PYRIMETHAMINE IN THE TREATMENT OF POLYCYTHAEMIA RUBRA VERA

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Polycythaemia rubra vera has been treated by venesection, haemolytic drugs, irradiation, and occasionally by alkylating agents. Irradiation by means of injected radiophosphorus (^{32}P) is probably the most widely used treatment at the moment. The introduction of chemical inhibitors of nucleic-acid synthesis has provided yet another method of suppressing the hyperactive erythron in this disease. This paper describes our experience of one such drug—pyrimethamine.

Hitchings *et al.* (1948) showed that many aminopyrimidines inhibit the growth of *Lactobacillus casei* by preventing the utilization of folic acid. The discovery of the association between antimalarial action and folic-acid antagonism in the case of proguanil (Falco *et al.*, 1949) led to the investigation of the aminopyrimidines as possible antimalarial agents. The most active aminopyrimidine was found to be 2-4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine (Falco *et al.*, 1951). This drug was called "daraprim" and has received the official name of pyrimethamine.

Pyrimethamine has proved to be a valuable agent in malarial prophylaxis (Vincke and Lips, 1952), and when combined with sulphadiazine is effective against toxoplasmosis (Frenkel and Hitchings, 1957).

Hamilton *et al.*, (1952) reported that the administration of diaminopyrimidines of this type to rats and dogs produced a megaloblastic anaemia with leucopenia. A detailed study of the pharmacology of pyrimethamine was reported by Schmidt *et al.* (1953), who found that the compound was extremely toxic in high doses, but their data indicated that erythropoietic depression might be obtained at doses low enough to avoid serious effects on other systems.

Isaacs (1954) was the first to report the use of pyrimethamine in the treatment of polycythaemia rubra vera. He produced depression of the red-cell count without leucopenia or thrombocytopenia in six patients receiving 12.5–25 mg. daily. A time lag of two to three weeks in the response of the red-cell count was noted. Subjective and objective improvement was obtained in all cases. Frost and Jones (1954) recorded the treatment of six patients receiving 15–25 mg. a day. Satisfactory clinical and haematological remissions occurred in all cases, and side-effects were not reported.

Wasserman (1955), however, condemned the use of pyrimethamine in the management of polycythaemia. He found the response to be slow, unpredictable, and unsatisfactory in at least 80% of cases, while side-

effects were prominent and troublesome. Leucopenia, thrombocytopenia, petechial and gastro-intestinal haemorrhage, nausea and vomiting, buccal ulceration, and bone-marrow aplasia were all reported.

Materials and Methods

This series consists of five patients presenting consecutively with polycythaemia rubra vera in relapse. Four had previously been treated with ^{32}P . One patient received two courses of pyrimethamine. The haemoglobin concentration and leucocyte count were measured at intervals of one to four weeks in Cases 1 to 3. Cases 4 and 5, and Case 3 during the second course of treatment, were subjected to more detailed study which included estimates of the haemoglobin concentration, packed cell volume, and red-cell, reticulocyte, leucocyte, and platelet counts. These were performed at weekly intervals on sequestered venous blood, using the techniques described by Dacie (1956), absolute erythrocyte values being calculated on each occasion. When the haemoglobin concentration had returned to normal, tests were carried out at two-weekly intervals. Red-cell mass, red-cell life, and plasma-iron-clearance rates were measured before and during treatment, using the ^{51}Cr and ^{59}Fe techniques described by Ebaugh *et al.* (1953) and Wetherley-Mein *et al.* (1956).

Initially, pyrimethamine was administered in 25-mg. scored tablets. Later, an elixir containing 6.25 mg./ml. became available and was used in Case 3(b).

Case 1

A man aged 60 presented in January, 1953, with dyspnoea, plethora and cyanosis, headaches, and giddiness. The tip of his spleen was palpable 1½ in. (3.8 cm.) below the costal margin. Investigations confirmed the diagnosis of polycythaemia rubra vera. Treatment was initially by venesection, but later two doses of ^{32}P were administered. However, in October, 1958, five and a half years later, he was again in relapse.

Investigations.—Haemoglobin, 21.9 g./100 ml.; erythrocytes, 7,500,000/c.mm.; leucocytes, 6,700/c.mm.; platelets, 260,000/c.mm.

Treatment.—Pyrimethamine was administered by mouth in doses ranging from 25 to 200 mg. a day. Fig. 1 demonstrates the difficulty experienced in establishing the daily dose of pyrimethamine necessary to maintain the haemoglobin concentration within normal limits. The leucocyte count tended to fall on a daily dosage exceeding 50 mg., and to rise above normal on sudden reduction of dosage. The time interval for leucocyte response was much shorter than that for haemoglobin concentration.

The patient became symptom-free after eight weeks' treatment and has remained so up to the time of writing,

Fig. 1.—Details of Case 1.

a period of two years. He experienced some epigastric discomfort at doses exceeding 100 mg. daily, and vomited once during the week in which he received 200 mg. a day. There were no other side-effects.

Case 2

A man of 56 presented in 1954 with a one-year history of progressive facial rubor and giddiness. He was found to be plethoric, with enlargement of the spleen to the umbilicus. Investigations confirmed the diagnosis of polycythaemia rubra vera. During the next three years the disease was controlled by three doses of ^{32}P ; but one year later, in 1958, he was again in relapse with severe giddiness and hepatosplenomegaly.

Investigations.—Haemoglobin, 20.8 g./100 ml.; erythrocytes, 8,000,000/c.mm.; leucocytes, 6,000/c.mm.; platelets, 300,000/c.mm.

Treatment.—Pyrimethamine was administered in daily doses of 25 to 75 mg. for one year, at which time he was lost to follow-up. Fig. 2 illustrates the satisfactory nature of the response obtained, the patient becoming asymptomatic in five weeks and remaining so throughout the treatment.

FIG. 2.—Details of Case 2.

It soon became clear that 50 mg. of pyrimethamine a day just exceeded the optimum dose for adequate control of erythropoiesis. Two brief interruptions of treatment at this dosage controlled the disease very satisfactorily for one year. He experienced some epigastric discomfort during treatment. This was unrelated to the dosage of pyrimethamine and was proved to be due to an exacerbation of long-standing peptic ulceration. It responded completely to antacids.

Case 3

A man aged 65 presented in October, 1954, with myocardial infarction. He was plethoric and his spleen had enlarged to the umbilicus, while the liver edge was palpable 1 in. (2.5 cm.) below the costal margin. The haemoglobin level was 18.2 g./100 ml. No treatment for the polycythaemia was given at this date. Eighteen months later he developed thromboses in his right upper and left lower limbs, and examination revealed other features characteristic of polycythaemia rubra vera. During the ensuing year he received two injections of ^{32}P with satisfactory results, but in 1959, two years after the second injection, he was once more in relapse with severe symptoms.

Investigations.—Haemoglobin, 18.3 g./100 ml.; erythrocytes, 6,300,000 c.mm.; leucocytes, 14,900/c.mm.; platelets, 350,000/c.mm.

Pyrimethamine 50 mg. daily was given for two weeks. This produced a profound fall in haemoglobin concentration, shown in Fig. 3, and a sharp depression of the leucocyte count. It will be noted that recovery of the haemoglobin concentration started two months later, but the leucocyte count recovered much more rapidly, producing a temporary leucocytosis.

Comments on Cases 1-3

A marked feature of these three cases is the wide range of doses necessary to maintain normal haemoglobin levels. Thus Case 1 required somewhere between 62.5 and 75 mg. a day, Case 2 just under 50 mg. a day, while the dose for Case 3 was evidently very much less than 50 mg. a day and probably less than 20 mg. It is also evident from Figs. 1-3 how difficult it is to establish the correct dosage from the slowly responding haemoglobin level. Although the leucocyte count tended to respond more rapidly it lacked sensitivity and was subject to variations independent of drug dosage.

In the normal subject the red-cell mass is regulated within 30% limits, but in polycythaemia rubra vera there is an excess of 30-200%. Since the life-span of the erythrocyte is unaltered the bone-marrow production rate must also be increased by 30-200%. If we consider a typical example where the red-cell mass is twice the normal value the object of treatment is twofold, firstly, to reduce the red-cell mass by 50%, and, secondly, to decrease red-cell production by 50%. If both of these objects are to be achieved by controlled suppression of nucleic-acid synthesis such that erythropoiesis is exactly halved, it follows that a period of time equal to the red-cell life (16 weeks) will elapse before the red-cell mass is normal. We have assumed that it is possible to adjust drug dosage precisely enough to achieve such an effect, but in fact, as we have seen, individual variation in dose response is so large that a measure of individual drug requirement is indispensable. A measure dependent on the red-cell mass—for example, the haemoglobin concentration—is not satisfactory because of the very long time interval which will elapse before a reliable estimate of the effectiveness of the drug dosage can be made.

In view of these problems it was decided to subject future cases to more detailed haematological study.

Case 4

A woman aged 69 presented with headache, tiredness, epistaxis, and facial rubor increasing over a period of three months. Physical examination showed marked plethora, with cyanosis of the face and lips, and congested conjunctival vessels. The spleen was palpable 1½ in. (3.8 cm.) below

FIG. 3.—Details of Case 3.

the costal margin. She was mildly hypertensive (B.P. 150/100) with grade 1 retinopathy.

Investigations.—Haemoglobin, 23.7 g./100 ml.; erythrocytes, 9,400,000/c.mm.; reticulocytes, 120,000/c.mm. (normal 20,000–100,000 c.mm.); leucocytes, 11,100/c.mm. (differential count normal); platelets, 400,000/c.mm.; red-cell mass, 82 ml./kg. (normal 20–40 ml./kg.); mean cell volume, 79 cubic microns; plasma iron clearance T/2 = 18 minutes (normal 70–130 minutes). Sternal marrow biopsy: normoblastic proliferation with hyperplasia of the myeloid series and megakaryocytes.

Treatment.—Pyrimethamine 25 mg. daily by mouth was administered for two weeks, after which the dose was increased to 50 mg. daily for a further two weeks. Fig. 4 shows that this depressed the reticulocyte and platelet counts to very low levels, and after two weeks the red-cell count and haemoglobin concentration began to fall. The erythrocytes showed marked anisocytosis with numerous macrocytes, causing the mean corpuscular volume to rise above normal. Thrombocytopenia was accompanied by slight epistaxis and several ecchymoses. Erythrocyte survival measured during the first month of therapy indicated a normal life-span (^{51}Cr T/2 = 30 days), and at the end of this period the red-cell mass had fallen to 73 ml./kg. and the iron clearance rate T/2 was 132 minutes. Pyrimethamine was withheld for the next two weeks, during which time the haemoglobin and erythrocyte levels continued to fall, but the reticulocyte and platelet counts recovered after one week. After a further week therapy was resumed at the lower dose rate of 12.5 mg. daily. Within two weeks the haemoglobin concentration and erythrocyte count were within normal limits, and after two months the red-cell mass was 40 ml./kg. Sternal bone-marrow biopsy revealed hyperplasia with many intermediate megaloblasts. The mean corpuscular haemoglobin concentration was not significantly altered by the treatment.

The patient began to feel better two weeks after starting treatment and was symptom-free within two months. The spleen decreased in size but remained palpable after seven months of treatment. Her drug requirement has been in the range of 6–12 mg. a day.

Case 5

A man aged 64 presented in March, 1956, with giddiness, plethora, and generalized pruritus. The spleen was not palpable, but investigation of the peripheral blood and bone-marrow confirmed the diagnosis of polycythaemia rubra vera. Satisfactory control was achieved with two doses of ^{32}P during the next four years, but in 1960 he was again in relapse.

Investigations.—Haemoglobin, 19.7 g./100 ml.; erythrocytes, 6,900,000/c.mm.; reticulocytes, 130,000/c.mm.; leucocytes, 6,100/c.mm. (differential count normal); platelets, 218,000/c.mm.; red-cell mass, 46.5 ml./kg.; mean cell volume, 93 cubic microns; plasma iron clearance T/2 = 33 minutes. Sternal marrow biopsy: normoblastic proliferation with hyperplasia of the myeloid series and megakaryocytes.

Treatment.—Pyrimethamine 50 mg. daily was given for one month. This produced a fall in the reticulocyte and platelet counts as shown in Fig. 5. The haemoglobin concentration began to fall after two and a half weeks. The peripheral blood then showed gross anisocytosis with many macrocytes, the mean cell volume having risen to 115 cubic microns. The dose was then reduced to 12.5 mg. a day, but the subsequent rise in reticulocyte count first to 34,000/c.mm., and then, one week later, to 120,000/c.mm., caused us to increase the daily dosage to 25 mg. During the ensuing three months the haemoglobin level and erythrocyte, leucocyte, and platelet counts remained within normal limits and the reticulocyte count between 40,000 and 60,000/

FIG. 4.—Details of Case 4.

FIG. 5.—Details of Case 5.

FIG. 6.—Details of Case 3(b).

c.mm. Sternal aspiration biopsy revealed a hyperplastic marrow with numerous intermediate megaloblasts. Erythrocyte survival measured during treatment was normal (^{51}Cr $T/2=29$ days) and the M.C.H.C. was not significantly altered. The plasma iron $T/2$ was prolonged to 71 minutes.

The patient remained well throughout the treatment, experiencing only minimal abdominal discomfort while taking 50 mg. of pyrimethamine daily. No other side-effects were noted.

Case 3(b)

This is the same patient as in Case 3, receiving a second course of pyrimethamine five months after his first treatment.

Investigations.—Haemoglobin, 17.7 g./100 ml.; erythrocytes, 5,000,000/c.mm.; reticulocytes, 100,000/c.mm.; leucocytes, 17,700/c.mm. (differential count normal); platelets, 415,000/c.mm.; red-cell mass, 43.5 ml./kg.; mean cell volume, 116 cubic microns; plasma iron clearance $T/2=15$ minutes. Sternal marrow biopsy: normoblastic proliferations with hyperplasia of the myeloid series and megakaryocytes.

Treatment.—Cautious treatment with pyrimethamine was resumed at a dosage of 12.5 mg. daily for three weeks. This depressed the reticulocyte count to 5,000/c.mm. as shown Fig. 6. The haemoglobin concentration and leucocyte and platelet counts were also depressed. Administration of the drug was continued for the next month at gradually reducing dosage and then stopped. The haemoglobin level continued to fall while the platelet and reticulocyte counts recovered.

In the seven months which have elapsed since this course of pyrimethamine three brief periods of drug treatment at doses of 3–6 mg. daily have been required to maintain a complete remission. The mean cell volume has remained between 110 and 120 cubic microns, the M.C.H.C. was within normal limits, and the life-span of his erythrocytes was normal during treatment (^{51}Cr $T/2=30$ days).

Comments on Cases 4, 5, and 3(b)

The reticulocyte count provided the earliest and most sensitive index of erythropoietic depression in these three cases. For maintenance purposes it seemed that the dose which maintained a count of 30,000 to 60,000/c.mm. (0.6–1.2%) resulted in a stable haemoglobin level. Although thrombocytopenia occurred initially in all three cases, it was not produced by this maintenance dose. Similarly, although epigastric discomfort and anorexia occurred at high dosage in Case 5, there were no side-effects at maintenance dosage in any patient.

Discussion

In each of the five cases continuous administration of pyrimethamine produced a steady fall in the haemoglobin concentration, packed cell volume, and red-cell count of peripheral blood after an initial time lag of two to three weeks. This effect was not due to haemolysis, since measurement of the red-cell life in three cases under treatment gave normal values. The early depression of the reticulocyte level and the slowing of the plasma-iron-clearance rate demonstrate conclusively that there is a marked reduction in red-cell output.

During treatment the erythrocytes showed gradually increasing anisocytosis and macrocytosis, with the mean cell volume rising to 110–120 cubic microns, and bone-marrow smears presented the picture of "maturation arrest" with numerous intermediate megaloblasts. These observations are consistent with the suggestion that the principal effect of this drug is to inhibit the

conversion of folic acid to folinic acid (Hitchings *et al.*, 1952). Megakaryocyte activity and myelopoiesis are also depressed at high dosage, but platelet and leucocyte levels were not affected by maintenance doses of pyrimethamine and side-effects were completely absent. Unlike Wasserman, we failed to observe a single instance of alopecia, buccal ulceration, gastro-intestinal haemorrhage, or bone-marrow aplasia, although particular attention was paid to these possibilities. Anorexia, epigastric discomfort, nausea, and vomiting were produced in two cases receiving higher doses. Similarly, ecchymosis and epistaxis occurred in one case receiving large doses of pyrimethamine.

The main problem has been that of deciding the correct dosage for each case—an important matter, since the side-effects which could be produced by excessive dosage are both unpleasant and dangerous. However, it has been practicable, using the rapidly responding reticulocyte count, to select the correct dose for each patient quite quickly, and it has been comforting to find that each patient behaved consistently according to his individual "sensitivity" and did not develop tolerance to the drug in the period under study. It is not clear whether there is a true variation in tissue sensitivity to the drug or whether the rate and extent of absorption are the variable factors. The animal studies of Schmidt *et al.* (1953) suggest that the rate of intestinal absorption may be one important variable. Once the patient's correct dose has been found, many weeks will elapse before a normal haemoglobin level is restored. It is dangerous to accelerate this process by using large doses of pyrimethamine, but, if the severity of the symptoms demand it, venesection may save valuable time.

Summary and Conclusions

Five cases of polycythaemia rubra vera treated with pyrimethamine for periods of seven months to two years are presented. Excellent clinical and haematological remissions were obtained and maintained in all cases with minimal side-effects. The wide variation in individual response to the drug is stressed and the value of the reticulocyte count as a rapid and sensitive index of drug requirement is emphasized.

Oral pyrimethamine provides an effective treatment for polycythaemia rubra vera, but its potential toxicity necessitates meticulous haematological control, particularly at the beginning of treatment. Side-effects have been consistently absent at the correct therapeutic dosage; in this respect our experience resembles that of Isaacs (1954) and of Frost and Jones (1954), and differs greatly from that of Wasserman (1955). It is indeed difficult to understand how such gross toxic effects could be produced at therapeutic dosage.

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SEASONAL INCIDENCE OF CLINICAL ONSET OF HODGKIN'S DISEASE

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In reviewing 269 cases of histologically proved Hodgkin's disease seen at the Royal Marsden Hospital since 1945, a high incidence of clinical onset in the month of December was observed.

Criteria for Selection

Since the mode of onset is so variable and the presenting symptoms may appear long after the true onset of the disease, a seasonal association could have significance only if the clinical onset were closely related in time to the apparent onset of the disease. In 106 cases there is reason to suppose this was so, and the following criteria were used in selecting them: (1) the month of onset was definitely stated; (2) lymphadenopathy was localized to one peripheral site at onset; (3) no evidence of generalized disease was present; (4) disease was confined to superficial lymph nodes with no clinical or radiological evidence of deeper involvement within six months of onset; and (5) patients were living in England at the time of onset.

Time of Onset.—In many cases the time of appearance of the first enlarged lymph node was remembered precisely by the patient, sometimes to within a few days. If the onset was remembered to within one calendar month the case was accepted for analysis, but when the description was less precise, as, for example, "about nine months ago," the case was excluded.

Site.—In 70 of the 106 patients the disease remained strictly localized to one region for six months or more. Of the remaining 36 patients, some developed enlarged nodes in the corresponding region on the opposite side, and a few had axillary involvement on the same side after initial cervical-node involvement. Provided the disease remained localized to these sites for at least six months, the case was included in the series. If the mediastinum, for instance, was the true site of onset, although the appearance of enlarged cervical nodes was

the first clinical evidence of disease, some evidence of a mediastinal lesion might have been expected to be manifest within six months.

Cases Excluded from the Series

Criteria for exclusion of cases were as follows. (1) Generalized peripheral lymphadenopathy at the time of clinical onset (3 cases). (2) Evidence of intrathoracic or intra-abdominal involvement at the time of presentation or within six months (37 and 9 cases respectively), or of paravertebral involvement (3 cases). (3) Localized disease for six months or more, but the month of onset not stated (24 cases). (4) Cases lost to follow-up within six months of the onset of the disease (1 case). (5) Onset of disease outside England (30 cases).

Constitutional symptoms such as fevers, sweats, lassitude, pruritus, or loss of weight were present in some cases, sometimes for several months, before Hodgkin's disease was diagnosed. These symptoms did not occur prior to the lymphadenopathy in the cases selected for the series.

A further 56 patients whose disease was clinically localized at the time of onset have been excluded from analysis because one or more of the following features were present: (1) peripheral lymphadenopathy in areas remote from the site of presentation within six months of clinical onset but without evidence of deeper involvement; (2) probable or early paratracheal or mediastinal spread not detected by serial x-ray films until five to six months after the first appearance of cervical nodes; (3) splenomegaly detected between three and six months after the onset of the disease; and (4) febrile episodes in the absence of other signs of spread of the disease within six months which, in retrospect, could have been due to subsequently discovered deep-seated disease not apparent at the time.

Although these 56 cases were not included in the series, they show almost exactly the same seasonal incidence as the 106 acceptable cases. If they were added to the 106, making a total of 162 cases, and the results compared with those of the 106 cases shown in Table I, the difference in incidence in any one month would be little more than $\pm 1\%$.

Many cases have thus been excluded because of the possibility that the disease was present for some time before the appearance of the first sign or symptom. Since rapidly progressive disease may develop from a focal lesion, some eligible cases might have been unnecessarily excluded.

Thus, of 269 case histories reviewed, 106 (39.5%) were regarded as suitable to be analysed for a possible seasonal association.

Results

Of the 106 patients selected, 23 (21.7%) had the clinical onset of Hodgkin's disease in the month of December. The results are shown in Table I.

Seasonal Incidence in the Two Periods 1945-53 and 1954-9.—To examine the seasonal incidence in different periods the 50 patients whose disease began in the period 1945-53 and the 56 whose disease began in 1954 to 1959 have been analysed separately. Table I shows that the seasonal incidence is essentially the same for each period and the whole period.

Incidence of Infection of the Upper Respiratory Tract at Clinical Onset of Hodgkin's Disease.—In the majority of cases the disease presented simply with painless