PAPERS AND ORIGINALS

Comparison of Azathioprine, Cyclophosphamide, and Gold in Treatment of Rheumatoid Arthritis

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Summary

Azathioprine, cyclophosphamide, and gold have been compared under double-blind conditions in the treatment of relatively early rheumatoid arthritis. Over 18 months the two "immunosuppressive" agents produced clinical improvement comparable to that achieved with gold, and they also facilitated a reduction in the dosage of corticosteroids and retarded radiological joint deterioration. Drug management was easiest with azathioprine. Cyclophosphamide was perhaps marginally the most effective drug but it produced azoospermia in males. If the long-term hazards of malignancy and mutagenesis prove to be acceptable then the antiproliferative agents provide a useful alternative to gold therapy and can with advantage be given relatively early in the course of rheumatoid arthritis.

Introduction

Numerous studies have established that various antiproliferative drugs can produce a modest but measureable improvement in patients suffering from rheumatoid arthritis (Mason

et al., 1969; Currey, 1971; Plotz, 1972; Steinberg, 1973). Though it is unclear how these agents act and certain long-term toxicity hazards remain to be evaluated many clinicians seem to be using these drugs extensively. For this reason we have investigated what we consider are two outstanding questions: What is the effect of using this therapy relatively early in the disease, at a stage when gold salts are usually considered? What are the relative merits of the different "immunosuppressive" agents available?

We therefore compared the effect of giving one of three drugs—azathioprine, cyclophosphamide, or gold—at a stage when a diagnosis of active, progressive, seropositive, and erosive rheumatoid arthritis is first established.

Methods

A double-blind between-patient trial was carried out at The London Hospital and the Royal National Hospital for Rheumatic Diseases at Bath.

Patients—Outpatients or inpatients at either centre were admitted to the trial if they were aged over 18 years and had definite or classical rheumatoid arthritis (American Rheumatism Association criteria) which was active and progressive with a positive test result for rheumatoid factor (latex test 1/80) and radiological evidence of joint erosions. Those taking corticosteroids were admitted only when the dose had been stable for the preceding three months. Patients were excluded if they had previously been treated with azathioprine, cyclophosphamide, or gold or had recorded a white blood cell count below 4,000/mm³, a neutrophil count below 2,000/mm³, a platelet count below 200,000/mm³, abnormal liver function test results, or blood urea above 50 mg/100 ml. Patients were also excluded if they had suffered from persistent dyspepsia or if they were considered to be at risk of becoming pregnant. Before entry each patient was warned about the general hazards of "immunosuppressive" therapy as these were understood at the time.

Drugs—Three treatments were compared: azathioprine 2.5 mg/kg daily in two or three divided doses; cyclophosphamide 1.5 mg/kg daily in two or three divided doses; gold (sodium aurothiomalate, Myocrisin) intramuscular injections

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of 10 mg, 20 mg, then 50 mg weekly to a total of 0.5 g followed by 50 mg every second week to a total dose of 1 g, thereafter 50 mg monthly. These doses were chosen as those which were then thought to represent the most satisfactory compromise between toxicity and therapeutic efficacy. Patients were randomly allocated to receive azathioprine, cyclophosphamide, or gold but were stratified according to sex and to whether or not they were taking corticosteroids. Patients allocated to the azathioprine or cyclophosphamide group received placebo injections designed to resemble gold injections in colour, while those allocated to the gold group received placebo tablets similar in appearance and taste to those containing the immunosuppressive drugs. Injections were given either in the clinic or at home by the general practitioner or district nurse. Only paracetamol or salicylates were allowed as analgesics and the consumption was recorded. Unused trial tablets were returned for counting at every visit.

Clinical Assessment.—At each visit the following assessments were made: the total number of limb joints (taking the hands and feet as single units) that were painful when put through the maximum possible active range; the duration of early morning stiffness; functional capacity (Steinbrocker, 1949); grip strength (sphygmomanometer cuff compression); overall joint pains (subjectively graded as nil, mild, moderate, or severe); joint size (proximal interphalangeal joints measured by Geigy ring gauge); walking time (over 12 metres).

Laboratory Investigations.—Blood counts were performed and erythrocyte sedimentation rates (E.S.R.) measured weekly for the first month, fortnightly for the next two months, and monthly thereafter. At each visit a sample of urine was tested for protein, glucose, and blood. Sheep cell agglutination tests, liver function tests, and x-ray examinations of hands and feet were carried out every six months.

Procedure.—After a run-in period of two weeks patients were seen every four weeks for assessment. Treatment was stopped if patients developed a white blood cell count below 3,500 mm³, neutrophil count below 2,000 mm³, or platelet count below 150,000 mm³, proteinuria, haematuria, pruritus, rash, persistent dyspepsia, or any other features which the clinician considered might have represented a serious toxic effect of any of the three drugs being tested. Depending on the subsequent course, and provided that the trial code was not broken, it was then at the discretion of the physician as to whether treatment could be restarted (taking into account the fact that the patient might be receiving any one of the three drugs).

Results

A total of 121 patients were admitted to the trial. Forty-four received azathioprine (25 female) 39 cyclophosphamide (21 female), and 38 gold (21 female). There were similar numbers of patients receiving corticosteroids in each group (6, 5, and 5 respectively). Sixty-two patients completed 48 weeks of trial treatment and 36 completed 72 weeks. Of the 85 who did not complete 72 weeks 53 were withdrawn because of what may have been drug toxicity (see below).

The mean age of all the patients was 52 years. Over half (55%) had had the disease for less than three years and two-thirds less than five years. The mean duration of disease of the whole group was five years, and that among patients given azathioprine was six years compared to four years for those given cyclophosphamide and four years for those given gold.

Though the gold group tended to be more severely affected than the other two groups, especially with regard to the number of joints affected (see table II) the differences in initial severity were not statistically significant. Bias in initial allocation of treatments was looked for and not found. For a brief period during the trial, however, the trial controller noted that there were significantly more patients receiving azathioprine or cyclophosphamide than gold. A careful

search failed to reveal any systematic cause for this effect, which disappeared as the trial went on and was not evident in the final analysis.

Double-blind conditions were maintained to the extent that in only two patients (both receiving gold) was the treatment allocation correctly guessed consistently by the physician.

There was a tendency for patients to reduce their intake of salicylate or paracetamol during the trial, but there was no significant difference between the three groups.

Analysis of the numbers of pills returned showed evidence in only one case that a poor clinical response might have been associated with failure to take the trial drug as prescribed.

CLINICAL ASSESSMENT

Over the 18 months of observation all three treatment groups showed steady improvement on the seven clinical criteria used.

Analysis of the data showed that there was a tendency for those with more severe disease (and hence more room for improvement by the criteria used here) to show more improvement than those with less severe disease, though this latter trend was less marked when considering percentage improvement. In other words, if percentage improvement was plotted against initial severity score as a graph this showed a definite slope with certain of the variables. The problem then was to discriminate between changes in score due to treatment and those attributable to the effect just described. This was particularly necessary as there was a tendency for the gold treated group to be more severely affected.

Discrimination was achieved by dividing the results into arbitrary groups based on equal segments of the initial severity score axis. Arbitrary transformations were then applied which were linear multiples of the grouped abscissal values. This had the effect of weighting the percentage improvements by amounts depending on the initial score; the weighting multiples were varied until trend dependent on initial scores was eliminated. Because the data were measured on a broad interval scale a non-parametric test for trend was used (Page's L test; Page, 1963). Once the trend had been eliminated, and in the data where no trend existed, differences between the treatments were looked for using the Mann-Whitney U test. Only two significant differences were apparent: both azathioprine and cyclophosphamide produced a greater improvement than gold in functional capacity at six months (table I), and cyclophosphamide produced a greater improvement than both azathioprine and gold in the number of joints affected at 18 months (table II). No significant differences

TABLE I—Functional Capacity at Six Months in Three Groups of Patients with Rheumatoid Arthritis

Treatment	Mean Initial Score	Mean % Reduction of Initial Score at 6 Months
Azathioprine	2.0	55*
Cyclophosphamide	1.9	65**
Gold	2.0	28

*Azathioprine was better than gold, $P < 0.05$ (Mann-Whitney U test).

**Cyclophosphamide was better than gold, $P < 0.01$ (Mann-Whitney U test).

TABLE II—Number of Joints Involved at 18 Months in Three Groups of Patients with Rheumatoid Arthritis

Treatment	Mean Initial Score	Mean % Reduction of Initial Score at 18 Months
Azathioprine	4.4	33
Cyclophosphamide	5.9	66*
Gold	10.2	45

*Cyclophosphamide was better than Gold, $P < 0.002$, and better than azathioprine, $P < 0.05$ (Mann-Whitney U test).

were found in early morning stiffness, grip strength, subjective assessment, joint size, or walking time at any stage.

X-RAY EXAMINATION

All x-ray pictures were scored for the severity of erosive changes they showed (graded 0 to 4) without knowledge of the patient's identity or treatment. Observers at the two centres recorded very similar results and interobserver differences proved negligible. Each six-monthly set of x-ray pictures was also compared with those taken six months previously and graded for improvement (1 to +2) or deterioration (0 to -2).

Predictably, very few radiographs showed evidence of improvement at any stage. Nevertheless, a significant difference emerged during the six-month period from 12 to 18 months after starting treatment in the frequency with which marked deterioration (-2) occurred. The gold treated group showed a higher proportion of patients with -2 grade deterioration (table III).

TABLE III—Radiological Changes between 12 and 18 Months

Treatment	No. of Patients with Score of ≥ -1	No. of Patients with Score of -2
Azathioprine	10	1*
Cyclophosphamide	14	0**
Gold	3	6

*Azathioprine was significantly better than gold, $\chi^2 P < 0.05$.

**Cyclophosphamide was significantly better than gold, $\chi^2 P < 0.01$.

ERYTHROCYTE SEDIMENTATION RATE

The E.S.R. fell in most patients, the maximum reduction occurring during the first six months (median values: azathioprine group 60-35 mm, cyclophosphamide group 55-37 mm, gold 60-31 mm). There were no significant differences between the groups at any stage.

RHEUMATOID FACTOR TESTS

The sheep cell agglutination test and latex test showed no significant differences between the groups. Differences in the techniques used at the two centres precluded analysis of the pooled data.

CHANGE IN STEROID REQUIREMENTS

Steroid requirements tended to fall in the azathioprine and cyclophosphamide groups but not in the gold group (table IV). With the small numbers involved, however, these differences reached significance only when cyclophosphamide and gold were compared at 48 weeks.

TOXICITY

There were no instances of irreversible bone marrow suppression, serious infection, apparent (biochemical) liver disturbance,

TABLE IV—Mean Reduction in Prednisolone in Three Groups of Patients with Rheumatoid Arthritis. Numbers of Patients are given in Parentheses

Treatment	Mean Dose at Entry (mg)	Mean Reduction (mg)	
		48 Weeks	72 Weeks
Azathioprine	8.0 (5)	1.5 (3)	4.5 (2)
Cyclophosphamide	8.5 (5)	3.5 (5)*	4.5 (5)
Gold	6.0 (5)	0 (4)	Increase of 0.5 (4)

*Cyclophosphamide treatment resulted in significantly greater reduction than gold at 48 weeks, $P < 0.03$ (Mann-Whitney U test).

malignancy, or death during the period of observation. The limitations imposed by the necessity to maintain "blindness" of the physician (who had to assume that any toxicity might be due to any of the three trial drugs), however, led to more withdrawals than would have been the case in normal treatment. This was especially the case with suppression of the white cell count. A degree of leucopenia or thrombocytopenia which would be acceptable in patients being treated with cyclophosphamide (and possibly azathioprine) might not be acceptable in those treated with gold. Consequently such patients had to be withdrawn irrespective of treatment. The number of patients in each treatment group withdrawn because of suspected adverse effects is shown in table V. Though there were fewer withdrawals amongst the azathioprine group than among those receiving cyclophosphamide or gold these differences did not reach statistical significance. Most withdrawals were due to an abnormal blood count, a rash, proteinuria, or gastrointestinal disturbance. Some patients showed more than one toxic feature at the time of withdrawal.

TABLE V—Incidence of Toxic Features leading to Withdrawal. Some Patients showed more than One Toxic Feature at Time of Withdrawal

	Azathioprine	Cyclophosphamide	Gold
Proteinuria	1	0	5
Skin rash	2	7	10
Marrow depression	7	11	4
Gastrointestinal disturbance	5	2	1
Herpes zoster	1	0	0
Haematuria	0	1	0
Alopecia	0	2	0
Amenorrhoea	0	1	0
Total No. of patients withdrawn due to toxicity	14 (31.8%)	20 (51.3%)	19 (50%)

AZOOSPERMIA

Another potential toxic effect, not leading to any withdrawals in this trial, is that of male sterility. When we became aware of this hazard (Fairley *et al.*, 1972) seminal analysis was performed on many male patients, some after they had been withdrawn from the trial (table VI). All of the six patients who had received cyclophosphamide had azoospermia, while none among the comparable numbers on azathioprine or gold showed this effect. One patient was azoospermic after only 14 weeks on cyclophosphamide, while another was azoospermic 74 weeks after stopping the drug. One woman on cyclophosphamide developed amenorrhoea.

Discussion

Ethical considerations prevented the inclusion of an "untreated" control group, but it is reasonable to assume that the overall improvement in the clinical criteria and the reduction in the E.S.R. seen in the gold-treated group reflected the modest efficacy of cryotherapy shown in previous controlled studies (Empire Rheumatism Research Council, 1961; American Rheumatism Association, 1973). If so this provides a familiar yardstick with which to judge azathioprine and cyclophosphamide. In the light of the clinical criteria used they emerged as about equal in effectiveness to gold when used in this way. The relatively small differences between the groups possibly suggest only a slight advantage for cyclophosphamide over the other two. The response to all these drugs was gradual, confirming the observation of Dixon *et al.* (1971) that azathioprine and cyclophosphamide do not have an immediate anti-inflammatory ("aspirin-like") action.

Two important therapeutic differences did, however, emerge. The first was that the azathioprine and cyclophosphamide groups showed less deterioration radiologically than the gold

TABLE VI—Results of Male Fertility Tests. Figures in Parentheses are Ranges

Treatment	No. Tested	Ages (Years)	Weeks of Treatment before Test	Weeks between stopping Treatment and Test	No. with Azoospermia
Azathioprine	5	52 (43-60)	47.2 (8-96)	8.8 (0-16)	0
Cyclophosphamide	6	50 (42-58)	42.1 (14-100)	15.0 (0-74)	6
Gold	6	56 (38-71)	24.6 (12-45)	20.8 (0-54)	0

group between 12 and 18 months. The second was that the two immunosuppressive groups showed some evidence of corticosteroid-sparing not shared by the gold group. With the small numbers involved the corticosteroid-sparing effects of both azathioprine and cyclophosphamide hardly reached statistical significance. Nevertheless, this tendency agrees with the previous experience of ourselves (Mason *et al.*, 1969) and others.

Altogether 55% of the patients entered this study when they had had their disease for less than three years, so we were dealing with relatively early cases. The data thus reflect what can be achieved with azathioprine and cyclophosphamide if they are introduced at a stage when many clinicians would start gold therapy.

Setting aside for the moment the long-term hazards of oncogenesis and mutagenesis this study confirmed our impression that, though toxic effects with azathioprine may be troublesome, this drug is if anything easier to manage than gold. The frequency of cyclophosphamide toxicity requiring withdrawal was comparable to that of gold though we were perhaps fortunate not to see more cystitis and alopecia. As already mentioned the high withdrawal rate because of toxicity was partly due to the design of the trial.

The effect of the antiproliferative drugs on the gonads of both sexes is a serious cause for anxiety (Fairley *et al.*, 1972; Kumar *et al.*, 1972). Our experience confirmed that cyclophosphamide causes azoospermia and may cause amenorrhoea. This was not the case for azathioprine or gold when tested in a comparable manner. More information is needed about this, but it could indicate a major advantage of azathioprine over cyclophosphamide.

This study was too short to provide useful information about the hazards of oncogenesis and mutagenesis. The former should be clarified by the Oxford study (Kinlen, 1973) but the latter will be difficult to establish in anything except the very long term. It is encouraging that the increased frequency of neoplasia (particularly lymphoma) among recipients of renal transplants given azathioprine (Penn *et al.*, 1971; Hoover and Fraumeni, 1973) has not yet been reported among patients given antiproliferative agents for rheumatic diseases though there have been isolated cases of malignancy (Tannenbaum and Schur, 1974). Schwartz (1972) has suggested reasons why graft recipients may be particularly at risk in this respect. With regard to the likelihood of teratogenesis and mutagenesis with azathioprine and cyclophosphamide, it seems that the alkylating agent is more prone than the antimetabolite to produce chromosomal changes (Tolchin *et al.*, 1973; Ginzler *et al.*, 1973) so on theoretical grounds there may be an advantage for azathioprine over the alkylating agents.

Chlorambucil, a drug not tested by ourselves but shown to be effective by others (Kahn *et al.*, 1971; Bontoux *et al.*, 1971) may perhaps offer a means of achieving the slightly greater therapeutic effect of an alkylating agent without the risks of cystitis and alopecia (Snaith *et al.*, 1973) though it is likely to produce gonadal suppression.

CONCLUSIONS

Our interpretation of these results is that cyclophosphamide and azathioprine given relatively early in the course of rheumatoid arthritis produce clinical improvement over 18 months

similar to that achieved with gold. If anything, cyclophosphamide is slightly more effective than the other two. Both azathioprine and cyclophosphamide showed advantages in reducing the rate of joint deterioration as seen radiologically and in steroid sparing not shared by gold. With all three drugs short-term toxicity was troublesome rather than serious, and azathioprine seemed the easiest drug to handle in practice. Men taking cyclophosphamide who were tested showed azoospermia and one woman on this drug developed amenorrhoea.

The role of the immunosuppressive drugs in the treatment of rheumatoid arthritis cannot be fully assessed until the results are known of surveys designed to assess the incidence of malignancy after such treatment (Kinlen, 1973) and the hazard of mutagenesis in younger patients. If these hazards prove to be acceptable then these drugs may provide a useful alternative to gold therapy. They can with advantage be given relatively early in the disease and are more effective than gold both in allowing corticosteroid dosage to be reduced and in protecting against joint deterioration.

Taken in conjunction with the results of others our data suggest that azathioprine is less toxic than cyclophosphamide particularly in regard to gonadal suppression, bladder pathology, and alopecia.

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