacodynamic parameters are shown in **Table 2**. The relationships between the time course effects of IFX derived from the pharmacodynamic
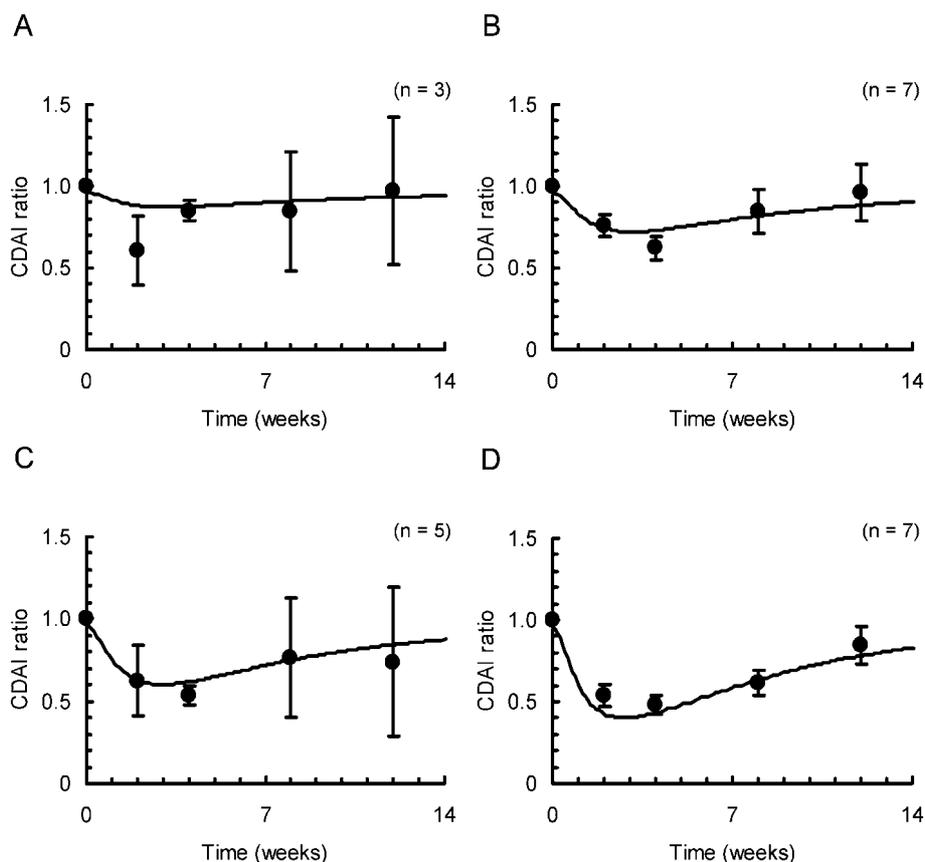
Fig. 4. Sequential changes of the Crohn's disease activity index (CDAI) scores following a single infusion at a dose of 1 mg/kg (A), 3 mg/kg (B), 5 mg/kg (C), and 10 mg/kg (D). Symbols were derived from data reported in literature[12] (mean ± SD). The regression lines are fitted curves.
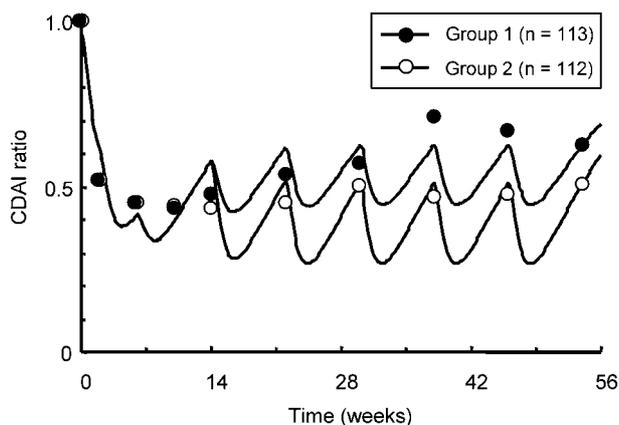
Fig. 5. Sequential changes of Crohn's disease activity index (CDAI) scores after repeated infusions. Symbols were derived from data reported in literature. The regression lines are fitted curves.

Table 2. Pharmacodynamic parameters for infliximab after a single infusion or repeated infusions

Parameter	Estimated value
k_{on} ($\mu M^{-1} \cdot day^{-1}$)	0.101 ± 0.009
k_{T1} (day^{-1})	0.0217 ± 0.003
k_{T2} (day^{-1})	0.0157 ± 0.0009
K_{max} (day^{-1})	177.6 ± 47.2
K' (day^{-1})	$1.047 \times 10^{-8} \pm 0.401 \times 10^{-8}$
k_T (day^{-1})	0.346 ± 0.091

model in the present study were matched to the reported data for CDAI ratios. No significant differences were observed between the calculated and observed CDAI ratios by χ^2-test (data not shown). The present model showed that the clinical effect of IFX reached maximum from 2 to 4 week after administration of IFX and was maintained for the next several weeks (**Fig. 4**).

Simulation of values representing sequential changes of CDAI following administration of IFX at the tested intervals:

The time courses of CDAI ratios following repeated infusions at a dose of 5 mg/kg IFX at the tested intervals were simulated from the estimated pharmacokinetic parameters (**Fig. 6**). With Schedules 1 and 2, more time was required to reach clinical remission than with Schedule 3.

Discussion

CD is a disease of unknown etiology, however, since
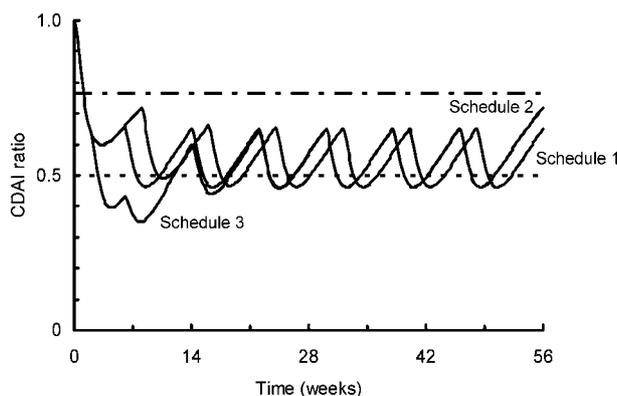
Fig. 6. Simulation of relationships between Crohn's disease activity index (CDAI) ratio and time after repeated infusion at tested intervals. Dashed line (----), upper limit of CDAI ratio corresponding to remission; chain line (-·-), upper limit of CDAI ratio corresponding to positive clinical response.

TNF-α levels are known to increase in patients with active CD, it is considered that chronic inflammation associated with the disease is related to TNF-α.[2-4] In the present study, the estimated K′ value was $1.047 \times 10^{-8} \pm 0.401 \times 10^{-8}$ day^{-1}, which was smaller than the K_{max} value (177.6 ± 47.2 day^{-1}) (**Table 2**), suggesting that TNF-α is mainly involved in the inflammation associated with CD, though the participation of other factors is possible. The estimated half-life value of TNF-α, calculated from k_{T1} and k_{T2}, was 31.9 and 44.1 days, respectively, which were both greater than the half-life of serum TNF-α, reported to be several minutes.[14,15] Therefore, it is considered that IFX does not interact only with soluble TNF-α, but also transmembrane TNF-α.[6] Transmembrane TNF-α is produced by monocytes, macrophages, and T-cells, and is released from cells as soluble TNF-α by metalloproteinase.[16] Although soluble TNF-α is rapidly inactivated and eliminated, transmembrane TNF-α would exhibit the same behavior with these cells whose lifetime has been reported to be several months.[17] Therefore, we considered that the estimated value of k_T from the present study consisted of the elimination rate constants of both transmembrane and soluble TNF-α.

The results of the present pharmacokinetic-pharmacodynamic model of the effects of IFX were in good agreement with the observed CDAI ratio data, which was considered to show the validity of our analysis. In addition, our findings indicated that the clinical effect of IFX reached a maximum level in 2 to 4 weeks after administration and was then maintained for the next several weeks. These results suggest that the standard dosage regimen of IFX, 5 mg/kg as a single intravenous infusion or that followed by additional 5 mg/kg doses 2 and 6 weeks later, is theoretically appropriate to achieve remission in the early stage of active CD. The results of

the simulation of regimen in Schedules 1 and 2, in which IFX was not re-administrated 2 weeks after the first infusion, showed the importance of another administration of IFX after 2 weeks to obtain remission in an early stage of active CD, if IFX is given as a repeated treatment.

In this study, we adapted the serum concentration of IFX and sequential change of CDAI in CD patients from previously published reports. Thus, the application limit of the present analytical model is following: (i) the model is only used for CD patients but not for the other disease such as rheumatoid arthritis; (ii) the dose of IFX is limited 1–20 mg/kg for sigle injection and 5–10 mg/kg for repeated administration, respectively; and (iii) CDAI value after the 46 week is not examined.

In conclusion, from our results, it was suggested that TNF-α was mainly involved in the inflammation associated with CD. Furthermore, it is thought that the rational dosage regimen of IFX for individual patient can be planned according to this analytical model. For instance, if a CDAI value after a first time administration of IFX is obtained, it is able to estimate the pharmacodynamic parameter for the individual patient by applying the CDAI value to the model. And it would become possible that an appropriate dosage can be set from the next administration. Thus, we considered that the present analytical model was useful to establish a rational dosage regimen of IFX for individual patients with CD.

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