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Defective Erythropoiesis in Bone Marrow is a Mechanism of Anemia in Children with Cancer

Evaluation of the mechanism of anemia in cancer patients might help to select patients for the more efficient use of erythropoietin (EPO, a growth factor for erythroid precursor cells). For this, we investigated whether the production of EPO responds to anemia and the bone marrow responds to EPO appropriately, and whether chronic inflammation is inhibitory to erythropoiesis in anemic cancer children. Serum levels of EPO, soluble transferrin receptor (sTfR), tumor necrosis factor (TNF)-α, and erythrocyte sedimentation rate (ESR) in anemic cancer children were measured by enzyme-linked immunosorbent assay and then the correlation coefficients between those parameters and hemoglobin (Hb) were determined. Both in leukemia and in solid tumor patients, there were significant inverse correlations between Hb and EPO (leukemia: tau=-0.547, p<0.0001; solid tumor: tau=-0.591, p<0.0001), and between sTfR and EPO (leukemia: tau=-0.223, p< 0.05; solid tumor: tau=-0.401, p<0.05). In contrast, sTfR showed a correlation with Hb in leukemia (tau=0.216, p<0.05) but not in solid tumor patients. sTfR was suppressed in 53% of anemic episodes of leukemia and 78% of those of solid tumor patients. Our results suggest that in cancer children, the EPO production is not defective and chronic inflammation is not inhibitory to erythropoiesis. Rather, the defective erythropoiesis itself is thought to be responsible for the anemia.

Key Words : *Erythropoiesis; Erythropoietin; Neoplasms; Anemia*

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INTRODUCTION

Anemia is one of the most common complications in cancer patients, resulting from the suppression of hematopoiesis by the cancer itself or therapeutic interventions, chronic nature of the disease itself, recurrent infections due to suppressed immunity, or impaired iron utilization (1, 2). Since anemia adversely affects not only the patients' daily performance status but also sensitivity to treatment and patients' prognosis (3, 4), correction of the anemia is mandatory. Recently the use of blood transfusion to correct anemia has been limited because it is related to allergic hypersensitivity, transmission of transfusion-mediated diseases, immune suppression, and even to recurrence of cancer (5).

Recombinant human erythropoietin (rhEPO) was first shown to be effective for anemia of patients with chronic renal failure in 1985, and since 1990, rhEPO has been tried for the prevention or treatment of anemia in cancer patients. In contrast to the chronic renal failure patients whose anemia is due to the decreased production of erythropoietin (EPO), anemic cancer patients responded to rhEPO only in 40-85% (2, 6, 7). Although there have been reports that higher doses of rhEPO could overcome the EPO resistance (2, 8), the economic burden of rhEPO usage urged us to select proper candidates for the use of rhEPO. Knowledge of the factors affecting hematopoiesis in anemic cancer patients might render the more efficient use of rhEPO and, if there are any factors that inhibit the EPO action, it might further suggest the way to overcome those inhibitions.

EPO is the most important factor for erythropoiesis produced in the kidney responding primarily to the tissue hypoxia and increases erythropoiesis by inducing proliferation of erythroid precursors (9). Since the serum EPO level reflects the accurate amount of EPO production (10), we can decide whether the inadequate EPO production is the cause of anemia by measuring the serum EPO. Soluble transferrin receptor (sTfR), one of the parameters reflecting erythropoiesis, rises before the increase of reticulocyte during active erythropoiesis, making it a useful index of early erythropoiesis (11). Furthermore, unlike ferritin, sTfR is not influenced by inflammation and therefore is useful to identify iron deficiency or to evaluate erythropoiesis in chronic disease- or inflammation-associated anemia (1). Although erythrocyte sedimentation rate (ESR) has low sensitivity and specificity in detecting the presence of disease, it is easy to measure and cost-effective, helping to screen the infection within a certain group (12). Anemia in cancer patients is most commonly associated with the chronic nature of cancer itself and the chronic

disease-associated anemia has known to be mediated by diverse cytokines (13). Increased cytokines in the chronic disease inhibits erythropoiesis by diverse ways from decreasing not only EPO production but also marrow response to EPO to inhibiting re-utilization of iron from the destroyed red cells (13, 14). One prototypic cytokine of those is tumor necrosis factor (TNF)-α (15).

The normal response to anemia of any cause is an increased production of EPO from the kidney, which stimulates erythropoiesis in the marrow to increase hemoglobin (Hb). If there is any block in this process, anemia cannot be adequately compensated. Since diverse factors interact within the normal erythropoiesis, multiple factors might be responsible for the anemia in cancer. In this study we determined the serum level of EPO, sTfR, TNF-α, and ESR in children with cancer with or without anemia, to investigate the mechanism of anemia and the factors responsible for the anemia in those children.

MATERIALS AND METHODS

Peripheral blood samples were collected from 32 cancer patients admitted to Korea University Medical Center for chemotherapy. Samples were drawn once or twice from a single patient if he/she was in different phases of treatment and the sampling interval was at least more than 4 weeks. Clinical characteristics of patients are shown in Table 1. Anemia was defined as Hb level of less than 10 g/dL in either sex. Hb was measured using Cell Dyn 4000 (Abbott, U.S.A.) in an EDTA tube and erythrocyte sedimentation rate (ESR) was measured using Sedimentic 100 (Analysis Instrument AB, Sweden) in a sodium citrate tube. Serum EPO, sTfR, and TNF-α were measured by commercially available enzyme immunoassay kits (EPO: Boehringer Mannheim, sTfR and TNF-α: R&D systems Inc.) following manufacturer's instructions. For statistical analyses of the data, Kendall rank correlation coefficients between Hb and those parameters were determined using StatView (Abacus Concepts, Inc.).

Table 1. Characteristics of the patients

Characteristics	No. of patients	No. of sampling
Sex		
Male	20	Hb<10 g/dL : 28
		Hb>10 g/dL : 20
Female	12	Hb<10 g/dL : 18
		Hb>10 g/dL : 12
Cancer type		
Leukemia		
ALL	21	Hb<10 g/dL : 28
		Hb>10 g/dL : 21
AML	2	Hb<10 g/dL : 4
		Hb>10 g/dL : 2
Solid tumor		
Neuroblastoma	4	Hb<10 g/dL : 4
		Hb>10 g/dL : 6
Brain tumor	2	Hb<10 g/dL : 5
		Hb>10 g/dL : 2
Lymphoma	1	Hb<10 g/dL : 1
		Hb>10 g/dL : 0
Pleuroblastoma	1	Hb<10 g/dL : 2
		Hb>10 g/dL : 0
Osteosarcoma	1	Hb<10 g/dL : 2
		Hb>10 g/dL : 1

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia

RESULTS

Correlation coefficients between Hb and EPO, sTfR, TNF-α, or ESR and between sTfR and EPO, TNF-α, or ESR in the leukemia and solid tumor patients are summarized in Table 2.

To investigate whether the EPO production is adequate in anemic cancer patients, the correlations between EPO and Hb were determined. There were moderate correlations between Hb and EPO both in leukemia (tau=-0.547, p<0.0001) and in solid tumor (tau=-0.591, p<0.0001) patients. sTfR, an index of erythropoiesis, showed a weak correlation with Hb in leukemia (tau=0.216, p<0.05) but not in solid tumor (tau=0.184, p=0.2) patients. Since cancer itself is a chronic disease and infection frequently occurs due to suppressed immunity in cancer, we investigated whether the chronic inflammation is inhibitory to the erythropoietic activity of the bone marrow in cancer patients. Neither TNF-α nor ESR, as an index of inflammation, showed a significant correlation with Hb, EPO, or sTfR in leukemia or solid tumor patients. sTfR

Table 2. Correlation coefficients between parameters

	Leukemia	Solid tumor
Hb, EPO	-0.547*	-0.591*
Hb, sTfR	0.216†	0.184
Hb, TNF-α	-0.095	-0.175
Hb, ESR	-0.122	-0.082
sTfR, EPO	-0.223†	-0.401†
sTfR, TNF-α	-0.016	0.029
sTfR, ESR	0.087	0.101

Hypothesized Correlation=0. *p-value<0.0001, †p-value<0.05.
Hb, hemoglobin; EPO, erythropoietin; sTfR, soluble transferrin receptor; TNF-α, tumor necrosis factor-α.

Table 3. Distribution of serum EPO and sTfR levels in anemic episodes (Hb<10) of leukemia and solid tumor patients

EPO	sTfR in			
	Leukemia		Solid tumor	
	Low*	High†	Low*	High†
Low‡	3	0	4	1
High§	14	15	7	2
Total	17	15	11	3

Low*: sTfR<20 nmol/L, High†: sTfR>20 nmol/L, Low‡: EPO<100 U/L, High§: EPO>100 U/L, EPO: erytropoietin; sTfR: soluble transferrin receptor.

representing erythropoietic activity inversely correlated with the level of EPO in leukemia (tau=-0.223, $p<0.05$) and in solid tumor (tau=-0.401, $p<0.05$) patients. To further characterize the erythropoiesis in cancer patients, responses of EPO and sTfR to anemia were determined. More than 100 U/L of EPO was defined as "EPO responding to anemia" (16, 17) and more than 20 nmol/L of sTfR was defined as "marrow responding to anemia" (18). The results are summarized in Table 3. In a total of 32 anemic episodes of leukemia, both EPO and marrow responded to anemia in 15 episodes (47%), neither EPO nor marrow responded to anemia in 3 episodes (9%), and EPO responded, but not marrow, in 14 episodes (43%). Conclusively in anemic leukemia patients, EPO responded in 29 episodes (90%) and the marrow did not respond in 17 episodes (53%). In a total of 14 anemic episodes of solid tumor, both EPO and marrow responded to anemia in 2 episodes (14%), neither EPO nor marrow responded in 4 episodes (28%), and EPO responded, but not marrow, in 7 episodes (50%). In anemic solid tumor patients, EPO responded in 9 episodes (64%) and the marrow did not respond in 11 episodes (78%).

DISCUSSION

For rhEPO to be effective in anemic cancer patients, prerequisites such as inappropriate EPO production, normal response of marrow to EPO, and no inhibitory effect of cytokines to marrow are needed (19). Inappropriate EPO production and/or impaired response of marrow to EPO have been suggested as mechanisms of anemia in cancer patients (20-24). Whether the EPO production in anemic cancer patients is appropriate and, if there is an inappropriate EPO production, whether the baseline serum EPO level can predict the effectiveness of rhEPO in the presence of inappropriate EPO production have been controversial (20-26). In our study, EPO increased significantly in response to the decrease of Hb in leukemia and solid tumor patients. The appropriateness of this EPO response in anemic cancer patients can be judged by comparing with the EPO level in iron deficiency anemia (19, 27). Although we could not perform this comparison, a significant moderate correlation between Hb and EPO in our study seems to reflect an appropriate EPO response. In contrast to EPO, sTfR showed no or only a weak correlation with Hb representing an impaired marrow response, in contrast with the finding of iron deficiency anemia.

The EPO response to anemia in cancer patients is shown to be diverse, i.e., high, normal, or low. Besides anemia itself, the level of EPO in cancer can be influenced by chemotherapy. Ozguroglu et al. showed that EPO increased but not adequately in anemic cancer patients and chemotherapy did not influence the level of EPO (27). In contrast, Ludwig et al. reported EPO increased during chemotherapy in leukemia and solid tumor patients and a higher rhEPO level was needed in the chemotherapy group than in the non-chemotherapy group to increase the same degree of Hb (26). Furthermore, Sawabe et al. described the increased level of absolute EPO during chemotherapy and they explained that this was due to the marrow suppressing effect from chemotherapy (28). Since our study was performed on the patients admitted for the chemotherapy, high EPO in our study might reflect the sum of marrow responses to chemotherapy and to anemia.

Pathophysiologically anemia can be divided into deficient response of marrow (low sTfR/high EPO) and deficient production of EPO (low sTfR/low EPO) (29). Since our study showed low sTfR and high EPO, the anemia in our cancer patients belongs to the former, coinciding with finding of Corraza et al. (22). In spite of a significant inverse relationship between Hb and EPO, Dowd et al. reported an inappropriately high or low EPO response to Hb in some cancer patients, suggesting a possibility of another control mechanism of EPO metabolism (23). Although the primary mechanism to control EPO is hypoxia due to low Hb, Cazzola et al. showed that the serum level of EPO in reduced erythroid mass is higher than that in the normal erythroid mass for the same degree of low Hb. They speculated that this was because of a difference in EPO use by erythroid mass, suggesting the amount of erythroid mass in the bone marrow as another mechanism to control serum EPO (30). Since sTfR reflects the amount of erythroid mass, our study showing low sTfR and high EPO can be due to decreased utilization of EPO by the cancer itself- or treatment-induced reduced erythroid mass as well as increased production of EPO by responding to anemia. This is further supported by correlations between EPO and sTfR both in leukemia and in solid tumor patients in our study.

One of the common causes of EPO resistance is infection or inflammation (31); and diverse inflammatory cytokines known to increase in cancer patient inhibit erythropoiesis (4, 32). In contrast to this general belief, TNF-α, a prototypic cytokine, and ESR, an index of inflammation, did not have any correlation with Hb or sTfR in our study, suggesting that the possibility of marrow suppression by TNF-α of chronic inflammation is unlikely.

Bone marrow suppression represented by low sTfR was shown in 53% of leukemia and 78% of solid tumor patients (Table 3). Interestingly, sTfRs in a single patient varied from high to low in different samples. If this is the case, the bone marrow suppression may be reversible and high sTfR means reactive bone marrow and deficient iron (18). Since 60% of total iron is stored in red blood cells, only the presence of anemia is enough to suggest the deficient storage of iron (26). In addition, functional deficiency of iron in cancer patients makes adequate iron supplementation mandatory to increase the effect of rhEPO if used.

In conclusion, our study demonstrated the EPO response to anemia is adequate and chronic inflammation is not inhibitory to erythropoiesis in children with cancer. Rather, the

suppressed marrow seems to be related to anemia in those children. Therefore the effectiveness of use of rhEPO without indication for the prevention or treatment of anemia in cancer is doubtful. For more effective use of rhEPO, careful patient selection and adequate iron supplementation are needed.

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