Influence of methotrexate on infliximab pharmacokinetics and pharmacodynamics in ankylosing spondylitis

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Infliximab pharmacokinetics in ankylosing spondylitis has been described but using sparse data.
- A previous work suggests that infliximab concentrations influence clinical response in ankylosing spondylitis, but the infliximab concentration–effect relationship is not known in this disease.
- Methotrexate was shown to influence infliximab pharmacokinetics in rheumatoid arthritis.

WHAT THIS STUDY ADDS

- This study is the first to describe infliximab pharmacokinetics in ankylosing arthritis using rich data.
- The infliximab concentration explains only a small part of interindividual variability in the response of ankylosing arthritis patients.
- Contrary to what is observed in rheumatoid arthritis, methotrexate influences neither infliximab pharmacokinetics nor concentration–response relationship in ankylosing spondylitis.

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Keywords

ankylosing spondylitis, infliximab, methotrexate, pharmacokinetics

Received

8 October 2010

Accepted

10 June 2011

Accepted Article

22 June 2011

AIMS

Infliximab, an anti-tumour necrosis factor α monoclonal antibody, has profoundly modified the treatment of several inflammatory diseases. The objective was to assess the influence of methotrexate on the variability of infliximab pharmacokinetics and concentration–effect relationship in axial ankylosing spondylitis (AAS) patients.

METHODS

Twenty-six patients with AAS were included in a prospective study. They were treated by infliximab 5 mg kg⁻¹ infusions at weeks 0, 2, 6, 12 and 18. Infliximab concentrations were measured before, and 2 and 4 h after each infusion, and at each intermediate visit (weeks 1, 3, 4, 5, 8, 10 and 14). Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was measured at each visit. Infliximab pharmacokinetics was described using a two-compartment model with first-order distribution and elimination constants. A population approach was used. Infliximab pharmacodynamics was described using the area under the BASDAI curve.

RESULTS

A total of 507 blood samples and 329 BASDAI measurements were collected. The following pharmacokinetic parameters were obtained (interindividual coefficient of variation): volumes of distribution for the central compartment = 2.4 l (9.6%) and peripheral compartment = 1.8 l (26%), systemic clearance = 0.23 l day⁻¹ (22%) and intercompartment clearance = 2.3 l day⁻¹. Methotrexate influenced neither pharmacokinetic nor BASDAI variability.

CONCLUSIONS

Using the present dosage, the clinical efficacy of infliximab is only weakly influenced by its serum concentrations. The results do not support the combination of methotrexate with infliximab in ankylosing spondylitis.

Introduction

Infliximab is a chimeric monoclonal immunoglobulin G1 antibody targeted against tumour necrosis factor α (TNF- α). This biopharmaceutical has profoundly modified the treatment of several inflammatory diseases and is currently approved for rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn's disease (CD), chronic ulcerative colitis, psoriatic arthritis and psoriasis. Infliximab was developed in AS because high concentrations of TNF- α are measured in AS patients [1].

The administration of infliximab leads to highly variable serum concentrations between patients [2]. This variability is relevant because infliximab concentrations were shown to influence the clinical response of RA [3], CD [4], AS [5, 6] and psoriatic patients [7]; however, to our knowledge, infliximab pharmacokinetics in AS has never been described using rich data.

Methotrexate may play a role in this pharmacokinetic variability because higher infliximab concentrations were observed in RA patients cotreated with methotrexate than in those treated with infliximab alone [8–10]. Immunosuppressive agents as a whole modify infliximab concentrations as reported in CD patients [4, 11]. The increase of clinical efficacy of infliximab together with its concentrations during methotrexate co-administration, as observed in the pivotal study of infliximab in RA, led to the approval of the combination of infliximab and methotrexate in this disease.

In axial ankylosing spondylitis (AAS), methotrexate alone has no efficacy [12, 13], but the combination of infliximab and methotrexate seems to be safe and effective [14]; however, data comparing infliximab with and without methotrexate are scarce and controversial [15]. Perez-Guijo *et al.* found a better clinical response when methotrexate is added to infliximab [16], whereas Breban *et al.* found a nonsignificant trend for a better response with the association [17]. In this latter study, Krzysiek *et al.* observed no influence of methotrexate on infliximab concentrations except for early relapsing patients, in whom infliximab concentrations were higher when methotrexate was associated [18]. However, these data are difficult to interpret, because infliximab was not administered on a regular basis in patients cotreated with methotrexate. Therefore, whether or not methotrexate influences infliximab pharmacokinetics or the concentration–effect relationship in AAS remains unclear.

The aim of this comparative randomized study was to describe infliximab pharmacokinetics and concentration–response relationship in axial ankylosing spondylitis, and to assess the influence of methotrexate on infliximab pharmacokinetics and concentration–response relationship.

Methods

Patients

This study, approved by the ethics committee of Tours University Hospital, was conducted in accordance with the Declaration of Helsinki. It was registered on clinicaltrials.gov as NCT00507403. Patients were recruited between January 2008 and April 2009. The study details were explained to all patients, and all of them provided written informed consent. To be eligible, patients had to be adult (18 years of age or older), with AS according to modified New York criteria [19], with an indication for anti-TNF- α treatment and no contraindication for either infliximab or methotrexate. Treatment with nonsteroid anti-inflammatory drugs before or during the study was allowed. Patients were excluded from the study if they were pregnant or anticipate getting pregnant at any time during the study, breast feeding, addicted to alcohol or drugs within 1 year prior to the inclusion or were participating in another clinical study; if they had been or were already being treated with infliximab or methotrexate; if they had white blood cells $<2000 \text{ mm}^{-3}$, haemoglobin $<9 \text{ g dl}^{-1}$ or platelets $<10^5 \text{ mm}^{-3}$, an active malignancy within the 5 years prior to the inclusion, severe or persistent infections requiring hospitalization or intravenous antibiotic treatment within 30 days prior to inclusion, severe chronic disease (hepatitis B or C, human immunodeficiency virus, active or latent tuberculosis, demyelinating disease, or renal, hepatic, haematological, endocrine, pulmonary, cardiac, neurological or brain degenerative disease), or a planned surgical intervention during the study period.

Study design

This 18 week comparative, prospective, randomized, open, bicentric study (Tours and Besançon, France) was designed to assess the influence of methotrexate on infliximab pharmacokinetics and concentration–response relationship in AAS. At the enrolment visit (week –2), patients were randomly assigned to infliximab alone (IFX alone arm) or to the combination of infliximab and methotrexate (MTX+IFX arm).

In both arms of the study, patients were assessed weekly between weeks 0 and 6, every 2 weeks between weeks 6 and 14, at week 18 and, if possible, at week 24. Infliximab 5 mg kg^{-1} infusions were administered at weeks 0, 2, 6, 12 and 18. Infusions of infliximab lasted for 2 h. In the IFX+MTX arm, oral methotrexate 10 mg per week was taken on the day before the weekly visits and every week afterwards.

Data

Infliximab concentrations At each visit, blood samples were collected to measure infliximab serum concentrations. At weeks 0, 2, 6 and 12, samples were also collected immediately after the end and 2 h after the end of the infu-

sion. Infliximab concentrations were measured using a validated enzyme-linked immunosorbent assay (ELISA) [20]. The limit of detection was 0.014 mg l^{-1} , and the lower and upper limits of quantification (between-assay precision, coefficient of variation %) were 0.04 (9.8%) and 4.5 mg l^{-1} (5.3%), respectively.

Other laboratory analyses At the enrolment visit and at each subsequent visit, erythrocyte sedimentation rate and C-reactive protein were measured, and antibodies towards infliximab (ATI) were detected. In each centre (Tours and Besançon, France) erythrocyte sedimentation rate was measured in the local laboratory, whereas C-reactive protein was measured in the laboratory of biochemistry of Tours university hospital. The serum concentration of ATI was measured by a double-antigen ELISA on the basis of capture by infliximab-coated microplates and detection by peroxidase-conjugated infliximab. This assay was standardized by the use of a mouse monoclonal antibody against human immunoglobulin G. Owing to interference by circulating infliximab, only sera with an infliximab concentration $<2 \text{ mg l}^{-1}$ were tested. The positive threshold of detection was 0.07 mg l^{-1} . A patient was considered ATI-positive if ATIs were detected on at least one visit. Positive ATI patients were removed from the pharmacokinetic analysis.

BASDAI score At each visit, patients answered the French version of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). This index provides a numerical rating scale (0–10) which measures the disease impact in terms of pain, fatigue and morning stiffness as a numerical rating scale [21].

Pharmacokinetic analysis

Software Pharmacokinetic and pharmacodynamic data were analysed with a population approach using the non-linear mixed-effects program MONOLIX 3.1 software [22], which combines the stochastic expectation-maximization (SAEM) algorithm and a Markov chain Monte-Carlo procedure for likelihood maximization. This software showed satisfactory performance in difficult analyses [23]. To ensure the best possible convergence, a large number of iterations (3000 for K1 and 1000 for K2) was performed. K1 and K2 refer to the SAEM procedure of Monolix, called 'iteration kernels'. During K1, the sequence of step sizes is constant, which allows the exploration of the parameter space. During K2, the step sizes decrease to ensure convergence. Five Markov chains were used, and simulated annealing was used to improve the convergence of the SAEM algorithm towards the global maximum of the likelihood. Each run was made three times to ensure that estimated parameters and likelihood remained stable. The random seed was changed between each of the three runs.

Structural model Infliximab concentrations were described using compartmental pharmacokinetic modelling.

One, two and three mammillary compartment models with first-order distribution constants were tested. Linear and nonlinear (Michaelis–Menten) eliminations were also tested. Structural models were compared using Akaike's information criterion (AIC) [24], defined as: $\text{AIC} = \text{OFV} + 2p$, where OFV is the value of the objective function and p is the number of model parameters to be estimated. The OFV was $-2 \times \ln(\text{likelihood})$ (-2LL). The model with the lowest AIC was chosen.

Interindividual and error model The interindividual variability of pharmacokinetic parameters was described using an exponential model: $\theta_i = \theta_{\text{TV}} \cdot \exp(\eta_i)$, where θ_i is the estimated individual parameter, θ_{TV} the typical value of the parameter and η_i the random effect for the i th patient. The values of η_i were assumed to be normally distributed with mean 0 and variance ω^2 . Correlations between random effects were tested. Additive, proportional and mixed additive–proportional residual error models were tested. For example, the combined additive–proportional model was implemented as follows: $Y_{O,ij} = Y_{P,ij}(1 + \varepsilon_{\text{prop},ij}) + \varepsilon_{\text{add},ij}$, where $Y_{O,ij}$ and $Y_{P,ij}$ are observed and predicted j th measurements for the i th patient, respectively, and $\varepsilon_{\text{add},ij}$ and $\varepsilon_{\text{prop},ij}$ are additive and proportional errors, with mean 0 and respective variances σ_{add}^2 and σ_{prop}^2 .

Covariates Owing to the relatively small number of patients, only a few covariates were tested, which were already shown to influence infliximab concentrations or efficacy. Binary covariates were sex and methotrexate cotreatment. Continuous covariates were age, height, weight and body surface area (BSA). The influence of a binary covariate on θ_{TV} was implemented as $\ln(\theta_{\text{TV}}) = \ln(\theta_{\text{CAT}=0}) + \beta_{\text{CAT}=1}$, where $\theta_{\text{CAT}=0}$ is the value of θ for the reference category and $\beta_{\text{CAT}=1}$ is a parameter which provides the value of θ_{TV} for the other category. Continuous covariates (COV) were centred on their median, as follows: $\theta_i = \theta_0[\text{COV}/\text{med}(\text{COV})]^{\beta_{\text{COV}}}$, where θ_0 is value of θ for the median value of COV, β_{COV} quantifies the influence of COV on θ and $\text{med}(\text{COV})$ is the median value of COV in the population.

Model comparison and covariate selection Interindividual, residual and covariate models were compared using -2LL and AIC. Of two models, that with the lowest significant -2LL value, assessed by a likelihood ratio χ^2 test (LRT), and the lowest AIC was selected. First, the individual influence of each covariate on each value was tested using the LRT test with $\alpha = 0.1$. If some covariates were redundant (e.g. weight and BSA), the most significant was kept. As the number of selected covariates at the first step was low, no stepwise forward/backward covariate selection was needed; each combination of covariates which influenced parameters was tested to obtain the final model. The covariates were kept in the final model if their influence was significant for $\alpha = 0.01$. The goodness of covariate description was inspected by visual inspection of random effects (i.e. ETA) vs. covariate plots.

Model goodness of fit and evaluation In general, goodness of fit for a given model was assessed by plots of population-predicted (PRED) and individual-predicted (IPRED) measurements vs. observed measurements [9], IPRED and observed concentrations vs. time, and by evaluating the residuals via graphical inspection of population (PWRES) and individual weighted residual (IWRES) distributions, and normalized prediction distribution errors (NPDE) [25].

Computation of the area under the concentration vs. time curve The area under the concentration vs. time curve from weeks 0 to 18 (AUC₁₈) was computed for each patient, which is the largest interval for which all concentrations and BASDAI measurements are available.

Pharmacodynamics

For each patient, the area under the BASDAI vs. time curve from weeks 0 to 18 (AUE) was calculated using the trapezoidal method. This value was standardized as AUE_s = (AUE/E₀) – 100, where AUE_s (%) is the standardized AUE and E₀ is the reference area, i.e. the individual area that would have been obtained if BASDAI remained at baseline between weeks 0 and 18. Negative or positive values for AUE_s corresponded to disease improvement or worsening, respectively.

Statistical analysis

The characteristics of the patients of each arm of the study were compared. Quantitative variables were compared using Student's unpaired *t*-test. Qualitative variables were compared using Pearson's χ^2 test; if the number of patients in one group was less than five, Fisher's exact test was used. All tests were two-tailed, with $\alpha = 0.05$. These analyses were carried out using R software 2.7.1 (Vienna, Austria) [26].

Results

Patients

Twenty-six patients were included in the study, 14 in the methotrexate and infliximab cotreatment (IFX+MTX) arm and 12 in the infliximab alone (IFX alone) arm. The majority of patients were men with active AAS and presented low inflammatory activity. Antibodies towards infliximab (ATI) were detected in only one patient. There was no significant difference between arms (Table 1).

Pharmacokinetic modelling

Patient no. 17 was ATI+ and was therefore removed from the analysis. A total of 484 infliximab serum concentrations were available for the 25 patients included. The best description of concentration data was obtained using a structural two-compartment model with first-order distribution and elimination constants, as follows:

$$\begin{aligned}\frac{dC_1}{dt} &= (ln(t) - CL \cdot C_1 - Q \cdot C_1 + Q \cdot C_2) / V_1 & C_1(0) &= 0 \\ \frac{dC_2}{dt} &= (Q \cdot C_1 - Q \cdot C_2) / V_2 & C_2(0) &= 0\end{aligned}$$

where $ln(t)$ is infliximab infusion rate (in milligrams per hour), C_1 (in milligrams per litre) and C_2 (in milligrams per litre) are concentrations of central and peripheral compartments, respectively, V_1 (in litres) and V_2 (in litres) are volumes of distribution of the central and peripheral compartments, respectively, CL (in litres per day) and Q (in litres per day) are systemic and distribution clearance, respectively.

The best residual model was combined additive-proportional. A third compartment was not identifiable, and a nonlinear elimination did not improve model fitting.

Table 1

Summary of patient characteristics at baseline

Patient characteristics	IFX+MTX (n = 14)	IFX alone (n = 12)	P value
Sex: men/women, n (%)	11/3 (79/21)	9/3 (75/25)	0.8
Age (years)	45.5 [29–55]*	42.5 [27–59]*	0.3
Height (m)	1.73 [1.63–1.81]*	1.71 [1.54–1.75]*	0.1
Weight (kg)	77 [60–123]*	70.5 [52–104]*	0.2
Body surface area (m ²)	1.92 [1.66–2.45]*	1.85 [1.54–2.28]*	0.2
Disease duration (years)	4.5 [1–19]*	4 [0–28]*	0.9
HLA B27+, n (%)	10 (71)	9 (75)	0.8
Sacroiliitis, n (%)	13 (93)	11 (92)	0.5
Amor score	8 [6–13]*	8 [4–12]*	0.5
NSAIDs cotreatment	12 (86)	8 (67)	0.5
BASDAI	7.0 [5.0–8.2]*	5.8 [3.9–8.4]*	0.2
ESR (mm)	8.5 [1–50]*	4 [1–25]*	0.4
C-reactive protein (mg l ⁻¹)	2.65 [0.5–31.2]*	3.6 [0.5–18]*	0.5

*Results are given as median [range]. Amor score is a clinical score that contains several items and is used to make the ankylosing spondylitis diagnosis (score > 6). Abbreviations: BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; ESR, erythrocyte sedimentation rate; IFX, infliximab; MTX, methotrexate; NSAIDs, nonsteroid anti-inflammatory drugs.

Figure 1

Observed concentration vs. population model-predicted values (PRED; A), individual predicted values (IPRED; B), log-PRED (C) and log-IPRED (D)

No significant correlation was found between the inter-individual distributions of the pharmacokinetic parameters. All diagnostic plots were obtained from the final model. Some concentrations measured within the 2 h following the end of an infusion ($>220 \text{ mg l}^{-1}$) were underpredicted by the model (Figure 1). Residual distribution and normalized prediction distribution error (NPDE) plots (Figure 2), and observed and predicted concentration vs. time plots (Figure 3), showed a good agreement of the model with the data. The pharmacokinetic parameters were estimated with good precision (Table 2).

Height, weight and BSA had a significant influence on V_1 , and BSA significantly influenced V_2 (Figure 4). As height, weight and BSA were correlated, only the influence of BSA on V_1 was kept in the model because it led to the strongest -2LL reduction (9.29). In the final model, covariates were BSA, V_1 and V_2 . Methotrexate cotreatment influenced none of the pharmacokinetic parameters. Patient no. 17 displayed increased infliximab clearance starting from week 2 compared with other patients (Figure 5), and this clearance seemed to increase with time. There was no significant difference of AUC_{18} between the IFX alone and IFX+MTX

groups ($P = 0.55$; Table 3 and Figure 6). For a median subject, the distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) were 0.3 and 14 days, respectively.

Pharmacodynamics

A total of 329 BASDAI values were available for the 26 patients. There was no significant difference of standardized AUE (AUE_s) between patients treated and not treated with methotrexate ($P = 0.63$; Table 3 and Figure 6). In addition, no relationship was detected between AUC and AUE (Figure 7).

Discussion

We used pharmacokinetic modelling to analyse the possible influence of methotrexate on infliximab pharmacokinetics or concentration–efficacy relationship in AAS. Indeed, population pharmacokinetic modelling is a powerful approach to study the influence of individual factors on dose–response variability. Compartmental modelling has already been used to describe infliximab pharmacokinetics

Figure 2

Distribution of population (left) and individual residuals (middle) and of normalized prediction distribution error (NPDE; right) vs. normal distribution (top), time (middle) and estimated concentrations (bottom). IWRES, individual weighted residual; PWRES, population weighted residual

[2, 20, 27–29], but in all these studies the concentration data were sparse, with only trough and peak samples. To our knowledge, this is the first analysis using rich data obtained in a study specifically designed for this purpose, and it may therefore provide a more accurate estimation of infliximab pharmacokinetic parameters than previous studies.

Infliximab concentrations were satisfactorily described using a two-compartment model, and pharmacokinetic parameters were accurately estimated. The increase of central volume of distribution (V_1) with BSA is in agreement with previous studies on infliximab [27–29] and on other monoclonal antibodies [30, 31]. However, the influence of BSA on peripheral volume of distribution (V_2) has not been reported before.

The absence of detection of a concentration–effect relationship (Figure 7) suggests that the lack of clinical improvement may not be due to underexposure to infliximab, but rather to its low clinical efficacy in these patients. Our results contrast with the study of de Vries *et al.*, which reported lower trough infliximab concentrations in nonresponder AS patients than in responders [6]. In their study, however, several nonresponders had trough concentrations close to 0, explained by the presence of ATI. In the

absence of ATI, trough concentrations may not predict the responder/nonresponder status of AS patients.

The absence of methotrexate influence on infliximab pharmacokinetics in AS patients is surprising, because this influence was reported in RA [9]. The pharmacokinetics of antibodies, including infliximab, is complex. It was shown that antibody clearance is increased when antigen burden (i.e. the amount of target to be neutralized) is high [24, 32–36]. Interestingly, for infliximab, the antigen burden (i.e. the concentration of circulating TNF- α) is lower in AS than in RA [1]. This is consistent with the reported higher infliximab elimination half-life in RA (10 days [2]) than in SA patients (16 days [29]). Methotrexate, acting as an anti-inflammatory drug, contributes to a significant decrease of TNF- α levels. Therefore, in RA, methotrexate may decrease TNF- α levels significantly, leading to a decrease in target-related clearance of infliximab. In AS, methotrexate may have a more limited effect because of the lower antigen burden, leading to a nonsignificant influence on infliximab pharmacokinetics. The definite evidence for the absence of influence of methotrexate on infliximab pharmacokinetics will be obtained using a bioequivalence study.

Figure 3

Observed (open circles) and model-predicted infliximab concentrations (continuous lines), and observed Bath Ankylosing Spondylitis Disease Activity Index (BASDAI; filled squares and dashed lines) in four representative patients. IFX, infliximab

Our pharmacokinetic model has some limitations. The interindividual variance of distribution clearance (ω^2_D) was removed from the pharmacokinetic model because a shrinkage (underestimation of variability) was observed. This is a common finding for monoclonal antibodies used in noncancer diseases [2, 28, 29], because a dense sampling protocol during the first days after an infusion is impractical. Although ATI were detected in only one patient (patient no. 17), their presence was associated with an increased clearance (Figure 5), as previously reported [27–29]. As this patient showed increasing clearance with time, which was difficult to describe using our model, this patient was removed from the population pharmacokinetic analysis.

It was suggested that the combination of methotrexate with infliximab decreased the risk for development of ATI [3]. This possible influence could not be investigated in the present study because only one patient developed ATI.

No significant difference in AUE_5 was observed between the IFX alone and MTX+IFX study arms. This suggests that methotrexate does not influence infliximab efficacy in AS. The AUE_5 was used to account for the whole follow-up of patients; however, this parameter may be less sensitive to treatment alteration than pharmacokinetic–

pharmacodynamic modelling. Pharmacokinetic–pharmacodynamic modelling was attempted to describe the relationship between infliximab concentrations and BASDAI score; however, no relevant model was obtained. Indeed, most pharmacokinetic–pharmacodynamic models require the pharmacodynamic marker to be at steady state before treatment. As, for some patients, BASDAI increased with time, the assumption of steady state did not hold. In addition, disease progression models, as described by Holford *et al.* [37] could not be identified. The influence of methotrexate on BASDAI was further investigated using repeated-measures ANOVA, which confirmed the nonsignificant influence of methotrexate. To assess the influence of methotrexate on the infliximab concentration–effect relationship in AS patients, new trials are needed. They should include a control arm (i.e. patients not treated with infliximab), or at least repeated BASDAI measurements should be made before treatment.

Our study used rich data to describe infliximab pharmacokinetics. Methotrexate does not influence infliximab pharmacokinetic variability and seems not to influence its concentration–response relationship in AAS. These results do not support the combination of methotrexate with infliximab in ankylosing spondylitis.

Figure 4 Central (A) and peripheral volumes of distribution (B) vs. body surface area. Random effects (ETA) for central (C) and peripheral (D) vs. body surface area

Table 2 Estimated pharmacokinetic parameters

Parameter (unit)	Estimate	RSE (%)
V ₁ (l)	2.3	3
Body surface area on V ₁	0.88	27
Systemic clearance (l day ⁻¹)	0.21	5
V ₂ (l)	1.8	6
Body surface area on V ₂	1.9	23
Intercompartment clearance (l day ⁻¹)	2.3	5
ω _{V1} (%)	14	16
ω _{CL} (%)	24	15
ω _{V2} (%)	22	21
ω _Q (%)	–	–
σ _{C,add} (mg l ⁻¹)	2.6	16
σ _{C,prop} (%)	0.18	5

Parameters are described in the text. The residual standard error (RSE) (%) is obtained as follows: RSE = (estimate/standard error) × 100. V₁, V₂: resp. central and peripheral volumes of distribution; CL, Q: resp. systemic and intercompartment clearances; ω: interindividual standard deviation of pharmacokinetic parameter distributions; σ: residual standard deviation. Abbreviations: ATI, antibodies toward infliximab; BSA, body surface area; PAT, previous anti-tumour necrosis factor α treatment.

Table 3 Influence of methotrexate on pharmacokinetic and pharmacodynamic variability

	MTX–	MTX+	P value
AUC ₁₈ (%)	169 242 (124 111–203 782)	164 222 (102 165–295 858)	0.55 (NS)
AUE ₅ (%)	–15.7 (–93.2 to 27.1)	–24.5 (–68.1 to 6.9)	0.63 (NS)

Results are presented as median (range). Abbreviations: AUC₁₈, area under the concentration vs. time curve from week 0 to week 18; AUE₅, standardized area under the BASDAI vs. time curve from week 0 to week 18.

Competing interests

Gilles Paintaud is involved in clinical studies sponsored by Roche, Innate Pharma and LFB (Laboratoire Français des Biotechnologies); his research team received donations from Abbott Pharma, Wyeth and Merck Serono. Daniel

Wendling received research support from Abbott, Aventis, Schering-Plough, Wyeth, Servier and Roche Chugai; he is a consultant for Abbott, Amgen, Wieth and Roche Chugai. Denis Mulleman received research support from Abbott. Philippe Goupille received research support from Abbott, Schering-Plough, Wyeth, Roche and Bristol-Meyers-Squibb; he is consultant for L.F.B., Roche, Schering-Plough and Wyeth. The other authors have no competing interests to declare.

Figure 5

Observed concentrations vs. time for patient no. 17 (continuous lines and filled circles), and 5th, 50th and 95th percentiles obtained by simulations (dashed lines). Stars are concentrations below the limit of quantification (0.014 mg l^{-1} ; dotted line)

Ethical considerations

This study, approved by the ethics committee of Tours University Hospital, was conducted in accordance with the declaration of Helsinki. All subjects received full written and oral information on the objectives and the procedure of the study and gave their written informed consent. This trial was registered on clinicaltrials.gov as NCT00507403.

This study was financed by the French Ministry of Health (regional PHRC 2004).

The authors thank Nelly Jaccaz-Vallée, Sergine Gosset, Fanny Teasdale and Hélène Bansard for blood sampling, Anne-Claire Duveau for technical assistance in infliximab

Figure 7

Standardized area under the BASDAI curve (AUE_3) vs. AUC in patients treated with infliximab alone (open circles) or infliximab combined with methotrexate (filled circles). AUC, area under the concentration

Figure 6

Distribution of AUC from 0 to week 18 (AUC_{18} ; A) and of (b) normalized area under the BASDAI curve (AUE_3) for infliximab alone (IFX alone) and infliximab combined with methotrexate (IFX+MTX) patients. AUC, area under the concentration

concentration measurements, Jean-Christophe Pagès, Eric Pivert and the biochemistry laboratory which measured C-reactive protein concentrations, Hervé Watier for carrying out the measurement of serum concentrations of antibodies towards infliximab and Wiebe De Jong for his technical support with the study protocol and data management.

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