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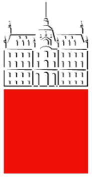

Population Pharmacokinetics of the Active Metabolite of Leflunomide in Patients with Rheumatoid Arthritis

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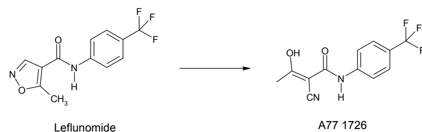
Objectives

Leflunomide is a disease-modifying antirheumatic drug of the isoxazole class. Following oral administration it is rapidly absorbed and almost completely presystemically metabolized to A77 1726 with immunosuppressive effects. In the first year of treatment 40 - 70% of patients are withdrawn from the therapy with leflunomide due to adverse drug reactions or lack of efficacy [1]. A study on microsomes suggested that CYP1A2, CYP2C19 and CYP3A4 may be involved in the metabolism of leflunomide to A77 1726 [2]. Genetic polymorphism of *CYP1A2* was associated with leflunomide toxicity [3]. Large inter-individual variability in leflunomide pharmacokinetics was reported, with A77 1726 steady-state plasma concentrations ranging from 3 to 176 mg/L [4]. Previous population pharmacokinetic studies demonstrated that some of the variability can be explained by variation in patient age, gender, body size, liver function and smoking status [4-6]. The aim of this study was to evaluate the influence of genetic polymorphisms of CYPs on interpatient variability in A77 1726 concentration and to explore the relationship between drug exposure, efficacy and toxicity.

Chemical structures of leflunomide and its major metabolite A77 1726.



Patients and study design

The study recruited 71 patients of whom 67 were diagnosed with rheumatoid arthritis and 4 with polyarthritis resembling rheumatoid arthritis and psoriasis. All patients were on maintenance therapy with leflunomide 10 or 20 mg/day. In the majority of patients therapy was initiated with a loading dose of 60 or 100 mg/day for 3 days and then continued with a recommended dose of 20 mg/day. A detailed history of leflunomide dosing, including the duration of leflunomide therapy and all other concomitant medications were collected from patients' charts. The study was cross sectional by design and A77 1726 pharmacokinetics, disease activity and other clinical measurements were assessed at one time in each patient. RF, anti-CCP, ALT, S-creatinine, ESR, and CRP were measured as part of the standard patient care. Glomerular filtration rate (GFR) was calculated by the Modified Diet in Renal Disease (MDRD) study equation. Treatment response was evaluated by DAS28, ESR and patients' assessment on visual analogue scale. Three steady-state blood samples (pre-dose, at 3 and 6 hours) were taken in each patient. A77 1726 concentration in plasma was measured with HPLC. A genotyping approach was used to determine C-163A in *CYP1A2* gene and SNPs that characterize *CYP2C19 *2, *3, *4*, and **17* alleles.

Descriptive data of patients included in the study.

Continuous data expressed as median (range).

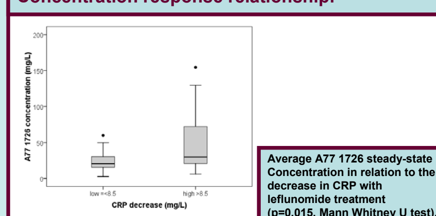
Patient characteristic		N (%)
Demographic and clinical data		
Sex	male	14 (19.7)
	female	57 (80.3)
Age [years]		59 (27-82)
BMI		25.0 (16.5-34.1)
Current smokers		17 (23.9)
Disease duration [months]		120 (7-480)
Presence of erosions		49 (75.4)
Treatment		
Duration of leflunomide treatment [months]		35.8 (6.9-120.0)
Loading dose (first 3 days of treatment)	100 mg/day	46 (68.7)
	60 mg/day	12 (17.9)
Maintenance dose	20 mg/day	65 (91.5)
	10 mg/day	6 (8.5)
Co-treatment	NSAID	31 (43.1)
	MTX	11 (15.5)
	Corticosteroids	23 (31.9)
	Proton pump inhibitors	11 (15.5)
Biochemical assessments		
RF and/or anti-CCP seropositivity		55 (78.6)
ALT [μkat/L]		0.35 (0.14-2.37)
GFR [mL/min]		72.8 (30.3-106.4)
ESR [mm/h]		22.0 (1.0-80.0)
CRP positivity (CRP ≥ 5 mg/L)		38 (54.3)
Disease activity (DAS28)		4.3 (1.3-7.8)

Distribution of *CYP1A2* and *CYP2C19* genotypes.

***PCR amplification for CYP1A2 C-729T and T-739G failed in 2 and patient respectively.**

Genotypes		n (%)
CYP1A2 C-163A	CC	4 (5.6%)
	CA	40 (56.3%)
	AA	27 (38.0%)
CYP1A2 C-729T*	CC	68 (98.5%)
	CT	1 (1.5%)
CYP1A2 T-739G*	TT	64 (94.1%)
	TG	4 (5.9%)
CYP2C19 genotypes	**1/*1*	22 (31.0%)
	**1/*2*	10 (14.1%)
	**1/*17*	28 (39.4%)
	**17/*17*	9 (12.7%)
	**2/*17*	2 (2.8%)

Concentration response relationship.



Average A77 1726 steady-state Concentration in relation to the decrease in CRP with leflunomide treatment (p=0.015, Mann Whitney U test).

Pharmacokinetic analysis

Population pharmacokinetic analysis was performed using NONMEM (version 6, level 2) and PSN (version 2.3). A77 1726 plasma concentration data were fitted by a one-compartment model with first-order absorption and elimination (ADVAN2,TRANS2). FOCEI was used for estimation of CL/F and V/F. Due to insufficient data, k_a was fixed to 1 h^{-1} based on the literature value of plasma elimination half-life of approximately 2 weeks and t_{max} of 6-12 hours. Exponential model was evaluated to describe the inter-individual variability, while additive, proportional and combination error models were evaluated to describe residual variability of A77 1726 concentration. During base model building, case deletion diagnostics was used to detect outliers in the data. The covariate effects tested were body weight, BSA, sex, age, GFR, ALT, duration of duration of leflunomide treatment, smoking status, co-treatment with NSAIDs, methotrexate, corticosteroids, and proton pump inhibitors, and genetic polymorphism of *CYP1A2* and *CYP2C19*. The final model was evaluated by the numerical predictive check and the bootstrap method. Bootstrap sampling method with replacement using 2000 replications was used to determine 2.5th and 97.5th percentile for each of the population parameters and were reported as 95% confidence intervals. To assess the effect of misspecification of k_a on other parameters, sensitivity analysis was conducted with k_a ranging between 0.2-5 h^{-1}.

Results

Due to very long elimination half-life, A77 1726 concentration profiles were very flat with little variation within an individual patient. The difference between maximum and minimum A77 1726 plasma concentration in an individual patient ranged between 1.2 and 18.2%. However, there was significant inter-individual variability, and A77 1726 trough plasma concentrations ranged between 1.9 and 156.9 mg/L, with a mean of 33.7 ± 28.9 mg/L. Six patients were suspected for poor drug compliance, but were nevertheless included in the initial pharmacokinetic analysis. A77 1726 plasma concentration profiles were best described with a one-compartment model with absorption rate constant fixed at 1 h^{-1}, exponential model for inter-individual variability in CL/F and a combination error model, comprising an additive and proportional component for residual, intra-individual variability. Inter-individual variability in V/F could not be estimated due to parameter shrinkage. Case-deletion diagnostics revealed that 2 of the 6 subjects previously suspected for poor drug compliance, are outliers with a covariance ratio of less than 0.4 and a Cook's score of more than 1. Consequently, these two subjects' data were excluded from the further analysis. With the base model developed on the remaining data from the 69 patients, CL/F was estimated at 0.0302 L/h and an inter-individual variability of 78%, while V/F was estimated at 8.55 L. Residual variability in A77 1726 concentration was 7.48% (proportional) and 0.250 mg/L (additive).

Summary of the univariate analysis of covariate relationships.

[a]Compared to CC genotype
[b]AA and CA combined against CC genotype
[c]Compared to **1/*1* genotype
[d]1/*2* and **2/*17* combined against **1/*1*, **1/*17* and **17/*17***

Effect	ΔOFV	d.f.	p value	Estimate	s.e.
Clearance (CL/F)					
age	-0.774	1	0.379	-0.334	0.454
weight	-0.848	1	0.357	0.477	0.559
BSA	-0.956	1	0.328	0.875	0.956
sex	-0.150	1	0.699	0.914	0.217
smoking	-0.304	1	0.581	1.13	0.185
disease duration	-0.471	1	0.493	0.0615	0.0894
duration of leflunomide treatment	0.000	1	1.000	0.00127	0.00233
ALT	-0.032	1	0.858	-0.0341	0.172
GFR	-5.115	1	0.024	0.901	0.374
NSAID	-0.061	1	0.805	0.953	0.169
MTX	-0.306	1	0.580	1.15	0.283
corticosteroids	-1.097	1	0.295	1.23	0.228
Proton pump inhibitors	-0.121	1	0.728	0.915	0.171
[a]*CYP1A2* C-163A	-0.917	2	0.338	CA: 1.28 AA: 1.44	0.440 0.519
[b]*CYP1A2* C-163A	-0.541	1	0.462	1.34	0.411
[c]*CYP2C19*	-7.415	4	0.006	**1/*2*: 1.89 **1/*17*: 1.32 **17/*17*: 0.945 **2/*17*: 2.36	0.476 0.302 0.229 0.421
[d]*CYP2C19*	-4.975	1	0.026	1.72	0.333
Volume of distribution (V/F)					
age	-1.075	1	0.300	0.366	0.227
weight	-6.606	1	0.010	1.62	0.544
BSA	-5.728	1	0.017	2.55	0.939
sex	-8.596	1	0.003	2.90	0.407
ALT	-1.706	1	0.192	0.366	0.371
NSAID	-0.991	1	0.319	1.34	0.429
MTX	-0.772	1	0.380	0.725	0.215
corticosteroids	-0.009	1	0.924	1.03	0.346
Proton pump inhibitors	-0.395	1	0.530	0.794	0.208

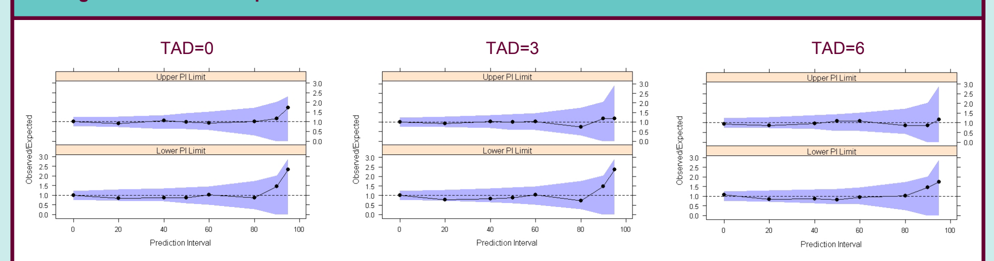
Influence of patients' glomerular filtration rate on oral clearance of A77 1726 estimated by the base model.

Post hoc estimates of individual patients' A77 1726 oral clearance by the base model According to *CYP1A2* C-163A and *CYP2C19* genotypes.

Parameters of the final model.

Parameter	Estimate	95% CI
Oral clearance		
CL/F [L/h]	0.0374	0.0235 – 0.0541
Effect of GFR	0.777	0.005 – 1.408
Effect of *CYP2C19*2* allele	1.71	1.25 – 2.63
Inter-individual variability [CV%]	73	58 – 86
Distribution volume		
V/F [L]	7.66	5.93 – 9.22
Effect of sex	2.76	1.67 – 5.80
Residual variability		
Proportional [%]	7.33	5.96 – 8.25
Additive [mg/L]	0.268	0.180 – 0.684

Coverage for the numerical predictive check.



Univariate analysis of covariate relationships performed by forward inclusion into the base model revealed that A77 1726 CL/F is affected by GFR and presence of *CYP2C19*2* allele, while V/F is affected by patients weight, BSA and sex. Due to the high correlation between patient weight and BSA, only the former was introduced into the full model. In the backward elimination step, removal of the influence of patient weight on V/F from the full model resulted in non-significant increase in OFV of 2.707, corresponding to p = 0.10. When final NONMEM run was repeated with altered k_a, fixed at five times lower and five times greater value, parameter estimates of A77 1726 CL/F changed by less than 2% and estimates of the effect *CYP2C19*2* genotype ranged between 1.68 and 1.71, while the exponent on GFR ranged between 0.775 and 0.791. On the other hand, as expected the influence on estimation of V/F was more pronounced. Coefficient on V/F ranged from 4.36 to 7.84 L and the effect of sex on V/F ranged between 2.70 and 4.81. Alteration of k_a resulted in ΔOFV in the range between -0.786 and +14.429.

Average steady-state A77 1726 concentrations were higher in patients with ΔCRP of more than 8.5 mg/L (49.7 ± 39.0 mg/L) than in patients with ΔCRP of less or equal to 8.5 mg/L (24.8 ± 13.7 mg/L, p = 0.015). Similar non-significant trends were observed with other measurements of disease activity. At the inclusion in the study 5 patients had a record of ADRs. During the one year follow-up 5 additional patients developed severe ADRs and were discontinued from leflunomide. However, A77 1726 C_{ss} was not different in patients experiencing ADRs (34.7 ± 23.1 mg/L) compared to those reporting no ADRs (36.4 ± 31.9 mg/L, p = 0.682).

Conclusions

Based on our observation of 71% higher CL/F in carriers of *CYP2C19*2* allele with lower enzyme activity, we assume that metabolic transformation of leflunomide to A77 1726 is decreased, leading to incomplete bioavailability. Our results correlate with the observation that rifampicin, inducer of many drug transporters and CYPs, including CYP2C19 was found to significantly increase A77 1726 area under the plasma concentration-time curve in patients co-treated with rifampicin compared to patients on monotherapy with leflunomide. Our results indicate that plasma concentrations of leflunomide metabolite are associated with the treatment response, but not with leflunomide-induced toxicity.

References

[1] Rozman B (2002) Clinical pharmacokinetics of leflunomide. *Clin Pharmacokinet* **41**:421-430.

[2] Kalgutkar AS, Nguyen HT, Vaz AD, Doan A, Dalvie DK, McLeod DG and Murray JC (2003) In vitro metabolism studies on the isoxazole ring scission in the anti-inflammatory agent lefluonomide to its active alpha-cyanoenol metabolite A771726: mechanistic similarities with the cytochrome P450-catalyzed dehydration of aldoximes. *Drug Metab Dispos* **31**:1240-1250.

[3] Bohanec Grabar P, Rozman B, Tomsic M, Suput D, Logar D and Dolzan V (2008) Genetic polymorphism of CYP1A2 and the toxicity of leflunomide treatment in rheumatoid arthritis patients. *Eur J Clin Pharmacol* **64**:871-876.

[4] Chan V, Charles BG and Tett SE (2005) Population pharmacokinetics and association between A77 1726 plasma concentrations and disease activity measures following administration of leflunomide to people with rheumatoid arthritis. *Br J Clin Pharmacol* **60**:257-264.

[5] Shi J, Kovacs SJ, Wang Y, Ludden TM and Bhargava VO (2005) Population pharmacokinetics of the active metabolite of leflunomide in pediatric subjects with polyarticular course juvenile rheumatoid arthritis. *J Pharmacokinet Pharmacodyn* **32**:419-439.

[6] van Roon EN, Jansen TL, van de Laar MA, Janssen M, Yska JP, Keuper R, Houtman PM and Brouwers JR (2005) Therapeutic drug monitoring of A77 1726, the active metabolite of leflunomide: serum concentrations predict response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* **64**:569-574.

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