Erythropoietic Effect of Plasma from Patients with Advanced Cancer[1]

Dincer Firat[2] and Jose Banzon[3]

Department of Medicine, Long Island Jewish Medical Center-Queens Hospital Center, Jamaica, New York 11432

SUMMARY

The erythropoietic effect of plasma from anemic and nonanemic patients with various cancers was determined by increase in percentage of incorporation of injected ^{59}Fe into circulating RBC's of protein-deprived CF_1 adult female mice in 48 hr. In 14 anemic patients with lymphoma or leukemia and in 12 anemic patients with solid tumors, the erythropoietic effect of plasma was significantly reduced compared to 12 normal plasmas but was increased compared to 0.9% NaCl solution controls ($p < 0.001$). In contrast, the erythropoietic effect of plasma in 8 nonanemic patients (4 lymphomas or leukemias and 4 solid tumors) was similar to normal controls but was significantly different from results in their anemic counterparts ($p < 0.001$).

INTRODUCTION

Anemia is a common finding in patients with cancers. In most cases, the anemia constitutes a part of the disease, and often it is due to decreased or ineffective erythropoiesis (2, 7, 10, 23). The reason behind this impairment of the marrow response to anemia is not clear, although decreased production or availability of erythropoietic hormone has been postulated (23). However, reports concerning plasma erythropoietin levels in anemia of cancer have been conflicting (8, 15, 22). The possibility of interference with action or production of erythropoietin by metabolic products of the tumor or by an antibody or inhibitor against erythropoietin or stem cells of the marrow has also been postulated (2, 6, 11, 12).

This study compares EEP[4] obtained from anemic and nonanemic cancer patients to that of normal plasma and explores the possibility that plasma of cancer patients interferes with the activity of exogenous erythropoietin.

MATERIALS AND METHODS

EEP from 26 anemic (Hb < 10 g/100 ml) and 8

nonanemic (Hb > 10 g/100 ml) patients with various cancers, as well as the EEP from 12 normal persons, has been studied (Table 1). Patients with anemia due to causes other than cancer were excluded by appropriate findings. The extent of disease was comparable in both groups, although anemic patients generally had more active disease. Patients in terminal states and those receiving transfusions or myelosuppressive therapy within 4 weeks of the study were excluded.

Complete hemograms and serum protein measurements were obtained for all patients. In addition, serum vitamin B_{12}, folic acid, and iron levels were determined in 18 patients with lymphoma or leukemia. Bone marrow aspirations were performed in 25 patients and were examined for possible infiltration with malignant cells, cellularity, and iron stores.

EEP was measured by a modified bioassay with the use of protein-deprived CF_1 adult female mice; EEP was expressed as percentage of injected ^{59}Fe (ferrous citrate, ^{59}Fe, Mallinckrodt Nuclear, St. Louis, Mo.) incorporated in 48 hr into circulating RBC's of mice. Animals weighing 20 to 25 g were fed a protein-free diet (Nutritional Biochemicals Corp. Cleveland, Ohio) for 4 days in order to reduce endogenous erythropoietin production, similar to starved, protein-deprived, or hypophysectomized rat assays (9, 20). They were then placed on a regular diet (Rockland mouse diet-Purina laboratory chow, Ralston Purina Co., St Louis, Mo.) for the rest of the experiments. Water was given *ad libitum.* On the 4th and 5th days of the experiment, mice were given i.p. injections of 0.5 ml plasma. Radioactive ^{59}Fe (0.5 μCi in 0.5 ml of 0.9% NaCl solution) was injected i.p. into each mouse on the 6th day; 48 hr later, the animals were briefly anesthetized with ether and sacrificed by exsanguination. The radioactivity of 0.5 ml of whole blood was then determined in a well-type scintillation counter and the percentage of incorporation of injected ^{59}Fe (standard solution) into circulating blood was calculated. The total circulating blood of the mouse after 4 days of protein-deprivation diet was calculated to be 5.5% of the body weight, based on determinations made by exsanguination. For each experiment, 5 to 6 animals were used, and 0.9% NaCl solution controls were included throughout.

The sensitivity of the assay animals to various doses of exogenous erythropoietin was tested by injecting 0.01 to 4 units of Step 3 sheep erythropoietin (Connaught Laboratories, Willowdale, Ontario, Canada).

In the 2nd part of this study, plasma from 4 anemic patients was injected into mice together with 0.1 unit of erythropoietin; as control, 0.1 unit of erythropoietin was injected alone.

Table 1
Diagnosis and bone marrow findings in
34 patients with advanced cancer

| | | | Bone marrow | | |
Diagnosis	No. of patients	Cellularity	*m/e* ratio	Infiltration	Iron
Lymphoma					
Anemic	8	Normal	Normal	0	2^+–4^+
Nonanemic	4	Normal	Normal	0	2^+–4^+
Leukemia, anemic	6	Increased	Increased	6	2^+–4^+
Breast cancer					
Anemic	4	Normal	Normal	2	2^+
Nonanemic	1	Normal	Normal	1	2^+
Prostate cancer, anemic	2	Normal	Normal	1	2^+
Other solid tumors					
Anemic	6	*a*	*a*	*a*	*a*
Nonanemic	3	*a*	*a*	*a*	*a*

a Bone marrow examination was not performed.

RESULTS

The average total serum protein in both groups of patients was 7 ± 1 g/100 ml. In 18 patients with lymphoma and leukemia, serum B_{12}, folic acid, and iron levels were within normal limits. The bone marrow, examined in 25 of 35 patients, revealed normal cellularity with normal or increased iron stores in all (Table 1). Infiltration of marrow by malignant cells was present in 6 patients with leukemia, 2 patients with breast cancer, and 1 patient with prostatic cancer.

The results of EEP from 34 cancer patients and 12 controls indicated that EEP was proportionately related to the blood Hb level (Chart 1). Although individual variations occurred, when EEP was plotted against Hb levels, there seemed to be 2 distinct groupings below and above the Hb level of 10 g/100 ml.

In 14 anemic patients with lymphoma or leukemia (mean Hb 8.7 g/100 ml) and 12 anemic patients with solid tumors (mean Hb 7.5 g/100 ml), EEP was significantly reduced ($8.7 \pm 2\%$ and $9.2 \pm 2.4\%$, respectively) compared to 12 normal plasmas ($15.4 \pm 1.7\%$, $p < 0.001$) but was increased compared to 0.9% NaCl solution controls ($2.6 \pm 2.1\%$, $p < 0.001$). In contrast, EEP in 8 nonanemic patients (4 lymphomas or leukemias and 4 solid tumors) was similar to normal controls ($16.2 \pm 2.8\%$ and $14 \pm 1.9\%$, respectively) but was significantly different from their anemic counterparts ($p < 0.001$) (Table 2).

There were no significant differences in EEP among diagnostic groups by sex or between those with or without evidence of bone marrow infiltration. Age did not influence the results.

The minimum amount of erythropoietin which could be detected by this assay was 0.02 or 0.1 unit with either 1 or 2 standard deviations (Table 2).

Average EEP of 4 additional plasmas from anemic cancer patients with and without addition of 0.1 unit of erythropoetin was $13.5 \pm 2\%$ and $7.6 \pm 1.6\%$, respectively, while erythropoietic effects of 0.1 unit of erythropoietin alone was $13.5 \pm 3\%$ (Chart 2).
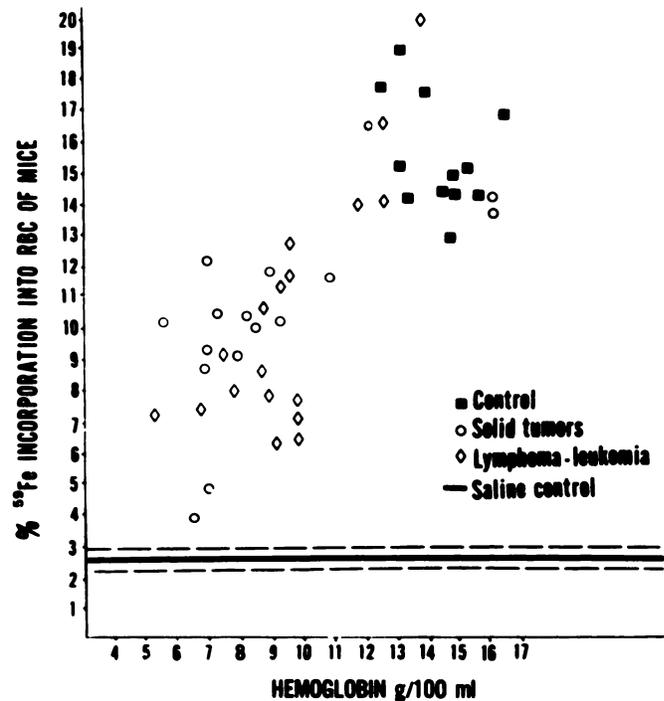

Chart 1. Scattergram illustrating erythropoietic effect of plasma against Hb level of each individual. - - -, 1 S.D. from 0.9% NaCl solution control.

DISCUSSION

Although complications of the underlying disease, such as infection, uremia, blood loss and myelosuppresive therapy or concomitant primary blood disorders, may play a role, the mechanism of the anemia of cancer is often difficult to assess. Exclusive of blood loss, the pathogenesis of such anemia must involve a decreased rate of red cell production, an increased rate of destruction, or a combination of both. Although decreased life-span of red cells has been documented in cancer patients, this finding is often absent and even when present is

rarely severe enough to overcome functional erythropoietic reserve of normal bone marrow and account for anemia (3, 10, 14, 23). Therefore, impairment of the marrow response to anemia must be present.

One reason for ineffective erythropoiesis in anemia of cancer may be the presence of inhibitors or antibodies against erythropoietin. Interference with action of erythropoietin by catabolic products of the tumor or by inappropriate secretion of a physiological inhibitor normally involved in regulation of red cell production has been postulated (2, 6, 11). A factor that suppressed production of marrow stem cells, but not the effect of erythropoietin, has been demonstrated in a patient with Hodgkin's disease (6). There was no evidence of an antibody. Others suggest the presence of an antibody in plasma which may neutralize or block the action of erythropoietin (11, 12). However, Ward *et al.* (22) were unable to show such an antibody against erythropoietin in 2 cancer patients with anemia. The plasma from 4 of our anemic patients neither suppressed the action of injected

erythropoietin nor showed an additive effect (Chart 2). Although this could not rule out the presence of an erythropoietin-antibody complex with little or no excess antibody, the interference with action of erythropoietin by a significant amount of the catabolic products of the tumor or inhibitors seems unlikely. Since erythropoietin and the antibodies against it are not species specific (16, 21), the possibility of a species-specific antibody blocking the action of erythropoietin at the cellular level (12) could also be ruled out. A more sensitive assay with smaller quantities of erythropoietin may reveal the presence of minute amounts of inhibitors or antibodies.

The lack of the additive effect of plasma and erythropoietin could be explained by a very low erythropoietin content of plasma undetectable by the method used. In fact, increased EEP of these patients compared to 0.9% NaCl solution may be attributed to nonspecific stimulation of the erythropoiesis in mice by foreign proteins (13).

Differences in EEP from anemic and nonanemic patients may be due to a variety of factors. Rapid replacement of proteins and B complex vitamins of test animals by the contents of injected plasma or nonspecific protein products (16) could influence the results. However, this could not account for the difference between anemic and nonanemic groups, since their total serum protein, B_{12}, folic acid, and iron levels were similar. Furthermore, neither vitamin B complex injections (Combex, 0.1 ml, Parke-Davis Co., Detroit, Mich.) nor 100 mg of blood hydrolysates (Nutritional Biochemicals) produced a significant increase of ^{59}Fe uptake in mice (2 and 2.2%, respectively). Loss of weight *per se* could not play a role, since in 2 markedly underweight patients without cancer or anemia, EEP was within normal limits (Table 3).

Another reason for low EEP in anemic cancer patients could be decreased production or availability of the erythropoietic hormone, erythropoietin. Earlier reports indicated that there was a normal or increased erythropoietin level in plasma of cancer patients (1, 8, 15). However, the details concerning possible contributory factors (*e.g.*, hemolysis, blood loss,

Table 2
Erythropoietic effect of plasma from patients with cancer

Type of plasma	No. of patients	Mean Hb g/100 ml	Mean ^{59}Fe incorporation into RBC of mice (%)
Normal controls	12	14.2 ± 1.1[a]	15.4 ± 1.7[a]
NaCl solution controls			2.6 ± 2.1[b]
Lymphomas	14	8.7 ± 1	8.7 ± 2
Leukemias	4	12.8 ± 0.8	16.2 ± 2.8
Solid tumors	12	7.5 ± 1	9.2 ± 2.4
	4	13.5 ± 2.3	14 ± 1.9
Erythropoietin (Step 3)			
0.02 unit			8.2 ± 2
0.1 unit			14 ± 2
0.2 unit			19.4 ± 2.4
1.0 unit			26 ± 3.6

[a] Mean ± S.D.
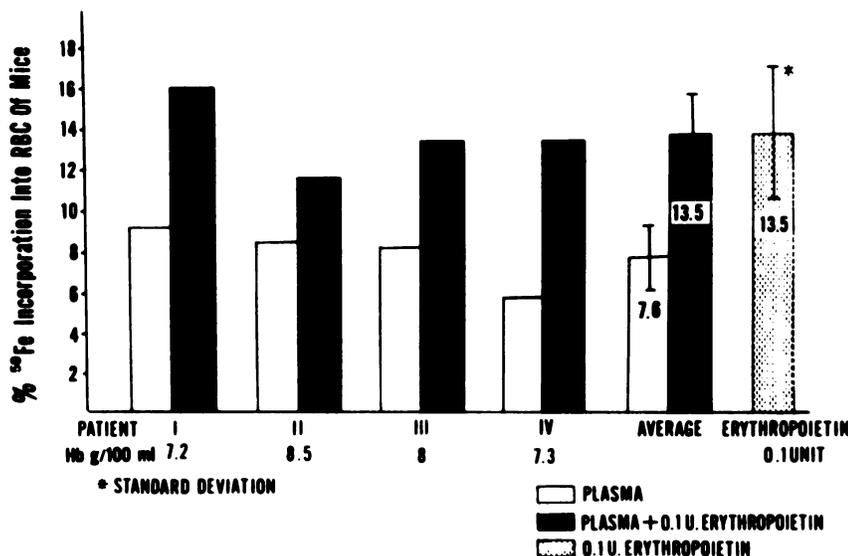[b] Represents mean value of 50 controls.

Chart 2. Erythropoietic effect of 0.1 unit of erythropoietin alone compared to effect of anemic plasma with or without addition of 0.1 unit of erythropoietin. Note lack of interference with activity of erythropoietin by anemic plasma from cancer patients.

Table 3

Erythropoietic effect of plasma from various patients without cancer

Diagnosis	Hb (g/100 ml)	Mean ^{59}Fe incorporation into RBC of mice (%)
Sickle cell disease (aplastic crisis)	6.5	23.7, 31.8[a]
Sickle cell disease	11	19.5[b]
Renal cyst (polycythemia)	16.8	22.4
Secondary polycythemia (congestive heart failure)	19	20.7
Cachexia (organic brain syndrome, arteriosclerotic heart disease)	11.7	15.5
Cachexia (organic brain syndrome, arteriosclerotic heart disease)	13	16.6
Polycythemia vera	18	8.5
Aplastic anemia	6	41

[a] Two assays performed 1 week apart.
[b] During recovery phase of aplastic crisis.

anoxia) and the degree of anemia were often not available. Increased level of another erythropoietic factor in plasma of anemic cancer patients has also been reported (18). It is known that certain animal and human tumors contain and secrete an excessive amount of erythropoietin and may produce polycythemia (1, 5, 17). Recently, decreased erythropoietin levels were demonstrated in 7 anemic cancer patients by a polycythemic CF_1 female mouse assay; in 5 others, serum erythropoietin levels were elevated (22). The sensitivity of our assay method compares favorably with the polycythemic mouse assay (0.02 to 0.1 and 0.05 unit can be detected, respectively) (4). Furthermore, plasma erythropoietin levels detected by our method in patients with other hematological disorders confirm those measured by other methods (Table 3). The starved rat assay used in earlier studies is a less sensitive assay (4).

We may therefore conclude that the erythropoietin level of plasma in anemic cancer patients is reduced. Whether utilization of erythropoietin or its precursors by the tumor tissue or a change in its renal excretion (rather than decreased production) is responsible cannot be stated since kinetic studies were not performed. However, these patients may have several factors causing decreased production of erythropoietin in spite of anemia and occasionally myelophthisis. Negative nitrogen balance, common in patients with cancer, has been shown to reduce production of erythropoietin (19). Another factor may be the relatively decreased oxygen need by tissues for slowed general metabolic processes in these patients, similar to that demonstrated during protein-free diets and starvation (20). Clinical observations lend support to the hypothesis that there is a new physiological level of Hb which is capable of carrying enough oxygen for reduced metabolic processes. This is supported by the observation that the Hb level in anemic cancer patients often returns to the original level when lowered by treatment or blood loss or when increased by multiple transfusions.

An impairment in the mechanism of release of mature RBC's from bone marrow may also be considered, since in the majority of patients the erythroid elements of the marrow were normal. Deficiency of such a factor, which has also been postulated in the etiology of anemia due to cancer, deserves further study (7).

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