

PAPER

Therapy with intermittent pulse cyclophosphamide for pulmonary hypertension associated with systemic lupus erythematosus

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The aim of this study was to compare the efficacy of intravenous cyclophosphamide (IVCYC) versus oral enalapril in mild or moderate pulmonary hypertension (PH) in systemic lupus erythematosus (SLE). Thirty-four patients with SLE who had systolic pulmonary artery pressure (SPAP) > 30 mmHg by Doppler echocardiography were randomized to receive IVCYC (0.5g/mt² body surface area, monthly), or oral enalapril (10mg/day) for six months. The primary outcome was the significant decrease in SPAP. An additional outcome measure included the improvement in the heart functional class (NYHA). Sixteen patients received cyclophosphamide and 18 enalapril. IVCYC decreased the median values of SPAP from 41 to 28 mmHg ($P < 0.001$), and enalapril from 35 to 27 mmHg ($P = 0.02$). IVCYC reduced more than twice as much SPAP than enalapril ($P = 0.04$). In those patients with SPAP ≥ 35 mmHg, cyclophosphamide decreased from 43 to 27 mmHg ($P = 0.003$), but enalapril was not effective ($P = 0.14$). The NYHA functional class improved only in those with cyclophosphamide ($P = 0.021$). Also IVCYC had a higher frequency of side effects including infections (RR = 1.6; 95% CI, 1.001–2.47), and gastrointestinal side effects (RR = 14.6; 95% CI, 2.15–99.68). We concluded that IVCYC was effective in mild and moderate PH associated with SLE. Further research is needed to evaluate its long-term efficacy. *Lupus* (2004) **13**, 105–112.

Key words: cyclophosphamide; pulmonary hypertension; SLE

Introduction

Pulmonary arterial hypertension (PH) is a severe complication in patients with systemic lupus erythematosus (SLE). Case reports and case series have considered PH as a rare entity.^{1,2} A review found only 46 patients with PH documented in the period 1952–1986.³ Nevertheless, more recent series using Doppler echocardiography described a prevalence of PH in SLE arising from 11 to 43%.^{4–6} These findings suggest an underestimation of the prevalence of PH in retrospective studies; this underestimation is partly due to the absence of relevant symptoms during the earlier

stages of PH. This delay in the diagnosis partially explains the observance of the increases in the risk for development of cardiac failure and early death in patients with PH associated with SLE.^{7,8} The National Institutes of Health classify as presence of pulmonary hypertension the finding of a pulmonary artery pressure > 25 mmHg at rest, or > 30 mmHg with exercise.⁹ Using Doppler echocardiography to evaluate pulmonary artery pressure, can increase the chance of an opportune diagnosis of PH. Some studies in SLE patients have documented PH with Doppler echocardiography in the presence of an increase of the systolic pulmonary artery pressure (SPAP) higher than 30 mmHg.^{5,6} Currently, the determination of this SPAP by Doppler is considered a reliable method to detect PH.^{10,11}

Many approaches for PH in SLE have been suggested. These treatments include vasodilator

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drugs, steroids, anticoagulants, and immunosuppressive drugs.¹² The evidence of the effectiveness of these approaches is not conclusive. Some studies describe a possible benefit of angiotensin converting enzyme inhibitors in both primary or secondary PH.^{12–15} However, because of the severity of PH in connective tissue disease, their efficacy is not conclusive.

Intermittent IVCYC, has become the gold standard therapy for nephritis in SLE,^{16,17} and several studies suggest its effectiveness in other manifestations including central nervous system involvement.^{18,19} Case reports and case series suggest a beneficial effect of cyclophosphamide in PH.^{12,20–22} However, these studies do not provide strong evidence about the efficacy, safety and effect size of this therapy. The aim of this study was to compare in a controlled clinical trial the efficacy of IVCYC versus oral enalapril in mild or moderate PH in SLE.

Material and methods

We included in the screening for PH, consecutive outpatients with diagnosis of SLE²³ between 18 and 55 years of age and prednisone doses of ≤ 15 mg/day. All the potentially eligible patients were evaluated by Doppler echocardiography and invited to participate in the trial if they had PH defined as SPAP > 30 mmHg.^{5,6,11,24} Those female patients who were not menopausal were required to have a negative pregnancy test result, and to be practising a reliable contraceptive method. We excluded those patients with overlapping syndrome, pregnancy, moderate or severe valvular disease, systemic hypertension, history of pulmonary embolism or myocardial infarction, chronic renal failure, or those patients with asthma, chronic obstructive pulmonary disease or pulmonary fibrosis. Also excluded were those patients receiving, within three months before the study, entry immunosuppressive therapy with cyclophosphamide or other immunosuppressive drugs, intravenous bolus of methylprednisolone, cyclosporine, oral calcium channel blockers or angiotensin converting enzyme inhibitors, or if they had active infections.

Clinical settings

Two different outpatient rheumatology clinics were sources for the patients: one secondary care hospital (Hospital General Regional 45, IMSS) and one university hospital (Hospital General de Occidente, SS) in Guadalajara, Mexico.

Study design

Since there is no gold standard for the treatment of PH in SLE, we originally planned to design a placebo

controlled study. However, according to modifications suggested by the Research and Ethics Committee in our hospitals, we finally decided to use enalapril as comparator based on several studies that have shown benefits with angiotensin converting enzyme inhibitors in secondary PH.^{12–14}

We conducted a randomized, parallel groups controlled trial. Patients were randomly assigned with a blocking factor of 4 to minimize the possibility that the researcher guessed the next treatment assignment. Each center was given a block allocation number.

Interventions

All patients were randomly assigned to receive one of two regimens: a) IVCYC, 0.5 g/mt² of body surface area administered monthly for six consecutive months; or b) oral enalapril, 10 mg/day administered in single daily doses for six months.

Cointerventions

During the trial the following drugs were allowed: a) prednisone in stable doses ≤ 15 mg/day, b) antimalarials, c) anti-inflammatory drugs, and d) antibiotics in the case of infections. Ondansetron was used for treatment of nausea and vomiting secondary to cyclophosphamide therapy.

The use of other immunosuppressive drugs, prednisone doses > 15 mg/day, beta-blockers, or calcium channel blockers was not allowed during the trial.

Measurements

Clinical evaluations included a structured questionnaire, physical examination, the New York Heart Association cardiovascular functional class, and disease activity using the Mex-SLEDAI index.²⁵ All the evaluations were made by the same evaluator (LGL) at the baseline and every month during the trial.

Laboratory determinations included complete blood cells count, platelet count, chemistry profile, urinalysis, electrolytes, and liver function tests and they were obtained every month during trial. Creatinine clearance and proteinuria were assessed at baseline, 3 and 6 months. Blood sera were tested for anticardiolipin antibodies (isotypes IgG and IgM) by ELISA.²⁶

Assessment by echocardiography

In order to minimize the chance of measurement bias, all the studies of SPAP were made by the same experienced cardiologist–echocardiographer, who performed the echocardiography at baseline (previous to

treatment) and at the end of the trial (after six months) in all the patients included. This investigator was blinded for variables determinant of the response such as treatment group and clinical variables except age, gender, height and weight.

Echocardiographic examinations were performed on all the patients lying in the supine decubitus position. Color-Doppler images were used to evaluate the presence of tricuspid regurgitation. The tricuspid regurgitation jet was recorded by means of continuous wave Doppler. Signals in the Doppler image for tricuspid regurgitation were considered adequate if they were pansystolic with a well-defined shape. Maximum velocity of regurgitation was used to calculate the transtricuspid pressure gradient using the modified Bernoulli equation ($4V^2$). The right atrial pressure was estimated using the maximum inferior vena cava diameter following the parameters described by Simonson *et al.*⁵ The SPAP was calculated as the sum of the transtricuspid pressure gradient plus the value of the right atrial pressure. Measurements of SPAP were made at baseline and at six months.

Outcome measures

The primary efficacy measure was the statistically significant reduction of SPAP from baseline to six months. Subsequently we arbitrarily classified three types of possible response: a) satisfactory if the decrease in SPAP was $\geq 20\%$ or if this pressure reached values below 30 mmHg, b) null response if the decrease in SPAP was $< 20\%$ and this pressure did not reach normal values, and c) worsening if there was an increase in SPAP.

Side effects

Patients were assessed for side effects at every visit. Infections were recorded as side effects and classified as: a) severe, when the patients required hospitalization for treatment, and b) mild, if they did not require hospitalization. Patients were dropped out of the study in the case of severe infections, severe hypotension, significant thrombocytopenia (platelets $< 80000\text{mm}^3$), leukopenia (leukocytes $< 2000\text{mm}^3$), moderate anaemia (haemoglobin $< 10\text{g/L}$), renal or bladder toxicity, or in the case of developing a severe relapse requiring drugs not allowed by the study protocol.

Statistical analysis

We used as primary outcome measure the improvement in SPAP. Data of SPAP are presented as medians and ranges. Since the pulmonary pressure was not normally distributed due to the sample size, we chose the use of

nonparametric statistics to compare the values of SPAP between and within groups. Mann–Whitney *U*-test was used to compare SPAP at baseline between the two treatment groups and to assess the differences in pulmonary pressure reduction between groups. Wilcoxon rank-sum test was used to compare within-group SPAP. We performed a separate subgroup analysis with those patients who had a SPAP $\geq 35\text{mmHg}$ at the study entry. An intention-to-treat analysis was performed assuming that the one single patient with IVCYC who withdrew had no changes (no response) in the pulmonary pressure at the end of the trial with respect to baseline. We computed the number of patients who would need to treat to obtain a satisfactory response in diminishing at least 20% of the basal values of the SPAP. We estimated the relative risks (RR) and their 95% confidence intervals (95% CI) for side effects. Statistical significance was set up at ≤ 0.05 level. All analyses were conducted by using SPSS 8.0.

Ethics

The study protocol was reviewed and approved by the Institutional Research and Ethics Committee, and each patient provided written informed consent.

Results

Selection and baseline characteristics

In total, 204 consecutive outpatients with SLE were screened with Doppler echocardiography. Thirty-six patients, all women with PH, were potentially eligible for the study (Figure 1). Two of them declined to participate; the first because she wished to become pregnant and the second because of her unwillingness to take the risk of receiving cyclophosphamide. A total of 34 patients with PH were included in the trial and after the randomization 16 of them were assigned to receive IVCYC and 18 to receive enalapril. All the patients were followed for six months. Table 1 shows the baseline clinical features of the patients. At the entry of the trial SPAP was statistically higher in the group with IVCYC compared with the group with enalapril (median 41 mmHg versus 35 mmHg respectively; $P = 0.04$).

Efficacy

Table 2 describes the changes in pulmonary pressure within group from baseline to six months and compares the differences obtained between groups. At the end of the study, both groups showed a significant decrease in pulmonary pressure. However, this decrease was higher

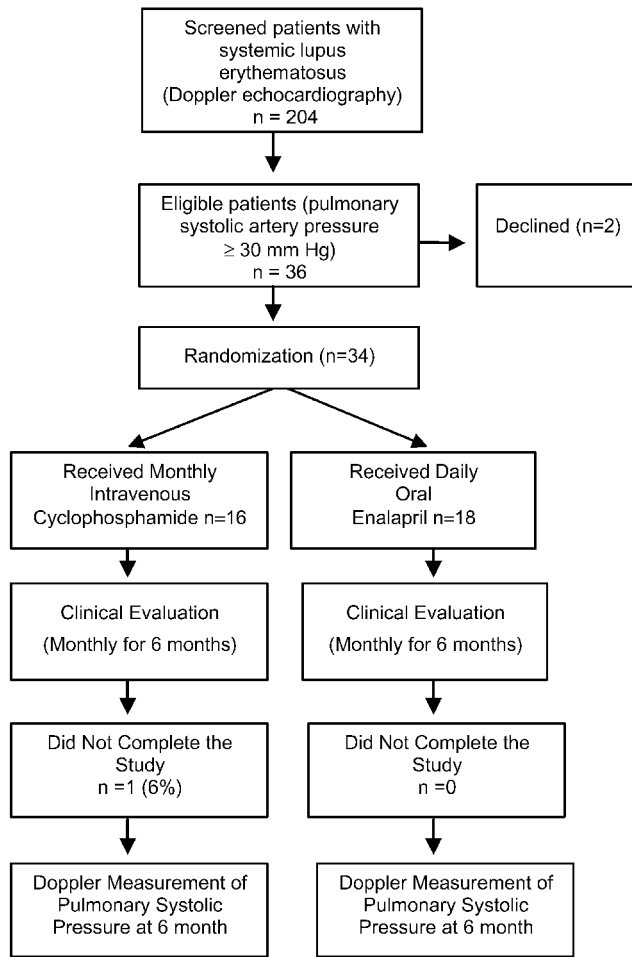


Figure 1 Study profile.

with IVCYC (from 41 to 28mmHg; $P < 0.001$) than with enalapril (from 35 to 27mmHg; $P = 0.02$). The differences in the decrease in SPAP were statistically higher with IVCYC than with enalapril (a decrease of 15mmHg compared with a decrease of 7mmHg respectively; $P = 0.04$). After evaluating the results of the subgroup with a cut-off of SPAP ≥ 35 mmHg at baseline, we observed a significant decrease only in the IVCYC group (change from 43 to 27mmHg; $P = 0.003$), but not in the enalapril group ($P = 0.14$).

NYHA functional class

Patients with IVCYC had a significant improvement in the NYHA functional class ($P = 0.02$), whereas no improvement was observed with enalapril ($P = 0.08$).

Withdrawals

During the trial, only one patient withdrew. This was a patient with IVCYC who developed pneumonia and

severe leukopenia that were attributable to toxicity of the immunosuppressive drug.

Intention-to-treat analysis

Table 3 describes the response in those patients who completed the trial and the results in the intention-to-treat analysis. A satisfactory response of decreasing pulmonary pressure was developed by 12/15 patients (80%) with IVCYC, one patient had a null response, and two had a worsening response. In the enalapril group, a satisfactory response was developed by 12/18 patients (67%), three patients had a null response, and three had a worsening response. Calculating that the number needing to be treated were patients with SPAP > 30 mmHg, only eight patients needed to be treated with IVCYC to obtain one additional benefit (satisfactory decrease of SPAP) with respect to enalapril. The benefit was higher in patients with SPAP ≥ 35 mmHg at baseline, where only two patients needed to treat to show clinical benefit. In the intention-to-treat analysis the number needed to treat increased to 15 patients when the treatment was used for any patient with SPAP > 30 mmHg, but remained at only two patients needing to be treated to show clinical benefit when SPAP at the inclusion was ≥ 35 mmHg.

Side effects

Both IVCYC and enalapril were adequately tolerated. Table 4 describes the side effects. Adverse clinical events occurred in 15 of 16 IVCYC users (94%) and 12 of 18 enalapril users (67%) (RR = 1.4; 95% CI = 0.99–1.99). Infections and gastrointestinal symptoms were the most common adverse drug reactions. Infections occurred in 87% IVCYC users and 55% enalapril users (RR = 1.6; 95% CI = 1.001–2.47). One patient receiving IVCYC developed severe infection (pneumonia) associated with leukopenia, 20 days after the first application of cyclophosphamide, requiring hospitalization and treatment with intravenous antibiotics. This was the only patient who dropped out of the trial. There were no observed deaths during the study.

Discussion

The present study showed that IVCYC is effective for decreasing the pulmonary pressure in PH associated with SLE. Along with the decrease in pulmonary pressure, an improvement was observed in the NYHA functional class. To the best of our knowledge, this is the first controlled trial using immunosuppressive drugs for this entity. These findings are consistent with

Table 1 Baseline clinical characteristics

Patients	Total (n = 34)	Cyclophosphamide (n = 16)	Enalapril (n = 18)	P-value ^b
Age				
Mean, years (SD)	38 (11)	36 (10)	41 (12)	
Median (range)	38 (18–55)	36 (19–54)	38 (18–55)	> 0.2
Duration of SLE				
Mean in years (SD)	7 (5)	9 (5)	5 (3)	
Median (range)	6 (1–18)	8 (1–18)	4 (1–10)	0.03
Mex-SLEDAI score				
Mean (SD)	2.1 (2.6)	3.1 (3.1)	1.1 (1.5)	
Median (range)	1.5 (1–10)	3 (0–10)	0 (0–4)	0.01
SPAP ^a				
Mean in mmHg (SD)	39 (6)	41 (7)	36 (4)	
Median (range)	36 (32–54)	41 (32–54)	35 (32–46)	0.04
Positive anti-dsDNA, n (%)	(33)	(33)	(33)	> 0.2
Positive anticardiolipin antibodies, n (%)	(56)	(51)	(62)	> 0.2

SD = standard deviations; SPAP = systolic pulmonary artery pressure.

^aSPAP was highest at the baseline for the group receiving cyclophosphamide ($P = 0.04$).

^bP-value: all the comparisons were made with Mann–Whitney *U*-test.

several case reports and case series that observed benefits of cyclophosphamide in PH associated with connective tissue diseases.^{12,20–22} In a recent report, Tanaka *et al.*²² described a dramatic response in three of their 12 cases with SLE and associated PH who were treated with cyclophosphamide and oral corticosteroids.

Instead of the effectiveness of cyclophosphamide, our results showed only a mild benefit of enalapril, but this effect was not significant in patients with SPAP ≥ 35 mmHg.

Most of the patients treated with IVCYC had non-severe adverse drug reactions. Overall, the frequency of mild infections in both groups was higher than that reported with cyclophosphamide for renal disease.^{16,27} The differences among the study populations need to be taken into account to explain these findings.

To explain the effects of cyclophosphamide in PH, it is necessary to examine some hypotheses about the role of immune mechanisms in the genesis of PH. Quismorio *et al.*²⁸ and others²⁹ have found evidence of deposits of antinuclear antibodies, anti-dsDNA,

rheumatoid factor, immunoglobulins, and complement fractions on the pulmonary vessels in PH. Fayemi³⁰ observed vasculitis of the pulmonary vasculature in 8 of 20 autopsies with SLE, whereas Yoshio *et al.*³¹ described elevation of anti-endothelial cell antibodies in PH. All these findings suggest a participation of the immune system for the development of PH in SLE. Cyclophosphamide exerts actions to decrease the synthesis of immunoglobulins and immunocomplexes.³² These immunosuppressive properties may explain their effects on pulmonary pressure observed in our study. Instead, whether or not the effects of enalapril in PH are mediated exclusively by their vasodilator properties or through a direct effect on the immune system, remains to be determined. An increase of the immunoreactivity for angiotensin converting enzyme has been observed in pulmonary vessels in primary PH.³³ Increase in angiotensin II is associated with endothelial apoptosis, developing a stimuli for the proliferation of smooth muscle cells in pulmonary arteries.³⁴ Captopril and enalapril exert effects on the immune system decreasing the

Table 2 Changes in systolic pulmonary artery pressure: comparisons within and between groups

Treatment	SPAP at the baseline (mmHg)	SPAP at 6 months (mmHg)	P-value (within group)	Decrease in mmHg	P-value ^a (between groups)
All patients evaluated					
Cyclophosphamide (n = 15), median (range)	41 (32 to 54)	28 (18 to 40)	< 0.001	15 (– 8 to 28)	
Enalapril (n = 18), median (range)	35 (32 to 46)	27 (18 to 58)	0.02	7 (– 20 to 20)	0.04
Response in those with SPAP ≥ 35 mmHg					
Cyclophosphamide (n = 11), median (range)	43 (36 to 54)	27 (21 to 34)	0.003	15 (5 to 28)	
Enalapril (n = 9), median (range)	38 (36 to 46)	28 (18 to 58)	0.14	10 (– 20 to 20)	0.04

SPAP = systolic pulmonary artery pressure comparison for differences in SPAP within group using Wilcoxon test.

^aComparison of the decrease of SPAP in mmHg between groups with Mann–Whitney *U*-test.

Table 3 Intention-to-treat analysis for obtaining a satisfactory response in decrease of pulmonary pressure

Outcome	Cyclophosphamide satisfactory response (n/t)	Enalapril satisfactory response (n/t)	Number needing to be treated
Response in patients who finished the trial			
SPAP > 30mmHg at baseline	12/15	12/18	8
SPAP ≥ 35mmHg at baseline	11/11	3/9	2
Intention-to-treat ^a			
SPAP > 30mmHg at baseline	12/16	12/18	15
SPAP ≥ 35mmHg at baseline	11/12	3/9	2

SPAP = systolic pulmonary artery pressure; n/t, number with satisfactory response/total of patients evaluated.

^aFor the intention-to-treat analysis failure in response was assumed for one patient with cyclophosphamide who withdrew from the study due to side effects.

production of tumor necrosis factor-alpha, interleukin 1 and endothelin, the last implicated in the pathogenesis of PH.^{35,37}

Currently it is being discussed whether patients with mild PH need to be treated. Severe PH in SLE is associated with cardiopulmonary failure and death, decreasing the time of survival to two years after its diagnosis.^{7,8} However, we did not observe deaths in the present study, although we have described previously a cohort study where patients with SLE and SPAP > 36mmHg had increased risk for developing cardiac failure.³⁸ Therefore, we consider that to treat patients with mild PH could contribute to decreasing progression to cardiac failure. Magliano *et al.*³⁹ have reviewed the current therapeutic options for PH in autoimmune rheumatic diseases. These therapies include vasodilators, anticoagulants, epoprostenol and prostacyclin analogues, endothelin receptor antagonists, inhaled nitric oxide and finally experimental drugs and lung transplantation. We propose that IVCYC should be included in the list of these therapeutic options for PH in SLE.

Our study has several limitations. First, the randomization was unable to assure that important

determinants of the outcome were evenly distributed between the two treatment groups. This drawback is a result of the small sample size. However, the randomization is still useful to decrease the likelihood of selection bias. Thereafter, we controlled the effects of the imbalance in the levels of SPAP at baseline, through two different strategies: a) performing an analysis of the differences in decrease of pulmonary pressure within group, and thereafter comparing these differences between groups; and b) performing a subgroup analysis with those patients with pulmonary pressure ≥ 35mmHg. The superiority of IVCYC remained after adjusting for these baseline differences. The second limitation in our study was the lack of blindness regarding the treatment possibly influencing the reporting of secondary endpoints such as NYHA or side effects. Nevertheless, we blinded the treatment group for the echocardiographer who evaluated SPAP. Therefore, we consider that this strategy minimized the likelihood of differential evaluation bias for our main outcome measure. The third limitation was that although we did not observe deaths during the trial, due to the short term of follow-up, any assumption made about the effects of IVCYC in decreasing

Table 4 Incidence of adverse events^a

	Cyclophosphamide (n = 16) (%)	Enalapril (n = 18) (%)	RR (95% CI)
Withdrawals	1 (6)	0	—
Side effects (total)	15 (94)	12 (67)	1.4 (0.99–1.99)
Specific side effects			
Infections total by group	14 (87)	10 (55)	1.6 (1.001–2.47)
Mild infections	13 (81)	10 (55)	1.2 (0.62–2.19)
Severe infections ^a	1 (6)	0	—
Nausea or vomiting	13 (81)	1 (6)	14.6 (2.15–99.68)
Arterial hypotension	0	8 (44)	—
Leukopenia ^a	1 (6)	0	—

^aNo thrombocytopenia or haematuria were observed during the trial.

^bSevere infection and leukopenia were present in one patient who withdrew from the study.

RR = relative risk; 95% CI = 95% confidence interval.

mortality secondary to cardiac failure needs to be made cautiously and further studies are required to evaluate the permanence of the efficacy of IVCYC after having stopped the therapy.

In summary, our findings suggest the efficacy of IVCYC for mild or moderate PH associated with SLE. Nevertheless, further studies are needed in order to demonstrate the consistency of our findings as well as to determine the long-term benefit of this therapy. One additional issue for further studies is the evaluation of the possible benefits offered by a combination of IVCYC and vasodilators for those cases without response or severe PH.

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