Twelve-month azathioprine as maintenance therapy in early diffuse systemic sclerosis patients treated for 1-year with low dose cyclophosphamide pulse therapy

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Received on September 21, 2006; accepted in revised form on January 18, 2007.

ABSTRACT

Objective. *To investigate the role of azathioprine in maintaining improvement after 1-year low-dose IV pulse CYC therapy in patients with early diffuse Systemic Sclerosis (dcSSc).*

Methods. *Thirteen patients with early dcSSc who had completed a year of treatment with low-dose IV pulse CYC underwent AZA treatment (100 mg/day) in a prospective 1-year study. Modified Rodnan skin score (mRss), Health Assessment Questionnaire-Disability Index (HAQ-DI), forced vital capacity (FVC), and diffusing lung capacity for CO (DLCO) were assessed as outcome measures. In addition, the nine organ/system Medsger et al. severity scores and the European Scleroderma Study Group (ESSG) activity index were evaluated.*

Results. *The improvement from a year of CYC therapy was maintained by AZA treatment. No outcome measures deteriorated (mRss 8.23 ± 2.9 vs. 6.38 ± 3.4; HAQ-DI 0.38 ± 0.4 vs. 0.32 ± 0.3; FVC 89.5 ± 13.2 vs. 89.4 ± 15.9; DLCO 73.6 ± 14.4 vs. 75.0 ± 19.5), nor were there any increases in any organ/system severity scores or ESSG activity index detected.*

Conclusion. *This study suggests a role of AZA in maintaining the improvement induced by low dose pulse CYC in early dcSSc, making it possible a short duration of treatment at a low cumulative dose of the drug. These results, however, await confirmation in controlled studies.*

Introduction

There is presently no drug or combination of drugs that have been definitely shown to affect the overall disease course in patients affected by Systemic Sclerosis (SSc) (1). Nevertheless, cyclophosphamide (CYC) in different therapeutic regimens has been reported to be effective in SSc interstitial lung disease and to favourably affect skin and lung involvement in patients with both early diffuse (dcSSc) and long-standing disease (2-9). In this regard, we have recently pointed out a significant improvement in skin thickening, lung function parameters and Health Assessment Questionnaire - Disability Index (HAQ-DI) in 12 early dcSSc patients treated with low-dose pulse CYC for 1 year (10).

The occurrence of side effects such as cytopenia, ovarian failure, infection, hemorrhagic cystitis, and particularly cancer prevents long-term CYC treatment as well as high cumulative doses of the drug (11-13). This evidence has prompted some investigators to assess the efficacy of azathioprine (AZA) in maintaining the improvement/remission induced by CYC in patients with different systemic autoimmune rheumatic diseases such as Systemic Lupus Erythematosus (SLE) (14) and ANCA associated vasculitis (15).

Here, we report the effects of an open uncontrolled study on AZA in early diffuse SSc patients who had been treated for a year with low-dose pulse CYC therapy.

Patients and methods

Patients
From January 1st 2002, fifteen patients with early dcSSc according to LeRoy et al. (16) have been treated at our Unit with low-dose intravenous CYC according to a drug regimen first proposed by Martin-Suarez et al. (17) and partially modified by us for patients with SSc (500 mg at days 1-8-15, then every 28 days for 1 year; cumulative dose 7.5 gm). The results of a one year IV CYC treatment obtained in 12 of these patients have been previously reported (10). Subsequently, all fifteen patients were invited to undergo a one year prospective study to assess the efficacy of AZA in maintaining the improvement obtained by CYC therapy. Two patients, both belonging to the original study group (10), were not enrolled: the first one was lost to follow-up for incompliance; the other one refused to consent to undergo AZA therapy. Thus, thirteen out of fifteen patients underwent the present open trial.

Treatment
Azathioprine. AZA (50 mg/day per os) was administered for 20 days. The dosage was increased thereafter to 100 mg/day for one year.
Other therapies. All the patients were also treated with aspirin (100 mg/daily)

Table 1. Epidemiological and clinical features of the 13 SSc patients investigated.

Sex, F : M	12 : 1
Age, years (median, range)	38 (23 – 53)
Disease duration, *months (median, range)	16 (5 – 21)
ACR criteria fulfilled	100%
Subset (15)	Diffuse
Autoantibody profile:	
ANA positive	13 (100%)
Anti-Scl 70 positive	12 (92.3%)
Anti-nucleolar	1 (7.7%)
Organ involvement	
Peripheral vascular	13 (100%)
Skin	13 (100%)
Joint / tendon	8 (61.5%)
Muscle	3 (23.1%)
GI tract	9 (69.2%)
Lung	9 (69.2%)
Heart	2 (15.3%)
Kidney	0 (0 %)

*At the start of CYC therapy, as evaluated from the appearance of the 1st symptom (either Raynaud's phenomenon or non Raynaud's symptom).

as antiplatelet agent, omeprazole (20–40 mg/day), domperidone (20 mg/day), nifedipine (30-60 mg/day) and low-dose steroids (10 mg/day, at 8.00 am) (as antinflammatory agent).

Assessment
Efficacy. Modified Rodnan skin score (mRss) (18); Health Assessment Questionnaire - Disability Index (HAQ-DI) (19); forced vital capacity (FVC) as analyzed by the pneumothoracograph Unit of ALTAIR 4000-COSMED (20) and diffusing lung capacity for CO (DLCO) as assessed by the single breath method (21) were investigated as primary outcome measures according to White *et al.* (22) and compared to the respective values detected at the end of a year of CYC treatment.
In addition, the nine organ/system Medsger *et al.* severity scale (23) and the European Scleroderma Study Group (ESSG) activity index (24) were as-

sessed both at the beginning and at the end of AZA therapy.
Safety. Complete blood cell counts, urinalysis, blood urea nitrogen, creatinine and liver enzymes were evaluated at 2-4-6-8 weeks after the beginning of AZA treatment, then monthly.
Ethics. The study protocol was approved by the local ethical Committee, and patients signed informed consent.

Statistical analysis
Data are presented as mean ± standard deviation (SD), unless indicated otherwise. Changes in outcome measures were analyzed by non-parametric tests. A p value less than 0.05 was considered significant. The statistical analysis was performed using SPSS software .

Results
Table I lists the main epidemiological and clinical features of the thirteen patients investigated as detected at the start of AZA treatment.
Table II shows the behaviour of the main outcome measures investigated, as evaluated at the beginning of CYC treatment, at the end of CYC treatment-starting point of AZA therapy and after one year of AZA treatment. None of the patients had any deterioration in any outcome measures investigated during the AZA treatment time.
Table III shows the nine organ/system Medsger *et al.* severity scale as detected during the year of AZA therapy. None of the patients had any increase in organ/system severity scores.
The ESSG activity index, which had resulted to be 5.04 ± 1.57 at the beginning of CYC therapy and was 0.85 ± 0.55 at the end of 1-year of CYC therapy ($p < 0.001$ versus baseline values), was 0.54 ± 0.43 at the end of 1-year of AZA treatment ($p > 0.05$ versus the 12-month values). The improvement in joint/tendon reflected the change in finger to palm distance, the decrease of which had to be ascribed to edema/arthritis and not to articular contractures. The comparison between the values in mRss and HAQ-DI registered in patients with a baseline DLCO < 70% (n = 8) and those detected in patients with a DLCO > 70% were not statistically significant.

Table II. Outcome efficacy measures in 13 early dcSSc patients undergoing 2 years of prospective Cyclophosphamide (CYC) – Azathioprine (AZA) therapy.

	CYC therapy	AZA therapy	
	Baseline	12 months	24 months
mRss	23.38 ± 6.12	8.23 ± 2.9*	6.38 ± 3.4**
HAQ-DI	0.66 ± 0.72	0.38 ± 0.4*	0.32 ± 0.3
FVC	86.38 ± 18.28	89.5 ± 13.2*	89.4 ± 15.9
DLCO	65.15 ± 18.54	73.6 ± 14.4***	75.0 ± 19.5

Values are mean ± standard deviation.
Wilcoxon test: *p: 0.001 baseline *vs.* 12 months; **p: 0.01 12 months *vs.* 24 months; ***p: 0.008 baseline *vs.* 12 months.

Table III. Medsger *et al.* organ/system severity scale in 13 dcSSc patients at baseline, after 1-year CYC and 1-year AZA therapy, respectively.

Organ system involvement	CYC		AZA	
	Baseline	12 months	24 months	p^*
General	1 (0 - 2)	0 (0 -1)	0 (0)	NS
Peripheral vascular	1 (1 - 3)	1 (1 - 2)	1 (1 - 2)	NS
Skin	2 (2 - 3)	1 (1 - 1)	1 (1 - 1)	NS
Joint / tendon	2 (0 - 4)	1 (0 - 4)	0 (0 - 3)	NS
Muscle	0 (0 - 1)	(0)*	(0)	NS
GI tract	1 (0 - 1)	1 (0 - 1)	1 (0 - 1)	NS
Lung	2 (0 - 3)	1 (0 - 2)*	1 (0 - 2)	0.08**
Heart	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)	NS
Kidney	0 (0)	0 (0)	0 (0)	NS

Values are median (range). *Wilcoxon's test: baseline *vs.* 12 months; **corrected for multiple analysis.

Discussion

Treatment of patients with SSc, particularly those with dcSSc, is still a challenge (1). Over the last 10 years, increasing evidence has supported a role of CYC in the treatment of interstitial lung disease (3, 4, 6, 7, 9) and of early diffuse disease (8, 10). A decrease in mRss, which is currently considered to be a valid outcome measure in patients with early disease, was detected by Apras *et al.* (8) in early dcSSc patients treated with CYC (2.5 mg/Kg/day per os) for one year. We also detected this outcome measure by administering a IV pulse regimen, planned in an attempt to reduce the burden of side effects by avoiding leukopenia as the treatment goal in the induction phase (17). In addition, we pointed out a nearly significant decrease in HAQ-DI [another validated measure of disease outcome according to Merkel *et al.* (25)], as well as no increase in any of the 9 scores of the Medsger *et al.* organ/system severity scale (23) and no change in the ESSG activity index (24).

Long-term CYC-treatment is not often used because of significant side effects (particularly cancer) that can emerge after several years. In 1979, Plotz *et al.* (11) reported bladder complications occurring in nine out of 54 patients (7 acute hemorrhagic cystitis, 2 transitional cell carcinoma; 28 and 60 months from drug withdrawal) treated with oral CYC for either SLE or rheumatoid arthritis (RA). They concluded that the late-occurring serious toxicities of CYC should limit the use of the drug in the treatment of non-malignant inflammatory conditions. Talar-Williams *et al.* (12) investigated the incidence of bladder toxicity in patients with Wegener granulomatosis. They estimated the incidence of bladder cancer to be 2% at 5 years, 5% at 10 years, and 16% at 15 years. Recently, Knight *et al.* (13) pointed out a doubling of bladder cancer risk for every 10 gram increase in the total cumulative dose and an eightfold increased risk for treatment durations longer than one year. This evidence has prompted a number of investigators to try to introduce azathioprine as maintenance therapy in patients with different autoimmune systemic conditions brought into remission by CYC (14, 15).

The concept of disease remission has not been formally addressed in SSc. Nevertheless, medically meaningful differences have been defined by a consensus among experts (22). At the end of 1-year CYC treatment, all of our patients had experienced a clinically meaningful response (*i.e.* ≥ 30% of mRss decrease); five out of 7 (71.4%) patients with an abnormal total HAQ-DI (*i.e.* HAQ > 0.5) had experienced a decrease of 0.25 in this parameter, that is greater than the minimally significant difference in SSc as recently evaluated by Khanna (26); all experienced either a significant increase (≥ 15% of predicted) (1) or a stabilisation of FVC and DLCO. At the end of 1-year AZA treatment, no patient underwent either an increase in mRss or HAQ-DI or a decrease in FVC, DLCO or a deterioration in any of the 9 scores of the Medsger et al. organ/system severity scale (23) or an increase in ESSG activity index (24). The change in ESSG activity index as well as in the 9 Medsger's severity scores are not validated measures of response to treatment. Nevertheless, the absence of any change in any of these measures, at least reflect a stabilisation of the disease.

From an efficacy point of view, our study presents same limitations.

1. It is an uncontrolled trial, therefore, until the results will be confirmed by a prospective controlled trial, one could wonder if they depend on the natural course of the disease. The clinician (G.V.) performing the modified Rodnan skin score measurement was aware of the current treatment.

   Nevertheless, the detection of a significant improvement in HAQ-DI and DLCO and of any deterioration in any organ/system involvement during a 2-year follow up of patients with early (< 2 years) diffuse disease (which excludes a natural course event) strongly suggest the efficacy of the drug regimen since the natural course of early dcSSc is known to be characterized by the increase in skin thickening and the involvement of target internal organs (27, 28).

2. The administration of low-dose corticosteroids as well as antiplatelet agents and calcium channel blockers might have played a role in the improvement detected in our patients.

3. The low values of HAQ-DI at the start of AZA therapy would suggest that the SSc patients investigated are affected by mild disease. However, these same 13 patients when enrolled in the first CYC trial presented a HAQ-DI reflecting a moderate-severe disease (0.66 ± 0.72).

4. The effects registered at the end of the 2-year trials (1-year of CYC, 1-year of AZA) could be ascribed to a late effect of CYC, since the efficacy of AZA in decreasing skin sclerosis has been questioned.

   Thiopurine methyltransferase levels were not assessed to ensure dose equivalence (29).

   Therefore, the mechanism of CYC-AZA regimen in early SSc, as well as in other autoimmune systemic rheumatic diseases (14, 15), is still unknown and it is difficult to predict the single patient who will get a significant benefit by the treatment.

From a safety point of view, however, our study suggests the possibility to reduce the duration of exposure to CYC and the cumulative dose of this drug, in order to improve its safety profile. In that regard, Tashkin *et al.* (9) have recently pointed out that the SSc patients undergoing 2 mg/Kg/daily CYC treatment for 1 year did not develop any deterioration in lung volumes 1 year after the end of CYC therapy. A significant percentage of the patients investigated by these authors, however, were affected by limited SSc and had a disease duration as long as 7 years.

Moreover, in the last few years, mycophenolate mofetil (MMF) has been increasingly used as a disease modifying agent in many connective tissue diseases including SSc (30). Its efficacy still awaits to be confirmed in controlled studies. At present, it is difficult to predict which drug (CYC, AZA or MMF) will eventually be the best choice in SSc patients.

In conclusion, our study suggests that a 24-month CYC-AZA regimen might alter the pace of the disease in pa-

tients with early dcSSc. A multicenter randomised placebo-controlled study should be carried out to confirm or reject our results.

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