Erythropoietin Concentration in Amphotericin B-Induced Anemia

ROB R. MacGREGOR,[1]* JOHN E. BENNETT,[2] AND ALLAN J. ERSLEV[3]

Infectious Diseases Section, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174[1]; Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Disease, Bethesda, Bethesda, Maryland 20014[2]; and The Cardeza Foundation, Thomas Jefferson University, Philadelphia, Pennsylvania 19107[3]

Amphotericin B was given to six patients with systemic fungal infections. A dose averaging 1.78 g, administered from 42 to 144 days, was associated with a fall in hematocrit to a mean value of 25.8%. Despite this degree of anemia, no elevation of erythropoietin concentrations in urine or serum could be detected. Thus, amphotericin appears to cause anemia by inhibiting erythropoietin production rather than by suppressing bone marrow activity directly.

Amphotericin B is an amphoteric polyene antibiotic which has been the mainstay of therapy against the systemic mycoses since the late 1950s (3). Although it can be a life-saving drug, its use is limited by significant toxic reactions: fever, malaise, anorexia, phlebitis, azotemia, hypokalemia, and anemia occur commonly (3–5, 11). The anemia is normocytic and normochromic, with a reticulocyte count inappropriately low for the degree of hemoglobin reduction (4). Although there is some decrease in erythrocyte survival associated with the early stages of amphotericin B treatment of systemic mycoses (4), the cause for the amphotericin-induced anemia appears to be reduced erythrocyte production. This poor erythroid production could result either from a direct toxic effect of amphotericin on the marrow, or secondary to a suppression of erythropoietin production by amphotericin.

In this study, we measured erythropoietin levels in serum and urine of patients at the nadir of their amphotericin-induced anemia. The degree of anemia was sufficient to stimulate elevated erythropoietin levels if the anemia had resulted from direct drug inhibition of marrow production. In contrast, if amphotericin caused anemia by inhibiting normal erythropoietin production, the levels would be low.

MATERIALS AND METHODS

The study subjects all received amphotericin B therapy for systemic mycoses, treatment occurring either at the Clinical Center, National Institutes of Health, or at the Hospital of the University of Pennsylvania. Table 1 lists their clinical characteristics. The average age was 43 (range 19 to 72). Only subject 1 had a marked anemia before therapy, having just entered remission of acute lymphocytic leukemia as a result of combination chemotherapy. Although his hematocrit was only 26% at the onset of amphotericin B therapy, his reticulocyte count was 5.6% and his marrow demonstrated erythroid hyperplasia with no evidence of leukemia. Underlying diseases in the study subjects included leukemia, alcoholism, and inactive tuberculosis; three subjects had no associated illnesses. The average total dose of amphotericin received up to the time of erythropoietin assay was 1.78 g (range 0.84 to 2.46), and the hematocrits on the day of assay averaged 25.8% (range 23 to 28). Serum urea nitrogen/creatinine averaged 12.3/0.87 mg/dl (range 8/0.6 to 21/1.2) before amphotericin, and at the time of study 23.7/1.52 mg/dl (range 14/1.1 to 36/2.0).

On the day of assay 5 ml of serum was obtained and frozen at −20°C, and a 24-h urine collection was begun. On completion of the collection, both specimens were shipped on dry ice to the Cardeza Foundation laboratory for assay.

The erythropoietin assay utilized has been described in detail elsewhere (8). Briefly, adult mice are rendered polycythemic by transfusion (to suppress endogenous erythropoietin production) and then injected subcutaneously with 1.0 ml of the material to be assayed. In the current study, this material consisted of undiluted serum or urine concentrated 50-fold by dialysis against carbowax. A 0.5-μCi dose of Fe^{59} is injected intraperitoneally and 66 h later blood is obtained to determine iron utilization. Using a curve relating various concentrations on an erythropoietin standard (Standard B International Reference Preparation, Medical Research Council Division of Biological Standards, National Institute for Medical Research, Mill Hill, London) to the 66-h utilization of Fe^{59}, one can estimate the concentration of erythropoietin per milliliter of material assayed.

The minimum concentration of erythropoietin which the assay can detect is >0.05 U/ml of serum and >1.5 U/24 h of urine. Only one of the patients (no. 3) had a concentration in serum or urine which exceeded this threshold (Table 1). His amphotericin treatment had ended 30 days before specimens were

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TABLE 1. Clinical characteristics of study subjects

Patient no.	Timing of test[a]	Duration of therapy (days)	Time of assay	Total dose (g)	Hematocrit	Retics	Blood urea nitrogen	Creatinine	Erythropoietin (U)[b]		Comments
									Urine	Serum	
1	Pre	54	Last day of amphotericin	2.4	26	5.6%	10	0.6	<1.5	<0.05	Leukemia just entered remission secondary to chemotherapy when cryptococcal meningitis appeared
	Post				23	0.1	32	1.1			
2	Pre	69	3 days after stopping amphotericin	1.9	38	1.2	14	1.0			Cryptococcal meningitis
	Post				27	0.5	36	2.0	Toxic	<0.05	
3	Pre	61	30 days after	1.67	39	0.5	12	1.2			Mediastinal histoplasmosis
	Post				26	0.2	15	1.6	1.5	0.18	
4	Pre	144	3 days after	2.46	33		8	0.7			Alcoholism with pancreatic pseudocyst; pulmonary sporotrichosis
	Post				26	0.5	14	1.8	<1.5	<0.05	
5	Pre	42	3 days after	0.84	36		9	0.8			Inactive silicotuberculosis; cryptococcal meningitis. 5-fluorocytocine (150 mg/kg per day also)
	Post				28		29	1.3	<1.5	<0.05	
6	Pre	82	Last day	1.4	40	0.3	21	0.9			Cryptococcal meningitis; 5-fluorocytocine (150 mg/kg per day also)
	Post				25	1.0	16	1.3	Toxic	<0.05	

[a] Pre, Pre-amphotericin; Post, immediately post-amphotericin.
[b] Assay sensitive to >1.5 U/24 h in urine, >0.05 U/ml in serum. Levels in normal individuals are below these values.

obtained for assay, a time interval during which hematocrit recovery often has begun (1). In all other patients, at a point where the degree of anemia would be expected to stimulate the production of high erythropoietin levels, none could be detected in either serum or 24-h urine collections.

DISCUSSION

Anemia occurs in more than 95% of patients requiring prolonged amphotericin B therapy (4, 10). In one study, the mean hematocrit fell from 41% before therapy to 27% at the completion of therapy (4). Defective iron reutilization is present in many systemic mycotic infections and may in part be responsible for their anemia of chronic infection (6, 7). However, the anemia of amphotericin B therapy is usually time related to drug administration and not to the underlying disease, and so far the characteristic combination of a low serum iron and increased iron stores in the bone marrow has not been described. Another pathogenic possibility is hemolysis with shortened erythrocyte survival caused by the direct action of amphotericin on circulating cells (9). However, Brandriss et al. have reported that patients had a slightly reduced erythrocyte survival prior to initiation of amphotericin B therapy (thought to be related to their systemic fungal infections), and no further decrease occurred whem amphotericin was begun (4). Furthermore, the hemolytic process would be expected to result in reticulocytosis instead of the reticulocytopenia usually seen. Consequently, it has been concluded that the anemia is caused by a drug-induced suppression of erythrocyte production rather than an increased rate of destruction. This conclusion is supported by the fact that the anemia is normocytic and normochromic, and that bone marrow examination, reticulocyte counts, and ferrokinetic studies fail to reveal a compensatory erythropoietic response to the anemia.

A direct toxic suppression of bone marrow cell production by amphotericin B is a possibility. However, the results reported here indicate that the anemia is associated with a reduced production of the stimulating hormone erythropoietin rather than with the increased production usually seen in primary hypoplastic anemias. None of our patients had a detectable level of erythropoietin in serum or urine at a time when the mean hematocrit value was 26%. A normal erythropoietin response at that degree of anemia raises the serum level to 0.3 to 1.0 U/ml and the daily excretion of erythropoietin in urine to about 10 to 30 U (1, 7, 8). The laboratory performing the tests in this report has observed such elevated levels of erythropoietin in essentially all patients with hematocrits in this range, unless renal impairment has been present. Con-

sequently, it appears that the anemia of amphotericin B therapy is caused by a relative failure of erythropoietin production. It has been reported variously that chronic active infection causes a severe reduction (12), a borderline reduction (7), and no reduction (2) in erythropoietin. However, at the time when the patients had developed anemia and had no measurable erythropoietin in their blood, they had no clinical evidence of active infection. Two of the patients were also receiving 5-fluorocytosine, a drug which can induce anemia by direct suppression of marrow production. Such suppression, however, should have been an additional stimulus for erythropoietin production and cannot explain our findings.

A direct inhibition of the assay by amphotericin in the serum or urine being assayed is unlikely because four of the patients tested had completed therapy 3 to 30 days before specimens were obtained for assay, so that the maximum possible amount of amphotericin injected was trivial.

The observed erythropoietin deficiency anemia mimics that of chronic renal failure and may be secondary to amphotericin's nephrotoxic action. Although Brandriss et al. found no correlation between the degrees of azotemia and anemia (4), Miller and Bates (10) reported that their patients with the most marked increase in blood urea nitrogen during therapy had the greatest decrease in hematocrit ($P < 0.01$). In our patients, the mean blood urea nitrogen of 25.2 and creatinine of 1.6 mg/dl were only mildly increased, and it seems unlikely that this degree of renal damage or uremia could be responsible for the severe anemia. However, it has to be conceded that we do not know the exact mode or site of erythropoietin production in the kidneys and it is possible that amphotericin exerts a selective inhibition of erythropoietin production there.

Regardless of the mechanism, erythropoietin was undetectable in our patients at the nadir of their anemia, and this deficiency appears to make a major contribution to the anemia.

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