It is known that actinomycin D blocks the action of parathyroid hormone on bone (Eisenstein *et al.*, 1964; Rasmussen *et al.*, 1964) while not interfering with its actions on renal calcium and phosphate handling. Actinomycin D does not prevent secretion of parathyroid hormone (Tashjian, 1965). It is possible that the RNA inhibitors act initially by preventing parathyroid hormone induced calcium release from bone, leading to a fall in serum calcium with a resulting increase in parathyroid hormone secretion. This extra parathyroid hormone (unable to act on bone) could be responsible for hypophosphataemia and hypocalciuria, by an action on tubular reabsorption. This aspect may be clarified somewhat by parathyroid hormone assay and studies of renal phosphate clearance.

The similarities between the effects of actinomycin D on our patients, and mithramycin (Ryan and Schwartz, 1969), and the actions of calcitonin in Paget's disease as described by Bijvoet and Jansen (1967) and Bijvoet *et al.* (1968), suggest that RNA inhibitors may act by potentiating or stimulating the action of calcitonin. Calcitonin assay may shed some light on this aspect.

A direct action of RNA inhibitors on osteoblasts appears quite likely, particularly in view of the findings of reduced formation of chondroitin sulphate in rats treated with actinomycin D (Hulth and Lindberg, 1968) and the finding of inhibition of bone growth in children treated with this agent for renal tumours (Aarskog and Hexeberg, 1968). However, the finding of such a pronounced fall in urinary calcium even after the serum calcium level has returned to normal suggests

that a humoral mechanism is also likely to be involved. Actinomycin D is a toxic drug, a factor which must be a major one in the limitation of the use of this drug in Paget's disease, where long-standing treatment would be required.

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Clinical Trial of Malaria Prophylaxis in Tropical Splenomegaly Syndrome

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Summary

A controlled, randomized, double-blind trial of malaria prophylaxis in tropical splenomegaly syndrome showed a significant reduction in spleen size and an improvement of anaemia and symptoms in patients treated with antimalarials compared with control subjects receiving placebo. This study confirms the observations from West Africa and provides further indirect evidence for a malarial aetiology of tropical splenomegaly syndrome.

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Introduction

Tropical splenomegaly syndrome refers to chronic splenomegaly of uncertain cause which has been reported from a number of tropical countries (Pitney, 1968). In Uganda the syndrome was formerly known as "big spleen disease" and has been associated with lymphocytic infiltration of the hepatic sinusoids (Hamilton *et al.*, 1965, 1966; Marsden *et al.*, 1965). Malarial infection has been implicated in its aetiology on the basis of several kinds of indirect evidence. There is a close geographic coincidence of tropical splenomegaly syndrome with malarious areas, and patients having it show high titres of malaria antibodies (Gebbie *et al.*, 1964; Hamilton *et al.*, 1965; Marsden *et al.*, 1967). Sick-cell trait, which confers considerable protection against severe *Plasmodium falciparum* malaria, occurs rarely if at all in these patients (Hamilton *et al.*, 1969). More compelling evidence is contained in the reports of improvement in hepatosplenomegaly and anaemia in patients with tropical splenomegaly syndrome following long-term malaria prophylaxis (Lowenthal and Hutt, 1968; Watson-Williams and Allan, 1968; Sagoe, 1970). In order to confirm these reports a double-blind randomized clinical trial using primaquine and chloroquine was carried out in 29 Ugandan patients with tropical splenomegaly syndrome. The results form the basis of the present report.

Patients and Methods

A weekly "big spleen clinic" was established at Mulago Hospital in November 1968. Thirty-one patients who met our criteria for tropical splenomegaly syndrome (Hamilton *et al.*, 1965) and who agreed to long-term treatment and follow-up were admitted to the study between November 1968 and September 1969. Two patients were lost to follow-up, leaving 29 evaluable patients: 14 received malaria prophylaxis and 15 received placebo. The two groups are comparable with respect to age, sex, and duration in the trial (Table I).

TABLE I—Comparison of Trial and Control Groups

	Antimalarial	Control
No. of patients	14	15
Male/female	7/7	6/9
Median age (years) .. .	30 (12-65)	27 (13-60)
Months on trial (median) .. .	10 (6-14)	10 (2-15)

All patients were admitted to Mulago Hospital, where a detailed clinical history was taken and a complete physical examination carried out. The laboratory evaluation included: haemoglobin, W.B.C., differential count, reticulocyte count, thick and thin blood film for malarial parasites, urine analysis, stool examination, liver function studies, malaria antibody and brucella agglutinin tests, haemoglobin electrophoresis, glucose-6-phosphate dehydrogenase (G6PD) determination, chest radiogram, electrocardiogram, and bone-marrow examination when indicated. After appropriate blood-coagulation studies, liver biopsy was performed with a Menghini needle, and the tissue was processed for imprint cytology, routine histology, and electron microscopy.

Criteria for admission to the treatment trial included chronic splenomegaly and sinusoidal lymphocytosis in the liver biopsy specimen. Other causes of splenomegaly (Marsden and Hamilton, 1969) were excluded by appropriate investigations. Patients under 12 years of age were excluded from the study. None of the patients had received previous malaria prophylaxis.

The treatment trial consisted of primaquine 15 mg daily for two weeks (if G6PD was not deficient) and chloroquine phosphate 300 mg base given weekly. The control group received placebo tablets. Tablets sufficient for a one-year trial for 40 patients (20 antimalarial, 20 placebo) were divided into 12-monthly pill containers and coded by random numbers from 1 to 40. Treatment packages were sequentially allocated to those included in the trial. Patients were carefully instructed in their own language on how to take the medicine, and were asked to repeat the instructions to ensure comprehension.

Patients were seen every two weeks during the first month of the trial and once a month thereafter. At each clinic visit the investigators recorded any symptoms of intercurrent illnesses and assured themselves that the drugs were being taken in accordance with instructions. Spleen size was recorded initially and at each clinic visit by measuring the vertical distance in centimetres from the costal margin at the midclavicular line to a transverse line tangential to the most distant point of the spleen. The percentage change in spleen volume was calculated on the assumption that a barely palpable spleen is a sphere of 9-cm diameter (400 g) (Bruce-Chwatt, 1956), and that the linear change in centimetres below the costal margin was a change in the diameter (not radius). Liver size was also recorded in centimetres below the right costal margin at the midclavicular line and below the xyphoid. Haemoglobin, W.B.C., differential count, and blood films for malaria parasites were routinely performed at each clinic visit, and other investigations were carried out as indicated.

Results

Preliminary results in 16 patients analysed in June 1969 (Wood *et al.*, 1970) showed no significant differences in spleen size reduction or symptomatic improvement between the treatment and control groups. By April 1970, however, 15 months after the trial was started, there were significant differences.

Spleen Size.—The changes in estimated spleen volume in all patients are given in Table II. All 14 patients receiving

TABLE II—Changes in Spleen Volume

Malarial Prophylaxis				Placebo			
Case No.	cm Splenomegaly below Costal Margin		% Change in Volume*	Case No.	cm Splenomegaly below Costal Margin		% Change in Volume*
	Before	After			Before	After	
30	10	0	-89	2	10	6	-50
18	12	2	-86	4	21	18	-37
9	8	0	-85	25	15	12	-32
22	18	6	-84	24	12	11	-14
3	6	1	-71	32	13	12	-13
17	16	8	-69	16	6	6	0
20	11	5	-66	11	2	2	0
10	12	6	-63	7	8	8	0
13	12	6	-63	15	16	17	+13
29	12	6	-63	27	10	11	+18
1	12	7	-56	12	8	10	+39
6	3	1	-42	23	10	12	+36
8	10	7	-40	21	10	12	+36
28	10	8	-28	26	11	14	+52
				14	3	7	+138

*Calculation of % spleen volume change as follows (also see text): cm splenomegaly after trial = S1; cm splenomegaly before trial = So.

$$\% \Delta V = \frac{\left(\frac{S1 + 9}{2}\right)^3 - \left(\frac{So + 9}{2}\right)^3}{\left(\frac{So + 9}{2}\right)^3} \times 100$$

malaria prophylaxis had significant reduction in splenomegaly; in 11 this was by more than 50% of the original spleen volume. No control patient's spleen was reduced by more than 50%. There were no sex differences in responsiveness to antimalarials, but younger patients responded significantly better than older ones (Table III) ($P < 0.05$,

TABLE III—Relationship of Spleen Size Reduction to Age in Patients Receiving Antimalarials

Case No.	Age	Sex	% Volume Reduction
22	12	M.	-84
9	13	F.	-85
10	14	F.	-63
17	18	M.	-69
30	21	F.	-89
18	29	F.	-86
20	30	M.	-66
13	35	F.	-63
28	38	F.	-28
29	45	F.	-63
8	48	M.	40
1	50	M.	56
6	60	M.	-42
3	65	M.	-71

Spearman rank correlation coefficient $r_s = 0.55$; $P < 0.05$

Spearman rank correlation coefficient $r_s = 0.55$). In addition, the younger patients tended to have larger spleens, though in this small group the relationship was not statistically significant ($P < 0.1$). Spearman rank correlation coefficient $r_s = 0.46$). Splenomegaly in the control patients decreased slightly in three, remained unchanged ($\pm 20\%$ in estimated volume change) in seven, and increased in volume in five (see Table II). The progression of splenomegaly in the control group was not related to age, sex, or duration of observation. The splenic volume reduction in patients receiving antimalarials is shown in the Chart. Though there is some asymptotic flattening of the curve, the spleens are continuing to become smaller. Hepatomegaly was not a prominent feature in this series of patients with tropical splenomegaly syndrome, and significant changes were not observed in liver size during the study.

Time course of spleen volume reduction in patients receiving antimalarials. Figures in parentheses refer to patients observed at each interval.

Symptoms.—At the end of the trial patients were asked whether they felt better, the same, or worse since the trial began. The assessment of symptoms included feeling of well-being, appetite, fatigue, weakness, fevers, and splenic pain. In general, patients initially sought help for two different reasons: most complained of chronic abdominal discomfort directly attributable to the large spleen, whereas some had symptoms of anaemia. Relief of symptoms (Table IV) was

TABLE IV—Symptomatic Improvement During the Study Period

	No. of Patients	
	Antimalarial	Control
Better	10 (71%)	2 (13%)
Same	4 (29%)	10 (67%)
Worse	0	3 (20%)
	14	15

significantly greater in the treatment group than in controls ($P < 0.01$ by χ^2).

Haemoglobin.—Changes in haemoglobin levels before and on completion of the trial showed improvement in the treatment group ($P < 0.05$, Mann Whitney U test) (Table V).

TABLE V—Changes in Haemoglobin Levels During Study Period

Changes in Haemoglobin (g/100 ml)	No. of Patients	
	Antimalarial	Control
Increase { 3+	3 (21%)	1 (7%)
1-2.9	5 (35%)	2 (14%)
No change ±1	5 (35%)	6 (40%)
Decrease 1-2.9	1 (7%)	6 (40%)

None of the patients in the study had G6PD deficiency. Haemoglobin levels in the control group did not improve, but tended to decline. None of the patients had significantly depressed W.B.C. or platelet counts initially and there were no changes with antimalarials.

Parasitaemia.—On initial examination 4 out of 14 who were put on malaria prophylaxis and 2 out of 15 in the control group had scanty *P. falciparum* parasitaemia. Occasional parasites were seen in the control group during the study, but in neither group were there clinical attacks of acute malaria.

Discussion

This study confirms the clinical effectiveness of prolonged malaria prophylaxis in the treatment of tropical splenomegaly syndrome. As in the experience of West African investigators (Watson-Williams and Allan, 1968; Sagoe, 1970) long-term

therapy is necessary before definite clinical responses are observed. Significant reduction in spleen size, substantial symptomatic relief, and improvement in haemoglobin levels were found. Further analysis of the patients who responded to malaria prophylaxis showed that younger patients responded better than older ones. The poorer response in the elderly may be related to secondary changes, such as fibrosis and adhesions in spleens which have been enlarged for many years.

Our study differed from that carried out in West Africa as follows: (1) primaquine and chloroquine rather than proguanil were used, (2) the design of the two trials was different, (3) the criteria for patient selection in our series included hepatic sinusoidal lymphocytosis, and (4) the degree of splenomegaly and haematological abnormalities was less pronounced than in the West African patients. Nevertheless, the studies have shown that two different malarial prophylactic regimens will produce substantial improvement in tropical splenomegaly syndrome.

As soon as the results of this trial were analysed and significant differences were observed, those receiving placebo tablets were placed on primaquine and chloroquine. Six thus far evaluated have already shown substantial reduction of spleen size over a period of six months. The spleen size reduction time curves of most of our treated patients were still continuing downward after 12 months of therapy, and it seems clear that continued malaria prophylaxis is required. The optimal duration of antimalarial administration required for control of tropical splenomegaly syndrome has yet to be established. Both in our experience and in that from West Africa patients have relapsed after discontinuing their medication even after long periods of successful treatment. It may be that patients require prophylaxis as long as they remain exposed to malarial infection.

The pathogenesis of tropical splenomegaly syndrome and its relation to malaria is still unclear. These patients appear to have generally normal immune responses (Ziegler *et al.*, 1969), but produce a large amount of macroglobulin (Wells, 1968). Electron microscopy studies of the liver have shown mature lymphocytes in close association with hypertrophied Kupffer cells, an appearance which is very suggestive of a cellular immune response (Hutt and Fluck, 1970). These observations have led to speculation that there is a persistent antigenic stimulus which results in a benign lymphoproliferative disorder predominately affecting the spleen and liver. The stimulus may be due to the malaria parasite itself, malaria antigen in an altered form, or possibly even to a form of malaria antibody. As malaria parasite frequency and density rates are very low in this condition and overt attacks of malaria are very rare, the immune response can be said to be effective in this respect.

In any event, if chronic malarial parasitaemia of a very low grade provides the antigenic stimulus leading to tropical splenomegaly syndrome, then the beneficial effect of malaria prophylaxis can be readily accounted for by the elimination of parasites from the blood. This seems to be the simplest explanation of the clinical observations at present.

The best treatment for tropical splenomegaly syndrome has not been fully settled. Splenectomy should be reserved, at least in the tropics, for special indications (Hamilton *et al.*, 1967; Paliwoda and Hutt, 1968). It is possible that more potent cytotoxic agents such as methotrexate or chlorambucil have a role in severe hypersplenism in which splenectomy cannot be performed. Prolonged therapy with antimalarial drugs (chloroquine or proguanil), however, seems to be the most reasonable and effective treatment for uncomplicated cases of tropical splenomegaly syndrome at the present time.

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Tropical Splenomegaly Syndrome in Zambia: Further Observations and Effects of Cycloguanil and Proguanil

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Summary

Nineteen Zambian patients with the tropical splenomegaly syndrome and sinusoidal lymphocytosis on liver biopsy were studied. The association of macrobulinaemia with the tropical splenomegaly syndrome has again been confirmed. Sixteen patients were treated with antimalarials—12 with cycloguanil pamoate alone, 3 with cycloguanil and proguanil, and 1 with proguanil alone. Twelve patients were observed for periods of sufficient length for the drug effect to be assessed, and in 11 there was a good response in terms of decrease in spleen size.

Cycloguanil pamoate may be of value both for prophylaxis and treatment in areas where tropical splenomegaly syndrome is endemic.

Introduction

The tropical splenomegaly syndrome has been the subject of several reviews during the past two years (Pitney, 1968; *British Medical Journal*, 1967, 1969). Previous studies in Zambia showed that tropical splenomegaly syndrome occurring there was similar to that described in other parts of Africa (Lowenthal et al., 1966) and also that long-term antimalarial prophylaxis with proguanil was beneficial (Lowenthal and Hutt, 1968). Similar results, with more complete remissions, have been found in two larger series of cases treated with proguanil in Nigeria (Watson-Williams and Allan, 1968; Sagoe, 1970). A double-blind trial on cases of tropical splenomegaly syndrome in Uganda with long-term chloroquine therapy has also shown significant improvement in patients' symptoms and spleen size (Stuiver et al., 1971). Earlier experience with long-term treatment with proguanil

showed that it was not always easy to ensure that the drug was continually taken over a long period of time. For this reason it was decided to treat cases of tropical splenomegaly syndrome with the depot injectable antimalarial cycloguanil pamoate (Camolar) either alone or in some cases combined with proguanil.

Patients and Methods

Nineteen patients (13 male and 6 female) who fulfilled the diagnostic criteria for tropical splenomegaly syndrome (Marsden and Hamilton, 1969) were admitted to the wards of the Ndola Central Hospital during the period December 1968 to March 1970. In all cases a detailed clinical history and physical examination were carried out. Spleen size was measured along the longest palpable axis of the organ. All clinical examinations were made by one of us (M.N.L.). Laboratory examinations included: haemoglobin, white count and differential, thick blood film for malarial parasites, urine analysis and stool examination for parasites in all cases, haemoglobin electrophoresis in 14 patients, and sternal marrow puncture in 12 patients. Percutaneous liver biopsy was performed in all 19 patients and the diagnosis of tropical splenomegaly syndrome with hepatic sinusoidal lymphocytosis was established according to previous criteria (Marsden et al., 1967). Repeat liver biopsies were done in five patients at intervals after treatment had started. Other investigations, including plasma protein levels, empirical liver function tests, agglutinations against brucella and salmonella organisms, serum iron levels, and chest x-ray examination, were done in most cases.

Immunological Studies.—IgM levels were measured in 17 cases by a modification of the method of Mancini et al. (1965). The assay was repeated in seven cases after therapy, and in three of these a third estimation was made after a further interval. For the purpose of providing control values IgM was assayed in two other groups of patients. The first included patients with moderate to massive splenomegaly due to chronic myeloid leukaemia (3 cases), primary myeloid metaplasia (2), schistosomiasis (5), tropical splenomegaly syndrome without hepatic sinusoidal lymphocytosis (4), hepatic cirrhosis (6), and one case each of tuberculosis, amyloidosis, and con-

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