Plasma Erythropoietin in Health and Disease*

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ABSTRACT

Erythropoietin is a hormone produced by the kidneys and by certain extrarenal tissues and released into the circulation in response to tissue hypoxia. Its study has provided new information about oxygen transport and bone marrow stem cell function and its determination in plasma can give valuable diagnostic clues as to the etiology and pathogenesis of anemias and polycythemias. The various methods used for such measurements are discussed, and it is recommended that the \textit{in vivo} bioassay in polycythemic mice be utilized until a workable radioimmune assay has been perfected. The results with the use of this \textit{in vivo} bioassay to measure plasma erythropoietin in patients with uncomplicated anemia, aplastic anemia, anemia of renal disease, anemia of chronic inflammatory or neoplastic disorder polycythemia vera, and secondary polycythemia are charted and their diagnostic significance discussed.

Introduction

Erythropoietin is a renal hormone released into the circulation in response to tissue hypoxia. Its study has provided us with new information about oxygen transport and bone marrow stem cell function and its determination in plasma can give valuable diagnostic clues to renal function and tissue oxygenation.

In mammals, the maintenance of a steady oxygen supply for cellular aerobic metabolism is accomplished by a transport chain which brings atmospheric oxygen to the pulmonary alveoli, across the alveolar-capillary membrane and then, reversibly bound to red cell hemoglobin, to the various tissues. In the tissues, the remaining pressure head of oxygen ensures that oxygen molecules diffuse far enough from the capillaries to make oxygen available at the threshold of each and every cell of the body. The transport chain is finely tuned to cellular demands for oxygen and each link: lungs, red cell mass, heart and vessels, can adapt over a wide

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range to changes in ambient oxygen tension or changes in the functional capacity of other links. This adaptability depends on the existence of a number of interlocking feedback circuits. Among these, the simplest is the feedback circuit adjusting the red cell mass. This is, of course, not surprising since red cells are quite simpleminded, designed almost exclusively for the purpose of carrying oxygen from the lungs to the tissues. In this circuit, the size of the red cell mass is controlled by the rate of bone marrow production of red cells which in turn is controlled by a renal hormone, named erythropoietin. Since the red cell mass affects the renal tissue tension of oxygen and in turn the production of erythropoietin, the components of a classical feedback circuit are shown in figure 1.

Although the feedback terminology is derived from our present technological culture, the concept of homeostatic controls has been around since Claude Bernard a century ago realized that optimal organ function demanded the existence of a stable constant milieu interieur.11 His student and successor to the chair of Physiology at the Faculté des Sciences in Paris, Paul Bert, understood that the size of the red cell mass was of crucial importance for the proper transport of oxygen, but failed to realize that the size was adaptable and could adjust to the availability of oxygen. His friend and benefactor, Dr. Dennis Jourdanet, also failed to find a connection despite the fact that Dr. Jourdanet, a physician working in the highlands of Mexico, had observed that the blood of his Mexican surgical patients was thicker and contained more red corpuscles than the blood of his old patients at sea level in Paris.

It was not until 1896 that these observations were connected by Friedrich Miescher and a viable feedback circuit constructed. At that time, Dr. Miescher, because of pulmonary tuberculosis, had been sequestered in a sanatorium in the high Alps and his fertile mind, until then preoccupied with the isolation and identification of deoxyribonucleic acid, turned to the problem of the effect of high altitude and hypoxia on red cell production. He proposed that the rate of red cell production is regulated by the oxygen tension of bone marrow, a proposal generally accepted until the 1940s when Berk and co-workers showed that erythropoietic activity and bone marrow oxygen tension are not directly connected.

The existence of an indirect connection via a factor released in response to hypoxia was first suggested by Carnot and
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Figure 3. The relation between hematocrit percentages and plasma erythropoietin titers of patients with anemias uncomplicated by renal or inflammatory features, of patients with polycythemia vera and of normal individuals.

Deflandre in 1906 but experimental support was not provided until 1953. Currently, it is accepted that the rate of red cell production is stimulated by a hormone, erythropoietin, released in response to hypoxia and that it is regulated by the previously mentioned feedback between the bone marrow and the kidney (figure 1).

Procedure

Erythropoietin is a polypeptide hormone with a molecular weight of about 40,000 and a sialic acid content of about 10 percent. It is defined according to its biologic activity, and one unit was supposed to equal the erythropoietic activity of 5 \( \mu \text{M} \) of cobalt chloride in a fasted rat. Since the activity of cobalt chloride in the polycythemic mouse assay system is quite variable, one unit is usually defined as that activity which corresponds to the activity of one unit of a standard erythropoietin maintained at the Bureau of Standards, National Institutes of Medical Research, London, England. In biochemical terms, one unit corresponds to about 14 ng of erythropoietin or 0.35 pM.

A number of tests have been designed to measure the level of erythropoietin in plasma or serum but the mainstay is still the laborious and capricious bioassay in the polycythemic mouse. The mice are first rendered polycythemic either by hypertransfusion or by a preparatory stay of about two weeks in a hypoxic chamber. It is unlikely that these animals are more sensitive to erythropoietin than normal animals but since the endogenous erythropoietin production is completely suppressed, it is easier to measure the erythropoietin content of any sample injected. The erythropoietic activity induced is usually measured by the incorporation of radioactive iron into red cells (figure 2) but obviously could equally well be assessed by enumerating reticulocytes. Polycythemic and less reliably starved rats have also been used as assay animals.

The use of in vitro tests for biologic activity of erythropoietin has a great deal of appeal but the accuracy and reproducibility of such tests have been disappointing. The rate of utilization of radioactive iron in heme synthesis, or the incorporation of glucosamine into red cell membranes have been used to measure erythropoietic stimulation in crude bone marrow suspensions. Recently, \(^{59}\text{Fe}\) uptake or \(^{125}\text{I}\) deoxyuridine uptake by suspensions of fetal mouse liver cells have been used quite successfully to measure erythropoietic activity. Since the results do not always correlate well with results obtained from in vivo assays, it appears as if serum or plasma contains substances in addition to erythropoietin with stimulating activity on fetal mouse livers.
On the other hand, it is possible that some of these discrepancies are due to partial desialization of the erythropoietin molecules. Desialated erythropoietin is removed almost immediately from the circulation in an assay animal and, therefore, will not register any erythropoietic activity while it retains its full activity in an in vitro assay. Recently, it has been possible to measure small amounts of erythropoietin in vitro by culturing bone marrow stem cells and enumerate erythropoietin induced colonies on the surface of semi-solid fibrin clots or agar cultures. This test which directly measures the response of erythropoietin on its target cells in bone marrow is most promising but still is a research tool.

Immunologic tests, from crude measures of agglutination to highly accurate radioimmune assays, have also been developed. A red cell agglutination inhibition test is being marketed as a kit; however, in our hands, at least, it has been found to be completely unreliable. A radioimmune assay based on highly purified, if not pure, erythropoietin is at work in several laboratories and holds great promise to become the definitive test for erythropoietin. However, the pure erythropoietin is only available in small quantities and both its biologic and immunologic activity may be impaired by iodination. Furthermore, the anti-erythropoietin antibody used is poorly standardized and it must be conceded that the radioimmune assay today has more promise than utility.

Consequently, it is still necessary to rely primarily on the in vivo bioassay of plasma or serum in polycythemic mice despite variability from animal to animal and a lower level of sensitivity of about 50 mU per ml. This latter limitation can be overcome by assessing the plasma content of erythropoietin indirectly through the measurement of the 24 hour urinary excretion of erythropoietin. However, this is an indirect measure dependent on the renal clearance of erythropoietin, and it also involves the assay of an often unsterile endotoxin-containing urine extract, all in all rendering this method not too attractive.

Actually, the importance of the urinary studies lies primarily in the fact that urine from very anemic individuals may provide us with a useful source for erythropoietin for isolation and purification. In some patients, the level of daily excretion can be in the thousands of units of erythropoietin, a considerable amount in view of the fact that one commercial house sells partly purified erythropoietin for one dollar a unit. Recently, it has been possible to overcome the low level of sensitivity by assaying a concentrate of plasma rather than the native plasma sample. Since
Figure 5. The relation between hematocrit percentages and plasma erythropoietin titers of anephric patients or patients with chronic renal disease. The cross hatched area corresponds to the range of values from figure 3.

Erythropoietin is quite heat stable, a five minute boiling of an acidified plasma in a water bath will only reduce the biologic activity by 50 percent while it will remove almost 99 percent of the total protein content. The plasma extract can then be concentrated against carbowax and assayed in a routine manner in polycythemic mice. In order to get the lower level of sensitivity down to about 3 mU per ml, it is necessary to concentrate about 200 ml of plasma extract 40 times. This usually causes no problems since it is possible to obtain large amounts of plasma from normal individuals by plasmapheresis and from patients with polycythemia by therapeutic phlebotomy.

Results

Results from erythropoietin assay of plasma from a great number of patients ranged from less than 3 mU per ml up to 18,000 mU per ml. In order to construct the so-called physiologic response curve, titers have been used of normal individuals, of patients with polycythemia vera and of patients with uncomplicated anemias (figure 3). Erythropoietin titers of normal individuals range from less than 3 mU per ml to 15 mU per ml with a mean of 6.5 mU per ml. Since erythropoietin production in patients with polycythemia vera apparently is unaffected by the disease process, the level in these patients should correspond to the level which would have been found in normal individuals if transfused to corresponding hematocrits (53 percent or higher). Erythropoietin titers of patients with anemias uncomplicated by renal disease, starvation, inflammatory reactions or major changes in oxygen affinity range from less than 3 mU/ml to 18,000 mU/ml.

Using these data, a rough physiologic range can be established. It has to be conceded, however, that the tremendous steepness of the curve makes it extremely difficult to provide close correlations between hematocrit levels and erythropoietin titers. The reason for this steep increase is unknown since in vitro studies of the responsiveness of erythropoietin sensitive stem cells (CFU-E) is linearly proportional to erythropoietin concentration. Although the activation of earlier erythropoietin sensitive stem cells (BFU-E) demands higher concentrations of erythropoietin, about 10 to 50 times as much, it still can not explain that the erythropoietin titer in an anemic patient may be up to 10,000 times as high as in a normal individual.

Erythropoietin titers in anephric patients or patients with chronic renal disease not surprisingly are below those of patients with uncomplicated anemia (figure 4). This striking relationship between renal function and erythropoietin production supports the many experimental and clinical observations indicating that erythropoietin is produced by the kidneys.
Nevertheless, erythropoietin has not been isolated convincingly from kidney extract and the exact intrarenal site or mechanism for its production is not known. At the present, it seems most likely that erythropoietin is synthesized in the renal parenchyma (medulla?) in an inactive form and is first activated at the time of release. The presence of small amounts of erythropoietin in the plasma of some anephric individuals probably reflects residual extrarenal production of this hormone by reticuloendothelial cells or hepatic parenchyma.

Although the production of erythropoietin from diseased kidneys is extremely low as compared with the level anticipated at the particular hematocrit, the level is actually about that of normal individuals and therefore should have been able to support a normal level of red cell production. Since this is not the case, it must be assumed that uremic erythropoietic toxins decrease both the rate of red cell production and the viability of red cells in peripheral blood.

Another anemia which is associated with subnormal erythropoietin levels is the anemia of chronic disease (figure 5). This is an anemia present in patients with chronic inflammatory or infectious disorders or with generalized neoplastic diseases and it is characterized by impaired iron utilization, erythropoietin production and erythropoietin response. A similar impairment in erythropoietin production is observed during starvation, and it seems possible that it is part of an adaptive host response aimed at reducing cellular catabolism of nutrients in short supply. This suggestion is supported by the fact that deiodination of thyroxine to the metabolically active triiodothyronine also is reduced during chronic diseases and starvation. Consequently, the reduction in erythropoietin production could be similar to that found in hypothyroid states, but further studies are needed in order to unravel the pathogenesis of this common but challenging anemia.

Supranormal erythropoietin titers have been claimed to be present in patients with aplastic anemia owing to decreased consumption of this hormone by the aplastic bone marrow. Since the evidence supporting such a concept is not very convincing, an explanation for the high levels of erythropoietin in plasma and urine has not been available. It is true that there may be a slightly higher oxygen affinity of the blood from patients with aplastic anemia than in blood from patients with hemolytic anemia owing to the absence of reticulocytes, but the oxygen affinity does correspond closely to that of normal blood. On examination of figure 6, it appears that the erythropoietin production in aplastic anemia actually falls within the so-called normal range, suggesting that lower levels found in many anemias of corresponding hematocrits may be due to complications and that the level of eryth-
Erythropoietin titers of patients with aplastic anemia should be taken as the norm for renal synthesis and release of this hormone.

Erythropoietin production in patients with classical polycythemia vera was reduced to less than 3 mU per ml in all of the 21 cases tested so far (figure 3). Consequently, an erythropoietin titer of less than 3 mU per ml probably should be added to the diagnostic criteria set forth by the Polycythemia Vera Group as necessary for the establishment of a definitive diagnosis of polycythemia vera.

Secondary appropriate and inappropriate polycythemia should theoretically be associated with measurable erythropoietin titers. Of 72 cases of suspected secondary polycythemia tested in our laboratory, 52 were found to have elevated levels, while 20 had low levels in the polycythemia vera range (figure 7). Of these latter, two have subsequently been found to have polycythemia vera but the other 18 have not as yet shown evidence of this disease. Consequently, an elevated level of erythropoietin in patients with hematocrits in excess of 53 percent appears in most cases to be a reliable indication for the presence of a secondary polycythemia, while levels below 3 mU per ml although suggestive of polycythemia vera so far can not be used as a pathognomic indication for the presence of this disease.

Erythropoietin measurements are slowly coming of age and are establishing themselves as important tools in the differential diagnosis of anemias and polycythemias. The in vivo and in vitro bioassays, unfortunately, are still too crude and laborious to be used in the routine hospital laboratory but when replaced by the radioimmune assay, it appears certain that erythropoietin measurements will become an integral part in the workup of hematologic patients.

References


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