CONCISE REPORT

Methotrexate polyglutamation in relation to infliximab pharmacokinetics in rheumatoid arthritis

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ABSTRACT

Objective The combination of methotrexate (MTX) with infliximab can modify infliximab pharmacokinetics and lower the incidence of antibodies against infliximab (ATIs). We hypothesised that the pharmacokinetic interaction between MTX and infliximab is related to activation of MTX to immunosuppressive MTX polyglutamates (MTXPGs).

Methods Adult patients with rheumatoid arthritis receiving weekly MTX with infliximab for more than 3 months were enrolled in a cross-sectional study. Blood was collected at trough before the infusion of infliximab. Red blood cell (RBC) MTXPGs were measured using liquid chromatography, and circulating levels of infliximab were measured using a cell-based assay. ATIs were measured using enzyme immunoassays. Statistical analyses consisted of multiple regression and Wilcoxon tests.

Results In the 61 patients enrolled in the study, ATIs were detected in 11 (18%). Regression analyses revealed that lower infliximab levels (median 3.3 μg/ml) were associated with the presence of ATIs and lower RBC MTXPG levels (median 28 nmol/l) (p<0.05). Logistic regression revealed that RBC MTXPG levels above 25 nmol/l were associated with a 4.7-fold lower likelihood of having ATIs (OR=4.7; 95% CI 1.1 to 20.8; p=0.02). None of the 12 patients with RBC MTXPG levels above 50 nmol/l tested positive for ATIs.

Conclusions These hypothesis-generating data indicate that MTXPGs are associated with infliximab pharmacokinetics and ATI formation.

INTRODUCTION

The introduction of anti-tumour necrosis factor-α (TNF-α) therapies, including the chimeric monoclonal antibody, infliximab, has been a major advance in the treatment of rheumatoid arthritis (RA).[1] Yet, a large proportion of patients with RA do not respond adequately to this chimeric antibody. One of the most well-established mechanisms impairing the pharmacokinetic and clinical efficacy of infliximab is the emergence of immunogenicity and formation of neutralising anti-drug antibodies, or antibodies against infliximab (ATIs).[2–4] This anti-drug antibody-mediated neutralisation of infliximab is detrimental to the achievement of adequate serum levels. As a result, shorter duration of response and secondary failure of treatment (ie, loss of efficacy) are observed. Furthermore, the formation of immune complexes between infliximab and ATIs increases the risk of idiosyncratic reactions and the appearance of infusion reactions.[2 5]

Currently, it is not possible to predict which patients will mount an immune response to infliximab, although the schedule of administration,[6] dose intensity,[7] and concomitant background methotrexate (MTX) treatment[7] can alter the propensity of infliximab to become immunogenic. However, the precise mechanism by which the combination of MTX with infliximab produces lower frequency of ATI formation compared with infliximab monotherapy has not been established.

In this study, we hypothesised that the activation of MTX to MTX polyglutamates (MTXPGs) may in part be responsible for the lower immunogenicity of infliximab when in combination with MTX.

METHODS

The study was cross-sectional using adult patients with RA at three study sites (Brigham and Women's, Boston, Massachusetts, USA, The Center for Rheumatology, Albany, New York, USA and Altoona Center for Clinical Research, Altoona, Pennsylvania, USA). All patients with RA (according to the 2010 ACR/EULAR criteria for RA[8]) enrolled had received a stable dose of MTX (for at least 4 months) in combination with infliximab for at least 3 months. Clinical variables and demographic data were collected at the time of a single study visit. The study was approved by an independent ethics committee, and informed consent was collected from all patients. Blood was drawn into an EDTA-containing tube and one serum separator tube at the time of the study visit. The blood was drawn just before the infliximab infusion (trough) and shipped overnight using refrigerated transportation kits. Disease activity was estimated using the Clinical Disease Activity Index (CDAI). Erythrocytes and serum were isolated on receipt of the blood specimen and stored frozen at −80°C. Red blood cell (RBC) MTXPGs, consisting of MTXPG3, were measured using a liquid chromatography assay developed and validated in our laboratory.[9] The quantification limit was 5 nmol/l RBCs. Circulating levels of infliximab in the serum were measured by Biomonitor (Copenhagen, Denmark) using a cell-based assay consisting of erythroleukaemic K562 cells transfected with a NFκB-regulated firefly luciferase reporter–gene construct.[10] ATIs were measured using a proprietary enzyme immunoassay technique (Biomonitor). The cut-off for positive ATI was 0.9 U/l. Anti-mutated citrullinated vimentin autoantibody levels (anti-MCVs) were measured using an ELISA (Orgentec Diagnostika, Mainz, Germany). Statistical analysis consisted of multivariate logistic/linear regression and Wilcoxon tests as appropriate.

Clinical and epidemiological research

RESULTS

A total of 61 patients with RA who received MTX (12 of them received parenteral MTX) with infliximab infusion were enrolled from June to December 2011. Patient characteristics are presented in table 1. In all patients, the primary indication for infliximab infusion was high disease activity despite MTX treatment. At the time of the clinical assessment, median RBC $MTXPG_3$ levels were 28 nmol/l (IQR 16–40), while median trough infliximab levels were 3.3 μg/ml (IQR 1.4–7.7). ATIs were detected in 11 patients (18%, ATI>0.9 U/l). As expected, higher MTX doses produced higher RBC $MTXPG_3$ levels (R^2=0.19; p<0.01).

Multivariate linear regression analyses revealed that lower infliximab trough levels were associated with lower infliximab doses, lower RBC $MTXPG_3$ levels, presence of ATIs and shorter duration of MTX therapy (table 2). Nearly 50% of the variance in infliximab trough levels could be explained (global R^2=0.49) by these four independent variables. Patients with ATIs presented with severalfold lower infliximab levels than those without (median 5.0 μg/ml (IQR 2.0–8.4) vs median < 0.5 μg/ml (IQR <0.5–1.4); p<0.001). Similar infliximab doses were administered in ATI-negative (median 6.0 mg/kg/8 weeks (IQR 4.2–8.4)) and ATI-positive (median 6.0 mg/kg/8 weeks (IQR 4.8–9.0)) patients (p>0.9). There was no difference in MTX doses between ATI-positive (median 17.5 mg/week (IQR 10–20)) and ATI-negative (median 13.5 mg/week (IQR 7.5–17.5)) patients (p=0.22). However, patients with ATIs had lower RBC $MTXPG_3$ (median 22 nmol/l) than patients without ATI (median 30 nmol/l) (p=0.037) (figure 1). Logistic regression revealed that RBC $MTXPG_3$ levels above 25 nmol/l were associated with a 4.7-fold lower likelihood of having ATIs (OR=4.7; 95% CI 1.1 to 20.8; p=0.02) and none of the 12 patients with RBC $MTXPG_3$ above 50 nmol/l tested positive for ATIs (figure 1). In contrast, three out of four patients (75%) with RBC $MTXPG_3$ levels below 5 nmol/l had ATIs. Patients with ATIs had higher disease activity (median CDAI 10.0 (IQR 14–31)) than those without ATIs (median CDAI 7.0 (IQR 3–12)), but the difference was not significant (p=0.39). Circulating levels of infliximab, body mass index, anti-MCV, RBC MTXPGs levels and smoking status were not associated with disease activity (data not shown).

DISCUSSION

In the past few years, a wealth of data have established that interindividual variations in serum levels of infliximab and ATI

Table 1 Patient characteristics

Characteristic	Value
Age (years)	64 (54–71)
Gender (female)	77%
Duration of disease (years)	11 (6–20)
BMI	27.4 (22.3–32.6)
Anti-MCV positivity (>20 U)	83.6%
Smokers, n (%)	7 (12%)
Duration of MTX therapy (months)	112 (65–150)
MTX dose (mg/week)	15 (10–20)
Infliximab dose (mg/kg/8 weeks)	6.0 (4.4–8.4)
Duration of infliximab therapy (months)	70 (29–107)
Previous anti-TNFs	36%
CDAI	8 (4–13)

Results are expressed as median (IQR) or percentage as appropriate.
BMI, body mass index; CDAI, Clinical Disease Activity Index; MCV, mutated citrullinated vimentin; MTX, methotrexate; TNF, tumour necrosis factor.

Table 2 Stepwise multivariate linear regression analysis for infliximab trough levels

Variable	Step	Global R^2	Partial R^2	Estimate	p Value
Intercept				0.189±2.1	
Infliximab (mg/kg/8 weeks)	1	0.364	0.364	3.48±0.62	<0.001
ATI positivity	2	0.436	0.072	−4.14±1.85	0.029
RBC $MTXPG_3$ (nmol/l)	3	0.469	0.033	0.08±0.04	0.032
Duration of MTX therapy (months)	4	0.494	0.025	−0.02±0.01	0.100

The dependent variable was infliximab trough levels expressed in μg/ml. Estimates given as mean±SD.
ATI, anti-infliximab antibody; MTX, methotrexate; MTXPG, methotrexate polyglutamate; RBC, red blood cell.

positivity are associated with variable therapeutic response to infliximab in RA.[3] [11] [12] While the determination of these pharmacokinetic and exposure markers are not currently standard of care, their potential for therapeutic decision making and individualisation of infliximab dosing is under intense debate. This interest is also further justified by the necessity to control costs and improve long-term disease control in RA.

MTX remains the cornerstone front-line treatment for RA and the most common disease-modifying drug prescribed with an anti-TNF drug such as infliximab. Our report adds to the body of evidence that concomitant MTX is beneficial to infliximab pharmacokinetics,[7] and it is the first to suggest that elevated RBC MTXPG levels maximise infliximab systemic exposure and lower the incidence of ATI formation. The precise mechanism by which MTXPGs produce lower immunogenicity is, however, not known, but we hypothesise that their potent effect on the aminoimidazole carboxamide ribonucleotide (AICAR) transformylase followed by de novo purine biosynthesis inhibition and suppression of T cell clonality or delayed T cell repopulation may explain the propensity of MTXPG to suppress infliximab immunogenicity.[13–15]

A recent report suggested that higher MTX doses were associated with lower incidence of antibody to another TNF blocker (adalimumab).[16] While in our study similar MTX doses were administered in patients with and without ATIs, higher MTX doses resulted in higher MTXPGs levels. It follows that our observations are consistent with the notion that optimal MTX dosing (and thus adequate MTXPG level) may facilitate immune tolerance to anti-TNFs prone to immunogenicity.[16]

It should also be recognised that all patients enrolled in the study were receiving infliximab because of inadequate disease control despite MTX monotherapy, and that the primary cause for the lack of MTX efficacy may have resulted from poor MTXPG exposure (eg, <25 nmol/l) or alternatively because of adequate MTXPG accumulation in the context of disease refractoriness to the antifolate effect (eg, <50 nmol/l). As such, the observation that none of the patients with MTXPG above 50 nmol/l were positive for ATIs suggests that the lack of anti-inflammatory effects does not preclude the achievement of adequate immunosuppression to promote tolerance to infliximab. Alternatively, the indication that 75% of patients with MTXPG below 5 nmol/l had ATIs suggests that poor MTX absorption or non-compliance with background MTX treatment may be important in maximising infliximab exposure.

We acknowledge that our study has limitations because of its cross-sectional design, and prospective studies are essential to establish whether MTXPG accumulation can maximise
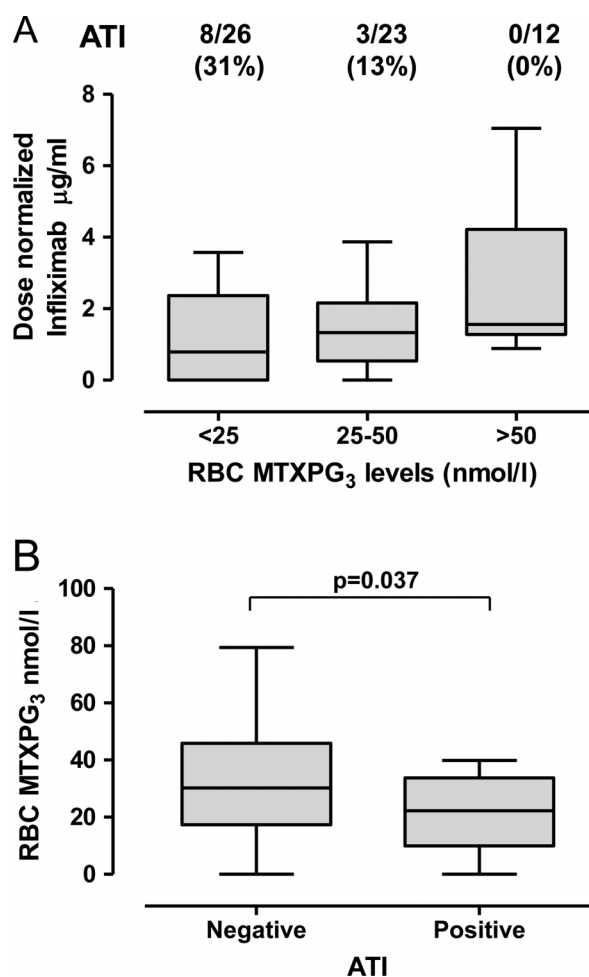
Figure 1 Effects of red blood cell (RBC) methotrexate polyglutamates (MTXPG3) on infliximab pharmacokinetics. (A) Dose-normalised infliximab levels (in μg/ml per mg infliximab every 8 weeks) as a function of RBC MTXPG3. The incidence of anti-infliximab antibodies (ATIs) for each category of MTXPG3 level is indicated. Four patients had MTXPG3 <5 nmol/l, and three of them had ATIs. Median methotrexate dose was 10 mg/week (IQR 7.5–15) in patients with RBC MTXPG3 below 25 nmol/l, 15 mg/week (IQR 12.5–17.5) in those with RBC MTXPG3 ranging from 25 to 50 nmol/l, and 20 mg/week (IQR 13.7–23.7) in those with RBC MTXPG3 >50 nmol/l. (B) RBC MTXPG3 in patients with (n=50) or without (n=11) ATIs. Bars represent median with IQR and min max values.

infliximab exposure and thus facilitate disease control. However, it is tempting to suggest that optimisation of background MTX therapy and achievement of adequate MTX exposure may potentiate the efficacy of infliximab in RA in addition to perhaps facilitating infliximab dosage-reduction strategies.

In conclusion, these hypothesis-generating data indicate that MTX exposure is associated with infliximab pharmacokinetics and formation of ATIs in RA.

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