Increased Hemolysis after Intramuscular Iron Administration in Patients with Paroxysmal Nocturnal Hemoglobinuria

Report of Six Occurrences in Four Patients, and Speculations on a Possible Mechanism

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Paroxysmal nocturnal hemoglobinuria (PNH) is a unique hemolytic anemia characterized by accelerated intravascular hemolysis during sleep. Cross-transfusion studies have shown that the defect responsible for hemolysis is intracorpuscular.1 Most available data are consistent with an abnormality of the red blood cell stroma.2-7 Despite extensive studies of PNH erythrocytes and the extracorpuscular factors necessary for their lysis, the basic mechanism responsible for hemolysis has not been clarified.

Hemosiderinuria, a more constant finding than hemoglobinuria, may account for iron losses as high as 20 mg. in 24 hours,8,9 and iron stores may be rapidly depleted during unusually active phases of the disease. During treatment of the superimposed iron deficiency, increased hemolytic activity has been noted in patients with PNH after administration of iron salts9 and injections of saccharated iron oxide.10

The purpose of this report is (1) to call attention to 4 patients with PNH whose disease activity (manifested by increased hemolysis) was accelerated by the intramuscular administration of iron-dextran, and (2) to postulate a responsible mechanism. All patients have been followed regularly by one of us (C. E. M.) after the diagnosis of PNH was made. Repeated observations of clinical status, routine hematologic indices, and degree of hemoglobinuria have been made at intervals varying from 1 week to 1 month in each patient, and occasionally during brief hospitalizations for observation or treatment.

Case Reports

Case 1

E. T., a 29-year-old woman, was first admitted to Duke University Medical Center in August, 1953, complaining of easy bruisability, shortness of breath, and malaise. Her physical examination at that time was not remarkable. The hemoglobin was 6.1 Gm. per cent, the white blood cell count was 3950/mm.3 with 180,000 platelets/mm.3, and the...
reticulocyte count was 4.1 per cent. The formed elements of the blood appeared normal in stained blood films. Bone marrow aspirates were cellular and contained an increased number of erythroid elements. Diagnostic studies regarding hemolysis or hypersplenism were inconclusive. The patient was not relieved by adrenocorticosteroid or folic acid therapy. In 1955 radical mastectomy for carcinoma of the right breast was performed and thereafter her hemoglobin level remained normal for 2 years. In 1958 the patient first noted that her urine was dark in the morning, and on examination she was found to have a positive Ham acid-serum hemolysis test. She was started on androgen therapy (Halotestin, 15 mg./day, orally) and, subsequently, had only mild early morning hemoglobinuria with infrequent episodes of accelerated disease activity during the next 3 years.

During the last several months she had been completely asymptomatic and denied noticeable hemoglobinuria. During this period the hemoglobin level decreased to 6 Gm. per cent and her erythrocytes became hypochromic. No stainable iron was found in marrow aspirates. She was started on iron-dextran, 200 mg./day, intramuscularly. She complained 4 days later of malaise, nausea, and mild generalized aching, and experienced marked hemoglobinuria, greater in the morning, which persisted throughout the day. Iron therapy was continued on an intermittent (200 mg. every 3-4 days) basis. She experienced malaise and fever and noted increased hemoglobinuria after each injection. Although iron therapy was stopped after 2 weeks, she continued to have increased hemoglobinemia and hemoglobinuria. The hemoglobin level increased to 8 Gm. per cent 3 weeks after the start of iron therapy and during this period her reticulocyte percentage increased from 12 to 40 per cent.

Increased hemoglobinuria persisted and during the next 4 months the hemoglobin level remained stable at 8 Gm. per cent while the reticulocyte count was consistently 35 per cent or greater. Thereafter, over a 3 month period the patient noted a gradual decrease of hemoglobinuria. During this time the hemoglobin fell to 6 Gm. per cent and her erythrocytes became hypochromic and microcytic. The reticulocyte count remained elevated at 35 per cent. Again, no stainable iron was present in marrow aspirates. Five days after starting daily injections of 200 mg. of iron-dextran, she again experienced fever, malaise, and a marked increase of hemoglobinuria. The hemoglobin level increased to 7.5 Gm. per cent over a 2 week period without a significant change of the reticulocyte percentage. The increased hemoglobinuria has persisted for several months.

Case 2

The details of this 15-year-old boy’s illness have been previously reported. He was found to have a positive Ham acid-serum hemolysis test and low erythrocyte acetylcholinesterase activity after a year of intermittent hemoglobinuria which had been preceded by aplastic anemia (pancytopenia and hypoplastic bone marrow). Because of persistent anemia Hb 5 Gm. per cent during a quiescent stage of his illness, hypochromia of erythrocytes in the stained blood film, and an absence of stainable iron in his marrow aspirates, he was started on iron-dextran 200 mg./day, given intramuscularly. Three days later he experienced malaise, low grade fever, nausea, and intense hemoglobinemia and hemoglobinuria (nocturnally accentuated). The reticulocyte percentage was unchanged at 12 per cent. Although this acute episode was controlled by infusion of 6 per cent Dextran, he continued to have a marked increase of hemoglobinuria for 3 months.

Case 3

A. G., a 37-year-old Negro laborer, was found to have a positive Ham acid-serum hemolysis test in 1960. In October, 1962, during an admission to another hospital, the hemoglobin level was 6 Gm. per cent, the erythrocytes were hypochromic and microcytic in stained blood films and bone marrow aspirates contained no stainable iron. The patient received iron-dextran, 100 mg./day, intramuscularly, for 10 days. Seven days after therapy was started, he developed malaise, aching, nausea, fever, and a marked increase
of hemoglobinuria. He was treated with washed red blood cells but continued to experience greater than usual hemoglobinuria thereafter for several months.

In January, 1964, he was admitted to this hospital because of progressive weakness and easy fatigability. The hemoglobin was 5.7 Gm. per cent, hematocrit 23 per cent, and reticulocyte count 17.2 per cent. The erythrocytes in stained blood films appeared hypochromic and bone marrow aspirates contained no stainable iron. He was started on iron-dextran, 200 mg./day, intramuscularly. Six days later he developed fever, malaise, and a marked increase of hemoglobinemia and hemoglobinuria. The hemoglobin which had risen to 6.5 Gm. per cent fell to 5.0 Gm. per cent and the hematocrit fell from 25 to 20 per cent. There was no significant change of the reticulocyte count during iron administration or after the onset of acute hemolytic episode (range 17 to 19 per cent). He received intravenous Dextran and 750 ml. of washed red cells. The acute hemolytic episode was stopped although increased hemoglobinuria (compared to that noted prior to iron therapy) persisted for several months.

Case 4

H. W., a 15-year-old Negro schoolboy, was found to have PNH in August, 1963, after 1 year of aplastic anemia (pancytopenia and hypocellular marrow). The hemoglobin remained at about 8.0 Gm. per cent and the reticulocyte count varied from 11 to 19 per cent. From January, 1964, until April, 1964, he had a gradual decrease of hemoglobinemia and hemoglobinuria, and during May and June there was no noticeable hemoglobinuria. Meanwhile, his hemoglobin had fallen to 6 Gm. per cent and erythrocytes in stained blood films became markedly hypochromic. Bone marrow aspirates contained no stainable iron. In July, 1964, he was started on iron-dextran, 200 mg./day, given intramuscularly. Five days later he acquired marked hemoglobinuria, increased hemoglobinemia, and malaise. The reticulocyte count was 13 per cent prior to iron administration and 16 per cent at the onset of hemolysis. Thereafter the reticulocyte count fluctuated between 14 and 17 per cent. Although the acute episode of hemolysis was controlled with intravenous Dextran, he has continued to have a definite increase of hemoglobinemia and hemoglobinuria compared to that prior to iron therapy.

DISCUSSION

Each of the patients with PNH described above acquired iron deficiency as evidenced by a fall of hemoglobin, progressive development of erythrocyte hypochromia and absence of stainable iron in marrow aspirates. In the absence of other causes for blood loss, this most likely resulted from urinary iron loss subsequent to persistent hemosiderinuria. Each patient received several intramuscular injections of iron-dextran (during a relatively quiescent phase of his illness). Three to 7 days after therapy was begun each patient experienced an increase in the activity of his disease evidenced by fever, malaise, aching, and marked accentuation of nocturnal hemoglobinemia and hemoglobinuria, the latter persisting for several months thereafter. The absence of other causes for increased hemolysis such as infection, the relative quiescence of the disease prior to parenteral iron administration and the similar timing of the hemolytic reaction in each of the patients linked the administration of iron to the accelerated hemolysis.

Since the onset of the hemolysis occurred at a time when reticulocytes would first be expected to appear in the circulation in significant numbers, we first assumed that the iron had stimulated erythropoiesis and supplied an additional increment of “defective” cells to the circulating red cell mass.
with a resultant increase of hemolysis. The observation that most of the intravenously\textsuperscript{12,13} or intramuscularly\textsuperscript{14} injected dose of radioiron reappears in circulating erythrocytes between 3 and 8 days would be consistent with this hypothesis. Studies have suggested the existence of two populations of erythrocytes in patients with PNH, one which was very susceptible to destruction and the other which had a normal survival.\textsuperscript{2} This phenomenon was also apparent from in vitro studies and there was some in vivo and in vitro evidence that PNH reticulocytes were more susceptible to destruction than older cells.\textsuperscript{15,16} In our patients, however, the reticulocyte percentage was noted to be significantly increased after iron therapy in only one of the five episodes of accelerated hemolysis where the determination was done. In addition, in case 1 the reticulocyte percentage increased in only one of the two episodes of iron-exacerbated disease.

The failure of the reticulocyte count to increase after administration of iron did not rule out the possibility of increased production of reticulocytes and increased reticulocyte destruction. The life span of “susceptible” reticulocytes may have been only a few moments and if so, they would not have been represented in samples of blood taken for counting.

The fact that we have never seen similar hemolytic reactions in any other patients who have received intramuscular iron-dextran therapy suggested a unique direct effect of iron on PNH erythrocytes. Iron carbohydrate complexes have been demonstrated to cause hemolysis in vitro.\textsuperscript{17} Although the concentrations of iron in those experiments were greater than those expected to occur during in vivo therapy, the data suggested that hemolysis was caused by ionized iron. Addition of ferrous ions enhanced hemolysis and the addition of chelating agents inhibited hemolysis.

While exploring the effects of iron-overloading in animals, Goldberg, et al.\textsuperscript{18} demonstrated increased levels of “lipid peroxides” in animal tissues injected with an iron-dextran complex. The role of iron in accelerating the formation of “lipid peroxides” from unsaturated fatty acids has been recognized for several years.\textsuperscript{19,20} Peroxidation of unsaturated fatty acids takes place as a nonenzymatic reaction in the presence of ferrous iron and oxygen.\textsuperscript{21,22} A scheme of this type of reaction is shown in figure 1. Tissues which contain an excess of unsaturated fatty acids or pro-oxidants or lack normal antioxidants should form unusually high quantities of “lipid peroxides” under proper conditions. Cell damage might then occur directly from oxidation of cellular lipid components or indirectly from the toxic effect of the peroxides formed on other metabolic systems.\textsuperscript{23,25}

Although not confirmed by all investigators, PNH erythrocytes have been reported to contain increased quantities of arachidonic and pentanoic acids and reduced quantities of oleic acid.\textsuperscript{6,7,27} If they contain unusually high quantities of the more unsaturated fatty acids, PNH erythrocytes would have an enhanced tendency to form “lipid peroxides” and the administration of iron in proper amounts might be expected to initiate lysis of susceptible erythrocytes. Similar effects could occur if PNH erythrocytes or plasma contained increased pro-oxidants or decreased anti-oxidants. The accelerated
hemolysis in our patients began at a time when iron replenished erythrocytes would be appearing in the circulation in significant numbers and when the serum iron concentration would be expected to be at its peak. Therefore, although no serum iron levels were determined in our patients, the onset of symptoms and increased hemolysis occurred when a rapid increase in the surrounding and internal iron concentration of the patient's erythrocytes would be expected.

The observation by Ham that the intravenous injection of 1 Gm. of ascorbic acid into a patient with PNH precipitated a severe hemolytic episode was of particular interest since current data support the concept that ascorbic acid (or similar reducing agents) reduces ferric iron to the ferrous state thus allowing the cycle of ferrous iron reaction with unsaturated fatty acid to continue. That erythrocytes from irradiated mice became unusually sensitive to acidified serum (a feature of PNH erythrocytes) further suggested a relationship between iron accelerated hemolysis in PNH and peroxidation of lipids since the latter has been demonstrated in tissues exposed to ionizing and ultraviolet radiation.

That PNH activity in our patients became quiescent as they became iron deficient and remained accelerated for several months when iron stores were repleted suggested a chronic (perhaps underlying) role of iron in the PNH hemolytic mechanism in addition to the acute effect shown in our patients.

Recent studies in this laboratory have shown that PNH erythrocytes were unusually sensitive to the lytic effect of agents capable of peroxidizing erythrocyte lipid (at pH 7.45), and did form greater quantities of lipid peroxides.
than normal erythrocytes. Similar changes were found in erythrocytes of mice after repeated injections of iron-dextran or ferrous iron salts.

The adverse in vitro effects of "lipid peroxides" on proteins, enzymes, and subcellular particles have been well documented.19,25,32 We have previously showed that hemolysis in mice and man during exposure to oxygen under high pressure was directly or indirectly caused by peroxidation of erythrocyte lipid.33-37

Our hypothesis could explain iron accelerated hemolysis in PNH and does provide new areas for further investigation of the basic PNH hemolytic mechanisms. It also emphasizes the need for further consideration of the in vivo role of lipid peroxidation in red cell damage.

SUMMARY

Four patients with PNH were described who developed accelerated activity of their disease after the intramuscular administration of iron-dextran for treatment of iron deficiency. This was considered to reflect a unique effect of iron on PNH erythrocytes. It was postulated that this effect resulted from cell damage caused by iron catalyzed peroxidation of erythrocyte lipids, a reaction to which PNH erythrocytes could be unusually susceptible by virtue of an increased content of unsaturated fatty acids or pro-oxidants or decreased content of antioxidants.

ACKNOWLEDGMENTS

We wish to express our grateful appreciation to Dr. R. Wayne Rundles for critical review of the manuscript, to Norma Lester and Sandra Humn for technical assistance, and to Patricia Johnson for aid in preparation of the manuscript.

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