Erythropoietin in Management of Infants

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Erythroid burst-forming units are present in large numbers in infants with the anemia of prematurity. The erythroid progenitors respond normally in vitro to recombinant human erythropoietin (rHuEpo) (1). Although the mean hematocrit is considerably lower in premature infants than in adults, the mean serum erythropoietin concentration in the infants may not be any different from that in the adults (1). Subsequently, erythropoietin-sensitive progenitors have also been found in bone marrow samples obtained from preterm infants (Fig. 1) (2). The above-mentioned studies provide a physiologic basis for therapeutical trials of rHuEpo in infants with the anemia of prematurity.

In an attempt to stimulate endogenous production of red blood cells and thus provide an alternative to red cell transfusions, Halperin et al. (3) have described their experience on the use of rHuEpo in the management of seven preterm infants with anemia of prematurity. The seven infants were free of common medical problems of prematurity, had a gestational age of between 30 and 33 weeks, and a birth weight of between 860 and 1800 g. The administration of rHuEpo was started at 21–33 days of life; it was given subcutaneously three times a week for 4 weeks in amounts of 25–100 IU/kg body weight per dose. The treatment was followed by at least a twofold increase in the absolute number of reticulocytes in all patients (Fig. 2). The authors estimated that the peak reticulocyte count appeared at least 2 weeks earlier than in their historical reference group. In this experiment, the rHuEpo medication was discontinued at the age when one would expect to detect the physiologic stimulation of erythropoiesis and rise in reticulocyte count (4). The data on reticulocyte counts (Fig. 2) indicate that these events were diminished or even abolished in most of the infants. This finding may indicate that release of endogenous erythropoietin may not occur immediately (5), and that one should also study the effect of a more prolonged course of rHuEpo medication. Hemoglobin concentrations, at least in the larger prematures with birth weight over 1000 g, usually catch up to the values in term infants by the age of 4 months (6). On this basis, the age of 4 months might be the time when rHuEpo medication should be discontinued. Halperin et al. (3) further observed that the 4-week rHuEpo medication period resulted in the correction of the anemia of prematurity in four of the seven patients and an avoidance of red cell
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...Marrow of five healthy adults

FIG. 1. Responsiveness of erythroid colony-forming units (CFU-E) to recombinant human erythropoietin (rHuEpo). Low-density marrow cells from 10 infants with anemia of prematurity were cultured in the presence of various concentrations of erythropoietin ranging from 0 to 2.0 IU/ml. The highest number of CFU-E/105 plated cells was taken as 100%, and all other values were expressed as a proportion of that maximum value. Bars represent SEM. For comparison, cells from bone marrow aspirates from five healthy adult volunteers (closed circles) and from five term neonates (open circles) were studied (2).

transfusions in all patients. The authors conclude that rHuEpo might play a role in the management of the anemia of prematurity (3). They also emphasized it would be advantageous to select the infants for whom rHuEpo might be most beneficial. In their opinion the selection should be done at around the age of 1 month, primarily by hematocrit level. That rHuEpo works if started at an earlier age, or even at birth, remains to be determined.

Of the limiting factors, lack of iron seems to be important (see chapters by E. F. Bell and by A. Zipursky, this volume). Of the seven preterm infants, those who remained iron-sufficient during the rHuEpo medication were the best responders (3). It is unlikely that clinical manipulation to improve the iron sufficiency during the first or second month of life would be effective in improving the putative response to rHuEpo in an individual patient. This view is based on the poor anticipated response to oral iron medication in conditions where iron stores are abundant (see chapter by E. F. Bell, this volume). Maternal iron medication is not the solution to the problem, even in preterm infants fed human milk, since neither the concentration of iron in breast milk nor its availability are improved by iron supplements (7). On the other hand, preterm infants have some iron reserves in their tissue stores. The limiting factor may be the rate of mobilization of iron stores soon after birth (8). It has been speculated that the high dose of rHuEpo increases the iron mobilization...
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300

FIG. 2. Reticulocyte count in seven premature infants treated with recombinant human erythropoietin (rHuEpo). Period of treatment (indicated at bottom left corner) extended from day 0 to day 28. rHuEpo was given subcutaneously in doses of 75–300 IU/kg body weight per week (3).

excessively relative to the stores available in very-low-birth-weight infants (3). This hypothesis is based on their finding that the two smallest infants of the group, both of whom received rHuEpo at a high dose of 300 IU per kilogram body weight weekly, developed evidence of iron deficiency (3). Another alternative might be parenteral administration of iron before rHuEpo medication to increase the iron stores for subsequent use. However, this is only a hypothetical possibility which is not supported by any appropriate clinical data in preterm infants.

In preterm infants, rHuEpo medication may specifically stimulate the production of fetal hemoglobin (Fig. 3) (3). This is in agreement with earlier studies in baboons (9). Furthermore, in anemic baboons, administration of low doses of erythropoietin had virtually no effect on production of fetal hemoglobin. When high doses were given, erythropoietin induced dose-dependent fetal cell production (Fig. 4) (10). The authors have speculated that the sudden increase in the level of serum erythropoietin in acute anemia may elicit severe kinetic distortions leading to a vigorous fetal cell response. On the other hand, a steady increase in erythropoietin, as in chronic anemia, may produce only a moderate kinetic distortion of the expanded erythropoiesis. This may result in only minor, if any, stimulation of fetal cells (Fig. 5) (10).

Congenital nephrosis is an inherited disease characterized by proteinuria starting from birth. It may prove to be a model for studying the mechanism regulating erythropoiesis during rHuEpo medication. Before nephrectomy, the patients are nephrotic and have protein deficiency due to protein losses into the urine which cannot be
FIG. 3. Percentage of fetal hemoglobin-containing cells in the infants shown in Fig. 2 (3).

FIG. 4. Induction of F cell formation by recombinant human erythropoietin (rHuEpo) with reference to dose (10).
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Normal

Acute Anemia

Chronic Anemia

FIG. 5. Model of Stamatoyannopoulos et al. of F-cell formation in acute anemia or sudden erythroid regeneration (middle) and in chronic anemia when the erythropoiesis is already expanded (right) (10).

totally compensated for by intravenous and nutritional means. On peritoneal dialysis after nephrectomy the infants initially grow fast, which results in a rapid rise in their blood volume. Thus erythropoiesis must be stimulated to maintain the concentration of hemoglobin. The rapid growth after nephrectomy may thus exceed the capacity of the bone marrow to maintain adequate erythropoiesis, and protein deficiency may continue because protein is needed for the rapid growth and some protein is lost continuously into the dialysate. Seven infants with congenital nephrosis were given rHuEpo, 20–50 IU/kg three times a week subcutaneously, for 14 weeks (11). By the end of this period the red blood cells present should have been produced during the study. Of the seven patients, two showed a response and increased their hemoglobin concentration from 7 to over 10 g/dl. Blood transfusions were not totally avoided. Although the response to rHuEpo, if estimated from the rise in hemoglobin concentration, was generally poor, the response measured as total erythrocyte volume was better (Table 1). In most patients the improvement in hemoglobin concentration was masked by enhanced growth (11). Although all infants were able to decrease their serum ferritin concentrations and also to maintain them within normal limits, most developed evidence of iron deficiency (Table 1). The mean weekly concentrations of serum albumin remained below 3.0 g/dl in five patients, although the average intake of protein was high, with a mean of 2.8 g/kg/day. The authors concluded that under these conditions the response to rHuEpo was not satisfactory, with protein deficiency and rapid growth being the main reasons. It will be interesting to learn whether protein intake also limits the rHuEpo response in the treatment of the anemia of prematurity. Small preterm infants fed human milk, in particular, may be in a similarly marginal state of protein nutrition, which is corrected by doubling the protein intake (12). This doubling of protein intake also prevents the anemia of prematurity in most cases (12). However, the requirement of protein for rHuEpo-stimulated erythropoiesis may be substantial and may compete with other needs for
TABLE 1. Influence of rHuEPO on iron-related blood values in seven infants with CNF who were undergoing peritoneal dialysis after nephrectomy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Hemoglobin (gm/dl)</th>
<th>Serum ferritin (µg/L)</th>
<th>MCV (M)</th>
<th>Serum iron* (mg/dl)</th>
<th>Erythrocyte protoporphyrin† (IU/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>At 14 wk</td>
<td>Baseline</td>
<td>At 14 wk</td>
<td>Baseline</td>
</tr>
<tr>
<td>1</td>
<td>7.5</td>
<td>10.4</td>
<td>180</td>
<td>11</td>
<td>85</td>
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<tr>
<td>2</td>
<td>7.0</td>
<td>7.9</td>
<td>66</td>
<td>34</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>7.6</td>
<td>6.4</td>
<td>61</td>
<td>21</td>
<td>81</td>
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<tr>
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<td>7.0</td>
<td>10.0</td>
<td>80</td>
<td>42</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>6.5</td>
<td>1100</td>
<td>500</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>7.4</td>
<td>7.8</td>
<td>350</td>
<td>42</td>
<td>87</td>
</tr>
<tr>
<td>7</td>
<td>7.6</td>
<td>7.1</td>
<td>780</td>
<td>400</td>
<td>94</td>
</tr>
</tbody>
</table>

Reference values: serum ferritin, 7 to 140 µg/L; mean corpuscular volume, 70 to 80 ft; serum iron, >40 mg/dl; erythrocyte protoporphyrin, <80 IU/dl; MCV, mean corpuscular volume.

* A mean of 14 values taken at 1-week intervals.
† A mean of 2 values at the beginning and end of the study.

protein. The authors speculated that body growth has priority over erythropoiesis with regard to protein use (11). The response in hemoglobin might have been better if they had used a higher dose of rHuEpo. However, it might also have resulted in progression of the general protein deficit and consequently in potential ill effects. Another study on 15 adults with a variety of glomerular diseases fed a high-protein diet containing an average of 162 g of protein per day resulted in increased serum and urinary erythropoietin levels and an increase in reticulocyte count compared to patients given 49 g of protein per day (13). Prostaglandins and plasma renin activity were also higher in patients on the high-protein diet and may have acted as direct mediators of the increased erythropoietin production. The authors speculated that a high-protein diet may effect these changes by increasing renal oxygen consumption (14).

There is increasing evidence that erythropoietin is not the only growth factor necessary for erythropoiesis. The hematopoietic growth factors which possess erythroid burst-promoting activity may include interleukin-3, interleukin-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (14–16). Ohls et al. (17) have detected erythroid burst-promoting activity in the sera of patients with the anemia of prematurity and anemia of end-stage renal disease. In the latter, the burst-promoting activity was completely ablated by anti-GM-CSF antibody. However, in infants with the anemia of prematurity, neither anti-GM-CSF nor anti-interleukin-3 ablated the activity (17). They conclude that these two anemias are not due to an overall deficiency of erythropoietic growth factors but are secondary to a specific deficiency of erythropoietin.

Androgens may also act by stimulating endogenous erythropoietin and by increasing the sensitivity of erythroid progenitors to the available erythropoietin (18). In eight males on chronic maintenance hemodialysis for at least 6 months, androgen
medication with 100 mg of nandrolone decanoate once a week potentiated the effect of rHuEpo medication in the treatment of anemia compared to seven men given only rHuEpo (19). In relation to the early stage of infancy, these findings may be of importance in males because androgens are normally secreted in considerable quantity soon after birth (20). Moreover, in uremic adults, administration of rHuEpo may also stimulate growth hormone release under conditions when growth-hormone-releasing hormone is given together with rHuEpo. This effect was not demonstrable in subjects with normal function (21). The authors conclude that this mechanism might explain the influence of rHuEpo on the growth of children with chronic renal failure (22).

Endogenous production of erythropoietin may continue in the kidneys or even increase in the liver in conditions when the rHuEpo medication is given in infants (23). Molecular charge heterogeneity of human erythropoietin differs in individual serum specimens and may serve as a method which is useful for detecting the presence of injected rHuEpo in the blood samples of patients with endogenous erythropoietin production (24). These techniques may prove to be particularly interesting in future studies on infants; for example, one would anticipate that the induction or reinduction of fetal erythropoiesis may be of significant magnitude in some individuals with anemia of prematurity or end-stage renal failure.

In adults, the cost–benefit analyses of use of rHuEpo suggest that the elimination of transfusions alone could offset from 25% to 50% of costs (25). However, such dollar estimates will only represent a part of the beneficial consequences of rHuEpo. Similar analyses of each specific indication for the use of rHuEpo in infants should also be developed.

REFERENCES


**DISCUSSION**

*Dr. Shaw:* Neonatologists are very interested in maintaining the hemoglobin without resorting to transfusion, so this presentation was of great interest. Is it known what regulates erythropoietin secretion in the fetus? Is there any evidence that it is regulated by the oxygen tension in the blood or oxygen delivery to the tissues?

*Dr. Siimes:* The answers to these questions are not known at present.

*Dr. Shaw:* Is there any evidence that the use of recombinant human erythropoietin stimulates antibody production?

*Dr. Siimes:* We looked for antibodies. No infant in our study developed any.

*Dr. Dallman:* In adults, failure to respond to erythropoietin has often been due to an insufficient supply of iron to support the increased erythropoiesis. This seemed also to be true in the study of preterm infants by Halperin *et al.* (1).

*Dr. Siimes:* The major difference between infants and adults is the rapid growth rate of infants. In our experience, often when we think that iron is the limiting factor in a particular
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We find there is another additional reason. There are many factors that can limit the effectiveness of erythropoietin under these conditions, though iron is the central one.

Dr. Dallman: Shannon and coworkers are conducting trials of erythropoietin in our medical center. The results of the initial trial with low-dose erythropoietin and iron were disappointing. A marked drop in ferritin and serum iron during treatment indicated that iron might have been the limiting factor. A subsequent protocol randomized infants to placebo or a higher dose of erythropoietin (500 units per week). Iron was given at a higher dose than before (6 mg/kg daily) in infants on full feedings and was well tolerated when given in three doses between feeds. The infants had a mean birth weight of about 900 g and were treated quite early—between 4 and 28 days—in order to prevent anemia rather than waiting for it to develop. The results so far are very encouraging, and there are discussions of a multicenter trial with a similar protocol.

Dr. Siimes: In our experience the infants with the lowest serum concentrations of albumin have the worst response, and these may be the infants with the poorest state of protein nutrition. I think that it would be important to study this in addition to examining the role of iron requirements in preterm infants during erythropoietin treatment. Growth should not be ignored since the variation in growth between individual premature infants is enormous, even among relatively healthy infants.

Dr. Ziegler: You suggested that erythropoietin use in preterm infants could be associated with a risk of producing protein deficiency. Could you explain the possible mechanism?

Dr. Siimes: It has been well documented that small preterm infants may have laboratory evidence of protein lack. They have low concentrations of serum albumin which is eliminated when human milk protein supplements are given. Such supplementation also increases hemoglobin concentrations to values close to those found in full-term infants. My worry is that by “artificially” increasing the need for nutritional protein by stimulating the synthesis of erythrocyte hemoglobin, one may produce a more general protein deficiency at a time when supply may be marginal.

Dr. Doyle: I'm not sure that the use of recombinant erythropoietin in preterm infants is justified. I don't think we have any idea of what outcome we should be assessing. I do not believe it is valid to consider a reduction in transfusions as the sole criterion for embarking on this type of treatment, nor do I think it is valid to promote this treatment since there is no evidence that a child with a higher hemoglobin is a healthier child. We have to be very careful about the measurement of outcome, which must relate to the infant's health.

There is the same concern over iron. Halperin's group is now pushing iron supplementation to the limit to try to get erythropoiesis going (1). There is even talk of intravenous iron, a mode of treatment which has not been well studied in this population.

With regard to side effects of erythropoietin, although the hypertensive effect has only been seen in adults with underlying renal disease, it seems likely that there will be some neonates who fall into this category, and the incidence of hypertension in the newborn has not been well studied. Another concern from Halperin's work is neutropenia. A number of Halperin's patients became neutropenic, the mechanism being unknown.

Dr. Shaw: On the subject of intravenous iron, we did a study some years ago using metabolic balance techniques and the chemical measurement of iron (2). The data obtained from this study are consistent with the interpretation that there is no control over iron absorption over the range of oral intakes that we studied. If they are given too much, preterm infants retain more than a fetus in utero, whereas if given too little they retain insufficient amounts. To maintain the hemoglobin mass that prevails in preterm infants without transfusion, a daily intake of about 2.0–2.5 mg/kg is needed. If the use of erythropoietin is aimed at maintaining
an "intrauterine" hemoglobin mass, they might need about 5 mg/kg/day. If more were re-
quired, for example to offset losses due to venesection, our data suggest that it is readily
provided by increasing the oral intake. The data do not support the notion that intravenous
iron would be necessary.

Dr. Zlotkin: I should like to comment on outcome. I disagree with Dr. Doyle that a reduction
in the number of transfusions is not of benefit to the individual infant. There is a definite
risk associated with every transfusion. Risks include viral infections—particularly hepatitis,
AIDS, and CMV—and the possibility of allergic reactions. Transfusions are also expensive.

Dr. Doyle: The risk of AIDS is fortunately very small but the risk of other infections is,
I agree, still real. The risk for allergic reactions in premature infants is virtually zero. Cost-
benefit analyses are always useful for society but should not be put above the individual
interests of a particular child until we can show that the alternative treatment is both safe
and useful.

Dr. Zipursky: I believe that the use of erythropoietin and possibly of iron in preterm infants
may be dangerous. Those of us who recall the experience with other "harmless" therapies
in premature infants—such as vitamin K, vitamin E, and oxygen—will recognize that we
must be very cautious in the use of any new agent in the neonate. With respect to eryth-
ropoietin, we should examine the problems that demand its use. It should be pointed out
that the majority of transfusions given to preterm infants are given in the first 2 weeks of
life, mainly in response to blood sampling. This being the case, erythropoietin could have
little effect on the overall need for transfusions. With regard to subsequent transfusions, we
could make better use of single donors if one large unit of blood were to be broken down
into multiple small units. In this way a single donor could provide blood for treatment during
the first 2 weeks and for any subsequent transfusions that might be required. It should then
not be necessary to use additional donors for infants over 2 weeks of age, and the risks of
transfusion would be proportionately reduced.

Dr. Shaw: Is it not true that levels of erythropoietin in fetal life are very high, so that all
one is trying to do is to reverse some of the effects of premature birth? The fetal marrow is
being driven by these high erythropoietin levels which decline immediately after birth. It
seems to me that erythropoietin should be a useful alternative to transfusion, so there are
good reasons for investigating its use. I agree with the view that we need to know more about
possible hazards.

Dr. Zipursky: It is dangerous to say that any unknown agent is "safe" for newborn infants.

Dr. Dallman: I agree that the transfusions currently given during the first 2 weeks could
not be avoided by erythropoietin treatment. However, many transfusions are given after 2
weeks, and in those infants who are at highest risk of developing anemia it seems unreason-
table to wait for clinically obvious hypoxia as an indication for erythropoietin treatment.

Dr. Doyle: In relation to the infection hazard of transfusions, there are many ways of
handling this in any blood bank. For example, CMV can be eliminated entirely by screening
or by freezing donated blood. However, I should like to ask why transfusions are being given.
We should examine carefully the reasons for transfusion in our units. In our blood bank we
introduced a form 2 years ago on which the indication for transfusion had to be entered. As
a result of this simple device, transfusion frequency fell. The indication for transfusion should
be used as the end point, not the number of transfusions.

Dr. Bell: Our ignorance in this area is vast. We have no good studies showing the efficacy
of any particular transfusion practice, or comparing them with other practices such as eryth-
ropoietin treatment. A lot of work needs to be done. In a nationwide survey of US nurseries
and blood banks the average number of blood transfusions given to babies under 1500 g was
between 9 and 10, most in the first 2–3 weeks. Dr. Zipursky’s suggestion of single donors is a good one. The safety of blood transfusions is obviously acceptable to many neonatologists, but they are not absolutely safe.

*Dr. Viteri:* What do you consider to be the indications for the use of erythropoietin in pediatrics at present?

*Dr. Siimes:* I believe there are three indications. The first is patients with renal failure, though it is clear that there are problems of protein nutrition in this group, particularly in infants with nephrosis. The second indication is the preterm infant, and we have already discussed the issues here. The third indication may be in patients with pancytopenia after chemotherapy. These three clinical indications are clearly all quite different.

*Dr. Finch:* A comment about the use of erythropoietin in infancy: The importance of iron supply in enabling erythropoietin to increase erythropoiesis in adults is well known; in infancy and early childhood, the decreased MCV and serum iron suggests that iron may already be limiting erythropoiesis. If this is the case, erythropoietin may be of limited use.

**REFERENCES**