Red cell aplasia following prolonged D-penicillamine therapy

J. L. GOLLAN, SONAY HUSSEIN, A. V. HOFFBRAND, AND SHEILA SHERLOCK

From the Departments of Medicine and Haematology, The Royal Free Hospital, Pond Street, Hampstead NW3 2QG

SYNOPSIS   Red cell aplasia developed in a case of Wilson's disease following an increase in D-penicillamine dosage after 14 years' treatment. *In vitro* study of the effect of D-penicillamine on ^{59}Fe incorporation by marrow cells did not suggest that the patient's erythropoiesis was particularly sensitive to D-penicillamine or determine the mechanism of drug toxicity. However, three weeks after the drug was withdrawn, evidence of marrow regeneration was apparent, and within 10 weeks the haemoglobin had returned to normal. The patient has subsequently remained asymptomatic on an alternative chelating agent, triethylenetetramine dihydrochloride.

Since Walshe (1956) first demonstrated the ability of D-penicillamine to promote the urinary excretion of copper in Wilson's disease, the drug has found a variety of therapeutic applications. With its value in rheumatoid arthritis recently established and studies in chronic active liver disease in progress a renewed interest in the drug has been stimulated. Adverse haematological effects of D-penicillamine, such as thrombocytopenia and neutropenia, occur frequently, and an aplastic anaemia may rarely develop. Selective suppression of the erythroid series has, to our knowledge, not previously been observed. We report here the development of a reversible red cell aplasia in a case of Wilson's disease after 14 years' D-penicillamine therapy.

Case Report and Investigations

In 1954 at the age of 4 years hepatosplenomegaly was observed during the course of a transient febrile illness. A liver biopsy was performed and reported as showing almost normal histology. In 1960 the patient developed jaundice with ascites and a mild anaemia. An open liver biopsy on this occasion revealed established cirrhosis. Wilson's disease was diagnosed by the presence of Kayser-Fleischer rings and by the family history of a brother who had died at the age of 10 years with cirrhosis. Urinary copper excretion was 280 μg/24 h (normal < 70 μg/24 h) but the serum copper and caeruloplasmin concentrations were normal. Treatment with D-penicillamine

HCl was initiated in a dose of 0·9 g daily with gradual resolution of the jaundice and ascites.

By 1965 (aged 15), the patient was asymptomatic, still with prominent Kayser-Fleischer rings and minimal hepatosplenomegaly, but with no features of chronic liver disease or abnormal neurological signs. The haemoglobin level was 14·0 g/dl and conventional liver function tests were normal. Several small opaque gallstones were observed on the abdominal *x*-ray. Hepatic biopsy showed an inactive cirrhosis with mild fat infiltration and a liver copper concentration of 620 μg/g dry weight (normal < 55 μg/g). The urinary excretion of copper was 1500-2000 μg/24 h on 0·9 g D-penicillamine daily. Two years later the dose was increased to 1·8 g daily and urinary copper excretion rose to 2300-2800 μg/24 h.

The serum caeruloplasmin on this occasion had fallen to 7·2 mg/dl (normal > 20 mg/dl). In 1968, she became pregnant but developed pre-eclamptic toxaemia, and intrauterine death resulted at the 12th week. Although D-penicillamine therapy was maintained throughout the pregnancy no fetal abnormalities were noted. A live male baby was born by Caesarean section in 1970, but in the following year a further pregnancy was terminated due to toxaemia, and a tubal ligation was performed.

In 1973, after 13 years of chelation therapy, the haemoglobin level was 14·2 g/dl with the red cell indices, total white cell and platelet counts normal. The urinary copper excretion on 1·2 g D-penicillamine HCl had diminished to 300-550 μg/24 h, and the Kayser-Fleischer rings were still visible on slit-lamp examination. In view of these findings, treat-

ment was changed to 2·0 g D-penicillamine base daily and the urinary copper clearance was increased to 800-1200 μg/24 h. Four months later the patient was admitted to hospital with a history of increasing lethargy, exertional dyspnoea, and giddiness of six weeks' duration. The haemoglobin concentration was 4·0 g/dl (figure) with 2% reticulocytes. The red cells were macrocytic with moderate poikilocytosis and some oval cells and a MCV of 106 fl; white cells and platelets were normal. The faeces were negative for occult blood and there was no evidence of blood loss from other sources. A ^{51}Cr-labelled red cell survival time was normal, with a negative Coombs' test and antinuclear factor, and a serum copper concentration of 5 μg/dl (normal 80-150 μg/dl). An assay for the measurement of plasma D-penicilla-mine level was not available at this time. The serum and red cell folate and serum vitamin B_{12} levels were normal with negative parietal cell and intrinsic factor antibody tests. Ham's test was negative. Pyridoxine deficiency was excluded by the measurement of urinary xanthurenic and kynurenic acid excretion before and after a tryptophan load. The results were normal. The serum zinc concentration was normal (125 μg/dl). Sternal bone marrow biopsy showed normal cellularity with an M:E ratio of 4:1. Granu-

lopoiesis and megakaryocytes were normal. Erythro-poiesis was normoblastic and some of the inter-mediate and late normoblasts showed nuclear distor-tion and karyorrhexis and a few contained Howell-Jolly bodies. Iron stores were increased but no ring sideroblasts were present. Liver function tests and clotting studies were normal. The hepatic copper concentration had decreased to 188 μg/g dry weight. The serum immunoglobulins were normal apart from an IgA level of 32 mg/dl (normal 100-450 mg/dl). The patient was transfused 7 units of packed cells and was discharged from hospital with a haemoglobin of 12·4 g/dl (figure) on 2·0 g D-penicillamine base and 100 mg pyridoxine daily.

The symptoms of anaemia returned, and eight weeks after transfusion the haemoglobin was 6·8 g/dl with an absolute reticulocyte count of 0·2% and a normal white cell and platelet count. A further sternal marrow examination showed no significant change.

SPECIAL STUDIES

In vitro bone marrow colony growth in agar-gel medium was carried out according to Pike and Robinson (1970) with and without addition of D-penicillamine HCl and D-penicillamine base. Bone marrow was incubated with D-penicillamine for 1·5 h before it was washed and cultured. There was no evidence of *in vitro* suppression of leucopoiesis by D-penicillamine HCl or D-penicillamine base (table I).

The *in vitro* effect of D-penicillamine on haem synthesis by marrow cells was studied by measuring ^{59}Fe uptake for haem synthesis by marrow erythro-blasts (Krantz *et al*, 1963). Marrow was obtained from the patient and from a healthy volunteer. The patient's plasma and normal ABO compatible plasma were used in the culture of the patient's marrow cells. The volunteer's plasma and ABO compatible plasma were used for culturing the volunteer's marrow cells. Each test was done with and without addition of D-penicillamine base. There was inhibition by D-penicillamine of ^{59}Fe incorporation into the patient's marrow erythroblast haem, but similar inhibition was observed in the normal control marrow erythroblasts (table II). Also there was no evidence that the patient's plasma
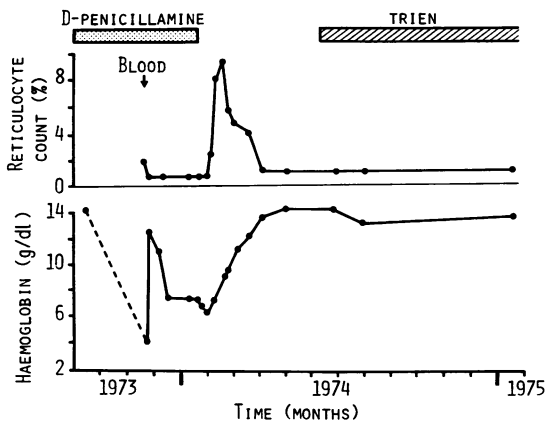

Figure *Haemoglobin concentration and reticulocyte count in relation to drug therapy.*

Material	Colonies/8 × 10⁴ Nucleated Cells	Colony-forming Units in Culture/1 × 10⁶
Bone marrow and saline	22·0	275
Bone marrow + D-penicillamine base (50 μg/ml of marrow)	17·4	217
Bone marrow + D-penicillamine HCl (50 μg/ml of marrow)	18·4	230

Table I *Effect of D-penicillamine on haemopoietic cell culture in agar*

D-Penicillamine Base	Counts/minute			Plasma
	⁵⁹Fe Total	*⁵⁹Fe in Haem*	*⁵⁹Fe in Protein*	
Without	98 440	308	586	
	90 977	138	325	
	95 136	174	366	Patient's
With (50 μg/ml of culture)	90 450	21	107	
	93 804	32	172	
	92 845	31	254	
Without	80 875	109	192	
	82 911	115	189	
	82 524	98	204	ABO compatible
With (50 μg/ml of culture)	82 117	30	174	
	78 810	29	89	
	82 205	29	108	
Without	97 178	118	239	
	99 400	139	241	
	102 248	142	255	Volunteer's
With (50 μg/ml of culture)	100 606	28	71	
	101 265	27	61	
Without	93 222	118	228	
	96 701	100	207	ABO compatible
With (50 μg/ml of culture)	98 072	26	75	

Table II *Effect of D-penicillamine on ⁵⁹Fe uptake* in vitro *by bone marrow cells*

contained an antibody or other inhibitory factor to haem synthesis in her own marrow.

Transformation of the patient's lymphocytes, assessed by ³H-thymidine uptake with phytohaemagglutinin (PHA) (Das and Hoffbrand, 1970), was much less than in a normal control but on addition of D-penicillamine base there was comparable inhibition of ³H-thymidine uptake into the patient's and control lymphocytes. There was no evidence that D-penicillamine base itself caused transformation of the patient's lymphocytes assessed morphologically and by ³H-thymidine uptake (table III).

Although these *in vitro* studies did not suggest that the patient's haemopoietic tissues were particularly susceptible to D-penicillamine, the drug was discontinued. Over the ensuing two weeks the haemoglobin concentration stabilized, and during the third week a reticulocyte response became evident (figure). This continued until 10 weeks later when the haemoglobin reached normal levels (13·6 g/dl).

Chelation therapy was discontinued for a total of 14 weeks during which time the patient remained asymptomatic with normal liver function tests and

a urinary copper concentration repeatedly in the normal range (14-45 μg/24 h). In view of the potential hazard of a permanent red cell aplasia, which may have been induced by the reintroduction of D-penicillamine, in June 1974, treatment was begun with the alternative chelating agent, triethylenetetramine dihydrochloride (Trien). The drug was prepared by the procedure of Dixon *et al* (1972) and was introduced over a four-week period to an optimum dose of 1·6 g daily. This produced an inital urinary copper excretion of 600 μg/24 h which subsequently has been maintained at approximately 300 μg/24 h. Urinary iron excretion has remained normal on triethylenetetramine dihydrochloride (less than 5 μg/24 h). Currently the patient is asymptomatic with a normal haemoglobin concentration. The serum IgA level remains low, but transformation of lymphocytes with PHA has reverted to normal.

Discussion

The moderate degrees of thrombocytopenia, neutropenia, and, less frequently, eosinophilia and thrombocytosis which may occur shortly after the initiation of D-penicillamine therapy are rarely severe enough to warrant withdrawal of the drug (Walshe, 1963; Jaffe, 1965; Fawcett *et al*, 1966; Deiss *et al*, 1971; Multi-Centre Trial Group, 1973). Agranulocytosis, which is observed more often in patients with rheumatoid arthritis than Wilson's disease, is usually reversible on cessation of the drug (Payne and Cahill, 1969; Golding *et al*, 1970; Lièvre *et al*, 1971), although the outcome may be fatal (Corcos *et*

	³H-Thymidine Uptake/10⁶ Cells (counts/min)		
	– PHA	*+ PHA*	*+ D-Penicillamine Base (50 μg/ml culture)*
Patient	92	482	163
Control	162	8412	177

Table III *Lymphocyte transformation with PHA and D-penicillamine*

al, 1964). Aplastic anaemia is a rare development, usually during the first year of therapy (Bird, 1974; Cramér *et al*, 1974) and, as with the other haematological effects of D-penicillamine, the reaction appears to be idiosyncratic rather than cytotoxic. Pyridoxine antagonism may be demonstrated in patients on D-penicillamine (Jaffe *et al*, 1964) and pre-existing pyridoxine deficiency may be exacerbated (Swaiman and Milstein, 1970). However, the antipyridoxine activity of the drug is slight and does not appear to cause clinical signs of deficiency or anaemia. It has been shown that the chelating action of the drug does not influence the serum iron concentration (Walshe and Patston, 1965), although deficiency may develop as the result of variceal haemorrhage from portal hypertension or following haematuria from glomerulonephritis, which is a rare adverse reaction to D-penicillamine therapy (Sternlieb *et al*, 1975). Finally, patients on the drug may develop a lupus-like syndrome with positive tests for antinuclear factor and LE cells. Haematological abnormalities, apart from those due to alimentary blood loss, may occasionally be observed in untreated cases of Wilson's disease. Intermittent haemolytic episodes may frequently pass unrecognized or precede other manifestations of the disease (McIntyre *et al*, 1967; Deiss *et al*, 1970; Iser *et al*, 1974), and pancytopenia has also been reported (Heinrich and Kaltenbach, 1965).

The development of anaemia, in this case, appeared to correspond with an increase in the dose of D-penicillamine and a change to the base form of the drug after 14 years' treatment without any adverse reaction on the hydrochloride. Within 10 weeks of this change, the patient was symptomatic and by 16 weeks the haemoglobin had fallen to 4 g/dl. D-penicillamine induced red cell aplasia was considered in view of the absent reticulocyte response and after investigations had excluded blood loss, haemolysis, a lupus-like reaction, and deficiencies of folate, vitamin B_{12}, and pyridoxine. No other drugs or potential marrow suppressive agents had been taken by the patient.

Pure red cell aplasia has been described following viral infections, complicating haemolytic anaemia and uraemia and with riboflavin deficiency in children with kwashiorkor. Many drugs and chemicals have also been implicated, but, in most cases, withdrawal of the agent resulted in remission (Mitchell, 1973). Immunological abnormalities have been observed in some cases in association with a thymoma and an IgG autoantibody directed against the patient's erythroblasts (Krantz, 1974). In the present study, D-penicillamine produced equal inhibition of ^{59}Fe incorporation into erythroblast haem of both the patient and control marrows. The

drug did not cause lymphocyte transformation and although a diminished response to PHA was observed during the period of red cell aplasia, the significance of this is uncertain with regard to pathogenesis. The mechanism of drug toxicity thus remains undetermined.

The presence of substantial numbers of red cell precursors in the bone marrow was surprising in view of the low reticulocyte count and severe anaemia. It may be explained by patchy loss of erythropoiesis in the marrow which resulted in a biopsy sampling error. It is also possible that erythroblast development was inhibited at a relatively late stage. Before ferrokinetic studies or ^{52}Fe marrow scans could be performed, however, marrow recovery occurred. The reticulocyte count rose three weeks after the cessation of D-penicillamine, and a normal haemoglobin level was achieved within 10 weeks. As a permanent red cell aplasia may well have resulted from the reintroduction of D-penicillamine, administration of the alternative chelating agent, triethylenetetramine dihydrochloride (Walshe, 1969), was begun and has been continued for over 12 months with the maintenance of a normal haemoglobin concentration and adequate urinary copper excretion.

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