Uremic toxicity and anemia

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ABSTRACT: Inappropriate erythropoietin production is the main defect responsible for the anemia of chronic renal failure. However, many other factors can contribute. There is support for the existence in uremic serum of substances that can inhibit erythropoiesis and cause hemolysis, but it is still debated how far uremic retention solutes contribute to the pathogenesis of anemia during chronic renal failure. This article looks at the role of uremic toxicity in exacerbating the anemia of chronic renal failure.

Key words: *Anemia, Chronic renal failure, Dialysis, Uremic toxins, Phosphatidylserine, Uremia*

INTRODUCTION

Anemia is common in patients suffering from chronic renal failure (CRF), and is one of the leading causes of increased cardiovascular morbidity and mortality in these patients. The anemia is normocytic and normochromic in origin, hypoproliferative with a low reticulocyte count (1, 2). It usually becomes manifest when creatinine clearance has dropped to approximately 40 mL / min / 1.73 m^2 of body surface area, subsequently worsening with the progressive deterioration in renal function (3). Though the hematocrit may vary considerably in patients with a comparable reduction in kidney function, except for polycystic kidney disease, the underlying primary renal disease appears to have no specific effect on the degree of anemia.

The main defect responsible for the anemia of CRF is absolute or relative erythropoietin (EPO) deficiency (4). The introduction of recombinant human erythropoietin (rHuEpo) has revolutionized the care of patients suffering from renal anemia. The availability of this treatment has almost completely eradicated the severe anemia of end-stage renal disease (ESRD) (5), as well as reducing left ventricular hypertrophy (6). However, despite increases in the use and average dose of rHuEpo, and a recommended target hematocrit above 33% during this therapy (7, 8), a substantial proportion of ESRD patients still fail to achieve a satisfactory hematocrit (9, 10).

The anemia of CRF is a complex disorder in which many factors other than EPO deficiency may play a role. These include hematinic deficiencies (iron, folic acid), inflammation, aluminium intoxication, hyperparathyroidism with myelofibrosis, external blood loss, as well as hemolysis and bone marrow suppression (11) probably induced by retained toxic metabolites. These factors can all contribute to anemia and blunt the response to rHuEpo, and need therefore to be evaluated.

How far uremic retention solutes contribute to the pathogenesis of anemia during CRF is disputed. The presence of toxic compounds suppressing erythropoiesis is supported by the elevated plasma levels of EPO (12-14) in some severely anemic ESRD patients (suggesting a suppressed bone marrow response to EPO), a frequent rise in hematocrit after the start of regular dialysis treatment (14, 15), and a dose-dependent inhibition of bone marrow cells in culture when exposed to uremic serum (16, 17). It has also been demonstrated that exposure to uremic serum shortens the survival of erythrocytes from healthy subjects (1, 2). However, though there is support for the existence of uremic retained substances that suppress erythropoiesis and cause hemolysis, the evidence that the anemia of CRF is primarily an endocrine deficiency (18, 19) has led to the view that uremic inhibition plays a minor role, if any (20). Studies showing that the rate of removal of waste products from the blood (dialysis "dose") is a major factor in the correction of anemia and its responsiveness to erythropoietin therapy (21-25), together with recent findings on the possible pathophysiological relevance to anemia of substances accumulated in uremic serum (26, 27), have renewed interest in the role of uremic "toxins".

This article reviews the role of uremic toxicity in exacerbating the anemia of CRF.

Uremic toxicity and inhibition of erythropoiesis

The presence of inhibitors of erythropoiesis in uremic plasma was postulated in the light of the report that anemia improves after hemodialysis is started (15). It was shown later that the hematocrit rises after the start of regular dialysis in spite of a significant drop in endogenous serum erythropoietin levels, suggesting that hemodialysis removes a bone marrow inhibitor (3, 14). There is also ample evidence that adequacy of dialysis is a key to correcting anemia and optimizing rHuEPO usage in a number of hemodialysis patients (28, 29).

The inhibitor theory is supported by a number of *in vitro* studies. Plasma from anemic uremic patients has been shown repeatedly to inhibit heme synthesis (16, 30-34). Bone marrow cells from healthy subjects and ESRD patients respond similarly to erythropoietin but total heme synthesis is significantly less in cultures prepared with uremic serum than normal serum (35). Low-molecular-weight inhibitors of heme synthesis have been found in serum (30, 31) and urine (32). Ohno et al. (36) found that human uremic serum contained an inhibitor of erythroid colony-forming units (CFU-E) and of erythroid burst-forming units (BFU-E).

Compared to control serum, serum from uremic patients cultured with normal human marrow caused a 72% decrease in BFU-E colony growth and an 82% decrease in CFU-E colony growth, neither HD nor peritoneal dialysis succeeding in removing the inhibitor (37). The inhibitory effect of human uremic serum on colony growth from progenitor cell types may be specific for the erythroid line, since there was no inhibition of the growth of granulocyte-macrophage CFU (38, 39). Significant correlations have been reported between hematocrit values of uremic patients and the degree to which serum from these patients inhibits erythroid colony formation in mouse marrow cultures and heme synthesis in normal rabbit marrow cultures (34).

These findings suggest that uremic serum contains substances that inhibit either the growth of erythroid progenitor cells or heme synthesis, supporting the concept that uremic suppression of erythropoiesis is a cause of the anemia of CRF (30, 36, 40, 41). These studies, however, have not been totally confirmed. While there appears to be a substance in serum from uremic patients that inhibits hematologic precursors of other species, when human instead of murine erythroid marrow is cultured, its growth is not inhibited by autologous uremic serum (20, 42). Some investigators have been unable to find any inhibition of BFU-E by uremic serum (43). In addition, uremic inhibition of *in vitro* erythropoiesis may lack specificity, since serum from uremic patients also inhibits the growth of both CFU-granulocyte-macrophages and CFU-megakaryocytes (44); yet no such inhibition of platelet or leukocyte production appears to exist *in vivo*.

Attempts to identify endogenous "uremic" inhibitor(s) of erythropoiesis have also led to conflicting results. Many compounds have been promoted as potential inhibitors; the most intensively investigated include polyamines, ribonuclease, and parathyroid hormone (PTH).

Polyamines (such as spermine, spermidine, putrescine, and cadaverine) are a series of organic cations that most (45-47), though not all (48), investigators have found elevated in uremia. Spermine levels are inversely correlated with the hematocrit in anemic ESRD patients (45). Higher concentrations of extracellular polyamines cause hypoproliferation of human erythroid precursors, particularly the CFU-E (49). There is reasonable evidence that polyamines have a specific inhibitory effect on erythropoiesis in uremia (49-52). However, there is doubt about this specificity since inhibition of granulopoiesis has also been observed (44, 53). Thus, it remains to be established just how specific the effect of polyamines is on erythropoiesis compared with granulopoiesis (54).

The significance of ribonuclease as a specific erythroid inhibitor – it is strikingly more active in uremic serum (55) – needs further study since it does not inhibit BFU-E, and the amount required to inhibit CFU-E *in vitro* far exceeds the levels found in uremic patients.

PTH is considered a major uremic toxin (56), but findings that crude extracts of the parathyroid gland significantly inhibited *in vitro* hematopoiesis (57) could not be reproduced with either the biologically active N-terminal fragment (aminoacids 1-34) or the intact PTH molecule (58-60).

Thus, still unknown uremic toxin(s) may be inhibiting erythropoiesis and contributing to the development of anemia in patients suffering from renal failure. Low-molecular-weight inhibitors may reasonably be supposed to be involved since anemia improves after cellulosic dialysis is started (28). Neither the concentration of urea per se, however, nor that of creatinine, is inhibitory *in vitro* (54). Medium-to-large molecular weight inhibitors may also be implicated. "Middle-molecule" uremic toxins have long been considered responsible for the inhibition of erythropoiesis in ESRD patients (17, 61-63). More efficient removal of middle molecules by the more porous peritoneal membrane (64-66) has been offered as one possible explanation for the less severe anemia found in patients treated with peritoneal dialysis than those on hemodialysis (67-69) before the days of rHuEPO.

The potential contribution of high-molecular-weight substances to the onset of uremic anemia is suggested

by studies on the characterization of compounds eliminated by protein-leaking hemodialyzers. Using a large-pore, highly permeable membrane (BK-F polymethylmethacrylate) it was shown that uremic serum contains a fraction whose estimated molecular weight lies between 500 and 1000 kilodaltons (70), which cannot be detected when using less permeable membranes (71). This fraction, called the KR4-O fraction, has considerable concentration-dependent inhibitory action on the formation of mouse bone marrow erythroid progenitor cells (70).
It has also been reported that polyaminated peptides and proteins can accumulate in the plasma of patients on hemodialysis (72). This could contribute to the toxicity related to high-molecular-weight toxins, since spermidine-protein conjugates contained in a fraction of molecular weight > 100 kilodaltons had a marked inhibitory effect on the proliferation of CFU-E (26). Interestingly, removal of these high-molecular-weight substances by highly permeable membranes improved the anemic status in some hemodialysis patients (70, 72, 73).
In the aggregate, the balance of evidence indicates that the state of uremia inhibits erythropoiesis *in vitro*, and probably also *in vivo*. However, the exact mechanism of this adverse effect remains obscure.

Uremic toxicity and shortened red blood cell survival

One documented abnormality of erythrocytes in uremia is that their survival time shortens once advanced renal failure develops. Decreased RBC survival in dialysis patients has been documented by isotope red cell tagging with ^{51}Cr (74), $DF^{32}P$ (75), ^{14}C-cyanate (76), and by measurements of carbon monoxide exhalation (77). Red cell survival averages approximately half-normal, ranging from one-third normal to normal (reviewed in 78). Variability in the frequency and degree of hemolysis may be explained by differences in the patient population or the method. Of interest is the finding that when quantitated by ^{51}Cr labeling in 1986, the mean red cell half-life in HD patients was 23 days (normal 28-32), which is significantly longer than when similar studies were done 10 to 20 years earlier (79).
The mild to moderate hemolysis that occurs in uremia may not happen in anemia, if the response of erythropoiesis is sufficient. However, erythropoiesis is depressed in chronic uremia (11). Thus, a reduced red cell lifespan is considered a contributory factor to the anemia of renal failure (75).
The accelerated destruction of RBC in uremia appears to be the result of the uremic environment. Cross-transfusion studies have shown that normal RBC have a shortened lifespan in uremic patients, whereas red cells from uremic patients have a normal survival time when transfused into healthy subjects (1, 2). RBC survival time in uremia may also be inversely correlated with the serum BUN concentration (80), suggesting a potential direct effect of uremic waste products on erythrocytes.
These results indicate that the primary cause of hemolysis in renal failure is the retention of one or more uremic solutes in plasma (11, 56). Some improvement is achieved when regular dialysis starts (74, 81), suggesting removal of the hemolytic factor(s), though red cell survival is usually not completely corrected (75, 82, 83). A role for parathyroid hormone has been claimed (84), but the nature of the extrinsic uremic compound leading to the qualitative RBC defect has still to be identified.
Various abnormalities have been put forward as potential contributors to the reduced RBC survival time in uremia. The inhibitory effects of uremic plasma on the activity of membrane sodium ATPase (85) and membrane calcium ATPase (86) have been suggested as contributing to hemolysis. Inhibition of plasma membrane Ca^{2+}-ATPase activity, induced by p-hydroxyhippuric acid (87), may raise intracellular levels of free Ca^{2+} and thus be toxic to RBC, since maintenance of a low Ca^{2+} content is essential to cell survival (88, 89). Alterations in the structure and function of erythrocyte plasma membrane including reduced membrane fluidity and impairment of metabolic parameters may also shorten the RBC lifespan in uremia (90-93). Moreover, there is evidence that excessive oxidative stress associated with CRF and exacerbated by HD (94, 95) may contribute to uremic anemia by shortening RBC survival.
Exogenous antioxidants such as glutathione and vitamin E may have positive effects (96-98). In addition, the use of vitamin E-containing membranes, which raise vitamin E levels and lower oxidative stress in plasma and blood cells, thereby reducing HD-related oxidant stress (reviewed in 95 and 99), can significantly improve the anemic status in chronic dialysis patients (100-102). The beneficial effect on anemia of the vitamin E-bonded membrane seems to be the consequence of enhanced erythrocyte survival in patients treated with this new antioxidant-based dialytic therapy (101). The combined use of a vitamin E-modified membrane and exogenous glutathione improves RBC survival and may be the most indicated antioxidant therapy to reduce the oxidative stress-related component of uremic anemia (102).
The shortened lifespan of circulating erythrocytes may result from enhanced recognition of these cells by circulating mononuclear phagocytes, leading to their removal from the circulation. A well-character-

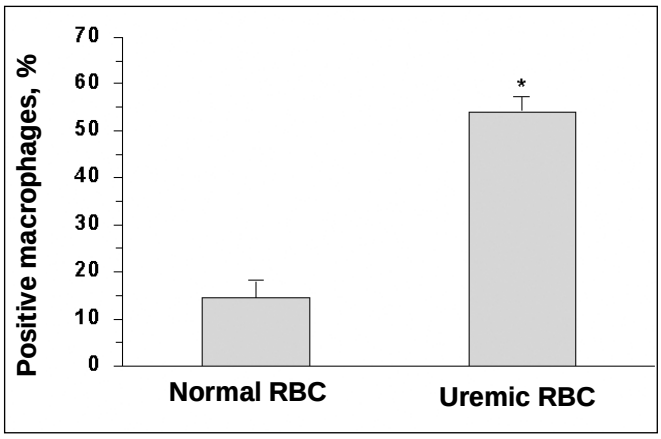

Fig. 1 - Phagocytosis by human monocyte-derived macrophages of erythrocytes from healthy subjects and chronic uremic patients. Values are the percentages of macrophages that phagocytosed one or more erythrocytes (Positive Macrophages) and are expressed as means ± SEM. * Significant difference from the normal control group (p<0.001).

ized mechanism leading to macrophage recognition of aged or damaged RBCs is the loss of plasma membrane phospholipid asymmetry, particularly the appearance of the aminophospholipid phosphatidylserine (PS) at the extracellular face of the erythrocyte membrane (103-107). PS is normally confined to the membrane's inner leaflet (108), and maintaining plasma membrane asymmetry, even at the expense of energy consumption (109), is of critical importance for cells since exposure of PS on the outer leaflet of the RBC membrane may have several pathophysiological implications (reviewed in 109 and 110).

In recent studies, we observed an increase in PS exposure in erythrocytes from chronic uremic patients, irrespective of whether the patients are on dialysis or not (27, 111, 112). While PS externalization normally occurs in aged erythrocytes to help get them cleared, in uremia the abnormality mainly affects red cells that are still young enough to be expressing a marker for reticulocytes (111). We found that the percentage of PS-positive erythrocytes increased with the progressive decline in renal function, and remained high on renal replacement therapy. The abnormal PS exposure in uremic RBCs seems to be related to inhibition of the ATP-dependent aminophospholipid translocase which specifically transports phosphatidylserine from the outer to the inner leaflet of the RBC plasma membrane, against the concentration gradient (113), thereby generating and maintaining membrane asymmetry.

In line with earlier observations of humoral inhibitors of the RBC Na^+-K^+-ATPase (85) and Ca^{2+}-ATPase in uremia (86), we found that uremic plasma strongly influences the exposure of PS in erythrocytes. The percentage of PS-positive normal RBCs increased when incubated in uremic plasma, reaching values comparable to those found in chronic uremic patients (111). Preliminary *in vitro* experiments indicate that the ability of uremic plasma to cause RBC PS exposure is associated with a molecular weight range between 10 and 20 kilodaltons and is strongly inhibited by boiling (111).

These observations suggest that the putative uremic factor(s) influencing the appearance of PS on the outer face of the red cell membrane is a large heat-labile molecule, possibly a protein or peptide, though a low-molecular weight substance behaving like a middle molecule due to high protein binding, or a synergism between several accumulated solutes, cannot be excluded at present.

Because erythrocyte surface-exposed PS may serve as an "eat-me" signal that specifically triggers macrophage recognition (103-107), we also investigated the relationship between the abnormal PS exposure in uremic erythrocytes and their propensity for phagocytosis by human monocyte-derived macrophages (27). Erythrophagocytosis was significantly higher in uremic patients than healthy controls (Fig. 1). Also, phagocytosed uremic RBCs appeared intact, suggesting they were identified before lysis through some surface changes recognized by the macrophages. Several observations suggest that surface-exposed PS are involved in the recognition of uremic RBCs in our model of erythrophagocytosis (27). Thus, a PS recognition mechanism may promote the susceptibility of uremic erythrocytes to phagocytosis and be involved in the shortened erythrocyte lifespan typical of uremia.

Conclusions

From current evidence it appears that uremic toxicity may affect the RBC mass by interfering with both production and the lifespan. However, specific waste products that are demonstrably active as hemolysins or inhibitors of erythropoiesis remain to be identified. Thus, the exact role of substances pathologically retained in the uremic organism in the anemia of CRF still needs to be fully explained. Nevertheless, many studies confirm that adequate dialysis can help correct anemia and foster responsiveness to rHuEPO by removing accumulated molecules that adversely affect the patient's hematological status. Over recent years, an increasing number of pathologically retained solutes have been suggested as potentially relevant to the genesis of anemia of CRF. Understanding their role may help define the exact contribution of uremic toxicity to anemia.

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