

EXTENDED REPORT

Therapeutic drug monitoring of A77 1726, the active metabolite of leflunomide: serum concentrations predict response to treatment in patients with rheumatoid arthritis

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Background: Leflunomide is the prodrug of the disease modifying antirheumatic metabolite A77 1726. More than 50% of patients withdraw from leflunomide treatment within one year, mainly because of adverse drug reactions. Therapeutic drug monitoring of A77 1726 may be useful in predicting the efficacy of leflunomide treatment.

Objective: To study the relation between A77 1726 steady state serum concentrations and disease activity using the 28 joint (DAS28) response.

Methods: Outpatients with rheumatoid arthritis on a stable leflunomide dose for >4 months were included. DAS28 score and adverse drug reactions were recorded. Blood samples were taken for determination of A77 1726 concentrations. The primary end point was the relation of serum A77 1726 concentrations with DAS28 response category

Results: Serum A77 1726 concentrations were determined in 52 patients. A receiver operating characteristic (ROC) curve showed an area under the curve (AUC) of 0.73 (95% confidence interval, 0.54 to 0.93) ($p < 0.05$). The sensitivity exceeded 99% at concentrations below 16 mg/l. DAS28 values at the point of sampling showed no relation with A77 1726 concentrations (AUC of the ROC curve = 0.50 (0.33 to 0.67) (NS)).

Conclusions: A77 1726 steady state serum concentrations show a relation with DAS28 response. Determination of serum A77 1726 concentrations in patients with insufficient response to treatment may help when decisions have to be made about continuation of treatment or dose adjustment.

Leflunomide is a disease modifying antirheumatic drug (DMARD) of the isoxazole class. After oral administration it is rapidly, non-enzymatically, and completely converted into its active metabolite A77 1726 (2-cyano-3-hydroxy-N-(4-trifluoromethylphenyl)-crotonamide).¹ A77 1726 has antirheumatic activity through inhibition of the enzyme dihydro-orotate dehydrogenase (DHODH). DHODH is a key enzyme in the de novo production of pyrimidines in T lymphocytes, a process essential for T lymphocyte proliferation. A77 1726 has a long mean plasma half life of 15.7 days (range 14 to 18) in patients with rheumatoid arthritis.^{1–3}

Leflunomide has antirheumatic activity comparable with methotrexate and sulfasalazine.⁴ Although an antirheumatic effect can be observed within a few weeks, in some patients onset of efficacy takes up to six months.⁵ Moreover, there is a high incidence of adverse events, possibly explained by the uniform dosing schedule with only few options for dose adjustment. These factors limit the efficacy of leflunomide in populations of patients with rheumatoid arthritis.^{6–8} Thus optimisation of leflunomide treatment is warranted.

Therapeutic drug monitoring based on steady state A77 1726 serum concentrations may allow individualised dose adjustment and consequently increase the clinical effectiveness. In phase II pharmacokinetic population modelling studies, a relation between steady state A77 1726 serum concentrations of less than 13 mg/l and a reduced probability of clinical success has been described.⁹

In this study we investigated the relation between rheumatoid arthritis disease activity and the steady state

serum concentrations of A77 1726 in patients treated with leflunomide.

METHODS

Patients

Patients with rheumatoid arthritis, visiting the outpatient departments of rheumatology in the four participating centres in the period from January 2003 to January 2004, and on fixed doses of leflunomide for at least the previous four months, were asked to participate. After obtaining written informed consent, a single venous blood sample was taken for determination of A77 1726. We also recorded patient demographic data, disease characteristics, drug treatment, and the four variables required for calculating the 28 joint disease activity score (DAS28): tender joint count, swollen joint count, erythrocyte sedimentation rate, and a 100 mm visual analogue scale for general health status as estimated by the patient. The DAS28 score at the time of starting on leflunomide was retrieved from the patients' records. All DAS28 variables were scored for each patient by the same rheumatologist at each visit. DAS28 was calculated from the four variables at the end of the study to prevent any influence of the DAS28 score on treatment decisions.

Abbreviations: AUC, area under the curve; DAS28, 28 joint disease activity score; DHODH, dihydro-orotate dehydrogenase; DMARD, disease modifying antirheumatic drug; EULAR, European League Against Rheumatism; ROC, receiver operating characteristic

Exclusion criteria were participation in another study and concomitant use of daily doses of >10 mg prednisone equivalents or pharmacokinetically interacting drugs. Interacting drugs were detected by checking medication histories for prescription of rifampicin, activated charcoal, or cholestyramine.³

As non-compliance with leflunomide treatment influences exposure to leflunomide and therefore the steady state serum concentrations of A77 1726, an effort was made to assess treatment compliance. For this purpose the patients' local pharmacies were asked for leflunomide refill data. Tracking of virtually complete prescription data is possible because in the Netherlands patients usually register with one pharmacy only, and local pharmacies keep a computerised record of all prescriptions dispensed. The refill rate is calculated from the refill data as:

$$\frac{[n \text{ tablets delivered} / n \text{ days since leflunomide start}] / n}{\text{prescribed tablets per day}}$$

Refill rates under 1.0 represent underconsumption, above 1.0 overconsumption. Refill rates from 0.9 to 1.1 were categorised as good compliance.¹⁰

The human medical ethics research committee approved the study.

Determination of A77 1726

Blood samples were analysed for A77 1726 by a validated high pressure liquid chromatographic method.¹¹ We calculated mean (SD) serum A77 1726 concentration and the percentage of patients with a steady state A77 1726 concentration of <13 mg/l, the previously reported cut off concentration for optimal clinical success.⁹

End points

The primary end point was to determine the relation between serum A77 1726 concentration and the DAS28 responder category. The DAS28 responder category was determined by comparing the DAS28 score at the start of leflunomide treatment with score at the point of sampling. Responders were categorised according to the EULAR criteria.^{12, 13} In clinical rheumatology practice a moderate response is insufficient as a treatment goal, so for our analyses we compared a good response with moderate or no response. The null hypothesis for this end point was that low A77 1726 serum concentrations will predict a poorer response. For determination of this end point we included all patients for whom we had available the serum A77 1726 concentrations, DAS28 at the start of leflunomide treatment, and DAS28 at the point of sampling. As disease activity is directly influenced by concomitant use of DMARDs, patients on leflunomide in combination with other DMARDs were excluded from this analysis.

The secondary end point was to determine the relation between serum A77 1726 concentration and disease activity at the point of sampling. DAS28 was categorised into low (≤ 3.2) or high (> 3.2) disease activity, according to the EULAR criteria.^{12, 13} The null hypothesis for this end point was that lower A77 1726 serum concentrations are associated with high disease activity. All patients in whom serum A77 1726 concentrations and the DAS28 score at the point of sampling were available were included for analysis of this end point. As for the primary end point, concomitant use of DMARDs other than leflunomide was an exclusion criterion for this analysis.

Statistical analysis

SPSS 12.0.1 for Windows was used for data collection, data validation, data selection, and statistical analysis. Kolmogorov–Smirnov analysis was used to test for normality of the distribution of serum A77 1726 concentrations per

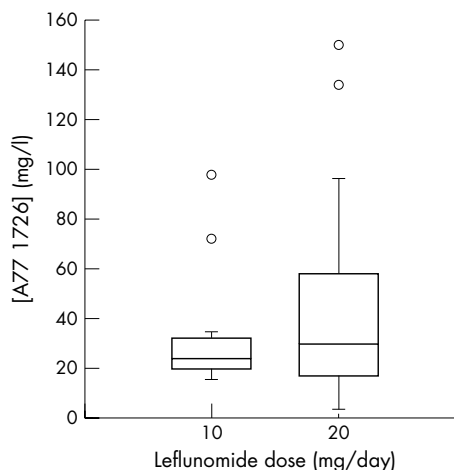


Figure 1 Box and whisker plots for serum A77 1726 concentrations with daily doses of 10 mg and 20 mg leflunomide. Circles indicate outlying values.

dose group. Differences in mean serum A77 1726 concentrations between the leflunomide dose groups were studied using Student's *t* test. Receiver operator characteristics (ROC) curves and χ^2 analysis were used to determine the relation of serum A77 1726 concentrations with disease activity and DAS28 responder category, respectively. The relation between disease activity or response and corticosteroid or NSAID use was tested using χ^2 analysis.

RESULTS

The steady state A77 1726 concentration was determined in 52 patients (table 1). These showed large interindividual variability, ranging from 3 to 150 mg/l (fig 1). In six patients (12%), A77 1726 concentrations were <13 mg/l; all these patients were on daily leflunomide doses of 20 mg. In two patients, serum A77 1726 concentrations exceed 100 mg/l; both these were on 20 mg leflunomide daily. The mean (SD) serum A77 1726 concentrations in patients on 10 and 20 mg doses were 33 (24) mg/l (range 15 to 98) and 42 (35) mg/l (range 3 to 150), respectively. The difference in mean serum A77 1726 concentrations in the two dose groups did not reach statistical significance ($p = 0.12$).

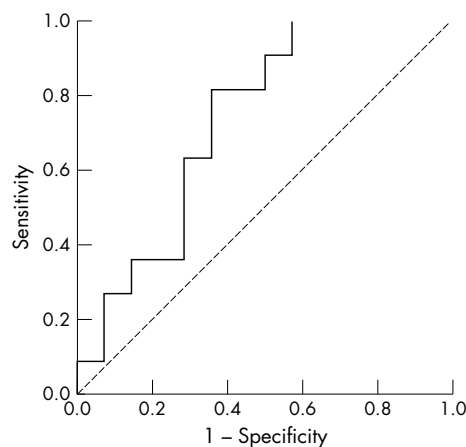


Figure 2 ROC (receiver operating characteristic) curve for the 28 joint disease activity score response versus A77 1726 concentration (AUC = 0.73 (95% confidence interval, 0.54 to 0.93)). The dashed line is the reference. AUC, area under the curve.

Table 1 Demographic and clinical characteristics

Characteristic	
Number of patients	52
Age (years)	69 (12)
Female	67%
Time since diagnosis of rheumatoid arthritis (years)	11 (10)
Rheumatoid factor positive	71%
Leflunomide prescribed as first DMARD	21%
DMARDs before leflunomide (n)	2.0 (1.7)
Range	0 to 6
>1 Year of leflunomide treatment	90%
>2 Years of leflunomide treatment	60%
Duration of leflunomide use (days)	921 (299)
Range	136 to 1327
Prescribed daily leflunomide dose	
10 mg	25%
20 mg	65%
Other*	10%
Concomitant corticosteroid use	44%
Concomitant other DMARD use	8%
Concomitant NSAID use†	58%

Values are per cent, n, or mean (SD) as indicated.

*Range 5 to 15 mg leflunomide daily.

†At least one active prescription at the point of sampling (low dose salicylates (<100 mg daily) not included).

DMARD, disease modifying antirheumatic drug; NSAID, non-steroidal anti-inflammatory drug.

Seventy one per cent of the patients showed compliance, with refill rates between 0.9 and 1.1. Refill rates varied between 0.56 and 1.35, with 22% of the population having a rate below 0.9 and 7% having a rate above 1.1. Refill rate and A77 1726 serum concentration were not correlated ($r = 0.008$). Patients with A77 1726 concentrations <13 mg/l or >100 mg/l showed compliance, with refill rates between 0.93 and 1.06.

Primary end point: DAS28 response v A77 1726 concentration

DAS28 values were recorded at the start of leflunomide treatment and at the point of sampling in 25 patients (table 2). Figure 2 shows the ROC curve for DAS28 response in relation to the A77 1726 concentration. The area under the curve is 0.73 (95% confidence interval (CI), 0.54 to 0.93) ($p < 0.05$). At a serum concentration of 18 mg/l A77 1726, sensitivity was 90.9%. At the 16 mg/l level the sensitivity was 100%; that is, patients with a good response according to the EULAR criteria were not present below this serum concentration of A77 1726.

Table 3 shows the 2×2 table for test results (positive test: A77 1726 serum concentration ≥ 16 mg/l) versus response (positive response: good responder according to DAS28 criteria). In χ^2 analysis there was significant dependence of DAS28 response on A77 1726 serum concentration, using 16 mg/l as the cut off point for the dichotomy ($p = 0.02$).

The positive predictive value of a high A77 1726 serum concentration (≥ 16 mg/l) for a good response according to DAS28 criteria was 56% (95% CI, 33% to 79%); that is, in 56% of the patients a serum A77 1726 concentration of 16 mg/l or more correctly predicted that they would respond well. The negative predictive value of a low A77 1726 serum concentration (<16 mg/l and "no response") was 100% (the 95% confidence interval was not calculated as there were no good responders at serum A77 1726 concentrations of less than 16 mg/l); thus serum A77 1726 concentrations of less than 16 mg/l were associated with non-response in all cases where such low concentrations were found. The likelihood ratio for a positive test result—that is, the ratio of a positive test result (A77 1726 serum concentration ≥ 16 mg/l) for good versus moderate or non-response—was 1.9 (95% CI, 1.1 to 2.9). The likelihood ratio for a negative test result—that is, the ratio of

Table 2 Demographic and clinical characteristics of the population used for the analysis of the 28 joint disease activity score response

Characteristic	
Number of patients	25
Age (y)	68 (13)
Female	72%
Period since diagnosis of rheumatoid arthritis (y)	8 (7)
Rheumatoid factor positive	60%
DAS28 at baseline	5.1 (0.9)
DAS28 >5.1 at baseline (n)	13
DAS28 >3.2 and ≤ 5.1 at baseline (n)	12
Leflunomide prescribed as first DMARD	24%
Concomitant corticosteroid use	40%
Concomitant other DMARD use	0%
Concomitant NSAID use*	38%

Values are per cent, n, or mean (SD) as indicated.

*At least one active prescription at the point of sampling (low dose (<100 mg daily) salicylates not included).

DAS28, 28 joint disease activity score; DMARD, disease modifying antirheumatic drug; NSAID, non-steroidal anti-inflammatory drug; y, years.

a negative test result (A77 1726 serum concentration <16 mg/l) for good versus moderate or non-response—was 0 (0.0 to 1.5).

The DAS28 response was not significantly related to corticosteroid use ($p > 0.1$) or NSAID use ($p > 0.1$).

Secondary end point: DAS28 versus A77 1726 concentration

Data from 45 of the 52 patients included in the study were used for this end point. Five patients were excluded because of combination treatment with a DMARD other than leflunomide (three with methotrexate, two with hydroxychloroquine), and two patients were excluded because missing data prevented the DAS28 from being calculated (VAS_{general health}). Figure 3 shows the ROC curve for DAS28 in relation to the A77 1726 concentration. The area under the ROC curve was 0.50 (95% CI, 0.33 to 0.67) ($p > 0.1$). Disease activity was not significantly related to corticosteroid use ($p = 0.1$) or NSAID use ($p > 0.1$).

DISCUSSION

Although our data showed no association between serum concentrations of A77 1726 and disease activity, none of the patients with low A77 1726 concentrations had a good response according to the EULAR criteria.

Criteria for therapeutic drug monitoring

The International Association for Therapeutic Drug Monitoring and Clinical Toxicology defines therapeutic drug monitoring as "... the measurement made in the laboratory of a parameter which, with appropriate interpretation, will directly influence prescribing procedures..."¹⁴ Ensom *et al*¹⁵ have defined criteria for situations in which therapeutic drug monitoring may be of value. These are: the drug has to be part of the standard care for the indication; the drug can easily be determined in biological matrices; the pharmacological effect of the drug is not directly measurable; the drug has a small therapeutic window; and treatment with the drug has to be continued for long enough to allow the effect of dose adjustments to be determined on the basis of therapeutic drug monitoring. Further, Ensom *et al* state that there should be substantial inter- or intraindividual variability in pharmacokinetics and that there has to be a relation between drug concentration levels and clinical efficacy. When considering these criteria in relation to our present data, it is apparent that therapeutic drug monitoring for leflunomide/

Table 3 2×2 table for response versus A77 1726 serum concentration

A77 1726 serum concentration	DAS28 response category		
	Good	Non- or moderate	Total
≥16 mg/l	10 (40%) ^a	8 (32%) ^b	18 (72%)
<16 mg/l	0 (0%) ^c	7 (28%) ^d	7 (28%)
Total	10 (40%)	15 (60%)	25 (100%)

Values are n (%).

PPV (positive predictive value) = $a/(a+b)$.

NPV (negative predictive value) = $d/(c+d)$.

Likelihood ratio for positive test result = $[a/(a+c)]/[b/(b+d)]$.

Likelihood ratio for negative test result = $[c/(a+c)]/[d/(b+d)]$.

DAS28, 28 joint disease activity score.

A77 1726 may be useful in improving treatment efficacy for several reasons: leflunomide is one of the DMARD options for the long term treatment of rheumatoid arthritis but with dose limiting toxicity; its efficacy cannot be determined early after initiation of treatment or after dose adjustment; and serum A77 1726 concentration can be determined by a relatively simple chromatographic technique.

In relation to the last criterion mentioned by Ensom *et al* (large variability in drug concentration levels), data from clinical studies are scarce. However, large interindividual variability of A77 1726 concentrations in a rheumatoid population was shown in a recently published study, where concentrations varied between 5 and 93 mg/l ($n = 12$).¹⁶ Large interindividual variability of A77 1726 serum concentrations was also reported by Mladenovic *et al*.² Our data correlate well with these results. We conclude that leflunomide does indeed meet that criterion.

It would be interesting to know whether the variability in A77 1726 concentrations can be reduced by individualised dosing based on patient characteristics. However, one study on the influence of demographic variables found that none of the variables studied affected steady state A77 1726 concentrations substantially.⁹ This leaves the possibility of adjusting leflunomide treatment post hoc on the basis of therapeutic drug monitoring.

In 1997, a pharmacokinetic/pharmacodynamic model for predicting therapeutically active serum concentrations of A77 1726 was published,⁹ based on data from phase I and II studies. Using follow up results after six months of leflunomide treatment and the Paulus criteria as the efficacy end point, the investigators concluded that the maximum

probability of clinical success would be obtained by a dose that maintains the steady state A77 1726 serum concentration above 13 mg/l. On the basis of this model they stated that a daily leflunomide dose of 20 mg would result in steady state A77 1726 concentrations above 13 mg/l in more than 99% of the patients. However, our data show that 12% of the patients have A77 1726 concentrations of less than 13 mg/l despite good compliance with treatment, defined as pharmacy refill rates between 0.9 and 1.1. Using the previously reported model,⁹ we conclude that in a significant proportion of the rheumatoid population a daily leflunomide dose of 20 mg does not lead to steady state A77 1726 concentrations with a maximum probability of clinical success.

On the other hand, our data support the conclusion⁹ that a certain steady state concentration has to be exceeded in order to obtain clinical success. The target concentration was previously determined to be 13 mg/l,⁹ but our results suggest a target concentration exceeding 18 mg/l for >90% sensitivity or exceeding 16 mg/l for >99% sensitivity. Twenty eight per cent of our patients had steady state A77 1726 serum concentrations of less than 16 mg/l, representing approximately 50% of the patients with an inadequate response—that is, moderate or non-responders according to the EULAR criteria.

Some comments on the current study and the interpretation of the results are required. Our study was retrospective in design and included a relatively small population for study of the relation between steady state A77 1726 serum concentrations and response. For this reason our results require confirmation in a larger prospective study. Whether the current design and sample size had an influence on the study findings, apart from considerations of statistical power, is unclear. There are indications that this may not be the case. Attending rheumatologists were not aware of the serum A77 1726 concentration at the time of blood sampling and or when collecting data for calculating the DAS28, making biased estimations of disease activity unlikely. Further, as discussed earlier, our data are in accordance with the results of Weber *et al*.⁹ Both studies found approximately the same serum A77 1726 concentration cut off point for response to treatment.

In our study 44% and 58% of the patients concomitantly used corticosteroids and NSAIDs, respectively. As both corticosteroids and NSAIDs may influence individual DAS28 scores this concomitant treatment may have affected our results. Although no relation between corticosteroid or NSAID use and disease activity or response was found, our study was not designed to correct for this potential confounder.

No correlation between disease activity at the point of sampling and serum A77 1726 concentrations was found. One possible explanation for this is that disease activity at the point of sampling was not stratified for baseline activity. A DAS28 response which combines disease activity at the point

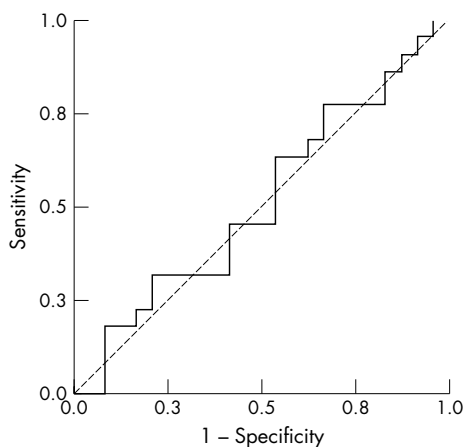


Figure 3 ROC (receiver operating characteristic) curve for the 28 joint disease activity score response versus A77 1726 concentration (AUC = 0.50 (95% confidence interval, 0.33 to 0.67)). The dashed line is the reference. AUC, area under the curve.

of measurement and the change in disease activity from baseline is an appropriate and validated end point for this analysis. We used this analysis in the absence of published data on a potential direct correlation between serum A77 1726 concentrations and disease activity.

Furthermore, in our study no specific efforts were made to optimise patient compliance with leflunomide treatment besides routinely exercising good clinical practice. Deviations from the prescribed daily dose are likely to affect the steady state A77 1726 serum concentration. However, no influence on the study results of deviations in treatment compliance—that is refill rates outside the range of 0.9 to 1.1—is to be expected, for several reasons. First, no correlation between refill rate and serum A77 1726 concentration over the complete concentration range was detected. Second, patients with serum A77 1726 concentrations <13 mg/l and >100 mg/l all showed refill rates of between 0.93 and 1.06. Therefore, low or high refill rates were not associated with serum A77 1726 concentrations in the lower or upper concentration ranges.

Clinical implications

Our data show that disease activity assessed by DAS28 criteria was not correlated with serum A77 1726 concentrations. When disease activity at baseline is taken into account, there is a measure of response that allows evaluation of leflunomide induced DAS28 responder categories. Our data on the relation between A77 1726 concentrations and DAS28 response suggest possible future clinical applications for therapeutic drug monitoring of A77 1726.

It would be interesting to know whether early decisions on treatment withdrawal or continuation are improved under non-blinded conditions—that is, whether decisions can be based on the combination of insufficient response and a low A77 1726 steady state serum concentration. Assuming compliance with treatment and stable dosing, there is a direct correlation between the duration of treatment, the serum A77 1726 concentration at the point of assessment, and the steady state A77 1726 serum concentration. This leads to the hypothesis that a non-steady-state serum A77 1726 concentration determined early in the course of leflunomide treatment—for example after four weeks—may well predict a patient's response to therapy later on. Applying this hypothesis to leflunomide treatment offers the theoretical opportunity to make early decisions based on non-steady-state A77 1726 concentrations, and may prevent delay before treatment is switched to more effective alternatives for the individual patient. To what extent this approach will lead to improvements in leflunomide treatment outcome has to be the subject of further studies.

Second, one could speculate whether patients with an inadequate clinical response to leflunomide treatment and a low, subtherapeutic A77 1726 serum concentration (<16 mg/l; 28% of the whole population in our study) will show an improved response at increased daily leflunomide doses. As A77 1726 shows linear pharmacokinetics (a linear relation between dose rate and steady state serum concentrations), an increased dose will lead to higher serum concentrations. With a positive predictive value of 56%, not all patients with A77 1726 concentrations \geq 16 mg/l will become good responders according to DAS28 response criteria. We can put into perspective the potential role of therapeutic drug monitoring on the basis of our results as follows: from the fraction of patients with a moderate or no response according to DAS28 criteria, approximately 50% will have a serum A77 1726 concentration of less than 16 mg/l. When increasing the dose for these patients, we expect 56% to become DAS28 good responders.

An additional point needs to be made about this approach. Comparative studies on leflunomide in rheumatoid arthritis have so far used a narrow dose range, varying between 5 and 25 mg daily.² Despite its increased efficacy, a daily dose rate of 25 mg is correlated with a higher incidence of adverse effects.^{2,9} Whether toxicity at leflunomide doses of more than 20–25 mg/day remains a problem when the doses are increased selectively in those with serum A77 1726 concentrations below 16–18 mg/l remains to be determined in clinical studies.

Information on higher doses is available from studies in the fields of rheumatology,^{17,18} transplantation medicine,¹⁹ and oncology.²⁰ Recently, results were published on 11 patients with rheumatoid arthritis treated with 40 mg leflunomide daily for at least three months.¹⁷ These patients previously tolerated the 20 mg daily dose but still had active disease. The investigators found that a daily dose of 40 mg increased efficacy of the treatment in six of the 11 patients. Four patients had mild and reversible adverse events after the dose increase. Metzler *et al*¹⁸ described a prospective study of leflunomide in 20 patients with Wegener's granulomatosis. Daily doses were increased stepwise to a maximum of 40 mg. These investigators concluded that the safety profile of leflunomide was comparable with that found in clinical trials despite the higher doses than those used in the treatment of rheumatoid arthritis. Williams *et al*¹⁹ described a retrospective review of 53 liver or kidney transplant recipients receiving leflunomide at maintenance doses of 40 to 60 mg daily, after receiving loading doses of 1200–1400 mg over seven days to achieve steady state A77 1726 serum concentrations of 100 mg/l. In their review leflunomide was well tolerated and dose limiting side effects occurred in fewer than 15% of the patients when serum drug levels were less than 80 mg/l. The investigators concluded, on the basis of more than 300 days of follow up, that patients can safely be given doses at this level. Although not directly applicable to a population with rheumatoid arthritis, these data are useful with regard to the tolerability of doses above 20 mg/day.

Conclusions

We have shown that in a steady state there is no association between disease activity and serum A77 1726 concentration. However, lower subtherapeutic A77 1726 serum concentrations are related to absence of response on leflunomide therapy. These results support the conclusion that determination of serum A77 1726 concentrations may influence prescribing procedures and provide the opportunity for optimising leflunomide treatment.

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