Long-term efficacy and toxicity of cyclosporin A + fluocortolone + methotrexate in the treatment of rheumatoid arthritis

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Abstract

Objective

The therapeutic efficacy and tolerability of the combination of cyclosporin A, methotrexate and fluocortolone was evaluated after 96 months of treatment in 140 patients with rheumatoid arthritis.

Methods

The initial dose of CyA was 5 mg/kg per day and was subsequently modified on the basis of the individual clinical response. Fluocortolone was initially administered at a dose that was sufficient to control disease activity (80-130 mg/week) and then was gradually tapered down to a maintenance dose of 15-20 mg/week. MTX was given intravenously at a dose of 15 mg once weekly for 4 consecutive weeks and then, after a 2-week interval, every 2 weeks or every month depending on the evolution of the disease.

Results

At the end of the study a statistically significant improvement was observed in both clinical (VAS, grip-strength, duration of morning stiffness, number of swollen joints, number of painful joints, Ritchie's index and Lee's functional index) and laboratory parameters: ESR ($p = 0.000$); α_2 globulins ($p = 0.000$); hemoglobin ($p = 0.000$); CRP ($p < 0.001$); and rheumatoid factor ($p = 0.000$). Radiological evalutation revealed little progression in anatomic lesions (Larsen score $p = 0.699$; number of erosions $p = 0.344$), thus suggesting that our protocol may be capable of showing down both bone resorption and cartilage loss. Renal toxicity, defined as an increase in plasma creatinine concentrations of more than 50% of the baseline value, was observed in 12 patients (8.5%), but the drug was discontinued in only one, who simultaneously presented high blood pressure.

Conclusion

The positive results so far achieved in our study must be interpreted as being due to the combined action of the individual drugs, which made it possible for them to be used at relatively low dosages that minimised the onset of their side effects while maintaining the efficacy of their suppressive action.

Key words

Rheumatoid arthritis, cyclosporin A, methotrexate, fluocortolone.

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Introduction

Rheumatoid arthritis (RA) is a multi-system inflammatory disease process characterized by chronic, proliferative and erosive synovitis (1). Progressive RA has a worse prognosis in certain patients who can be identified by their high joint counts and poor functional status (2). Although the triggering process is not known, T cells are thought to play a central role in modulating the inflammatory process (3). RA synovium predominantly contains T memory and helper/inducer (CD4+, 4B4+) lymphocytes (4). CD4+ cells recognize HLA class II antigens and are activated to proliferate, differentiate and secrete soluble lymphokines, including interferon-γ and interleukins 2 (IL-2), 3 (IL-3) and 4 (IL-4) (5).

In the context of these observations, the target of RA therapy has been to suppress active T-lymphocyte inflammation in synovial tissue (6). Some authors have recently reported that cyclosporin A (CyA) is efficacious in the treatment of various autoimmune diseases, including RA (7, 8), psoriasis (9), nephrotic syndrome (10), inflammatory bowel disease (11), and severe endogenous uveitis (12), at considerably lower doses than those which have so far been used in organ transplant anti-rejection therapy.

Cyclosporin A is an immunomodulating agent that specifically but reversibly blocks the action of CD4+ cells (13). Furthermore, CyA inhibits the spontaneous proliferation of synovial T cells *in vitro* (14). Another possible agent for use in rheumatoid arthritis is methotrexate (MTX), whose efficacy and tolerability have recently been reported in a large number of patients (15). However, dissatisfaction with the effects of monotherapy (16) has led to increasing interest in the possibilities of combination therapy (17 - 19).

As we have obtained encouraging preliminary results with relatively low doses of CyA combined with fluocortolone and MTX in the treatment of a number of autoimmune diseases (12, 20), we decided to extend the use of this combination to patients with rheumatoid arthritis. This article reports the efficacy and toxicity of combined CyA, fluocortolone and MTX treatment in 140 RA patients unresponsive to conventional therapy.

Patients and methods

Patients

One hundred and forty patients (98 women, 42 men), aged 16-87 years (mean 47.44 ± 12.64), who fulfilled the American Rheumatism Association criteria for definite or classic RA (21), whose disease could be classified as anatomical and functional stage II or III according to the criteria of Steinbrocker (22), and whose disease duration was at least 6 months (Table I), were recruited for this study after their informed consent had been obtained. The mean disease duration was 15.21 ± 8.61 years (range 2 - 25 yrs.). Fifty of the patients had previously been treated with NSAIDs, 52 with gold salts, 54 with cortisone, 16 with penicillamine, 18 with hydroxychloroquine, 20 with levamisole and 12 with sulfasalazine (Table I). None of them had shown a therapeutic response to traditional drugs during the previous six months. Some benefit had been obtained from the administration of prednisone at a dose of 1 mg/kg body weight (which usually corresponded to 40 - 70 mg), but the onset of side effects not only made it impossible to use higher doses but also required a progressive reduction in posology that led to a clinical relapse after 6

Table I. Demographic and clinical characteristics of the 140 patients at study entry.

Females/males	98/42
Age (yrs.)	47.44 (12.64; 16 - 87)*
Duration of illness (yrs.)	15.21 (8.61; 2 - 25)*
ARA functional class	
II	68
III	22
Rheumatoid factor positive	86
HLA-DR4 positive	44
Previously failed treatment regimen	
Gold salts	53
and/or NSAIDs	103
and/or steroids	83
and/or hydroxychloroquine	80
and/or D-penicillamine	17
and/or levamisole	21
and/or salazopyrine	15
Previously received DMARDs (%)	
≥ 4	14 (10)
≥ 3	35 (25)
≥ 2	62 (44)

* mean (SD; range)

to 7 weeks. The interval from the last intra-articular injection of corticosteroids to the start of cyclosporine therapy was set at one month.

Exclusion criteria included: significant renal disease (defined as a creatinine concentration > 120 (mol/l) or detectable hepatic disease (an increase in liver enzymes to twice the baseline level or a bilirubin concentration above baseline); leukopenia (total white cell count < 3500 cells/mm^3 or a polymorphonuclear cell count < 1500 cells/mm^3) and/or thrombocytopenia (platelet count < 150,000 cells/mm^3); a history or the presence of malignancy; the presence of chronic infection; the presence of any disease or abnormal condition potentially capable of compromising the absorption, metabolism or excretion of CyA; pre-existing bone marrow hypoplasia; major surgery during the two months preceding study entry (including joint surgery); insulin dependent diabetes; overt obesity; the need for concomitant therapy with any other experimental drug; the use of anticoagulant therapy; or unwillingness or inability to cooperate and/or give informed consent.

Women of childbearing potential were also excluded.

Drug administration protocol

All of the patients were given combined treatment with CyA, fluocortolone and MTX; in less severe cases the treatment was started after at least one week of total therapeutic washout.

The initial dose of CyA was 5 mg/kg per day in two divided doses at 12 hr intervals, usually at 8 a.m. and 8 p.m. (in the case of overweight patients, the dose was based on their ideal body weight). By the third day of treatment blood cyclosporin levels were between 100 and 200 mcg/l in all of the patients. As soon as a clear improvement in clinical signs and instrumental and laboratory results was observed, CyA treatment was reduced to 6 (then 5, then 4) days per week on the basis of the individual response. The reason for reducing the number of days of CyA administration per week without changing the daily dosage was to decrease the risk of renal damage without modifying the immunological activity of the drug. Specifically, the interruption of CsA administration on Thursdays and Sundays was expected to bring the cyclosporinemia values down to zero (thus eliminating the possibility of renal overload and consequent damage), but was considered to be too short an interruption to inhibit its action on lymphocytes and therefore its immunomodulatory activity.

The corticosteroid fluocortolone was used because of its lack of effect on sodium retention and the fact that it has less Cushing-like activity than prednisolone (23). It was initially administered at a dose sufficient to control disease activity (i.e., 80 - 130 mg/week administered on 5 days out of 7), and was then gradually tapered depending on the course of the disease down to a maintenance dose of 15 - 20 mg/week administered on 3 days.

All of the patients also received MTX, which has been proven to have an immunosuppressive action in autoimmune diseases, to induce the rapid abatement of fever, and to have an equally rapid anti-inflammatory effect (24). MTX was given intravenously at a dose of 15 mg once weekly for 4 consecutive weeks and then, after a 2-week interval, every 2 weeks or every month depending on the evolution of the disease. In addition, the patient was given folinic acid 15 mg for 6 days after each MTX administration.

Clinical efficacy criteria

The evaluation of patient benefit was based on the following variables: the duration of morning stiffness in minutes; the Ritchie and Lee indexes (25, 26); the number of swollen joints; the number of painful joints; and the grip strength in each hand measured using a sphygmomanometer cuff. The visual analog scales (VAS) used to measure pain consisted of a 10 cm vertical line with “no pain” at the bottom and “maximum imaginable pain” at the top (27).

Laboratory assessments

Cyclosporin treatment was monitored by periodically evaluating biological parameters, including the erythrocyte sedimentation rate (ESR by Westergreen), C-reactive protein (CRP, laser nephelometry), α_2-globulins, immunoglobulins (IgA, IgG, IgM) and IgM rheumatoid factor (Rose Waaler), as well as of hemopoiesis, blood coagulation, liver and renal function, and blood pressure. Whole blood CsA radioimmunologic assays were performed using specific monoclonal antibodies (28) on samples drawn 12 hrs after the last dose was administered.

Radiological evaluations of the patient’s wrists, hands, ankles and feet were made at baseline and after every 12 months. The radiographs were evaluated by one of us, a radiologist, who was not aware of the patient’s treatment or whether the films were taken at baseline or at the end of study. The number of erosions and the Larsen score (29) were recorded.

Physical examination, as well as the hemato-biochemical tests, were carried out every two weeks for the first month, monthly until the 6th month, then every three months until the 12th month, and then every six months.

After every six months of treatment a global assessment of efficacy was made by the patient and the investigator using a five-point scale (5= excellent; 4 = good, 3 = moderate; 2 = slight; 1 = none).

The results were analyzed using the Wilcoxon-Mann-Whitney U test. P values < 0.05 were considered significant.

Results

A significant improvement in the disease activity indices was observed after six months of treatment and was maintained throughout the period of follow-up (Tables II and III). After 12 months of treatment, there was a 92% reduction in the Ritchie index ($p = 0.000$), an 88% reduction in pain ($p = 0.000$), and a 94% increase in function ($p = 0.000$).

The results of the laboratory tests are summarised in Table III. After 6 months of treatment, the mean CRP and ESR values of 0.64 mg/dl and 31.75 mm/h respectively, were significantly lower than at baseline ($p < 0.005$ and $p = 0.000$, respectively). After 24 months, the mean CRP level was 90% less than at baseline ($p < 0.005$) and the mean ESR was 67% less ($p = 0.000$). The mean levels of CRT remained stable throughout the period of observation, whereas the mean ESR progressively diminished until it reached 7 mm/hr at the end of the study ($p = 0.000$) (Table III).

Table II. Changes in the clinical parameters of 140 patients with rheumatoid arthritis during combined cyclosporin A + steroid + MTX treatment.

Variable	0 mos. (n = 140)	6 mos. (n = 140)	D (%)	12 mos. (n = 140)	D	24 mos. (n = 138)	D	36 mos. (n = 138)	D	48 mos. (n= 138)	D	96 mos. (n= 135)	D
VAS (cm)	77.5 (40 - 100)	15 (0 - 100)	71	5 (0 -40)	91	0 (0 -40)	92	0* (0 - 20)	94	0* (0 - 20)	95	0 (0 - 8)	95
Grip strength (mm Hg)													
Left hand	62.5 (14 - 115)	115 (53 - 158)	66	122.5 (58 - 164)	72	122.6 (101 - 168)	73	125 (102-168)	77	125* (102-150)	77	125 (102-168)	77
Right hand	66 (15 - 128)	122.5 (65 - 177)	69	141 (72 - 191)	84	142.5 (73 - 195)	86	154 (92 - 216)	98	154 (92 - 216)	98	154 (92 - 216)	98
Lee's index	19 (0 - 38)	6 (0 - 19)	67	0 (0 - 8)	94	0 (0 - 8)	95	0 (0 - 8)	95	1* (0 - 8)	95	3.5 (1 -6)	95
Morning stiffness (min.)	240 (0 - 480)	30 (0 - 250)	83	5 (0 - 200)	93	5 (0 - 120)	94	5* (0 - 20)	98	2.5* (0 - 10)	99	2.5 (0 - 5)	99
Number of swollen joints	20 (0 - 42)	1 (0 - 22)	92	0 (0 - 3)	99	0 (0 - 2)	99	0* (0 - 1)	99	0* (0 - 1)	99	0 (0 - 1)	99
Number of painful joints	19.5	2	84	1	88	0.5	94	0*	94	0*	94	0	94
Ritchie's index	23 (5 - 66)	2.5 (0 - 20)	82	1 (0 - 13)	92	1 (0 - 13)	95	1* (0 - 2)	97	0.5* (0 - 2)	97	0.5 (0 - 2)	97
Overall efficacy assessment**													
Patient	0.3 (0.0 - 1.0)	3.0 (0.0 - 4.0)		3.2 (1.0 - 4.0)		3.5 (1.5 - 4.0)		3.5 (3.0 - 4.0)		3.6 (3.0 - 4.0)		3.7 (3.0 - 4.0)	
Investigator	0.4 (0.0 - 1.0)	3.0 (0.0 - 4.0)		3.3 (1.0 - 4.0)		3.4 (1.0 - 4.0)		3.5 (2.5 - 4.0)		3.5 (2.5 - 4.0)		3.7 (3 - 4)	

p= 0.000; *p < 0.06

Table III. Changes in the laboratory parameters of 140 patients with rheumatoid arthritis during combined cyclosporin A + steroid + MTX treatment.

Variable	0 mos. (n = 140)	6 mos. (n = 140)	D (%)	12 mos. (n = 140)	D (%)	24 mos. (n = 138)	D (%)	36 mos. (n = 138)	D (%)	48 mos. (n = 138)	D (%)	96 mos. (n = 135)	D (%)
ESR (mm/hr)	64.81 (28.05) (18 - 100)	31.75 (17.23)* (10 - 70)	- 49	25.54 (14.08)* (6 - 65)	- 61	21.53 (13.31)* (4 - 42)	- 67	16.37(10.13)* (6 - 40)	- 75	15.40 (11.24)* (6 - 40)	- 76	7.00 (1.41)* (6 - 8)	- 89
a_2-Globuins (g/l)	11.41 (3.80) (4.70 - 18.60)	7.87 (2.49)* (4 - 16.20)	- 31	6.52 (2.35)* (3.60 - 13.16)	- 43	5.59 (1.58)* (3.80 - 12.70)	- 51	5.20 (1.12)* (4.00 - 8.60)	- 54	5.04 (0.69)* (4.10 - 6.20)	- 56	5.05 (0.14)* (4.10 - 6.22)	- 56
Hemoglobin (g/dl)	10.96 (1.39) (8.80 - 14.50)	12.73 (0.95)* (10.00 - 14.70)	+ 16	13.03 (0.61)* (10.90 - 14.30)	+ 19	13.25 (0.52)* (12.00 - 14.60)	+ 21	13.25 (0.55)* (12.20 - 14.60)	+ 21	13.32 (0.61)* (12.20 - 14.60)	+ 22	14.25 (0.49)* (13.90 - 14.60)	+ 30
CRP (mg/l)	5.72 (9.77) (0.33 - 11.70)	0.64 (0.86)* (0.12 - 6.27)	- 89	0.58 (0.07)* (0.30 - 0.60)	- 90	0.57 (0.08)* (0.30 - 0.60)	- 90	0.54 (0.10)* (0.40 - 0.60)	- 91	0.48 (0.12)* (0.30 - 0.60)	- 92	0.48 (0.10)* (0.30 - 0.60)	- 92
RF (IU/ml)	237.47 (232.32) (11.50 - 1640.00)	120.44 (169.64)* (11.40 - 1000.00)	- 49	47.00 (53.21)* (11.00 - 300.00)	- 80	36.35 (30.02)* (10.00 - 140.00)	- 85	36.25 (26.28)* (24.00 - 130.00)	- 85	36.20 (25.64)* (28.00 -120.00)	- 85	36.30 (28.15)* (28.00 - 120.00)	- 88
Platelets (x 10^9)	380 (100.15) (298 - 480)	352 (102.15)^ (254 - 455)	- 7	330 (98.67)* (229 - 430)	- 13	292 (103.35)* (189 - 396)	- 16	280 (99.55)* (185 - 370)	- 20	278 (100.35)* (184 - 382)	- 21	279 (98.13)* (182 - 378)	- 21

Mean (SD) and (minimum - maximum).
*p = 0.000; ^0.01 < p < 0.05.

The hemoglobin levels determined after six months of treatment were significantly higher than at baseline (p = 0.000), and reached 13.25 g/dl after 36 months (an increase of 20.89%: p = 0.000).

The efficacy of the treatment was judged to be “good” or “very good” by 85% of the patients and by 85% of the physicians after 12 months of treatment, increasing to respectively 92% (p= 0.000) and 91% (p = 0.000) by the end of the study (Table II).

Tolerability

Table V summarises the adverse events observed during the study; the most frequent was hypertrichosis, which affected 50 (35.7%) of the patients. Fifteen patients (10.71%) developed arterial hypertension (defined as a diastolic blood pressure of more than 90 mmHg), two of whom had had mild essential hypertension (DBP < 95 mmHg) well controlled by calcium-antagonists at the beginning of the study. Only two patients developed both renal toxicity and hypertension, one of whom had to discontinue the study treatment despite the concomitant administration of calcium antagonists (which controlled the hypertension in the remaining 14 patients).

In the other 125 patients, the mean systolic pressure increased from 136.15 ± 14.34 mmHg at baseline to 137.94 ± 10.47 after six months, 138.50 ± 8.90 after 12 months, and 140.00 ± 8.70 after 90 months (p = 0.011). The mean diastolic pressure was 75.65 ± 3.28 mmHg at baseline, 75.52 ± 3.84 after six months, 76.08 ± 6.00 after 12 months (p < 0.05), and 76.50 ± 4.30 after 96 months (p = 0.079).

Renal toxicity

An increase in creatinine levels of more than 50% above baseline values was observed in 12 of the 140 patients (8.5%), but prompted the withdrawal of the study drug in only one case. Comparison of the clinical characteristics of the patients experiencing renal toxicity with those of the patients who did not showed that renal toxicity was more frequent in patients aged > 60 years, in those with a plasma creatinine concentration > 90 µmol/L at baseline, and in those with a history of urinary tract diseases (Table VI).

Table IV. Plasma creatinine, GRF, BUN, uric acid, serum potassium, serum magnesium and bood pressure values observed before, during and at the end of follow-up

Variable	0 mo. (n= 140)	6 mos. (n= 140)	12 mos. (n= 140)	24 mos. (n= 138)	36 mos. (n= 138)	48 mos. (n= 138)	96 mos. (n= 135)
Plasma creatinine (μmol/l)	82.67 (11.81) (59 - 106)	84.65 (11.66)^^ (61 - 107)	85.50 (11.77)^ (63 - 109)	86.00 (12.50)^ (63 - 110)	87.12 (14.54)° (66 - 109)	87.90 (15.60)° (68 - 107)	89.50 (17.68)* (77 - 102)
Creatinine clearance (ml/min)	118.56 (6.43) (100 - 128)	116.56 (6.54)^ (96 - 126)	115.93 (6.53)* (96 - 126)	115.35 (7.16)* (96 - 126)	112.06 (7.22)* (96 - 120)	115.20 (4.92)^ (107 - 120)	108.00 (2.83)* (106 - 110)
BUN (g/l)	17.46 (1.98) (13 - 22)	18.15 (1.83)° (14 - 22)	18.46(1.80)* (15 - 22)	18.35 (2.09)* (14 - 22)	17.56 (1.86)* (14 - 20)	16.80 (1.26)* (14 - 19)	6.50 (3.54)° (14 - 19)
Serum potassium (mEq/l)	4.20 (0.15) (3.8 4.40)	4.18 (0.15)^^ (3.80 - 4.50)	4.18 (0.16)^^ (3.80 - 4.50)	4.22 (0.17)^^ (3.80 - 4.50)	4.22 (0.19)^^ (3.80 - 4.50)	4.18 (0.15)^^ (3.90 - 4.40)	4.18 (0.21)^ (3.80 - 4.10)
Uric acid (mg%)	5.06 (0.39) (4.20 - 6.00)	5.08 (0.45)** (4.20 - 6.30)	5.15 (0.46)** (4.30 - 6.50)	5.16 (0.54)** (4.40 - 6.90)	5.16 (0.72)^^ (4.50 - 7.10)	5.17 (0.96)^^ (4.50 - 7.00)	5.20 (0.14)* (4.30 - 4.50)
Mg+ (mmol/l)	0.81 (0.05) (0.74 - 0.83)	0.82 (0.02)^ (0.79 - 0.83)	0.82 (0.01)^ (0.80 - 0.83)	0.82 (0.03)^ (0.80 - 0.83)	0.82 (0.02)^ (0.80 - 0.84)	0.83 (0.01)* (0.81 - 0.85)	0.83 (0.01)* (0.81 - 0.84)
Systolic pressure (mm/Hg)	136.15 (14.34) (120 - 185)	137.00 (11.37)** (120 - 170)	139.50 (9.90)^ (120 - 160)	139.96 (9.71)^ (125 - 160)	140.50 (7.58)∞ (130 - 155)	141.50 (7.62)* (135 - 160)	140.00 (9.70)°° (140 - 140)
Diastolic pressure (mm/Hg)	76.25 (4.28) (60 - 95)	76.32 (4.84)** (60 - 90)	76.56 (8.00)** (65 - 85)	76.56 (8.00)** (65 - 85)	76.77 (4.28)^^ (65 - 80)	76.77 (6.36)^^ (65 - 80)	76.77 (5.30)^^ (65 - 80)

Mean (SD) and (minimum - maximum).
**NS; *p = 0.000; °0.001 ≤ p ≤ 0.005; °°0.005 ≤ p ≤ 0.01; ^ 0.01 ≤ p ≤ 0.05; ^^ 0.1 ≤ p ≤ 0.5.

The mean plasma creatinine levels increased from 82.67 ± 11.81 μmol/L at baseline to 85.50 ± 11.77 after 12 months ($p < 0.05$), and 89.50 ± 17.68 after 96 months ($p = 0.000$). In the patient who discontinued CyA treatment, the concentration of plasma creatinine had increased from 92 to 145 (mol/L, and was accompanied by an increased in blood pressure (Table IV). The increase in creatinine levels occurred after one month of treatment; after discontinuation of the drug the creatinine level returned to 90 μmol/L and the blood pressure normalised.

NSAID therapy was discontinued in all of the patients when they entered the study, at which point the mean plasma creatinine level in the patients as a whole was 82.67 ± 11.81 μmol/L: 85.30 ± 10.20 in the patients who had been receiving NSAIDs and 82.20 ±11.15 μmol/L in those who were not.

Six of the 140 patients complained of paresthesias, none of whom required the discontinuation of treatment: these disturbances began to appear during the first month and, despite the continuation of treatment, disappeared by month 5. These patients tended to present a low level of magnesium (Mg+) before the beginning of the therapy (0.76 ± 0.05 vs 0.81 ± 0.05 mmol/L; $p = 0.018$). Among the patients as a whole, serum Mg+ levels declined from 0.81 ± 0.05 mmol/L before treatment to 0.79 ± 0.07 mmol/L after one month ($p = 0.006$). During the same period magnesium levels were lower in the patients with paresthesias: 0.73 ± 0.03.

Table V. Adverse reactions in the 140 patients with rheumatoid artritis treated with combined cyclosporin A + steroid + MTX.

Side effect	Frequency No.	Frequency %	Cause of withdrawal No.	Cause of withdrawal %
Hypertrichosis	50	35.70	0	0
Renal toxicity	12	8.57	1	0.71
Paraesthesia	6	4.28	0	0
Nausea	10	7.14	0	0
Hypertension	15	10.71	1	0.71
Epigastric discomfort	12	8.57	0	0
Gingivitis	4	2.85	0	0
Fatigue	8	5.70	0	0
Tremor	2	1.42	0	0
Benign gynaecomastia	2	1.42	0	0
Nervousness	2	1.42	0	0
Headache	2	1.42	0	0
Skin rash	2	1.42	0	0
Herpes zoster infection	8	5.70	0	0
Tumor	3	2.14	3	2.14

Table VI. Predisposing factors for renal toxicity (defined as an increase in plasma creatinine concentrations of 50% above baseline or > 150 µmol/l) in the 140 patients with RA treated with a combination of cyclosporin A + steroid + MTX.

Characteristics	Renal toxicity		
	Yes	No	p value#
Number of patients	12	128	
Age (years)	63.87 (8.7)*	46.56 (12.03)	0.000
Patients > 60 years	11	3	0.012
Plasma creatinine concentration at entry (µmol/l)	93 (19.3)	82.04 (11.81)	0.016
Plasma creatinine concentration at entry > 88 µmol/l (pts.)	10	5	< 0.003
Concomitant use of Ranitidine (pts.)	5	24	NS
Previous renal diseases (pts.)	7	1	NS
Concomitant NSAID† intake (pts.)	7	21	NS

#Statistical analysis: $\chi 2$ test for qualitative variables and Mann-Whitney bilateral U test for quantitative variables
*Mean (SD); †NSAID: non-steroidal anti-inflammatory drug.

Nausea and epigastric discomfort were transient side effects probably not related to CyA administration.
The fact that the dose was not decreased in the case of the majority of the side effects shows that they were considered clinically unimportant.

The development of malignancy was reported in three patients; these were all solid tumours (two lung cancers and one prostate carcinoma). Table VIII summarises these 3 cases, including their age, sex, outcome, previous therapy, and the dose and duration of exposure to the individual study drugs until the time that the malignancies were diagnosed. All of these patients received the same therapy as those who did not develop neoplasms. In all 3 patients, CyA and fluocortolone were administered until the diagnosis of the neoplasms. The duration of treatment before diagnosis ranged from 2 to 3 years; the two patients developing lung cancer were both heavy smokers.
Eight patients (5.6%) developed herpes zoster infection, which in all cases arose at the same time as a flu-like syndrome; these infections resolved after seven days of treatment with aciclovir 1.2 mg/die and did not require hospitalisation.
The results of the radiological evaluation are shown in Table VII; after 96 months of treatment, neither the Larsen scores nor the number of erosions showed any statistically significant differences vs baseline (p = 0.699 and 0.344, respectively).
The favourable results made it possible to reduce the CyA treatment from 6 to 5 administrations per week after the first six months, and to 4 administrations per week during the second year. The initial

Table VII. Radiological evaluation at baseline, and after 12, at 24 and 96 months in the 140 patients with rheumatoid artritis who underwent combination therapy with cyclosporin A + steroid + MTX.

	Baseline (n = 140)	12 months (n = 140)			24 months (n = 138)			96 months (n = 135)		
Outcome measure	Mean ± SD	Mean ± SD	% Chge.	P	Mean ± SD	% Chge.	P	Mean ± SD	% Chge.	P
Larsen score (max. 5) (max. - min.)	1.62 ± 0.57 (1 - 2)	1.61 ± 0.75 (0 - 3)	- 0.61	0.858	1.60 ± 0.81 (0 - 3)	-1.23	0.810	1.59 ± 0.81 (0 - 3)	-1.85	0.699
No. of erosions (max. - min.)	5.17 ± 0.39 (4.60 - 5.83)	5.18 ± 0.40 (4.60 - 6.00)	0.19	0.896	5.19 ± 0.40 (4.60 - 6.00)	0.38	0.639	5.21 ± 0.40 (4.60 - 6.00)	0.77	0.344

The statistical significance of the values was calculated using the t-test for paired data.

Table VIII. Cases of malignancies.

		CsA		MTX (mg.)		Fuocortolone		Previous		Outcome
Malignancy	Age/sex	mg/kg†	Dur.*	Total	Dur.	mg/wk	Dur.	therapy	Dur.	
Lung carcinoma	72/M	5	36	720	12 (24)#	80	36	Gold, hydroxychlo.	8	Died (liver metastases)
Lung carcinoma	68/M	5	28	480	10 (18)	80	28	Gold, hydroxychlo.	10	Died (liver, brain metastases)
Prostate carcinoma	73/M	5	34	420	8 (26)	80	34	Gold , salaz. hydroxychlo.	14	Improved

*Dur.: duration in months.
† Suspended for one day per week; # Number in parentheses: months after discontinuation of therapy to diagnosis of tumour.
Gold: gold salts; Hydroxychl: hydroxychloroquine; Salaz: salazopyrine.

dose of 80-180 mg/week of fluocortolone was reduced to 80 - 60 mg/week after six months, to 50 - 35 mg/week after 12 months, and to 35 - 25 mg/week at the end of the period of observation.
Given the optimum results immediately obtained, MTX administration was reduced to once a fortnight after the first six months, and subsequently to once a month. In all of the patients the drug was discontinued between the 12th and 18th month of observation.

Discussion

The results of this study of the treatment of RA with the association of CsA, fluocortolone and MTX appear to be encouraging. One of the most interesting findings is the fact that our protocol may have effects on the evolution of the disease, as is shown by the absence of progression in the radiological lesions even after 96 months of treatment. Førre *et al.* (8) have previously reported reduced progression in the radiological manifestations in RA patients treated with CyA for 48 weeks, thus confirming the finding in rats that CyA treatment suppresses both RA-induced bone resorption and the loss of cartilage (30). This absence of progression is probably the consequence of an interruption of local inflammation (30). On this point it has been suggested that a plateau may be reached as early as 6 years after the disease onset, and certainly by 10 years (31-33). Therefore, the absence of radiographic progression may simply reflect the fact that patients have accumulated as much damage as will be seen.
The therapeutic strategy adopted made it possible to achieve our target of obtaining the maximum possible benefit in terms of remission and subsequent disease control, without the occurrence of any side effects attributable to the use of the drugs.
Monotherapy rarely leads to lasting remission in RA (17). Clinical studies of other combinations (with anti-malarials, gold salts, azothioprine or penicillamine), as well as of MTX alone, have shown equivocal benefits (17, 34, 35). However, the combination of CyA, fluocortolone and MTX immediately led to highly favourable result in our patients, up to the point of total and lasting disease remission.
Our data confirm the results of other studies which suggested that the combination of cyclosporine and methotrexate was more effective than methotrexate alone in patients showing a partial response to methotrexate (18, 19). It would appear that MTX exerts its favourable action during the initial phase of treatment by inhibiting macrophage and monocyte function, and the synthesis and secretion of IL-1 on the part of mononucleate cells (36).
The complete absence of exacerbations and/or relapses would seem to be due to the combined action of CyA and cortisone, which are known to act on humoral and cell-mediated immunity after antigen stimulation to the point of inhibiting the proliferation of T-helper and B lymphocytes, and the production of γ-interferon (37). These last elements play a determining role in the proliferation, activation and formation of the synovial pannus (14). CyA probably also inhibits the process of DNA-RNA transcription necessary for lymphokine production (38).
This observation is supported by the surprising therapeutic effect of the drug not only in terms of the rapidity of the symptomatic articular response, but above all in terms of the alterations in the blood chemistry test parameters. In this regard, it is worth underlining the interesting finding of the significant decrease in the ESR (from 64.8 to 7.00 mm/hr; $p = 0.000$), which persisted throughout the study period. This finding is in contrast with the reports of other authors using CyA alone who, although they observed a reduction in CRP levels, did not find any significant change in the ESR (39, 40). The reason for this difference is not known.
In our protocol MTX was discontinued after 20 months, but there are several reports in the literature indicating that MTX may be continued for long periods with encouraging results (41).
One of the limitations emerging from the present study is the difficulty of treating patients with refractory RA. Almost all of our patients were affected by severe disease and had not responded to a large number of previous monotherapies.
Another encouraging element is that no significant or unexpected toxic effects were observed in the short term or, more importantly, in the long term. Our combination protocol did not demonstrate any greater toxicity than that which would be expected from the use of MTX, CyA or cortisone alone. Our study shows that the administration of the combination of the three drugs did not seriously damage renal function in most of our patients. It is also worth underlining the fact that, precisely in order to evaluate the real nephrotoxic potential of the drug doses used in this study, we deliberately excluded all patients with pre-existing signs of chronic renal insufficiency. On this subject, a important data emerging from our study is that the serum creatinine level was not changed in statistically significant fashion. This is in contrast with other studies and could be partially explained by interruption of all FANs therapy.
Hypertrichosis was the most frequently recorded undesired effect, and this could represent a considerable problem for female patients. However, it is known that CyA is not without toxic side effects, particularly when administered for long periods of time, and so it is our intention to continue its administration in combination with other immunosuppressant drugs (in addition to MTX and fluocortolone) with the precise aim of identifying those combinations that will allow a further reduction in the dose of CyA itself.
The long-term risks of cancer due to the combined administration of CyA and MTX require further study (42, 43), but it is already known that the risk of cancer in RA patients is 5 times higher than that in the general population, and may even be 10 times higher in the case of patients treated with azathioprine (44). Other drugs, such as chlorambucil and cyclophosphamide, are also associated with an increased cancer risk (45). The reason for the higher risk of cancer in RA patients is not known: it is possible that RA patients may be more susceptible to neoplasm; RA and cancer may have similar extrinsic causes; or RA treatment may facilitate the development of neoplasm (46). Reversible lymphomas have been observed in patients who received either MTX or cyclosporine

alone. The results of the present study do not allow us to formulate any hypotheses concerning the role that the drugs may have had in the onset of neoplasms. Nevertheless, although a facilitating role of CyA in the two patients who presented lung cancer cannot be excluded, the fact that they were both heavy smokers must be borne in mind

A recent study of 1000 RA patients treated with CyA (47) found the onset of 17 cases of neoplasms after treatment with the drug. CyA does not have any intrinsic carcinogenic or mutagenic potential (48, 49), but its use is associated with an increased risk of malignancies due to its immunosuppressive properties. In relation to the possible carcinogenic role of CyA, it is important to underline the fact that no significant difference in the incidence of malignancies or lymphoproliferative disorders has been found between transplanted patients treated with traditional immunosuppressive agents, such as azathioprine alone or in combination with steroids, and those treated with CyA (50, 51).

It is also worth remembering that the positive results so far achieved in our study must be interpreted as being due to the combined action of the individual drugs, which made it possible for them to be used at relatively low dosages that minimised the onset of their side effects while maintaining an efficacious suppressive action. The use of the same drugs in monotherapy would have required much higher doses that, sooner or later, would inevitably have led to the undesired side effects known to be associated with the more or less prolonged use of such high doses of the same drugs.

References

1. HARRIS ED JR: Clinical features of rheumatoid arthritis. *In* KELLY WN, HARRIS ED JR, RUDDY S, and SLEDGE CB (Eds.): *Textbook of Rheumatology*. 4th ed., vol. 1, Philadelphia, W.B. Saunders, 1993: 874-911.
2. PINCUS T, CALLAHAN LF: What is the natural history of rheumatoid arthritis ? *Rheum Dis Clin North Am* 1993; 19: 123-51.
3. BUCHT A, OKSENBERG JR, LINDBOLD S, GRONBERG A, STEINMAN L, KLARESKOG L: Characterization of T-cell receptor repertoire in synovial tissue from different temporal phases of rheumatoid arthritis. *Scand J Immunol* 1992; 35: 159-63.
4. HOWELL MD, DINELY JP, LUNDEN KA: Limited T-cell receptor b-chain heterogeneity among interleukin-2 receptor-positive synovial T-cell suggest a role for superantigen in rheumatoid arthritis. *Proc Nat Acad Sci USA* 1991; 88: 10921-7.
5. PANAYI GS: The immunopathogenesis of rheumatoid arthritis. *Br J Rheumatol* 1993; 32 (Suppl. 1): 4-14.
6. EPSTEIN FH: Rheumatoid arthritis: Pathophysiology and implications for therapy. *N Engl J Med* 1990; 322: 1277-88.
7. LANDEWE RBM, GOEI THE HS, van RIJTHOVEN AWAM, RIETVELD JR, BREEDVELD FC, DIJKMANS BAC: Cyclosporine in common clinical practice: An estimation of the benefit/risk ratio in patients with rheumatoid arthritis. *J Rheumatol* 1994; 21: 1631-6.
8. FØRRE Ø and the Norwegian Arthritis Study Group: Radiologic evidence of disease modification in rheumatoid arthritis patients treated with cyclosporine. *Arthritis Rheum* 1994; 37; 1506-12.
9. LOWE NJ: Systemic treatment of severe psoriasis. The role of cyclosporine. *N Engl J Med* 1991; 324: 277-84.
10. BOREL JF: Mechanism of action of cyclosporin A and rationale for use in nephrotic syndrome. *Clin Nephrol* 1991; 35 (Suppl. 1): 523-30.
11. SANDBORN WJ, TREMAINE WJ: Cyclosporin treatment of inflammatory bowel disease. *Mayo Clin Proc* 1992; 67: 981-90.
12. PASCALIS L, PIA G, ARESU G, FRONGIA T, BARCA L: Combined cyclosporin A-steroid-MTX treatment in endogenous non-infections uveitis. *J Autoimmunity* 1993; 6: 467-80.
13. LUQMANI R, GARSON C, BACON P: Clinical pharmacology and modification of autoimmunity and inflammation in rheumatoid disease. *Drugs* 1994; 47: 259-85.
14. RUSSELL RGG, GRAVELEY R, SKJODT H: The effect of Cyclosporin A on bone and cartilage. *Br J Rheumatol* 1993; 32 (Suppl. 1): 42-6.
15. RAU R, SCHLEUSSER B*et al*.: Longterm treatment of destructive rheumatoid arthritis with methotrexate. *J Rheumatol* 1997; 24: 1981-9.
16. PINCUS T, CALLAHAN LF: Remodeling the pyramid or remodeling the paradigms concerning rheumatoid arthritis - Lesson from Hodgkin's disease and coronary artery disease. *J Rheumatol* 1990; 17: 1582-5.
17. FELSON DT, ANDERSON JJ, MEENAN RF: The efficacy and toxicity of combination therapy in rheumatoid arthritis: A meta-analysis. *Arthritis Rheum* 1994; 37: 1487-91.
18. TUGWELL P, PINCUS T, YOCUM D: Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis. *N Engl J Med* 1995; 333: 137-41.
19. STEIN CM, PINCUS T, YOCUM D: Combination treatment of severe rheumatoid artritis with cyclosporine and methotrexate for forty-eight weeks. *Arthritis Rheum* 1997; 40: 1843-51.
20. PIA G, PASCALIS L, ARESU G, ROSETTI L, LEDDA MA: Evaluation of the efficacy and toxicity of the Cyclosporine A. *Sarcoidosis* 1996; 13: 146-52.
21. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
22. STEINBROCKER O, TRAEGER CH, BATTERMAN RC: Therapeutic criteria in rheumatoid arthritis. *JAMA* 1949; 140: 315-24.
23. MIESCHER PA, BERIS PH: Immunosuppressive therapy in the treatment of autoimmune diseases. *Springer Semin Immunopathol* 1984; 7: 69-72.
24. MIESCHER PA, HUANG YP, ZUBLER RH: New approaches to the treatment of rheumatoid arthritis. *Springer Semin Immunopathol* 1988; 11: 1-12.
25. RITCHIE DM, BAYLE J, MCINNES J, GRIEVESON P, BUCHANAN W: Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968; 37: 393-406.
26. LEE P, JASANI MK, DICK WC, BUCHANAN W: Evalution of a functional index in rheumatoid arthritis. *Scand J Rheumatol* 1973;2:71-7.
27. HUSKISSON EC: Measurement of pain. *J Rheumatol* 1982; 5: 768-69.
28. DONATSCH P, ABISCH E, HOMBERGER M, TROBER R, TRAPP M, VOGES RA: Radioimmunoassay to measure cyclosporin A in plasma and serum samples. *J Immunoassay* 1981; 2: 19-23.
29. LARSEN A: Radiological grading of rheumatoid arthritis: An interobserver study. *Scand J Rheumatol* 1973; 2: 136-8.
30. RUSSELL G, GRAVELY R, SEID J, AL-HUMIDAN AK, SKJODT H: Mechanism of action of cyclosporin and effects on connective tissues. *Semin Arthritis Rheum* 1992; 21 (Suppl. 3): 16-22.
31. FERRACCIOLI GF: Radiographic assessment of rheumatoid arthritis. Controversies an perspectives. *Clin Exp Rheumatol* 1990;8:529-30.
32. FUCHS HA, PINCUS T: Radiographic damage in rheumatoid arthritis: Description by nonlinear models. *J Rheumatol* 1992; 19: 1655-8.
33. KUPPER IH, VAN LEEUWEN MA, VAN RIEL PLCM, SLUITER WJ, HOUTMAN NM, CATS HA, VAN RIJSWIJK MH: Influence of a ceiling effect on the assessment of radiographic progression in rheumatoid arthritis during the first 6 years of disease. *J Rheumatol* 1999; 26: 268-76.
34. TUGWELL P, BOERS M: Long-acting drug combinations in rheumatoid arthritis: Updated overiew. *In* WOLFE F and PINCUS T (Eds.): *Rheumatoid Arthritis: Pathogenesis, Assessment, Outcome, and Treatment*. New York, Marcel Dekker 1994: 357-71.
35. BENSEN W, TUGWELL P, ROBERTS R: Combination therapy of Cyclosporine with methotrexate and gold in rheumatoid arthritis (2 pilot studies). *J Rheumatol* 1994; 21: 2034-8.
36. KREMER JM, PETRILLO GF, LAWRENCE DA: Methotrexate (MTX) induces significant changes in IL-1, IL-2, IL-6, and IL-8 but not lymphocyte markers in patients (pts) with rheumatoid arthritis (RA). *Arthritis Rheum* 1993; 36 (Suppl.): S77 (abstract).
37. RUSSELL RGG, GRAVELEY RM, COXON F: Cyclosporin A. Mode of action and effects on bone and joints tissue. *Scand J Rheumatol* 1993; 32 (Suppl. 1): 42-52.
38. LANDEWÉ RBM, MILTENBURG AMM, BREEDVELD FC, DAHA MR, DIIJKMANN BAC: Cyclosporine and chloroquine synergistically inhibit the interferon-gamma production by CD4 positive and CD8 positive syno-

vial T cell clones derived from a patient with rheumatoid arthritis. *J Rheumatol* 1992; 19: 1353-7.

39. TUGWELL P: Cyclosporin in the treatment of rheumatoid arthritis. *J Autoimmunity* 1992; 5 (Suppl. A): 231-40.
40. LANDEWÉ RBM, GOEI THE HS, VAN RIJTHOVEN AWAM, BREEDVELD FC, DIJKMANS BAC: A randomized, double bind, 24-week controlled study of low dose cyclosporine versus chloroquine for early rheumatoid arthritis. *Arthritis Rheum* 1994; 37: 637-43.
41. WEINBLATT ME, KAPLAN H, GERMAIN BF: Methotrexate in rheumatoid arthritis: A five-year prospective multicenter trial. *Arthritis Rheum* 1994; 37: 1994-2002.
42. KAMEL OW, VAN DE RIYN M, LEBRUN DP, WEISS LM, WARNAKE RA, DORFMAN RF: Lymphoid neoplasms in patients with rheumatoid arthritis and dermatomyositis: Frequency of Epstein-Barr virus and other features associated with immunosuppression. *Hum Pathol* 1994; 25: 638-43.
43. FERRACCIOLI GF, CASATTA L, BARTOLI E: Epstein-Barr virus-associated Hodgkin's lymphoma in a rheumatoid arthritis patient treated with methotrexate and cyclosporin A. *Arthritis Rheum* 1995; 38: 867-8.
44. KWONG YL, AU WY, LIANG RH: Acute myeloid leukemia after azathioprine treatment for autoimmune diseases: Associaton with -7/7q. *Cancer Genet Cytogenet* 1998; 104: 94 -7.
45. ARNOLD M, SCHRIEBER L, BROOKS P: Immunosuppressive drugs and corticosteroids in the treatment of rheumatoid arthritis. *Drugs* 1988; 36: 340-63.
46. BAECKLUND E, EKBOM A, SPAREN P, FELTELIUS N, KLORESCKOG L: Disease activity and risk of lymphoma in patients with rheumatoid arthritis: Nested case-control study. *BMJ* 1998; 317: 180-1.
47. ARELLANO F, KRUPP P: Malignancies in rheumatoid arthritis patients treated with cyclosporin A. *Br J Rheumatol* 1993; 32 (Suppl. 1): 72-5
48. ZWANENBURG TSB, SUTER W, MATTER BE: Absence of genotoxic potential for Sandimmun in experimental systems. *Transpl Proc* 1988; 20 (Suppl. 3): 931-3.
49. MIESCHER PA, FAVRE A, LEMOINE R: The place of cyclosporin in the treatment of rheumatoid arthritis. *In* BONOMO L (Ed.): *Cyclosporin in Autoimmune Diseases*. Rome, Il Pensiero Scientifico Editore 1990: 119-32.
50. DIJKMANS BA: Safety aspects of cylosporin in rheumatoid arthritis. *Drugs* 1995; 50 (Suppl. 1): 41-7.
51. ABRAMCZUK A, KOHLER CO, ENGELMANN A, BACK D, OPELZ G: Influence of blood transfusions and imunosuppressive therapy on malignancies in renal transplant recipients. *In* SCHMAHL D and PENN I (Eds.): *Cancer in Organ Transplant Recipients*, Proceedings, Heidelberg, 28-29 May 1990. Berlin, Springer 1991; 132-8.