Azathioprine in the Treatment of Systemic Lupus Erythematosus

A Controlled Study

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Sixteen patients with systemic lupus erythematosus have been observed for from 1 to 4 years while taking prednisone plus azathioprine in a dose of 2.5 mg/kg/day and compared to 19 patients with systemic lupus erythematosus managed with prednisone alone for a similar period. Patients receiving azathioprine showed a decreased mortality and morbidity, required less daily prednisone than the control group, exhibited no increased tendency to develop infection, had fewer exacerbations of their disease and maintained better renal function. Discontinuation of the azathioprine was associated with severe exacerbations of disease. The toxicity of azathioprine was minimal.

Purine analogs and the cytotoxic drugs originally devised for use in neoplastic disease were soon found to have immunosuppressive activity. From this observation, clinical trials in diseases in which immunologic abnormalities are prominent inevitably followed. The use of both types of agents became widespread after case reports of their benefit in such diseases were published. The present report describes the use of one such drug, azathioprine (Imuran)*, in one disease, systemic lupus erythematosus, in a controlled study. The study confirms the previous impressions of the usefulness of the drug in this disease, and raises questions as to the precise manner in which the drug is best employed.

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MATERIALS AND METHODS

During the 3-year period, June 1966 to June 1969, patients with systemic lupus erythematosus were entered into a controlled, but nonblind study for the evaluation of azathioprine in the management of this illness. The study was performed with FDA approval and informed consent was obtained in all cases. All patients who met the following criteria were included: 1) either a positive LE cell preparation or a positive test for antinuclear antibodies (immunofluorescent technic with guinea pig liver as substrate and serum used in a 1:10 dilution); 2) objective evidence of at least two of the following manifestations of systemic lupus erythematosus: a) arthritis, b) rash, c) serositis, d) myocarditis, e) thrombocytopenic purpura or a white blood cell count less than 3500/cu mm or hemolytic anemia, f) renal disease as defined by an endogenous creatinine clearance of less than 75 ml/min or proteinuria greater than 0.5 g/day; and 3) no history of having received immunosuppressive or cytotoxic drugs in the past.

All of the patients had positive tests for antinuclear factors. Once the diagnosis of systemic lupus

erythematosus was established, a previously prepared list was used to assign patients randomly to either the control group or the "treatment" group. Patients assigned to the control group were treated by the house staff, as considered appropriate, in consultation with the authors. Patients in the treatment group were treated in the same way with the exception that they also received azathioprine in a dose of 2.5 mg/kg/day. After discharge from the hospital, all patients were observed personally by the authors. At each clinic visit, an attempt was made to taper or discontinue the dose of prednisone consistent with a feeling of well-being on the part of the patient. Symptoms controlled with salicylate (eg, joint pain or fever) or laboratory manifestations of disease activity (eg, elevated ESR, proteinuria, leukopenia) were not considered to be contraindications to continued reduction of prednisone in the absence of symptoms of disease activity.

Twenty-one patients were entered into each group. Seven patients, 2 in the control group and 5 in the treatment group, did not survive the initial hospitalization and will not be considered further except to note that: a) these patients all died of either severe renal or cerebral disease not reversible by up to 1000 mg of prednisone per day or of infection coincident with the administration of these high doses of prednisone, and b) azathioprine did not demonstrate any usefulness in the management of these acute, severe exacerbations of systemic lupus erythematosus.

There were thus 16 "treatment" and 19 control patients who were observed for periods between 1 and 4 years. The pertinent data on these patients are included in Table 1. In all parameters, the two groups are comparable: the duration of disease prior to entry into the study, the frequency of functional renal impairment, and (not shown) the type of disease in terms of organ system involvement, degree of anemia, serum gamma globulin levels, etc.

RESULTS

Deaths

Six deaths occurred among the 19 control patients. Patients C-1, C-7 and C-10 died of renal insufficiency; patient C-2 died suddenly at home after either a massive hematemesis or hemoptysis (no autopsy obtained); patient C-8 died of meningococcal meningitis; patient C-11 died of gram-negative sepsis. There were no deaths among the 16 patients receiving azathioprine.

Morbidity

One parameter of intercurrent morbidity during the follow-up period is the frequency of required re-hospitalizations. After the initial hospitalization, 9 of the 19 control patients were hospitalized versus 3 of the 16 patients receiving azathioprine (P 0.04 by Fisher's exact probability method). Total required hospitalizations for active systemic lupus erythematosus were 30 in the control group versus 6 in the "treatment" group ($P < .01$ by chi-square, two-tailed).

The circumstances associated with renewed activity of the disease in the azathioprine-treated group are of particular interest. Patient T-1 was hospitalized on 3 occasions with leg pain, pleuritic chest pain, cough with sputum (twice with hemoptysis) and fever. Three lung scans with aggregated ^{131}I albumin and one pulmonary angiogram were normal. All symptoms resolved in a few days with or without raising the maintenance dose of prednisone. While these three hospitalizations were included as representing episodes of lupus activity, they may not have been since her usual manifestations of activity (arthritis, rash and serositis) were not present. Patient T-6, who had a history of rather mild Raynaud's phenomenon, became pregnant and the azathioprine was discontinued. She subsequently aborted, but because she wished to become pregnant again, azathioprine was not restarted. Four months later she developed pain and cyanosis in all fingertips except the thumbs, which went on to dry gangrene in three fingers, despite intravenous heparin, and bilateral cervical sympathectomy. The third patient who was receiving azathioprine and who required readmission for

Table 1. Pertinent Data on 19 Control Patients and 16 Patients Treated with Azathioprine

Control patients												Treatment patients										
Patient, age, sex	Disease duration (yr)	Follow-up	BUN 1	BUN 2	24-hr urine protein 1	24-hr urine protein 2	C_{Cr} 1	C_{Cr} 2	Prednisone	Hosp SLE	Patient, age, sex	Disease duration (yr)	Follow-up	BUN 1	BUN 2	24-hr urine protein 1	24-hr urine protein 2	C_{Cr} 1	C_{Cr} 2	Prednisone	Hosp SLE
C- 1: 29F	1	1½*	25	27	0.62	2.30	21	30	20	1	T- 1: 27F	11	4	19	13	5.10	0.12	88	122	10	3
C- 2: 19F	7	2*	8	13	0.03	0.36	37	70	10	0	T- 2: 31F	2	4	15	11	0.30	0.22	42	100	0	0
C- 3: 21F	5	4	10	5	0.09	0.49	93	?	20	4	T- 3: 48F	3	3½	14	19	0.50	0.20	70	110	0	0
C- 4: 29F	4	4	8	10	0.04	0.41	44	116	30	3	T- 4: 19F	6	3½	20	24	3.00	0.00	100	108	0	0
C- 5: 17F	½	4	9	11	0.07	0.11	56	100	40	2	T- 5: 18F	½	3½	22	18	0.07	0.24	78	86	15	0
C- 6: 30F	15	4	18	14	0.21	0.20	63	110	7½	1	T- 6: 22F	11	3	20	14	1.90	1.40	121	88	20	1
C- 7: 45F	1	2*	18	100	0.13	4.50	109	7	20	5	T- 7: 42F	16	3	8	8	0.07	0.90	74	100	0	0
C- 8: 28F	4	½*	16	?	0.00	?	55	?	10	0	T- 8: 18F	½	2½	20	14	2.20	0.25	42	60	7½	0
C- 9: 41F	½	3½	20	16	0.22	0.11	52	109	10	0	T- 9: 27F	½	2½	10	12	1.90	0.26	97	109	0	0
C-10: 15F	3	1½*	12	300	2.30	0.50	100	5	15	2	T-10: 43F	10	2½	12	17	0.10	0.28	30	83	7½	0
C-11: 37F	16	2*	15	11	0.06	0.10	70	50	15	0	T-11: 41F	1	2	10	13	1.50	0.25	55	123	0	0
C-12: 22F	2	3	10	11	1.80	0.65	120	100	2½	0	T-12: 31F	1	2	15	37	0.33	1.66	106	70	10	0
C-13: 39F	10	2½	13	11	0.19	0.41	93	103	10	0	T-13: 32F	½	1½	14	17	0.15	0.19	118	110	0	1
C-14: 20F	4	2½	35	14	4.00	0.48	80	61	0	0	T-14: 28F	1	1½	15	11	0.12	0.00	65	100	12½	0
C-15: 44M	½	2½	16	27	0.02	0.10	110	67	30	1	T-15: 17F	½	1	13	13	0.10	0.27	75	115	5	0
C-16: 20F	15	1½	12	10	0.24	0.12	93	100	0	2	T-16: 31F	1	1	13	17	?	0.35	?	99	5	0
C-17: 24F	½	1½	14	17	0.13	0.26	98	70	7½	0											
C-18: 32F	4	1	16	16	0.02	0.50	57	72	2½	0											
C-19: 28F	2	1	8	16	2.70	2.00	120	105	30	0											
Mean 28	5	2½	15		0.68	0.76	77		14.7	1.1	30	4	2½	15	16	1.15	0.41	77	99	5.8	0.3

Key: Age = age in years at time of entry into the study. Disease duration = time in years between occurrence of first symptoms of systemic lupus erythematosus and time of entry into the study. Follow-up = number of years between entry into the study and either the time of the last evaluation of the patient or the death of the patient (asterisks. BUN, 24 hour urine protein, and C_{Cr} = blood urea nitrogen in mg %, 24-hour urinary protein excretion in grams, and endogenous creatinine clearance in ml/min. 1 and 2 = measurements made at time of entry into the study (1) and at the time of the last evaluation (2). Prednisone = mean daily dose in milligrams of prednisone during the six-month period prior to the final evaluation of the patient. Hosp SLE = the number of hospitalizations required by the patient during the follow-up period for exacerbations of systemic lupus erythematosus.

The mean figures are self-explanatory, but are omitted for the control patients for the final BUN and creatinine clearance because of the skew produced by the two uremic patients (see text for discussion and statistical analyses).

activity of her disease was doing well but stopped coming to the clinic and stopped taking the drug. Three months later she was again ill with her usual manifestations of lupus activity. Azathioprine was restarted in both patients.

Thus, of the patients receiving azathioprine and whose disease became active enough to require hospitalization, in one instance it is questionable whether or not the patient was truly suffering from active systemic lupus erythematosus, and in the other two cases the renewed activity occurred several months after azathioprine was discontinued.

Eight control patients and two "treatment" patients required hospitalization for treatment of infectious disease (P 0.01 by Fisher's exact probability test). Four cases of active tuberculosis were recognized in the control group; none in the treatment group. There were two deaths due to infection in the control group (gram-negative sepsis and meningococcal meningitis); there were none in the treatment group. There was thus no evidence that azathioprine increased the susceptibility of patients to infection.

Renal Function

Most manifestations of systemic lupus erythematosus can be controlled with adrenocorticosteroids. The response of the renal disease is less predictable. Using the criterion that more than 0.5 g of urinary protein per day or a creatinine clearance of less than 75 ml/min is abnormal, 13 of the 19 control patients (68%) and 11 of the 16 treatment patients (69%) had renal disease at the time they were admitted into the study.

At the time of the final evaluation, renal function in the two groups differed. Among the control patients, Patient C-1, who entered with decreased renal function, died in uremia 1½ years later. Patient C-7, who originally had no evidence of renal involvement, died in uremia 2 years later. Patient C-10 had a 3-year history of proteinuria when entered into the study and died in uremia a year later. Patients C-14 and C-15 have shown some decrease in creatinine clearance (Table 1).

Of the patients who have been treated with azathioprine, only patient T-12 has shown significant progression of renal disease which occurred while she was not taking the drug. She became pregnant, was operated on for a ruptured tubal pregnancy and did not restart azathioprine because she wished to become pregnant again. Seven months later, her blood urea nitrogen was 37 mg%, creatinine clearance was 70 ml/min and she was excreting 1.66 g of protein per day. The azathioprine was begun again.

For the entire azathioprine-treated group there was a tendency for renal function to improve: the mean creatinine clearance increased from 77 (30–121) to 99 ml/min (70–123) and the 24-hour urinary protein decreased from 1.15 (0.07–5.10) to 0.41 g/day (0.00–1.66). Such improvement did not occur in the control group.

Serum Factors

The test for antinuclear antibodies was positive in all patients in both groups at both the beginning and end of the study. The two groups did not differ with respect to the frequency with which rheumatoid factor or serum γ-globulin levels were present at either the beginning or the end of the study.

Pregnancy

No patient in the control group has become pregnant, whereas three patients taking azathioprine have become pregnant.

One aborted and one had a ruptured tubal pregnancy. The third, who unlike the first two did not discontinue azathioprine, went on to deliver a normal child and to maintain a state of well-being.

Prednisone Requirement

If azathioprine has any usefulness in suppressing manifestations of systemic lupus erythematosus, the effect should have been reflected in a lower steroid requirement by the patients receiving azathioprine Such was found to be true. Seven of the 16 patients receiving this drug remain well and take no steroid drugs. Only 2 of the 19 control patients do not require prednisone (P 0.03 by Fisher's exact test). The mean maintenance dose of prednisone in each group for the 6-month period prior to the final evaluation was 5.8 mg/day (range: 0–20) in the treated group and 14.7 mg/day (range: 0–40) in the control group ($P < .01$ by Student's t-test, two-tailed).

Azathioprine Toxicity

Azathioprine was begun at a dose of approximately 2.5 mg/kg/day, so that most patients were taking 100, 125 or 150 mg/day in divided doses. The criterion for discontinuing or decreasing the dose was a granulocyte count of less than 1000 cells/cu mm. White counts were done weekly for the first 4 weeks, and every 4 to 6 weeks thereafter. In two patients, this level occurred within 2 weeks after the drug was started; the drug was discontinued and then restarted at 50 mg/day less. Both patients have continued to take the drug at this lower dose without difficulty. One patient, T-5, after taking azathioprine for 2½ years, developed thrombocytopenia and ecchymoses, leukopenia and a hematocrit of 16%. The drug was stopped and the abnormalities reverted to normal. She was restarted on a smaller dose. No other toxicity has been noted thus far in any patient. At the time of the final evaluation, all patients in the azathioprine-treated group were taking the drug.

DISCUSSION

The two theoretical rationales for the use of azathioprine in the management of patients with systemic lupus erythematosus relate to two of its known actions—immunosuppression and anti-inflammatory effects. Even accepting that the production of antibody to nuclear components or to cytoplasmic RNA is etiologically related to this disease, there is little evidence to suggest that azathioprine is effective in suppressing antibody production, other than when given simultaneously with a new antigen (1–3). Plasma cells and the long-lived lymphocytes are resistant to 6-mercaptopurine (and presumably to azathioprine) (4), and it is these cells that are probably responsible for maintaining established immunity. Azathioprine does not produce changes in parameters of immuno-competence regularly in patients, and any changes, when they do occur, do not correlate with clinical improvement or lack of it (3, 5–8). Nor did azathioprine have any effect on immune complex deposition in the kidney of NZB mice with lupus-like renal disease (9). The anti-inflammatory effect of 6-mercaptopurine has been well demonstrated in experimental animals (10). These data suggest that if azathioprine is useful in the management of patients with systemic lupus erythematosus, it is primarily as an anti-inflammatory drug or by some other unknown mechanism.

The present study confirms the impression of previous case reports in which azathioprine was used either alone or with small doses of prednisone (3, 5, 6, 8). Our patients receiving azathioprine have had a

more stable course than the control group, have required less manipulation of the steroid dosage from month to month, and have had fewer severe exacerbations of the disease. The data also confirm previous impressions that the prednisone requirement of patients receiving azathioprine is less than that of control subjects. There was no evidence that the drug was acting as an immunosuppressive agent, as measured by immunoglobulin levels, changes in antinuclear factors, or by an increased risk of infection. Furthermore, the data suggest that, as has been noted elsewhere (11), azathioprine may inhibit the occurrence or progression of renal disease.

It is difficult to explain a salutary effect of azathioprine on the course of the renal disease of systemic lupus erythematosus by invoking a purely anti-inflammatory effect. In one reported study, there was a good response to azathioprine in 6 patients, as measured by improved renal function, a decrease in proteinuria, improvement in the appearance of renal biopsies, and less staining of glomeruli with fluorescein-tagged anti-γ-globulin. These patients, however, also received about 60 mg of prednisone per day (12). In another report, of 4 patients with lupus nephritis and the nephrotic syndrome treated with azathioprine, 3 were said to have improved (13). One other report has noted that in 2 patients with lupus nephritis treated with azathioprine, while there was no change in the titers of antinuclear antibodies, the complement levels rose to normal (8). It is conceivable, therefore, that in humans azathioprine is able to inhibit the formation of antigen-antibody complexes and their subsequent deposition on glomerular basement membrane.

While azathioprine toxicity in this study was minimal, long-term toxicity remains an unknown. In 6 patients treated with total doses varying from 1 to 18 g, structural chromosomal abnormalities during metaphase were uniformly noted in bone marrow cells while the patient was receiving the drug (14). Although none of these abnormalities persisted after azathioprine was discontinued, the authors nevertheless observed that "there is considerable experimental evidence which indicates that the administration of agents with a chromosomal breaking effect in vivo may involve a potential carcinogenic or teratogenic risk."

Since our data suggest that leukopenic doses of azathioprine are not necessary to achieve a therapeutic effect (mean WBC at the end of the study was 6,900 with a range of 3,200–19,400) and the above authors have noted that in the absence of leukopenia chromosome breaks do not occur, a dosage of 2.5 mg/kg/day would appear to be a reasonable upper limit.

Severe relapses and progression of disease coincident with discontination of azathioprine were observed in 3 of our patients. Indeed, this may not be a problem restricted to patients with systemic lupus erythematosus, as 3 patients with non-lupus chronic renal disease have been reported in whom renal function deteriorated when azathioprine was discontinued (15).

The patients included in this study are probably not representative of the general population with systemic lupus erythematosus since they were entered into the study at the time of a hospital admission and tended therefore to have rather serious disease. This is reflected in the high overall death rate—13 of 42. While this fact does not affect the validity of the results in this study with these patients, it does affect the answer to the question: should *all* patients with systemic lupus erythematosus be treated with azathioprine? What is true for

patients with visceral involvement and persistent disease may not be true for patients with milder disease. Whether a patient whose disease is controlled on salicylates or small doses of prednisone should also be receiving azathioprine—either as treatment or as prophylaxis against more serious future involvement—cannot now be answered.

Finally, the present study has demonstrated that patients with systemic lupus erythematosus taking azathioprine do better than patients with systemic lupus erythematosus taking small doses of prednisone often insufficient to suppress all parameters of disease activity. The relative benefits and risks of azathioprine vs larger doses of prednisone sufficient to induce and maintain complete remissions remain a problem for future study.

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