PREDNISONE AND AZATHIOPRINE COMPARED TO PREDNISONE PLUS LOW-DOSE AZATHIOPRINE AND CYCLOPHOSPHAMIDE IN THE TREATMENT OF DIFFUSE LUPUS NEPHRITIS

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A 1-year double-blind crossover study comparing prednisone and azathioprine to prednisone plus low-dose azathioprine and cyclophosphamide was carried out in 14 patients with diffuse lupus nephritis. Low-dose triple therapy had no apparent therapeutic advantage over prednisone plus azathioprine. Cyclophosphamide-induced ovarian failure and hematuria were not avoided by its use in low dose.

High-dose ($\geq$ 50 mg/day) prednisone therapy of active diffuse proliferative lupus nephritis has been reported to result in improved long-term survival when compared to low-dose prednisone therapy (1). Nevertheless the renal disease in many such patients appears to be refractory to much higher, extremely toxic doses of steroids.

In two recent studies treatment of systemic lupus erythematosus with long-term azathioprine in addition to steroids has been reported to result in decreased mortality and morbidity, as well as in the maintenance of better renal function, when compared to steroids alone (2,3). However azathioprine has not been shown to be effective in treating the acute exacerbation of lupus nephritis (4,5).

Short-term cyclophosphamide in doses averaging 2.2 mg/kg/day has been associated with improvement in urinary sediment and proteinuria, but it has not been shown to increase the effectiveness of steroids in improving creatinine clearance (6). This dose of cyclophosphamide has been associated with considerable toxicity, including alopecia, bone marrow depression, hemorrhagic cystitis, sterility, and the long-term risk of increased incidence of malignancies (7–10).

In the NZB/W mouse model of human SLE, a low-dose combination of the three drugs—methylprednisolone, azathioprine, and cyclophosphamide—has been associated with longer survival and improved renal function when compared to any single or double drug regimen at higher doses (11–12). Although the triple drug regimen was synergistic with regard to therapeutic effect, it was not associated with increased toxicity.

For these reasons a study was undertaken to assess the combination of azathioprine and cyclophosphamide, each in low dose, along with prednisone, as an alternative regimen for the treatment of lupus nephritis. This regimen was compared to the prednisone plus azathioprine regimen previously used at this institution (3).

MATERIALS AND METHODS

Fourteen consecutive patients who fulfilled the following criteria were entered into the study.

1. The presence of at least four criteria for the diag-

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nosis of SLE as outlined by the American Rheumatism Association (13), *and*

2. Active renal disease as manifested by *either*

a. The *new* appearance of hypocomplementemia, azotemia (serum creatinine greater than 1.2 mg%), urinary protein excretion greater than 200 mg/24 hours, cellular casts or more than 10 red blood cells per high-power field in the urine sediment, or hypertension, *or*

b. Deterioration in renal status in a patient with previously known renal disease, including either the *new* development of any of the above manifestations, or a 50% increase in serum creatinine, or a 200% increase in urinary protein excretion, *and*

3. A renal biopsy demonstrating diffuse proliferative or membranous glomerulonephritis

Patients with a serum creatinine greater than 3.0 mg% were excluded from the study because their immediate prognosis was considered to be very poor. Patients with previous exposure to cytotoxic drugs were also excluded.

All patients gave their informed consent in accord with a protocol and consent form approved by the Health Sciences Review Committee.

The study was double-blind with crossover to the opposite drug regimen under certain conditions. Initially all patients received prednisone as treatment for their active nephritis at a minimum dose of 1 mg/kg/day for 3 weeks. They were then randomly assigned to one of two groups, one receiving azathioprine, 2.5 mg/kg/day, and the other receiving azathioprine and cyclophosphamide, each in a dose of 1.25 mg/kg/day. Patients were reevaluated at 4-month intervals by predetermined criteria for therapeutic success or failure. Patients successful on the starting regimen were continued on that regimen, whereas patients termed therapeutic failures were crossed to the alternate drug regimen. Similarly, the fulfilling of predetermined criteria for drug toxicity at any time resulted in discontinuation of that regimen and crossover to the alternate regimen.

Patients were seen at 1- to 4-week intervals, and the steroid dose was tapered by a maximum of 5-mg decrements at each clinic visit, in accordance with parameters of clinical disease activity, including urine sediment, protein excretion, serum complement (C3), and serum creatinine, as well as extrarenal manifestations of SLE. A patient was considered to be a therapeutic success at the end of each 4-month interval if, on 20 mg/day of prednisone or less, the serum creatinine, complement, and urine protein were normal or stable and red cells were absent from the urine. Failure to suppress the active renal disease or to reduce the prednisone dose to 20 mg/day resulted in crossover to the alternate regimen. Toxicity of the immunosuppressive regimen sufficient to warrant its discontinuation was defined as the development of neutropenia of less than 1200/mm^3 or the appearance of gross hematuria. After recovery from drug toxicity, patients were crossed to the alternate drug regimen.

RESULTS

Fourteen patients participated in the 1-year study, 6 patients initially receiving azathioprine and 8 the combination of low-dose azathioprine and cyclophosphamide. On entrance into the study, the patients in the two groups were not significantly different with respect to age, duration of SLE, serum creatinine, or degree of proteinuria (see appendix). One patient in each group had pure membranous nephritis on renal biopsy; all others had a diffuse proliferative lesion.

Thirty-seven 4-month treatment periods were completed during the study. There were eighteen periods on azathioprine, of which twelve were successful, including three of six initial periods. Twelve of nineteen were successful on the combined regimen, including five of eight initial periods. Eleven patients completed a year of the study, 5 finally receiving azathioprine and 6 the combination of azathioprine and cyclophosphamide (Table 1). Of the 6 patients beginning the study on azathioprine, 2 were therapeutic successes throughout the study. Two patients were crossed to the combined regimen for therapeutic failure, and 1 of these was a success on that regimen. Azathioprine was discontinued in 2 patients for toxicity—neutropenia in one and aplastic anemia in the other (Table 2).

Eight patients initially received low-dose azathioprine plus cyclophosphamide (Table 2). Two were therapeutic successes throughout the study. Three patients were crossed to full-dose azathioprine because of therapeutic failure; 2 of these 3 did well on that regimen. The combined regimen was withdrawn for toxicity in 2 patients starting on that regimen. One, with neutropenia, was then a success on azathioprine. The other developed neutropenia and hemorrhagic cystitis just before the conclusion of the study. The final patient withdrew from the study after 4 months with her renal disease in remission; subsequently it exacerbated and could not be suppressed. She is receiving maintenance hemodialysis.

There was no difference between the two drug regimens as measured by the mean change in the param-

Table 1. *Comparison of Drug Regimens by Treatment Periods*

	Azathioprine	Cyclophosphamide Plus Azathioprine
Total treatment periods	18	19
Number successful	12 (67%)	12 (63%)
Initial treatment periods	6	8
Number successful	3 (50%)	5 (62%)
Final drug regimen	5	6

Table 2. *Course of Patients Based on Initial Drug Regimen*

	Azathioprine	Cyclophosphamide Plus Azathioprine
Initial drug regimen	6	8
Therapeutic success for entire year	2	2
Crossed to alternate regimen for therapeutic failure	2	3
Successful on alternate regimen	1	2
Failure on alternate regimen	1	1
Initial regimen withdrawn for toxicity	2	2
Voluntary withdrawal from study	0	1
Deaths	1	1

eters of renal disease activity from the beginning to the end of each treatment period (Table 3). The degree of proteinuria and the serum C3 level improved modestly with both regimens, but neither regimen produced significant improvement in serum creatinine. The mean steriod dose at the end of each interval was also similar for the two regimens (Table 3).

Toxicity was seen with both drug regimens. Neutropenia of less than 1200/mm^3 necessitated withdrawal of the combined regimen three times and of azathioprine twice, once in association with sudden, fatal aplastic anemia. Gross hematuria appeared once during the study in a patient receiving cyclophosphamide. Amenorrhea, with ovarian failure confirmed by laparoscopy, occurred in 3 patients following 4, 8, and 11 months of the cyclophosphamide plus azathioprine regimen. There were three episodes of severe infection, all in patients receiving 80–100 mg/day of prednisone for unsuppressed active lupus.

Two of these infections accounted for the only two deaths during the study. Cytomegalovirus pneumonia was seen in a patient receiving azathioprine, after crossover from the combined regimen for therapeutic failure. The other patient, also receiving azathioprine, developed staphylococcal pneumonia and subsequently sudden bone marrow aplasia, which resulted in her death. A third patient, receiving the combined cyclophosphamide and azathioprine regimen, developed extensive cellulitis and osteomyelitis of the leg, which was successfully treated.

Table 3. *Interval Changes in Laboratory Parameters and Mean Steroid Dose with Treatment*

	Azathioprine	Cyclophosphamide Plus Azathioprine
Number of treatment periods	18	19
Mean change in renal function*		
Creatinine (mg%)	−0.1 ± 0.2‡	0 ± 0.1
Urine protein (g/24 hours)	+0.8 ± 0.4	+0.7 ± 0.8
C3 (mg%)	5.6 ± 7.0	7.3 ± 5.7
Mean prednisone dose (mg/day)	22.5 ± 5.1	24.4 ± 7.3

* + = improvement; − = deterioration.
‡ SEM.

DISCUSSION

Within the limitations of the design of the study, there appeared to be no therapeutic advantage of one drug regimen over the other. The double-blind crossover trial was designed to compare the efficacy of the low-dose triple therapy regimen (prednisone plus azathioprine plus cyclophosphamide) with prednisone plus azathioprine treatment, without committing patients to long-term use of an apparently unsuccessful drug regimen. If one regimen were superior to the other by being more efficacious or less toxic, more patients should have been receiving that regimen by the end of the study. However the total number of treatment periods on each regimen was similar, as was the number of patients completing the study on each regimen.

It is possible that treatment periods of longer than 4 months might have revealed an advantage of the triple therapy regimen over prednisone plus azathioprine. Patients whose active renal disease had been suppressed by the end of the first 4-month interval, but whose prednisone dose had not yet been tapered to 20 mg/day, were considered to be therapeutic failures and were crossed to the alternate regimen. Continuation of the initial regimen during further tapering of the steroid dose might have resulted in continued disease suppression, thus showing an increased therapeutic advantage for that regimen. In fact suppression of renal disease occurred in only 2 such patients, 1 on each of the initial treatment regimens. In addition previous data suggest that the benefit of cyclophosphamide added to steroids that is seen after 10 weeks of therapy is no longer apparent after 2 years of its continued use, while the toxicity of the drug increases (6,14).

A persisting effect of either immunosuppressive drug after its discontinuation and crossover to the other regimen is unlikely to have blurred any distinction be-

tween the two regimens, because improvement rates and final outcome were similar for both starting regimens.

The likelihood of demonstrating a difference between the two treatment regimens is limited by the small number of patients entered into the study. Although not significantly statistically different with regard to any parameter, the patients randomly assigned to receive cyclophosphamide plus azathioprine initially tended to have their SLE and clinically apparent renal disease for a longer period of time, and the initial mean serum creatinine was somewhat higher than that in the azathioprine group. It is possible that these differences could have masked a therapeutic advantage of the combined regimen. However only 1 patient in each initial treatment group had renal disease for more than 1 year—3 years for a patient on the combined regimen and 8 years for a patient on azathioprine. Similarly, 2 patients in each initial treatment group entered the study with a serum creatinine greater than 1.2 mg%.

Drug toxicity during the study was attributable both to the high doses of corticosteroids necessary to suppress active disease and to the particular cytotoxic drugs employed. Severe infection, often fatal, was seen only in patients receiving large doses of prednisone. Isolated leukopenia occurred on both drug regimens. Sudden bone marrow aplasia, a previously observed complication of azathioprine therapy (3), occurred in 1 patient receiving the full-dose azathioprine regimen, but it was not observed in patients receiving cyclophosphamide. The particular problems of cyclophosphamide-induced ovarian failure and hemorrhagic cystitis were not avoided by the use of a low dose of this drug.

Appendix. *Clinical and Laboratory Parameters for Individual Patients by Treatment Periods*

Serum Creatinine (mg%)	C3 (mg%)	Urine Protein (mg/24 hr)	Urine Microscopic	Extrarenal SLE	Prednisone Dose (mg/day)	Drug Regimen	Comments
Patient 1. Age 29. Membranous nephritis. SLE × 7 Years. Renal disease × 1 year.							
0 1.0	100	1,500	5–7 wbc	Pericarditis	60	—	
1 0.9	103	621	20–25 rbc	—	12.5	C + A	
2 0.8	107	661	20–30 wbc, 0–1 rbc	—	7.5	C + A	
3 0.9	120	98	10–15 wbc	—	10	C + A	Amenorrhea at 9 months
Patient 2. Age 26. Diffuse nephritis. SLE × 3 years. Renal disease × 3 years.							
0 2.8	37	12,720	0–3 wbc, 5–6 rbc	Arthritis, rash, fever	120	—	
1 0.9	59	3,911	8–10 wbc, 2–3 rbc	Malar rash	17.5	C + A	Withdrew voluntarily
2 0.9	40	1,593	0–2 wbc, 2–3 rbc	Malar rash, fever, arthritis, leukopenia	40	—	
3 8.0	29	5,500	Many rbc, rbc casts	Pericarditis, fever	100	—	Anasarca, hemodialysis
Patient 3. Age 25. Diffuse nephritis. SLE onset. Renal disease onset.							
0 0.7	70	433	4–5 wbc, 8–10 rbc	Leukopenia, fever, LE peritonitis, cerebritis	80	—	
1 0.6	100	0	1–2 wbc, 5–6 rbc	—	5	C + A	
2 0.7	76	0	5–7 wbc	—	0	C + A	
3 0.8	120	0	5–6 wbc	—	0	C + A	Amenorrhea at 11 months
Patient 4. Age 20. Diffuse nephritis. SLE × 7 years. Renal disease onset.							
0 0.8	50	7,500	8–10 wbc, 12–15 rbc	Fever, myocarditis	80	—	
1 1.2	69	2,820	8–10 wbc, 1–2 rbc	Arthritis, fever, myocarditis	40	C + A	840 PMNs, cellulitis; *crossed for toxicity and therapeutic failure*

Appendix (Continued). *Clinical and Laboratory Parameters for Individual Patients by Treatment Periods*

	Serum Creatinine (mg%)	C3 (mg%)	Urine Protein (mg/24 hr)	Urine Microscopic	Extrarenal SLE	Prednisone Dose (mg/day)	Drug Regimen	Comments
2	0.7	99	0	5–6 wbc, 1–2 rbc	—	20	A	
3	0.8	100	0	negative	—	0	A	
Patient 5. Age 33. Diffuse nephritis. SLE × 3 years. Renal disease × 1 year.								
0	1.0	53	1,613	6–7 wbc, 10–20 rbc, 0–1 rbc cast	Arthritis	60	—	
1	1.3	61	1,500	3–4 wbc, 0–1 rbc	Arthritis	30	C + A	*Crossed for therapeutic failure*
2	3.0	44	3,536	3–4 wbc, many rbc	Arthritis, fever, cutaneous vasculitis	80	A	*Withdrawn for therapeutic failure*
3	>10	30	—	Many rbc	Diffuse systemic vasculitis	120	—	IV cyclophosphamide, hemodialysis, GI hemorrhage, died of cytomegalovirus pneumonia
Patient 6. Age 20. Diffuse nephritis. SLE × 1 year. Renal disease onset.								
0	2.0	31	1,271	8–10 wbc, 15–16 rbc	Pleuritis, pericarditis	60	—	
1	1.2	93	600	0–1 wbc, 0–1 rbc	—	15	A	
2	3.0	60	500	16–18 wbc, 0–3 rbc, 2–3 wbc casts	Pericarditis	60	A	*Crossed for therapeutic failure*
3	4.5	40	7,500	Many rbc, 0–2 wbc cast	Pericarditis	80	C + A	Hemodialysis started 2 months later
Patient 7. Age 35. Membranous nephritis. SLE × 4 years. Renal disease onset.								
0	0.9	52	0	1–2 wbc, 10–12 rbc	Peripheral neuropathy	50	—	
1	0.8	94	0	18–20 wbc, 1–2 rbc	—	15	A	
2	0.8	48	0	16–18 wbc	—	5	A	
3	0.8	47	0	Negative	—	5	A	
Patient 8. Age 33. Diffuse nephritis. SLE onset. Renal disease onset.								
0	1.4	44	1,500	8–10 wbc, 2–3 rbc	Arthritis, fever, myocarditis, pericarditis	60	—	
1	2.8	60	800	3–5 wbc, 2–3 rbc	Fever, myocarditis, pericarditis, seizures	105	A	Staph pneumonia, sudden aplastic anemia, *withdrawn for toxicity;* died with DIC
Patient 9. Age 16. Diffuse nephritis. SLE × 6 years. Renal disease × 1 year.								
0	1.2	70	3,900	12–15 wbc, many rbc	—	25	—	
1	0.8	120	824	8–10 wbc, 0–1 rbc	—	20	A	1,200 PMNs; *withdrawn for toxicity*
2	1.2	95	0	12–15 wbc	Cerebritis, myositis, fever	100	—	IV meds only
3	1.0	81	500	0–2 wbc	Myositis	20	C + A	600 PMNs; *withdrawn for toxicity*

Appendix (Continued). *Clinical and Laboratory Parameters for Individual Patients by Treatment Periods*

Serum Creatinine (mg%)	C3 (mg%)	Urine Protein (mg/24 hr)	Urine Microscopic	Extrarenal SLE	Prednisone Dose (mg/day)	Drug Regimen	Comments
Patient 10. Age 15. Diffuse nephritis. SLE onset. Renal disease onset.							
0 1.0	61	1,795	3-4 wbc, 10-20 rbc	Arthritis, fever, alopecia, pleuritis	50	—	
1 0.8	98	0	Negative	—	20	C + A	
2 0.9	67	0	Negative	—	10	C + A	
3 0.9	82	0	Many rbc	—	0	C + A	Gross hematuria; *withdrawn for toxicity*
Patient 11. Age 28. Diffuse nephritis. SLE × 8 years. Renal disease × 8 years.							
0 0.9	50	9,000	8-10 wbc, 4-5 rbc	Alopecia, cutaneous vasculitis	60	—	
1 1.0	53	2,987	1-2 wbc	Alopecia	15	A	
2 0.9	57	500	3-4 wbc	—	5	A	
3 0.9	48	1,476	1-2 wbc	—	10	A	
Patient 12. Age 33. Diffuse nephritis. SLE onset. Renal disease onset.							
0 0.8	60	1,000	15-18 wbc, 3-4 rbc	Fever, rash, arthritis, pleuritis, oral ulcers	75	—	
1 1.0	64	0	3-4 wbc	Alopecia, malar rash, fever, cutaneous vasculitis	35	C + A	*Crossed for therapeutic failure*
2 0.9	69	0	negative	—	10	A	
3 0.7	66	0	negative	—	0	A	
Patient 13. Age 25. Diffuse nephritis. SLE onset. Renal disease onset.							
0 1.8	53	4,400	3-4 wbc, 1-2 rbc cast	Thrombocytopenia, fever, seizures	60	—	
1 1.2	102	4,400	2-4 wbc, 4-6 rbc	Hypertension, fever, seizures	20	C + A	
2 1.9	46	2,000	8-10 wbc, many rbc, granular casts	Hypertension	60	C + A	*Crossed for therapeutic failure*
3 1.1	79	1,500	5-10 wbc, 2-3 rbc	—	20	A	
Patient 14. Age 37. Diffuse nephritis. SLE × 12 years. Renal disease onset.							
0 0.9	69	985	1-2 rbc, 10-15 granular casts	Hypertension	50	—	
1 0.7	60	910	Negative	—	35	A	*Crossed for therapeutic failure*
2 0.8	65	1,061	Negative	—	20	C + A	Amenorrhea at 4 months of cyclophosphamide
3 0.8	65	500	Negative	—	20	C + A	

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