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Azathioprine in Primary Biliary Cirrhosis: A Preliminary Report of an International Trial

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The effects of azathioprine on the course of primary biliary cirrhosis were studied prospectively in a multinational, double-blind randomized clinical trial involving 236 patients, of which 124 received azathioprine and 112 placebo. No significant effects were seen on survival, clinical course, hepatic histologic features, hepatic tests, or immunologic abnormalities after a median follow-up period of 18 mo, but most of the trends observed were in favor of azathioprine. The results obtained so far indicate that the effect of azathioprine as a single treatment is limited and probably of little clinical importance, but more years of follow-up will be needed to provide a definite conclusion.

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Primary biliary cirrhosis (PBC) is characterized by destruction of the small intrahepatic bile ducts with progressive cholestasis, leading ultimately to cirrhosis and hepatocellular failure. Although the etiology of the disease remains obscure, immune reactions appear to be involved in the pathogenesis of the bile duct damage. Cell-mediated immune responses to bile antigens have been demonstrated in 80% of cases[1] and between 30% and 50% of cases have lymphocytes in peripheral blood, which are cytotoxic for liver cells in vitro.[2,3] Studies by others have pointed to the presence of high concentrations of circulating immune complexes in 95% of cases,[4,5] and it has been suggested that these could be involved in the pathogenesis of the bile duct damage.[6] Thus, some benefit might be expected from immunosuppressive drugs. The effect of corticosteroids have not been tested in a controlled trial, presumably because they may accelerate the development of osteoporosis,[7] and to date there is no convincing evidence that corticosteroids have any effect on the progression of the disease.

Azathioprine in a dosage of 2 mg/kg body wt has been tested in a controlled trial involving 45 symptomatic precirrhotic patients.[8] Initially, the results of hepatic tests improved, but long-term follow-up demonstrated that azathioprine did not prevent the development of cirrhosis, nor did it improve survival in the 22 treated patients when compared with the 23 untreated controls.[9] At the time of the initial report in 1971,[8] it was decided to set up a multicenter,

double-blind, randomized clinical trial of azathioprine vs. placebo in primary biliary cirrhosis, and the present report describes the results of this trial to date.

Patients and Methods

Patients in whom the clinical picture and the histologic features were diagnostic of or compatible with PBC and in whom the serum alkaline phosphatase activity was greater than twice the upper limit of normal were included, and they were admitted to the trial regardless of duration of the disease and the histologic stage. Patients were randomized to azathioprine or placebo separately for each center and for each sex by using the sealed envelope technique. The tablets, which looked identical, contained 50 mg of either azathioprine or lactose. Patients of 40 kg or less received 6 tablets/wk. For each 10-kg increment in body wt, the dosage was increased by 2 tablets/wk to a maximum dosage of 14 tablets/wk. Thus each patient received the equivalent of 1 mg/kg body wt daily to a maximum dosage of 100 mg/day. For the first 2 wk, half the indicated dose was given. Leucocyte and platelet counts were done every 2 wk for 2 mo and monthly thereafter. If the leucocyte count dropped below 2000 cm^{-3} or if the platelet count below 20,000 cm^{-3}, treatment was temporarily discontinued.

Each patient had a detailed clinical assessment at entry and at 6 monthly intervals thereafter. This included an estimate of the degree of incapacitation based on the number of days in the previous 100 spent in each of the following categories: (a) normal health; (b) reduced wellbeing; (c) without capacity for work, but out of bed; (d) at home in bed; and (e) in bed in hospital. This was calculated as the Incapacitation Index, (0a+1b+2c+3d+4e)/4. Hepatic tests and investigations for immunoglobulins and autoantibodies were also performed every 6 mo.

Before the entry, and at yearly intervals thereafter, a liver biopsy was performed in each patient. The specimen was examined by one histopathologist (HP) without knowledge of the clinical state or treatment. Each biopsy was assessed with regard to histologic stage, the presence or absence of bile ducts, and other histologic features. The criteria that were used for defining the histologic stage of the disease were decided upon at the start of the trial and were adhered to thereafter.

Stage I: "Chronic destructive nonsuppurative cholangitis" was applied when this typical lesion was found without any other histologic alteration. This particularly implied that the limiting plate around the portal tract was intact, indicating that the process had not extended into the lobular parenchyma.

Stage II: "Portal inflammation with ductular proliferation and destruction" was usually associated with septa formation without distortion of the lobular architecture.

Stage III: "Scarring" (fibrosis) was diagnosed when the fibrosis predominated over the inflammation. This was often associated with central-portal fibrotic or necroinflammatory bridges and occasionally nodules, but in general the hepatic vein tributaries were clearly defined.

Stage IV: "Cirrhosis" was diagnosed when most of the nodules characteristic of cirrhosis were devoid of hepatic veins, indicating advanced destruction of the lobular architecture.

The occurrence rate after entry into the study of death (the death rate), and clinical, laboratory, and histologic features in patients *without* these features at entry was analyzed by the life-table method using the log-rank test for statistical comparison of groups.[10] The treatment effect on the development of each abnormal feature was summarized as the *occurrence rate ratio* (relative risk). This was calculated as $(O_A/E_A)/(O_P/E_P)$, where O_A is the number of patients *observed* to develop a particular abnormal feature and E_A is the number of patients *expected* to develop the feature in the azathioprine group during the observation period. O_P and E_P are the corresponding values for the placebo group. The calculation of E_A and E_P utilizes the temporal pattern of the occurrences and assumes that risk of occurrence is the same in the two groups.[10] Thus an occurrence rate ratio of 0.5 would mean that the feature considered is half as likely to occur during azathioprine treatment as during placebo treatment. The implementation of this method in the analysis of continuous variables demands the definition of a level separating "occurrence" from "no occurrence." We have arbitrarily chosen a value corresponding to the "abnormal" (upper or lower) 15th percentile of the distribution at entry as the limit beyond which a value constitutes an occurrence. This common level permits a comparison of the tendency of different variables to move towards more abnormal values, and a reasonable number of patients are allowed to "cross" the limits.

Between October, 1971 and December, 1977, 236 patients have been entered into the trial from seven different national centers. Of the total, 131 have been followed in London and Glasgow, 61 in Copenhagen, 13 in New York, 11 in Barcelona, 9 in Clichy, 8 in Leuven, and 3 in Sydney. In the random allocation, 124 received azathioprine and 112 placebo. The number allocated to each of the treatment groups was not significantly different in any of the participating centers. Table 1 shows the data at the entry into the trial of patients in the two groups which are similar except for the activity of alkaline phosphatases and the percentage with stage IV and piecemeal necrosis. Of the 236 patients, 211 were females. The median age was 54.5 yr (range 25–78) and the median duration of history 2.3 yr (range 0–12). Mitochondrial antibodies were found in 91% of the patients. More than one-fourth of the patients had already progressed to the cirrhotic stage (stage IV).

A review of the follow-up period is given in Table 2. For each year, the number of patients with predicted incomplete follow-up due to recent entry, the number lost to follow-up, and the number of deaths are indicated. Patients lost to follow-up included both withdrawals and dropouts. Six patients receiving azathioprine were withdrawn because of complaints of nausea and vomiting attributed to the drug. Two patients, 1 on placebo and 1 on azathioprine, were withdrawn because they were considered suitable for treatment by liver transplantation. Fifty-two patients dropped out, in all cases due to lack of cooperation. Twenty-two of these (42%) were in the azathioprine group. Analysis of the follow-up period—in-

Table 1. Data at Start of Trial

Variable	Azathioprine $n = 124$	Placebo $n = 112$
General		
Median age (yr)	54	55
Percentage of males	11%	13%
Median duration of history (yr)	2.0	2.2
Clinical		
Pruritus	75%	75%
Jaundice	61%	58%
Pigmentation	53%	56%
Hepatomegaly	57%	51%
Splenomegaly	35%	37%
Xanthomata	28%	26%
GI-bleeding	18%	17%
Ascites	6%	8%
Collapse of vertebrae	2%	5%
Median incapacitation index (0)	0	3
Cholestyramine treatment	37%	28%
Diuretic treatment	10%	17%
Laboratory		
Median bilirubin (μmol/l) (3-20)	33.9	30.3
Median alkaline phosphatase (IU/l) (3-85)[a]	695	454
Median alanineaminotransferase (IU/l) (7-40)	111	102
Median cholesterol (mmol/l) (3.0-8.3)	8.14	8.10
Median albumin (g/l) (35-50)	35.0	34.9
Median immunoglobulin G (g/l) (5-16)	14.8	14.0
Median immunoglobulin A (g/l) (1.25-4.25)	3.0	2.6
Median immunoglobulin M (g/l) (0.5-2.0)	3.3	3.3
Median mitochondrial antibody titre (0)	320	310
Mitochondrial antibodies	92%	88%
Smooth muscle antibodies	37%	29%
Antinuclear antibodies	31%	29%
Thyroid antibody (TRC)	24%	28%
Histologic		
Stage I	13%	14%
Stage II	43%	37%
Stage III	24%	15%
Stage IV[a]	20%	34%
Intralobular hepatitis	26%	33%
Intralobular inflammation	49%	39%
Granulomas	24%	26%
Bile duct destruction	31%	32%
Proliferation and destruction of ductules	79%	80%
Piecemeal necrosis[a]	62%	76%
Lymphoid follicles	38%	41%
Fibrosis without cirrhosis	63%	51%
Cirrhosis	25%	36%
Central cholestasis	16%	15%
Peripheral cholestasis	38%	43%

[a] Values significantly different ($P < 0.05$). Data have been compared using the Chi-square test (discontinuous variables) or the Mann-Whitney nonparametric test (continuous variables).

cluding life-table analysis taking incomplete follow-up, withdrawal, or dropping out as an occurrence—revealed no significant differences between the two groups. The median follow-up period was 18 mo.

Results

Life-Table Analysis

The survival curves (Figure 1) of the azathioprine group and the placebo group were not significantly different ($P = 0.42$). Table 3 shows the effect of azathioprine on the occurrence rates of "abnormal" (as defined in Methods) clinical, laboratory, and histologic features after entry. The effect of azathioprine did not reach statistical significance for any variable, although the effect on IgG, thyroid antibody, and albumin was close to statistical significance. All variables with a *P*-value less than 0.5 except two, i.e., serum IgA > 4.6 g/liter and fibrosis, showed trends in favor of azathioprine. Table 3 also shows 95% confidence limits and type II error risks (β) of overlooking an effect of azathioprine reducing the occurrence rate with 25% and 50%, respectively, compared with placebo. The probability that azathioprine reduces the occurrence rate with 25% is rather high for many variables, whereas a 50% reduction is not very likely. However, a harmful effect of azathioprine (occurrence rate ratio above 1) cannot be excluded as indicated by the 95% confidence limits, but such an effect is less likely except in the case of 2 variables.

The causes of death are shown in Table 4. The tendency towards more frequent death from hepatic failure and GI bleeding in the placebo group was not significant. Death from malignant neoplasms or infections was not more common among patients receiving azathioprine treatment.

Separate analysis of subgroups (defined by nationality, sex, age, duration of history, and the histologic stage) failed to reveal a response to azathioprine treatment that differed from that found for the whole material.

Discussion

Primary biliary cirrhosis is a rare disease. Estimates of its incidence range from 1:50,000[12] to 1:1.6 million.[13] Only a multicenter trial can provide information on a sufficient number of patients within a reasonable time. The inherent tendency towards greater heterogeneity of patient selection in a multicenter trial was counteracted by using a common protocol and clinical proforma, and by central evaluation of histologic and immunologic data.

In this report, emphasis is placed upon the results obtained by the life-table method, which allows for

Table 2. Analysis of Patient Follow-up

	Year of follow-up					
	1	2	3	4	5	6
Azathioprine						
Number at risk	124	80	56	31	20	10
Number of predicted incomplete follow-ups	18	13	10	11	6	5
Number of unpredicted losses	15	4	9	0	1	0
Number of deaths	11	7	6	0	3	2
Placebo						
Number at risk	112	65	39	26	10	2
Number of predicted incomplete follow-up	22	13	7	9	2	1
Number of unpredicted losses	18	4	1	4	4	0
Number of deaths	7	9	5	3	2	0

incomplete follow-up. This method also avoids the biases inherent in a conventional transectional analysis in which deteriorating trends may be masked by loss of the most severely ill patients by death or drop-out. Although the life-table method only uses the time to the *first* occurrence of an abnormal feature and disregards later fluctuations between "normal" and "abnormal," the method is unbiased, and the statistical test for comparison of occurrence rates (the log-rank test) is valid.[10]

The implementation of this method in the analysis of continuous variables demanded definition of levels separating "occurrence" from "no occurrence." An arbitrary level (corresponding to the "abnormal"—upper or lower—15th percentile at entry—see Methods) was chosen. The double-blind design of the trial allows interpretation of subjective variables such as the degree of incapacitation.

The results to date fail to show any statistically significant beneficial effect of azathioprine compared with placebo. Survival was practically the same in the two groups. However, the trends noted concerning incapacitation, pruritus, need for cholestyramine treatment, jaundice, hepatomegaly, fall in serum albumin, rise in bilirubin, alkaline phosphatase, and cholesterol indicate that azathioprine may to some extent inhibit the progress of the disease. The trends in serum IgG levels and the titer of mitochondrial antibodies might be mediated by an inhibitory effect of azathioprine on pathologic immune processes in the liver. The trend in the development of granulomas, intralobular inflammation, and bile duct destruction supports this view. However, the influence on progression to stages III and IV was very small and far from statistical significance.

The tendency to a greater increase in the serum level of IgA and in fibrosis in the liver biopsy do not fit into this pattern, but, when a large number of

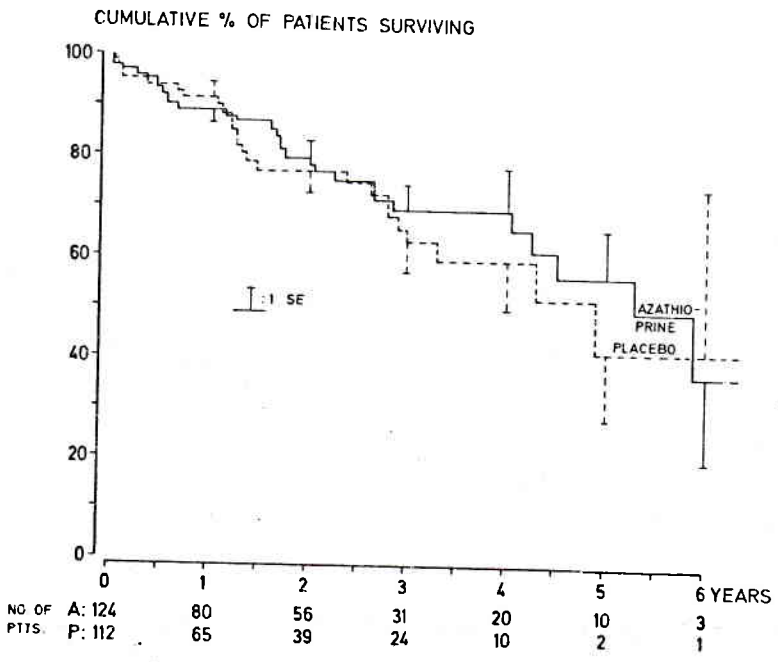

Figure 1. Cumulative survival curves of azathioprine and placebo groups ($P = 0.42$).

Table 3. Effect of Azathioprine vs. Placebo on the Occurrence Rate of Abnormal Clinical, Laboratory, and Histologic Features After Entry[a]

Variable	Numbers at risk		O/E-ratio		Occurrence rate ratio (ORR) (with 95% confidence limits)[b]		*P*-value (2 α)	Type II error risk (β)[c] of: ORR ≤0.75	Type II error risk (β)[c] of: ORR ≤0.50
	Aza.	Plac.	Aza.	Plac.					
IgG > 24 g/liter	109	94	10/14.3	14/9.7	0.49	(0.22–1.07)	0.075	0.86	0.53
Thyroid antibody (TRC)	43	28	13/17.0	12/8.0	0.51	(0.24–1.09)	0.082	0.84	0.49
Albumin < 29 g/liter	109	93	20/25.1	21/15.9	0.61	(0.33–1.11)	0.104	0.76	0.27
Hepatomegaly	53	55	16/20.7	21/16.2	0.60	(0.31–1.14)	0.12	0.76	0.29
Mitochondrial antibody titer > 1200	71	65	15/18.9	17/13.1	0.61	(0.31–1.20)	0.15	0.73	0.29
Pigmentation	59	50	16/19.6	16/12.4	0.63	(0.33–1.24)	0.18	0.69	0.24
Cholestyramine treatment	78	81	20/24.0	22/18.0	0.68	(0.38–1.23)	0.20	0.63	0.15
Incapacitation index > 30	108	95	22/26.0	21/17.0	0.69	(0.39–1.24)	0.21	0.61	0.14
Bile duct destruction	86	77	12/14.9	13/10.1	0.63	(0.29–1.35)	0.23	0.67	0.28
Intralobular inflammation	63	68	24/27.8	26/22.2	0.74	(0.42–1.27)	0.27	0.53	0.085
Peripheral Cholestasis	77	64	25/28.4	21/17.6	0.73	(0.41–1.30)	0.29	0.53	0.095
Bilirubin > 105 μmol/liter	101	99	16/18.8	16/13.2	0.70	(0.35–1.38)	0.30	0.58	0.17
Stage 3 or 4	70	56	32/35.5	25/21.5	0.78	(0.46–1.29)	0.33	0.45	0.046
IgA > 4.6 g/liter	105	98	20/17.3	12/14.7	1.40	(0.70–2.81)	0.34	0.039	0.0018
Piecemeal necrosis	47	27	42/45.7	28/24.3	0.80	(0.50–1.27)	0.34	0.39	0.023
Granulomas	95	83	16/18.3	14/11.7	0.73	(0.36–1.48)	0.39	0.52	0.14
Fibrosis (without cirrhosis)	46	54	28/25.1	19/21.9	1.28	(0.72–2.26)	0.40	0.034	0.0006
Death	124	112	29/32.0	27/24.0	0.81	(0.48–1.36)	0.42	0.39	0.038
Stage 4	99	73	26/28.6	22/19.4	0.81	(0.46–1.40)	0.44	0.40	0.047
Intralobular hepatitis	92	75	23/25.1	16/13.9	0.80	(0.43–1.49)	0.48	0.42	0.070

[a] Only variables with *P*-values less than 0.5 are presented. [b] Calculated as antilog (log ORR ± 1.96 × $SD_{(\log ORR)}$), where $SD_{(\log ORR)} = \sqrt{(\log ORR)^2/\chi^2}$ [11]; χ^2 is calculated as described by Peto et al.[10] [c] Calculated using U (standardized deviate of normal distribution) = (log 0.75 − log ORR)/$SD_{(\log ORR)}$ and U = (log 0.50 − log ORR)/$SD_{(\log ORR)}$, respectively.

variables are being analyzed, some aberrant changes may occur by chance, especially when the differences between the groups are small.

The patients at a precirrhotic stage of the disease (i.e., stages I and II) were expected to derive greatest benefit from azathioprine. However, in this respect the present observations confirm the results of the previous trial, which included only precirrhotic cases.[9] In the latter study, the dosage of azathioprine was twice as high as the present trial. Nevertheless, the beneficial trends seen were of a similar magni-

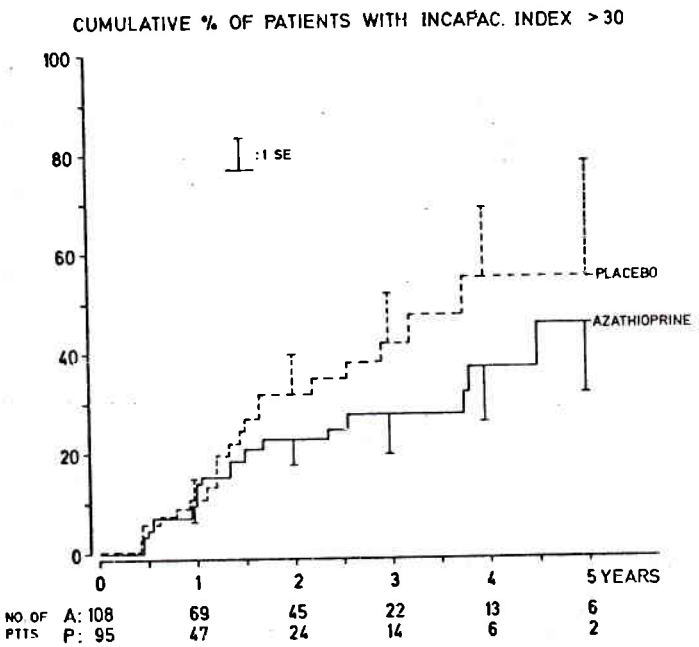

Figure 2. Cumulative percentage of patients developing incapacitation index > 30 after entry in azathioprine and placebo groups (P = 0.21).

Table 4. Causes of Death

Cause	Number of patients: Azathioprine	Number of patients: Placebo
Hepatic failure	8	7
Hepatic failure plus hemorrhage	8	13
Hemorrhage	3	1
Bronchopneumonia	2	1
Sepaticemia	2	0
Miliary tuberculosis	0	1
Pneumococcal meningitis	0	1
Renal failure	1	1
Myocardial infarction	2	0
Pulmonary embolus	1	0
Bronchogenic carcinoma	0	1
Hepatocellular carcinoma	1	0
Automobile accident	1	0
Esophageal perforation	0	1
Total	29	27

tude in both trials. In spite of the lower dose used in the present trial, 6 patients had to be withdrawn because of side effects.

The number and the causes of death were similar in the azathioprine and the placebo group, and also similar to those published in other large series.[14,15] To date, azathioprine did not appear to have an oncogenic effect, nor did it increase the frequency of infections or gastrointestinal bleeding, but such effects should be carefully looked for during the continued follow-up of the patients.

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