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EDITORIAL REVIEW

Do polyamines play a role in the pathogenesis of the anemia of end-stage renal disease?

A normochromic normocytic anemia with a hypoproliferative bone marrow is invariably associated with the latter stages of end-stage renal disease (ESRD) [1–3], and has been the focus of much research over the past few years. The anemia of ESRD is multifactorial in origin. Possible causes of this anemia include erythropoietin deficiency, [4] hemolysis [5] and blood loss from various causes including chronic dialysis [6–8], inhibitors of erythropoiesis [9–11], and poor nutritional status [12].

Two major factors have been implicated in the pathogenesis of the anemia of ESRD. First and foremost, there is a deficiency of erythropoietin production in ESRD, in that serum erythropoietin concentrations, although often found to be normal [9, 13] or elevated [10, 13], may not be high enough relative to the severity of the anemia [4]. Secondly, polyamines, which are organic cations that play various roles in normal cellular proliferation and differentiation [14–17], accumulate in plasma and body fluids of patients suffering from ESRD [18], and have been reported to reduce the proliferation and maturation of erythroid cells [11–12]. However, the relative role of polyamines in this anemia is controversial.

A review of the anemia of ESRD appeared in this journal in 1985 emphasizing the role of Ep deficiency as the primary etiology of this disease [19]. The purpose of this paper is to review current knowledge regarding this anemia, and to consider the question, do polyamines play any role in the pathogenesis of the anemia of ESRD? The reason for considering this question is that even though erythropoietin levels in plasma are normal or elevated, the uremic patient in ESRD remains anemic. These plasma erythropoietin levels in uremic patients with anemia, although elevated, are low for the degree of anemia when compared with plasma Ep levels in patients with sickle cell or iron deficiency anemias (Fig. 1) [20]. However, improvement in the anemia has been reported with the use of continuous ambulatory peritoneal dialysis [21–23]. This suggests the existence of dialyzable substances present in the sera of uremic patients that act as inhibitors of erythropoiesis. These substances could be polyamines. There are three major areas of controversy regarding the role of polyamines in this anemia. First, are levels of polyamines significantly elevated in the sera of uremic patients, and if so, is there a correlation between the degree of uremia and anemia with serum levels of the polyamines? Secondly, do polyamines exert a specific inhibitory effect on erythropoiesis, or do they exert a generalized nonspecific cellular surpressive effect disrupting both myelopoiesis and erythropoiesis? Finally, do polyamines contribute to the anemia of ESRD by inhibiting the bioactivity of erythropoietin itself?

The first controversial point to be addressed is whether polyamines accumulate in the plasma and body fluids of patients suffering from ESRD. Crucial to establishing a role for polyamines in the pathogenesis of the anemia of ESRD is the demonstration of elevated levels of polyamines in the sera of uremic patients as well as a correlation between polyamine levels with the degree of uremia and anemia. Several studies [18, 24–26] have demonstrated elevated levels of polyamines in uremia. Swendseid, Panaqua and Kopple [25] using an amino acid analysis technique reported elevated levels of free spermine and spermidine in erythrocytes, and increased putrescine in the urine of patients in renal failure. These investigators [25] postulated that an increase in polyamines in blood was involved in the mechanism of the anemia of ESRD. Similarly, using a radioimmunoassay technique Campbell et al [24] reported that the polyamines spermine and spermidine were increased up to six times normal levels in the sera of patients with renal failure and on regular hemodialysis. However, the values reported by this group [24] were obtained by a radioimmunoassay method which may not be specific since the spermine antibody employed in this study could have a 22% cross reactivity with spermidine. Even though the validity of this work was questioned [27], and this radioimmunoassay may not have been specific for spermine, it is still possible that the assay did detect polyamines as a class. In contrast to the results obtained in this study [24], Spragg and Hutchings [26] using high performance liquid chromatographic analysis of the predialysis sera of nine anemic and severely uremic patients, found only serum putrescine levels to be elevated to two times that of normal values, while spermine and spermidine levels were not increased. Conversely, Saito et al [18] also using high-performance liquid chromatography and the same fluorometric technique, with a five times larger sample population, found a statistically significant elevation in serum levels of the polyamines, cadaverine, putrescine, spermine, and spermidine in the sera of uremic patients. In addition, spermine levels have been shown to be reciprocally correlated with hematocrit values in patients suffering from anemia of ESRD [18]. These investigators [18] have also reported a significant correlation between the increase in serum creatinine and the elevations in serum spermidine and putrescine in patients with ESRD. Thus, these reports demonstrate that serum levels of polyamines are elevated in ESRD, and there appears to be a correlation between the levels of some polyamines with the degree of anemia and uremia. It would be interesting to determine with the use of multiple linear regression analysis, as was done with PTH in similar studies [28], whether, after controlling for the effect of creatinine, the

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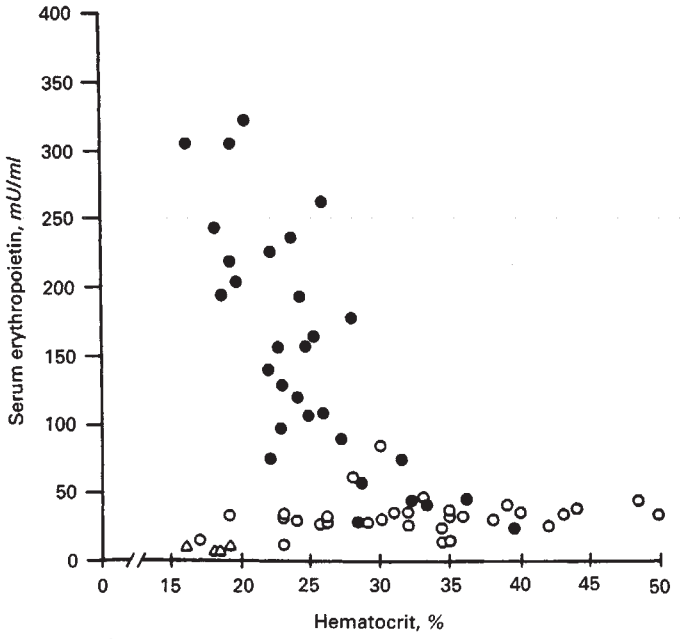

Fig. 1. *Relationship between serum erythropoietin concentration and hematocrit levels in children with normal renal function and varying degrees of anemia due to sickle cell disease or iron deficiency (●), in children with end-stage renal disease (ESRD) (○) and in anephric children (△).* The mean serum Ep levels in 35 children with ESRD was 32.4 ± 2.4 μ/ml; 30 children with anemia of similar severity with sickle cell disease or iron deficiency ranged between 28.7 to 327 μEp/ml; the mean serum Ep titers in 4 anephric children was 9.1 ± 0.75 μ/ml; the mean for 20 normal children was 19.6 ± 1.5 μ/ml. Used with permission from *Textbook of Nephrology* [20]. Based on data from *J Lab Clin Med* 105:449–458, 1985 [35].

polyamines are a significant predictor of hematocrit in patients with anemia and uremia in ESRD.

The second controversial point to be addressed is whether or not elevated levels of polyamines exert a specific inhibitory effect on erythropoiesis. Since a normochromic-normocytic anemia is characteristic of ESRD, whereas leukopenia or a generalized myelosuppression is not typical, it is essential to establish that polyamines are specific inhibitors of erythropoiesis. Evidence in support of polyamines exerting an erythroid-specific inhibitory effect comes from the work of several investigators [29–32]. Radtke et al [11] first reported that spermine was an inhibitor of erythropoiesis using a fetal mouse liver erythroid colony system. Recently it has been shown that the polyamines, spermine, and spermidine are potent inhibitors of erythropoietin-stimulated incorporation of ^{59}Fe into newly synthesized heme by fetal mouse liver cells in short-term culture, suggesting one possible mechanism by which the polyamines may exert an erythroid-specific inhibitory effect [29].

It is known that the anemia of ESRD progresses in the course of renal insufficiency to the point where dialysis becomes necessary [9, 21–29, 33] and then the anemia improves after several weeks of adequate dialysis therapy [8, 13]. Since it has already been shown that polyamine levels are elevated in the sera of uremic patients, it should stand to reason that if polyamines are pathogenic in the anemia of ESRD, then the sera of uremic patients should inhibit erythropoiesis to a greater extent than granulopoiesis if tested in vitro in bone marrow cultures. We have recently demonstrated that sera of predialysis uremic patients exert a more selective dose-related inhibition of erythroid colony formation than of granulocyte-macrophage colony growth in murine bone marrow cultures [31]. Similarly, recent work using sera from uremic patients and ABO-matched human bone marrow cultures showed a selective inhibition of erythropoiesis; albeit, a stimulatory effect on granulopoiesis was reported [30]. This finding is in accord with previous observations that the sera of uremic patients may support granulopoiesis more efficiently than sera from normal subjects [34, 35]. In contrast, Delwiche et al [36] found that sera from uremic patients lacked in vitro specificity. In comparison with normal human serum, increasing concentrations of sera from uremic patients induced inhibition of the growth of erythroid (CFU-E), granulocyte-macrophage (CFU-GM) and megakaryocytic (CFU-Meg) colonies [36]. However, Pavlovic-Kentera [37], also using the mouse bone marrow erythroid colony (CFU-E) assay, reported inhibition of CFU-E colony formation in 34 of 35 hemodialysis patients with ESRD. None of the sera of these ESRD patients produced a significant inhibition of CFU-GM [37]. Also, it is worth noting that a transient neutropenia regularly develops in some patients with ESRD undergoing hemodialysis [38]. However, studies of the pathogenesis of this phenomenon suggests that this decrease in blood levels of neutrophils and monocytes is due to sequestration of these cells in the pulmonary vasculature of the dialysis patient. It has been suggested [38] that this neutropenia results from the activation of complement by the dialysis coil. There are no reports of a suppression of leukopoiesis in patients with ESRD caused by specific uremic toxins.

Spermine and spermidine were recently found to inhibit erythropoiesis to a greater extent than granulopoiesis in a dose-related manner [32]. This work is in contrast to the findings of Segal, Stueve and Adamson [39], who reported that these polyamines lacked in vitro specificity. The reason for this discrepancy is not known, however, differences may exist in culture technique. One notable difference is that although both groups used greater than physiological concentrations of polyamines, Kushner et al [32] used a tenfold greater concentration of polyamines at their highest test dose. Even at this high concentration of polyamines neither erythroid nor granulocyte-macrophage colony growth was totally eliminated. In contrast, Segal, Stueve and Adamson [39] showed a complete elimination of both erythroid and granulocytic-macrophage colony growth at a polyamine concentration thirty times lower than the peak concentration used by Kushner et al [32]. The reasons for these differences are not known. Further work is needed to definitively establish whether or not the polyamines as a group, or one polyamine in particular, exerts an inhibitory effect specifically on erythropoiesis.

Finally, in considering mechanisms for the polyamines in the pathogenesis of the anemia of ESRD, if indeed they are involved in the mechanism of this anemia, an interaction between the polyamines and erythropoietin at the level of the bone marrow is possible. For example, as mentioned previously it has recently been shown that the polyamines spermine and spermidine are among the most potent inhibitors of erythropoietin-stimulated incorporation of ^{59}Fe into newly synthesized heme by fetal mouse liver cells in short-term culture [29],

suggesting a possible pathogenic mechanism. However, it is not clear whether this inhibition of polyamines on ^{59}Fe incorporation into heme is due to an effect directly on Ep, erythroid cells or heme synthesis itself. It seems clear that inadequate production of erythropoietin by the diseased kidney is a major contributing factor to the anemia of uremia [4, 10, 13, 40, 41]. In addition, it has been suggested that the most important factor leading to the anemia of ESRD is a dysregulation of erythropoietin synthesis in response to anemic hypoxia [4]. In contrast, it has been shown that the anemia of ESRD progresses in the course of renal failure to the point where dialysis becomes necessary [9], and then the anemia improves after several weeks of adequate dialysis therapy [33]. Improvement of the anemia has been demonstrated in patients on dialysis despite a significant drop in serum erythropoietin concentrations [9, 33], while at the same time there is an increase in hemoglobin concentration [33]. Also, in an early report only 2 of 16 patients with advanced renal disease on dialysis therapy for as long as six years had higher than normal Ep levels when the sera were assayed in hypoxic protein-starved mice [42]. However, more recently it has been shown that serum erythropoietin levels in uremic patients, although inappropriately low for the degree of anemia, are often above the levels present in normal nonanemic individuals [33, 43, 44]. These observations provide indirect support for a pathogenic role of inhibitors or toxic factors which inactivate erythropoietin, and which accumulate in the sera of uremic patients during the course of ESRD, which are removed, at least in part, by dialysis therapy. Further support for this hypothesis comes from three recent reports on the effects of different modes of dialysis on serum erythropoietin levels [22, 45, 46]. In these studies [22, 45, 46] as in previous work [21, 23], higher post-dialysis hematocrits and erythropoietin levels were seen with continuous ambulatory peritoneal dialysis than with hemodialysis, suggesting that more efficient modes of dialysis effectively remove some substance or substances that accumulate in the sera of uremic patients which reduce both the immunologic and biologic activity of erythropoietin. Similarly using a radioimmunoassay technique, McGonigle et al [35] demonstrated that the sera of normal human subjects were capable of enhancing the immunoreactivity of human urinary erythropoietin, whereas sera from uremic patients either failed to enhance or slightly reduced the immunoreactivity of Ep. Also, in this report the sera of uremic patients incubated with Ep were shown to block its biological reactivity in a fetal mouse liver erythroid colony assay. However, it is not clear whether the inhibition was due to the inactivation of Ep itself or was the result of inhibition of the effects of Ep on erythroid colony formation at the cellular level. It has been shown that a dose responsive increase in hematocrit level can be induced in patients with the anemia of ESRD with the administration of increasing concentrations of purified human recombinant Ep [47]. However, the plasma levels of Ep in dialysis patients treated with human recombinant Ep to maintain a normal hematocrit are well above physiological levels [48]. Thus, this may indicate that a competitive inhibition of the effects of Ep on hematocrit can be overcome by elevated levels of this hormone, and support the existence of circulating uremic toxins which either inactivate Ep directly or block its effect at a cellular level. Overall, there is considerable support in favor of substances accumulating in the sera of uremic patients which may interact with erythropoietin leading to anemia in ESRD, but there is no direct evidence that these substances are polyamines. However, there is strong indirect support for implicating the polyamines as uremic toxins. Further work is necessary to definitively determine if a specific role for polyamines exists in the mechanism of this anemia.

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