Clinical Biochemistry 69 (2019) 8–14

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Relationships between concomitant biologic DMARDs and prednisolone administration and blood tacrolimus exposure or serum CYP3A4/5-related markers in rheumatoid arthritis patients

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ARTICLE INFO

Keywords:
Tacrolimus
Rheumatoid arthritis
Biologic DMARDs
4β-hydroxycholesterol
25-hydroxyvitamin D
CYP3A5

ABSTRACT

Background: This study aimed to evaluate the relationships between concomitant biologic disease-modifying anti-rheumatic drugs (DMARDs) and prednisolone administration and blood tacrolimus exposure or serum CYP3A4/5-related markers in rheumatoid arthritis (RA) patients without severe disease activity.

Methods: Forty-six RA patients treated with oral tacrolimus once daily for maintenance of clinical remission to moderate disease activity were enrolled. The blood concentrations of tacrolimus and its major metabolite were determined at 12 h after the evening dosing. Blood samples for determination of serum markers including 4β-hydroxycholesterol (4β-OHC), 25-hydroxyvitamin D (25-OHD) and interleukin-6 (IL-6), and CYP3A5 genotype were collected.

Results: Most enrolled patients had RA with clinical remission to mild disease activity. Concomitant tocilizumab or low-dose prednisolone administration did not alter the blood tacrolimus exposure. Serum 4β-OHC level was lower in tocilizumab co-treated patients than in the biologic DMARD non-treated patients. The blood tacrolimus concentration was inversely correlated with the serum level of 25-OHD, but not 4β-OHC and IL-6. The serum level of 4β-OHC was positively associated with that of 25-OHD. No correlations were observed between the serum levels of CYP3A4/5 activity markers and IL-6. The patients with the homozygous *CYP3A5*3* had the higher blood tacrolimus concentration, while *CYP3A5*3* allele was not associated with the serum levels of 4β-OHC and 25-OHD.

Conclusions: Concomitant use of tocilizumab or low-dose prednisolone had no effect on the pharmacokinetics of tacrolimus, while tocilizumab lowered serum 4β-OHC. Blood tacrolimus exposure was negatively associated with serum 25-OHD in RA patients with clinical remission to moderate disease activity.

1. Introduction

Tacrolimus, a macrolide calcineurin inhibitor, is an immunomodulator that acts by inhibition of cytokine production and cytokine-stimulated T-cell activation [1]. Several clinical studies have demonstrated the excellent efficacy of tacrolimus therapy in rheumatoid arthritis (RA) patients [2,3]. The main route of tacrolimus elimination is 13-*O*-demethylation by hepatic cytochrome P450 (CYP) 3A in humans [4]. CYP3A5 is the most significant genetic contributor to interindividual variation in tacrolimus clearance [5,6]. In RA patients who had the homozygous *CYP3A5*3* allele, a higher blood exposure of tacrolimus was observed in a previous report [7].

In active RA, abundant proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α are secreted into the circulation [8]. The cytokines reduce hepatic CYP3A4 expression and drug transporter activity [9–11]. Chronic inflammation in RA potentially leads to the reduction of CYP3A4/5 activity [12]. In patients with chronic and cancer inflammation, the serum IL-6 level negatively

Abbreviations: RA, rheumatoid arthritis; CYP, cytochrome P450; IL-6, interleukin-6; CRP, C-reactive protein; DMARDs, disease-modifying anti-rheumatic drugs; PXR, pregnane X receptor; 4β-OHC, 4β-hydroxycholesterol; 25-OHD, 25-hydroxyvitamin D; LLOQ, lower limit of quantification

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https://doi.org/10.1016/j.clinbiochem.2019.05.003
Received 29 December 2018; Received in revised form 19 April 2019; Accepted 6 May 2019
Available online 08 May 2019

associated with hepatic CYP3A4-mediated drug clearance [13,14]. Few clinical reports have been published on the relationships between the pharmacokinetics of tacrolimus and serum IL-6 in RA patients.

RA management using biologic disease-modifying anti-rheumatic drugs (DMARDs) is often used in combination with glucocorticoids and conventional DMARDs including tacrolimus. Biologic DMARDs and glucocorticoids are potential modifiers of CYP3A4/5 activity in humans [15–17]. The pregnane X receptor (PXR) was reported to be the key nuclear receptor regulating expression of CYP enzymes and glucocorticoid induction of CYP3A4 occurs through activation of the PXR [18,19]. In RA patients, suppressed CYP3A activity was regained after initiation of IL-6 receptor antibody drugs such as tocilizumab and sarilumab [15,20]. These antibodies against IL-6 receptor, and other antibody drugs such as tumor necrosis factor-α inhibitors also potentially alter CYP3A4/5 activity in tacrolimus-treated RA patients.

Several blood endogenous markers of CYP3A4/5 activity have been reported, including serum 4β-hydroxycholesterol (4β-OHC) and 25-hydroxyvitamin D (25-OHD) in humans [21,22]. 4β-OHC is produced principally by CYP3A4/5 metabolism of cholesterol, and serum 4β-OHC correlates with hepatic CYP3A4/5 activity [21]. In contrast, the expression of CYP3A4 in the intestine is regulated by the nuclear vitamin D receptor in addition to PXR [22]. Serum 25-OHD, a major circulating form of vitamin D, is considered as the indicator of vitamin D skin synthesis and nutritional intake [23]. Serum 25-OHD has the seasonal variation caused by exposure to sunlight [24]. Serum 4β-OHC as a non-P-glycoprotein activity marker has been employed increasingly as a useful tool for biomarker studies of CYP3A4/5-mediated drug clearance and for studies of CYP3A5 genotype-phenotype relationships [25–27]. Serum 4β-OHC has been shown to be relatively a poor predictor of tacrolimus dose requirements in organ transplant recipients [28]. A few earlier studies investigated the relationships between serum markers of CYP3A4/5 activity and serum cytokine or biologic DMARDs co-treatment in RA patients [29,30]. In RA patients co-treated with biologic DMARDs or glucocorticoids, the pharmacokinetics of tacrolimus and serum markers of CYP3A4/5 activity remain to be characterized.

Low-dose of tacrolimus is commonly used for the treatment of RA. In RA patients co-treated with CYP3A4/5 modifiers, blood tacrolimus is potentially predictable using the serum markers of CYP3A4/5 activity. The aim of this study was to evaluate the relationships between concomitant biologic DMARD and prednisolone administration and blood tacrolimus exposure or serum CYP3A4/5-related markers in RA patients without severe disease activity.

2. Materials and methods

2.1. Ethics

The present study was conducted in accordance with the principles of the Declaration of Helsinki and its amendments, and the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The Ethics Committee of Hamamatsu University School of Medicine approved the study protocol (approval number, 15–034). The patients were briefed about the scientific aim of the study and each patient provided written informed consent.

2.2. Patients and study schedule

This observational study enrolled 46 Japanese RA patients treated with oral tacrolimus for maintenance of clinical remission to moderate disease activity at Hamamatsu University Hospital (Hamamatsu, Japan). They received tacrolimus immediate release capsules or tablets once daily in the evening and its adjusted dose was fixed for at least 2 weeks. Exclusion criteria were as follows: (1) patients who were being co-treated with a strong inducer or inhibitor of CYPs, including azole antifungals, macrolide antibiotics, rifampicin, and carbamazepine [31]; (2) patients who had withdrawn from biologic DMARD administration, including a monoclonal antibody or Fc-fusion protein drug within the past 8 weeks; (3) patients who were undergoing short-term administration (< 8 weeks) of biologic DMARDs; (4) patients who were receiving administration of a glucocorticoid other than oral prednisolone; (5) patients who were receiving vitamin D supplementation; (6) patients who had an active infectious disease; (7) patients with hepatic dysfunction (serum total bilirubin > 2.0 mg/dL); (8) patients with renal dysfunction (serum creatinine > 2.0 mg/dL); and (9) patients with poor medication adherence based on interviews and medical records. These exclusion criteria minimize the blood tacrolimus variation caused by non-target factors. Blood specimens were drawn into tubes with and without EDTA disodium salts once at 12 h after the evening dosing during the study period at each patient.

2.3. Concomitant biologic DMARD and prednisolone administration

This study investigated the presence or absence of concomitant use of biologic DMARDs including a monoclonal antibody or Fc-fusion protein drug or prednisolone in the enrolled patients. The biologic DMARDs investigated in this study were tocilizumab and non-anti-IL-6 receptor antibodies including infliximab, adalimumab, golimumab, and certolizumab pegol or Fc-fusion protein drugs including etanercept and abatacept that had obtained pharmaceutical approval for RA in Japan during the study period. The biologic DMARD co-treated patients were divided into the following groups: tocilizumab co-treatment and non-treatment.

2.4. Determination of blood tacrolimus and its 13-O-demethylated metabolite

Tacrolimus and its 13-*O*-demethylated metabolite in whole blood were determined by an LC-MS/MS method [32]. The calibration curves of blood tacrolimus and its 13-*O*-demethylated metabolite had linearity at ranges of 0.5–20 ng/mL ($r > 0.999$) and 0.1–5 ng/mL ($r > 0.999$), respectively. The intra- and inter-day accuracies for tacrolimus and its 13-*O*-demethylated metabolite were 94–109% and 99–108%, and 98–109% and 99–107%, respectively, while their imprecisions were < 12% and 9%, and < 13% and 10%, respectively. The lower limits of quantification (LLOQs) for tacrolimus and its 13-*O*-demethylated metabolite in whole blood were 0.5 and 0.1 ng/mL, respectively. As blood disposition parameters, blood exposure and metabolism of tacrolimus were estimated as the dose-normalized concentration and the metabolic ratio as its ratio to 13-*O*-demethyltacrolimus, respectively.

2.5. Determination of serum markers of CYP3A4/5 activity

Saponified 4β-OHC in serum was determined by an LC-MS/MS method [33]. The calibration curve of 4β-OHC was linear over the serum range of 5–200 ng/mL ($r > 0.999$). The intra- and inter-day accuracies of 4β-OHC were 104–114% and 95–107%, while its intra- and inter-day imprecisions were < 9% and 14%, respectively. The LLOQ for serum 4β-OHC was 5 ng/mL. 25-OHD in serum specimens was measured using a commercially available enzyme-linked immunosorbent assay kit (25(OH)-Vitamin D direct day, Immundiagnostik AG, Bensheim, Germany). The LLOQ for serum 25-OHD was 2.6 ng/mL.

2.6. Determination of serum inflammatory markers

The serum level of IL-6 was measured using a commercially available enzyme-linked immunosorbent assay kit (Legend Max Human IL-6, BioLegend Inc., San Diego, CA, USA). The LLOQ for serum IL-6 was 1.6 pg/mL. The serum IL-6 level in patients with less than LLOQ was considered to be 1.6 pg/mL. The serum level of C-reactive protein (CRP) was measured by a latex agglutination immunoassay method (Nanopia CRP, Sekisui Medical Co., Ltd., Tokyo). The LLOQ for serum CRP was 0.01 mg/dL.

2.7. Genotyping of CYP3A5

Genomic DNA was isolated from the mixture of buffy coat and erythrocytes of each patient using a commercially available kit (DNA Extractor WB, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The primer sequences and PCR-RFLP conditions for *CYP3A5*3* analyses were as described previously [34]. The CYP3A5 genotypes were divided into the following groups: **1* allele carrier group (**1/*1 + *1/*3*) and homozygous **3* allele group (**3/*3*).

2.8. Statistical analyses

All statistics were analyzed using IBM SPSS Statistics ver. 22 (IBM Japan Ltd., Tokyo). Since the biologic DMARD (not including tocilizumab)-treated group had a small number of patients (n = 4), the statistical comparison was performed between the patients with biologic DMARD non-treatment and tocilizumab co-treatment. The effects of concomitant biologic DMARD and prednisolone administration on the blood disposition parameters of tacrolimus and serum markers of CYP3A4/5 activity and inflammation were evaluated using the Mann-Whitney *U* test. The nonlinear correlations between the serum markers of CYP3A4/5 activity or serum IL-6 and blood disposition parameters of tacrolimus were evaluated using Spearman's rank correlation test. The differences in the blood disposition parameters of tacrolimus and serum markers of CYP3A4/5 activity between the CYP3A5 genotypes were analyzed using the Mann-Whitney *U* test. A $p < .05$ was considered to indicate statistical significance.

3. Results

3.1. Patient characteristics

Table 1 shows the patient characteristics of the study population. In enrolled RA patients, the medians of serum CRP and IL-6 levels were 0.24 (interquartile range, 0.09–0.73) mg/dL and 13.7 (6.2–65.2) pg/mL, respectively. Most enrolled RA patients had clinical remission to mild disease activity (median Disease Activity Score 28-CRP and erythrocyte sedimentation rate were 2.37 and 2.94, respectively). The enrolled patients maintained clinical remission with a tacrolimus dose range from 0.5 to 3 mg (median, 1.8 mg). Seven patients received tocilizumab, 3 patients abatacept, and 1 patient etanercept. Twenty-three patients received oral prednisolone (median and range, 5 and 1–15 mg). Among the enrolled patients, 16 were co-treated with low-dose methotrexate (median and range, 7 and 2–14 mg per week). The patients received conventional DMARDs including mizoribine (n = 5), iguratimod (n = 4), bucillamine (n = 4), salazosulfapyridine (n = 2), and mycophenolate mofetil (n = 2). Two of 11 patients treated with biological DMARDs received low-dose prednisolone (< 5 mg).

Table 1
Patient characteristics in the study population.

Gender, male/female	16/30
Age, years	67 (60–75)
Body weight, kg	52.0 (47.4–58.2)
Serum creatinine, mg/dL	0.77 (0.65–0.94)
Blood urea nitrogen, mg/dL	16.6 (13.5–22.2)
Serum total protein, g/dL	7.0 (6.8–7.5)
Serum albumin, g/dL	4.0 (3.7–4.3)
Total bilirubin, mg/dL	0.7 (0.6–0.9)
AST, IU/L	19 (16–22)
ALT, IU/L	14 (10–16)
Serum CRP, mg/dL	0.24 (0.09–0.73)
Serum IL-6, pg/mL	13.7 (6.2–65.2)
DAS28-CRP	2.37 (1.56–3.11)
DAS28-ESR	2.94 (2.40–3.50)

Data are expressed as the median and interquartile range in parentheses.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; IL-6, interleukin-6; DAS28-CRP, Disease Activity Score 28-CRP, and DAS28-ESR, Disease Activity Score 28-erythrocyte sedimentation rate.

3.2. Influence of concomitant biologic DMARD and prednisolone administration

Concomitant tocilizumab or prednisolone administration had no effect on the blood disposition parameters of tacrolimus (Table 2). The serum 4β-OHC level in tocilizumab co-treated patients was significantly lower than that in the biologic DMARD non-treated patients (P = .008). The median levels of serum IL-6 in the biologic DMARD non-treated patients and tocilizumab co-treated patients were 13.3 and 73.1 pg/mL, respectively, although no significant difference was observed. The serum CRP level in the tocilizumab co-treated patients was significantly lower than that in the biologic DMARD non-treated patients (P = .007). Serum levels of 25-OHD and total cholesterol in the prednisolone co-treated patients tended to be higher than those in the non-treated patients (P = .106 and P = .063, respectively). No differences were observed in the serum levels of the other serum markers between the patients with and without prednisolone co-treatment. In exploratory data analyses, body mass index and gender also did not affect blood tacrolimus and serum CYP3A4/5-related markers in RA patients with clinical remission to moderate disease activity (data not shown).

3.3. Relationships between the serum markers and blood tacrolimus

Fig. 1 shows the correlations between the blood disposition parameters of tacrolimus and serum markers of CYP3A4/5 activity or serum IL-6. The serum level of 25-OHD was negatively correlated with the blood concentration of tacrolimus (Spearman's rank correlation coefficient (r_s) = −0.444, P = .002). No correlations were observed between the blood concentration of tacrolimus and the serum levels of 4β-OHC (r_s = −0.040, P = .794) or IL-6 (r_s = 0.070, P = .644). In contrast, the metabolic ratio of tacrolimus had no relationship with the serum levels of 4β-OHC (r_s = 0.121, P = .422), 25-OHD (r_s = 0.069, P = .651), and IL-6 (r_s = 0.039, P = .796). Concomitant use of tocilizumab or prednisolone did not strongly affect the negative correlation between the serum 25-OHD level and the blood tacrolimus concentration (Figs. S1 and S2).

3.4. Inter-relationships between serum markers

Fig. 2 shows the correlations between serum markers of CYP3A4/5 activity and serum IL-6 in RA patients. The serum 4β-OHC level was significantly correlated with that of 25-OHD (r_s = 0.334, P = .023). There were no correlations between the serum levels of IL-6 and 4β-OHC (r_s = −0.071, P = .641) or 25-OHD (r_s = −0.238, P = .111). Concomitant use of prednisolone did not affect the positive tendency of correlation between the serum levels of 4β-OHC and 25-OHD (Fig. S3), while concomitant use of tocilizumab impaired the correlations (Fig. S4).

3.5. Influence of CYP3A5 genotypes

Two patients were homozygous wild type (*CYP3A5*1/*1*), 17 were heterozygous mutant type (**1/*3*), and 27 were homozygous mutant type (**3/*3*). The allele frequency of *CYP3A5*3* in this population was 0.77. Table 3 shows the influence of CYP3A5 genotype on the blood disposition parameters of tacrolimus and serum markers of CYP3A activity. The blood concentration of tacrolimus was significantly 60% higher in patients with the homozygous *CYP3A5*3* allele than in those with the **1* allele (P ≤ .001). No differences were observed in the serum levels of 4β-OHC and 25-OHD between the CYP3A5 genotypes. Concomitant use of tocilizumab or prednisolone did not alter the

Table 2
Relationships between concomitant biologic DMARD (A) and prednisolone (B) administration and the blood disposition parameters of tacrolimus or serum CYP3A4/5-related markers in patients with rheumatoid arthritis.

(A) Concomitant biologic DMARD

Concomitant drug	Biologic DMARD non-treatment	Tocilizumab co-treatment	P value	Biologic DMARD co-treatment (not including tocilizumab)
Number of patients	35	7		4
Blood tacrolimus, ng/mL per mg/kg	108 (70–174)	96.2 (74.7–123.6)	0.597	89.6 (87.9–93.2)
Metabolic ratio of tacrolimus	0.167 (0.094–0.216)	0.131 (0.115–0.153)	0.257	0.173 (0.145–0.193)
Serum 4β-OHC, ng/mL	105 (76–145)	58.8 (50.6–83.6)	0.008	89.9 (83.5–104.4)
Serum total cholesterol, mg/dL	170 (150–203)	165 (111–171)	0.122	164 (143–174)
Serum 25-OHD, ng/mL	9.12 (5.62–12.01)	5.56 (4.88–5.94)	0.106	12.1 (9.4–15.7)
Serum IL-6, pg/mL	13.3 (6.7–60.8)	73.1 (12.1–83.9)	0.272	5.8 (3.8–12.9)
Serum CRP, mg/dL	0.34 (0.13–0.94)	0.02 (0.02–0.09)	0.007	0.20 (0.15–0.33)

(B) Concomitant prednisolone

Concomitant drug	Prednisolone non-treatment	Prednisolone co-treatment	P value
Number of patients	23	23	
Blood tacrolimus, ng/mL per mg/kg	106 (84–158)	94.3 (72.3–127.6)	0.410
Metabolic ratio of tacrolimus	0.157 (0.117–0.186)	0.167 (0.091–0.210)	0.809
Serum 4β-OHC, ng/mL	92.0 (65.2–129.4)	97.8 (73.8–134.9)	0.386
Serum total cholesterol, mg/dL	163 (144–179)	178 (159–206)	0.063
Serum 25-OHD, ng/mL	6.59 (5.37–9.83)	10.3 (6.5–14.0)	0.106
Serum IL-6, pg/mL	16.1 (6.1–69.6)	11.9 (6.5–43.9)	0.628
Serum CRP, mg/dL	0.15 (0.07–0.50)	0.43 (0.18–0.80)	0.150

Data are expressed as the median and interquartile range in parentheses. The influence of concomitant drug administration on the parameters was analyzed using the Mann-Whitney *U* test. With respect to concomitant biologic DMARDs, statistical comparison was performed between the biologic DMARD non-treatment and tocilizumab co-treatment.
DMARDs, disease-modifying anti-rheumatic drugs; 4β-OHC, 4β-hydroxycholesterol; 25-OHD, 25-hydroxyvitamin D; IL-6, interleukin-6; and CRP, C-reactive protein.

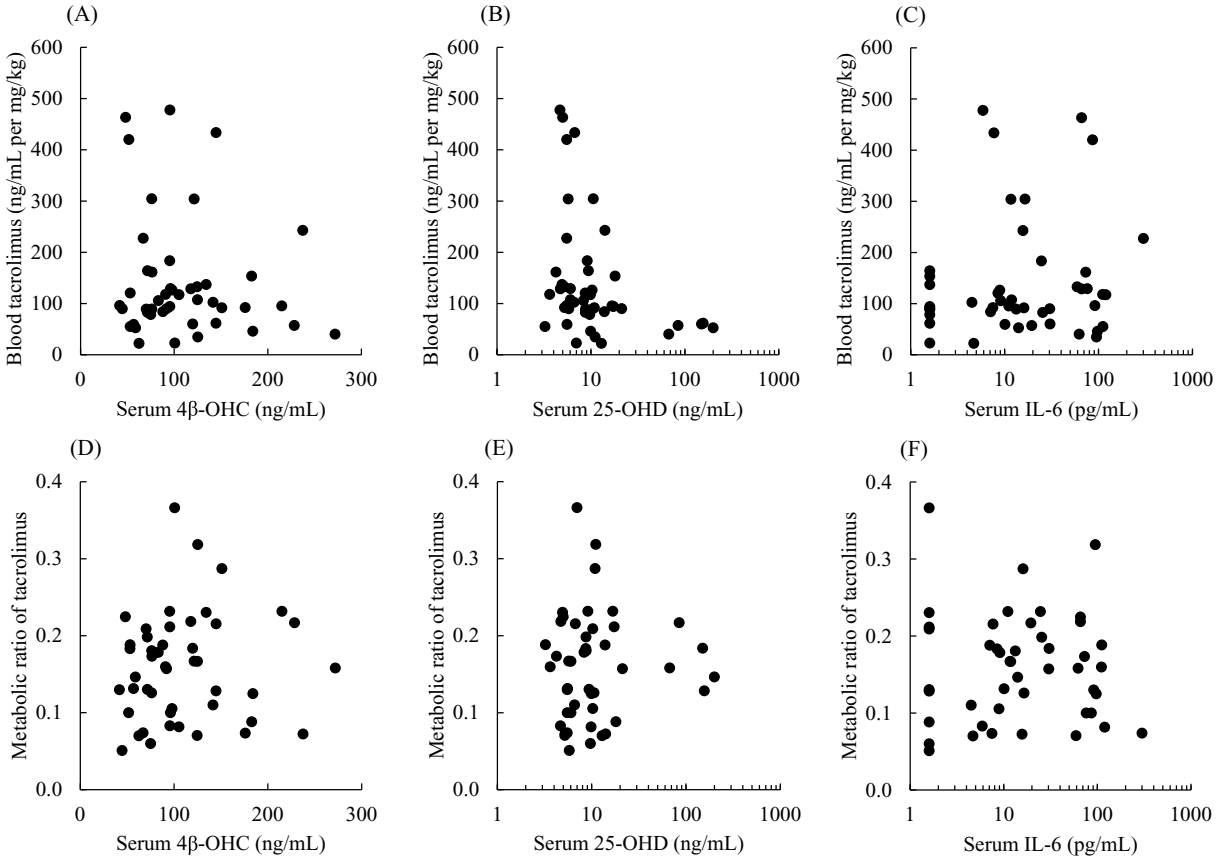

Fig. 1. Correlations between the serum markers of CYP3A4/5 activity or serum IL-6 and blood disposition parameters of tacrolimus in rheumatoid arthritis patients. The correlations between (A) serum 4β-hydroxycholesterol (4β-OHC), (B) serum 25-hydroxyvitamin D (25-OHD), or (C) serum interleukin-6 (IL-6) and the blood concentration of tacrolimus, and between (D) serum 4β-OHC, (E) serum 25-OHD, or (F) serum IL-6 and the metabolic ratio of tacrolimus were evaluated using Spearman's rank correlation test.

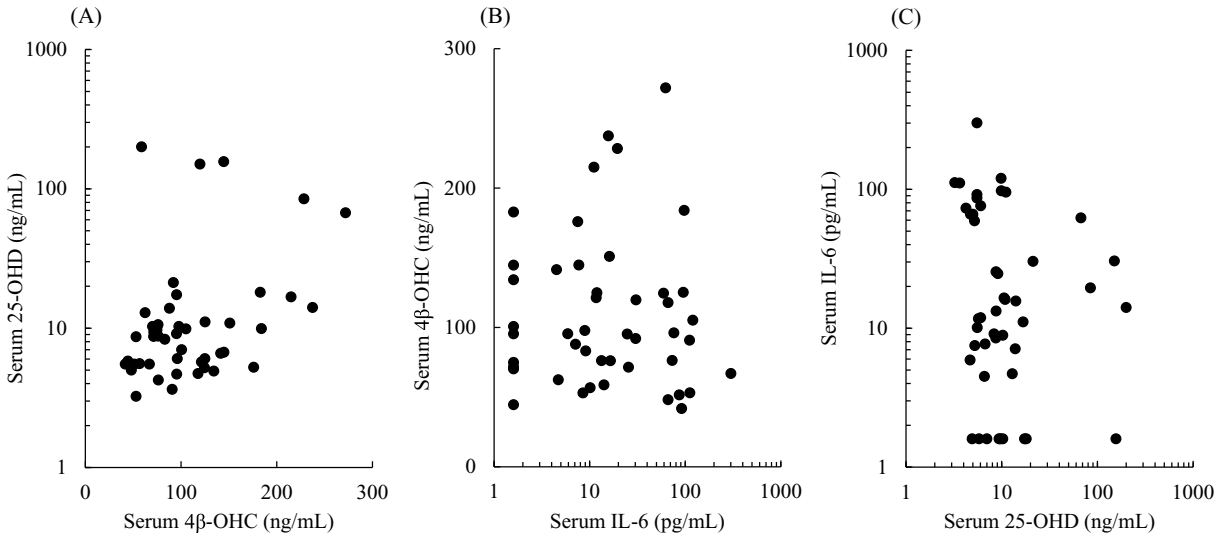

Fig. 2. Correlations between the serum markers of CYP3A4/5 activity and serum IL-6 in rheumatoid arthritis patients. The correlations between (A) serum 4β-hydroxycholesterol (4β-OHC) and 25-hydroxyvitamin D (25-OHD), (B) serum interleukin-6 (IL-6) and 4β-OHC, or (C) serum 25-OHD and IL-6 were evaluated using Spearman's rank correlation test.

impact of CYP3A5 genotype on the blood concentration of tacrolimus (Table S1).

4. Discussion

In RA patients with biologic DMARD co-treatment, the tacrolimus pharmacokinetics and serum markers of CYP3A4/5 activity remain to be characterized. This study investigated the relationships between concomitant use of biologic DMARDs and prednisolone and tacrolimus pharmacokinetics or serum CYP3A4/5-related markers in RA patients without severe disease activity. Our findings suggest that adjustment of the tacrolimus dose is not needed in RA patients without severe disease activity after starting tocilizumab or low-dose prednisolone co-treatment. To the best of our knowledge, this is the first report to characterize the pharmacokinetics of tacrolimus and endogenous CYP3A4/5 markers including serum 25-OHD in RA patients with biologic DMARD co-treatment.

Concomitant use of tocilizumab or low-dose prednisolone had no effect on the pharmacokinetics of tacrolimus in this study. RA patients co-treated with tocilizumab tended to have lower serum levels of 4β-OHC and 25-OHD than those without biologic DMARDs. Under a severe inflammatory state, lower plasma exposure of CYP3A4 substrates was observed after starting IL-6 receptor inhibitors [15,16]. In contrast, biologic DMARDs did not alter the serum 4β-OHC in RA patients with moderate inflammation [29]. RA patients without biologic DMARDs had higher serum levels of 4β-OHC (median, 105 ng/mL) in the present study, while RA patients co-treated with tocilizumab had lower serum levels of 4β-OHC (median, 58.8 ng/mL). The median plasma level of 4β-OHC in healthy women was 38.7 ng/mL in a previous report [33]. Development of RA is related to that of metabolic syndrome and its related abnormality of lipid metabolism [35,36]. Although the reduction mechanism of serum 4β-OHC by tocilizumab is unclear, tocilizumab administration led to more normal serum levels of 4β-OHC in RA patients without severe disease activity.

A negative correlation was found between serum 25-OHD and blood tacrolimus exposure in this study. Since the nuclear vitamin D receptor regulates the expression of CYP3A4 in intestine [22], serum 25-OHD associates with CYP3A4-mediated intestinal metabolism. CYP3A4 activity regulated by vitamin D in intestine potentially affects the oral bioavailability of tacrolimus. The serum level of 25-OHD had no relationship to the metabolic ratio of tacrolimus, therefore, it may strongly affect the first-pass effect of oral tacrolimus. In contrast, serum 4β-OHC did not associate with the tacrolimus pharmacokinetics in the present study. Earlier studies also reported that serum 4β-OHC did not predict the dose requirement of tacrolimus in kidney transplant recipients [28,37]. The prediction of tacrolimus pharmacokinetics using serum 4β-OHC was difficult in our study population.

Blood tacrolimus exposure did not associate with serum IL-6 in this study. IL-6 released during an inflammatory response was found to reduce CYP3A4 expression [9,10]. The mRNA levels of CYP3A4 and CYP3A5 in human hepatocytes were reported to be suppressed by IL-6, with half maximal effective levels of 3.2 and 51.0 pg/mL, respectively [38]. Our study enrolled RA patients who maintained clinical remission to moderate disease activity with medications and the median serum level of IL-6 in biologic DMARD non-treated RA patients was 13.3 pg/mL. Serum IL-6 may affect the activity of CYP3A4 but not CYP3A5,

Table 3
Relationships between CYP3A5 genotype and the blood disposition parameters of tacrolimus or serum markers of CYP3A4/5 activity in patients with rheumatoid arthritis.

CYP3A5 genotypes	*1 allele carrier (n = 19)	Homozygous *3 allele (n = 27)	P value
Tacrolimus dose, mg/kg	0.034 (0.028–0.042)	0.022 (0.019–0.036)	0.058
Blood tacrolimus, ng/mL per mg/kg	82.9 (54.0–95.8)	133 (93–235)	< 0.001
Metabolic ratio of tacrolimus	0.178 (0.139–0.193)	0.126 (0.082–0.214)	0.096
Serum 4β-OHC, ng/mL	87.9 (66.3–122.4)	96.1 (75.5–143.1)	0.332
Serum 25-OHD, ng/mL	10.3 (6.5–15.3)	6.72 (5.37–10.26)	0.138

Data are expressed as the median and interquartile range in parentheses. The influence of CYP3A5 genotypes on the parameters was analyzed using the Mann-Whitney *U* test.
4β-OHC, 4β-hydroxycholesterol; and 25-OHD, 25-hydroxyvitamin D.

which is predominantly involved in tacrolimus elimination, in this study. Tocilizumab raised the serum IL-6 in RA patients and its mean level was 89.7 pg/mL [39], while the median level in our study was 73.1 pg/mL. In tocilizumab co-treated patients, serum IL-6 did not bind to its receptor [40], resulting in failure to function. These data suggest that serum IL-6 is not a suitable marker for determining blood tacrolimus exposure in RA patients.

Serum 4β-OHC was positively correlated with serum 25-OHD in this study. In patients receiving a high dose of vitamin D for 12 months, no correlation was observed between serum levels of 25-OHD and 4β-OHC [41]. Patients enrolled in this previous study had much higher serum levels of 25-OHD than those in our study. Our results may suggest a positive correlation between serum levels of 25-OHD and 4β-OHC under physiological conditions. The inflammatory response triggered by serum IL-6 reduced CYP3A4 expression [9,10]. In our study, no correlation was found between the serum levels of CYP3A4/5 activity markers and IL-6. There was a negative correlation between plasma 25-OHD and IL-6 in samples obtained from a health survey [42]. In tocilizumab-treated patients, the higher serum level of ineffective IL-6 may be responsible for the lack of correlations between the serum markers.

Patients with the homozygous *CYP3A5*3* allele had a higher blood exposure of tacrolimus in RA patients without severe disease activity. Our previous study also showed that RA patients lacking CYP3A5 had a higher blood exposure of tacrolimus under non-treatment of biologic DMARDs [7]. CYP3A5 genotypes strongly affected the blood tacrolimus exposure in the study population, including biologic DMARD co-treated patients. In contrast, no difference was observed in the serum 4β-OHC level between the CYP3A5 genotypes in the present study. Markers of CYP3A4/5 activity could be used as a substitute for CYP3A5 genotype. In RA patients without severe disease activity, the CYP3A5 genotype was not a determinant of the serum 4β-OHC level. The serum 25-OHD level also did not associate with CYP3A5 genotype. Serum 25-OHD as a vitamin D status marker is determined by the environmental factors including skin synthesis, nutritional intake of vitamin D, and season with varying exposure to sunlight [23,24]. Our data support the finding that serum 25-OHD is an independent marker that is not correlated with CYP3A5 genotype.

This study has several limitations which should be pointed out. First, this study enrolled RA patients with clinical remission. In RA patients with severe inflammation, CYP3A4 activity is suppressed by high serum IL-6 [12]. Our findings can be applied to RA patients with remission to mild disease activity. Tacrolimus is metabolized predominantly by CYP3A5. The reduction of CYP3A4 activity by inflammation would not strongly affect the blood tacrolimus exposure. In severe active RA with high serum IL-6 (> 51.0 pg/mL [38]), initiation of tocilizumab may regain CYP3A5 activity and decline blood tacrolimus exposure. Second, our study included only 7 tocilizumab co-treated patients. Concomitant tocilizumab does not seem to significantly affect blood tacrolimus exposure based on the distribution of data. Further studies including longitudinal analysis or a greater number of tocilizumab-treated patients would confirm our findings. Third, this study did not investigate the genotype of drug transporters because we aimed to clarify the relationships with endogenous CYP3A4/5-related markers. Tacrolimus is a substrate of P-glycoprotein and its ABCB1 genotype affected the blood tacrolimus exposure in RA patients [7]. In addition, the present study did not evaluate the blood exposure profile after dosing. Analyses including ABCB1 genotype and blood exposure profile would improve the predictability of tacrolimus pharmacokinetics in RA patients.

In this study population, concomitant use of tocilizumab or low-dose prednisolone did not alter blood tacrolimus exposure. These results indicate that adjustment of the dose of tacrolimus is not required in RA patients without severe disease activity after starting tocilizumab or low-dose prednisolone. Additionally, our findings suggest the predictability of tacrolimus pharmacokinetics using serum 25-OHD in RA patients without severe disease activity. In RA patients without severe inflammation, serum 25-OHD may be a better CYP3A4/5 metric, in addition to CYP3A5 genotype, for achieving a target blood level of tacrolimus.

5. Conclusions

Concomitant use of tocilizumab or low-dose prednisolone had no effect on the pharmacokinetics of tacrolimus, while tocilizumab lowered serum 4β-OHC. Blood tacrolimus exposure was inversely with serum 25-OHD in RA patients with clinical remission.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.05.003.

Acknowledgments

This work was supported by Japan Society for the Promotion of Science KAKENHI Grant Numbers 17H00474 and 18H00420.

Declaration of conflicting interests

The authors declare there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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