CONCISE REPORT

An open label, single dose study to evaluate the safety, efficacy, and effects on CD25 expression of ciclosporin in patients with active rheumatoid arthritis despite treatment with methotrexate and infliximab

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Objective: To explore the safety, efficacy, and lymphocyte activation of a triple therapeutic regimen with infliximab, methotrexate (MTX), and ciclosporin A (CsA) by an open label, pilot study.

Patients and methods: 19 patients (mean age 52.9 years) with active rheumatoid arthritis (mean DAS28 7.3) after a mean of 16.8 infliximab infusions and dose adjustments of both infliximab and MTX were enrolled. CsA was added to a stable therapeutic regimen. Disease activity was evaluated by the DAS28. Lymphocyte activation was evaluated by assessing CD25 expression on peripheral blood mononuclear cells (PBMCs). Primary end points were safety and efficacy according to the EULAR response criteria at 24 weeks.

Results: Eight patients (42%) discontinued treatment: adverse events (3), inefficacy (2) or non-compliance (2). One patient had a stroke and died. 5/11 (45%) patients who completed 24 weeks' treatment were moderate responders. CD25 expression, both on unstimulated and phytohaemagglutinin stimulated PBMCs in five patients assessed, was reduced (mean (SD) values from 37 (34)% to 15 (10)% and from 50 (15)% to 29 (20)%, respectively).

Conclusion: In this group of patients with refractory, highly active disease, addition of CsA reduced lymphocyte activation, and resulted in a modest response and a high rate of discontinuation. In such patients, other new approaches need to be explored.

Rheumatoid arthritis (RA) is a chronic inflammatory disease, which leads to progressive destruction of joints, diminishes quality of life, and may reduce life expectancy.¹ Methotrexate (MTX), an anti-inflammatory, immunomodulating, and antiproliferative agent, assumed a dominant role in the 1990s.² However, remissions of disease in patients receiving MTX are rare and the drug, although considerably slowing down the formation of erosions, is far from halting them.³

The clinical application of biological agents that selectively inhibit tumour necrosis factor α (TNF α) and interleukin (IL) 1 β represents a major therapeutic advance.^{4–5} Infliximab, a chimeric monoclonal antibody that binds to free and cell bound TNF α , is widely used in clinical practice, usually in combination with MTX.⁴ However, in a proportion of patients with RA even the combination of infliximab with MTX is

inadequate to control disease, despite achieving response criteria. Significant continuing disease activity despite anti-TNF treatment is becoming increasingly apparent in clinical practice.^{6–8}

Ciclosporin A (CsA) exerts its immunomodulatory effect through IL2 and T lymphocytes. Combination of MTX with CsA offers additional benefit to patients with incomplete response to MTX alone, without serious adverse events.⁹

In animal models of arthritis, combining T cell with cytokine directed treatments is synergistic.¹⁰ In a pilot study of patients unable to receive MTX, infliximab in combination with CsA was shown to be effective.¹¹ Because current treatment protocols combine infliximab with MTX in most patients, we sought to explore the efficacy and safety of this therapeutic paradigm in a pilot, proof of concept clinical trial.

PATIENTS AND METHODS

Patients and treatment

Patients with RA according to the revised American College of Rheumatology (ACR) criteria,¹² with active disease despite treatment with infliximab and MTX for ≥ 6 infliximab infusions, were included in the study. Active disease was defined as ≥ 6 swollen and ≥ 6 tender joints and at least one of the following: erythrocyte sedimentation rate > 30 mm/1st h, or C reactive protein (CRP) > 20 mg/l, or pain on a 100 mm visual analogue scale (VAS) > 50 mm or patient's global assessment on a VAS > 50 mm. Patients had to be receiving stable treatment with infliximab (every 6 weeks), MTX (≥ 15 mg/week), and/or prednisone (≤ 10 mg/day) for at least 1 month before inclusion in the study and continue with a stable dose throughout study period. CsA was introduced in a dose of 2.5–3 mg/kg. Non-steroidal anti-inflammatory drugs were not allowed.

Clinical evaluation

Clinical evaluation was performed at study enrolment and on every infliximab infusion. We applied the 28 joint count Disease Activity Score (DAS28) based on four variables to assess disease activity at each visit. Response to treatment

Abbreviations: ACR, American College of Rheumatology; CRP, C reactive protein; CsA, ciclosporin A; DAS28, 28 joint count Disease Activity Score; DMARDs, disease modifying antirheumatic drugs; EULAR, European League Against Rheumatism; HAQ, Health Assessment Questionnaire; IL, interleukin; MTX, methotrexate; PBMCs, peripheral blood mononuclear cells; PHA, phytohaemagglutinin; RA, rheumatoid arthritis, TNF α , tumour necrosis factor α ; VAS, visual analogue scale

Table 1 Patients' characteristics at study enrolment

<i>Demographics</i>	
Age (years)	52.9 (25–72)*
Female sex (%)	68
Disease duration (years)	9.9 (1.5–23)
Rheumatoid factor positive (%)	63
<i>Treatment</i>	
Infliximab	
Infusion number	16.8 (6–23)
Dose (mg/kg/infusion)	4.2 (3–5.6)
Methotrexate dose (mg/week)	17.1 (15–20)
Concomitant corticosteroids (%)	26
Prednisolone dose (mg/day)	6.5 (5–10)
<i>Disease characteristics, mean (SD)</i>	
Tender joints (28)	18.4 (9.7)
Swollen joints (28)	17.8 (6)
DAS28	7.3 (1.2)
C reactive protein (mg/l)	22 (26)
Haemoglobin (g/l)	125 (16)
Patient's global assessment (1–10)	6.7 (2.9)
Physician's global assessment (1–10)	7 (1.1)
HAQ (0–3)	1.1 (0.7)
VAS for pain	6.7 (2.7)

*Mean (range).

was assessed by the European League Against Rheumatism (EULAR) response criteria.¹³

Laboratory evaluation

Patients were evaluated at baseline and before each infliximab infusion by complete blood count with differential and platelet count, biochemistry, erythrocyte sedimentation rate, and CRP.

Evaluation of lymphocyte activation

Peripheral blood mononuclear cells (PBMCs) were isolated on a Ficoll gradient (sigma 1077-1), washed twice in phosphate buffered saline, and stimulated in RPMI/10% fetal calf serum/2 µg/ml phytohaemagglutinin (PHA) for 24 hours at a concentration of 10⁶ cells/ml. Activated PBMCs (5×10⁵) were stained with phycoerythrin labelled anti-CD25 (0479, Immunotech) for 20 minutes at 4°C, washed, and fixed. Flow cytometric data were acquired on an EpicsElite Analyzer (Coulter). Analyses were performed at baseline and after 12 weeks of treatment. Cell activation status was determined assessing IL2Rα (CD25) expression by fluorescence activated cell sorting (FACS) analysis before and after PHA stimulation.

Statistical analysis

Statistical evaluation within the group was done by the use of a paired Student's *t* test. A *p* value <0.05 (two tailed) was considered significant.

RESULTS

Patients' demographics and disease characteristics

A total of 19 patients were enrolled (table 1). All had longstanding RA and several disease modifying antirheumatic drugs (DMARDs; mean 3.1) had failed. At study entry, they had active disease characterised by a high number of swollen (mean (SD) 17.8 (6)) and tender joints (18.4 (9.7)). They had received multiple infliximab infusions (mean 16.8) with a mean infliximab dose of 4.2 mg/kg (range 3–5.6 and 7/19 at 5 mg/kg) every 6 weeks. Most of them had secondary treatment failures after an initial response. All patients were receiving concomitant MTX treatment (mean 17.1 mg/week, range 15–20 mg/week), while five (26%) were receiving prednisolone (6.5 mg/day).

Side effects: withdrawals

Eight patients (42%) discontinued treatment during the 24 weeks. A 60 year old patient had a stroke and died after 18 weeks on triple treatment. Two patients discontinued because of infection: one had a community acquired pneumonia (10th week) and one developed extrapulmonary tuberculosis (cervical lymph node involvement) after 3 weeks of triple treatment having received 18 infliximab infusions. Two patients stopped because of non-compliance, one because of gastrointestinal discomfort (6th week), and two because of inefficacy after 18 weeks of triple treatment (both had DAS28 >5.1).

Efficacy

Of 11 patients who completed 24 weeks of treatment, five (45%) were moderate responders according to the EULAR response criteria. In those 11 patients significant improvements (*p*<0.05) in the mean values of swollen joints, Health Assessment Questionnaire (HAQ), patient's assessment of pain and disease activity, and physician's assessment were seen (table 2). However, only 2/11 (18%) patients had DAS28 <5.1 (the cut off limit for high disease activity) at the 24th week. During the 24 weeks of treatment, the DAS28 value of five (45%) patients dropped lower than 5.1 at some time point, while 8/11 (73%) patients satisfied EULAR criteria for moderate response. When the ACR criteria were applied to assess response to treatment, 4/11 (36%) completers fulfilled the ACR20 response criteria and 1/11 (9%) the ACR50 response criteria. No clinical or laboratory characteristics at baseline could predict response to CsA.

Lymphocyte activation

CD25 expression was determined on PBMCs from five patients. A reduction in CD25 expression both in unstimulated and PHA stimulated PBMCs was detected in four of five patients after treatment. More specifically, on unstimulated lymphocytes the mean CD25 expression of these five patients was reduced from 37% at baseline to 15% at the 12th week, while after PHA stimulation the mean expression was reduced from 50% to 29%, respectively. Figure 1 shows

Table 2 Disease activity in the 11 patients completing 24 weeks of treatment

Variable	Baseline	24th Week	p Value
DAS28	7.3 (1.1)	6.1 (1.1)	0.023
DAS28 (CRP)	6.1 (1)	4.85 (1)	0.013
Tender joints (0–28)	21.9 (8.5)	14.9 (9.8)	NS
Swollen joints (0–28)	19.2 (6.3)	12.2 (7.3)	0.025
HAQ (0–3)	1.44 (0.7)	0.73 (0.6)	0.019
Pain (0–100)	74.1 (25.8)	47.3 (28.3)	0.031
Patient's global assessment (0–100)	71.8 (25.7)	44.1 (28.8)	0.027
Physician's global assessment (0–100)	74 (9.7)	41.8 (22.4)	0.001

Results are shown as mean (SD).
NS, non-significant

Figure 1 Down regulation of CD25 expression on PBMCs after PHA stimulation in patient number 5 after 12 weeks of treatment. Both a reduction in relative cell number and mean fluorescence intensity can be seen. (A) IL2R antibody. (B) Isotypic control on PBMCs from the same patient at the same time points. (The y axis shows the relative cell number and the x axis the log₁₀ fluorescence intensity).

representative data for patient number 5. Among those five patients evaluated, only one was a responder and exhibited a reduction of CD25 expression from 67.2% at baseline to 39.4% at the 12th week (41.4% reduction). The remaining four non-responders showed a comparable mean reduction (from 45.8% at baseline to 26.9% at the 12th week, 41.3% mean reduction).

DISCUSSION

Despite the effectiveness of the combination of infliximab and MTX in clinical practice, there is a subgroup of patients with RA who have continuing disease activity. In this pilot, proof of concept study, we sought to investigate the potential efficacy of adding a T cell directed agent to the treatment of patients refractory to anti-TNF and MTX. The doses of both infliximab (mean 4.2 mg/6 weeks) and MTX (mean 17.1 mg/week) used in our cohort, are generally accepted by the community as adequate for disease control.^{14,15} Our results suggest a modest response and a high rate of discontinuation. However, these results should be interpreted cautiously because of the open label design of the study.

During 24 weeks of follow up, 8/19 (42%) patients discontinued treatment mainly because of side effects or incomplete compliance. Infections, a major concern when combining immunosuppressive agents, occurred in two patients. A community acquired bacterial pneumonia was successfully treated with a course of antibiotics. One patient developed lymph node tuberculosis 3 weeks after the initiation of CsA and after having received 18 infliximab infusions. Tuberculosis was successfully treated, while he continued methotrexate for RA. In this patient, infliximab was started before the establishment of routine tuberculosis screening before infliximab treatment.

In this group of patients with highly active disease (mean DAS28 at baseline 7.3), 5/11 (45%) patients who completed 24 weeks of treatment were moderate responders (mean DAS28 improvement 2.1, range 1.2–3.6). Although in these 11 patients improvement in the mean DAS28 value was significant from the 12th week of treatment (*p* values 0.03, 0.05, and 0.02 at weeks 12, 18, and 24, respectively), the DAS28 dropped below 5.1 in only two patients (mean DAS after treatment 5.9, range 4.1–7.2). All clinical measures (except tender joint count) were significantly improved from the 12th week of treatment (table 2). Interestingly, 80% of the patients showed clinically significant improvement in HAQ score (improvement >0.22) from the 6th week of treatment, underlining the improvement of the functional status shortly after CsA introduction.

An alternative approach when dealing with patients refractory to anti-TNF is to switch from one biological agent

to another.^{6,8} Data from a Swedish registry reported significant improvement (*p*<0.02) in the mean DAS28 when switching from etanercept to infliximab or from infliximab to etanercept.⁶ Similarly, a group from Spain showed improvement (*p* = 0.012) in the mean DAS28 in 12 patients who were switched from infliximab to etanercept.⁸ Whether switching to another anti-TNF agent or adding another DMARD is more effective in controlling disease activity in patients refractory to anti-TNF treatment is not known at present.

In summary, patients not responding to adequate doses of infliximab and MTX represent a group of patients with refractory disease. In this group of patients with RA, addition of CsA resulted in clinically significant improvement in patients' functional status and statistically significant improvements in clinical variables. However, the response was modest and was associated with a high rate of discontinuation. These data suggest that in patients with highly refractory disease, combinations of anti-cytokine treatment with various DMARDs are unlikely to be sufficient. For these patients new approaches, including B cell depleting treatments or autologous stem cell treatment, need to be further explored.

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